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**Short Communication**

# Use of highway culverts by the water opossum (*Chironectes minimus*) in southeastern Brazil

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***Abstract:*** The water opossum (*Chironectes minimus*) is a semi-aquatic mammal that is infrequently sampled in Atlantic rainforest areas in Brazil. Here we report on new records of *C. minimus* in the state of São Paulo, southeastern Brazil, and comment on its behavior and ecology. We placed nine camera traps in culverts and cattle boxes under a highway, between 2017 and 2019. From a total of 6,750 camera-trap-days, we obtained 16 records of *C. minimus* (0.24 records/100 camera-trap-days) in two cameras placed in culverts over streams. Most of the records were made between May and August, in the dry season and in the first six hours after sunset. The new records are from a highly degraded area with some riparian forests. The records lie approximately 30 km away from the nearest protected area where the species is known to occur. We suggest that *C. minimus* has some tolerance to degraded habitats, as long as the water bodies and riparian forests are minimally preserved. The new records presented here also fill a distribution gap in western São Paulo state.
**Keywords:** Atlantic rainforest; camera traps; Didelphidae; wildlife passages.

## Uso de drenagens fluviais sob rodovias pela cuíca d'agua (*Chironectes minimus*) no sudeste do Brasil

***Resumo:*** A cuíca d'agua (*Chironectes minimus*) é um mamífero semiaquático pouco amostrado em áreas de Mata Atlântica do Brasil. Neste estudo apresentamos novos registros de *C. minimus* no estado de São Paulo, sudeste do Brasil, e comentamos sobre seu comportamento e ecologia. Nós instalamos nove armadilhas fotográficas em drenagens fluviais e passagens de gado sob uma rodovia, entre 2017 e 2019. De um total de 6.750 armadilhas-fotográficas-dia, obtivemos 16 registros de *C. minimus* (0,24 registros/100 armadilhas-fotográficas-dia) em duas armadilhas fotográficas instaladas em drenagens fluviais. A maioria dos registros foram feitos entre maio e agosto, na estação seca, e nas seis primeiras horas da noite. Os novos registros são de uma área altamente degradada que possui algumas matas ciliares. Os registros estão a aproximadamente 30 km da área protegida mais próxima onde a espécie já foi registrada. Nós sugerimos que *C. minimus* possui certa tolerância a hábitats degradados, contanto que os corpos d'agua e as matas ciliares estejam minimamente preservados. Os novos registros aqui apresentados também preenchem uma lacuna amostral no oeste de São Paulo.
**Palavras-chave:** armadilhas fotográficas; Didelphidae; Mata Atlântica; passagens de fauna.

## Introduction

Our understanding of the ecology (Galliez et al. 2009, Fernandez et al. 2015) and distribution (Ardente et al. 2013, Brandão et al. 2015) of the water opossum (*Chironectes minimus*) has substantially improved in recent years. It is now assumed that *C. minimus* is continuously distributed from southern Mexico southwards to northern Argentina and southern Brazil (Stein & Patton 2008, Astúa 2015). In Brazil, *C. minimus* occurs in every biome, except in the dry forests of the Caatinga (Melo & Sponchiado 2012, Brandão et al. 2015). In the Atlantic Forest biome, most of the locality records are near the Atlantic coast (Stein & Patton 2008, Melo & Sponchiado 2012). In São Paulo state, southeastern Brazil, it is known from one historical (Pelzeln 1883) and five recent localities (Carvalho 1965, Nogueira et al. 2004, Prada 2004, Breviglieri & Pedro 2010, Faria & Pires 2010), in the Cerrado and Atlantic Forest biomes.

Most of the information on the ecology of *C. minimus* comes from studies carried out in Rio de Janeiro, southeastern Brazil, which have shed light on the home length, population density, use of shelters, and activity pattern of the species (Galliez et al. 2009, Leite et al. 2013, Fernandez et al. 2015, Leite et al. 2016). These and other reports show that *C. minimus* uses water bodies with sandy or stony substrates, fast-flowing water, and with well-preserved riparian forests (Palmeirim et al. 2014, Voss et al. 2001, Bressiani & Graipel 2008). Although vehicle collisions are rare, a single roadkilled *C. minimus* was recorded among 444 roadkilled mammals in southern Brazil (Coelho et al. 2008), and in São Paulo state one *C. minimus* was found among 184 roadkilled mammals (Prada 2004). A likely explanation for this relatively low number of roadkills is that, due to its association with water bodies, *C. minimus* rarely ventures across dry and usually elevated paved highways. Therefore, we expect that culverts under highways that have perennial water flow may increase the permeability of *C. minimus* in anthropized landscapes.

Recent papers have demonstrated that camera trapping methods can document a greater abundance of some mammals than what might be detected using conventional survey methods, such as transects and active search for footprints (Tobler et al. 2008, O'Brien et al. 2003, Gregory et al. 2015). In the case of *C. minimus*, specimen capture requires unconventional live traps set at specific places, such as narrow streams (Bressiani & Graipel 2008). Given its elusiveness, camera trapping at strategic places might reveal more data on the ecology of the species (Oliveira-Santos et al. 2008). In road ecology studies, camera traps set at underpasses are often used in conjunction with tracks and roadkill monitoring (Grilo et al. 2008, González-Gallina et al. 2018). Therefore, such studies, besides assessing the highway impacts on mammals, might also be important to record poorly known species.

Here we report on a new locality of *C. minimus* for the state of São Paulo in southeastern Brazil, comment on its behavior as revealed in the camera trap videos and discuss on the importance of highway underpasses for the species persistence in anthropized areas.

## Material and Methods

The study site is in western São Paulo state, southeastern Brazil, in the counties of Caiuá and Presidente Venceslau (Figure 1).

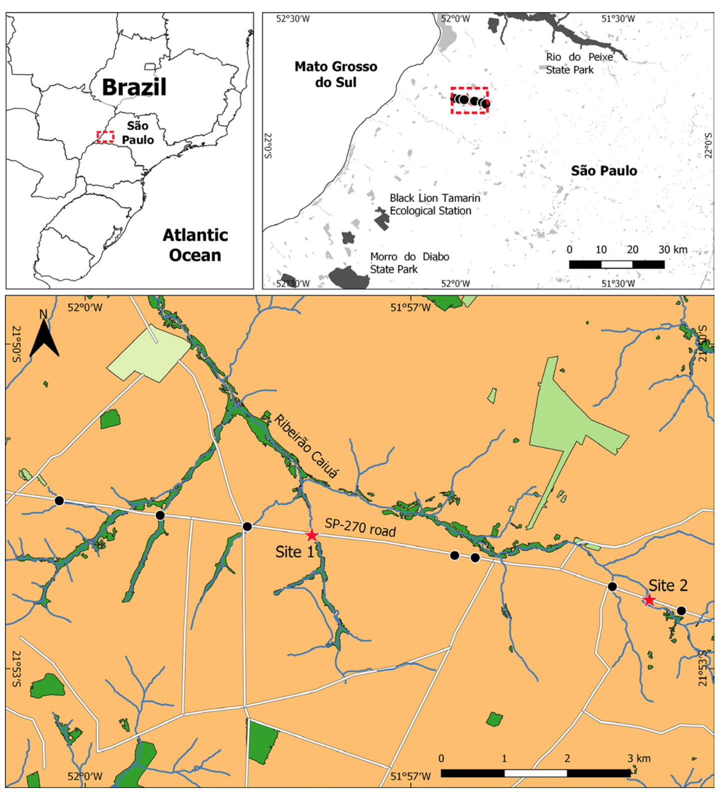


**Figure 1.** Study site, showing its location in Brazil and in the state of São Paulo (protected areas are in dark gray and unprotected forest fragments are in light gray). A total of nine camera-trap stations (black dots and red stars) were implemented, of which two (red stars) recorded *C. minimus* (Site 1 – 21°51'34.18"S; 51°57'54.69"W and Site 2 – 21°52'10.05"S; 51°54'48.16"W). Camera traps were placed in underpasses over streams that fall in the Ribeirão Caiuá. In the larger map, orange represent anthropized areas, light green are eucalyptus plantations, dark green are native forest fragments, blue lines are streams, and white lines represent the road network.

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Originally, the area was predominantly composed of seasonal forests of the Atlantic rainforest, considered one of the world's biodiversity hotspot (Mittermeier et al. 2011). However, most of the original forest has been cut down and the remaining forest fragments are generally small (< 100 ha), restricted to riparian forests, and interspersed with sugarcane plantations and pasture (Uezu 2006, Ribeiro et al. 2009, Rezende 2014). The site is near four large protected areas (> 6,000 ha each), to the north there are the Rio do Peixe State Park (ca. 33 km to the northeast) and the Aguapeí State Park (ca. 80 km to the northeast) (Faria & Pires 2010). Approximately 47 km to the southwest is the Black Lion Tamarin Ecological Station (6,600 ha; Valladares Padua 2007), and further south (ca. 70 km) is the Morro do Diabo State Park (33,000 ha), the largest fragment of seasonal forest in São Paulo state (Faria & Pires 2006, Rezende 2014).

This study is part of a faunal monitoring project aimed at investigating the impact of highways in wildlife. In order to verify the use of small streams by wildlife, we monitored nine underpasses, including cattle boxes and culverts, along 10.7 kilometers of the SP-270 state highway (Figure 1, Table S1). Out of the nine monitored structures, *C. minimus* was recorded in two culverts, hereafter Site 1 (21°51'34.18"S; 51°57'54.69"W), and Site 2 (21°52'10.05"S; 51°54'48.16"W). The underpasses in Sites 1 and 2 were circular structures with width and height of approximately 2 meters. Following the rivers, the two sites with *C. minimus* records are 6.7 km apart and are both crossed by streams which form the Ribeirão Caiuá (main Caiuá river), a small stream that falls in the large Rio Paraná, approximately 19 km to the northwest. The two sampled streams have flowing water, sandy substrate, and a depth of water varying from approximately 0.2 m to 1 m along different seasons.

We placed nine Bushnell™ Aggressor No-Glow 20 MegaPixel camera traps in the middle of the passage structures aiming to detect wildlife crossing under the road. All cameras were in the field full time on and once per month the memory cards and batteries were replaced. Survey period was from November 2017 to November 2019. The highway was monitored for roadkilled animals during the same period.

All novel data on *Chironectes minimus* presented here were gathered from camera-traps. Records were considered as independent if the time between consecutive videos was more than 1 hour apart, following Tobler et al. (2008). To evaluate the activity period of *C. minimus*, data from the whole sampling period were pooled by hours after sunset and divided in four different periods of the night, following Galliez et al. (2009).

## Results

There were 16 independent camera trapping records (19 movies) of *Chironectes minimus* over 6,750 camera-trap-days (750 in each of the nine stations), for a rate of 0.24 records/100 camera-trap-days (Table 1). Other animals observed in the videos that captured *C. minimus* were capybaras (*Hydrochoerus hydrochaeris*), and unidentified bats and frogs, but they did not interact with the water opossum. The records of *C. minimus* at Sites 1 and 2 occurred between 19:00 and 4:00 hours, with 10 out of 16 (62%) made in the first six hours after sunset (Figure 2A). Most of the records (81%; 13 of 16) were made in the dry season (INMET 2019), between May and August (Figure 2B). No intraspecific interactions were observed, as only a single animal was present in every video. In the highway monitoring, no roadkilled *C. minimus* were recorded. Behaviors recorded include walking on dry substrate (N=14), walking on shallow water (N=9), swimming (N=4), entering water from dry substrate (N=5), and eating (N=1) (Figure 3; Table 1).

**Table 1.** Date, time, and observed behavior of the *Chironectes minimus* individuals filmed at Ribeirão Caiuá, São Paulo, Brazil. EAT = eating; ENT = entering water form dry substrate; SHA = walking on shallow water; SWI = swimming; WAL = walking on dry substrate.

| Camera trap station | Date | Time | Behavior(s) observed |
|---|---|---|---|
| Site 1 | May 8, 2018 | 22:46 | EAT, SHA |
| Site 1 | May 8, 2018 | 23:05 | ENT, SHA, WAL |
| Site 1 | May 13, 2018 | 03:57 | SHA, WAL |
| Site 1 | May 13, 2018 | 21:29 | ENT, SHA, WAL |
| Site 1 | May 17, 2018 | 01:21 | SHA, SWI |
| Site 1 | May 17, 2018 | 01:25 | SHA, SWI |
| Site 1 | May 18, 2018 | 21:29 | SHA, WAL |
| Site 1 | May 18, 2018 | 22:22 | SWI |
| Site 1 | Jun 4, 2018 | 21:04 | WAL |
| Site 1 | Jul 6, 2018 | 20:53 | WAL |
| Site 1 | Aug 5, 2018 | 20:53 | WAL |
| Site 1 | Aug 6, 2018 | 01:32 | WAL |
| Site 1 | Aug 6, 2018 | 02:12 | ENT, SWI, WAL |
| Site 1 | Aug 6, 2018 | 20:15 | WAL |
| Site 1 | Aug 11, 2018 | 00:04 | ENT, WAL |
| Site 1 | Aug 11, 2018 | 02:49 | ENT, SHA, WAL |
| Site 1 | Nov 16, 2019 | 00:47 | WAL |
| Site 1 | Nov 18, 2019 | 20:46 | WAL |
| Site 2 | Mar 4, 2018 | 19:47 | SHA |

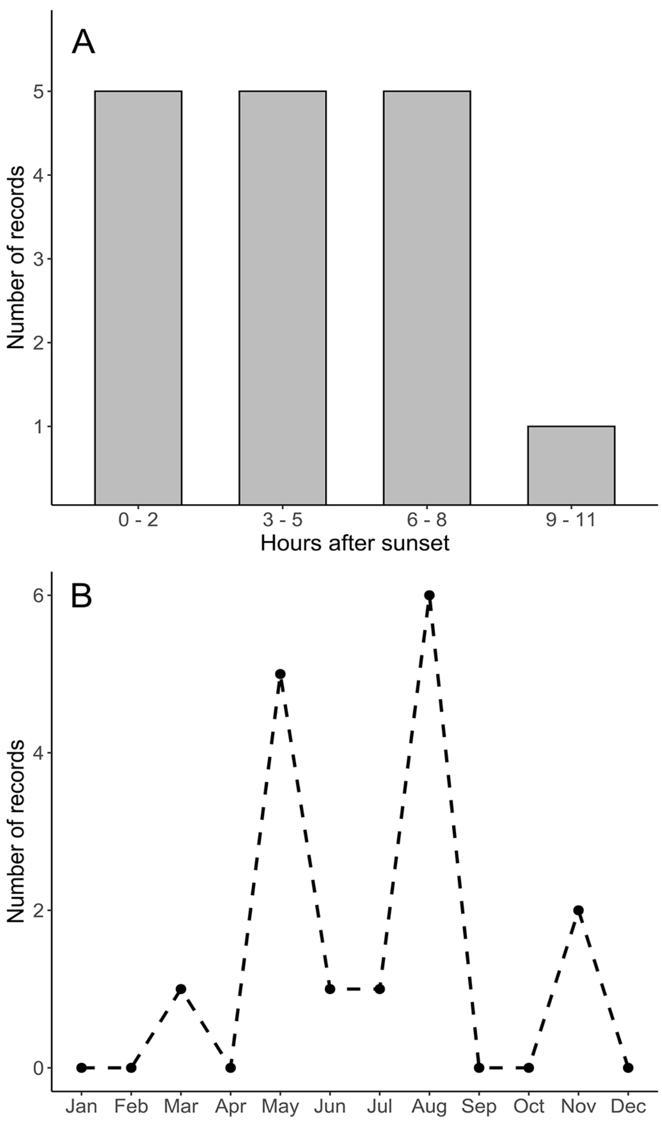


**Figure 2.** A) Activity pattern of *Chironectes minimus* in the Ribeirão Caiuá, São Paulo, Brazil. Number of records are based on camera trapping between November 2017 and November 2019; B) Monthly camera trap records of *Chironectes minimus* in Ribeirão Caiuá, São Paulo, Brazil, obtained between November 2017 and November 2019.

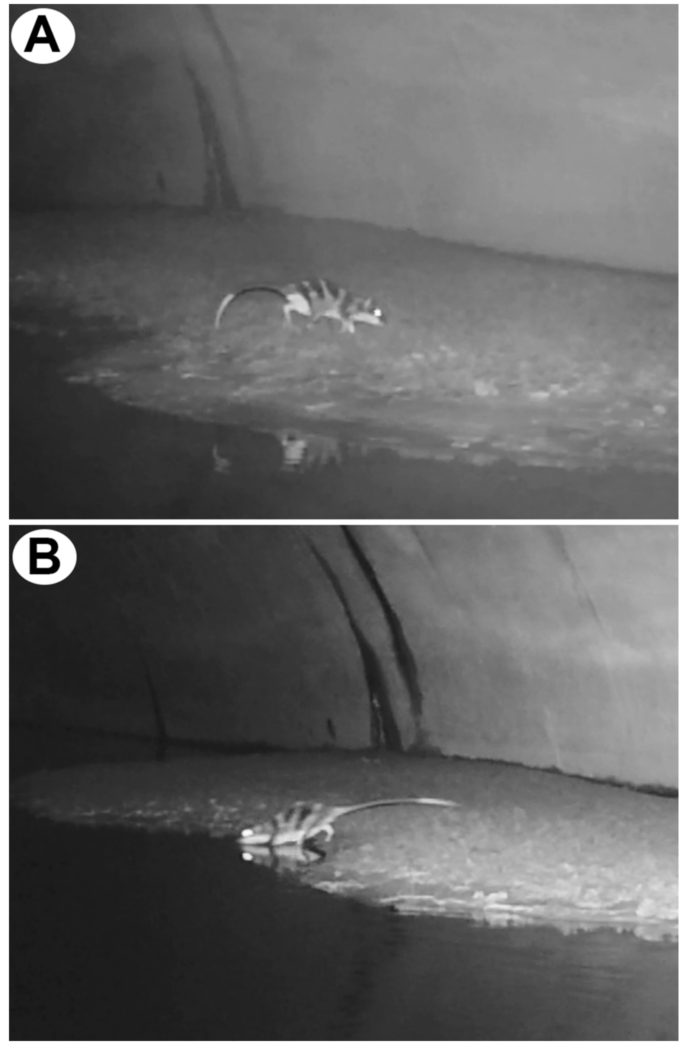


**Figure 3.** Behaviors recorded for *Chironectes minimus*: walking on dry substrate (A) and entering water (B).

## Discussion

Activity period inferred from the camera trap records agree with Galliez et al. (2009) and Leite et al. (2013), who recorded most of the activities of *Chironectes minimus* in the first half of the night. Leite et al. (2013) also observed a difference in seasonal activity, with males more active than females during the dry season. Here, records were more frequent in the dry season, but we could not determine the sex of the filmed individuals. Considering that the home length of *C. minimus* may vary between 0.8 and 9.6 km but few individuals have home lengths larger than 6.7 km (Fernandez et al. 2015, Leite et al. 2016), it is likely that the records reported herein represent at least two individuals.

The closest previous records of *C. minimus* are from relatively well-protected areas in Rio Pardo (state of Mato Grosso do Sul), approximately 37 km to the west (Melo & Sponchiado 2012), and Rio do Peixe State Park (São Paulo state), approximately 30 km to the north (Faria & Pires 2010). However, the geographical coordinates given for the Rio Pardo locality (21°46'S, 52°09'W; Stein and Patton, 2008) refer to the mouth of the river, and the actual record could have come from further upstream. Therefore, the only reliable closest record is the one from Rio do Peixe State Park, which was confirmed by the authors based on a photograph of a specimen from "Córrego do Prado", a tributary of the Rio do Peixe (Nelson Gallo, *in litt.*).

The absence of roadkill records of *C. minimus* is congruent with previous studies (Prada 2004, Coelho et al. 2008, Caires et al. 2019, Magioli et al. 2019), even though the recorded individuals frequently used areas adjacent to the studied highway. Vehicle collision rate seems to be low for *C. minimus* even when compared with other semi-aquatic mammals such as the capybara (*Hydrochoerus hydrochaeris*) and the Neotropical otter (*Lontra longicaudis*) (Coelho et al. 2008, Huijser et al. 2013, Magioli et al. 2019). A plausible explanation for the rarity of roadkills is that *C. minimus* seems to never leave watercourses during its daily movements (Leite et al. 2016).

Although *C. minimus* is currently listed as "Least Concern" by the IUCN Red List (Pérez-Hernandez et al. 2016), mainly due to its wide distribution, the species may be locally rare and vulnerable (e.g. Conselho Estadual de Política Ambiental 2010) and some of the populations seem to be decreasing (Pérez-Hernandez et al. 2016).

Therefore, we suggest that streams crossing highways through culverts can mitigate highway impacts on *C. minimus* by promoting connectivity and reducing the mortality risk of the species.

Our results indicate that *C. minimus* can tolerate a moderate level of disturbance, as long as the water bodies and riparian forests are minimally preserved. Conversely, the relatively close protected areas may act as refuges for the species in the region, even though there are no direct river connections between the study area and the Rio do Peixe State Park, the nearest protected area. Finally, we reinforce the importance, in road ecology studies, of setting camera traps at strategic locations when sampling for taxa that have specific habitat requirements.

## Supplementary Material

The following online material is available for this article:

Table S1 - Geographical coordinates of the nine camera-trap stations placed at underpasses of the SP-270 highway in São Paulo state, Brazil.

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## Author Contributions

Paula Ribeiro Prist: 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.

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

Fernanda Delborgo Abra: 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.

Thais Pagotto: Contribution to data collection.

Osnir Ormon Giacon: Contribution to data collection.

## Conflicts of Interest

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

## Ethics

This study did not involve animal experimentation or collecting.

## Data Availability

Every information necessary to replicate this study is present in the manuscript text.

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**Inventory**

# Inventory of the fish fauna from Laranjinha River, Paranapanema River system, Brazil

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***Abstract:*** This work is the most comprehensive survey of the Laranjinha River´s fishes, a tributary of the Cinzas River, Paranapanema River basin. Throughout its course, there is only a low-height dam, including a transposition system located 98 km from its mouth. The sampling was carried out in nine locations, from the source to the mouth, with six field incursions in each location, using different fishing gear. A total of 11,924 fish were collected, distributed in seven orders, 27 families, and 100 species. The most representative order in the number of species was Siluriformes, followed by Characiformes. As for the families, Loricariidae comprised 21% and Characidae 14% of species richness. *Phalloceros harpagos* was the species with the highest absolute abundance, representing 11.3% of the total, followed by *Hypostomus ancistroides* with 9.8%. However, considering the average abundance and frequency of occurrence, *Hypostomus ancistroides* was the most abundant species, followed by *Hypostomus* cf. *paulinus*, *Psalidodon* aff. *paranae* and *Phalloceros harpagos*. Among the collected species, the *Apteronotus acidops*, *Brycon orbygnianus*, *Brycon nattereri*, *Crenicichla jupiaensis*, and *Rhinelepis aspera* were classified as endangered on the most recent IUCN Red List. Also, from the total sampled fish, 9.8% are considered non-native species. Among the native species recorded, 10 species are large migratory species, which indicates that the Laranjinha River is a route for spawning and maintenance of species diversity in the middle Paranapanema River. Therefore, the Laranjinha River is a heritage of fish diversity and deserves special attention in its preservation.
***Keywords:*** *Checklist; fish diversity; freshwater; upper Paraná River.*

## Inventário da ictiofauna do rio Laranjinha, sistema do rio Paranapanema, Brasil

***Resumo:*** Este é o levantamento mais abrangente de peixes do rio Laranjinha, um afluente do rio das Cinzas, bacia do rio Paranapanema. Ao longo de sua rota, existe apenas uma pequena barragem com um sistema de transposição localizado 98 km de sua nascente. A amostragem foi realizada em nove locais, desde a nascente até a foz, com seis incursões de campo em cada local, com o auxílio de diferentes artes de pesca. Foram coletados 11.924 indivíduos, distribuídos em sete ordens, 27 famílias e 100 espécies. A ordem mais representativa foi Siluriformes, seguida por Characiformes. Quanto às famílias, Loricariidae compôs 21% e Characidae 14% da riqueza de espécies. *Phalloceros harpagos* foi a espécie com maior abundância absoluta, representando 11,3% do total, seguida por *Hypostomus ancistroides*, com 9,8%. No entanto, considerando a abundância média e a frequência de ocorrência, *Hypostomus ancistroides* foi a espécie mais abundante, seguida por *Hypostomus* cf. *paulinus*, *Psalidodon* aff. *paranae* e *Phalloceros harpagos*. Entre as espécies coletadas, *Apteronotus acidops*, *Brycon orbygnianus*, *Brycon nattereri*, *Crenicichla jupiaensis* e *Rhinelepis aspera* estão listadas em categorias de ameaça na Lista Vermelha da IUCN mais recente.

Além disso, do total de indivíduos amostrados, 9,8% são considerados espécies não nativas. Entre as espécies nativas registradas, 10 espécies são migratórias de grande porte, o que indica que o rio Laranjinha é uma rota de desova e manutenção da diversidade de espécies no médio rio Paranapanema. Portanto, o rio Laranjinha é um patrimônio da diversidade de peixes e merece atenção especial em sua preservação.

***Palavras-chave:*** *Água doce; alto rio Paraná; diversidade de peixes; lista de espécies.*

## Introduction

In the last few decades, human actions have caused numerous environmental changes, which emphasizes the need to know the local biodiversity, in order to reduce harmful activities. To this extend, the creation of inventories contribute to the discovery and description of new species before their extinction, also contributing to the creation of new records, to the knowledge about geographical distribution, the documentation of non-native species, the defininition of biogeographic patterns, and the establishment of suitable conservation strategies (Costello et al. 2011, Ota et al. 2015, Frota et al. 2019).

The fishes of the upper Paraná River basin are the most studied in Brazil. The basin has more than 310 fish species (Langeani et al. 2007), a number currently outdated, considering new records and the recent discovery of new species (e.g. Frota et al. 2016, Froehlich et al. 2017, Cavalli et al. 2018, Ota et al. 2018, Jarduli et al. 2020). The Paranapanema River, a major tributary of the upper Paraná River, is currently fragmented by several hydroelectric dams (Britto & Carvalho 2006), and therefore, its dam-free tributaries are of great importance for the maintenance of fish species (Hoffmann et al. 2005, Silva et al. 2017, Galindo et al. 2019, Lansac-Tôha et al. 2019). Besides, the Cinzas River is an important tributary of the Paranapanema River basin, which is the main watercourse of the region known as "Norte Pioneiro" (Pioneer North) of the Paraná state, and it is essential for maintaining ichthyofauna biodiversity of the Capivara reservoir (Vianna & Nogueira 2008, Orsi 2010).

Fish inventories were carried out in the Paranapanema River tributaries, including the Cinzas, Jaguariaíva and Tibagi rivers (e.g., Shibatta et al. 2002, Castro et al. 2003, Cetra et al. 2012, Cionek et al. 2012, Costa et al. 2013, Hoffmann et al. 2005, Cetra et al. 2016, Frantine-Silva et al. 2015, Almeida et al. 2018, Jerep & Shibatta 2017, Claro-García et al. 2018, Frota et al. 2020, Jarduli et al. 2020, Garcia et al. 2020). However, a comprehensive checklist of the Laranjinha River ichthyofauna has not been published yet, except for a small streams (e.g., Costa et al. 2013), and the scientific knowledge of its fish species remains unknown. Furthermore, projects of Small Hydropower Plants (SHPs) are being developed, which represents a potential threat to the species of this river (Galindo et al. 2019). Thus, this study aimed to provide the first inventory of the Laranjinha River fish fauna.

## Material and Methods

### *1. Study area*

The Laranjinha River is entirely situated in the northeastern portion of the state of Paraná, and it is the main tributary of the left bank of the Cinzas River, which pours to the left bank of the middle Paranapanema River (Figure 1 and 2). The headwater is in the municipality of Ventania (24°14'43.43"S; 50°14'32.78"W), at 984 m of altitude, and the mouth is situated 4 km from the town of Itambaracá (23°01'03.51"S; 50°24'22.68"W), at 348 m of altitude.

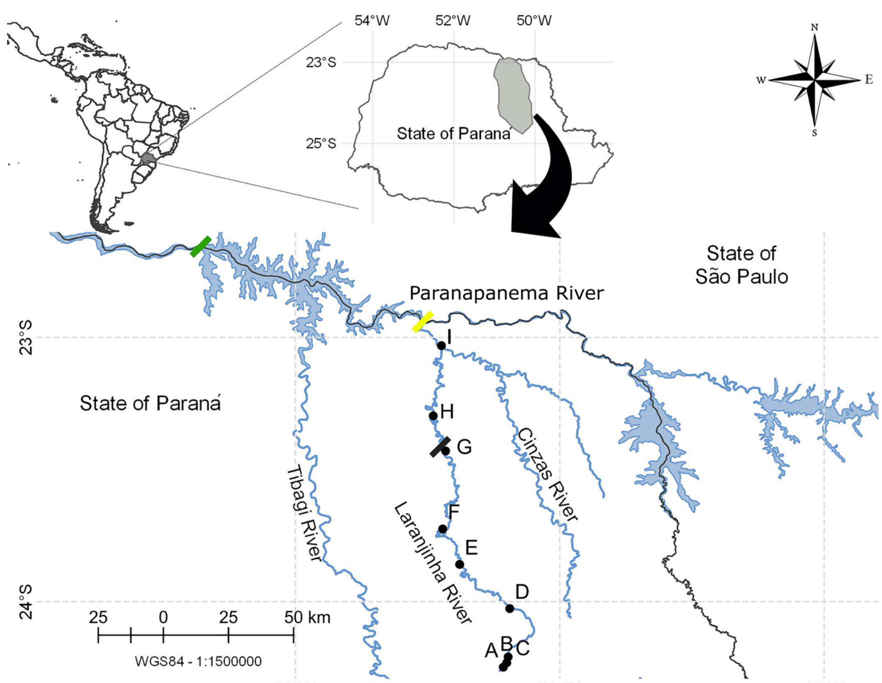


**Figure 1.** Map of the points sampled along the Laranjinha river (black circles). The green rectangle represents the Capivara reservoir, and the yellow rectangle represents the Canoas I reservoir, both on the Paranapanema River basin.


**Figure 2.** Partial view of collection points along the Laranjinha River, illustrating the environments and marginal vegetation. Points near source (a-c), points in the middle portion (d-f), and places near the mouth (g-i).

The Laranjinha River, with its meanders, extends through 350 km long, and it is situated in the sedimentary basin of the state of Paraná, with its source and mouth in the second and third plateaus, respectively (Santos et al. 2006). Between the years 1956 and 1960, a Small Hydropower Plant (SHP) was built 98 km from the mouth of the river. This dam never went into operation, and in 2006, a fish pass system was built in it (Schwartz 2006; Figure 2G).

## 2. *Data collection*

Nine sites for sampling were distributed from the source to the mouth of the Laranjinha River (Figures 1 and 2; Table 1). Six collections were carried out in each site, with the aid of different fish gears (seines, gill nets, cast nets and sieves), from October 2010 to April 2012. The collections were authorized by SISBIO (Sistema de Autorização e Informação em Biodiversidade), Ministério do Meio Ambiente, under the nº 23315-1.

After the capture of the fish, the specimens were anesthetized with 10% benzocaine. This substance promotes a reduction in gill ventilation due to the depression of spinal respiratory centers, promoting a decrease in blood flow through the gills (Mattson & Riple 1989; Tytler & Hawkins 1981). After performing the opercular movements, the fish were fixed in 10% formalin and preserved in 70% ethanol in the Laboratório de Genética e Conservação of the Universidade Estadual do Norte do Paraná (GECON/UENP-CCP) and identified in species-level. The validity of the species was checked using Fricke et al. (2019), and the classification was based using Van der Laan et al. (2020). The species were identified following Ota et al. (2018), Jarduli et al. (2020), Terán et al. (2020), and in the lack of information about the species from the authors before mentioned, personal communication with specialists took place (i.e., FC Jerep, CDCM de Santana, CAM Oliveira and CH Zawadzki).

The record of *Poecilia reticulata* Peters 1859 and *Imparfinis schubarti* (Gomes 1956) were based on Costa et al. (2013). Non-native species were based in Orsi & Agostinho (1999), Lobón-Cerviá & Bennemann (2000), Langeani et al. (2007), Júlio-Júnior et al. (2009), Britton & Orsi (2012), Ortega et al. (2015), Azevedo-Santos et al. (2016), Ota et al. (2018), Pelicice et al. (2018) and Jarduli et al. (2020) (Table 2). Vouchers of all species were deposited in the Museu de Zoologia of the Universidade Estadual de Londrina (MZUEL). The abundances of orders, families, and species were conducted using the Statistica 7.0 software (StatSoft Inc. 2011). The Kendeigh index of abundance (1944) of each species was calculated as KI = √FO.M; where KI is the abundance index, and FO is the frequency of occurrence calculated as the number of sites where the species were captured, and divided by the total number of sites multiplied by 100, and M is the mean number of specimens of each species. The classification of species was done by sorting the values in descending order. The dominance index and evenness were calculated with the program PAST v. 2.17c (Hammer et al. 2001).

**Table 1.** Description of the sampled sites in the Laranjinha river, a tributary of the Paranapanema River, Paraná. "Localities from which coordinates were not originally georeferenced were determined through Google Earth, and are approximate."

| Site | Locality | City | Coordinates | Environment Description |
|---|---|---|---|---|
| A | Pedreira | Ventania | 24°15'8.55"S 50°12'1.35"W | The riparian vegetation is well-preserved on both river banks; substrate predominantly sandy and slabs; average width of approximately 4m. Near pastureland and agriculture areas. |
| B | Cachoeira | Ventania | 24°14'37.57"S 50°12'24.48"W | The riparian vegetation is well-preserved on both river banks; substrate predominantly rocks and sand; average width of approximately 4m. Eight kilometers above receives a massive load of domestic effluents. |
| C | SANEPAR | Ventania | 24°13'24.56"S 50°11'57.88"W | The riparian vegetation is well-preserved on the right river bank, a predominance of grass in the left river bank; mostly sedimentary and clayey substrate; average width of approximately 5m; great anthropic disturbance by the presence of a bridge and rural road. |
| D | Distrito da Moquém | Ventania | 24°01'36.6"S 50°11'21.6"W | The riparian vegetation is well-preserved on both river banks (showing the best conditions among all stretches studied); some rocky walls, and predominantly sandy bottom; average width of approximately 13m. Stretch including a lake between a rapid and a small waterfall. It is inserted in a private property for exotic tree planting for logging. |
| E | Figueira | Figueira | 23°51'34.73"S 50°22'43.04"W | The riparian vegetation is absent on both river banks (mostly grass); mainly rocky and sandy substrate; average width of approximately 14m. It is located in pastureland and agriculture areas, upstream of a thermal power plant. |
| F | Ibaiti | Ibaiti | 23°43'31.88"S 50°26'34.42"W | The riparian vegetation is reduced on the left bank (significantly modified by agricultural activity) while the right bank is well-preserved; average width of approximately 40m; some rapids and predominantly rocky and sandy substrate. It is located upstream of a sugar and ethanol plant and surrounded by agriculture areas. |
| G | Barragem da Corredeira | Ribeirão do Pinhal | 23°17'49.95"S 50°28'43.27"W | Riparian vegetation is present on both river banks, well-preserved, alternating sections with grass predominance stretches; includes a dam separating an upstream lake (average width of approximately 63m) and downstream stretch including rapids and some deeper wells (average width of 48m); sand and clayey substrate in the lake and a rocky substrate along downstream stretch; surrounded by pastureland and agriculture areas. |
| H | Santa Amélia | Santa Amélia | 23°24'53.06"S 50°27'8.60"W | The riparian vegetation is poorly preserved on both river banks; large rapids and some deeper wells; predominantly rock substrate; average width of approximately 55m; surrounded by pastureland and agriculture areas. |
| I | Foz | Bandeirantes | 23°1'53.90 S 50°26'51.90"W | The riparian vegetation is poorly preserved on both river banks; some upstream rapids, however, a calm water segment prevails until the confluence between Laranjinha and Cinzas Rivers, including a rocky and sandy (primary) substrate; average width of approximately 48m. It is surrounded by pastureland and agriculture areas. |

**Table 2.** List of species captured along the Laranjinha River (sites A to I, see Figures 1 and 2, and Table 1 for location and characterization), Paraná, Brazil, and their respective catch abundance. ♠ Non-native species, according to, Langeani et al. (2007), Júlio-Júnior et al. (2009), Ota et al. (2018), Jarduli et al. (2020) and Orsi personal communication. ♦ Additional species recorded in the basin by Costa et al. (2013).

| | Sampled Sections | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Táxon** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **Total** | **FO** | **FO%** | **Mean** | **FO%*Mean** | **KI** | **Voucher** |
| **CYPRINIFORMES** | | | | | | | | | | | | | | | | |
| **Cyprinidae** | | | | | | | | | | | | | | | | |
| *Cyprinus carpio* Linnaeus 1758♠ | | | | | | | 2 | | | 2 | 1.0 | 11.1 | 0.2 | 2.46 | 1.6 | MZUEL 10559 |
| **CHARACIFORMES** | | | | | | | | | | | | | | | | |
| **Crenuchidae** | | | | | | | | | | | | | | | | |
| *Characidium* aff. *zebra* | | | | | | 4 | 19 | 1 | | 24 | 3.0 | 33.3 | 2.7 | 88.88 | 9.4 | MZUEL 09350 |
| **Erythrinidae** | | | | | | | | | | | | | | | | |
| *Hoplias* gr. *malabaricus* | | | 1 | 6 | 17 | 62 | 6 | 2 | 29 | 123 | 7.0 | 77.8 | 13.7 | 1062.96 | 32.6 | MZUEL 19806 |
| **Parodontidae** | | | | | | | | | | | | | | | | |
| *Apareiodon ibitiensis* Campos 1944 | | 1 | | 4 | | 1 | 10 | | 2 | 18 | 5.0 | 55.6 | 2.0 | 111.11 | 10.5 | MZUEL 16063 |
| *Apareiodon piracicabae* (Eigenmann 1907) | | | | 35 | 45 | 44 | 91 | 79 | 143 | 437 | 6.0 | 66.7 | 48.6 | 3237.03 | 56.9 | MZUEL 09257 |
| *Parodon nasus* Kner 1859 | | | | | | | 25 | 2 | | 27 | 2.0 | 22.2 | 3.0 | 66.66 | 8.2 | MZUEL 09365 |
| **Serrasalmidae** | | | | | | | | | | | | | | | | |
| *Metynnis lippincottianus* (Cope 1870) ♠ | | | | | | | | 1 | 6 | 7 | 2.0 | 22.2 | 0.8 | 17.28 | 4.2 | MZUEL 09519 |
| *Piaractus mesopotamicus* (Holmberg 1887) | | | | | | | 3 | | 6 | 9 | 2.0 | 22.2 | 1.0 | 22.22 | 4.7 | MZUEL 09471 |
| *Serrasalmus maculatus* Kner 1858 | | | | | | | 32 | 1 | 14 | 47 | 3.0 | 33.3 | 5.2 | 174.07 | 13.2 | MZUEL 16059 |
| **Anostomidae** | | | | | | | | | | | | | | | | |
| *Leporellus vittatus* (Valenciennes 1850) | | | | | | | 2 | 2 | | 4 | 2.0 | 22.2 | 0.4 | 9.87 | 3.1 | MZUEL 09556 |
| *Leporinus amblyrhynchus* Garavello & Britski 1987 | | | | 20 | 23 | 9 | 43 | 11 | 15 | 121 | 6.0 | 66.7 | 13.4 | 896.29 | 29.9 | MZUEL 09262 |
| *Leporinus friderici* (Bloch 1794) | | | | | | | 3 | 9 | 43 | 55 | 3.0 | 33.3 | 6.1 | 203.70 | 14.3 | MZUEL 09455 |
| *Leporinus octofasciatus* Steindachner 1915 | | | | 5 | 3 | 12 | 10 | | 16 | 46 | 5.0 | 55.6 | 5.1 | 283.95 | 16.9 | MZUEL 09440 |
| *Leporinus striatus* Kner 1858 | | | | | | 15 | | 17 | 10 | 42 | 3.0 | 33.3 | 4.7 | 155.55 | 12.5 | MZUEL 09520 |
| *Megaleporinus obtusidens* (Valenciennes 1837) | | | | | | | 10 | 1 | 7 | 18 | 3.0 | 33.3 | 2.0 | 66.66 | 8.2 | MZUEL 09431 |
| *Schizodon borellii* (Boulenger 1900) | | | | | 4 | 1 | | 1 | 79 | 85 | 4.0 | 44.4 | 9.4 | 419.75 | 20.5 | MZUEL 09467 |
| *Schizodon nasutus* Kner 1858 | | | | | 25 | 29 | 49 | 2 | 16 | 121 | 5.0 | 55.6 | 13.4 | 746.91 | 27.3 | MZUEL 09356 |
| **Curimatidae** | | | | | | | | | | | | | | | | |
| *Cyphocharax modestus* (Fernández-Yépez 1948) | | | | 27 | 4 | 75 | 1 | 1 | 17 | 125 | 6.0 | 66.7 | 13.9 | 925.92 | 30.4 | MZUEL 09448 |
| *Steindachnerina insculpta* (Fernández-Yépez 1948) | | | | 2 | | 12 | 109 | 12 | 37 | 172 | 5.0 | 55.6 | 19.1 | 1061.72 | 32.6 | MZUEL 09451 |
| **Prochilodontidae** | | | | | | | | | | | | | | | | |
| *Prochilodus lineatus* (Valenciennes 1837) | | | | | 120 | 40 | 29 | 4 | 4 | 197 | 5.0 | 55.6 | 21.9 | 1216.04 | 34.9 | MZUEL 09437 |
| **Triportheidae** | | | | | | | | | | | | | | | | |
| *Triportheus nematurus* (Kner 1858) ♠ | | | | | | | | 1 | 43 | 44 | 2.0 | 22.2 | 4.9 | 108.64 | 10.4 | MZUEL 09458 |

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| Táxon | Sampled Sections A | B | C | D | E | F | G | H | I | Total | FO | FO% | Mean | FO%*Mean | KI | Voucher |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bryconidae** | | | | | | | | | | | | | | | | |
| Bryconinae | | | | | | | | | | | | | | | | |
| *Brycon nattereri* Günther 1864 | | | | 82 | 5 | | | | | 87 | 2.0 | 22.2 | 9.7 | 214.81 | 14.7 | MZUEL 09470 |
| *Brycon orbygnianus* (Valenciennes 1850) | | | | | | | 50 | | | 50 | 1.0 | 11.1 | 5.6 | 61.728 | 7.9 | MZUEL 09485 |
| Salmininae | | | | | | | | | | 0 | | | | | | |
| *Salminus brasiliensis* (Cuvier 1816) | | | | | | | | 1 | | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09527 |
| *Salminus hilarii* Valenciennes 1850 | | | | | | | 1 | | | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09518 |
| **Acestrorhynchidae** | | | | | | | | | | | | | | | | |
| *Acestrorhynchus lacustris* (Lütken 1875) | | | | | | | 56 | 38 | 61 | 155 | 3.0 | 33.3 | 17.2 | 574.07 | 24.0 | MZUEL 09543 |
| **Characidae** | | | | | | | | | | | | | | | | |
| Stethaprioninae | | | | | | | | | | | | | | | | |
| *Astyanax lacustris* (Lütken 1875) | | | 1 | 18 | 42 | 96 | 242 | 106 | 134 | 639 | 7.0 | 77.8 | 71.0 | 5522.22 | 74.3 | MZUEL 09538 |
| *Hyphessobrycon eques* (Steindachner 1882) ♠ | | | | | | | | | 12 | 12 | 1.0 | 11.1 | 1.3 | 14.81 | 3.8 | MZUEL 09464 |
| *Moenkhausia* aff. *intermedia* | | | | | | | | | 157 | 157 | 1.0 | 11.1 | 17.4 | 193.82 | 13.9 | MZUEL 09457 |
| *Oligosarcus paranensis* Menezes & Géry 1983 | | | | 45 | 13 | 18 | | | | 76 | 3.0 | 33.3 | 8.4 | 281.48 | 16.8 | MZUEL 09534 |
| *Psalidodon bockmanni* (Vari & Castro 2007) | | | 1 | 36 | 70 | 7 | 4 | 21 | 17 | 156 | 7.0 | 77.8 | 17.3 | 1348.14 | 36.7 | MZUEL 09354 |
| *Psalidodon* aff. *fasciatus* | | | | 201 | 9 | 11 | 86 | 11 | 14 | 332 | 6.0 | 66.7 | 36.9 | 2459.25 | 49.6 | MZUEL 09541 |
| *Psalidodon* aff. *paranae* | 571 | 134 | 70 | 14 | 9 | 22 | 2 | 1 | | 823 | 8.0 | 88.9 | 91.4 | 8128.39 | 90.2 | MZUEL 09441 |
| Characinae | | | | | | | | | | | | | | | | |
| *Galeocharax gulo* (Cope 1870) | | | | | | | 118 | 42 | 43 | 203 | 3.0 | 33.3 | 22.6 | 751.85 | 27.4 | MZUEL 09259 |
| Aphyocharacinae | | | | | | | | | | | | | | | | |
| *Aphyocharax* cf. *dentatus* Eigenmann & Kennedy 1903 ♠ | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09463 |
| Cheirodontinae | | | | | | | | | | | | | | | | |
| *Odontostilbe weitzmani* Chuctaya, Bührnheim & Malabarba 2018 | | | | | 7 | 3 | | | | 10 | 2.0 | 22.2 | 1.1 | 24.69 | 5.0 | MZUEL 09557 |
| *Serrapinnus notomelas* (Eigenmann 1915) | | | | | 26 | 48 | 2 | | | 76 | 3.0 | 33.3 | 8.4 | 281.48 | 16.8 | MZUEL 09435 |
| Stevardiinae | | | | | | | | | | | | | | | | |
| *Bryconamericus* aff. *iheringii* (Boulenger 1887) | | | | 13 | 112 | 19 | 18 | 98 | | 260 | 5.0 | 55.6 | 28.9 | 1604.93 | 40.1 | MZUEL 09528 |
| *Piabarchus* aff. *stramineus* | | | | | 2 | 4 | 2 | 15 | | 23 | 4.0 | 44.4 | 2.6 | 113.58 | 10.7 | MZUEL 09433 |
| *Piabina argentea* Reinhardt 1867 | | | | | | | 2 | 5 | | 7 | 2.0 | 22.2 | 0.8 | 17.28 | 4.2 | MZUEL 09348 |
| **GYMNOTIFORMES** | | | | | | | | | | | | | | | | |
| **Apteronotidae** | | | | | | | | | | | | | | | | |
| *Apteronotus acidops* Triques 2011 | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | |
| *Apteronotus* aff. *albifrons* (Linnaeus 1766) | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09558 |
| *Apteronotus* cf. *caudimaculosus* Santana 2003 ♠ | | | | | | | | 3 | | 3 | 1.0 | 11.1 | 0.3 | 3.70 | 1.9 | MZUEL 09538 |

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| Táxon | A | B | C | D | E | F | G | H | I | Total | FO | FO% | Mean | FO%*Mean | KI | Voucher |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sampled Sections | | | | | | | | | | | | | | | |
| **Sternopygidae** | | | | | | | | | | | | | | | | |
| *Eigenmannia* sp. | | | | | 3 | 2 | 4 | 3 | 11 | 23 | 5.0 | 55.6 | 2.6 | 141.97 | 11.9 | MZUEL 09552 |
| *Sternopygus macrurus* (Bloch & Schneider 1801) | | | | | | | 1 | 5 | 3 | 9 | 3.0 | 33.3 | 1.0 | 33.33 | 5.8 | MZUEL 09454 |
| **Gymnotidae** | | | | | | | | | | | | | | | | |
| *Gymnotus sylvius* Albert & Fernandes-Matioli 1999 | | | 2 | | 1 | 10 | 9 | 4 | 7 | 33 | 6.0 | 66.7 | 3.7 | 244.44 | 15.6 | MZUEL 09546 |
| *Gymnotus omarorum* Richer-de-Forges, Crampton & Albert 2009 | | | | | | 10 | | | | 10 | 1.0 | 11.1 | 1.1 | 12.34 | 3.5 | MZUEL 09517 |
| **SILURIFORMES** | | | | | | | | | | | | | | | | |
| **Trichomycteridae** | | | | | | | | | | | | | | | | |
| *Cambeva diabola* (Bockmann, Casatti & de Pinna 2004) | 26 | 169 | 8 | 9 | 3 | | | | | 215 | 5.0 | 55.6 | 23.9 | 1327.16 | 36.4 | MZUEL 09516 |
| **Callichthyidae** | | | | | | | | | | | | | | | | |
| *Callichthys callichthys* (Linnaeus 1758) | | | | | | | 1 | | | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09535 |
| *Corydoras aeneus* (Gill 1858) | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09547 |
| *Hoplosternum littorale* (Hancock 1828) | | | | | | | 3 | | | 3 | 1.0 | 11.1 | 0.3 | 3.70 | 1.9 | MZUEL 09544 |
| **Loricariidae** | | | | | | | | | | | | | | | | |
| Rhinelepinae | | | | | | | | | | | | | | | | |
| *Rhinelepis aspera* Spix & Agassiz 1829 | | | | | | | | | 12 | 12 | 1.0 | 11.1 | 1.3 | 14.81 | 3.8 | MZUEL 09549 |
| Loricariinae | | | | | | | | | | | | | | | | |
| *Loricariichthys platymetopon* Isbrücker & Nijssen 1979 ♠ | | | | | | | | 10 | 38 | 48 | 2.0 | 22.2 | 5.3 | 118.51 | 10.9 | MZUEL 09460 |
| *Proloricaria prolixa* Isbrücker & Nijssen 1978 | | | | | | | | 22 | 15 | 37 | 2.0 | 22.2 | 4.1 | 91.35 | 9.6 | MZUEL 09525 |
| *Rineloricaria latirostris* (Boulenger 1900) | | | | 3 | 2 | 2 | | 1 | | 8 | 4.0 | 44.4 | 0.9 | 39.50 | 6.3 | MZUEL 09530 |
| *Rineloricaria pentamaculata* Langeani & de Araujo 1994 | | | 1 | | | | | 1 | 2 | 4 | 3.0 | 33.3 | 0.4 | 14.81 | 3.8 | MZUEL 09545 |
| Hypoptopomatinae | | | | | | | | | | | | | | | | |
| *Curculionichthys insperatus* (Britski & Garavello 2003) | | | | | 45 | | | | | 45 | 1.0 | 11.1 | 5.0 | 55.55 | 7.5 | MZUEL 09469 |
| *Neoplecostomus yapo* Zawadzki. Pavanelli & Langeani 2008 | 35 | 29 | 5 | 2 | | | | | | 71 | 4.0 | 44.4 | 7.9 | 350.61 | 18.7 | MZUEL 09542 |
| *Otothyropsis biamnicus* Calegari, Lehmann A. & Reis 2013 | | | | 23 | 2 | | | | | 25 | 2.0 | 22.2 | 2.8 | 61.72 | 7.9 | MZUEL 09071 |
| Hypostominae | | | | | | | | | | | | | | | | |
| *Ancistrus* cf. *cirrhosus* (Valenciennes 1836) | | | | | | | 4 | 3 | 2 | 9 | 3.0 | 33.3 | 1.0 | 33.33 | 5.8 | MZUEL 09522 |
| *Hypostomus albopunctatus* (Regan 1908) | | | | 11 | 1 | | 88 | 19 | | 119 | 4.0 | 44.4 | 13.2 | 587.65 | 24.2 | MZUEL 09362 |
| *Hypostomus ancistroides* (Ihering 1911) | | | 299 | 305 | 232 | 140 | 113 | 30 | 51 | 1170 | 7.0 | 77.8 | 130.0 | 10111.11 | 100.6 | MZUEL 09450 |
| *Hypostomus* cf. *paulinus* (Ihering 1905) | | | 6 | 246 | 91 | 86 | 194 | 244 | 141 | 1008 | 7.0 | 77.8 | 112.0 | 8711.11 | 93.3 | MZUEL 09480 |
| *Hypostomus* cf. *topavae* (Godoy 1969) | | | 1 | 1 | 1 | 40 | 32 | 74 | 56 | 205 | 7.0 | 77.8 | 22.8 | 1771.60 | 42.1 | MZUEL 09355 |
| *Hypostomus hermanni* (Ihering 1905) | | | | | | | 73 | 166 | 162 | 401 | 3.0 | 33.3 | 44.6 | 1485.18 | 38.5 | MZUEL 09363 |
| *Hypostomus iheringii* (Regan 1908) | | | | | | 2 | | | 13 | 15 | 2.0 | 22.2 | 1.7 | 37.03 | 6.1 | MZUEL 09473 |
| *Hypostomus nigromaculatus* (Schubart 1964) | | | | 29 | 5 | 6 | 9 | 9 | 7 | 65 | 6.0 | 66.7 | 7.2 | 481.48 | 21.9 | MZUEL 09361 |
| *Hypostomus regani* (Ihering 1905) | | | | | | | 11 | 5 | 5 | 21 | 3.0 | 33.3 | 2.3 | 77.77 | 8.8 | MZUEL 09524 |
| *Hypostomus* cf. *strigaticeps* (Regan 1908) | | | | 267 | 77 | 93 | 210 | 119 | 36 | 802 | 6.0 | 66.7 | 89.1 | 5940.74 | 77.1 | MZUEL 09366 |
| *Megalancistrus parananus* (Peters 1881) | | | | | | | | 5 | 5 | 10 | 2.0 | 22.2 | 1.1 | 24.69 | 5.0 | MZUEL 09526 |
| *Pterygoplichthys ambrosettii* (Holmberg 1893) ♠ | | | | | | | | 1 | 7 | 8 | 2.0 | 22.2 | 0.9 | 19.75 | 4.4 | MZUEL 09555 |

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| Táxon | A | B | C | D | E | F | G | H | I | Total | FO | FO% | Mean | FO%*Mean | KI | Voucher |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sampled Sections | | | | | | | | | | | | | | | |
| **Aspredinidae** | | | | | | | | | | | | | | | | |
| *Bunocephalus larai* Ihering 1930 | | | | | | | | 3 | 1 | 4 | 2.0 | 22.2 | 0.4 | 9.87 | 3.1 | MZUEL 09521 |
| **Auchenipteridae** | | | | | | | | | | | | | | | | |
| *Ageneiosus militaris* Valenciennes 1835 | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09459 |
| *Glanidium cesarpintoi* Ihering 1928 | | | | | | | | 1 | | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09539 |
| *Tatia neivai* (Ihering 1930) | | | 1 | | | 1 | 3 | 11 | 2 | 18 | 5.0 | 55.6 | 2.0 | 111.11 | 10.5 | MZUEL 09533 |
| **Doradidae** | | | | | | | | | | | | | | | | |
| *Rhinodoras dorbignyi* (Kner 1855) | | | | | | | | | 13 | 13 | 1.0 | 11.1 | 1.4 | 16.04 | 4.0 | MZUEL 09550 |
| **Heptapteridae** | | | | | | | | | | | | | | | | |
| *Cetopsorhamdia iheringi* Schubart & Gomes 1959 | | | | | | | 3 | | | 3 | 1.0 | 11.1 | 0.3 | 3.70 | 1.9 | MZUEL 09483 |
| *Imparfinis mirini* Haseman 1911 | | | | 1 | 2 | | 13 | 3 | 1 | 20 | 5.0 | 55.6 | 2.2 | 123.45 | 11.1 | MZUEL 09553 |
| *Imparfinis schubarti* (Gomes 1956)♦ | | | | | | | | | | | | | | | | |
| *Phenacorhamdia tenebrosa* (Schubart 1964) | | | | | | | 2 | | | 2 | 1.0 | 11.1 | 0.2 | 2.46 | 1.6 | MZUEL 09484 |
| *Pimelodella meeki* Eigenmann 1910 | | | | 1 | 3 | 8 | | | | 12 | 3.0 | 33.3 | 1.3 | 44.44 | 6.7 | MZUEL 09532 |
| *Rhamdia quelen* (Quoy & Gaimard 1824) | | | 36 | 5 | 4 | 3 | 4 | 2 | 14 | 68 | 7.0 | 77.8 | 7.6 | 587.65 | 24.2 | MZUEL 09548 |
| **Pimelodidae** | | | | | | | | | | | | | | | | |
| *Iheringichthys labrosus* (Lütken 1874) | | | | 4 | 16 | 37 | 24 | 16 | 44 | 141 | 6.0 | 66.7 | 15.7 | 1044.44 | 32.3 | MZUEL 09551 |
| *Megalonema platanum* (Günther 1880) | | | | | | | | | 5 | 5 | 1.0 | 11.1 | 0.6 | 6.17 | 2.5 | MZUEL 09474 |
| *Pimelodus maculatus* Lacépède 1803 | | | | 1 | | | 14 | 8 | 28 | 51 | 4.0 | 44.4 | 5.7 | 251.85 | 15.9 | MZUEL 09445 |
| *Pimelodus microstoma* Steindachner 1877 | | | | 57 | 6 | 9 | 103 | 39 | 7 | 221 | 6.0 | 66.7 | 24.6 | 1637.03 | 40.5 | MZUEL 09360 |
| *Pimelodus paranaensis* Britski & Langeani 1988 | | | | | | | 8 | 2 | | 10 | 2.0 | 22.2 | 1.1 | 24.69 | 5.0 | MZUEL 09438 |
| *Pinirampus pirinampu* (Spix & Agassiz 1829) | | | | | | | | | 4 | 4 | 1.0 | 11.1 | 0.4 | 4.93 | 2.2 | MZUEL 09475 |
| *Pseudoplatystoma corruscans* (Spix & Agassiz 1829) | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09461 |
| *Sorubim lima* (Bloch & Schneider 1801) ♠ | | | | | | | | | 3 | 3 | 1.0 | 11.1 | 0.3 | 3.70 | 1.9 | MZUEL 09462 |
| **Pseudopimilodidae** | | | | | | | | | | | | | | | | |
| *Rhyacoglanis paranensis* Shibatta & Vari 2017 | | | | | | | 115 | | | 115 | 1.0 | 11.1 | 12.8 | 141.97 | 11.9 | MZUEL 14120 |
| **SYNBRANCHIFORMES** | | | | | | | | | | | | | | | | |
| **Synbranchidae** | | | | | | | | | | | | | | | | |
| *Synbranchus marmoratus* Bloch 1795 | | | | | | | 1 | | | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09439 |
| **CICHLIFORMES** | | | | | | | | | | | | | | | | |
| **Cichlidae** | | | | | | | | | | | | | | | | |
| *Australoheros tavaresi* Ottoni 2012 | | | | | 1 | | | | | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09447 |
| *Crenicichla britskii* Kullander 1982 | | | | | | | | 4 | 9 | 13 | 2.0 | 22.2 | 1.4 | 32.09 | 5.7 | MZUEL 09453 |
| *Crenicichla jaguarensis* Haseman 1911 | | | | | | | 7 | 8 | 22 | 37 | 3.0 | 33.3 | 4.1 | 137.03 | 11.7 | MZUEL 09261 |
| *Crenicichla jupiaensis* Britski & Luengo 1968 | | | | | | | | | 1 | 1 | 1.0 | 11.1 | 0.1 | 1.23 | 1.1 | MZUEL 09468 |
| *Geophagus iporangensis* (Haseman 1911) | | | 11 | 182 | 238 | 183 | 3 | 10 | | 627 | 6.0 | 66.7 | 69.7 | 4644.44 | 68.2 | MZUEL 09353 |
| *Oreochromis niloticus* (Linnaeus 1758) ♠ | 2 | 1 | | | | | 3 | 7 | 1 | 14 | 5.0 | 55.6 | 1.6 | 86.41 | 9.3 | MZUEL 09482 |

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| Táxon | A | B | C | D | E | F | G | H | I | Total | FO | FO% | Mean | FO%*Mean | KI | Voucher |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sampled Sections | | | | | | | | | | | | | | | |
| **CYPRINIDONTIFORMES** | | | | | | | | | | | | | | | | |
| **Poeciliidae** | | | | | | | | | | | | | | | | |
| *Phalloceros harpagos* Lucinda 2008 | 278 | | 1043 | | 27 | 3 | | | | 1351 | 4.0 | 44.4 | 150.1 | 6671.60 | 81.7 | MZUEL 09429 |
| *Poecilia reticulata* Peters 1859♦♠ | | | | | | | | | | | | | | | | |
| Number of species | 5 | 5 | 15 | 31 | 38 | 38 | 57 | 60 | 64 | | | | | | | |
| Dominance | 0.49 | 0.42 | 0.54 | 0.12 | 0.1 | 0.07 | 0.06 | 0.08 | 0.05 | | | | | | | |
| Evenness | 0.49 | 0.52 | 0.17 | 0.39 | 0.39 | 0.49 | 0.43 | 0.34 | 0.46 | | | | | | | |

## Results

A total of 11.934 specimens were collected, comprising seven orders, 27 families, and 100 species (Table 2; Appendix 1 - 5). Among these, the most representative order was Siluriformes (44.5%), followed by Characiformes (38.6%), Gymnotiformes (6.9%), and Cichliformes (5.9%). Cyprinodontiformes, Cypriniformes, and Synbranchiformes were represented by only one specimen each. Regarding the families, Loricariidae had 20 species (21%) and Characidae 16 species (14%). Together, these families showed the highest species richness (representing 35% of total richness), followed by Pimelodidae and Anostomidae, both composed of eight species each (8%), Heptapteridae and Cichlidae, both with six species each (6%), and Bryconidae with four species (4%; Figure 3).

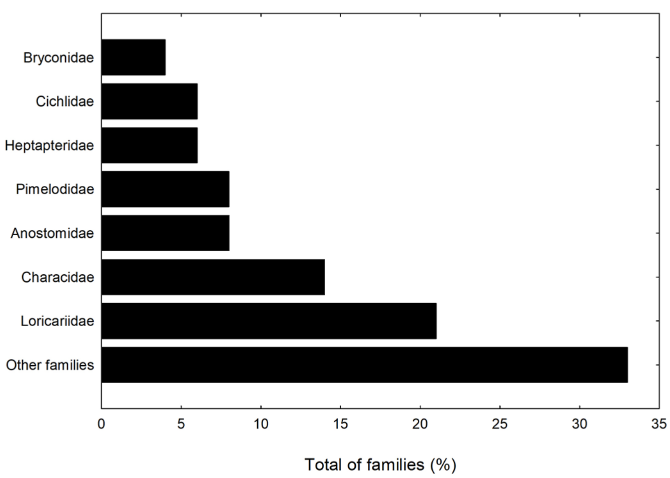


**Figure 3.** Percentage composition of representative families of the ichthyofauna from the Laranjinha River, Upper Parana River basin, Paraná State, Brazil.

Among the sampling sites, A and B showed the lowest number of species (five species each), while sampling site I had a higher number of species (64 species). There is a progressive tendency of species richness from the source to the mouth ($r^2 = 0.964$). The dominance (D) is higher in the upper Laranjinha River (sites A, B, and C showing D = 0.49, 0.42, and 0.54, respectively), and this dominance drops expressively on the sites D (0.12) until I (0.05).

The highest absolute abundance was observed in *Phalloceros harpagos* Lucinda 2008 (11.3%), *Hypostomus ancistroides* (Ihering 1911) (9.8%), *Hypostomus* cf. *paulinus* (Ihering 1905) (8.4%), *Psalidodon* aff. *paranae* (6.9%), *Hypostomus* cf. *strigaticeps* (Regan 1908) (6.7%), and *Astyanax lacustris* (Lütken 1875) (5.3%; Table 2). Concerning the frequency of occurrence, the most frequent species of Laranjinha River were *Psalidodon* aff. *paranae* (FO = 88.8%), *Hoplias* gr. *malabaricus*, *Astyanax lacustris*, *Psalidodon bockmanni* (Vari & Castro 2007), *Rhamdia quelen* (Quoy & Gaimard 1824), *Hypostomus ancistroides*, *Hypostomus* cf. *paulinus*, and *Hypostomus topavae* (Godoy 1969) (FO = 77.7%). The classification of species from Kendeigh abundance index highlights the following ranking: *Hypostomus ancistroides*, *Hypostomus* cf. *paulinus*, *Psalidodon* aff. *paranae*, *Phalloceros harpagos* Lucinda 2008, *Hypostomus* cf. *strigaticeps*, and *Astyanax lacustris*. Among these species, *Psalidodon* aff. *paranae* and *Phalloceros harpagos* were frequent and dominant in the headwater region (sites A, B, and C), while the others were frequent downstream those sites.

From all recorded species, 9.8% were non-native: *Aphyocharax* cf. *dentatus* Eigenmann & Kennedy 1903, *Apteronotus* cf. *caudimaculosus* Santana 2003, *Cyprinus carpio* Linnaeus 1758, *Hyphessobrycon eques* (Steindachner 1882), *Loricariichthys platymetopon* Isbrücker & Nijssen 1979, *Metynnis lippincottianus* (Cope 1870), *Oreochromis niloticus* (Linnaeus 1758), *Poecilia reticulata* Peters 1859, *Pterygoplichthys ambrosettii* (Holmberg 1893), *Sorubim lima* (Bloch & Schneider 1801), and *Triportheus nematurus* (Kner, 1858). Of these species, two were exotic: *Cyprinus carpio*, which is a carp from Asia, and *Oreochromis niloticus*, know as Nile-tilapia, from Africa.

Ten long-distance migratory species were recorded in the Laranjinha River basin: *Brycon orbygnianus* (Valenciennes 1850), *Megaleporinus obtusidens* (Valenciennes 1837), *Piaractus mesopotamicus* (Holmberg 1887), *Pinirampus pirinampu* (Spix & Agassiz 1829), *Prochilodus lineatus* (Valenciennes 1837), *Pseudoplatystoma corruscans* (Spix & Agassiz 1829), *Rhinelepis aspera* Spix & Agassiz 1829, *Salminus brasiliensis* (Cuvier 1816), and *Salminus hilarii* Valenciennes 1850. Additionally, *Leporinus friderici* (Bloch 1794), *Pimelodus maculatus* Lacepède 1803, *Rhamdia quelen*, and *Schizodon nasutus* Kner 1858, which are considered short-migratory species, were also recorded herein (Agostinho et al. 2007, Oliveira et al. 2015).

Among the collected species, *Apteronotus acidops* Triques 2011, *Brycon orbygnianus*, *Brycon nattereri* Günther 1864, *Crenicichla jupiaensis* Britski & Luengo 1968, and *Rhinelepis aspera* Spix & Agassiz 1829 are classified as endangered in the most recent IUCN Red List. It is worth mentioning that two species are possibly new to science, *Piabarchus* aff. *stramineus* (*sensu* Frota et al., 2016), and *Eigenmannia* sp.

## Discussion

The results presented in this study showed a high diversity of fishes (100 species) among the fish collected in the Laranjinha River. Such results follow the pattern found in the Neotropical regions, showing the dominance of Siluriformes and Characiformes (Agostinho et al. 1997, Lowe-McConnell 1999, Jarduli et al. 2020). Furthermore, among Paranapanema River tributaries, the number of species is lower if compared to the Tibagi River basin, where 158 species were registered (Lobón-Cerviá & Bennemann 2000, Bennemann et al. 2006, 2011, Shibatta & Cheida 2003, Hoffmann et al. 2005, Oliveira & Bennemann et al. 2005, Jerep et al. 2006, Sant'Anna et al. 2006, Shibatta et al. 2002, 2006a, 2006b, 2007, 2008, Galves et al. 2007, Vieira & Shibatta 2007, Orsi 2010, Raio & Bennemann 2010, Garcia et al. 2014, 2015, Silva et al. 2015, Frantine-Silva et al. 2015, Almeida et al. 2018, Jerep & Shibatta 2017, Claro-Garcia et al. 2018), and to the Cinzas River, with 114 species (Hoffmann et al. 2005, Vianna & Nogueira, 2008, Orsi 2010, Bennemann et al. 2011, Costa et al. 2013, Frantine-Silva et al. 2015, Almeida et al. 2018).

It is noteworthy that the most frequent species of Laranjinha River was *Psalidodon* aff. *paranae*, captured in eight sites. The higher abundance were in the uppermost site A, and along the downstream, the number of specimens decreased until site H, which corroborate the hypothesis that this species has a preference regarding the headwater region (Britski 1972), but it is not restricted to that. *Psalidodon* aff. *paranae* is an insectivorous species, feeding mainly of allochthonous resources from the riparian forest. However, it can gather enough food resources from environments with different levels of degradation (Ferreira et al. 2012).

In the upper region of Laranjinha River, *Phalloceros harpagos*, a native species, was the most abundant one, being dominant in the site C. The value of dominance index (D=0.54) and evenness (E=0.17) of that site, reflected the disturbance in the observed environment. Biological features of *P. harpagos* like constant reproduction (Wolff et al. 2007), adaptability, tolerance to heat, variations in salinity (Nascimento & Gurgel 2000), and high trophic plasticity (Casatti et al. 2009, Rocha et al. 2009) may be related to the success of the species at that location. This species has a wide geographical distribution (Thomaz et al. 2019), which may also be related to its ecological plasticity.

*Hypostomus ancistroides*, *H.* cf. *paulinus,* and *H.* cf. *strigaticeps* were abundant and frequent from C to I sites. A factor that possibly has favored these rheophilic species is the presence of running water in the Laranjinha River (Cecilio et al. 1997; Garcia et al. 2020). Several species of *Hypostomus* were collected in running waters showing a substrate with pebbles and rocks (Garavello & Garavello 2004; Perez-Junior & Garavello 2007), but in Laranjinha River these species also occur in some places with a sand bottom. *Hypostomus ancistroides*, the most abundant of these species, had higher abundance in the site C, which decreased until site I, and such data demonstrates that this species is the least rheophilic one among congeners.

In general, the non-native fish species recorded in this study were introduced from other drainages in Brazil. All these species are associated with human activities like aquarium trade (possibly *Aphyocharax* cf. *dentatus*, *Apteronotus albifrons*, *Hyphessobrycon eques* and *Poecilia reticulata*), fish ladders (possibly *Metynnis lippincottianus*, *Pterygoplichthys ambrosetti* and *Triportheus nematurus*), fish farming (certainly *Cyprinus carpio*, *Oreochromis niloticus* and *Sorubim lima*), and control of insect larvae (possibly *Poecilia reticulata*). It is worth mentioning that among these non-native species, two of them are considered exotic (*Cyprinus carpio* and *Oreochromis niloticus*). These records are alarming, as *C. carpio* is known to promote bioturbation by continually revolving the sediment (Ritvo et al. 2004). However, only two individuals were sampled, being exclusive on site G, an area surrounded by pastures and agriculture. On the other hand, *O. niloticus* was more abundant and widely distributed, being sampled in five sites, which corresponds to an area surrounded by pasture and agriculture, and specifically in site B with the discharge of effluents. In addition, this species changes the environment due to the excess of nitrogen excreta, thus favoring the proliferation of algae, which decreases light and dissolved oxygen (Britton et al. 2007, Vicente & Fonseca-Alves 2013). Besides that, *Poecilia reticulata* was registered by Costa et al. (2013) in the study of the Penacho stream, a small tributary on the right bank of the Laranjinha River that flows into the Cinzas River. The high abundance of *P. reticulata* indicates an instability of the environment, including factors such as the lack of food resources for other species and the low level of dissolved oxygen (De Souza & Tozzo 2013).

The occurrence of 13 medium- to large-size migratory fishes highlights the importance of Laranjinha River for the maintenance of the diversity and viability of the ichthyofauna of the middle Paranapanema River. Other studies also highlight the Cinzas River basin and the Laranjinha River as migratory routes to the fish fauna from the Capivara dam (Dias et al. 2004, Lopes et al. 2007, Vianna & Nogueira 2008, Orsi 2010). As the Laranjinha River has only a little dam, including a fish pass system built in it, it extends a long stretch without dams, enabling migratory fish species to use its free-flowing stretches to complete their reproductive cycles. Long-distance migratory species with high commercial value have been using tributaries of rivers intensely affected by dams as migratory routes (Agostinho et al. 2008). The pressure caused by power plant dam constructions is intense in freshwater systems (Agostinho et al. 2005), because the transformation of lotic areas into lentic ones interrupts the displacement of migratory fish (Agostinho et al. 2008, Pelicice et al. 2018). The impact is not restricted to migratory species, and it also impairs the local fauna that depends on the tributaries for the viability of spawning and survival of early life stages (Oliveira et al. 2015). Studying the dynamics of eggs and larvae in the Cinzas River, Vianna & Nogueira (2008), found that the fishes of the middle Paranapanema River use this tributary to spawn. In general, biological communities are under heavy pressure due to environmental instability caused by habitat fragmentation and loss of natural environments, which affect species abundance and richness (Pusey & Arthington 2003, Di Giulio et al. 2009, Shandas & Alberti, 2009).

Furthermore, the conservation of the Laranjinha River is essential to preserve the species already categorized as threatened by extinction in the IUCN's Red List (*Apteronotus acidops*, *Brycon orbygnianus* and *Crenicichla jupiaensis* as Endangered; *Brycon nattereri* as Vulnerable; and *Rhinelepis aspera* as Near Threatened) (ICMBio 2018). The fact that some of these fish appear on a list of endangered species is concerning and it reinforces that the focused area needs appropriate conservation strategies (Simic et al. 2007). Studies on *B. nattereri* reinforce the importance of Laranjinha River conservation, since this migratory and threatened species has been able to maintain a satisfactory population genetic diversity due to the quality of that environment (Galindo et al. 2019).

Finally, the Laranjinha River is a heritage of fish diversity due to the presence of large numbers of species (among migratory, threatened, and new species to science). Thus, it deserves special attention regarding its preservation.

## Supplementary Material

The following online material is available for this article:

Appendix 1

Appendix 2

Appendix 3

Appendix 4

Appendix 5

## Acknowledgments

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## Author Contributions

Bruno Ambrozio Galindo: 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.

Renata Rúbia Ota: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Thiago Deruza Garcia: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Raul Henrique Cardoso do Nascimento: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

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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**

## Anurans of a remnant of Mixed Rainforest in southern Brazil

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***Abstract:*** The Brazilian Atlantic Forest is one of the world's most biodiverse biomes, with large numbers of endemic and threatened species. However, this biome has suffered extensive deforestation and habitat fragmentation, with a drastic reduction of its original vegetation cover. The compilation of data on the occurrence patterns of anurans and their natural history is important for the development of effective conservation strategies. Here, we present the results of a survey of the anuran fauna of Parque Estadual do Papagaio Charão (PEPC) in Rio Grande do Sul state, southern Brazil, providing information on species endemism, conservation status, and reproductive modes. We collected data on the local anurans between March 2018 and February 2019 using active searches and pitfall traps. We recorded 26 anuran species distributed in seven families, with eight different reproductive modes. The largest number of species (20) was found at the forest edge, followed by the interior of the forest and open area, each with 16 species. The most abundant species were *Leptodactylus plaumanni* (41.7% of records), *Physalaemus cuvieri* (27.1%), and *P. carrizorum* (16.5%). Greater species richness and abundance were recorded during the rainier months, while temperature influenced only the abundance of the anurans. *Rhinella henseli, Rhinella icterica, Vitreorana uranoscopa, Aplastodiscus perviridis, Boana curupi, Boana leptolineata* and *Proceratophrys brauni* are all endemic to the Atlantic Forest. *Melanophryniscus devincenzii* is classified as Endangered by the IUCN, and *Proceratophrys bigibbosa* as Near Threatened. *Boana curupi* is considered to be Endangered in Rio Grande do Sul state, and Vulnerable in Brazil, while *V. uranoscopa* is Near Threatened in Rio Grande do Sul. Our findings emphasize the importance of protected areas, such as the PEPC, for the maintenance of anuran populations and communities in the Mixed Rainforest formations of southern Brazil.
***Keywords:*** Atlantic Forest; species list; community structure; reproductive modes; seasonality; conservation.

## Anuros de um remanescente de Floresta Ombrófila Mista no sul do Brasil

***Resumo:*** A Mata Atlântica abriga a maior biodiversidade do planeta, com elevados números de endemismos e espécies ameaçadas de extinção. Entretanto, esse bioma tem sofrido extensa perda e fragmentação do habitat, com redução drástica da sua cobertura vegetacional original. Suprir lacunas sobre a história natural e os padrões de ocorrências de anuros auxiliam no desenvolvimento de estratégias de conservação para esse grupo. Neste estudo apresentamos a anurofauna do Parque Estadual do Papagaio Charão (PEPC), no estado do Rio Grande do Sul, sul do Brasil, com informações sobre endemismos, *status* de conservação e modos reprodutivos das espécies. Nossas amostragens em campo ocorreram entre março de 2018 e fevereiro de 2019 e utilizamos procura ativa e *pitfalls traps* para a coleta dos anuros. Registramos 26 espécies de anuros distribuídas em sete famílias e oito modos reprodutivos. Um número maior de espécies foi encontrado na borda (20 espécies), seguida da floresta e área aberta (16 espécies cada). As espécies mais abundantes foram *Leptodactylus plaumanni* (41,7%), *Physalaemus cuvieri* (27,1%) e *P. carrizorum* (16,5%). Maior riqueza e abundância foram registradas no período com maior pluviosidade e a temperatura influenciou somente a abundância dos anuros. *Rhinella henseli, R. icterica, Vitreorana uranoscopa, Aplastodiscus perviridis, Boana curupi, B. leptolineata* e *Proceratophrys brauni* são endêmicas da Mata Atlântica. *Melanophryniscus devincenzii* está classificada como "Em perigo" e *Proceratophrys bigibbosa* como "Quase ameaçada" pela IUCN.

*Boana curupi* é considerada "Em perigo" no estado do Rio Grande do Sul e "Vulnerável" no Brasil. *Vitreorana uranoscopa* consta como "Quase ameaçada" no Rio Grande do Sul. Nossos resultados mostram a importância de áreas protegidas, como o PEPC, para a manutenção das populações e comunidades de anuros da Floresta Ombrófila Mista.
***Palavras-chave:*** Mata Atlântica; lista de espécies; estrutura da comunidade; modos reprodutivos; sazonalidade; conservação.

## Introduction

The considerable biodiversity and endemism of the Brazilian Atlantic Forest, combined with its extensive deforestation, has resulted in the extensive decimation of this biome, with only 11.7% of the original vegetation now remaining (Ribeiro et al. 2009). Ecological processes are altered in human-modified landscapes due to the progressive reduction in the size and quality of remnant areas of natural vegetation, which provokes changes in both biotic and abiotic factors, such as resource availability and temperature (Saunders et al. 1991, Laurence 2008). Habitat fragmentation degrades these ecosystems continuously, reducing the occurrence of species, the richness of communities, and the dispersal of both fauna and flora (Haddad et al. 2015). In southern Brazil, the Mixed Rainforest is an Atlantic Forest formation characterized by the presence of the gymnosperm *Araucaria angustifolia* (Bertol.) Kuntze., (Oliveira-Filho et al. 2013). This forest formation has been widely devastated, with the remnants now surviving in protected areas, either in the private domain or on private land (Sonego et al. 2007)

Anuran amphibians may be influenced directly or indirectly by anthropogenic impacts in a number of different ways, due in particular to the physiological characteristics of these animals, such as their moist and permeable skin, and the features of their life cycle (Wells 2007), with habitat loss being considered the principal factor responsible for the worldwide decline in anuran populations (Blaustein & Kiesecker 2002, Whittaker et al. 2013). The principal factors contributing to this threat include the loss, degradation, and fragmentation of habitats, edge effects, and the influence of the surrounding matrix (Becker et al. 2007, Almeida-Gomes & Rocha 2014, Schneider-Maunoury et al. 2016, Pfeifer et al. 2017, Ferrante et al. 2017, Ribeiro et al. 2018).

A total of 7,245 anuran amphibian species are currently recognized, worldwide (Frost 2020), of which, 1,093 are known to occur in Brazil (Segalla et al. 2019), that is, 15.1% of the total number. More than half (625 species or 57.2%) of Brazilian anurans are found in the Atlantic Forest, and most (485 or 77.6%) of these species are endemic to this biome (Rossa-Feres et al. 2017). The Mixed Rainforest is home to 109 species of anurans, and 26 (24%) are endemic to this formation (Rossa-Feres et al. 2017).

The anuran amphibian community has been the focus of many studies in Brazil (e.g. Martins et al. 2014, Leivas & Hiert 2016, Santos-Pereira et al. 2016, Andrade et al. 2017, Ceron et al. 2017, Silva et al. 2018, Foerster & Conte 2018, Fiorillo et al. 2018). Surveys typically provide important insights into the characteristics of an anuran community and its populations (Rocha et al. 2003). The compilation of a list of the species present in a given area is an essential first step in the development of effective conservation measures (Toledo & Batista 2012, Vasconcelos et al. 2014). More detailed studies, including the influence of environmental heterogeneity (Silva et al. 2012, Santos & Conte 2014) or the variation in climate, on anuran diversity (Costa et al. 2012, Titon & Gomes 2015, Vasconcelos & Nascimento 2016), provide increasingly valuable insights into the ecology of anuran communities.

In the present study, we investigate the species richness, spatial distribution (forest edge, interior, and open area), and the seasonal variation in the characteristics of an anuran community in a remnant of the Mixed Rainforest formation of the southern Atlantic Forest of Brazil.

## Materials and Methods

### 1. Study area

Our study was conducted in the Parque Estadual do Papagaio Charão – PEPC (27º54'49" S, 52º48'52" W, 503 m a.s.l.), located in the municipality of Sarandi, in northern Rio Grande do Sul state, southern Brazil, which is part of the Upper Uruguay physiogeographic region. This state park has an area of 1,000 hectares, and is representative of the Mixed Rainforest phytophysiognomy (SEMA 2020) of the Atlantic Forest biome (Figure 1). The region's climate is subtropical humid, *Cfa* in the Köppen-Geiger classification system. The mean annual temperature is 19.4ºC and mean annual rainfall is 1,765 mm (Wrege et al. 2012).

### 2. Sampling methods

We collected anurans using two methods. One method was active searching (Crump & Scott-Jr 1994), during which at least two collectors surveyed transects at night (18–23h) during three consecutive days per month (except in June and July). For this, we used two preexisting transects located within the three different environments sampled – forest edge, interior, and open area. We searched carefully for anurans along each transect, examining bromeliads, the leaf litter, tree trunks, rocks, marshes, streams, ponds, floodplains, and other habitats. A total of 459 hours of active searching was conducted during the present study, including 144 hours in the forest edge, 180 hours in the forest interior, and 135 hours in the open area.

The second method was pitfall trapping using interception traps interconnected by drift-fences (Corn 1994). These traps were used in pairs, with one set being deployed at the edge of the PEPC forest fragment (27º54'50" S, 52º48'57" W) and the other in its interior (27º54'49" S, 52º49'21" W). Each set of traps consisted of two straight lines of 40 m in length, separated by a distance of 30 m. The two sets of traps were separated by a distance of at least 500 m, in an attempt to avoid spatial autocorrelation. Each trap line consisted of five plastic 60-L buckets, which were buried in the ground and connected by a 60 cm-tall drift fence. Overall, 12 buckets were deployed in each habitat type. We perforated the bucket bottoms to permit rainwater runoff. We opened the pitfall traps on four consecutive days each month for 12 months, with a total of 48 days of sampling between March 2018, and February 2019, which is the equivalent of 1,152 bucket-days.

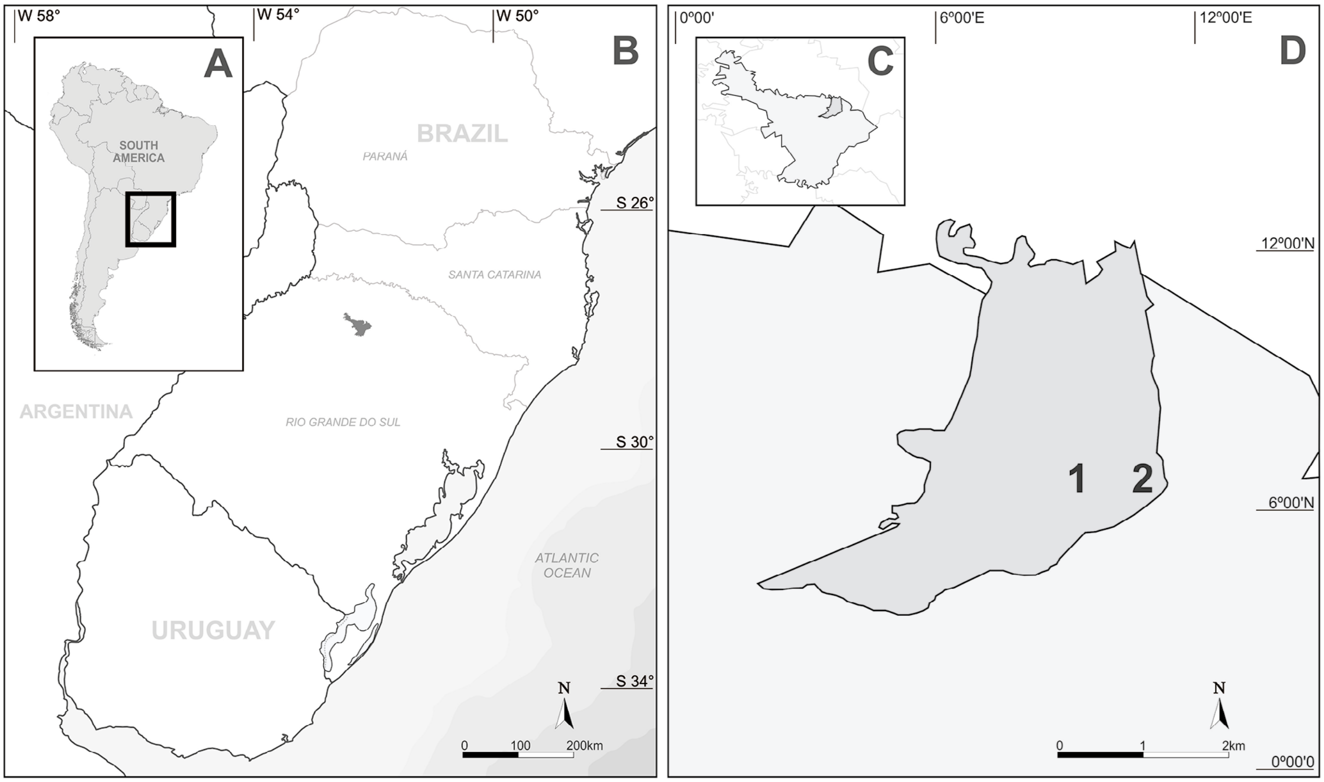


**Figure 1.** Location of the pitfall traps in the Parque Estadual do Papagaio Charão, in the municipality of Sarandi, Rio Grande do Sul, Brazil: 1 – forest interior; 2 – forest edge.

The traps were checked daily during sampling, and when not in use, the traps were sealed off, to avoid the unintentional capture, and potential death, of terrestrial vertebrates.

We collected the abiotic data (temperature, humidity, and rainfall) using a thermo-hygrometer and digital pluviometer, which were installed in the PEPC. We deposited voucher specimens in the Amphibian Collection of the University of Passo Fundo, CAUPF, under permits SISBIO no. 26826-1; SEMA/RS no. 49/2017; CEUA no. 11/2018 (Appendix 1). We also considered previous records of anuran species in the PEPC obtained by the team of the UPF Herpetology Laboratory (unpublished data).

### 3. *Data analysis*

We evaluated the sampling efficiency using a rarefaction curve, based on the matrix of the monthly records of anuran species (one sample = one day) by both active searches and the pitfall traps. We ran the nonparametric Bootstrap estimator in EstimateS9.0 (Colwell 2013) with 1,000 randomizations.

Prior to the statistical analyses, the data were tested to verify the satisfaction of the assumptions of normality and homoscedasticity of variances (Zuur et al. 2010). We used the *t* test to determine the significance of the difference in the mean species richness and abundance between the forest edge and interior, using only the pitfall trap data, to standardize the analysis. We applied a simple linear regression and *G* test (Zar 1999) to verify the possible relationship between anuran community parameters (species richness and abundance) and climatic variables (rainfall and temperature). We ran all the statistical tests in the BioEstat 5.3 software (Ayres et al. 2007). The correlations were considered significant when $P \leq 0.05$. We excluded the exotic species *Lithobates catesbeianus* (Shaw 1802) from all the analyses.

The anuran nomenclature adopted in the present study was based on Frost (2020), and we verified the conservation status of the species in the International Red List of Endangered Species (IUCN 2020), the Brazilian Red Book of Threatened Animal Species (ICMBIO 2018), and the List of Endangered Anurans of the state of Rio Grande do Sul (Rio Grande do Sul 2014). We classified the reproductive modes of the anuran species as in Haddad et al. (2013), and defined their degree of endemism according to Rossa-Feres et al. (2017).

## Results

We recorded 26 anuran species in the PEPC, representing 14 genera and seven families (Table 1, Figure 2). The Hylidae was the most diverse family, with nine species (n=9 species), followed by the Leptodactylidae (n=8 spp.), Odontophrynidae (n=3 spp.), Bufonidae (n=3 spp.), and the Brachycephalidae, Centrolenidae, and Microhylidae, each represented by a single species (Figure 2). We recorded the greatest species richness, 20 species (76.9% of the total), in the edge of the fragment, while we collected 16 species (61.5% of the total) in both the interior of the forest and the open area (Table 1). Three anuran species (11.5% of the total) were exclusive to both the forest and edge habitat, while two (7.7%) were found only in the open area (Figure 3). Eight anuran species (30.7%) occurred in all three environments, that is, the open area, and the forest edge and interior (Table 1). Five species (19.2%) were found in both the edge and open area, and four (15.3%) were recorded in both types of forest, while only one species (3.8%) was found in both the forest interior and the open area (Figure 3).

**Table 1.** Anuran species recorded in Parque Estadual do Papagaio Charão, in the municipality of Sarandi, Rio Grande do Sul, Brazil. Sample methods: pitfall trapping (PT) and active search (AS). The reproductive mode (RM) of each species is designated by a number, following the classification of Haddad et al. (2013). The conservation status of each species is defined according to the Brazilian (ICMBio 2018) and international (IUCN 2020) red lists. LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered, and DD = Deficient Data. [1]Endemic to the Atlantic Forest and [2]Endemic to the Mixed Rainforest domain of the southern Atlantic Forest (*sensu* Rossa-Feres et al. 2017). [3]Recorded previously.

| Species | Location | | | Method | RM | Red Lists | |
|---|---|---|---|---|---|---|---|
| | Edge | Forest | Open Area | | | Brazil | IUCN |
| **Bufonidae** | | | | | | | |
| *Melanophryniscus devincenzii* Klappenbach, 1968 | X | | | PT | 2 | LC | EN |
| *Rhinella henseli* (Lutz, 1934)[1,3] | | X | | AS | 1 or 2 | LC | LC |
| *Rhinella icterica* (Spix, 1824)[1] | X | X | X | PT/AS | 1 or 2 | LC | LC |
| **Brachycephalidae** | | | | | | | |
| *Ischnocnema henselii* (Peters, 1870) | X | X | | PT/AS | 23 | LC | LC |
| **Centrolenidae** | | | | | | | |
| *Vitreorana uranoscopa* (Müller, 1924)[1] | X | X | | AS | 25 | LC | LC |
| **Hylidae** | | | | | | | |
| *Aplastodiscus perviridis* (Lutz, 1950)[1] | X | X | X | AS | 5 | LC | LC |
| *Boana curupi* (Garcia, Faivovich & Haddad, 2007)[1,2] | | X | X | AS | 2 | VU | LC |
| *Boana faber* (Wied-Neuwied, 1821) | X | | X | AS | 1 or 4 | LC | LC |
| *Boana leptolineata* (Braun & Braun, 1977)[1,2] | X | | X | AS | 1 or 2 | LC | LC |
| *Dendropsophus minutus* (Peters, 1872) | X | X | X | AS | 1 | LC | LC |
| *Dendropsophus sanborni* (Schmidt, 1944) | X | | X | AS | 1 | LC | LC |
| *Ololygon aromothyella* (Faivovich, 2005) | X | X | | AS | 1 | LC | DD |
| *Scinax fuscovarius* (Lutz, 1925) | X | X | X | AS | 1 | LC | LC |
| *Scinax granulatus* (Peters, 1871) | X | | | AS | 1 | LC | LC |
| **Leptodactylidae** | | | | | | | |
| *Physalaemus biligonigerus* (Cope, 1861) | | | X | AS | 11 | LC | LC |
| *Physalaemus cuvieri* Fitzinger, 1826 | X | X | X | PT/AS | 11 | LC | LC |
| *Physalaemus carrizorum* Cardozo & Pereyra, 2018 | X | X | X | PT/AS | 11 | LC | LC |
| *Leptodactylus fuscus* (Schneider, 1799) | X | | | PT/AS | 30 | LC | LC |
| *Leptodactylus gracilis* (Duméril & Bibron, 1840)3 | X | | X | AS | 30 | LC | LC |
| *Leptodactylus latrans* (Steffen, 1815) | X | | X | AS | 11 | LC | LC |
| *Leptodactylus mystacinus* (Burmeister, 1861) | X | X | X | PT/AS | 30 | LC | LC |
| *Leptodactylus plaumanni* Ahl, 1936 | X | X | X | PT/AS | 30 | LC | LC |
| **Microhylidae** | | | | | | | |
| *Elachistocleis bicolor* (Guérin-Méneville, 1838) | | | X | AS | 1 | LC | LC |
| **Odontophrynidae** | | | | | | | |
| *Odontophrynus americanus* (Duméril & Bibron, 1841) | X | X | | PT | 1 | LC | LC |
| *Proceratophrys bigibbosa* (Peters, 1872) | | X | | PT/AS | 2 | LC | NT |
| *Proceratophrys brauni* (Kwet & Faivovich, 2001)[1,2] | | X | | PT | 2 | LC | LC |
| **Total richness of species** | **20** | **16** | **16** | | | | |

Reproductive modes: (1) exotrophic eggs and tadpoles in standing water; (2) exotrophic eggs and tadpoles in running water; (4) eggs and early larval stages in natural or manmade pools, after flooding, exotrophic tadpoles in streams or puddles; (5) eggs and early larval stages in underground "nests", after flooding, exotrophic tadpoles in streams or puddles; (11) eggs laid in floating foam nests and exotrophic tadpoles in puddles; (23) direct development of eggs on ground; (25) after hatching, the exotrophic tadpoles drop into running water; (30) foam nest with eggs and early larval stages in chambers built underground, after flooding, exotrophic tadpoles in lentic water. Adapted from Haddad et al. (2013).


**Figure 2.** Anurans recorded in the Parque Estadual do Papagaio Charão, in Sarandi, Rio Grande do Sul, Brazil. (A) *Rhinella henseli*; (B) *Leptodactylus mystacinus*; (C) *Elachistocleis bicolor*; (D) *Dendropsophus minutus*; (E) *Boana curupi*; (F) Ololygon *aromothyella*; (G) *Physalaemus biligonigerus*; (H) *Ischnocnema henselii;* (I) *Odontophrynus americanus*; (J) *Boana leptolineata*; (K) *Vitreorana uranoscopa*; (L) *Proceratophrys brauni*; (M) *Rhinella icterica*; (N) *Leptodactylus fuscus*; (O) *Leptodactylus plaumanni*; (P) *Aplastodiscus perviridis;* (Q) *Physalaemus carrizorum*; (R) *Proceratophrys bigibbosa*; (S) *Dendropsophus sanborni*; (T) *Melanophryniscus devincenzii*; (U) *Scinax granulatus*. Photographs: A, D, E, J, K, M, N, O, P, E, R, S, T, and U by N. Zanella; B, C, G, H, and I by L. A. P. Potrich and M. Santos-Pereira.

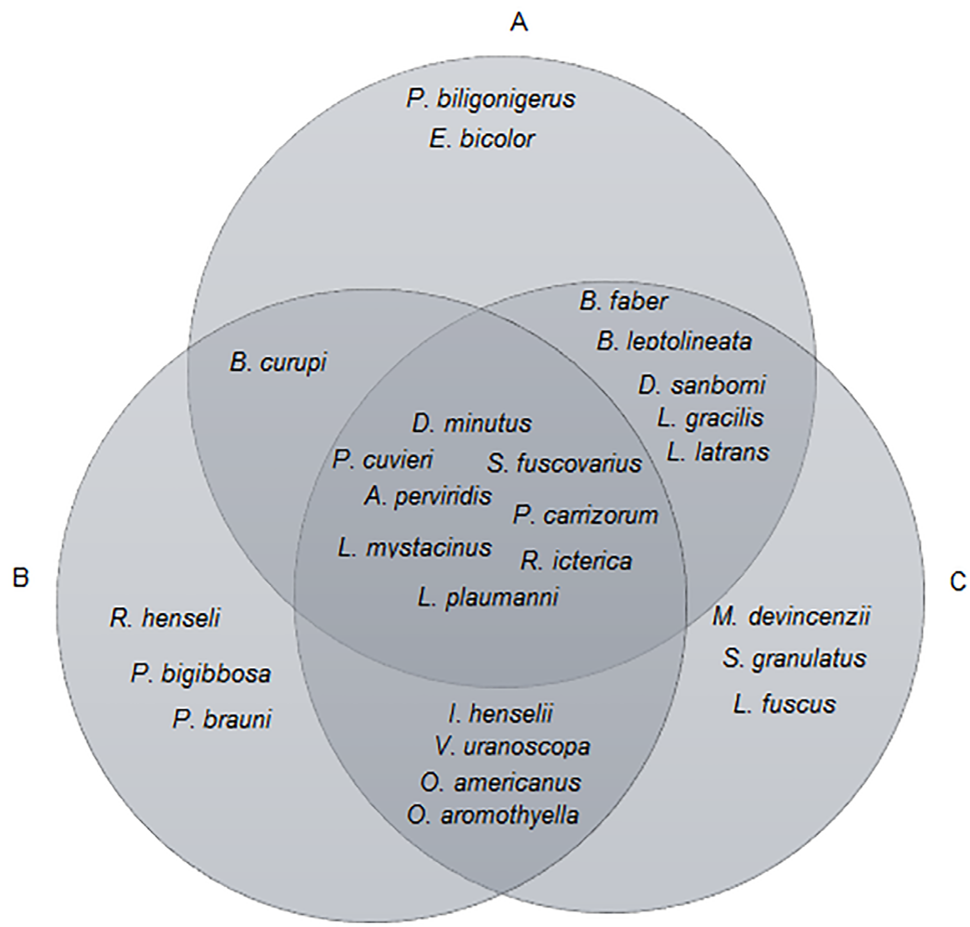


**Figure 3.** Venn Diagram of the intersection of the composition of anuran species in the three habitat types found in the Parque Estadual do Papagaio Charão, in Sarandi, Rio Grande do Sul, Brazil. A = Open area, B = Forest interior, and C = Forest edge.

A total of 11 anuran species were captured in the pitfall traps, including nine species in the edge and nine in the interior of the forest (Table 1). *Melanophryniscus devincenzii* and *Leptodactylus fuscus* were captured exclusively in the edge traps, while *Proceratophrys bigibbosa* and *Proceratophry brauni* were captured only in the interior of the forest. The most abundant species captured in the pitfall traps (Figure 4) were *Leptodactylus plaumanni* (n=53), *Physalaemus cuvieri* (n=32), and *Physalaemus carrizorum* (n=21). Neither anuran species richness (p=0.2667) nor abundance (p=0.1320) varied significantly between the forest edge and the interior. *Physalaemus cuvieri* dominated the forest interior, and *L. plaumanni* was the most abundant species in the edge habitat. By contrast, some species, i.e., *Leptodactylus fuscus* and *P. brauni*, were recorded only once during the study (Figure 4).

The anuran species richness estimated using the Bootstrap procedure was 25.50 species, with 21.79 species being estimated specifically for the forest edge, 16.63 species for the forest interior, and 15.77 for the open area. The shape of the curve (Figure 5) and the predicted species richness indicate that sampling effort was broadly adequate for all three types of habitat sampled in the PEPC, with only an additional two species being expected from further sampling.

We identified eight anuran reproductive modes in the present study (Table 1). The most frequent mode was mode 1, found in 11 species (42.3%), followed by mode 2, in seven species (26.9%), mode 11 and 30, both recorded in four species (15.3%), with modes 4, 5, 23, and 25, each being found in a single anuran species (3.8%).

Seven (27%) of the anuran species recorded in the PEPC are endemic to the Atlantic Forest, with three (11.54%) being endemic to the Mixed Rainforest formation (Table 1). *Melanophryniscus devincenzii* is classified as EN (Endangered) and *P. bigibbosa* as NT (Near Threatened) by the IUCN (2020). *Boana curupi* is considered EN (Endangered) in the state of Rio Grande do Sul (Rio Grande do Sul 2014) and VU (Vulnerable) in Brazil (ICMBIO 2018), while *Vitreorana uranoscopa* is classified as Near Threatened in Rio Grande do Sul (Rio Grande do Sul 2014).

Anuran species richness did not vary significantly with temperature ($p = 0.0653$), although abundance was significantly ($p < 0.0001$) higher in the warmer months in comparison with the cooler months of the year. Significant correlations were found between rainfall and both anuran species richness ($R = 0.3273$, $p < 0.0001$, $n = 42$) and abundance ($R = 0.3670$, $p < 0.0001$, $n = 42$).

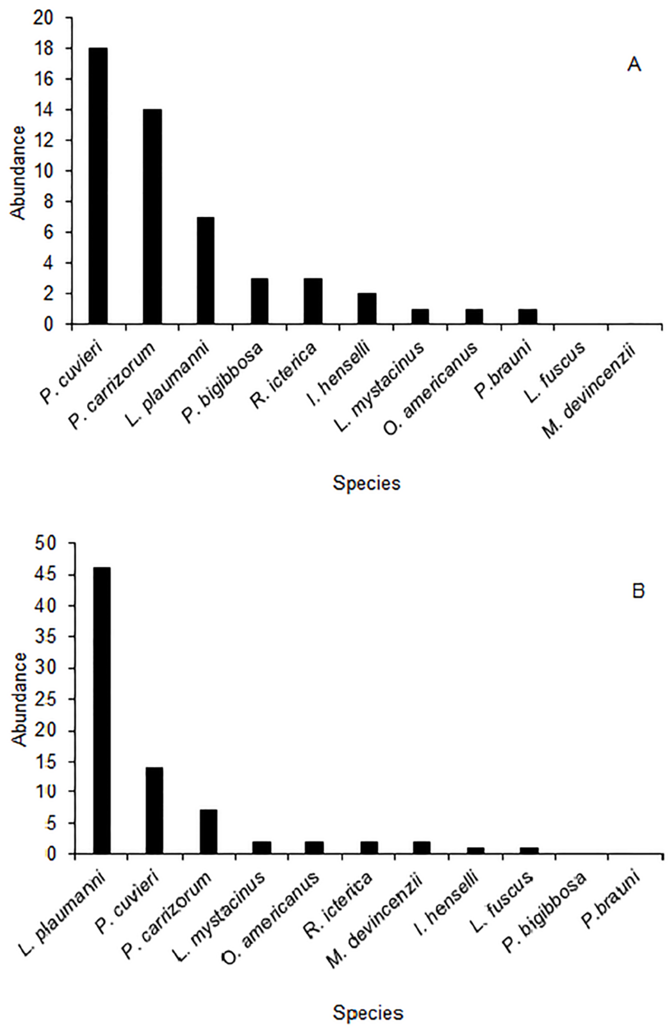


**Figure 4.** Abundance of anurans captured between March 2018 and February 2019 in the Parque Estadual do Papagaio Charão, in Sarandi, Rio Grande do Sul, Brazil, in two environments: A) the interior of the forest, and B) the edge of the fragment, considering only the pitfall traps data.

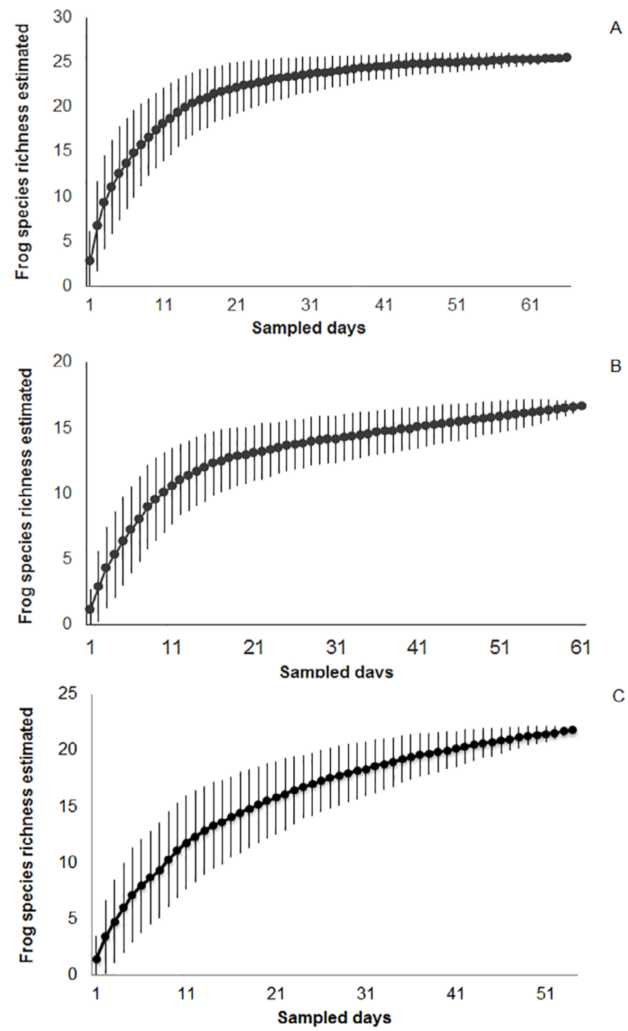


**Figure 5.** Rarefaction curves (Bootstrap estimator) of the anuran species recorded in the Parque Estadual do Papagaio Charão, in Sarandi, Rio Grande do Sul, Brazil, using active searches and pitfall traps between March 2018 and February 2019. A = General (all data); B = Forest interior only; C= Forest edge only.

## Discussion

The anuran species recorded by us in the PEPC represent 0.36% of total anuran diversity, worldwide (Frost 2020), and 2.3% of the Brazilian anuran fauna (Segalla et al. 2019). We also recorded 23.8% of the species known to occur in the Mixed Rainforest formation of the Atlantic Forest biome (Rossa-Feres et al. 2017). The anuran species richness recorded in the study represented 94.1% of that estimated, which indicates that the sample area was sampled adequately.

Our findings on the anuran community of the PEPC were similar to those of previous studies conducted at other sites within the Mixed Rainforest formation of the southern Atlantic Forest biome. For example, Zanella et al. (2013) recorded 23 anuran species in the Parque Municipal de Sertão, 60 km east of the PEPC, including 20 (76.9%) of the species recorded in the present study. Lucas & Fortes (2008) inventoried 29 anuran species (28 native and one exotic) in the Floresta National de Chapecó, 120 km north of the PEPC, including 17 (60.7%) of the species recorded at this site. Bastiani & Lucas (2013) recorded 22 native species in the Parque Estadual Fritz Plaumann, in the municipality of Concórdia, in Santa Catarina, southern Brazil, of which, 14 (53.8%) were recorded in the present study. Slightly further afield, 152 km northwest of the PEPC, in the semideciduous seasonal forest of the Parque Estadual do Turvo, Iop et al. (2011) recorded 30 anurans, including 21 of the species (70%) registered in the present study. This degree of homogeneity in the anuran fauna of the different sites is probably related to the broad phytophysiognomic similarities of the Atlantic Forest domain.

A predominance of hylid species is typical of the Neotropical region (Duellman 1999), in particular in the Atlantic Forest (e.g. Araujo & Almeida-Santos 2013, Santos-Pereira et al. 2016, Ceron et al. 2017). The family Leptodactylidae is also a prominent component of the anuran fauna of most Atlantic Forest sites (e.g. Conte & Rossa-Feres 2006, Lucas & Marocco 2011, Zanella et al. 2013). The leptodactylids are also a prominent group in the Pampa (e.g. Lipinski & Santos 2014, Bolzan et al. 2016), a grassland biome in Rio Grande do Sul, and neighboring areas of Uruguay and Argentina. As the Mixed Rainforest is adjacent to grassland (which is not the Pampa) in the PEPC (Overbeck et al. 2009, SEMA 2020), the composition of its anuran fauna may be influenced by both forest and grassland environments, which may account for the fact that the Hylidae and the Leptodactylidae were the most prominent families in the study area. This ecotonal scenario may also account for the presence in the PEPC of species endemic to the Atlantic Forest biome, i.e., *Aplastodiscus perviridis*, *Vitreorana uranoscopa*, *Rhinella henseli*, and *R. icterica*, and those with associate distributions within to the Mixed Rainforest phytophysiognomy, that is, *Boana curupi*, *B. leptolineata*, and *Proceratophrys brauni* (Rossa-Feres et al. 2017). *Proceratophrys brauni* has also been recorded in neighboring areas of Paraguay, Argentina, and Uruguay (Frost 2020).

We recorded a greater species richness in edge areas in comparison with the forest interior and the open area. This forest edge represents the transition between natural habitats which, modified by fragmentation, results in the formation of structurally distinct types of vegetation (Murcia 1995). As the PEPC is located with the transition zone between the Mixed Rainforest and grassland, this edge habitat may be similar to a natural ecotone. The greater species richness found at the forest edge may reflect the ecology of some anurans, which reproduce in open areas, but forage or seek shelter within the forest (Conte & Rossa-Feres 2007, Lucas & Fortes 2008, Quesnelle et al. 2015). The intercalation of pastures and forest formations permits different patterns of habitat use, which favor the occurrence of species specialized for the exploitation of both open areas and forested areas (Crivellari et al. 2014). In environments with different phytophysiognomies, such as forest and open areas, species richness is often greatest in areas of ecotone (Maragno et al. 2013). Previous studies have also found greater species richness at the edge of forest fragments (e.g. Urbina-Cardona et al. 2006, Zanella et al. 2013), indicating that anuran communities may be favored by transitional environments. While pitfall traps were not deployed in open area in the present study, the number of species recorded in this habitat was similar to that recorded in the interior of fragment, although it is important to remember that the number of species may be underestimated in open area given that many species may be less detectable using the traditional visual search approach (Cechin & Martins 2000).

Some anurans, such as *Dendropsophus minutus*, *P. cuvieri*, *A. perviridis*, *Leptodactylus mystacinus*, *L. plaumanni*, *Scinax fuscovarius*, *P. carrizorum*, and *R. icterica*, occurred in all three of the environments sampled in the present study. This reflects the ecological adaptations of these species, which are generalists with ample geographic ranges (Haddad et al. 2013, Vancine et al. 2018). By contrast, *Proceratophrys biggibosa*, *P. brauni* and *Rhinella henseli* were recorded only in the forest interior. These *Proceratophrys* species inhabit mountainous areas in subtropical rainforest ranging in altitude from 300 m to 1200 m a.s.l. (Kwet Di-Bernardo 1999, Kwet & Faivovich 2001), where they are typically found in rocky streams at the edge or in the interior of dense forests (Santos et al. 2009). *Rhinella henseli*, in turn, inhabits the forest and, during the breeding season, it is found in small streams, temporary pools (Kwet et al. 2010, Haddad et al. 2013, Lucas et al. 2018). Species found in both the forest and the edge (Table 1) are typically less demanding of habitat, allowing them to exploit different environments.

*Leptodactylus plaumanni* was recorded in all three environments, but was captured more frequently in the pitfall traps deployed in the edge of the forest. This common species is found in both forest and grassland (Kwet et al. 2010), and in Brazil, it is limited to the southern extreme of the country (de Sá et al. 2014, Frost 2020). *Physalaemus cuvieri* was the most abundant species in the interior of the forest fragment at PEPC, as recorded by Maragno et al. (2013). This species is widely distributed in South America (Frost 2020), and is typical of open areas (Kwet et al. 2010). The location of the PEPC within the Mixed Rainforest domain, which was originally formed by a mosaic of forest and grassland, may account for its presence in the forest at this site. The ecological characteristics of a species may often depend on its ability to adapt to a given environment (Urbina-Cardona et al. 2006).

A majority of the anurans recorded in the PEPC present reproductive mode 1 (42.3%), in which the eggs are deposited in water, where the exotrophic tadpoles develop, or mode 2 (26.9%), where the eggs are deposited in lotic water, where the tadpoles develop. Laying eggs directly on the surface of the water (mode 1) is the most primitive and generalized anuran reproductive mode (Duellman & Trueb 1994), and a large number of studies have shown that this mode is the most common in most anuran communities (e.g. Leivas et al. 2015, Nazaretti & Conte 2015, Santos-Pereira et al. 2016). The diversity of reproductive modes in an anuran community tends to reflect the heterogeneity of the environment, rather than phylogenetic relationships (Duellman & Trueb 1986). The occurrence of eight reproductive modes in the PEPC is typical of Atlantic Forest anurans, given the high humidity and ample diversity of microhabitats found in this forest (Haddad & Prado 2005). Areas with prolonged rains, such as the PEPC, tend to provide conditions for a broad range of reproductive modes, in contrast with more arid areas, with seasonal climates, that tend to restrict reproduction to more specialized modes that are more resistant to desiccation (Silva et al. 2012).

*Vitreorana uranoscopa* is a small arboreal anuran, which is listed as Vulnerable in Rio Grande do Sul (Rio Grande do Sul 2014). Recent studies have shown that the distribution of this species is expanding (Machado et al. 2010, 2014), with it being recorded increasingly in small fragments of forest (Savaris et al. 2011). At PEPC, the species was encountered only in the forest, including the edge. The occurrence of *V. uranoscopa* at the edge of the forest in the PEPC may be related to its ecological characteristics, given that it is found in the streams that run from the interior of the forest fragment to its edges, resulting in the dispersal of this anuran throughout the area. For many amphibian species, in fact, the presence of specific reproductive habitats may be the primary determinant of the occurrence of a species at a given site, irrespective of the other habitats available in the area (Zimmerman & Bierregaard 1986). *Boana curupi*, which is also endangered, is typically found in forested vegetation at the margins of shallow streams (Garcia et al. 2007, Iop et al. 2009, Lucas & Garcia 2011, Fontana et al. 2017), and in the present study, it was found vocalizing in a marsh, in an open area near the study forest.

In the present study, precipitation had a significant influence on anuran species richness and abundance in the PEPC, whereas temperature influenced only abundance, as observed in previous studies (Both et al. 2008, Conte & Rossa-Feres 2007, Santos & Conte 2014, Goyannes-Araújo et al. 2015). Amphibian reproduction is influenced primarily by climatic factors (Duellman & Trueb 1986), and in Brazil, a number of studies have shown that precipitation is the principal factor determining anuran species richness, in addition to the diversity of reproductive modes (Vasconcelos et al. 2010, Maffei et al. 2011).

*Lithobates catesbeianus*, an exotic and invasive species, was recorded in all three environments surveyed in the PEPC. This species is considered to be one of the principal threats to native anurans, given its capacity to adapt to different environments and compete with native anurans (Rocha et al. 2011, Almeida et al. 2020), which often in the decline of resident populations (Both et al. 2011, Silva et al. 2011, Santos-Pereira & Rocha 2015). The presence of *L. catesbianus* in a protected area the size of the PEPC reflects its capacity to invade novel environments, which is a potential cause for concern over the long term.

The anuran species recorded here in the Parque Estadual do Papagaio Charão appear to be a reliable estimate of the diversity of anurans found in this protected area in Rio Grande do Sul. Our findings indicate that the anuran diversity of the area may be the result of the interface of the forest ecosystems and grassland environments found in the park. The presence of threatened species in the PEPC reinforces the importance of this protected area for the conservation of the anuran communities of the Atlantic Forest biome. This is especially important in the context of the study region, where the Mixed Rainforest has suffered extensive impact and loss of cover over the past few years.

## Supplementary Material

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

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## Author Contributions

Ana Paula Potrich: Contributed to the collection, analysis, and interpretation of the data, and wrote the manuscript.

João Paulo Soares, Carlos Toffolo and Thaís Ascoli-Morrete: Contributed to data collection.

Manuela Santos-Pereira and Noeli Zanella: Contributed to the preparation of the manuscript, reviewed it critically, and added intellectual content.

## Conflicts of Interest

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

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**Short Communication**

# What do different landscapes of the Atlantic Forest reveal about the occurrence of *Discothyrea* Roger, 1863 (Formicidae: Proceratiinae)?

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***Abstract:*** *Discothyrea* is a genus composed of specialist predatory species rarely recorded and with little known biology. Specimen collection is usually associated with preserved native vegetation. In this work, we explore the landscape of sites with occurrence of *Discothyrea* seeking to improve knowledge about the natural history of this genus. Species of *Discothyrea* were recorded in ten Atlantic Forest sites. We analyzed the landscape around the place of occurrence of each species using a 500-m buffer. We classified the landscape as heterogeneous and homogeneous according to the percentage of natural (native vegetation), urban, and rural areas. We found 67 specimens of *Discothyrea*; 59 of them were *D. sexarticulata*, occurring in 88% of the fragments. There were also eight specimens of *D. neotropica* occurring in 12% of the fragments. The results show that *D. sexarticulata* can be found in homogeneous landscapes with anthropic influence (0-51% of rural area and 0-68% of urban area). *Discothyrea neotropica* is found in heterogeneous landscapes with a dominant presence of native vegetation (between 74-95%). The results improve knowledge on the biology of *Discothyrea* mainly in relation to the vicinity of occurrence sites. In addition, the results indicate that regional studies are important to understand species ecology.
***Keywords:*** *Hypogeic species; mosaic of landscapes; cryptic habit; conservation.*

## O que diferentes paisagens da Floresta Atlântica nos mostram sobre a ocorrência de *Discothyrea* Roger, 1863 (Formicidae: Proceratiinae)?

***Resumo:*** *Discothyrea* é um gênero composto por espécies predadoras especialistas, raramente registradas e com biologia pouca conhecida. A coleta de espécimes geralmente está associada à vegetação nativa preservada. Neste trabalho exploramos a paisagem de locais com ocorrência de *Discothyrea*, buscando incrementar o conhecimento sobre a história natural do gênero. Espécies de *Discothyrea* foram registradas em dez áreas de Mata Atlântica. A paisagem ao redor do local de ocorrência de cada espécie foi analisada, usando um *buffer* de 500 m. Classificamos a paisagem em heterogênea e homogênea de acordo com a porcentagem de área natural (vegetação nativa), urbana e rural. Encontramos 67 espécimes de *Discothyrea*; 59 de *D. sexarticulata*, em 88% dos fragmentos. E oito espécimes de *D. neotropica*, em 12% dos fragmentos. Nossos resultados mostram que *D. sexarticulata* pode ser encontrada em paisagens homogêneas e sob influência antrópica, com 0-51% de área rural e 0-68% de área urbana; e *D. neotropica* em paisagens heterogêneas, com presença dominante de vegetação nativa (entre 74-95%). Nossos resultados trazem um aporte de conhecimento à biologia de *Discothyrea*, principalmente em relação às adjacências do local de ocorrência. Além disso, nossos resultados indicam que estudos regionais são importantes ferramentas para o conhecimento da ecologia das espécies.
***Palavras-chave:*** *Espécie hipogeica; mosaico de paisagens; hábito críptico; conservação.*

## Introduction

The genus *Discothyrea* Roger, 1863, has 50 species distributed in tropical areas of the southern hemisphere. The occurrence area extends from the midwestern and southeastern United States to northern Argentina. It is found mainly in the Neotropical Region, especially in tropical forest areas (Antweb 2020, Antwiki 2020). There are eight species recorded in the Neotropical Region (Sosa-Calvo & Longino 2007, Antmaps 2020), five in Brazil [*Discothyrea denticulata* Weber, 1939, *D. horni* Menozzi, 1927, *D. humilis* Weber, 1939, *D. sexarticulata* Borgmeier, 1954 and *D. neotropica* Bruch, 1919], and two in the State of São Paulo [*D. sexarticulata* and *D. neotropica* (Delabie et al. 2015, Antmaps 2020)].

The species are considered rare in litter probably because (1) the colonies have few individuals (Dejean & Dejean 1998, Katayama 2013, Delabie et al. 2015), (2) there is a limitation of sampling techniques (Hita- Garcia et al. 2019), and (3) the workers are very small, between 0.2 and 0.5 mm (Brandão et al. 2009). The species have a cryptic habit and the nests are generally inconspicuous (Zacharias & Dharma Rajan 2004), located in litter interstices, decaying logs (Brown 1958; Delabie et al. 2015), and under rocks (Bharti et al. 2015). Twigs from the fragmentation of tree branches are important for many ant species, especially for nesting. However, it is probably not a type of resource used by *Discothyrea* species in litter (see Fernandes et al. 2019a, b; 2020).

The species are specialist predators feeding almost exclusively on arthropod eggs, especially centipedes and spiders (Brown 1957, 1958, Dejean et al. 1999, Baccaro et al. 2015), but also on *Tenebrio molitor* Linnaeus, 1758, larvae (Wazema, personal communication). *Discothyrea* specimens can be collected in leaf litter using pitfall traps (Morini et al. 2007) and mainly in samples of leaf litter in forests with different phytophysiognomies (Vasconcelos & Delabie 2000, Feitosa & Ribeiro 2005, Suguituru et al. 2013, Wazema et al. 2019) and at different altitudes (Santos 2008). There are records in *Pinus elliottii* (Pachedo et al. 2009) and eucalyptus crops (Mentone et al. 2011, Suguituru et al. 2011), but these sites were surrounded by native vegetation. Lassau & Hocholi (2004) analyzed the response of ant communities to various physical and biological factors that occur in undisturbed places and recorded species of *Discothyrea* in low- and high-complexity habitats. In fragments of Atlantic Forest in the state of São Paulo, records of *Discothyrea* spp. are usually associated to preserved native vegetation (Suguituru et al. 2013, 2015).

The Brazilian Atlantic Forest has been reduced to immense archipelagos of tiny and widely separated forest fragments (Joly et al. 2014). Moreover, urban areas surround most fragments (Tabarelli et al. 2005), as well as areas with other anthropogenic activities (Ribeiro et al. 2009), such as extensive agriculture areas and eucalyptus and sugarcane crops. In a natural environment, changes caused by different land uses alter the landscape structure (e.g., by loss of biotic and abiotic resources), which in turn affect ant communities (Crist 2009). Ants are important components of the edaphic fauna (Decaëns 2010). They are considered good ecological indicators (Ribas et al. 2012, Casimiro et al. 2019), as their communities are influenced both on a local and a regional scale (Spiesman & Cumming 2008, Cumming 2011). Smaller and isolated fragments are more susceptible to species extinction and invasion by generalist species (Schoereder et al. 2004). Generalist species affect habitats in sites with high proportions of matrix habitats in the surrounding landscape (Spiesman & Cumming 2008).

As the structure of vegetation and soil and related abiotic factors influence ant communities, the analysis of landscape fragments and their surroundings may help the implementation of conservation management plans or environmental planning (Lindenmayer et al. 2008, László et al. 2014). This is true especially when species are considered rare, as they play a fundamental role in the evolutionary adaptation of communities to changing land uses (László et al. 2014). In this work, we evaluated the landscape in the vicinity of *Discothyrea* occurrence sites seeking to fill gaps in knowledge about the biology of this genus. We hope to find *Discothyrea* in fragments of the Atlantic Forest surrounded mainly by native vegetation, as *Discothyrea* species are considered rare and specialized.

## Materials and Methods

This study was conducted in ten sites in the following cities: São Paulo (Previdência Park), Mogi das Cruzes (Kimberly-Clark Reserve, Francisco Affonso de Mello Municipal Natural Park, Private Natural Heritage Reserve - Botujuru, and Leon Feffer Park), Mogi das Cruzes/Bertioga (Neblinas Park), Biritiba-Mirim (Biritiba-Mirim Dam), and Salesópolis (Ponte Nova Dam, Paraitinga Dam, and Ribeirão do Campo Dam) (Figure 1). All sites are part of the Atlantic Forest Domain in Southeast Brazil (Fiaschi & Pirani 2009, Colombo & Joly 2010). According to the Köppen classification, the region's climate is mesothermal with dry winters (Cwb). The annual rainfall accumulation is 1,500 mm (Cptec-Inpe 2020).

Ants were collected on the litter between 2001 and 2019 using techniques such as mini-Winkler extractors (Suguituru et al. 2013, Wazema et al. 2019) and pitfalls (Morini et al. 2007). The identification was carried out using keys specific to this group (Borgmeier 1949, Fernández 2003, Jiménez et al. 2008, Eguchi et al. 2014, Xu et al. 2014, Bharti et al. 2015) and by comparison with specimens deposited at the Reference Collection of the Alto Tietê Myrmecology Laboratory (LAMAT-UMC) (Suguituru et al. 2015) of the University of Mogi das Cruzes, São Paulo, Brazil, where the vouchers of this work are deposited.

The landscape was characterized using a 500-m buffer for each species occurrence site (Figure 1). Each collection period has its own methods. Aerial images were obtained using the Landsat 8 Satellite (Bing aerial - Bing 2020 Microsoft Corporation Earthstar Geographics SIO, ©Microsoft Corporation). Each buffer (n = 10) was categorized in (1) native vegetation, (2) rural, and (3) urban areas. Each class was quantified in $m^2$. The landscape of the surroundings of each collection site was classified as heterogeneous [area of native vegetation ≥ 50% (Figure 2a)] and homogeneous area [percentage of rural and urban areas ≥ 50% (Figure 2b)] (Moreira et al. 2015). The scale was 1:3,000. The software QGIS, version 2.18.19, was used (QGIS Development Team 2018). The linear models (GLM) with Poisson distribution (software R) were used to test differences in species occurrence among areas. The analyses were performed using the software Rstudio (R, version 3.6.1, R Core Team 2019) at a 5% significance level.

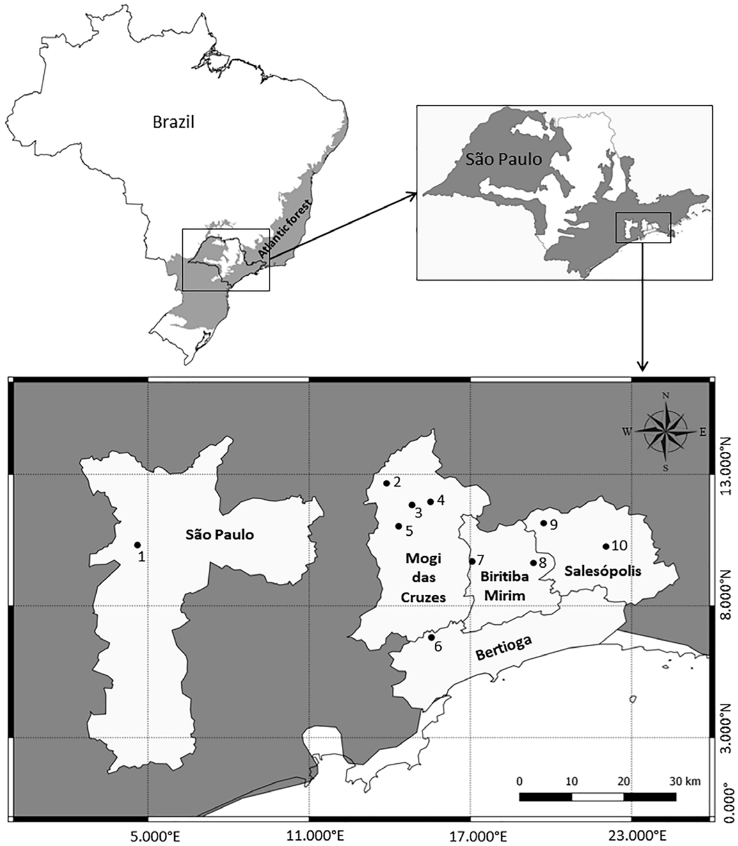


**Figure 1.** Location of collection sites. 1 - Previdência Park, 2 - Kimberly-Clark Reserve, 3 - Francisco Affonso de Mello Municipal Natural Park, 4 - Private Natural Heritage Reserve - Botujuru, 5 - Leon Feffer Park, 6 - Neblinas Park, 7 - Biritiba-Mirim Dam, 8 - Ponte Nova Dam, 9 - Paraitinga Dam, and 10 - Ribeirão do Campo Dam.

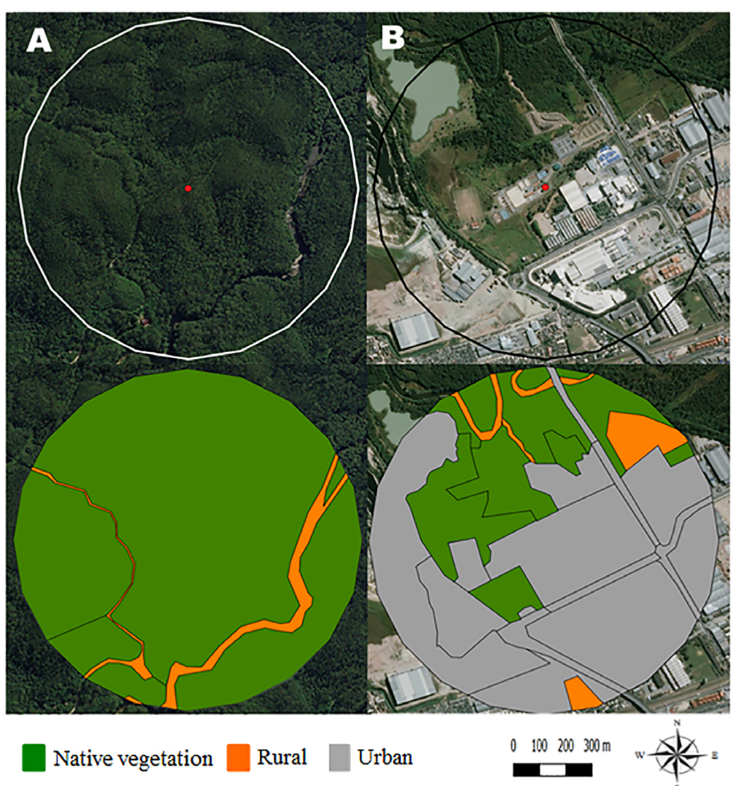


**Figure 2.** Characterization of the 500 m buffer and classes of the location where the species were collected. a - heterogeneous landscape (Neblinas Park, São Paulo city); and b – homogeneous landscape (Leon Feffer Park, São Paulo city).

## Results and Discussion

The results show that *D. sexarticulata* is more common than *D. neotropica* in the Atlantic Forest areas of the São Paulo state. We collected 67 specimens belonging to the *Discothyrea*. *Discothyrea sexarticulata* (59 specimens) occurred in 88% of the sites, and *D. neotropica* (eight specimens) occurred in 12% (Table 1). This is probably due to the resilience of *D. sexarticulata*. The data also show that this species occurs in fragments where the surroundings have 27-92% of native vegetation, 0-51% of rural areas, and 0-68% of urban areas (Table 1). We thus suggest that *D. sexarticulata* inhabits fragments with heterogeneous surroundings comprising a higher percentage of native and homogeneous vegetation and where anthropogenic changes (e.g., urban areas and crops) are more marked. In contrast, *D. neotropica* was recorded in fragments with more preserved surroundings, with 74-95% of native vegetation, 5-26% of rural areas, and 0-1% of urban areas (Table 1). The results suggest that this species occurs in fragments of the Atlantic Forest with a heterogeneous adjacency and a higher percentage of native Atlantic Forest vegetation. However, Arcusa & Cicchino (2017) reported that *D. neotropica* also inhabits pastures in the Pampas Region, which are considered areas of low environmental complexity.

The species of *Discothyrea* are tiny and its eyes have only one ommatid (Brandão et al. 2009, Delabie et al. 2015). These characteristics and the presence of small legs (Brandão et al. 2009) should limit mobility to small extensions (Yates & Andrew 2011). Therefore, the location where the nest is found should be relevant to these species, especially a location with a greater variety of interstices (e.g., litter), which contributes to less energy expenditure during foraging (Kaspari & Weiser 1999). Adjacent areas must affect the fragment's temperature and humidity (Lima-Ribeiro 2008), especially in areas considered small (Magnago et al. 2015). Changes in the natural environment can affect *Discothyrea* species at (1) a local scale, where the lack of humidity is a limiting factor for small species that forage in interstices of leaf litter (Kaspari 1996), and (2) a landscape scale, as landscape changes affect rare species more than common species (László et al. 2014). In this context, we suggest that *D. sexarticulata* may be less sensitive to changes than *D. neotropica*. However, the results of the analyses (Table 2) show that a larger number of samples is necessary, especially of *D. sexarticulata*.

**Table 1.** Collection site, landscape composition, and number of specimens according to *Discothyrea* species.

| Sites | Sites characterization | Coordenates | | Landscape composition (%) | | | Landscape types | Number of specimens | |
|---|---|---|---|---|---|---|---|---|---|
| | | Latitude | Longitude | Native vegetation | Rural area | Urban area | | *D. neotropica* | *D. sexarticulata* |
| 1-Previdência Park | Urban park | 23°34'00"S | 46°43'00"W | 37 | 0 | 63 | Homogeneous | – | 1 |
| 2-Kimberly-Clark Reserve | Anthropized fragment of Atlantic Forest | 23°26'52"S | 46°14'48"W | 59 | 8 | 33 | Heterogeneous | – | 9 |
| 3-Francisco Affonso de Mello Municipal Natural Park | Conservation units | 23°29'22"S | 46°11'55"W | 92 | 8 | 0 | Heterogeneous | – | 1 |
| 4-Private Natural Heritage Reserve - Botujuru | Conservation units | 23°28'59"S | 46°09'49"W | 49 | 51 | 0 | Homogeneous | – | 21 |
| 5-Leon Feffer Park | Urban park with Atlantic Forest native vegetation | 23°31'49"S | 46°13'26"W | 27 | 5 | 68 | Homogeneous | – | 1 |
| 6-Neblinas Park | Conserved Atlantic Forest area | 23°44'40"S | 46°09'43"W | 95 | 5 | 0 | Heterogeneous | 5 | 3 |
| 7-Biritiba Mirim Dam | Conserved Atlantic Forest fragment | 23°35'54"S | 46°05'06"W | 74 | 26 | 0 | Heterogeneous | – | 4 |
| 8-Ponte Nova Dam | Conserved Atlantic Forest fragment | 23°36'04"S | 45°58'10"W | 80 | 19 | 1 | Heterogeneous | – | 14 |
| 9-Paraitinga Dam | Conserved Atlantic Forest fragment | 23°31'28"S | 45°57'01"W | 51 | 49 | 0 | Heterogeneous | 2 | 4 |
| 10-Ribeirão do Campo Dam | Conserved Atlantic Forest fragment | 23°34'10"S | 45°49'57"W | 74 | 26 | 0 | Heterogeneous | 1 | 1 |
| **Specimes total** | | | | | | | | **8** | **59** |

**Table 2.** Summary of GLM with a transect-level random intercept (ten transects, one in each area). Landscape classes selected according to landscape composition (%) of areas: NV-U (Native vegetation + urban area), NV-R-U (Native vegetation + rural area + urban area) and NV-R (Native vegetation + rural area).

| Model: Occurrence of *D. sexarticulata* ~ Area + (1 \| transect) | | | | |
|---|---|---|---|---|
| Predictors | Estimated | Standard Error | Z | *p* |
| Intercept | -2.438 | 1.000 | 0.000 | 1.000 |
| NV-U | 6.280 | 1.414 | 0.000 | 1.000 |
| NV-R-U | 1.846 | 1.026 | 1.799 | 0.072 |
| NV-R | 1.482 | 1.022 | 1.449 | 0.147 |
| Model: Occurrence of *D. neotropica* ~ Area + (1 \| transect) | | | | |
| Predictors | Estimated | Standard Error | Z | *p* |
| Intercept | -1.830 | 5.718 | -0.003 | 0.997 |
| NV-U | -4.912 | 8.086 | 0.000 | 1.000 |
| NV-R-U | 1.790 | 5.718 | 0.003 | 0.998 |
| NV-R | 1.848 | 5.718 | 0.003 | 0.997 |

Therefore, this study significantly contributes to the knowledge on the biology of *Discothyrea*. There are few studies on the natural history and behavior of species of this genus possibly because they have cryptic habits and small sizes. The results show that *D. sexarticulata* and *D. neotropica* occur in fragments with different surroundings, suggesting a greater resilience of *D. sexarticulata*. Our study indicates that landscape scales can be important structuring forces on local communities. We report relevant technical knowledge for future studies on landscape ecology and ant communities in Atlantic Forest areas. Furthermore, we demonstrate the importance of regional studies as a tool for understanding species ecology. As most of the Atlantic Forest is composed of forest fragments smaller than 50 ha (Ribeiro et al. 2009), corresponding to vegetation islands within a matrix with several types of anthropogenic activity (e.g., crops, paved roads, dense buildings, railroads, and mining), studies of this nature are highly relevant for the conservation of species.

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## Author Contributions

Claudia Tiemi Wazema: Substantial contribution in the concept and designer of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content.

Victor Hideki Nagatani: Substantial contribution in the concept and designer of the study; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content.

Débora Rodrigues de Souza-Campana: Substantial contribution in the concept and designer of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content.

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Maria Santina de Castro Morini: Substantial contribution in the concept and designer of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content.

## Conflicts of Interest

The authors declare no conflicts of interest in the publication of this manuscript.

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**Article**

# Population structure of the shrimp *Palaemon pandaliformis* (Stimpson, 1871) (Caridea: Palaemonidae) in the river Rio Itapemirim, in Southeastern Brazil

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***Abstract:*** The present study analyzes the population structure of *Palaemon pandaliformis*, based on sex ratio, the frequency distribution in defined size classes and the reproductive biology of samples captured in seasonal collections (winter 2011 to autumn 2012) with sieves under marginal vegetation. A total of 1,043 specimens were obtained, 222 juveniles, 465 males and 356 females, being that of this total of females, 58.42% were in the ovigerous condition. The average size of the carapace for both sexes varied between 4 and 6 mm ($p > 0.05$) only the ovigerous females had higher averages ($p < 0.0001$). The sex ratio differed with 1:0.76 for males ($p = 0.0001$), although juvenile individuals and females were present in all sampled periods, except ovigerous females in autumn. Highest abundances were observed during the winter and spring period ($p = 0.0001$), decreasing in the periods with higher water temperature and salinity (summer and autumn). Was observed a continuous recruitment due to the presence of juveniles in all seasons, with greater abundance in winter and spring. Thus, based on the presence of ovigerous females in almost all seasons and juveniles in all, the reproduction for *P. pandaliformis* can be defined as seasonal-continuous with peak in winter period.
***Keywords:*** freshwater prawns; population dynamics; sex ratio; reproductive period.

## Estrutura populacional do camarão *Palaemon pandaliformis* (Stimpson, 1871) (Caridea: Palaemonidae) no Rio Itapemirim, sudeste do Brasil

***Resumo:*** O presente estudo analisou a estrutura populacional de *Palaemon pandaliformis*, com base na razão sexual, distribuição de frequência em classe de tamanho e biologia reprodutiva de amostras capturadas em coletas sazonais (inverno/2011 a outono/2012), com peneiras sob a vegetação marginal. Um total de 1.043 exemplares foi obtido, sendo 222 juvenis, 465 machos e 356 fêmeas, sendo que deste total de fêmeas, 58.42 % estavam na condição ovígeras. O tamanho médio da carapaça, para ambos os sexos, variou entre 4 e 6 mm ($p>0,05$), apenas as fêmeas ovígeras apresentaram maiores médias ($p<0,0001$). A razão sexual diferiu em 1:0,76 para machos ($p=0,0001$), embora os indivíduos jovens e as fêmeas estiveram presentes em todos os períodos amostrados, exceto as fêmeas ovígeras no outono. As maiores abundâncias foram observadas durante o período de inverno e primavera ($p=0,0001$), diminuindo nos períodos de maior temperatura e salinidade da água (verão e outono). Foi observado um recrutamento contínuo devido a presença de juvenis em todas as estações, com maior abundância no inverno e primavera. Assim, com base na presença de fêmeas ovígeras em quase todas as estações e juvenis em todas, a reprodução de *P. pandaliformis* pode ser definida como sazonal-contínua, com pico no período do inverno.
***Palavras-chave:*** camarão de água doce; dinâmica populacional; razão sexual; período reprodutivo.

## Introduction

Population studies of crustaceans provide relevant information on the dynamics and preservation of the studied species. This knowledge and a clear characterization of species are fundamental for measures aimed at the maintenance and conservation of natural resources and the understanding of their ecological stability. Variations in the different aspects of ecological stability, such as birth-death-ratio, density, sex ratio, reproductive aspects or growth and migration, are commonly observed and quantified in population studies (Costa & Negreiros-Fransozo 2003.

The families Atyidae and Palaemonidae are the most common ones among freshwater carideans. In Brazil, the palaemonids are mainly represented by the genera *Macrobrachium* (Spence Bate, 1868) and *Palaemon* (Weber, 1795) known as freshwater prawns (Carvalho et al. 1979, Bond-Buckup & Buckup 1989, Valenti et al. 1989).

Representatives of the species *Palaemon pandaliformis* (Stimpson, 1871) are small carideans prawns found in fresh and salt water environments from Guatemala to southern Brazil (Melo 2003, Ferreira et al. 2010). Individuals of this species, as well as a large part of the Palaemonidae, are osmoregulators (Freire et al. 2003). They can support variations in salinity, which allows them to explore environments from freshwater to estuaries, often found next to aquatic or marginal vegetation (Mortari & Negreiros-Fransozo 2007).

Although *P. pandaliformis* is not officially used for commercial purposes nor makes part of the human diet, this species is still widely exploited as live bait in artisanal fisheries (Müller et al. 1996). However, it is a species that is also an important part of the trophic chain, serving as food for various invertebrates, fish and birds (Mortari & Negreiros-Fransozo 2007).

Despite its important ecological role, the work related to the biology of the species is still unsatisfactory. Only few studies have been published addressing its reproductive biology (Lima & Oshiro 2002, Mortari et al. 2009, Rosa et al. 2015), ecological aspects (Müller et al. 1996, Mortari & Negreiros-Fransozo 2007) and sexual maturity (Anger & Moreira 1998, Paschoal et al. 2013). In Southeastern Brazil and especially in the State of Espírito Santo, a deeper analysis of the species dynamics of the shrimp *P. pandaliformis* are necessary in order to understand the importance of this species for the balance of an ecosystem of rivers and estuaries. Thus, the objective of this study was to analyze the population structure of these shrimps by surveying and studying data regarding their abundance, frequency distribution in size classes, sex ratio, seasonal recruitment of juveniles and the reproductive period based on the presence of ovigerous females.

## Material and methods

The shrimps associated with partially submerged marginal vegetation were captured in seasonal collections (winter: September 2011; spring: November 2011; summer: March 2012; autumn: June 2012). Two samplings were performed per period, in order to cover a larger area of capture and obtain replicates, totaling eight samples.

The collection points were set on a river arm close to the mouth of the river Rio Itapemirim (20°59'48.0"S; 40°48'53.8"W), in the municipality of Marataízes, located at the south coast of the State of Espírito Santo, Southeast Brazil (Figure 1). The collections were carried out during low tide (between 0.0 and 0.1m) during the day and were performed actively by a collector with the aid of a 2 mm mesh sieve for 25 minutes in each sampling.

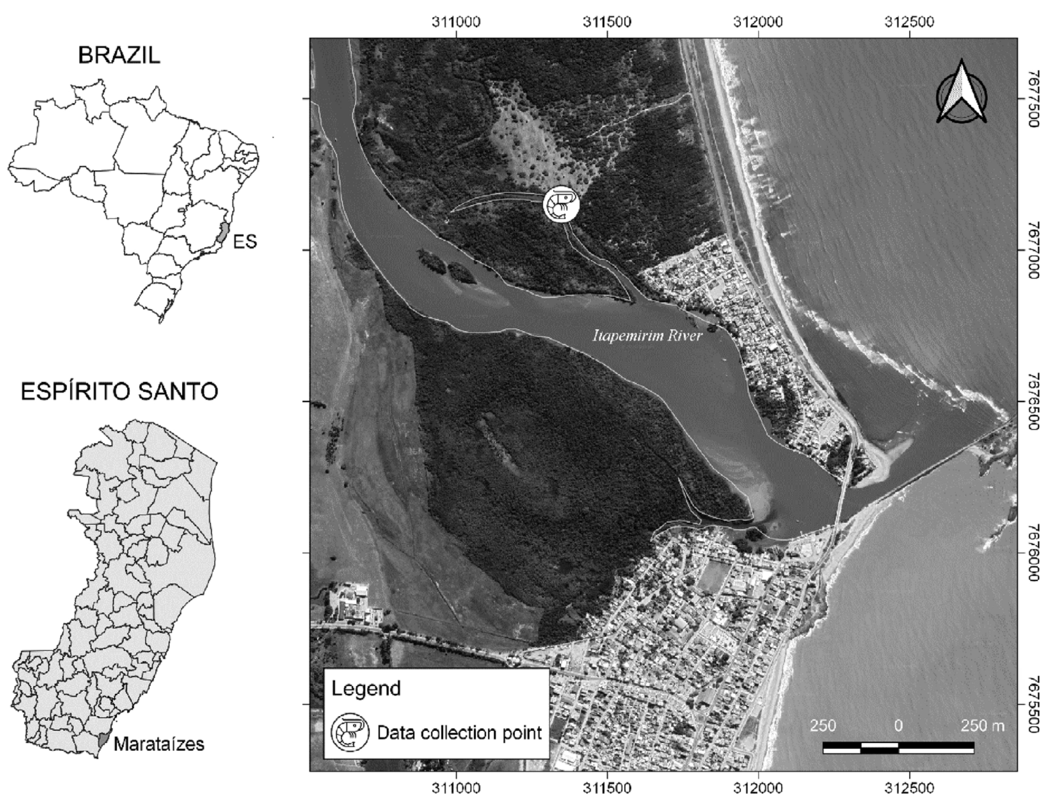


**Figure 1.** Collection point of Palaemonid shrimps in the river Rio Itapemirim/Marataízes, Espírito Santo, located in the southeast of Brazil between winter 2011 and autumn 2012.

The following environmental factors were analyzed during all collections: water temperature (°C), water salinity and depth were measured using a mercury thermometer, manual refractometer (‰) and measuring tape, respectively.

The samples of each point were grouped for a better analysis, considering proximity and the similarity of the environmental parameters in each sampling.

In the laboratory, the specimens were screened and identified (Melo 2003). Sexing was performed based on two characters: 1. the location of male and female gonopores located at the base of the 5th and 3rd pair of pereiopods, respectively; and 2. the morphology of the secondary sexual characters (in male individuals, the presence of the male appendix in the endopodite of the second pair of pleopods). In addition, females with eggs adhered to the pleopods located in the abdomen were registered (Bauer 2004).

For each collected individual, we measured the length of the carapace (CC, distance from the posterior margin of the orbit to the posterolateral margin of the carapace) with a digital caliper (0.01 mm). Following the work of Rosa et al. (2015), we separated juveniles from adult individuals (males, non-ovigerous females and ovigerous females) based on the size of the smallest ovigerous female we collected. Subsequently, we grouped the palaemonids into size classes with an amplitude of 1 mm, and visualized their frequency distribution for each sex with a histogram.

The average carapace length of the palaemonid shrimp was compared statistically using the Mann-Whitney test (Hammer et al. 2001). An analysis of variance (one-way ANOVA) was used to verify the existence of a possible difference between the abiotic factors (temperature and salinity) and between the constituent groups of the population (juvenile, males, non-ovigerous females and ovigerous females). Another analysis (two-way ANOVA) verified the average catches of palaemonid shrimp throughout the seasons. In the correlation between abiotic data (temperature and salinity) and data related to the abundance of palaemonids capture, we used the Pearson's coefficient and considered $p \leq 0.05$ to be significant.

The sex ratio of the population (1:1) was determined by sampling the collected adult individuals, followed by the Chi-square test ($X^2$) ($\alpha = 5\%$) (Zar 2010). Recruitment was verified through the proportion of juveniles in relation to the total of sampled adult individuals, whereas the reproductive period was analyzed through the percentage of ovigerous females in relation to the total of adult females registered in each season. The analyses of the tests were performed using the PAST statistical package at the level of 5% of significance (Hammer et al. 2001).

## Results

The water temperature presented a seasonal fluctuation (one-way ANOVA, p = 0.015), varying between 25 and 27°C in winter, spring and autumn, and being stable at 29°C during summer. The same seasonality was found for the water salinity (one-way ANOVA p <0.001), which showed a variation of up to 2 PSU over the seasons, except in summer when 9 PSU were registered (Figure 2). The correlation analysis showed a strong positive linear association between temperature and salinity variables (r = 0.782; p = 0.021). The depth of the collection site did not vary (average of 1.3 meters, one-way ANOVA, p> 0.05).

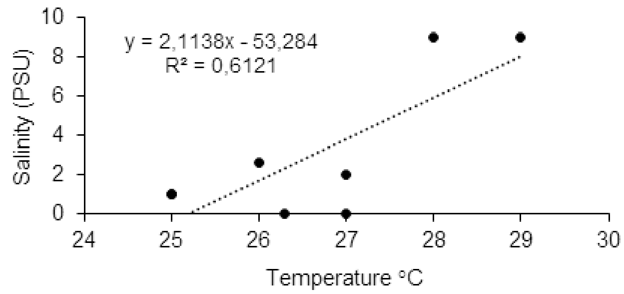


**Figure 2.** Seasonal variation and correlation of temperature (°C) and salinity (‰) of the water from the river Rio Itapemirim/Marataízes, Espírito Santo, located in the southeast of Brazil between winter 2011 and autumn 2012.

During the study period, a total of 1,043 individuals of *P. pandaliformis* were obtained: 222 juveniles (21.28%), 465 males (44.58%) and 356 females total (34.14%), of which 208 had eggs adhered to their pleopods (ovigerous females – 58.42%) (one-way ANOVA; p> 0.05). The largest numbers of shrimp were caught in winter and spring of 2011 (two-way ANOVA; p = 0.0001) (Figure 3). The results of the Pearson's correlation showed no significant relationship between the variation of temperature (r = -0.66; p = 0.069) and salinity (r = -0.46; p = 0.24) with the abundance of *P. pandaliformis* (Figure 4).

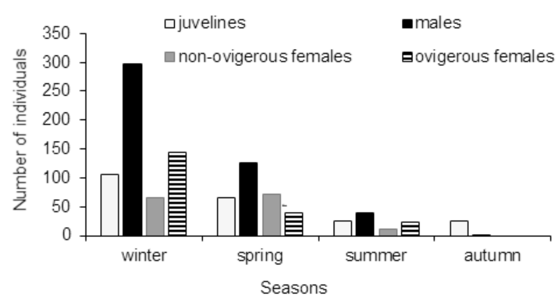


**Figure 3.** Number of individuals of *Palaemon pandaliformis* collected seasonally in an estuary in southeast Brazil between winter 2011 and autumn 2012.

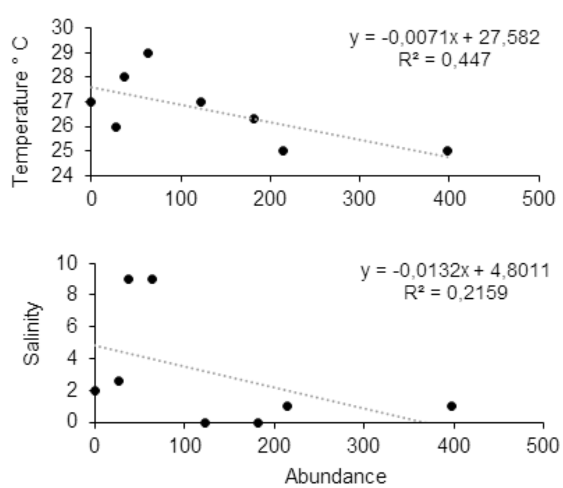


**Figure 4.** Correlation of abiotic factors (temperature and salinity) with the abundance of *Palaemon pandaliformis* collected seasonally in an estuary in southeast Brazil between winter 2011 and autumn 2012.

Most of the captured male individuals (39%) as well as the non-ovigerous females (12%) presented a carapace length varying between 4 to 6 mm CC, with no significant difference between the average size between the sexes (U = 32251; p = 0.2487) (Figure 5). For the ovigerous females, a greater amplitude in the carapace length was observed, with preferential distribution between classes 4-5 and 6-7 mm CC, differentiating it from the means of the male individuals (U = 21526; p <0.0001) and non-ovigerous females (U = 7173; p <0.0001). Juvenile individuals were abundant in the smaller size classes (<2 and 3-4 mm CC) (Figure 6).

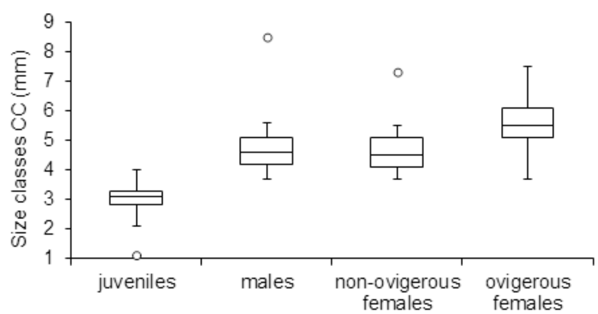


**Figure 5.** Average values of carapace length for each group of interest of *Palaemon pandaliformis* collected in an estuary in southeast Brazil between winter 2011 and autumn 2012. * Box and bar represent the quartiles, the highlighted line is the median and the point shows outliers.

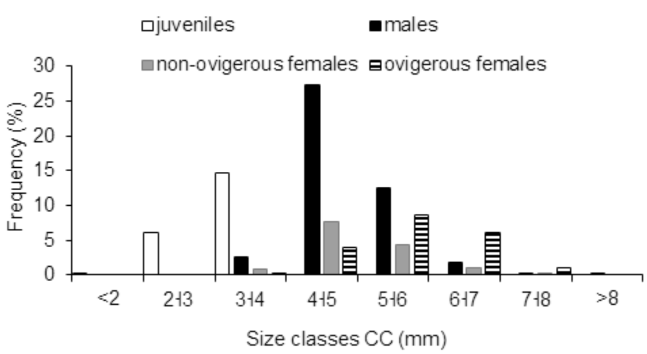


**Figure 6.** Frequency distribution of individuals in carapace length (CC) classes, for *Palaemon pandaliformis* collected in an estuary in southeast Brazil between winter 2011 and autumn 2012.

The sex ratio observed in the population of *P. pandaliformis* differed from that we expected (1:1) in favoring male individuals (♂1:0.76♀) ($X^2$ = 14.47; GL = 1; p = 0.0001).

Throughout the seasons that comprised this study, we observed continuous recruitment through the presence of juveniles in all seasons (Figure 7a). Despite the large proportion of juveniles individuals in autumn 2012, this was also the season with the lowest total number of observed *P. pandaliformis* (26 juveniles and 1 adult). Ovigerous females were present in all seasons of the year, except for autumn 2012, a season in which we observed no female individuals (Figure 7b).

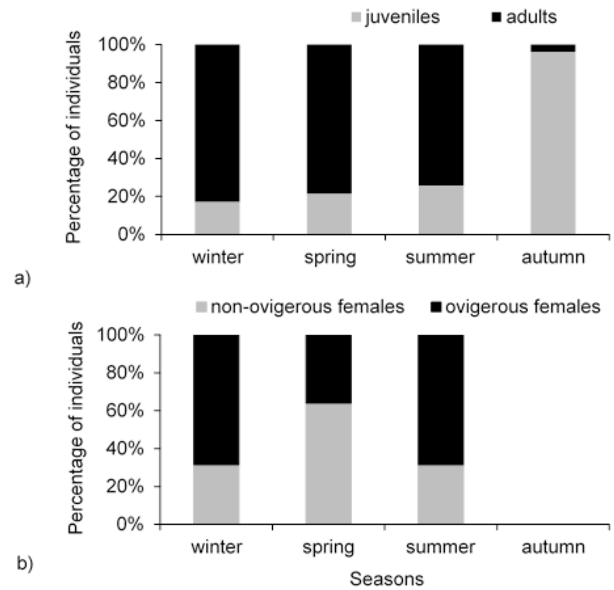


**Figure 7.** Percentage variation of juvenile and adult individuals (a). Percentage variation of non-ovigerous and ovigerous females (b) in the population of *Palaemon pandaliformis* collected seasonally in an estuary in southeast Brazil between winter 2011 and autumn 2012.

## Discussion

The present study characterized the population structure of *P. pandaliformis* an environmentally important species of caridean shrimp, on which to date no work had been published in the state of Espírito Santo, Southeastern Brazil. The environmental characteristics of the estuary in the present study showed a positive association between water temperature and water salinity, with a gradual increase in these values throughout the winter (September 2011) and spring (November 2011) until reaching the highest levels in summer (March 2012). This pattern is typical of estuarine dynamics due to their geomorphology and differences in hydrodynamics, which result in constant changes of water temperature and salinity (Miranda et al. 2002).

The highest abundance of *P. pandaliformis* occurred in winter 2011 and spring 2011, decreasing as the water temperature increased over the collection periods. Rosa et al. (2015) observed for subtropical environments in a salt marsh in Paraná, peaks in number of caught individuals of *P. pandaliformis* in December and October, with no correlations with water temperature. This pattern was also observed for species of the genus *Macrobrachium* in the studies carried out by Mossolin & Bueno (2002) and Fransozo et al. (2004). For subtropical and temperate environments, a pattern is described in which macrofaunal organisms have higher density values associated with the hottest months of the year (Emmerson 1985, Rosa & Bemvenuti 2006).

When comparing the relationship of the abundance of *P. pandaliformis* in two rivers (Rio Ubatumirin and Rio Comprido) with environmental factors, Mortari & Negreiros-Fransozo (2007) found a correlation with temperature only in the population of the Rio Comprido. Mortari et al. (2009) reported a correlation between salinity and abundance of palaemonids, especially in relation to ovigerous females during the reproductive period. Rosa et al. (2015) did not observe a correlation between abundance of this species and water temperature, but described a negative correlation with salinity. In this sense, although the present study has not shown a correlation between abundance and environmental factors, it is clear that the abundance of this group of organisms is related to environmental factors, since salinity generally functions as an important regulator of the abundance of these macrocrustaceans in estuarine environments (Teixeira & Sá 1998). On the other hand, temperature is another dynamic factor in this environment, which occasionally influences abundance, as it directly interferes with the physiology of crustaceans (Wear 1974, Melo &Veloso 2005), especially since this species is distributed both in fresh water and in estuaries (Bond-Buckup & Buckup 1989), having a great osmoregulatory capacity (Freire et al. 2003).

The sex ratio observed in the population of *P. pandaliformis* differed from what we expected (1:1), with a predominance of male individuals, similar to the findings in tropical freshwater environments documented by Paschoal et al. (2013) and Paschoal et al. (2016). Müller et al. (1999), Lima & Oshiro (2002), Mortari et al. (2009) and Rosa et al. (2015) on the other hand found a predominance of females in the populations they observed – a pattern also documented in studies of other freshwater carideans (Mossolin & Bueno 2002) on *Macrobrachium olfersii* (Wiegmann, 1836), Mantelatto & Barbosa (2005) with *M. brasiliense* (Heller, 1862), Sampaio et al. (2007) with *M. amazonicum* (Heller, 1862) and Soares et al. (2015) with *M. jelskii* (Miers, 1877). Rosa et al. (2015) suggested that the predominance of females seems to be a common characteristic for the populations of *P. pandaliformis*. However, for Wenner (1972) and Kim (2005), the difference in sex ratio in the population may be related to the mortality rates between males and females. Other factors can also influence this ratio, such as the rate of seedlings, dispersion, reproduction (Botelho et al. 2001), migration, habitat preference (Collins et al. 2006) and even predation by birds and fish, who aim especially for non-ovigerous and ovigerous females palaemonids (Soares et al. 2015).

In the comparison of the carapace length, the ovigerous females of *P. pandaliformis* had the highest averages, differing statistically from the males. Larger sizes in females are also reported in studies by Müller et al. (1996), Lima & Oshiro (2002), Paschoal et al. (2013) and Rosa et al. (2015). The males of *P. pandaliformis* do not defend the females and are not considered territorial, so that there is no need for energy expenditure for a body size increase, leaving more energy to produce sperm for reproduction (Paschoal et al. 2013). In females, on the other hand, the larger size of the abdomen and pleura contribute to a larger incubator chamber for the protection of their eggs during the incubation period (Paschoal et al. 2013).

The occurrence of sexually undifferentiated and immature (juvenile) individuals in the smaller size classes as well as the marked presence of adults from the medium size classes indicate a continuous population growth (Mattos & Oshiro 2009). Another pattern we observed was the occurrence of juveniles during all seasons, but with a predominance in the winter months, where a lower water temperature was recorded. Mattos & Oshiro (2009), who studied *M. potiuna* (Müller, 1880), suggested that recruitment is related to water temperature: since the colder waters exhibit higher levels of oxygenation, which favors high metabolic rates, they provide a better environment for the development of these caridean, while in periods of higher temperatures, there may be a reduction or absence of juveniles in the population – a pattern we also found in the present study.

Females with eggs adhered to their pleopods were observed in almost all seasons of the year, with the exception of autumn, presenting higher catch frequencies in winter and spring – this suggests a pattern of seasonal-continuous reproduction of the population of *P. pandaliformis* in the river Rio Itapemirim. According to Pinheiro & Fransozo (2002), a reproduction pattern is defined as seasonal-continuous when crustacean ovigerous females are recorded throughout the year, but with higher peaks in certain months; on the other hand, if these ovigerous females with or without developed gonads occur only in certain seasons and are absent in others, it is considered seasonal reproduction. The seasonal-continuous pattern has been observed in the estuarine regions of southeastern Brazil by Lima & Oshiro (2002) and Mortari et al. (2009) as well as in a tropical river system by Paschoal et al. (2013). On the other hand, Müller et al. (1996) and Rosa et al. (2015) observed a seasonal reproductive pattern for the same species, associating it to the hottest periods of the year in subtropical regions.

In this sense, we can conclude that the river Rio Itapemirim presents favorable conditions for the population establishment of *P. pandaliformis*. The abundance of this species was more accentuated in the period between September and November. The population was in balance with a constant recruitment of juveniles in the smallest size classes and presence of mature individuals from the intermediate classes, thus suggesting a continuous population growth, in addition, we observed the presence of ovigerous females in almost all seasons, thus, with based these results, suggesting a pattern of seasonal-continuous reproduction. This study is the first of its kind on this species in the state of Espírito Santo, and contributes to a better understanding of the species on a regional scale, as well as of the region of southeastern Brazil as a whole. We provide basic information on the behavior and adaptation of these shrimp in different habitats. In addition, these studies can serve as a tool for conservation and ecosystem management, since the shrimp are an important part of the trophic chain in rivers and estuaries, which are often integrated into large urban centers.

## Author Contributions

Pedro Laino: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual contente.

Joelson Musiello-Fernandes: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Adriane Araujo Braga: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

## Conflicts of interest

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

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Article

# Freshwater fishes of the Bahia State, Northeastern Brazil

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***Abstract:*** This work was carried out from the assessment of the conservation status of the freshwater ichthyofauna from Bahia State. The inventory data and species distribution were obtained from the specialized scientific literature and representative ichthyological collections. A total of 281 native species was recorded in Bahia State, distributed in the Northeastern Mata Atlantica (NMA) and São Francisco (SFR) freshwater ecoregions. There was a larger number of species in the NMA (187 spp.), composed by several coastal basins, than in the SFR (134 spp.), composed by São Francisco river basin. Among the 30 families recorded, Characidae and Rivulidae were the most representative, with 53 and 48 species, respectively. The conservation status of 214 species was assessed and 33 of them (15%) were included in the IUCN threat categories. Of these, 11 species were classified as vulnerable (VU), 12 as endangered (EN), and 10 as critically endangered (CR). Most threatened species (n = 14) belongs to the family Rivulidae. The larger number of threatened species in the NMA: (n = 23) is mainly related to the high endemism of restricted-range species associated with the human occupation impacts along the coastal regions. In the SFR, most of threatened species are annual killifishes, which are locally disappearing due to increasing degradation of their temporary habitats.
***Keywords:*** *biodiversity, threatened species, endemism, Northeastern Mata Atlantica ecoregion, São Francisco river basin.*

**Peixes de água doce do Estado da Bahia, Nordeste do Brasil**

***Resumo:*** Este trabalho foi realizado a partir da avaliação do estado de conservação da ictiofauna de água doce do Estado da Bahia. Os dados de inventário e distribuição das espécies foram obtidos a partir da literatura científica especializada e de coleções ictiológicas representativas. Um total de 281 espécies nativas foi registrado no Estado da Bahia, distribuídas nas ecorregiões de água doce Mata Atlântica Nordeste (NMA) e São Francisco (SFR). A riqueza de espécies foi maior na NMA (187 spp.), composta por diversas bacias costeiras, do que na SFR (134 spp.), composta pela bacia do rio São Francisco. Das 30 famílias registradas, as mais representativas foram Characidae e Rivulidae, com 53 e 48 espécies, respectivamente. O estado de conservação de 214 espécies foi avaliado e 33 (15%) destas foram classificadas em alguma categoria de ameaça da IUCN. Destas, 11 foram classificadas como vulneráveis (VU), 12 em perigo (EN) e 10 criticamente em perigo (CR). A maioria das espécies ameaçadas (n = 14) pertence à família Rivulidae. O elevado número de espécies ameaçadas na NMA: (n = 23) está associado ao alto endemismo das espécies com distribuição restrita e aos impactos da ocupação humana ao longo da região costeira. Na SFR, a maioria das espécies ameaçadas é de peixes anuais, que estão localmente desaparecendo devido à degradação crescente de seus habitats temporários.

***Palavras-chave:*** *biodiversidade, espécies ameaçadas, endemismo, Ecorregião Mata Atlântica Nordeste, bacia do rio São Francisco.*

## Introduction

The Neotropical region harbors the most diverse freshwater fish fauna in the world, with more than 5,700 described species, although the final number may exceed 8,000 species (Albert et al. 2011, Reis 2013, Bertaco et al. 2016, Reis et al. 2016). Putting this number into perspective, Neotropical freshwater fishes account for about one in five of the world's fish species, or approximately 10% of all vertebrate species (Albert & Reis 2011). According to Ribeiro (2006), the reasons for such a marked diversity are likely to be both historical and ecological, a result of millions of years of evolution from the breakup of Gondwana to the present day.

It is often claimed that freshwater ecosystems are the most endangered in the world (Sala et al. 2000, Dudgeon et al. 2006, Nogueira et al. 2010). As in other freshwater regions, South American fishes are threatened by overexploitation, flow modification, habitat destruction, species invasion, pollution, eutrophication, and siltation (Lévêque et al. 2008). Several fish conservation initiatives are underway in South America, including an ambitious program that assesses the conservation status of all Brazilian fish species (Reis 2013). This program resulted in the Brazilian list of threatened fishes and aquatic invertebrates, known as the Brazilian Red List (MMA 2014). Along with this national task, some parallel regional projects of conservation assessment of continental fishes have also been conducted (e.g., Rosa et al. 2003, Alves & Leal 2010, Reis et al. 2016).

In 2013, one of these regional initiatives was carried out in Bahia State, northeastern Brazil, and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) in collaboration with the Secretaria do Meio Ambiente do Estado da Bahia (SEMA), and several ichthyologists completed a comprehensive conservation assessment of the freshwater ichthyofauna in the state. It is important to highlight that the results of this regional workshop became the basis for the publishing of the list of threatened freshwater fish species reported from Bahia state (SEMA 2017). Bahia, one of the 27 federative units of Brazil, is the fifth largest state by area, with more than 564.000 km$^2$ and 417 municipalities (IBGE 2019). This state partially encompasses two freshwater ecoregions as proposed by Abell et al. (2008), the São Francisco (SFR, ecoregion 327) and Northeastern Mata Atlantica (NMA, ecoregion 328) ecoregions (Figure 1). According to these authors, a freshwater ecoregion is defined as a large area encompassing one or more freshwater systems with a distinct assemblage of natural freshwater communities and species.

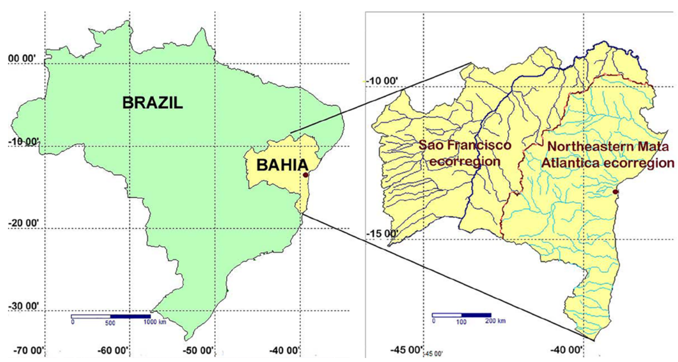


**Figure 1.** Localization and river drainages across Bahia state. Parts of the São Francisco (in dark blue) and Northeastern Mata Atlantica (in light blue) freshwater ecoregions in Bahia State, Northeastern Brazil, are shown. Red dotted line indicates the boundaries between Northeastern Mata Atlantica ecoregion and São Francisco ecoregion.

Although political geographic boundaries are useless for biological organisms, national or regional species lists are important for monitoring and conservation planning efforts (Silveira et al. 2010, Hortal et al. 2015). In this sense, any conservation initiative starts from the identification of the species that will be protect by such initiative. However, that apparently simple and basic information is absent or incomplete for diverse regions of Brazil. For example, the procedure of compilation of fish species that occur in rivers that drain Bahia state to evaluation of conservation status took several weeks of hard work previously to 2013 workshop, and involved the participation of several experts.

Before that effort, the knowledge about the species that occur in Bahia rivers was dispersed in some studies which focused on description of new species (e.g., Garavello 1977, Malabarba et al. 2004, Costa 2007, Zanata & Serra 2010), or representing the fish species inventory of some basins or part of them (e.g., Sato & Godinho 1999, Godinho & Godinho 2003, Rosa et al. 2003, Santos 2005, Sarmento-Soares et al. 2007, Barbosa & Soares 2009, Burger et al., 2011).

To make available the results of the list of threatened freshwater fishes of Bahia State elaborated during the aforementioned 2013 workshop, we report here the first checklist of freshwater fish species of the Bahia state. This list includes species from the SFR and NMA ecoregions described at present, as well the conservation status for those evaluate in 2013 Workshop. Without the pretense of being a definitive list, it is a starting point that should be broadened and updated in order to compile the scattered information available.

## Material and methods

### *1. Study area*

Freshwater fish species from stretches and tributaries in the SFR and NMA ecoregions of Bahia State (Figure 1) were analyzed. The SFR ecoregion is equivalent to the São Francisco basin, the third largest Neotropical hydrographic basin which is enclosed in an area of more than 630,000 km$^2$ (Sato & Godinho 1999, Kohler 2003, Langeani et al. 2009). The São Francisco is the longest river running entirely in Brazil, corresponding to more than 7.5% of the Brazilian territory (Godinho & Godinho 2003, Pompeu & Godinho 2006). With more than 2,500 km of extension (Godinho & Godinho 2003, Langeani et al. 2009), the São Francisco river and its tributaries drain three biomes (Caatinga, Cerrado and, peripherally, Atlantic Forest) crossing six states (from its source to mouth: Minas Gerais, Goiás, Bahia, Pernambuco, Alagoas, and Sergipe) plus the Federal District (Paiva 1982, Godinho & Godinho 2003, Langeani et al. 2009). From its headwaters in the Serra da Canastra, located in the central-western part of Minas Gerais State (at about 1,200-1,500 m above sea level), to its mouth, between Alagoas and Sergipe, the river drains more than 500 municipalities (Kohler 2003, Sato & Godinho, 2003). Although not entirely located in Bahia State, the São Francisco is the largest and one of the most important rivers of the state.

The NMA, in turn, comprises all coastal drainages in eastern Brazil, from the Sergipe river in the north to the Itabapoana river in the south (Camelier & Zanata 2014a). To the west, the NMA ecoregion is limited by the watershed border with the São Francisco river basin, along the Serra do Espinhaço up to the north, and by the Paraíba do Sul river basin in the south (Hales & Petry 2013). This ecoregion includes more than 25 isolated basins (Camelier & Zanata 2014a), draining the eastern slopes of the Brazilian crystalline shield directly into the Atlantic Ocean. From north to south, the main drainages of the NMA in Bahia State are: Vaza-Barris, Real, Itapicuru, Inhambupe, Pojuca, Paraguaçu, Jequiriçá, Contas, Cachoeira, Almada, Una, Pardo, Jequitinhonha, Buranhém, Frades, Jucuruçu, Itanhém, Peruípe, and Mucuri (Langeani et al. 2009, Camelier & Zanata 2014a). These drainages, along with several other smaller isolated basins of the NMA, are separated by the scarped, mountainous landscapes of the eastern margin of the Brazilian shield (Ribeiro 2006). The NMA is mainly inserted in the Atlantic Forest biome, although the northern drainages from the upper Contas to the Vaza-Barris river basins are partially under the influence of the semiarid Caatinga (Rosa et al. 2003, Camelier & Zanata 2014a, Lima et al., 2017).

### *2. Species inventory and list compilation*

The species inventory and information about their distributions were obtained by consulting different sources in the reliable specialized scientific literature (i.e. original descriptions, taxonomic reviews, phylogenetic studies, and species catalogues) and representative ichthyological collections such as Instituto Nacional da Mata Atlântica (formerly Museu de Biologia Professor Mello Leitão-MBML), Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Museu Nacional do Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu de História Natural da Bahia (UFBA), and Universidade Federal do Rio Grande do Norte (UFRN). Data were obtained from these fish collections or by searches in the national system of information on ichthyological collections (e.g., SIBIP/NEODAT III 2019, CRIA 2019) and, eventually, checked by consulting specialists. Taxonomic classification and species naming were determined mainly according to Fricke et al. (2020); for the species of Characidae, according to Mirande (2010).

To elaborate the list supporting this study, only freshwater fish species formally described until December 2019 were considered. Despite the fact that freshwater species were defined as those known to spend a significant part of their life cycle in low salinity (<0.5 ppt) continental waters (Myers 1949; Berra 2001), in the present study only primary freshwater fishes were considered.

The International Union for Conservation of Nature (IUCN) criteria were used to asses the conservation status of the species (IUCN 2012). The conservation status of species already evaluated according to these criteria was updated to the state of Bahia, which represented the regional assessment. Since the regional workshop for the evaluation of endangered freshwater fish species in Bahia occurred in November 2013, species described after this period did not have their conservation status assessed. The only exception is *Rhamdiopsis* sp., an undescribed troglobitic species whose description process is well advanced (M. E. Bichuette pers. comm.). Subterranean ecosystems and their biota pose special problems for conservation due to their intrinsic fragility and the distinctive features of subterranean communities, such as a high degree of endemism and morphological, ecological and behavioral specialization (Trajano & Bichuette 2010). Therefore, the assessment of the conservation status of *Rhamdiopsis* sp. has become necessary.

Species whose known distribution is restricted to the stretches of the basins draining Bahia State were classified as endemic. It should be noted that endemic species from the ecoregions analyzed (SFR or NMA), which are known to occur in other states (e.g., Sergipe, Minas Gerais, Espírito Santo), were not classified as endemic. Non-native species recorded in basins of the Bahia State were included in the list, but their conservation status was not evaluated.

## Results

### 1. Ichthyofauna composition

A total of 281 native freshwater fish species was recorded along the drainages of Bahia State (Table 1), accounting for about 9% of all species (3,116) known to occur in Brazil (ICMBio 2014). Of the taxa listed in this study, one subfamily (Copionodontinae), nine genera (*Copionodon*, *Glaphyropoma*, *Hirtella*, *Kalyptodoras*, *Mucurilebias*, *Myxiops*, *Ophthalmolebias*, *Prorivulus*, and *Pseudotatia*), and 126 species are only known from river basins draining Bahia State. Most of the native species are from the NMA, with 187 species occurring in Bahia. In stretches of the SFR draining the state, 134 native species were listed (Table 1).

**Table 1.** List of freshwater fish species of Bahia State and their conservation status according to state (SEMA 2017) and national assessments (MMA 2014), IUCN criteria and ecoregion (NMA, Northeastern Mata Atlantica; SFR, São Francisco), as well as their respective indication of origin (X, native species but not endemic to the Bahia part of the ecoregion; END, endemic to the Bahia part of the ecoregion; NNA, non-native species).

| ORDER/Family/*Species* | SEMA 2017 | | MMA 2014 | | SFR | NMA | Voucher / Source |
|---|---|---|---|---|---|---|---|
| | Status | IUCN Criteria | Status | IUCN Criteria | | | |
| **CLUPEIFORMES** | | | | | | | |
| **Engraulidae** | | | | | | | |
| *Anchoviella vaillanti* (Steindachner 1908) | LC | | LC | | X | | MZUSP 54604, MCP 16611 |
| **CHARACIFORMES** | | | | | | | |
| **Acestrorhynchidae** | | | | | | | |
| *Acestrorhynchus britskii* Menezes 1969 | LC | | LC | | X | | MZUSP 83828, MZUSP 54683 |
| *Acestrorhynchus falcatus* (Bloch 1794) | LC | | LC | | | X | MNRJ 21754, MZUSP 102488, MZUSP 102557, UFBA 4507 |
| *Acestrorhynchus lacustris* (Lütken 1875) | LC | | LC | | X | X | MZUSP 57172, MZUSP 28770, UFBA 2647, UFBA 4946 |
| **Anostomidae** | | | | | | | |
| *Hypomasticus mormyrops* (Steindachner 1875) | VU | B1 ab(iii) | LC | | | X | UFBA 4827, UFBA 5665 |
| *Leporellus pictus* (Kner 1850) | NE | | NE | | X | | UNT 13927; UNT 9561 |
| *Leporellus vittatus* (Valenciennes 1850) | LC | | LC | | X | | MZUSP 54659, UFBA 833 |
| *Leporinus bahiensis* Steindachner 1875 | LC | | LC | | | END | UFBA 2645, UFBA 2954, UFBA 1946, UFBA 4456, UFBA 4258, UFBA 4483 |
| *Leporinus copelandii* Steindachner 1875 | LC | | LC | | | X | MZUSP 88513, UFBA 2828, UFBA 5123, UFBA 5666 |
| *Leporinus melanopleura* Günther 1864 | LC | | LC | | | END | BMNH 1863, MZUSP 111246, UFBA 4282, UFBA 5103, UFBA 8052 |
| *Leporinus melanopleurodes* Birindelli, Britski & Garavello 2013 | NT | | NT | | | END | MZUSP 100987, MZUSP 109769, MZUSP 111227 |
| *Leporinus piau* Fowler 1941 | LC | | LC | | X | X | MZUSP 54667, MZUSP 14470, UFBA 4231 |
| *Leporinus steindachneri* Eigenmann 1907 | LC | | LC | | | X | MZUSP 63464, MZUSP 87868, UFBA 4415, UFBA 4715, UFBA 5056 |
| *Leporinus taeniatus* Lütken 1875 | LC | | LC | | X | X | MZUSP 83818, MZUSP 54662, UFBA 1948, UFBA 3564 |
| *Megaleporinus brinco* (Birindelli, Britski & Garavello 2013) | LC | | LC | | | END | MZUSP 105166, MZUSP 109762, UFBA 4843 |
| *Megaleporinus conirostris* (Steindachner 1875) | LC | | LC | | | X | MZUSP 63463, UFBA 5038 |
| *Megaleporinus elongatus* (Valenciennes 1850) | NE | | NE | | X | | MZUSP 2823, MZUSP 28773 |
| *Megaleporinus garmani* (Borodin 1929) | DD | | LC | | | X | MZUSP 87848, UFBA 5238 |
| *Megaleporinus obtusidens* (Valenciennes 1837) | LC | | LC | | X | | UFBA 200, UFBA 280, UFBA 835, UFBA 836, UFBA 966, UFBA 1005 |

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|---|---|---|---|---|---|---|---|
| *Megaleporinus reinhardti* (Lütken 1875) | LC | | LC | | X | X | MZUSP 54670, MZUSP 58224, UFBA 2868, UFBA 3386 |
| *Schizodon knerii* (Steindachner 1875) | NE | | NE | | X | | MZUSP 54676; MCP 16747, UFBA 6582 |
| **Bryconidae** | | | | | | | |
| *Brycon devillei* (Castelnau 1855) | EN | B1 ab(i,iii) | EN | B1 ab(i,iii) | | X | MNHN 4517 |
| *Brycon ferox* Steindachner 1877 | DD | | LC | | | X | MZUSP 53305, MZUSP 93920, UFBA 5039, UFBA 5060, UFBA 5095 |
| *Brycon orthotaenia* Günther 1864 | LC | | LC | | X | | MZUSP 70220, MCP 16676 |
| *Brycon vonoi* Lima 2017 | NE | | NE | | | X | MCZ 2416 |
| *Henochilus wheatlandii* Garman 1890 | NE | | NE | | X | | HU-Zoo 21109 |
| *Salminus franciscanus* Lima & Britski 2007 | LC | | LC | | X | | MZUSP 28797, UFBA 6577 |
| *Salminus hilarii* Valenciennes 1850 | LC | | LC | | X | | UNT 12431, UNT 12457 |
| **Characidae** | | | | | | | |
| *Astyanax* aff. *bimaculatus* (Linnaeus 1758) | LC | | LC | | | X | MZUSP 54839, MZUSP 54840, MCP 17920, MCP 36779, MBML 6407, MBML 4055, MHNCI-Peixes 11468 |
| *Astyanax brucutu* Zanata, Lima, di Dario & Gerhard 2017 | NE | | NE | | | END | ZUEC 12765, MZUSP 120743, UFBA 8167 |
| *Astyanax burgerai* Zanata & Camelier 2009 | DD | | DD | | | END | MZUSP 101245, UFBA 4346 |
| *Astyanax epiagos* Zanata & Camelier 2008 | DD | | DD | | | END | MZUSP 89568, UFBA 2792 |
| *Astyanax fasciatus* (Cuvier 1819) | LC | | LC | | X | X | MZUSP 28768, MZUSP 58823, MZUSP 28789, MZUSP 47378, MZUSP 80106, MBML 5357, MBML 6530, MBML 4103 |
| *Astyanax hamatilis* Camelier & Zanata 2014 | NE | | NE | | | END | MZUSP 49232, MCP 47663, UFBA 3675, UFBA 6987, |
| *Astyanax jacobinae* Zanata & Camelier 2008 | DD | | DD | | | END | MZUSP 89570, UFBA 2793 |
| *Astyanax lacustris* (Lütken 1875) | LC | | LC | | X | | MZUSP 58840, MZUSP 80125, MZUSP 80128 |
| *Astyanax lorien* Zanata, Burger & Camelier 2018 | NE | | NE | | | END | MZUSP 123398, UFBA 5393, UFBA 8109, UFBA 8359 |
| *Astyanax pelecus* Bertaco & Lucena 2006 | LC | | LC | | | END | UFBA 5634, UFBA 5638 |
| *Astyanax rivularis* (Lütken 1875) | LC | | LC | | X | X | UFBA 246 |
| *Astyanax rupestris* Zanata, Burger & Camelier 2018 | NE | | NE | | | END | MZUSP 89567, MZUSP 38537, MCP 53156, UFBA 2789 |
| *Astyanax sincora* Burger, Carvalho & Zanata 2019 | NE | | NE | | | END | MZUSP 120747, MNRJ 48346, UFBA 8201 |
| *Astyanax varii* Zanata, Burger, Vita & Camelier 2019 | NE | | NE | | | END | MZUSP 121062, MCP 54205, UFBA 7046 |
| *Astyanax vermilion* Zanata & Camelier 2009 | LC | | LC | | | END | MZUSP 101243, UFBA 4343 |
| *Compsura heterura* Eigenmann 1915 | LC | | LC | | X | X | MZUSP 54626, MZUSP 58801, UFBA 3005, UFBA 3301 |
| *Hasemania piatan* Zanata & Serra 2010 | EN | B1 ab(iii,v) | EN | B1 ab(iii,v) | | END | MZUSP 104538, MZUSP 104539, UFBA 4298 |

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|---|---|---|---|---|---|---|---|
| *Hasemania nana* (Lütken 1875) | NE | | NE | | X | | MZUSP 84072 |
| *Hemigrammus brevis* Ellis 1911 | LC | | LC | | X | X | MZUSP 58922, UFBA 3081 |
| *Hemigrammus gracilis* (Lütken 1875) | LC | | LC | | X | | MZUSP 84102, MZUSP 84035, UFBA 2866 |
| *Hemigrammus marginatus* Ellis 1911 | LC | | LC | | X | X | MZUSP 28802, UFBA 1942, UFBA 2721, UFBA 4127 |
| *Hyphessobrycon bifasciatus* Ellis 1911 | LC | | LC | | | X | MZUSP 54792, UFBA 4971, UFBA 5079 |
| *Hyphessobrycon brumado* Zanata & Camelier 2010 | LC | | LC | | | END | MZUSP 101246, UFBA 4340, UFBA 4341 |
| *Hyphessobrycon diastatos* Dagosta, Marinho & Camelier 2014 | NE | | NE | | X | | MZUSP 114030 |
| *Hyphessobrycon itaparicensis* Lima & Costa 2001 | LC | | LC | | | X | UFBA 4618, UFBA 5454, UFBA 7558, UFRN 207 |
| *Hyphessobrycon micropterus* (Eigenmann 1915) | LC | | LC | | X | X | MZUSP 58425, UFBA 2843 |
| *Hyphessobrycon negodagua* Lima & Gerhard 2001 | DD | | DD | | | END | MZUSP 53898, UFBA 4360, UFBA 5392 |
| *Hyphessobrycon parvellus* Ellis 1911 | LC | | LC | | | X | UFBA 2897, UFBA 3128, UFBA 3334, UFBA 3418, UFBA 4320 |
| *Hyphessobrycon santae* (Eigenmann 1907) | LC | | LC | | X | | MZUSP 40178 |
| *Hyphessobrycon vinaceus* Bertaco, Malabarba & Dergam 2007 | LC | | LC | | | END | UFBA 4607, UFBA 4608 |
| *Kolpotocheirodon figueiredoi* Malabarba, Lima & Weitzman 2004 | CR | B2 ab(iii) | CR | B2 ab(iii) | | END | MZUSP 700731, UFBA 7068 |
| *Lepidocharax diamantina* Ferreira, Menezes & Quagio-Grassioto 2011 | EN | B2 ab(iii,iv) | EN | B2 ab(iii,iv) | | END | MNRJ 37509, MZUSP 160499, UFBA 7816 |
| *Mimagoniates microlepis* (Steindachner 1877) | LC | | LC | | | X | MZUSP 93866, MZUSP 55012, USNM 249895, |
| *Mimagoniates sylvicola* Menezes & Weitzman 1990 | EN | B1 ab(iii,iv) | EN | B1 ab(iii,iv) | | END | MZUSP 93867, UFBA 3040, UFBA 4819, UFBA 5780, UFBA 7004 |
| *Moenkhausia costae* (Steindachner 1907) | LC | | LC | | X | X | MNHN 1907, UFBA 2837, UFBA 3571 |
| *Moenkhausia diamantina* Benine, Castro & Santos 2007 | LC | | LC | | | END | MZUSP 49233, MZUSP 49233, UFBA 3664 |
| *Moenkhausia sanctaefilomenae* (Steindachner 1907) | LC | | LC | | X | | MZUSP 58294, MZUSP 58272 |
| *Moenkhausia vittata* (Castelnau 1855) | LC | | LC | | | X | MZUSP 54776, MZUSP 54778, MZUSP 51804, UFBA 4945, UFBA 5063, |
| *Myxiops aphos* Zanata & Akama 2004 | LC | | LC | | | END | MZUSP 81026, UFBA 7798 |
| *Nematocharax varii* Barreto, Silva, Batalha-Filho, Affonso & Zanata 2018 | NE | | NE | | | END | MZUSP 123176, MCP 53336, UFBA 8439, |
| *Nematocharax venustus* Weitzman, Menezes & Britski 1986 | LC | | LC | | | X | UFBA 3762, UFBA 4259, UFBA 4494, UFBA 4280, UFBA 3823, UFBA 4378, UFBA 4913 |
| *Oligosarcus acutirostris* Menezes 1987 | LC | | LC | | | X | UFBA 5117, UFBA 4911 |

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|---|---|---|---|---|---|---|---|
| *Oligosarcus macrolepis* (Steindachner 1877) | LC | | LC | | | X | MZUSP 93871, UFBA 5113, UFBA 4894, UFBA 4501, UFBA 5098 |
| *Orthospinus franciscensis* (Eigenmann 1914) | LC | | LC | | X | | MZUSP 54679, MZUSP 54678, UFBA 4741 |
| *Phenacogaster franciscoensis* Eigenmann 1911 | LC | | LC | | X | X | MZUSP 28795, MZUSP 47376, UFBA 3141 |
| *Phenacogaster julliae* Lucena & Lucena 2019 | NE | | NE | | X | | MZUSP 123642, MCP 53629 |
| *Piabina argentea* Reinhardt 1867 | LC | | LC | | X | X | MZUSP 58878, UFBA 28, UFBA 138, UFBA 5318, UFBA 5338, UFBA 5356, UFBA 6121, UFBA 1941, |
| *Psellogrammus kennedyi* (Eigenmann 1903) | LC | | LC | | X | X | MBML 6539, MZUSP 58822, UFBA 2835 |
| *Rachoviscus graciliceps* Weitzman & Cruz 1981 | EN | B2 ab(iii,iv) | EN | B2 ab(iii,iv) | | END | MZUSP 93252 |
| *Roeboides xenodon* (Reinhardt 1851) | LC | | LC | | X | | MZUSP 54684, MCP 16920 |
| *Serrapinnus heterodon* (Eigenmann, 1915) | LC | | LC | | X | X | MZUSP 58794, MZUSP 47359, MZUSP 49251, MZUSP 54770, UFBA 1943, UFBA 3051, UFBA 3332, |
| *Serrapinnus piaba* (Lütken 1875) | LC | | LC | | X | X | MZUSP 58797, MZUSP 58796, MZUSP 54751, MZUSP 49252, MZUSP 54782, UFBA 2838, UFBA 2857 |
| *Tetragonopterus franciscoensis* Silva, Melo, Oliveira & Benine 2016 | NE | | NE | | X | X | MZUSP 90886, LBP 11552, MZUSP 90905 |
| **Crenuchidae** | | | | | | | |
| *Characidium* aff. *zebra* Eigenmann 1909 | LC | | LC | | | X | UFBA 5742 |
| *Characidium bahiense* Almeida 1971 | DD | | DD | | X | X | MCP 17013, UFBA 2975, UFBA 2847, UFBA 3333, UFBA 4348 |
| *Characidium clistenesi* Melo & Espíndola 2016 | NE | | NE | | | END | MZUSP 120530 |
| *Characidium cricarense* Malanski, Sarmento-Soares, Silva-Malanski, Lopes, Ingenito & Buckup 2019 | NE | | NE | | | X | MBML 12879, MBML 3846 |
| *Characidium deludens* Zanata & Camelier 2015 | NE | | NE | | | END | MZUSP 115000, UFBA 7563 |
| *Characidium helmeri* Zanata, Sarmento-Soares & Martins-Pinheiro 2015 | NE | | NE | | | END | UFBA 8709, UFBA 8711, UFBA 8715 |
| *Characidium kamakan* Zanata & Camelier 2015 | NE | | NE | | | END | MZUSP 115009, UFBA 7720 |
| *Characidium samurai* Zanata & Camelier 2014 | NE | | NE | | | END | MZUSP 108188, UFBA 7259 |
| **Curimatidae** | | | | | | | |
| *Curimatella lepidura* (Eigenmann & Eigenmann 1889) | LC | | LC | | X | X | MZUSP 54646, MZUSP 47363, UFBA 3519 |
| *Cyphocharax gilbert* (Quoy & Gaimard 1824) | LC | | LC | | X | X | MZUSP 1358, MZUSP 58881, MZUSP 51808, UFBA 4505, UFBA 4624, |
| *Cyphocharax pinnilepis* Vari, Zanata & Camelier 2010 | LC | | LC | | | END | MZUSP 93453, UFBA 4885 |

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|---|---|---|---|---|---|
| *Steindachnerina elegans* (Steindachner 1875) | LC | LC | X | X | MZUSP 58290, MZUSP 54697, MZUSP 54700, UFBA 3417, UFBA 3230, UFBA 3328, UFBA 3209, UFBA 4848, UFBA 4447, UFBA 4247, UFBA 4377 |
| **Erythrinidae** | | | | | |
| *Erythrinus kessleri* Steindachner 1877 | DD | DD | | END | MZUSP 93891, MZUSP 44018 |
| *Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829) | LC | LC | X | X | MZUSP 57392, MZUSP 38539, MZUSP 2801, UFBA 2899, UFBA 2966, UFBA 3044 |
| *Hoplias brasiliensis* (Spix & Agassiz 1829) | LC | LC | | X | MZUSP 45483, MZUSP 40174, MZUSP 40170, MZUSP 40270, UFBA 5429 |
| *Hoplias intermedius* (Günther 1864) | LC | LC | X | | MZUSP 94050, MZUSP 94434 |
| *Hoplias malabaricus* (Bloch 1794) | LC | LC | X | X | MZUSP 62574, MZUSP 84071, UFBA 3020, UFBA 384, UFBA 3104, UFBA 2947, UFBA 1985, UFBA 3839, UFBA 3717, UFBA 4916, UFBA 4808 |
| **Iguanodectidae** | | | | | |
| *Bryconops affinis* (Günther 1864) | LC | LC | X | | MZUSP 57922, MZUSP 54641, MZUSP 57924 |
| **Lebiasinidae** | | | | | |
| *Nannostomus beckfordi* Günther 1872 | DD | DD | | X | UFBA 1855, UFBA 1887, UFBA 1890, UFBA 1897, UFBA 3742, UFBA 4675, UFBA 4680, UFBA 7412 |
| **Parodontidae** | | | | | |
| *Apareiodon hasemani* Eigenmann 1916 | LC | LC | X | | MCP 17045 |
| *Apareiodon itapicuruensis* Eigenmann & Henn 1916 | LC | LC | | END | UFBA 0234, UFBA 4156 |
| *Parodon hilarii* Reinhardt 1867 | LC | LC | X | | MZUSP 58286, MZUSP 28780 |
| **Prochilodontidae** | | | | | |
| *Prochilodus argenteus* Spix & Agassiz 1829 | LC | LC | X | | MZUSP 28778, MZUSP 2040 |
| *Prochilodus brevis* Steindachner 1875 | LC | LC | | X | UFBA 3436, UFBA 1947, UFBA 5669, UFBA 4628, UFBA 4629 |
| *Prochilodus costatus* Valenciennes 1850 | LC | LC | X | X | MZUSP 54756, MZUSP 21549, UFBA 2825 |
| *Prochilodus hartii* Steindachner 1875 | LC | LC | | X | MZUSP 42677, UFBA 5272 |
| *Prochilodus vimboides* Kner 1859 | LC | LC | | X | UFBA 5094, UFBA 5061, UFBA 5181 |
| **Serrasalmidae** | | | | | |
| *Colossoma macropomum* (Cuvier 1816) | - | - | NNA | | Bezerra et al. 2008 |
| *Metynnis maculatus* (Kner 1858) | - | - | NNA | NNA | Rodrigues et al. 2018; MCP 36973 |
| *Myleus micans* (Lütken 1875) | LC | LC | X | X | MZUSP 58277, MZUSP 20460 |
| *Pygocentrus piraya* (Cuvier 1819) | LC | LC | X | NNA | MZUSP 57552, |
| *Serrasalmus brandtii* Lütken, 1875 | LC | LC | X | X | MZUSP 58296, MZUSP 57550, MZUSP 49254, MZUSP 57508, UFBA 3018, UFBA 2823 |

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| **Triportheidae** | | | | | |
| *Lignobrycon myersi* (Miranda Ribeiro 1956) | NT | NT | | END | UFBA 4260, UFBA 5179 |
| *Triportheus guentheri* (Garman 1890) | LC | LC | X | | MCP 16706, MCP 16680 |
| *Triportheus signatus* (Garman 1890) | DD | LC | | X | UFBA 3173, UFBA 4836 |
| **GYMNOTIFORMES** | | | | | |
| **Gymnotidae** | | | | | |
| *Gymnotus bahianus* Campos-da-Paz & Costa 1996 | LC | LC | | END | MZUSP 102453, MZUSP 48949, UFBA 4452, |
| *Gymnotus capitimaculatus* Rangel-Pereira 2014 | NE | NE | | END | UFRJ 9785, UFRJ 9964 |
| *Gymnotus carapo* Linnaeus 1758 | LC | LC | X | X | MBML 6548, MZUSP 86104, UFBA 3041, UFBA 2651, UFBA 4455 |
| *Gymnotus* cf. *pantherinus* (Steindachner 1908) | NE | NE | | X | MZUSP 104746 |
| *Gymnotus interruptus* Rangel-Pereira 2012 | DD | DD | | END | UFRJ 8218 |
| **Hypopomidae** | | | | | |
| *Brachyhypopomus menezesi* Crampton, Santana, Waddell & Lovejoy 2016 | NE | NE | X | | MZUSP 87147, MZUSP 40190 |
| **Sternopygidae** | | | | | |
| *Eigenmannia besouro* Peixoto & Wosiacki 2016 | NE | NE | END | | MZUSP 57890 |
| *Eigenmannia virescens* (Valenciennes 1836) | LC | LC | X | | MZUSP 84036, MZUSP 57891 |
| *Sternopygus macrurus* (Bloch & Schneider 1801) | LC | LC | X | | MZUSP 2644, MZUSP 84045 |
| **SILURIFORMES** | | | | | |
| **Auchenipteridae** | | | | | |
| *Glanidium botocudo* Sarmento-Soares & Martins-Pinheiro 2013 | DD | DD | | X | MNRJ 32538 |
| *Pseudauchenipterus affinis* (Steindachner 1877) | LC | LC | | X | MZUSP 51762, MZUSP 51750 |
| *Pseudauchenipterus jequitinhonhae* (Steindachner 1877) | LC | LC | | X | MZUSP 51735, UFBA 05398 |
| *Pseudotatia parva* Mess 1974 | NE | NE | END | | FMNH 70580 |
| *Tatia bockmanni* (Sarmento-Soares & Buckup 2005) | DD | LC | X | | MZUSP 82351 |
| *Trachelyopterus galeatus* (Linnaeus 1766) | LC | LC | X | X | MZUSP 90280, UFBA 93, UFBA 130, UFBA 163, UFBA 848, UFBA 872, UFBA 2819, UFBA 3046, UFBA 05119, UFBA 6648 |
| *Trachelyopterus striatulus* (Steindachner 1877) | LC | LC | | X | MZUSP 52627, MZUSP 93912, UFBA 4712, UFBA 4992, |
| **Callichthyidae** | | | | | |
| *Aspidoras kiriri* Oliveira, Zanata, Tencatt & Britto 2017 | NE | NE | | END | MNRJ 47400, UFBA 7352 |

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|---|---|---|---|---|---|---|---|
| *Aspidoras maculosus* Nijssen & Isbrücker 1976 | DD | | DD | | | END | MZUSP 88170, UFBA 3291 |
| *Aspidoras psammatides* Britto, Lima & Santos 2005 | DD | | DD | | | END | MZUSP 67194 |
| *Aspidoras virgulatus* Nijssen & Isbrücker 1980 | LC | | LC | | | X | MZUSP 39124, MBML 2030 |
| *Callichthys callichthys* (Linnaeus 1758) | LC | | LC | | X | X | UFBA 159, UFBA 2650, UFBA 3422, UFBA 5262, |
| *Corydoras costai* Ottoni, Barbosa & Katz 2016 | NE | | NE | | X | | UFRJ 7790, MCP 48169 |
| *Corydoras garbei* Ihering 1911 | LC | | LC | | X | | MZUSP 5319, MZUSP 5324 |
| *Corydoras lacerdai* Hieronimus 1995 | EN | B1 ab(iii) | EN | B1 ab(iii) | | END | MZUSP 47682, MZUSP 47683 |
| *Corydoras lymnades* Tencatt, Vera-Alcaraz, Britto & Pavanelli 2013 | DD | | LC | | X | | MNRJ 22370 |
| *Corydoras multimaculatus* Steindachner 1907 | LC | | LC | | END | | MZUSP 57405, MZUSP 57404 |
| *Corydoras nattereri* Steindachner 1876 | LC | | LC | | | X | MZUSP 51796, UFBA 4728, UFBA 2839 |
| *Hoplosternum littorale* (Hancock 1828) | LC | | LC | | X | X | MZUSP 57551, UFBA 1611 |
| *Scleromystax prionotos* (Nijssen & Isbrücker 1980) | LC | | LC | | | X | MCP 23650, MBML 1470, MBML 1546, UFBA 5073 |
| **Clariidae** | | | | | | | |
| *Clarias gariepinus* (Burchell 1822) | - | | - | | | NNA | Rocha et al 2008, MNRJ 28363 |
| **Doradidae** | | | | | | | |
| *Franciscodoras marmoratus* (Lütken 1874) | LC | | LC | | X | | MZUSP 2201, UFBA 208 |
| *Kalyptodoras bahiensis* Higuchi, Britski & Garavello 1990 | EN | B2 ab(iii) | EN | B2 ab(iii) | | END | MZUSP 38565, UFBA 3171, UFBA 7108, UFBA 7455 |
| *Wertheimeria maculata* Steindachner 1877 | LC | | LC | | | X | UFBA 5667 |
| **Heptapteridae** | | | | | | | |
| *Acentronichthys leptos* Eigenmann & Eigenmann 1889 | EN | B1 ab(iii) | LC | | | X | MZUSP 93856, UFBA 6019 |
| *Cetopsorhamdia iheringi* Schubart & Gomes 1959 | LC | | LC | | X | X | UFBA 4370, UFBA 5121, UFBA 6097 |
| *Imparfinis borodini* *Mees* & Cala 1989 | NE | | NE | | X | X | MBML 10327, MBML 10729 |
| *Imparfinis minutus* (Lütken 1874) | LC | | LC | | X | X | UFBA 935, UFBA 4958, UFBA 5071, UFBA 4987, UFBA 5053 |
| *Phenacorhamdia tenebrosa* (Schubart 1964) | DD | | LC | | | X | MCP 36935, MCP 36723, UFBA 7114 |
| *Pimelodella harttii* (Steindachner 1877) | DD | | DD | | | X | MZSUP 5141, UFBA 5647 |
| *Pimelodella itapicuruensis* Eigenmann 1917 | DD | | LC | | | X | MZUSP 88169 |
| *Pimelodella lateristriga* (Lichtenstein 1823) | LC | | LC | | | X | MZUSP 93864, MZUSP 54763, MZUSP 51798, UFBA 4973, UFBA 4984, |
| *Rhamdia enfurnada* Bichuette & Trajano 2005 | LC | | LC | | END | | LIRP 5643 |

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|---|---|---|---|---|---|---|---|
| *Rhamdia jequitinhonha* Silfvergrip 1996 | DD | | VU | | | X | MZUSP 102718 |
| *Rhamdia quelen* (Quoy & Gaimard 1824) | LC | | LC | | X | X | MCP 16658, MZUSP 83796, MZUSP 93851 MZUSP 54006, MZUSP 101358, UFBA 3006, UFBA 2894, UFBA 03353,UFBA 4263, UFBA 4858, , UFBA 4909, UFBA 4956 |
| *Rhamdiopsis krugi* Bockmann & Castro 2011 | VU | B1ab(iii) | VU | B1ab(iii) | | END | LIRP 5929, LIRP 5931, LIRP 5930 |
| *Rhamdiopsis* sp. | VU | D2 | VU | D2 | END | | M. E. Bichuette (comm. pers.) |
| **Loricariidae** | | | | | | | |
| *Harttia longipinna* Langeani, Oyakawa & Montoya-Burgos 2001 | DD | | DD | | END | | DZSJRP003666, MZUSP 57168 |
| *Hirtella carinata* Pereira, Zanata, Cetra & Reis 2014 | NE | | NE | | | END | ANSP 198032, MCP 48127, UFBA 5655 |
| *Hypostomus brevicauda* (Günther 1864) | DD | | DD | | | X | BMNH1864 |
| *Hypostomus francisci* (Lütken 1874) | LC | | LC | | X | | UFBA 4191, UFBA 6641 |
| *Hypostomus jaguar* Zanata, Sardeiro & Zawadzki 2013 | LC | | LC | | | END | MZUSP 110603, UFBA 6501 |
| *Hypostomus johnii* (Steindachner 1877) | NE | | NE | | X | | MCZ 7863, NMW 44192 |
| *Hypostomus leucophaeus* Zanata & Pitanga 2016 | NE | | NE | | | END | MZUSP 119822, UFBA 2993 |
| *Hypostomus lima* (Lütken 1874) | NE | | NE | | X | | UFBA 2046 |
| *Hypostomus macrops* (Eigenmann & Eigenmann 1888) | NE | | NE | | X | | MBML 11304, MBML- 10813, NUP 20383 |
| *Hypostomus unae* (Steindachner 1878) | DD | | LC | | | X | BMNH 1861, NMW 44259 |
| *Hypostomus vaillanti* (Steindachner 1877) | NE | | DD | | X | | NMW 44273 |
| *Hypostomus velhochico* Zawadzki, Oyakawa, Britski 2017 | NE | | NE | | X | | MCP 16689, MZUSP 94586, MZUSP 120452 |
| *Hypostomus wuchereri* (Günther 1864) | DD | | DD | | | X | BMNH1863 |
| *Megalancistrus barrae* (Steindachner 1910) | LC | | LC | | END | | NMW 48019 |
| *Otocinclus xakriaba* Schaefer 1997 | LC | | LC | | X | | MZUSP 51103, MCP 16877 |
| *Otothyris travassosi* Garavello, Britski & Schaefer 1998 | LC | | LC | | | X | MZUSP 94020, MZUSP 51435, MZUSP 94021, MZUSP 39095, MZUSP 51439, MZUSP 51438, MZUSP 51803, UFBA 4931, |
| *Pareiorhaphis bahianus* (Gosline 1947) | LC | | LC | | | END | UFBA 4486, UFBA 4555, UFBA 5100 |
| *Pareiorhaphis lophia* Pereira & Zanata 2014 | NE | | NE | | | END | MCP 47711, MCP 47712, MCP 48004, MZUSP 86089, MZUSP 86154, MZUSP 88163, UFBA 6188, UFBA 7026, UFBA 7063, UFBA 7350 |
| *Parotocinclus adamanteus* Pereira, Santos, de Pinna & Reis, 2019 | NE | | NE | | | END | MZUSP 124560, MCP 54151, MZUSP 93274 |
| *Parotocinclus arandai* Sarmento-Soares, Lehmann & Martins-Pinheiro 2009 | LC | | LC | | | END | MBML 2135, MBML 1486 |

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|---|---|---|---|---|---|---|---|
| *Parotocinclus bahiensis* (Miranda Ribeiro 1918) | LC | | LC | | | END | MZUSP 99753, UFBA 2978, UFBA 3103, UFBA 3349, UFBA 3118 |
| *Parotocinclus cristatus* Garavello 1977 | LC | | LC | | | END | MZUSP 102611, MZUSP 102634, UFBA 4451 |
| *Parotocinclus jimi* Garavello 1977 | LC | | LC | | | END | MZUSP 24531, UFBA 3875 |
| *Parotocinclus minutus* Garavello 1977 | DD | | DD | | | END | MCP 40034 |
| *Pogonopoma wertheimeri* (Steindachner 1867) | LC | | LC | | | X | MZUSP 51779 |
| *Pterygoplichthys chrysostiktos* (Birindelli, Zanata & Lima 2007) | LC | | LC | | | END | MZUSP 88157 |
| *Pterygoplichthys etentaculatus* (Spix & Agassiz 1829) | LC | | LC | | X | | MCP 16709 |
| *Rhinelepis aspera* Spix & Agassiz 1829 | DD | | LC | | X | | MZUSP 83660, MZUSP 2219 |
| **Pimelodidae** | | | | | | | |
| *Bergiaria westermanni* (Lütken 1874) | NE | | LC | | X | | UNT 12428, UNT 12470 |
| *Conorhynchos conirostris* (Valenciennes 1840) | EN | A2ac | EN | A2ac | X | X | MNHN A-9413 |
| *Duopalatinus emarginatus* (Valenciennes 1840) | LC | | LC | | X | | MZUSP 24871, MZUSP 2287 |
| *Pimelodus fur* (Lütken 1874) | LC | | LC | | X | | MZUSP 1078 |
| *Pimelodus maculatus* Lacepède 1803 | LC | | LC | | X | | MZUSP 54637, MZUSP 57549 |
| *Pimelodus pohli* Ribeiro & Lucena 2006 | LC | | LC | | X | | MCP 16661, MCP 16671 |
| *Pseudoplatystoma corruscans* (Spix & Agassiz 1829) | NT | | NT | | X | | UFBA 268 |
| **Pseudopimelodidae** | | | | | | | |
| *Cephalosilurus fowleri* Haseman 1911 | DD | | LC | | X | | FMNH 54254 |
| *Lophiosilurus alexandri* Steindachner 1876 | VU | A2 cd | VU | A2 cd | X | | MZUSP 1160, MZUSP 53261 |
| *Microglanis pataxo* Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon 2006 | LC | | LC | | | X | MZUSP 51790, UFBA 4985 |
| **Trichomycteridae** | | | | | | | |
| *Ammoglanis multidentatus* Costa, Mattos & Santos 2019 | NE | | NE | | | END | MNRJ 51340, UFRJ 12088 |
| *Copionodon elysium* Pinna, Burger & Zanata 2018 | NE | | NE | | | END | MZUSP 120631, UFBA 8100 |
| *Copionodon exotatos* Pinna, Abrahão, Reis & Zanata 2018 | NE | | NE | | | END | MZUSP 123522, MZUSP 121656 |
| *Copionodon lianae* Campanario & de Pinna 2000 | NT | | NT | | | END | MZUSP 81034 |
| *Copionodon orthiocarinatus* de Pinna 1992 | NT | | NT | | | END | MZUSP 42463, UFBA 3688 |
| *Copionodon pecten* de Pinna 1992 | NT | | NT | | | END | MZUSP 42461, UFBA 5289 |
| *Glaphyropoma rodriguesi* de Pinna 1992 | DD | | DD | | | END | MZUSP 42465 |
| *Glaphyropoma spinosum* Bichuette, de Pinna & Trajano 2008 | VU | B1 ab(iii) | VU | B1 ab(iii) | | END | MZUSP 99742 |

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| *Ituglanis agreste* Lima, Neves & Campos-Paiva 2013 | LC | | LC | | | END | MNRJ 40196, UFRN 29 |
| *Ituglanis cahyensis* Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon 2006 | EN | B1 ab(iii) | EN | B1 ab(iii) | | X | MNRJ 28404, MNRJ 28406 |
| *Ituglanis paraguassuensis* Campos-Paiva & Costa 2007 | DD | | DD | | | END | MZUSP 63138 |
| *Microcambeva draco* Mattos & Lima 2010 | EN | B1ab(iii) | EN | B1ab(iii) | | END | MCP 17796, MCP 47695 |
| *Stegophilus insidiosus* Reinhardt 1859 | LC | | LC | | X | | MBML 9078 |
| *Trichomycterus bahianus* Costa 1992 | LC | | LC | | | END | MZUSP 38636, MBML 1580 |
| *Trichomycterus payaya* Samento-Soares, Zanata & Martins-Pinheiro 2011 | DD | | DD | | | END | MBML 2560, UFBA 5284 |
| *Trichomycterus pradensis* Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon 2005 | LC | | LC | | | X | MBML 1480, MBML 1520 |
| *Trichomycterus rubbioli* Bichuette & Rizzato 2012 | VU | D2 | VU | D2 | END | | MZUSP 110977, MZUSP 110978, MZUSP 110984 |
| *Trichomycterus tete* Barbosa & Costa 2011 | LC | | LC | | | END | UFRJ 7774, UFRJ 7775, UFRJ 7776, UFRJ 8082 |
| **CYPRINODONTIFORMES** | | | | | | | |
| **Poeciliidae** | | | | | | | |
| *Pamphorichthys hollandi* (Henn 1916) | LC | | LC | | X | X | MZUSP 58869, MZUSP 57507, UFBA 2892, UFBA 3037, |
| *Phalloceros mikrommatos* Lucinda 2008 | DD | | DD | | | END | UFPB 5370, UFPB 2688 |
| *Phalloceros ocellatus* Lucinda 2008 | LC | | LC | | | X | MZUSP 93985, MZUSP 51794 |
| *Phalloptychus eigenmanni* Henn 1916 | CR | B2 ab(iii,iv) | CR | B2 ab(iii,iv) | | END | UFBA 3416, UFBA 7726 |
| *Poecilia reticulata* Peters 1859 | - | | - | | NNA | NNA | MZUSP 86110, MZUSP 58884, MCP 18128, MBML 5380, UFBA 5402 |
| *Poecilia vivipara* Bloch & Schneider 1801 | LC | | LC | | X | X | MZUSP 93991, MZUSP 51810, UFBA 3021, UFBA 2860, UFBA 3331, UFBA 3121, UFBA 4165, UFBA 4257, UFBA 4239, UFBA 4382, |
| **Rivulidae** | | | | | | | |
| *Anablepsoides bahianus* (Huber 1990) | DD | | DD | | | END | UFBA 3423, UFBA 2951 |
| *Atlantirivulus depressus* (Costa 1991) | NT | | NT | | | END | UFPB 2213, UFRJ 2118 |
| *Atlantirivulus unaensis* (Costa & de Luca 2009) | DD | | DD | | | END | UFRJ 6597 |
| *Cynolebias altus* Costa 2001 | LC | | LC | | END | | MZUSP 62564, MZUSP 62565 |
| *Cynolebias attenuatus* Costa 2001 | DD | | DD | | END | | MZUSP 62566, MZUSP 62567 |
| *Cynolebias elegans* Costa 2017 | NE | | NE | | END | | UFRJ 9431, UFRJ 6890 |
| *Cynolebias gibbus* Costa 2001 | DD | | DD | | END | | MZUSP 62568, UFRJ 4796 |
| *Cynolebias gilbertoi* Costa 1998 | DD | | DD | | END | | MZUSP 52304, MZUSP 52305 |
| *Cynolebias itapicuruensis* Costa 2001 | DD | | DD | | | END | UFBA 2626 |

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| *Cynolebias leptocephalus* Costa & Brasil 1993 | DD | | DD | | END | | MZUSP 43676, UFRJ 687 |
| *Cynolebias obscurus* Costa 2014 | NE | | NE | | END | | UFRJ 6774 |
| *Cynolebias ochraceus* Costa 2014 | NE | | NE | | END | | Costa 2014 |
| *Cynolebias oticus* Costa 2014 | NE | | NE | | END | | UFRJ 9437 |
| *Cynolebias paraguassuensis* Costa, Suzart & Nielsen 2007 | DD | | DD | | | END | UFRJ 6454, UFRJ 6455 |
| *Cynolebias porosus* Steindachner 1876 | DD | | DD | | END | | MZUSP 41378, MZUSP 41379 |
| *Cynolebias rectiventer* Costa 2014 | NE | | NE | | END | | UFRJ 8896 |
| *Cynolebias roseus* Costa 2014 | NE | | NE | | END | | UFRJ 9330, UFRJ 9331 |
| *Cynolebias vazabarrisensis* Costa 2001 | DD | | DD | | | END | MZUSP 62561, MZUSP 62560 |
| *Hypsolebias adornatus* (Costa 2000) | VU | D2 | VU | D2 | END | | MZUSP 54563, UFRJ 4805 |
| *Hypsolebias carlettoi* (Costa & Nielsen 2004) | CR | B2ab(i,ii,iii,iv) | CR | B2ab(i,ii,iii,iv) | END | | MCP 34089, UFRJ 5945 |
| *Hypsolebias caeruleus* Costa 2013 | NE | | LC | | END | | UFRJ 6855 |
| *Hypsolebias faouri* Britzke, Nielsen & Oliveira 2016 | NE | | NE | | END | | ZUEC 10796 |
| *Hypsolebias flagellatus* (Costa 2003) | NE | | NA | | X | | MCP 28578, UFRJ 4788 |
| *Hypsolebias fulminantis* (Costa & Brasil 1993) | CR | B2ab(i,ii,iii,iv) | CR | B2ab(i,ii,iii,iv) | END | | MZUSP 43674, UFRJ 685 |
| *Hypsolebias gardneri* Costa, Amorim & Mattos 2018 | NE | | NE | | X | | UFRJ 11859 |
| *Hypsolebias ghisolfii* (Costa, Cyrino & Nielsen 1996) | CR | B2ab(i,ii,iii,iv) | CR | B2ab(i,ii,iii,iv) | END | | MZUSP 49403, UFRJ 3526 |
| *Hypsolebias gilbertobrasili* Costa 2012 | NE | | NT | | END | | UFRJ 8325 |
| *Hypsolebias guanambi* Costa & Amorim 2011 | NE | D2 | VU | D2 | END | | UFRJ 6861 |
| *Hypsolebias harmonicus* (Costa 2010) | NE | D2 | VU | D2 | END | | UFRJ 6696 |
| *Hypsolebias igneus* (Costa 2000) | CR | B2ab(i,ii,iii,iv) | CR | B2ab(i,ii,iii,iv) | END | | MZUSP 56254, UFRJ 4857 |
| *Hypsolebias lopesi* (Nielsen, Shibatta, Suzart & Martín 2010) | NE | D2 | VU | D2 | END | | MZUSP 103102 |
| *Hypsolebias mediopapillatus* (Costa 2006) | VU | D2 | VU | D2 | END | | UFRJ 5406, MCP 40139 |
| *Hypsolebias nitens* Costa 2012 | NE | | LC | | END | | UFRJ 8289 |
| *Hypsolebias nudiorbitatus* Costa 2011 | NE | | DD | | | END | UFRJ 6837 |
| *Hypsolebias picturatus* (Costa 2000) | VU | D2 | VU | D2 | END | | MZUSP 59228, UFRJ 5053 |
| *Hypsolebias pterophyllus* Costa 2012 | NE | | LC | | END | | UFRJ 8376 |
| *Hypsolebias shibattai* Nielsen, Martins, Araujo & Suzart 2014 | NE | | NA | | END | | ZUEC 7648 |
| *Hypsolebias trifasciatus* Nielsen, Martins, Araújo, Lira & Four 2014 | NE | | NA | | END | | ZUEC 8302 |
| *Kryptolebias hermaphroditus* Costa 2011 | NE | | NE | | | X | UFRN 4344 |
| *Melanorivulus decoratus* (Costa 1989) | NT | | NT | | END | | MZUSP 39982, MZUSP 39983 |

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| *Mucurilebias leitaoi* (Cruz & Peixoto 1992) | CR | B2 ab(ii,iii,iv,v) | CR | B2 ab(ii,iii,iv,v) | | END | MNRJ 11646, MNRJ 11647 |
| *Ophthalmolebias bokermanni* (Carvalho & Da Cruz 1987) | CR | B2 ab(iii) | CR | B2 ab(iii) | | END | MZUSP 91519 |
| *Ophthalmolebias ilheusensis* (Costa & Lima 2010) | CR | B1 ab(iii) | CR | B1 ab(iii) | | END | UFRJ 6690, UFBA 5297 |
| *Ophthalmolebias perpendicularis* (Costa, Nielsen & de Luca 2001) | CR | B2 ab(i,ii,iii,iv,v) | CR | B2 ab(i,ii,iii,iv,v) | | END | MZUSP 62570 |
| *Ophthalmolebias rosaceus* (Costa, Nielsen & de Luca 2001 | VU | D2 | VU | seD2 | | END | MZUSP 62572 |
| *Ophthalmolebias suzarti* (Costa 2004) | VU | D2 | VU | D2 | | END | MZUSP 91518 |
| *Prorivulus auriferus* Costa, Lima & Suzart 2004 | DD | | DD | | | END | UFRJ 5932, UFRJ 5933 |
| *Xenurolebias myersi* (Carvalho 1971) | EN | B2ab(iii) | EN | B2ab(iii) | | X | UFRJ 249, UFRJ 377, UFRJ 1921 |
| **SYNBRANCHIFORMES** | | | | | | | |
| **Synbranchidae** | | | | | | | |
| *Synbranchus marmoratus* Bloch 1795 | LC | | LC | | X | X | MZUSP 83815, MZUSP 2667, UFBA 3010, UFBA 2832, UFBA 4009, UFBA 5646 |
| **PERCIFORMES** | | | | | | | |
| **Cichlidae** | | | | | | | |
| *Astronotus ocellatus* (Agassiz 1831) | - | | - | | NNA | NNA | MBML 7173, UFBA 5458 |
| *Cichla* spp. | - | | - | | NNA | NNA | MBML-Peixes 10405, UNT 10429, MZFS 13845, MZFS 11497 |
| *Cichlasoma sanctifranciscense* Kullander 1983 | LC | | LC | | X | X | MZUSP 58926, MZUSP 84085,UFBA 2977, UFBA 386, UFBA 3444, UFBA 3131, UFBA 4457 |
| *Crenicichla lacustris* (Castelnau 1855) | LC | | LC | | | X | UFBA 4709, UFBA 4824 |
| *Crenicichla lepidota* Heckel 1840 | LC | | LC | | X | X | MZUSP 84229, MZUSP 57903, UFBA 388 |
| *Geophagus brasiliensis* (Quoy & Gaymard 1824) | LC | | LC | | | X | MZUSP 87890, MZUSP 39110, MZUSP 54850, MZUSP 49240 |
| *Geophagus diamantinensis* Mattos, Costa & Santos 2015 | NE | | NE | | | END | UFRJ 8833 |
| *Geophagus itapicuruensis* Haseman 1911 | DD | | DD | | | END | FMNH 54365, FMNH 54204 |
| *Geophagus multiocellus* Mattos & Costa 2018 | NE | | NE | | | X | UFRJ 11764, MNRJ32263 |
| *Geophagus obscurus* (Castelnau 1855) | DD | | DD | | | END | MNHN A-9511 |
| *Geophagus rufomarginatus* Mattos & Costa 2018 | NE | | NE | | | X | UFRJ 9994, UFRJ 9519 |
| *Geophagus santosi* Mattos & Costa 2018 | NE | | NE | | | X | UFRJ 11765, UEFS 10336 |
| *Oreochromis niloticus* (Linnaeus 1758) | - | | - | | | NNA | MNRJ 28647, LIRP 5724, MCP 36707 |
| *Coptodon rendalii* (Boulenger 1897) | - | | - | | NNA | NNA | MZUSP 86156, Rodrigues et al 2018 |
| **Sciaenidae** | | | | | | | |
| *Pachyurus adspersus* Steindachner 1879 | DD | | DD | | | X | LIRP 1150, LIRP 1149 |
| *Pachyurus francisci* (Cuvier 1830) | LC | | LC | | X | | MZUSP 2498, MCP 16632 |
| *Pachyurus squamipennis* Agassiz 1831 | LC | | LC | | X | | UNT 12455 |
| *Plagioscion squamosissimus* (Heckel 1840) | - | | - | | | NNA | MZUSP 75093 |

Only 40 (14%) native species are shared between the two ecoregions. Eleven non-native species were listed in Bahia, which accounted for less than 5% of all species analyzed. Among the 30 families recorded (excluding those related only to non-native species), the most representative were Characidae and Rivulidae, with 53 and 48 species, respectively, followed by Loricariidae with 28 species (Figure 2). Many species were described in the last 20 years, 75 from the NMA and 41 from the SFR, corresponding to 27% and 15% of all species listed in this study, respectively (Table 1).

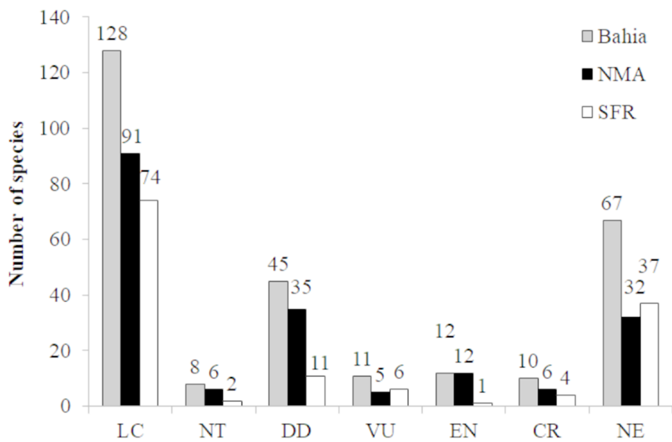


**Figure 2.** Number of freshwater fish species recorded per family in Bahia State and separately for each ecoregion studied (NMA, Northeastern Mata Atlantica; SFR, São Francisco).

## 2. *Assessment of conservation status*

In this study, 214 freshwater fish species were evaluated. Most of them were classified as 'Least Concern' (LC), 45 as 'Data Deficient' (DD), and eight as 'Near Threatened' (NT), which are not considered threatened categories according to the IUCN criteria (IUCN 2012) (Table 1, Figure 3). Thirty-three freshwater fish species (26 of them endemic to Bahia) were included in the threatened categories: 11 as 'Vulnerable' (VU), 12 as 'Endangered' (EN), and 10 as 'Critically Endangered' (CR) (Table 1, Figure 3). The Bahia State Red list has been already published by the Secretaria do Meio Ambiente do Estado da Bahia (SEMA 2017), although it contains only the threatened species and their conservation status.

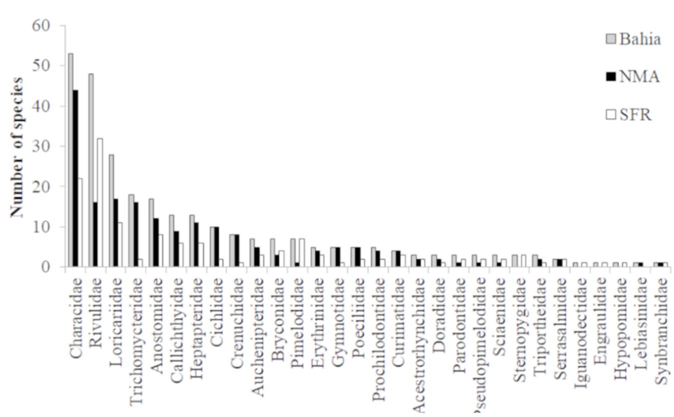


**Figure 3.** Number of freshwater fish species in Bahia State classified according to the categories proposed by IUCN (2012): LC, least concern; NT, near threatened; DD, data deficient; VU, vulnerable; EN, endangered; CR, critically endangered. NMA, Northeastern Mata Atlantica freshwater ecoregion; SFR, São Francisco freshwater ecoregion.

Most of the threatened species are in the NMA (23 species, 69.7%), while 10 (30.3%) endangered species are found in the SFR (Figure 3). Most of these belong to the family Rivulidae, with 14 species (42.4 % of threatened species), seven in each ecoregion. In addition, several small fish species of the Characidae, Heptapteridae, and Trichomycteridae families were also representative in the list of endangered species. These fishes are typical of first- and second-order streams, which may suffer more intensely from the effects of human activities (Oliveira & Bennemann 2005). Among tricomicterids and heptaperids, three troglobitic species (*Glaphyropoma spinosum*, *Rhamdiopsis krugi*, and *Rhamdiopsis* sp.) stand out among all others because of their low population density and restricted distribution in some caverns (Bockmann & Castro 2010, Bichuette et al. 2008, M. E. Bichuette pers. comm.), which are not protected in any conservation unit. Taken together, these factors can chronically devastate these populations and compromise species survival.

# Discussion

## 1. *Species composition*

In this study, 281 native freshwater fish species were recorded in Bahia State, distributed in coastal drainages of the NMA ecoregion and the São Francisco river basin draining the state. The composition of the Bahia State species partially agrees with the overall pattern of the Brazilian fish fauna, in which most species belong to Characidae, followed by Loricariidae (Bizerril 1994, Gonçalves & Braga 2012, Camelier & Zanata 2014a, Ferreira et al. 2014). Curiously, Bahia is one of the Brazilian states with the largest records of Rivulidae in its basins (see Frick & Eschmeyer 2020). Among the remaining families occurring in the state, more than half are represented by five or fewer species.

The number of Rivulidae species is higher in the SFR ecoregion, mainly due to the presence of several annual fish species of the genera *Hypsolebias* and *Cynolebias* that inhabit temporary pools in the tributaries at the right margin of the middle São Francisco river basin (Costa 2014, Costa et al. 2014). In the drainages of the NMA ecoregion, Rivulidae is mainly represented by the annual fish species of the genus *Ophthalmolebias*, most of them endemic to Bahia, presenting a narrow distribution, usually restricted to a single basin (Costa & Lima 2010). In this ecoregion, Rivulidae and Loricariidae have almost the same number of species, differing from the general pattern found in the other Brazilian coastal drainages cited above.

The river basins included in the NMA and SFR freshwater ecoregions which draining the state of Bahia exhibit a distinct composition, with only 40 native species shared between them. Most species from the NMA are characterized by a small size, possibly related to the large number of small streams of this ecoregion. According to several authors (e.g., Weitzman & Vari 1988, Castro 1999, Casatti et al. 2001, Abilhoa et al. 2011), the ichthyofauna of these freshwater ecosystems is mainly composed of small-sized species. In the São Francisco river basin, in addition to small fish species, larger species, most of them important for artisanal fisheries, are found, including migratory species such as *Conorhynchos conirostris*, *Prochilodus argenteus*, *Pseudoplatystoma corruscans*, and *Salminus franciscanus* (Sato & Godinho 2003, Godinho & Kynard 2006).

However, the number of species gathered in the present study is likely underestimated due to a range of factors. Some undescribed species were not counted, the only exception being *Rhamdiopsis* sp. from the Chapada Diamantina region (SFR). Some nominal widespread species may represent species complexes that need revisionary studies to solve the confusing taxonomy and the definition of the new species described (e.g., *Astyanax bimaculatus*, *A. fasciatus*, *Hoplias malabaricus*, *Geophagus brasiliensis*, *Rhamdia quelen*). In addition, some sub-basins were not sampled (e.g., most tributaries of the middle São Francisco in southwestern Bahia and headwaters of various river basins of the NMA).

More than 20 years ago, Menezes (1996a, b) cited the lack of knowledge about the fish fauna of drainages in Brazil's Northeastern region. Although a series of studies increasing that knowledge were conducted at that time, the results were far from satisfactory and Northeast Brazil is still pointed out as a region of scarce available information about freshwater fish records in a global database compilation (Tedesco et al. 2017). Current evidence of the lack of knowledge about the ichthyofauna in this region is the large number of taxa considered "sp." or accompanied by the terms "cf." or "aff." in taxonomic inventories (e.g., Sarmento-Soares et al. 2009a, Cetra et al. 2010, Burger et al. 2011, Ramos et al. 2014). Although numerous freshwater fish species from the Bahia river basins have been described in the last two decades (e.g., Bertaco & Lucena 2006, Ribeiro & Lucena 2006, Lima & Britski 2007, Benine et al. 2007, Zanata & Camelier 2008, 2009, 2010, 2014, 2015, Bichuette et al. 2008, Sarmento-Soares et al. 2009b, Sarmento-Soares et al. 2011, Ferreira et al. 2014, Vari et al. 2010, Bichuette & Rizzato 2012, Oliveira et al. 2013, Sarmento-Soares & Martins-Pinheiro 2013, Camelier & Zanata 2014b, Zanata et al. 2015, 2017, 2018, Mattos et al. 2015, Peixoto & Wosiacki 2016, Zanata & Pitanga 2016, Zawadzki et al., 2017; Barreto et al. 2018, Mattos & Costa, 2018, Burger et al. 2019, Lucena & Lucena 2019), ichthyological explorations continue to reveal unknown species, especially in small tributaries and in upper drainage areas. The increase in the number of species recently described from the São Francisco river basin has already been documented in the literature (see Alves et al. 2011 and Barbosa et al. 2017).

## 2. *Conservation concerns*

Overall, the results of the state and national conservation status assessments were remarkably similar, with divergences only for two species: *Acentronichthys leptos* and *Hypomasticus mormyrops*. In Bahia State, both species were included in threatened categories (VU and EN, respectively) since they occur at few impacted localities with continuing decline in the habitat quality. However, these species were classified as LC in the national assessment (MMA 2014) since their total distribution was considered wider, including river basins outside Bahia where these species are more abundant and are not threatened (e.g., Paraíba do Sul, Doce, plus small drainages in the states of São Paulo and Rio de Janeiro).

The conservation status of some species which occur in Bahia State changed in the last years. Some examples are the catfish 'pacamã' *Lophiosilurus alexandri* and the killifish *Hypsolebias adornatus*, both endemic to the SFR ecoregion, which were classified before as LC (MMA 2004) are now VU in both lists (SEMA 2017, MMA 2014). Several subpopulations of *L. alexandri* were probably locally extinct, reducing their population size by at least 30% (ICMBio 2018). On the other hand, *L. alexandri* was introduced in the Doce river, where became an important resource for artisanal fishing (Alves et al. 2007). Additionally, this species is cultivated in tanks as a commercial species for food near Colatina, Espírito Santo State (L. M. Sarmento-Soares pers. obs.). Nowadays, *H. adornatus* is also VU because one of its few known localities were destroyed, leading to local extinction. Moreover, this species is much appreciated as an aquarium fish, posing an additional and severe threat to this species. The category of three other annual killifishes, *Hypsolebias fulminantis*, *H. ghisolfii*, and *Ophthalmolebias perpendicularis* changed from VU (MMA 2004) to CR (MMA 2014). The first two, endemic to Bahia (SFR), are sympatric and were commonly found in temporary floodplain pools of Rio das Rãs, a tributary of the São Francisco basin (Costa 2007). However, their distribution is now restricted to a few localities as a consequence of the agricultural development and potteries established in the region. The case of *O. perpendicularis* is even more concerning since the species is currently classified as CR, the highest extinction risk category. In fact, this annual rivulid species may already be extinct since it was only known from the type locality in the Jequitinhonha river basin (NMA ecoregion), which was destroyed, and there has been no record of this species since 2000 (ICMBio 2018).

The large number of threatened freshwater fish species in the NMA ecoregion (23) is possibly due to the remarkable endemism of its ichthyofauna (Bizerril 1994, Ribeiro 2006, Camelier & Zanata 2014a) associated with the significant human occupation and alteration of the coastal region (Langeani et al. 2009). In the SFR ecoregion, most threatened species are annual killifishes, whose populations are disappearing due to the strong degradation and grounding of their habitats (see Costa 2002, ICMBio 2018). Eight species were classified as NT and may soon move to the list of endangered species if no conservation measures are adopted. In addition to the increasing human exploitation, the relatively small number of conservation units and protected areas in Bahia may be insufficient for the preservation of its fish fauna. Only 11.6% of the state area is protected as Conservation Units and less than 20% of these are in the higher protective level (Allen 2015). Additionally, we point out that most of these protect areas act as biodiversity islands, since they are surrounded by diverse monocultures, pastures and urban centers. One way to reduce these negative effect is the adoption of public polices that favor more environmentally appropriate agricultural practices in the buffer zones of Conservation Units, such as agroforestry systems (Sarmento-Soares & Martins-Pinheiro, 2017; Ewert et al. 2013).

The National Action Plans (Planos de Ação Nacional, PANs, in Portuguese) coordinated by the ICMBio, which have as main mission conserving Brazilian biodiversity, are public policies identifying and guiding priority actions to combat threats that endanger populations of species or environments (Polaz 2014). Therefore, the success of the PAN depends of both taxonomic information and data of the assessment of the conservation status of the species, such as provided in the present study. There are three PAN designed for freshwater fishes whose actions directly affect the conservation of the ichthyofauna in the state of Bahia: (1) Action Plan for the threatened species from the São Francisco watershed (Ordinance ICMBio nº 34, 27 May 2015), aiming mainly to improve the knowledge about threatened species and mitigate human impacting activities, to promote the conservation and recovery of aquatic fauna in the São Francisco river in five years (2015-2020);

(2) Action Plan for the threatened fish species from the Atlantic Forest rivers (Ordinance ICMBio nº 370, 1 August 2019), objecting to increase the conservation status and popularization of fishes, rivers, and streams of the Atlantic Forest in five years (2019-2024); and (3) Action Plan for the threatened species of Rivulidae family, with the general objective of establishing mechanisms to protect the rivulids and canceling the loss of habitat of the focal species, in five years (2013-2018); the second cycle of this PAN (2020-2025) was approved and it is in preparation, awaiting publication of the new ordinance.

Finally, it is expected that these joint actions (e.g., taxonomic studies, lists of species, conservation status of species, public policies) will contribute not only to the increase of the knowledge of the fish fauna but also to the conservation of these species and the environments inhabited by them.

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Ricardo Jucá Chagas: Substantial contribution in the concept and design of the study.

Alexandre Clistenes Santos: Contribution to data analysis and interpretation.

Angela Maria Zanata: Contribution to critical revision, adding intellectual content.

Beatriz Kawamura Rodrigues: Contribution to data analysis and interpretation.

Carla Natacha Marcolino Polaz: Contribution to critical revision, adding intellectual content.

Carlos Bernardo Mascarenhas Alves: Contribution to critical revision, adding intellectual content.

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

## Conflicts of Interest

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

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**article**

# New records of ants (Hymenoptera: Formicidae) for Colombia

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***Abstract:*** Even though Colombia has high levels of ant species richness in the Neotropical region, this richness continues to increase. New records of the ant subfamilies Amblyoponinae, Dolichoderinae, Dorylinae, Myrmicinae, and Ponerinae are presented. Two species of *Fulakora*, two species of *Azteca*, one species of *Cylindromyrmex*, 25 species of Myrmicinae belonging to 12 genera (*Acanthognathus, Basiceros, Daceton, Eurhopalothrix, Hylomyrma, Mycetomoellerius, Mycetophylax, Mycocepurus, Octostruma, Pheidole, Rogeria,* and *Talaridris*), and one species of *Leptogenys* are registered for the first time for Colombia. Five species are new records for South America. For each species, the geographical distance of the record closest to the Colombian locality is offered. Several factors, such as access to previously unexplored conserved areas, sampling techniques that cover heterogeneous microhabitats such as leaf litter, and many more taxonomic researches have allowed the knowledge of ant fauna in Colombia to continue growing.
***Keywords:*** Distribution range; Neotropical region; *Pheidole*; Species richness; South America.

## Nuevos registros de hormigas (Hymenoptera: Formicidae) para Colombia

***Resumen:*** Aunque Colombia tiene altos niveles de riqueza de especies de hormigas en la región neotropical, esta riqueza continúa aumentando. Se presentan nuevos registros de las subfamilias de hormigas Amblyoponinae, Dolichoderinae, Dorylinae, Myrmicinae y Ponerinae. Se registran por primera vez para Colombia, dos especies de *Fulakora*, dos especies de *Azteca*, una especie de *Cylindromyrmex*, 25 especies de Myrmicinae pertenecientes a 12 géneros (*Acanthognathus, Basiceros, Daceton, Eurhopalothrix, Hylomyrma, Mycetomoellerius, Mycetophylax, Mycocepurus, Octostruma, Pheidole, Rogeria,* y *Talaridris*), y una especie de *Leptogenys*. Cinco especies son nuevos registros para América del Sur. Para cada especie, se ofrece la distancia geográfica del registro más cercano a la localidad colombiana. Varios factores, como el acceso a áreas conservadas previamente inexploradas, las técnicas de muestreo que cubren microhábitats heterogéneos, como la hojarasca, y muchas más investigaciones taxonómicas han permitido que el conocimiento de la fauna de hormigas en Colombia continúe creciendo.
***Palabras clave:*** Ámbito de distribución; Región Neotropical; *Pheidole*; Riqueza de especies; América del Sur.

## Introduction

Globally, 337 valid genera and 13,809 valid species of ants are known (Bolton 2020). With 105 genera and more than 1100 species, the ant fauna in Colombia is one of the richest in the World (Fernández et al. 2019). Some ant genera, however, are poorly represented in Colombia (e.g., *Fulakora* Mann, 1919) while others, such as *Pheidole* Westwood, 1839 exhibit high diversity (Fernández et al. 2019). Other taxonomic groups, such as the subfamily Martialinae Rabeling & Verhaagh, 2008, and the genus *Bothriomyrmex* Emery, 1869, have yet to be found there. Because of this, Colombia ranks as the second most diverse Neotropical country, with a slightly lower ant genus richness than Brazil (105 vs 112 genera, respectively). The Colombian diversity, however, is relatively larger than Costa Rica's, whose myrmecofauna is much better known (Guerrero et al. 2018).

Recent field trips and routine curatorial activities in some major insect collections are helping to increase the knowledge of the ant fauna of this country. The recent ceasefire in Colombia has allowed field activities in areas that were previously too dangerous or areas under the protection of the National Parks Unit (Guerrero et al. 2018). This access to previously unsampled areas as the Colombian Amazon and forests in the Sierra Nevada de Santa Marta (northeastern Colombia) has allowed us to find 31 species of ants that we are registering for the first time for Colombia, and five of those ones are new records for South America.

## Materials and Methods

The examined specimens come from the following collections: CATAC, Colección de Artrópodos Terrestres de la Amazonía Colombiana, Instituto SINCHI, Leticia, Colombia; CBUMAG, Colecciones Biológicas de la Universidad del Magdalena, Santa Marta, Colombia; ICN, Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá D.C., Colombia; IAvH, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Colombia; and MPUJ, Colección entomológica de la Universidad Pontificia Javeriana, Bogotá D.C., Colombia. The samples were mounted and examined using diverse stereomicroscopes (Leica Wild m3c, Motic SMZ-168 and Carl Zeiss Stemmi 305) at magnifications of 60-80x.

The distance between the record in Colombia and the one in the nearest country was calculated with https://www.gps-coordinates.net/distance by calculating the straight line spacing between the geographic coordinates of both records. When a species has more than one record for Colombia, the Colombian record closest to the record in the neighboring country was used. The closest record to Colombia was extracted from electronic resources, such as antmaps.org (Janicki et al. 2016) and www.antweb.org, and in some cases from the original descriptions (e.g., Lattke, 1992). In the case of Antweb information, we record the specimen identifier (e.g., the FMNHINS0000095938 specimen of *Eurhopalothrix schmidti* (Menozzi, 1936) is the closest record to Colombia). In all cases, the distance is presented in kilometers.

## Results

### 1. *Taxa richness*

We identified 31 species of ants not reported from Colombia. These species are distributed among five subfamilies and 16 genera. The subfamily that contained the largest number of new records was Myrmicinae, with 12 genera, of which *Pheidole* with ten species, was the richest. The other subfamilies had only one or two new recorded species. We found only one genus in each of the following subfamilies: Amblyoponinae, Dolichoderinae, Dorylinae and Ponerinae.

### 2. *Checklist of new ant records for Colombia*

**Amblyoponinae**
***Fulakora agostii* Lacau & Delabie, 2002**

**Material Examined**. 1 worker. **Colombia: Quindío:** Armenia: Quindío University reserve, 4°33'15"N 75°39'40"W, 28/30-VIII-2009, squid bait, Martínez col. (ICN); 1 worker. **Colombia: Cundinamarca:** Quipile: El Tiber. Fca. Venecia. 04°42'12" N 74°33'56" W. 1523 m. Winkler Cafetal. 15-IX.2011. J. Cepeda, C. Cantor, R. Martinez Leg. 2 workers. **Colombia: Cundinamarca:** Quipile: El Tiber. Fca. La Aldea. 04°43'20" N 74°33'30" W. 1663 m. Winkler Cafetal. 17-IX.2011. J. Cepeda, C. Cantor, R. Martinez Leg.

**Comments.** Lacau & Delabie (2002) described *Fulakora agostii* based on 12 workers collected in Bahia, Brazil. Posteriorly Munoz (2018) recorded this species in Paraná, Brazil. The present record extends the distribution of *F. agostii* 4592 Km to the Northwest from the northernmost record in Bahia by Lacau & Delabie (2002).

***Fulakora armigera* Mayr, 1887**

**Material Examined**. 1 queen. **Colombia: Amazonas:** Leticia: Community Monifue Amena, BTF, 120 m, 4°08'30"S 69°55'23"W, 12-XII-2002, floor forest, Rodríguez col. (MPUJ).

**Comments.** Mayr (1887) first described *F. armigera* from Santa Catarina, Brazil. This species has since been sampled extensively in the southeast coast in Brazil and has been reported for Argentina (Bruch 1921). The present record extends the distribution of *F. armigera* 1984.47 km to the West from the closest record in Brazil recorded by Lattke (1985). The Venezuelan records (Lattke 1985) for *F. armigera* are a case of mistaken identity (Lattke, pers. comm.) as they later were identified as an undescribed species, *F. lurilabes* (Lattke 1991).

**Dolichoderinae**
***Azteca quadraticeps* Longino, 2007**

**Material Examined**. 2 queens. **Colombia: Magdalena:** Santa Marta: Universidad del Magdalena campus, 11°13'30"N 74°11'06"W, 2017, manual sampling, R. J. Guerrero col. (CBUMAG); 2 males. Same data (CBUMAG).

**Comments**. This species was described from Costa Rica and later reported in Nicaragua (Longino 2007, 2013c). This is the first record of *A*. *quadraticeps* for South America, in the urban area of Santa Marta, northern Colombia. The present record extends the distribution of *A*. *quadraticeps* 1021 Km to the East from the closest record in Limón, Costa Rica, reported by Longino (2007).

***Azteca snellingi*** **Guerrero *et al*. 2010**

**Material Examined**. 4 major workers. **Colombia: Magdalena:** Santa Marta: Bonda, Paso del Mango, Reserva Caoba, 300 m, 11°11'50.28"N 74°06'5.4"W, 09-XI-2019, manual sampling, M. Escárraga col. (CBUMAG); 1 minor worker. Same data (CBUMAG).

**Comments**. This species was described and exclusively known from Panama (Guerrero et al. 2010, Basset et al. 2012, Ribeiro et al. 2013). This is the first record of *A*. *snellingi* for South America. The present record in an area of dry forest in the foothills of the Sierra Nevada de Santa Marta, extends the distribution of *A*. *snellingi* 677 Km to the Northeast from the type locality.

**Dorylinae**

***Cylindromyrmex brevitarsus*** **Santschi, 1925**

**Material Examined**. 1 queen. **Colombia: Huila:** PNN Cueva de los Guácharos, Cedros, 1°42'29.959"N 76°08'51.521"W, XII-2006, W. Hernández col. (CBUMAG).

**Comments**. This species was previously known from Brazil, Costa Rica, Ecuador, Guatemala, México, Perú, Uruguay, and Venezuela (DeAndrade 2001, Branstetter & Saenz 2012, Bezdeckova et al. 2015, Fernández & Sendoya 2004). The present record is located 248 Km to the North from the closest record in Sucumbíos (Ecuador) reported by de Andrade (2001).

**Myrmicinae**

***Acanthognathus lentus*** **Mann, 1922**

**Material Examined**. 5 workers. **Colombia: Vaupés:** Mitú: km 2 via Monfort, 186 m, 1°14'05.1"N 70°12'57.8"W, 21-VII-2019, Winkler, N. Mazzi and L. Martínez col. (ICN).

**Comments**. This species was previously known from Brazil, Guyana, Honduras, and Suriname (Mann 1922, Brown & Kempf 1969, Brandão 1991, Galvis & Fernández 2009). The present record is located 1233 Km to the Northwest from the closest record in Amazonas, Brazil reported by Kempf (1975).

***Basiceros militaris*** **Weber, 1950**

**Material Examined**. 1 worker. **Colombia: Vaupés:** Villa Fátima, Cerro La Mujer, 273 m, 1°01'29.0"N 69°58'34.8"W, 17/22-VIII-2019, Winkler, G. Fiorentino, A. Meneses and S. Ramírez col. (ICN).

**Comments**. This species was previously known from Venezuela, Ecuador, and Brazil (Bolton 1995, Brown 1960). The present record is located 710 Km to the East from the closest record in the Yasuní National Park, Ecuador, reported by Mertl et al. (2012).

***Daceton boltoni*** **Azorsa & Sosa-Calvo, 2008**

**Material Examined**. 1 worker. **Colombia: Amazonas:** Leticia: Monifue Amena, 80 m, 4°6'S 69°55'W, 24-IX-2003, corner sampling on tree, Vargas col. (MPUJ); 1 worker. Leticia: Monifue Amena, 70 m, 4°6'S 69°55'W, 24-III-2004, Pitfall, D. Nariño col. (MPUJ); 1 worker. Leticia: Monifue Amena, 70 m, 4°6'S 69°55'W, 24-X-2004, manual sampling, E. Daza col. (MPUJ); 1 worker. Leticia: Monifue Amena, 70 m, 4°6'S 69°55'W, 23-X-2004, manual sampling, M. Gallejo col. (MPUJ); 2 workers. Leticia: Monifue Amena, 70 m, 4°6'S 69°55'W; 04-X-2005, shaking, Beltrán col. (MPUJ); 1 worker. Leticia: Monifue Amena, 70 m, 4°6'S 69°55'W, 04-X-2005, pitfall, E. Daza col. (MPUJ).

**Comments**. This species was previously known from Iquitos (Peru), Manaus, and Mato Grosso, Brazil (Azorsa & Sosa-Calvo 2008, Vicente et al. 2011). Therefore, this is the first record of *D. boltoni* in Colombia. This new record is in accordance with Azorsa & Sosa-Calvo's (2008) presumption about *Daceton* species distribution patterns. The present record is located 340 Km to the East from the type locality in Peru.

***Eurhopalothrix schmidti*** **(Menozzi, 1936)**

**Material Examined**. 1 worker. **Colombia: Santander:** Encino: RN Encino, 2000 m, 06°04'N 73°07'W, Winkler, E. González col. (IAvH). No collection date.

**Comments**. *Eurhopalothrix schmidti* was previously known from Costa Rica, Nicaragua, and Panama (Longino 2013a, c, Antweb 2020). The present record is located 886 Km to the Southeast from the closest record in Panama (Antweb: FMNHINS0000095938). This is the first South American record for this species.

***Eurhopalothrix xibalba*** **Longino, 2013**

**Material Examined**. 2 workers. **Colombia: Quindío:** Filandia: Vda. Cruces, Fca. El Brasil, Corredor Pavas Bosque, 1850 m, 4°41'17.41"N 75°36'32.81"W, Winkler, E. Jiménez and E.L. Franco E.L. col. (IAvH); Filandia: Vda. Cruces, Fca. Los Micos Cañada 1 transecto, 1750 m, 4°41'14.42"N 75°38'51.93"W, Winkler, E. Jiménez and E.L. Franco col. (IAvH). No dates for these localities.

**Comments**. This species has been reported from Mexico (Oaxaca), Guatemala, Honduras, Nicaragua, Costa Rica, Panama (Longino 2013a, Longino & Branstetter 2018, Antweb 2020). The present record is located 555 Km to the Southeast from the closest record in Panama (Antweb: CASENT0640577, CASENT0633025). This species is recorded for South America for the first time.

***Hylomyrma longiscapa* Kempf, 1961**

**Material Examined**. 2 workers. **Colombia: Guainía**: Inirida, Caño Sardina, 93 m, 3°57’20.1”N 68°01’15.0”W, 19-III-2019, Winkler in mainland forest, D. Castro col. (ICN). 1 worker. Same data (CATAC).

**Comments**. This species is known from Brazil (Souza et al. 2015), Ecuador (Salazar et al. 2015), French Guiana (Fernández & Sendoya 2004), Guyana (Fernández & Sendoya 2004), and Suriname (Fernández & Sendoya 2004). The present record is located 1078 Km to the closest record in Ecuador (Kempf 1975a).

***Mycetomoellerius relictus* (Borgmeier, 1934)**

**Material Examined**. 2 workers. **Colombia: Vaupés:** Mitú, Trubon, 196 m, 1°12’19.5”N 70°03’70.5”W, 24-III-2019, Flood Forest, D. Castro col. (ICN). 1 worker. Same data (CATAC).

**Comments**. This species has been previously reported multiple times in Brazil, French Guiana (Fernández & Sendoya 2004), Guyana (Fernández & Sendoya 2004), Peru, Suriname (Borgmeier 1934), Trinidad and Tobago (Weber 1968), and Venezuela (Mayhe-Nunes et al. 2002). The present record is located 1012 Km to the Southwest from the closest record in Venezuela.

***Mycetophylax strigatus* Mayr, 1887**

**Material Examined**. 1 worker. **Colombia: Nariño:** Orito: Territorio Kofán, 700 m, 0º30’N 77º13’W, manual sampling, E. González col. (IAvH). No date.

**Comments**. This species was previously known from Panama and Brazil (Wheeler 1949, Kempf 1964). The present record is located 999 Km to the Southeast from the closest record in Panama, reported by Wheeler (1949) (Although this record was considered as questionable by Kempf [1964]), and 1939 Km to the Northwest from the closest Brazilian record in the state of Amazonas, reported by Harada & Adis (1998).

***Mycocepurus goeldii* Forel, 1893**

**Material Examined**. 6 workers. **Colombia: Cauca:** Piamonte: Miraflor, 291 m, 1°1’46.25”N 76°26’31.18”W, VI-VII.2017, manual sampling, secondary forest, G. Delgado col. (ICN).

**Comments**. This species was previously known from Bolivia, Brazil, and Argentina (Kempf 1963, Mackay et al. 2004). The present record is located 1573 Km to the Northwest from the closest record in the state of Acre, Brazil (Oliveira et al. 2009).

***Octostruma batesi* (Emery, 1894)**

**Material Examined**. 2 workers. **Colombia: Vaupés:** Mitú: Trubon, 196 m, 1°12’19.5”N 70°03’70.5”W, 24-III-2019, leaf litter in floodplain forest, D. Castro col. (ICN). 1 queen. Same data as worker (ICN). 1 worker. Same data as worker and queen (CATAC).

**Comments**. This species was previously known from Bolivia (Longino 2013b), Brazil (Fernández & Sendoya 2004), Ecuador (Longino 2013b), French Guiana (Fernández & Sendoya 2004), Guyana (Fernández & Sendoya 2004), Guatemala (Fernández & Sendoya 2004), Panama (Fernández & Sendoya 2004), Suriname (Fernández & Sendoya 2004). The present record is located at approximately 788 Km to the East from the closest record in Ecuador.

***Octostruma betschi* Perrault, 1988**

**Material Examined**. 1 worker. **Colombia: Vaupés:** Mitú: Trubon, 196 m, 1°12’19.5”N 70°03’70.5”W, 24-III-2019, leaf litter in floodplain forest, D. Castro col. (ICN).

**Comments**. This species was previously known from Bolivia, Brazil, French Guiana (Perrault 1988) and Peru (Longino 2013b). The present record is located at approximately 1286 Km to the West from the closest record in the state of Amazonas, Brazil.

***Octostruma excertirugis* Longino, 2013**

**Material Examined**. 1 worker. **Colombia: Santander:** Cimitarra: Hacienda Ecológica Paraíso, 6°27’00.5”N 74°17’07.0”W, 16/18-VII-2019, Winkler. (ICN). No collector data.

**Comments**. This species was previously reported from Belize, Mexico, Guatemala, Honduras, Nicaragua, Costa Rica, Panama, and Ecuador (Longino 2013b, Antweb 2020). The present record is located 682 Km to the East from the closest record in Panama (Antweb: MCZ-ENT00511424).

***Pheidole bruchi* Forel, 1914**

***Material Examined***. 1 soldier. **Colombia: Cauca:** Santa Rosa: Polígono 146, 2005 m, 1°66’14.7”N 76°57’24.7”W, 22727-XI-2013, en tronco, Y.A Mera, D. Delgado, L. Ortiz, R. Sinisterra y C. Arturo col. (Unicauca); 2 workers. Same data (Unicauca); 2 soldiers. Santa Rosa: Polígono 146, 2020 m, 1°66’56.2”N 76°57’0.7”W, 22/27-XI-2013, en tronco, Y.A. Mera, D. Delgado, L. Ortiz, R. Sinisterra y C. Arturo col. (Unicauca); 1 worker. Same data (Unicauca).

**Comments**. This species was previously known for Argentina, Brazil, and Paraguay (Cuezzo 1998, Wild 2007, Calixto 2013). The present record extends the distribution of *Pheidole bruchi* 2924 Km to the North from the northernmost record in Santa Catalina, Argentina, reported by Bruch (1914).

***Pheidole bruesi* Wheeler, 1911**

***Material Examined***. 1 queen. **Colombia: Putumayo**: territorio Kofán, 1000 m, 23-IX-1998, E. González col. (IAvH). 4 workers. Same data (IAvH). 1 soldier. territorio Kofán, 1000 m, 13-VIII-1998, E. González col. (IAvH). 1 worker. **Vaupés**: Caparú, Estación Mosiro-Itajura, 60 m, 1°4’N 69°3’W, 4/11-III-2003, malaise, J. Pinzón col. (IAvH). 1 soldier. **Nariño**: Ipiales: Territorio Kofán, 700 m, 0°30’7”N 77°13’43”W, 27-IX-1998, manual sampling with tuna, E. González col. (IAvH).

**Comments**. This species was previously known for Brazil, French Guiana, and Lesser Antilles (Oliveira et al. 2009, Groc et al. 2013, Wilson 2003). The present record extends the distribution of *Pheidole bruesi* 990 Km to the Northwest from the closest record in Amazonas, Brazil, reported by Vasconcelos et al. (2010).

***Pheidole cataractae* Wheeler, 1916**

***Material Examined***. 1 soldier. **Colombia: Valle del Cauca:** Dagua: 3°39'25.59"N 76°41'24.52", 14/16-V-2015 (CBUMAG). No more data.

**Comments**. *Pheidole cataractae* was previously known from Brazil, Ecuador, Guyana, and Peru (Wheeler 1916, Mertl et al. 2009, Bezdeckova 2015, Souza et al. 2015,). The present record extends the distribution of this species 480 Km to the North from the closest record in Ecuador, reported by Mertl et al. (2009).

***Pheidole dolon* Wilson, 2003**

**Material Examined**. 1 soldier, 1 worker. **Colombia: Amazonas:** Leticia: vía Tarapacá Varzea, 2°53'21.93"S 69°44'30.50"W, 2002, Barriga col. (ICN).

**Comments**. *Pheidole dolon* was previously known from Bolivia, French Guiana, and Peru (Economo et al. 2015, Groc et al. 2009, Prado et al. 2019, Wilson 2003). The present record is located 1069 Km to the North from the type locality in Peru, reported by Wilson (2003).

***Pheidole kuna* Wilson, 2003**

***Material Examined***. 1 worker. **Colombia: Cauca:** Piamonte: 300m, 1°7'12.57"N 76°19'19.43"W, 19/22-V-2014, winkler, Y.A. Mera, D. Delgado, L. Ortiz, R. Sinisterra and C. Arturo col. (Unicauca). 2 soldiers. Piamonte: Vda. La Leona, 284 m, 1°6'24.51"N 76°15'52.26"W, 19/22-V-2014, manual sampling, Y.A. Mera, D. Delgado, L. Ortiz, R. Sinisterra and C. Arturo col. (UniCauca). 1 soldier. Piamonte: 300 m, 13/23-V-2014, manual sampling, Y.A. Mera, D. Delgado, L. Ortiz, R. Sinisterra and C. Arturo col. (Unicauca). 1 worker. Piamonte: 300 m, 1°7'12.57"N 76°19'19.43"W, 13/23-V-2014, manual sampling, Y.A. Mera, D. Delgado, L. Ortiz, R. Sinisterra and C. Arturo col. (Unicauca).

**Comments**. This species was previously known from Panama (Wilson 2003). The present record extends its distribution 979 Km to the South from the closest record in Panama, reported by Wilson (2003). This is the first record of *Pheidole kuna* for South America.

***Pheidole leonina* Wilson, 2003**

***Material Examined***. 1 soldier, 1 worker. **Colombia: Amazonas:** Río Ayo, 97 m, 2°6'45.82"S 69°46'40.32" W, 01-IV-2002, F. Quevedo col. (ICN). 1 soldier. same data (ICN)

**Comments**. *Pheidole leonina* was previously known from Brazil and Peru (Oliveira et al. 2009, Wilson 2003). The present record extends the distribution of this species 916 Km to the North from the most northern record of the species in Acre, Brazil, reported by Oliveira et al. (2009).

***Pheidole socrates* Forel, 1912**

***Material Examined***. 1 soldier. **Colombia: Nariño**: Barbacoas: RNN El Pangán, 1469m, 1°19'43" N 4°55' W, 06-VIII-2006, manual sampling, A. Miranda and O. Reyes col. (IAvH); 4 workers. Barbacoas: RNN El Pangán, 1189 m, 1°20'8" N 78°5'20" W, 30-VII/01-VIII-2006, pitfall, A. Miranda and O. Reyes col. (IAvH).

**Comments**. *Pheidole socrates* was previously known from Bolívia, Brazil, Ecuador, French Guiana, Guyana, Lesser Antilles, Mexico, Panama, Suriname, and Trinidad and Tobago (Forel 1912, Fernández & Sendoya, 2004, LaPolla & Cover 2005, Mamani-Mamani et al. 2012, Guenard et al. 2017). The present record is located 201 Km to the North from the closest record in Ecuador, reported in antmaps.org (Guénard et al. 2017).

***Pheidole midas* Wilson, 2003**

***Material Examined***. 1 worker, 1 soldier. **Colombia: Bolívar:** San Juan Nepomuceno: 9°56'56.49"N 75°5'1.47"W, 06/08-XI-2014 (CBUMAG); 3 soldier, 4 workers. **Colombia: Bolívar:** San Juan Nepomuceno: Arroyo Grande, 176 m, 9°56'23.532"N 75°10'7.622"W, 03-II-2016, pitfall, R. Achury col. (CBUMAG); 1 worker, 1 soldier. **Antioquia:** San Luis: El Refugio, Parque Ecológico Cañón del Río Claro, 515 m, 5°54'3.39"N 74°51'23.85"W, 10-IV-1998, A. Amarillo col. (ICN).

**Comments**.

*Pheidole midas* was previously known from Brazil, Ecuador, French Guiana, Lesser Antilles, Panama, Peru and Venezuela (Alonso *et al*. 2001, Basset *et al*. 2012, Fichaux *et al*. 2019, Filho *et al*. 2003, Mertl *et al*. 2010, Prado *et al*. 2019, Wilson 2003). The record from San Juan Nepomuceno is located 526 Km to the Southeast from the closest record in Panama reported by Guénard et al. (2017).

*Pheidole midas* was previously identified as *P. veletis* Wilson, 2003, as both species are differentiated by very subtle traits that can lead to misidentification. However, *Pheidole midas* can be differentiated by the dorsal surface of the head that is largely smooth and relatively shining, with notable foveas and a large-celled patch of rugoreticulum present to the side and behind each antennal fossa (Wilson 2003), while *P. veletis* with the dorsal surface of the head dull and rough, and carinulae originating on frontal lobes extend halfway between level of eye and occiput.

***Pheidole wallacei* Mann, 1916**

***Material Examined***. 4 workers. **Colombia: Amazonas:** Río Ayo, 1-V-2002, primary forest, F. Quevedo col. (ICN). No more data.

**Comments**. *Pheidole wallacei* was previously known from Brazil and French Guiana (Mann 1916, Groc et al. 2009). The present record is located about 1000 Km to the Northwest from the closest record in Rondonia, Brazil (Mann 1916).

***Pheidole zoster* Wilson, 2003**

***Material Examined***. 1 soldier, 3 workers. **Colombia:** Cauca: Sabanetas, La Romelia-El Tambo, El Ensueño, Km 2, 1938 m, 2°33'09.9"N 76°51'40.3"W. No more data. (IAvH).

**Comments**. *Pheidole zoster* was previously known from Brazil and Peru. The present record is located at approximately 1312 Km to the North from the closest record in Peru.

***Rogeria procera* Emery, 1896**

**Material Examined**. 2 workers. **Colombia: Guaviare:** Calamar Chiribiquete Cerro Campana, 306 m, 01°17'10.5"N 72°37'32.1"W, 3/6-III-2018, Pitfall white sand savannah, Tree trunk, D. Luna and A. Pinzon col. (ICN); 1 worker. Calamar Chiribiquete Cerro Campana, 306 m, 01°16'49.9"N 72°37'53.2"W, 3/6-III-2018, Winkler, Varillal, rocky leaf litter, D. Luna and A. Pinzón col. (ICN).

**Comments**. This species was previously known from Guyana (Lapolla and Fisher 2006), Brazil (Pará and Amazonas), and Salta, Argentina (Badano et al. 2005). The present record is 1483 Km to the West from the closest record in Manaus, Brazil (Kugler 1994).

***Rogeria subarmata* (Kempf, 1961)**

**Material Examined**. 1 worker. **Colombia: Vaupés:** Cgto. Pacoa, Comunidad Morroco, Cuenca Rio Cauauari, Cerro Morroco, 195 m, 00°08'19.2"N 70°57'01.3"W, 27-II-2018, manual firm floor forest, leaf litter, D. Luna and W. Gómez col. (ICN).

**Comments**. This species has been extensively reported for Brazil (Pará, Bahia, Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo). Furthermore, it has been recorded for French Guiana (Gibernau et al. 2007), Venezuela, and Ecuador (Ryder et al. 2010). The present record is located 583 Km to the Northeast from the closest record in Ecuador (Ryder et al. 2010).

***Talaridris mandibularis* Weber, 1941**

**Material Examined**. 1 worker. **Colombia: Caquetá:** PNN Serranía de Chiribiquete, 0°42'N 72°42'W, 30-I-2000, Winkler, F. Quevedo col. (ICN).

**Comments**. This species was previously known from Brazil, French Guiana, Guyana and Venezuela (Brown & Kempf 1960, Weber 1941). This species is listed in Fernández et al. (2019) without precise locality; this is the first geographical report and confirmation of the species in the country. The present record is located 794 Km to the West from the closest record in Venezuela (Lattke 1992).

### Ponerinae

***Leptogenys rasila* Lattke, 2011**

**Material Examined**. 1 worker. **Colombia: Cauca:** Popayán, Centro de estudios vegetales La Rejoya, 1770 m, 2°31'02"N 76°35'34"W, 21,22-IX-2019, Winkler, D. Cubillos col. (CBUMAG).

**Comments**. This species was previously known from Ecuador (Lattke 2011). The present record is located 463 Km to the northeast from the closest record in Ecuador (Salazar et al. 2015).

## Discussion

In the Neotropical region, the number of genera and species currently known is 131 and 3463, respectively (Fernández et al. 2019), and approximately 81% of these genera are registered in Colombia (Fernández et al. 2019). The thirty-one new ants registered here raise the number of specific taxa known from Colombia to 1197 species. This species richness represents more than one third (~34%) of the known Neotropical species.

In the "Hormigas de Colombia" book, Fernández et al. (2019) reported 50 genera and 535 Myrmicinae species for Colombia, but a recent phylogenetic work added two more genera, *Mycetomoellerius* Solomon et al., 2019 and *Paratrachymyrmex* Solomon et al., 2019 replacing the former genus *Trachymyrmex* Forel, 1893 (Solomon et al. 2019). The thirty-one new records we provide here increase the Myrmicinae composition to 560 species. This species richness is relatively higher than that of countries such as Costa Rica (535 native species *sensu* antmaps.org (Janicki et al. 2016) or 539 according to Antweb), whose forests have been widely sampled resulting in a much better-known ant fauna. Within the Myrmicinae, the genus *Pheidole* is the most species-rich, with more than 1100 species throughout the world. Currently, there are 620 species of *Pheidole* known for the Neotropical region, with just two invasive species, *Pheidole indica* Mayr, 1879 and *Pheidole megacephala* (Fabricius, 1793). After Guerrero et al (2018), the species richness of *Pheidole* was 118 species, including *Pheidole indica* (there reported for the first time for Colombia) and *Pheidole megacephala* (previously reported by Chacón de Ulloa & Achury 2011). Here, we provide ten new species records elevating the species richness of *Pheidole* in Colombia up to 128 species. The species richness of *Pheidole* in Colombia is almost half of the specific richness reported for the Mesoamerican wet forests (Longino 2019), however, the records for Colombia could equal or exceed those numbers, due to the study of *Pheidole* specimens coming from different types of forests, both from lowlands (e.g., tropical dry forest and sub-xerophytic formations) as Andean and sub-paramun forests.

Among the thirty-one new ants for Colombia, we found species whose updated distributions may be the basis for present or future biogeographic questions. We recorded for the first time five species that were previously known from Central America, *Azteca quadraticeps*, *Azteca snellingi*, *Eurhopalothrix schmidti*, and *Eurhopalothrix xibalba* and *Pheidole kuna*. The two *Azteca* species extend their distribution to the northernmost area of South America, both with populations inhabiting the lowlands of the Sierra Nevada de Santa Marta (northern Colombia).

In general, *Azteca* species have great dispersal capacity, which for both cases could have allowed them to reach a wide distribution dispersing through the Darien mountain range and the favorable habitat that extends along the Caribbean plain, until reaching the lowlands of the mountainous massif. In the case of the *Eurhopalothrix* species registered here, both present an Andean distribution, one in the eastern Cordillera (*Eurhopalothrix schmidti*) and the other in the central Cordillera (*Eurhopalothrix xibalba*). *Eurhopalothrix schmidti* was collected at 2000 m of altitude, matching the altitude distribution in Mesoamerica that ranges from 1100 m to 2200 m, while *Eurhopalothrix xibalba* was collected at high elevations, above 1700 m within the altitudinal range of this species in Central America, from 50 m to above 1600 m (Longino 2013a). On the other hand, *Pheidole kuna*, spreading through the Chocoan biogeographic region, adding to other arthropods from the Chocó-Darién province (Morrone 2014).

The records of *Fulakora agostii* in Colombia expands its distribution from Brazil to almost 4500 km north-west of South America. The first records of *Fulakora agostii* come from the Brazilian Atlantic Forest (Lacau & Delabie 2002), however, the *Fulakora agostii* population in Colombia comes from the Andean landscape at 1500 m altitude. The Colombian Andean and the Brazilian Atlantic forest populations are allopatric, with a large discontinuity in its distribution, as currently there are no records in the Amazon basin, despite the abundant sampling in the Brazilian Amazon. Several hypotheses may explain the current distribution range of this species, but only two will be outlined. First, its occurrence in those two kinds of forests could suggest wide ecological plasticity to adapt to contrasting habitats. The second hypothesis would take into account the taxonomic validity of the Andean population, in this sense, this Andean population could correspond to an undescribed different evolutionary lineage. Although the diagnostic characteristics in this population match completely those offered by Lacau & Delabie (2002) it is necessary to carry out molecular analysis at the population level in *F. agostii* to try and differentiate between these hypotheses.

Recently, Guerrero et al. (2018) indicated that the growing knowledge of Colombian ant diversity may be due to factors such as the possibility of sampling in forests under the protection of the National Parks Unit, using sampling techniques that produce large volumes of biological material (e.g. Winkler extractors). However, the possibility of sampling in areas that were previously in armed conflict has also had a positive effect on myrmecological studies in the country. In this case, 50% of the new reports here come from forested areas where the armed conflict has completely ceased (e.g., all *Octostruma* species and five species of *Pheidole* recorded here); the other 50% correspond to ants that were collected in forests protected by the network of national parks or inside indigenous reserves. These factors expose the importance of collecting in unsampled areas for 1) better resolution of the distribution of biota and to support stronger biogeographic hypotheses, 2) access to more populations of known species to have a clearer picture of their genetic variability, tokogenetic relationships of these populations (i.e., phylogeography) and understanding the phenotypic variability of some species, and 3) increase records within national biological collections to help complement biodiversity inventories and decision-making by government entities at different spatial scales.

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## Author Contributions

Emira I. García contributed in the preparation of the manuscript, as well as to the identification of several ant species and the collection of distribution data from museums and biological collections. Also, she critically reviewed the different versions of this manuscript, incorporating arguments and hypotheses contained in the latter.

María C. Tocora contributed to the preparation of the manuscript, as well as to the identification of ant species and the collection of distribution data from museums and biological collections. She reviewed and corrected the different versions of this manuscript.

Gian P. Fiorentino contributed to the preparation of the manuscript, as well as to the identification of ant species and the collection of distribution data from museums and biological collections. He reviewed and corrected the different versions of this manuscript.

Fernando Fernández contributed to the preparation of the manuscript, as well as to the identification of ant species and the collection of distribution data from museums and biological collections. He reviewed and corrected the different versions of this manuscript.

Mayron E. Escárraga contributed in the preparation of the manuscript, as well as in the identification of some ant species. He was responsible for managing electronic resources to calculate geographic distances and to refine the geographic coordinates of all records; he critically revised the first draft and the final version of the manuscript.

Roberto J. Guerrero contributed substantially in the preparation of the manuscript, as well as to the identification of several ant species and the collection of distribution data from museums and biological collections. He wrote and added intellectual arguments and hypotheses contained in the manuscript; moreover, critically reviewed and corrected the different versions of this one.

## Conflicts of Interest

The authors of this manuscript declare that they have no conflict of interest regarding the publication of this manuscript. Likewise, each of them agrees with the opinions contained in this manuscript.

## Ethics

All authors declare to be in compliance with all ethical stablished guidelines.

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**Short Communication**

# A case of complete albinism in the catfish *Cambeva guareiensis* (Siluriformes: Trichomycteridae)

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***Abstract:*** Albinism has been recorded in Neotropical freshwater fishes, mostly for nocturnal or cryptobiotic species. We report herein a case of albinism in the catfish *Cambeva guareiensis* (Trichomycteridae) from the Guareí River basin, Upper Paraná River basin, southeastern Brazil. The albino fish was caught with seven individuals with typical color pattern of the species. The features of the albino fish in life and shortly after preservation are described and illustrated.
***Keywords:*** *Albinism; color anomaly; fish; Guareí River basin; Neotropical region; Paranapanema River basin.*

## Um caso de albinismo completo no bagrinho *Cambeva guareiensis* (Siluriformes: Trichomycteridae)

***Resumo:*** Albinismo tem sido registrado em peixes de água doce Neotropicais, principalmente em espécies noturnas ou criptobióticas. Relatamos aqui um caso de albinismo no bagrinho *Cambeva guareiensis* (Trichomycteridae) da bacia do Rio Guareí, bacia do Alto Rio Paraná, sudeste do Brasil. O peixe albino foi capturado juntamente com sete indivíduos com padrão de cor típico da espécie. As caracteristicas do peixe albino em vida e logo após a preservação são descritas e ilustradas.
***Palavras-chave:*** *Albinismo; anomalia de cor; bacia do Rio Guareí; bacia do Rio Paranapanema; peixe, região Neotropical.*

## Introduction

Like several other vertebrate groups, fishes may display different types of physical abnormalities (*e.g.*, Bhagat & Kumar 2014; Catelani et al. 2017), among them those related to color such as different types of albinism (*e.g.*, Nobile et al. 2016). A completely albino individual is characterized by pinkish or yellowish color and pink or red eyes (*e.g.*, Sazima & Pombal-Jr. 1986; Oliveira & Foresti 1996; Silva et al. 2013). Currently, a small number of cases of complete albinism is recorded for Neotropical freshwater fishes, mostly in epigeal habitats (*e.g.*, del Barco & Panattieri 1980; Oliveira & Foresti 1996; Brito & Caramaschi 2005; Silva et al. 2013), although albinism is known in hypogeal habitats as well (Carvalho & de Pinna 1986). To date, reports of complete albinos among trichomycterid catfishes remain restricted to the cave-dwelling *Trichomycterus itacarambiensis* Trajano & de Pinna, 1996 (Carvalho & de Pinna 1986; Trajano 1997).

During a field work (Azevedo-Santos et al. 2020) at the Guareí River basin, an important tributary of the Upper Paranapanema River, Brazil, a complete albino specimen of the catfish *Cambeva guareiensis* Katz & Costa 2020 (Trichomycteridae) was caught together with normal individuals. Herein we describe and illustrate the case.

## Material and Methods

*Cambeva guareiensis* individuals were collected on 20 September 2017 in the Corrente stream (-23.434932°-48.388694°), in the Guareí River basin, Upper Paranapanema River, São Paulo State, southeastern Brazil (Azevedo-Santos et al. 2020). The Corrente stream presents clear waters along its course. In the sampled site the substrate is composed mostly of rocks; the riparian vegetation is relatively well preserved, especially if compared to other streams in the Guareí River basin. Specimens of this trichomycterid catfish were collected in a stretch above a waterfall, with rapid waters and rocky substrate. Collection methods (hand net), euthanasia, fixation and preservation of the specimens are fully described in Azevedo-Santos et al. (2020).

The eight specimens caught in the same stretch had their standard length (SL, 0.1 mm) measured under stereomicroscope and were deposited in the collection of the Departamento de Zoologia e Botânica, UNESP, São José do Rio Preto, SP, Brazil (DZSJRP 23090). Names for the varied forms of albinism followed mostly Henle et al. (2017) for amphibians, as we found no comparable definitions and descriptions for fishes.

## Results

The complete albino individual of *Cambeva guareiensis* has 28.3 mm SL and the normally pigmented individuals measure between 30.1 and 56.0 mm SL. The body proportions of the albino are similar to those of the normal specimens. In addition, meristics are within the range expected for normal specimens. In life, the albino had pink eyes, a yellowish color dorsally and pink laterally, and the opercle and the region immediately after pectoral fins were red (Figure 1a-b). Due to its small size and the photographic perspective, the eyes are best viewed on the right side of the albino in dorsal view (Figure 1b). Shortly after fixed in formalin, the specimen showed a yellowish color dorsally and laterally. The ventral region was slightly yellow to white. The eyes and the operculum were whitish, the rays of all fins were light yellow (Figure 2a). Pigmented individuals had brown spots dorsally and laterally, with a midline composed of larger spots. The ventral region was yellowish to white (Figure 2b).

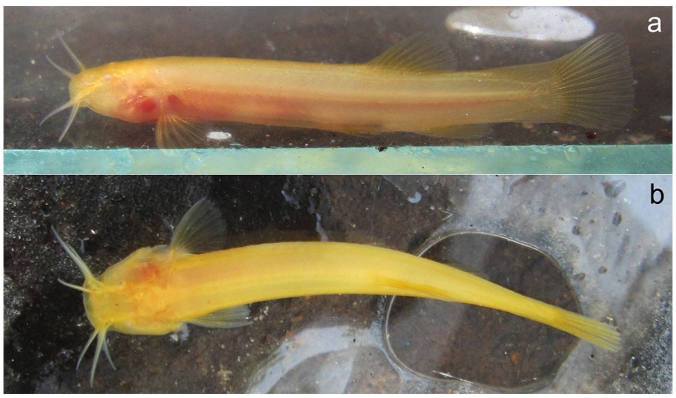


**Figure 1.** *Cambeva guareiensis* complete albino in life: (a) left side and (b) dorsal view (DZSJRP 23090).


**Figure 2.** *Cambeva guareiensis* individuals shortly after preservation: (a) complete albino (28.3 mm SL) and (b) normal color pattern (30.1 mm SL) (DZSJRP 23090).

## Discussion

The lack of pigmentation and pink eyes qualify the atypical *Cambeva guareiensis* individual as a complete albino (*sensu* Henle et al. 2017). The yellowish tinge and red eyes are typical traits of complete albinos (Sazima & Pombal-Jr. 1986; Oliveira & Foresti 1996; Silva et al. 2013). Therefore, the color of our specimen should not be confused with xanthism or flavism (*sensu* Henle et al. 2017), since xanthic individuals have pigmented eyes.

*Cambeva guareiensis*, a recently described species (Katz & Costa 2020), seems to be the second complete albino trichomycterid recorded to date. In a population of the cave-dwelling *Trichomycterus itacarambiensis* studied by Trajano (1997), there are depigmented individuals with red eyes (Carvalho & de Pinna 1986), which qualify them as complete or true albinos. However, it is difficult to qualify complete albinism in species that lack eyes, a typical trait in troglobiont organisms. This is the case of eyeless fish dwellling in caves and other types of hypogeal waters (Shibatta et al. 2007; Felice et al. 2008).

Complete or true albinos are rarely reported for the Neotropics, and most of them are catfishes (Siluriformes) belonging to several families including Callichthyidae, Doradidae, Heptapteridae, Loricariidae, and Trichomycteridae (del Barco & Panattieri 1980; Carvalho & de Pinna 1986; Sazima & Pombal-Jr. 1986; Burgess 1989; Brito & Caramaschi 2005; Manoel et al. 2017). There are two records of complete albinos in Gymnotidae (Campos-da-Paz & Caramaschi 1994; Oliveira & Foresti 1996) and one in Erythrinidae (Silva et al. 2013). The gymnotid and erythrinid fishes have nocturnal behavior, as is the case of most catfishes, a trait that may favor the survival of albino individuals in a natural environment (Sazima & Pombal-Jr. 1986). *Cambeva guareiensis*, like most trichomycterid species (Arratia & Huaquín 1995; de Pinna & Wosiacki 2003) is mostly nocturnal and cryptic, which favors its survival face to diurnal, visually hunting predators, as previously suggested for small albino catfishes in general (Sazima & Pombal-Jr. 1986; Brito & Caramaschi 2005; Manoel et al. 2017).

## Addendum

We refrained here from labelling our report on *Cambeva guareiensis* as a first case of albinism within the genus, even if it seems to be the only record of an albino of this species, and the second case of complete albinism within Trichomycteridae (Carvalho & de Pinna 1986; Trajano 1997). To illustrate our point, an albino described by Sazima & Pombal Jr (1986) was identified as *Rhamdella minuta* Lütken, 1874, according to the taxonomic knowledge of this group at the time of publication (H. A. Britski pers. comm.). However, I.S. later recognized the specimen as *Imparfinis mirini* Haseman, 1911 (Figure 3). Unaware of this situation, Manoel et al. (2017) reported on a first case of albinism in *I. mirini*, when the supposed *R. minuta* was, indeed, the first record of albinism the former species.

**Figure 3.** *Imparfinis mirini* complete albino, identified as *Rhamdella minuta* in Sazima & Pombal (1986). Adult specimen 46.4 mm SL (ZUEC 1438).

We take the opportunity to clear the facts about *I. mirini* albinos (Sazima & Pombal Jr. 1986; Manoel et al. 2017), and recommend that the word "first" should be used with care whatever the case or occurrence reported.

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## Author Contributions

Valter M. Azevedo-Santos: Collected and identified the specimen and wrote the first draft.

Fernando M. Pelicice: Collaborated with the writing process.

Ivan Sazima: Collaborated with the writing process.

Raoul Henry: Collaborated with the writing process.

## Conflicts of interest

None.

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**Inventory**

# Composition and richness of monogonont rotifers from La Plata River Basin, South America

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***Abstract:*** We present here the first study that analyzed the composition and richness of rotifers of the entire La Plata River basin, the second largest in South America, based on simultaneous and standardized sampling. Fifteen large reservoirs and eight river stretches were selected in the upper, middle, and lower portions of the Paraná, Paraguay, and Uruguay Rivers, which are the major rivers of the La Plata basin. We took a total of 86 samples (open water habitats) in 2010. A mean of 27±11 species per sub-basin was found, with the highest richness in the Lower Paraná (41 species), followed by the Paranapanema (40 species) and Lower Uruguay (38 species). Low richness was observed in the Middle Uruguay and Middle Paraná. We found 106 species belonging to 21 families and two orders. The family with the highest number of species was Lecanidae (21), followed by Brachionidae (20), Trichocercidae (9), and Synchaetidae (8). The species with higher occurrences were *Conochilus dossuarius*, *Kellicottia bostoniensis*, *Keratella americana*, *Keratella cochlearis* and *Hexarthra mira*. New occurrences of rotifers were registered for Brazil (*Colurella adriatica*), São Paulo State (*Enteroplea lacustris*), and Argentina (*Gastropus hyptopus*, *Harringia rousseleti* and *Lecane thienemanni*). Spearman correlation between the number of species and physical and chemical variables demonstrated positive correlation with chlorophyll and temperature, and negative correlation with dissolved oxygen. We extend the distribution list for some native (*Lecane ludwigii*) and non-native species of rotifers (*K. bostoniensis*). We also list the monogonont rotifer species found at the sampling stations.
***Keywords:*** *Biodiversity; Rotifera; Survey; New records; Lotic; Lentic environments.*

## Composição e riqueza de rotíferos Monogononta da Bacia do Rio da Prata, América do Sul

***Resumo:*** Apresentamos aqui o primeiro estudo que analisou a composição e riqueza de rotíferos de toda a bacia do Rio da Prata, a segunda maior da América do Sul, com amostragens simultâneas e padronizadas. Quinze grandes reservatórios e oito trechos lóticos foram selecionados nas porções alta, média e baixa dos rios Paraná, Paraguai e Uruguai, que atuam como os principais formadores da bacia do Prata. Coletamos um total de 86 amostras (habitats de águas abertas) em 2010. Foi encontrada uma média de 27 ± 11 espécies por sub-bacia, com maior riqueza no Baixo Paraná (41 espécies), seguido por Paranapanema (40 espécies) e Baixo Uruguai (38 espécies). Uma baixa riqueza foi observada no Médio Uruguai e no Médio Paraná. Encontramos 106 espécies pertencentes a 21 famílias e duas ordens. A família com maior número de espécies foi Lecanidae (21), seguida por Brachionidae (20), Trichocercidae (9) e Synchaetidae (8). As espécies com maior ocorrência foram *Conochilus dossuarius*, *Kellicottia bostoniensis*, *Keratella americana*, *Keratella cochlearis* e *Hexarthra mira*. Novas ocorrências de rotíferos foram registradas para o Brasil (*Colurella adriatica*), Estado de São Paulo (*Enteroplea lacustris*) e Argentina (*Gastropus hyptopus*, *Harringia rousseleti* e *Lecane thienemanni*). A correlação de Spearman entre o número de espécies e as variáveis físicas e químicas demonstrou correlação positiva com clorofila e temperatura, e correlação negativa com oxigênio dissolvido. Estendemos a lista de distribuição para algumas espécies nativas (*Lecane ludwigii*) e não-nativas de rotíferos (*K. bostoniensis*). Disponibilizamos também uma lista de espécies de rotíferos Monogononta encontrados nas estações amostradas.
***Palavras-chave:*** *Biodiversidade; Rotifera; Levantamento; Novos Registros; Lótico; Ambientes lênticos.*

## Introduction

Species inventories are important tools for conservation measures and management, especially in areas imperiled by human actions. It is also useful to show gaps in the scientific knowledge about zooplankton diversity and directions for future research.

There have been surveys of Rotifera diversity in the La Plata River basin, the second largest in South America. However, these surveys have focused on regions such as in the Upper Paraná floodplain (Lansac-Tôha et al. 2009), waterbodies of São Paulo State (Souza-Soares et al. 2011), and a few tributaries (Neschuk et al. 2002, Kuczynski 2017). There have been no basin-wide surveys that included all the countries drained by the basin.

The La Plata River basin has very distinct environments, with extensively dammed and undammed reaches. For example, there are reservoirs in more than half of the upper reaches in the Paraná River basin, leaving few truly lotic reaches; the opposite occurs in its middle and lower reaches (Agostinho et al. 2007). The situation is very similar for the Uruguay River. However, there are no reservoirs in the Paraguay River (Perbiche-Neves et al. 2016). This results in different habitats with distinct limnological features, which may favor differences in rotifer species composition among lotic and lentic regions.

There have been multiple studies of rotifer richness and distribution in Brazilian and Argentinian waters of the La Plata River basin. For example, Garraffoni & Lourenço (2012) surveyed rotifer species throughout Brazil. Other rotifer surveys were less extensive, such in Mato Grosso do Sul (Roche & Silva 2017), São Paulo (Souza-Soares et al. 2011), the Upper Tietê River basin (Lucinda et al. 2004), and Paranoá Reservoir (Padovesi & Andreoni 2011). Despite those surveys, the number of rotifer surveys are underrepresented (Souza et al. 2018), when compared to other groups of zooplankton such as copepods (Silva et al. 2009, Matsumura-Tundisi & Tundisi 2011, Perbiche-Neves et al. 2014).

For Argentina, José de Paggi (1990) listed 279 rotifer taxa. Most rotifer surveys have been in the Paraná River floodplains (Aoyagui & Bonecker 2004) and La Plata River tributaries (Macluf et al. 1998, Modenutti 1998, Bazzuri et al. 2020). Recently, Ferrando & Claps (2016) updated the checklist of Argentinian Rotifera, including a reporting 35 species of monogonont rotifers. According to the authors, "[...] the majority of reports were restricted to the provinces of Santa Fe (68% of the total records), Corrientes and Buenos Aires (50% of the total records), Río Negro and Formosa (30% of the total records)" (Ferrando & Claps 2016; p.2). The rotifer species which are more commonly found in Argentinian Paraná River reaches and La Plata River tributaries were *Keratella cochlearis* (Gosse, 1851), *K. americana* Carlin, 1943 and *Brachionus calyciflorus* Pallas, 1776 (Modenutti 1998, Bonetto & Wais 2006).

Knowledge of the diversity and distribution of rotifers in the Paraguay River basin is scarce and concentrated in Brazil and Argentina, including rivers in the Pantanal (Branco et al. 2018, Brito et al. 2020) and those joining the Paraná River (Frutos et al. 2006). Similarly, few rotifer surveys have been conducted in the Uruguay River basin (e.g., José de Paggi 1978; Picapedra et al. 2019).

Therefore, we provide for the first time a spatially extensive survey of Rotifera species found in the lentic and lotic stretches of the La Plata River basin to characterize its species diversity patterns. In addition, we have expanded the distribution of some Rotifera species not yet reported in the literature, thus contributing to the general knowledge of the diversity of the group in the region.

## Materials and Methods

### 1. *Study area*

The La Plata River basin has an area of 3.1 million km$^2$ (Cuya et al. 2013) and drains portions of five countries: Brazil, Paraguay, Uruguay, Argentina, and Bolivia. The main sub-basins are the Paraná, Paraguay, and Uruguay River basins. The Paraná basin is the largest, covering 48.7% of the basin, followed by the Paraguay (35.3%) and Uruguay (11.8%) basins (Cuya et al. 2013).

### 2. *Sampling*

A total of 86 samples were collected at 43 stations, including 15 reservoirs (in dam and upriver zones) and 13 lotic stretches distributed in the three main sub-basins of La Plata River (Figure 1, Table 1). Sites (open water - littoral habitats were not included) were sampled in January (summer - wet season) and July (winter- dry season) 2010. Ten water quality variables were measured at each sampling station during each visit following Perbiche-Neves et al. (2016) and Pessotto & Nogueira (2018): total phosphorus and nitrogen, temperature, transparency, turbidity, conductivity, pH, dissolved oxygen, depth, and total chlorophyll.

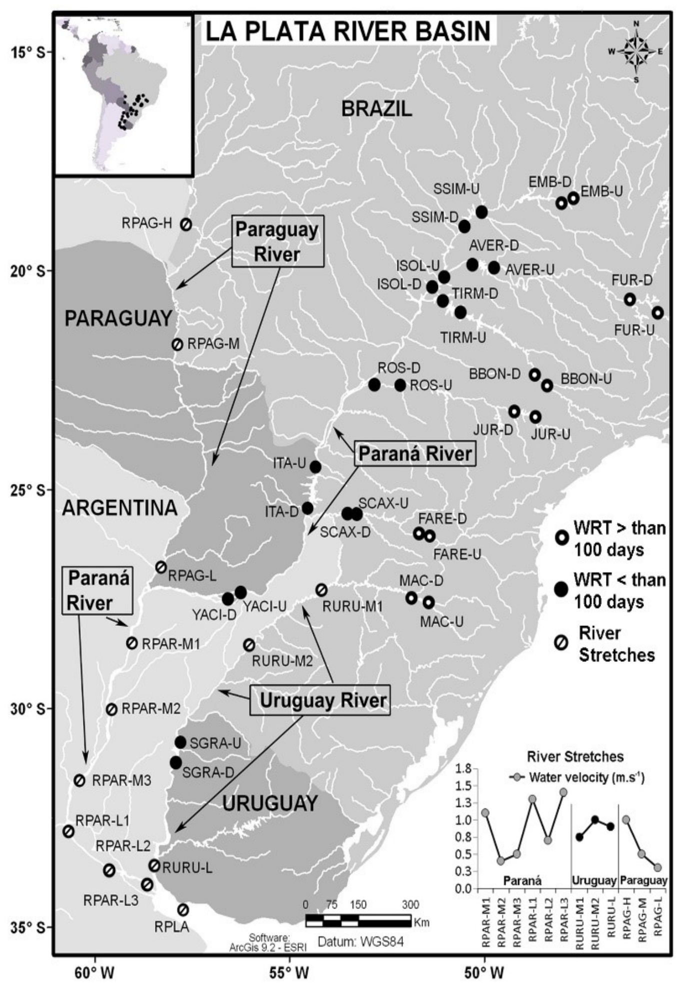


**Figure 1.** Locations of the 43 sites in La Plata River basin, with data of water retention time (WRT) and water velocity of the river stretches. For codes see Table 1. Adapted from Perbiche-Neves et al. (2016).

**Table 1.** Acronyms of the sites, sub-basin, geographical coordinates and habitat type sampled in the La Plata River basin. Number (n°) represents the sampling stations in the basin. Codes: ARG – Argentina, BRA – Brazil, PAR – Paraguay, URU – Uruguay.

| Site | Sub-basin | Coordinates | Acronyms | N° | Habitat |
|---|---|---|---|---|---|
| Emborcação HPP – MG/GO – BRA | Paranaíba | 18º26'28.43"S<br>47º58'59.59"W | EMB-U<br>EMB-D | 1<br>2 | Lentic<br>Lentic |
| São Simão HPP – MG/GO – BRA | Paranaíba | 19º00'04.51"S<br>50º29'47.69"W | SSIM-U<br>SSIM-D | 3<br>4 | Lentic<br>Lentic |
| Furnas HPP – MG – BRA | Grande | 20º39'38.30"S<br>46º18'01.65"W | FUR-U<br>FUR-D | 5<br>6 | Lentic<br>Lentic |
| Água Vermelha HPP – MG/SP – BRA | Grande | 19º51'58.67"S<br>50º19'11.62"W | AVER-U<br>AVER-D | 7<br>8 | Lentic<br>Lentic |
| Ilha Solteira HPP – SP/MS – BRA | Upper Paraná | 20º21'43.24"S<br>51º21'14.53"W | ISOL-U<br>ISOL-D | 9<br>10 | Lentic<br>Lentic |
| Barra Bonita HPP – SP – BRA | Tietê | 22º31'23.48"S<br>48º31'56.30"W | BBON-U<br>BBON-D | 11<br>12 | Lentic<br>Lentic |
| Três Irmãos HPP – SP – BRA | Tietê | 20º39'32.50"S<br>51º16'56.16"W | TIRM-U<br>TIRM-D | 13<br>14 | Lentic<br>Lentic |
| Jurumirim HPP – SP - BRA | Paranapanema | 23º13'02.15"S<br>49º13'26.89"W | JUR-U<br>JUR-D | 15<br>16 | Lentic<br>Lentic |
| Rosana HPP – SP/PR - BRA | Paranapanema | 22º36'02.03"S<br>52º51'07.39"W | ROS-U<br>ROS-D | 17<br>18 | Lentic<br>Lentic |
| Itaipu HPP – BRA/PAR | Upper Paraná | 25º24'21.09"S<br>54º34'02.38"W | ITA-U<br>ITA-D | 19<br>20 | Lentic<br>Lentic |
| Foz do Areia HPP – PR – BRA | Iguaçu | 26º00'23.84"S<br>51º39'45.76"W | FARE-U<br>FARE-D | 21<br>22 | Lentic<br>Lentic |
| Salto Caxias HPP – PR - BRA | Iguaçu | 25º32"25.00"S<br>53º29'30.72"W | SCAX-U<br>SCAX-D | 23<br>24 | Lentic<br>Lentic |
| Yaciretá HPP – Ituzaingó - ARG | Middle Paraná | 27º25'28.83"S<br>56º37'37.50"W | YACI-U<br>YACI-D | 25<br>26 | Lentic<br>Lentic |
| Paraná River – Bella Vista - ARG | Middle Paraná | 28º30'04.81"S<br>59º02'58.21"W | RPAR- M1<br>RPAR-M2<br>RPAR-M3 | 27<br>28<br>29 | Lotic<br>Lotic<br>Lotic |
| La Plata River – Rosário - ARG | Lower Paraná | 32°53'08.12"S<br>60°40'48.69"W | RPAR-L1<br>RPAR-L2<br>RPAR-L3 | 30<br>31<br>32 | Lotic<br>Lotic<br>Lotic |
| La Plata River –- URU/ARG | Lower Paraná | 34°00'51.25"S<br>58°19'21.84"W | RPLA | 33 | Lotic |
| Machadinho HPP – SC - BRA | Upper Uruguay | 27°31'12.35"S<br>51°47'05.01"W | MAC-U<br>MAC-D | 34<br>35 | Lentic<br>Lentic |
| Porto Xavier – RS - BRA | Middle Uruguay | 27°'52'17.26"S<br>55°07'25.49"W | RURU-M1<br>RURU-M2 | 36<br>37 | Lotic<br>Lotic |
| Salto Grande HPP – URU | Middle Uruguay | 31°15'44.17"S<br>57°55'47.34"W | SGRA-U<br>SGRA-D | 38<br>39 | Lentic<br>Lentic |
| Uruguay River - Fray Bentos – URU | Lower Uruguay | 33°21'02.20"S<br>58°25'49.97"W | RURU-L | 40 | Lotic |
| Paraguay River– Corumbá - BRA | Upper Paraguay | 18°59'40.76"S<br>57°39'12.53"W | RPAG-H | 41 | Lotic |
| Paraguay River – Assunción - PAR | Middle Paraguay | 25°28'24.65"S<br>57°33'40.53"W | RPAG-M | 42 | Lotic |
| Paraguay River – Paso de la Patria – PAR | Lower Paraguay | 27°15'38.43"S<br>58°35'39.79"W | RPAG-L | 43 | Lotic |

We sampled rotifers through vertical hauls by using a 50 µm mesh conical plankton net. In deep sites, the maximum depth hauled was 40 m (Perbiche-Neves et al. 2019). The sampled rotifers were subsequently packed, labeled, and fixed with 4% formalin solution. Identifications were conducted with an optical microscope (Zeiss Axio Imager.A2m) and by using species keys (Edmondson 1959, Koste 1978, Nogrady et al. 1995, Segers & Dumont 1995, Smet & Pourriot 1997, Nogrady 2002, Wallace et al. 2019). Voucher specimens were deposited in the Laboratory of Continental Waters Ecology, Institute of Biosciences of Botucatu at the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Brazil. The number of species was correlated with water quality variables by using non-parametric Spearman correlation and a logarithmic transformation in R Cran Project 3.3.0 (2016) using the Hmisc package of R.

## Results

The mean rotifer richness was 27±11 species. The sub-basins with higher richness were the Lower Paraná (41 species), followed by the Paranapanema (40 species) and Tietê (35 species). The basins with lower richness were the Middle Paraná and Lower Uruguay (Figure 2A).

The Rotifera fauna of the La Plata River basin was composed of 106 species, distributed in 21 families and 37 genera (Table 2, Figure 2B). The most representative family in the basin is the Lecanidae (21 species), followed by the Brachionidae (20), Trichocercidae (9), and Synchaetidae (8) (Figure 2B). The most speciose genera are *Lecane* Nitzsch, 1827 and *Brachionus* Pallas, 1766 with 21 and 10 species, respectively. We found 44 rotifer species in summer and 17 in winter. These seasonal periods share a combined 45 rotifer species (Figure 3).

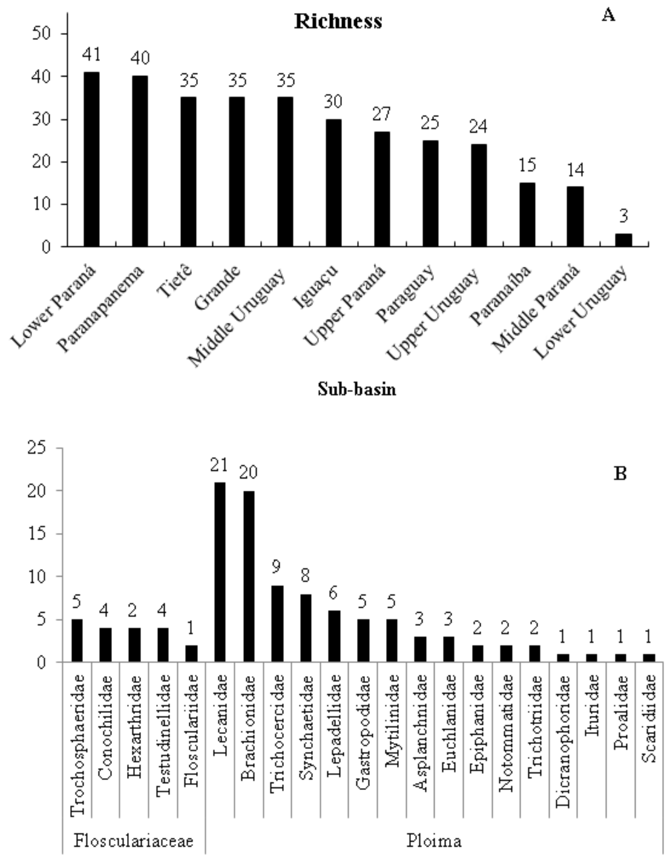


**Figure 2.** Rotifer richness per basin for species (A) and family (B).

**Table 2.** Rotifer species collected in lotic and lentic habitas in the La Plata River basin, South America.

| Order/Family | Species | Sites | Frequency (%) |
|---|---|---|---|
| Order Flosculariaceae | | | |
| Family Conochilidae | *Conochilus coenobasis* (Skorikov, 1914) | 4 | 2.32 |
| | *Conochilus dossuarius* Hudson, 1885 | 1, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 28, 29, 30, 31, 33, 34, 36, 43 | 65.11 |
| | *Conochilus natans* (Seligo, 1900) | 7, 8, 15, 34 | 9.3 |
| | *Conochilus unicornis* Rousselet, 1892 | 1, 2, 3, 4, 6, 7, 8, 12, 14, 15, 18, 28, 30, 32, 33, 34, 35, 39 | 41.86 |
| Family Flosculariidae | *Ptygura* sp. Ehrenberg, 1832 | 15 | 2.32 |
| Family Hexarthridae | *Hexarthra intermedia* (Wiszniewski, 1929) | 9, 16, 22 | 6.97 |
| | *Hexarthra mira* (Hudson, 1871) | 2, 5, 7, 8, 9, 10, 12, 15, 17, 21, 22, 23, 24, 27, 28, 30, 39, 43 | 41.86 |
| Family Testudinellidae | *Pompholyx triloba* Pejler, 1957 | 16, 32 | 4.65 |
| | *Testudinella mucronata* (Gosse, 1886) | 15, 31, 32, 39, 41, 43 | 13.95 |
| | *Testudinella ohlei* Koste, 1972 | 11 | 2.32 |
| | *Testudinella patina* (Hermann, 1783) | 12, 15, 18, 19, 28, 30, 32, 39, 40 | 20.93 |
| Family Trochosphaeridae | *Filinia limnetica* (Zacharias, 1893) | 21, 22 | 4.65 |
| | *Filinia longiseta* (Ehrenberg, 1834) | 7, 12, 21, 22, 30, 34 | 13.95 |
| | *Filinia opoliensis* (Zacharias, 1898) | 6, 7, 12, 16, 39, 43 | 13.95 |
| | *Filinia saltator* (Gosse, 1886) | 41 | 2.32 |
| | *Filinia terminalis* (Plate, 1886) | 5, 6, 7, 8, 11, 12, 13, 16, 21, 33, 34, 39 | 27.91 |
| Order Ploima | | | |

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|---|---|---|---|
| Family Asplanchnidae | *Asplanchna priodonta* Gosse, 1850 | 12 | 2.32 |
| | *Asplanchna sieboldii* (Leydig, 1854) | 7, 12, 18, 22, 24, 25, 30, 34, 35, 39, 43 | 25.58 |
| | *Harringia rousseleti* de Beauchamp, 1912 | 28 | 2.32 |
| Family Brachionidae | *Anuraeopsis fissa* Gosse, 1851 | 12, 21 | 4.65 |
| | *Anuraeopsis navicula* Rousselet, 1911 | 5, 15, 19 | 6.98 |
| | *Brachionus angularis* Gosse, 1851 | 30 | 2.32 |
| | *Brachionus budapestinensis* Daday, 1885 | 7 | 2.32 |
| | *Brachionus calyciflorus* Pallas, 1766 | 2, 5, 7, 8, 11, 12, 13, 15, 16, 18, 30, 31, 33, 34, 35, 39 | 37.21 |
| | *Brachionus caudatus* Barrois & Daday, 1894 | 29, 30, 31, 32, 37, 38, 39, 43 | 18.6 |
| | *Brachionus dolabratus* Harring, 1914 | 5, 6, 8, 13, 16, 25, 34, 35, 37, 39 | 23.25 |
| | *Brachionus falcatus* Zacharias, 1898 | 3, 5, 6, 7, 11, 12, 16, 23, 34, 35, 38, 39, 42, 43 | 32.56 |
| | *Brachionus mirus* Daday, 1905 | 10, 11, 12, 14, 21, 30, 31, 34, 39, 42, 43 | 25.58 |
| | *Brachionus quadridentatus* Hermann, 1783 | 32, 43 | 4.65 |
| | *Brachionus urceolaris* Müller, 1773 | 11, 39 | 2.32 |
| | *Brachionus zahniseri* Ahlstrom 1934 | 41 | 2.32 |
| | *Kellicottia bostoniensis* (Rousselet, 1908) | 4, 5, 6, 11, 12, 15, 16, 21, 22, 23, 24, 25, 29, 32, 33, 34, 35, 36, 38 | 44.19 |
| | *Keratella americana* Carlin, 1943 | 1, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 21, 22, 24, 35, 36, 38, 39, 43 | 44.19 |
| | *Keratella cochlearis* (Gosse, 1851) | 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 18, 21, 22, 26, 34, 35, 38, 39, 41, 42 | 51.16 |
| | *Keratella lenzi* Hauer, 1053 | 5, 8, 10, 11, 12, 13, 15, 34 | 18.61 |
| | *Keratella tropica* (Apstein, 1907) | 3, 4, 5, 6, 11, 12, 13, 14, 15, 16, 17, 21, 22, 35, 37, 38 | 37.21 |
| | *Plationus patulus* (Müller, 1786) | 11, 12, 13, 17, 21, 25, 26, 30, 32, 38, 39, 40, 42, 43 | 32.56 |
| | *Platyias leloupi* (Gillard, 1957) | 12, 17, 19, 27, 29, 30, 31, 32, 42, 43 | 23.25 |
| | *Platyias quadricornis* (Ehrenberg, 1832) | 17, 28, 29, 30, 43 | 11.63 |
| Family Dicranophoridae | *Dicranophoroides caudatus* (Ehrenberg, 1834) | 10 | 2.32 |
| Family Epiphanidae | *Epiphanes clavulata* (Ehrenberg, 1832) | 1, 11, 12, 15, 30, 43 | 13.95 |
| | *Epiphanes macroura* (Barrois & Daday, 1894) | 35 | 2.32 |
| Family Euchlanidae | *Beauchampiella eudactylota* (Gosse, 1886) | 30 | 2.32 |
| | *Dipleuchlanis propatula* (Gosse, 1886) | 41 | 2.32 |
| | *Euchlanis dilatata* Ehrenberg, 1832 | 11, 12, 19, 25, 27, 32, 37, 41 | 16.28 |
| Family Gastropodidae | *Ascomorpha agilis* Zacharias, 1893 | 39 | 2.32 |
| | *Ascomorpha ovalis* (Bergendal, 1892) | 34 | 2.32 |
| | *Ascomorpha saltans* Bartsch, 1870 | 5, 9, 11, 15, 17 | 11.63 |
| | *Gastropus hyptopus* (Ehrenberg, 1838) | 30, 39 | 4.65 |
| | *Gastropus stylifer* (Imhof, 1891) | 17 | 2.32 |
| Family Ituridae | *Itura aurita* (Ehrenberg, 1830) | 19 | 2.32 |

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|---|---|---|---|
| Family Lecanidae | *Lecane amazonica* (Murray, 1913) | 32 | 2.32 |
| | *Lecane bulla* (Gosse, 1851) | 12, 19, 21, 15, 30, 43 | 13.95 |
| | *Lecane cornuta* (Müller, 1786) | 43 | 2.32 |
| | *Lecane curvicornis* (Murray, 1913) | 11, 12, 17, 18, 19, 30, 32, 33, 39, 41, 42, 43 | 27.91 |
| | *Lecane elsa* Hauer, 1931 | 40, 41 | 4.65 |
| | *Lecane haliclysta* Harring & Myers, 1926 | 21, 41 | 2.32 |
| | *Lecane hornemanni* (Ehrenberg, 1834) | 7, 8, 15, 25, 34 | 11.63 |
| | *Lecane leontina* (Turner, 1892) | 30, 39 | 4.65 |
| | *Lecane ludwigii* (Eckstein, 1883) | 33 | 2.32 |
| | *Lecane luna* (Müller, 1776) | 7, 19, 29, 21, 24, 32, 35 | 16.28 |
| | *Lecane lunaris* (Ehrenberg, 1832) | 20, 30, 39 | 6.98 |
| | *Lecane obtusa* (Murray, 1913) | 2, 6, 15 | 4.65 |
| | *Lecane papuana* (Murray, 1913) | 7, 39, 42 | 6.98 |
| | *Lecane proiecta* Hauer, 1956 | 5, 7, 8, 12, 19, 39 | 13.95 |
| | *Lecane quadridentata* (Ehrenberg, 1830) | 19, 30 | 2.32 |
| | *Lecane rhytida* Harring & Myers, 1926 | 17 | 2.32 |
| | *Lecane robertsonae* Segers, 1993 | 6 | 2.32 |
| | *Lecane stenroosi* (Meissner, 1908) | 8 | 2.32 |
| | *Lecane subtilis* Harring & Myers, 1926 | 18 | 2.32 |
| | *Lecane thienemanni* (Hauer, 1938) | 19, 21, 28 | 4.65 |
| | *Lecane ungulata* (Gosse, 1887) | 5, 42 | 2.32 |
| Family Lepadellidae | *Colurella adriatica* Ehrenberg, 1831 | 21 | 2.32 |
| | *Colurella obtusa* (Gosse, 1886) | 21, 30 | 2.32 |
| | *Lepadella cristata* (Rousselet, 1893) | 38 | 2.32 |
| | *Lepadella donneri* Koste, 1972 | 18 | 2.32 |
| | *Lepadella ovalis* (Müller, 1786) | 42 | 2.32 |
| | *Lepadella patella* (Müller, 1786) | 41 | 2.32 |
| Family Mytilinidae | *Lophocharis oxysternon* (Gosse, 1851) | 5 | 2.32 |
| | *Mytilina acanthophora* Hauer, 1938 | 7 | 2.32 |
| | *Mytilina bisulcata* (Lucks, 1912) | 27 | 2.32 |
| | *Mytilina mucronata* (Müller, 1773) | 5, 7, 14, 19, 29 | 11.63 |
| | *Mytilina ventralis* (Ehrenberg, 1830) | 7, 8, 9, 12, 15, 16, 22, 27, 32, 35, 39 | 25.58 |
| Family Notommatidae | *Enteroplea lacustris* Ehrenberg, 1830 | 13 | 2.32 |
| | *Monommata maculata* Harring & Myers, 1930 | 30 | 2.32 |
| Family Proalidae | *Proalinopsis staurus* Harring & Myers, 1924 | 7 | 2.32 |
| Family Scaridiidae | *Scaridium longicaudum* (Müller, 1786) | 30 | 2.32 |
| Family Synchaetidae | *Ploesoma lenticulare* Herrick, 1885 | 21, 22, 24, 30 | 9.3 |
| | *Ploesoma truncatum* (Levander, 1894) | 5, 9, 15, 20, 21, 22, 23, 24, 34, 39 | 23.25 |
| | *Polyarthra dolichoptera* Idelson, 1925 | 5, 7, 8, 10, 11, 12, 13, 15, 16, 18, 21, 22 | 27.91 |
| | *Polyarthra remata* Skorikov, 1896 | 2, 9, 15, 21, 22, 25, 29, 35 | 18.6 |
| | *Polyarthra vulgaris* Carlin, 1943 | 10 | 2.32 |
| | *Synchaeta oblonga* Ehrenberg, 1832 | 7, 9, 17, 18, 39 | 11.63 |
| | *Synchaeta pectinata* Ehrenberg, 1832 | 5, 6, 7, 12, 15, 18, 30 | 16.28 |
| | *Synchaeta stylata* Wierzejski, 1893 | 7, 26, 30, 34 | 9.3 |

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|---|---|---|---|
| Family Trichocercidae | *Trichocerca bicristata* (Gosse, 1887) | 19, 42 | 2.32 |
| | *Trichocerca capucina* (Wierzejski & Zacharias, 1893) | 1, 2, 6, 21, 8, 12, 15, 37, 39 | 20.93 |
| | *Trichocerca chattoni* (de Beauchamp, 1907) | 9, 21, 22, 23, 24, 34, 39 | 16.27 |
| | *Trichocerca cylindrica* (Imhof, 1891) | 5, 8, 12, 15 | 9.3 |
| | *Trichocerca elongata* (Gosse, 1886) | 30, 33 | 4.65 |
| | *Trichocerca gracilis* (Tessin, 1890) | 6, 12, 25 | 4.65 |
| | *Trichocerca heterodactyla* (Tschugunoff, 1921) | 39 | 2.32 |
| | *Trichocerca longiseta* (Schrank, 1802) | 22 | 2.32 |
| | *Trichocerca rattus* (Müller, 1776) | 27 | 2.32 |
| Family Trichotriidae | *Trichotria tetractis* (Ehrenberg, 1830) | 20, 42 | 4.65 |
| | *Macrochaetus collinsii* (Gosse, 1867) | 37, 42 | 4.65 |

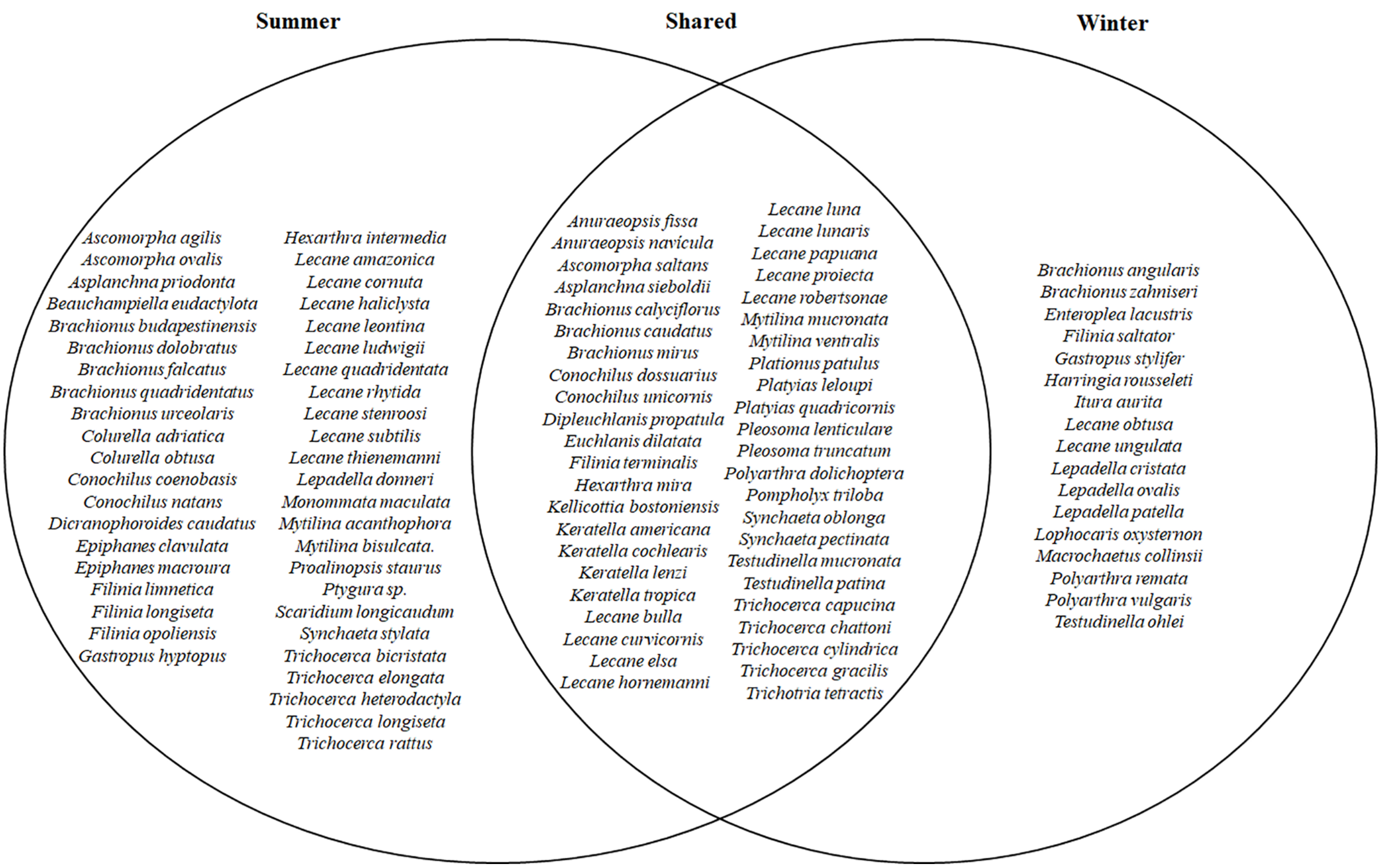


**Figure 3.** Species collected in the summer, winter and shared in both seasons.

Regarding individual sites, we found a wide range in species richness. Barra Bonita Reservoir (BBON-D; 12) in the Tietê River had the greatest species richness (22). The lowest richness was observed in the Lower (RURU-L; 40) and Middle (RURU-M1; 36) Uruguay River (3 species each; Table 2). The species occurring in >40% of the lotic and lentic sites evaluated were *Conochilus dossuarius* Hudson, 1885, *Kellicottia bostoniensis* (Rousselet, 1908), *Keratella americana* Carlin, 1943 *K. cochlearis* (Gosse, 1851) and *Hexarthra mira* (Hudson, 1871).

Our results indicate greater distribution ranges for several species. *Colurella adriatica* Ehrenberg, 1831, from the Foz do Areia Reservoir, is a new record for Brazil. *Gastropus hyptopus* (Ehrenberg, 1838) in the La Plata River and *Harringia rousseleti* de Beauchamp, 1912 and *Lecane thienemanni* (Hauer, 1938) in the Paraná River, are their first reports in Argentina. Finally, we expand the range of *Enteroplea lacustris* Ehrenberg, 1830, in São Paulo State (Brazil) and *Lecane ludwigii* (Eckstein, 1883) in Buenos Aires Province (Argentina).

Almost all the species are native, except *Kellicottia bostoniensis* which occurred in a new locality. Seven other species are Neotropical endemics (Table 2): *Brachionus dolabratus* Harring, 1914*, B. mirus* Daday, 1905*, B. zahniseri* Ahlstrom 1934*, K. americana, Lecane amazonica* (Murray, 1913), *L. proiecta* Hauer, 1956 and *Testudinella ohlei* Koste, 1972.

The mean ± standard deviations of water quality variables (Table 3) stratified by sub-basin reveals that the Tietê River has higher levels of total nitrogen, phosphorus, chlorophyll, and electrical conductivity.

The Lower Paraná River also demonstrates high values for these variables except for nitrogen. Higher temperatures were found in the Paraguay and Iguaçu Rivers. The lowest levels of dissolved oxygen occurred in the Paraguay River. Spearman correlations indicated that total chlorophyll and water temperature were positively correlated with species richness; dissolved oxygen demonstrated a negative correlation (Table 4).

## Discussion

We found a total of 106 rotifer species in the La Plata basin. Our data represent 14% of the rotifer species richness known to Brazil (Garraffoni & Lourenço 2012), 37% of that for São Paulo State (Souza-Soares et al. 2011), 30% of that for the Upper Paraná (Lansac-Tôha et al. 2009), and 40% for the Upper Paraguay River (Branco et al. 2018). Data from other inventories show that the rotifer fauna in the La Plata River basin is richer than what was demonstrated in our study, possibly because we sampled in few Uruguay and Paraguay River stretches, and exclusively in open water habitats, not in littoral. Therefore, as recommended by Ferrando & Claps (2016), further investigations should be carried out to expand the distribution list of species in the La Plata River Basin.

The most diverse families were Lecanidae (21 spp.) and Brachionidae (20 spp.). These two families compose most rotifer species throughout Brazil and Argentina (Garraffoni & Lourenço, 2012; Ferrando & Claps, 2016), supporting our findings.

The higher summer (wet season) rotifer richness may be associated with the concentrated rainfall events that occur during this season.

**Table 3.** Means ± standard-deviations of water quality variables by sub-basin.

| Sub-basin | Total Nitrogen (μg.L$^{-1}$) | Total Phosphorus (μg.L$^{-1}$) | Chlorophyll (μg.L$^{-1}$) | Depth Max. (m) | Transparency (m) |
|---|---|---|---|---|---|
| Paranaíba | 196.1±43.38 | 9.60±3.08 | 1.21±0.67 | 61.61±16.29 | 4.13±1.64 |
| Grande | 311.78±53.54 | 8.53±1.16 | 2.45±1.60 | 44.32±25.01 | 3.31±1.42 |
| Tietê | 2131.05±1373.24 | 58.19±45.73 | 7.19±6.54 | 25.93±6.85 | 2.70±1.45 |
| Paranapanema | 463.92±115.72 | 16.94±5.21 | 1.51±0.72 | 21.66±6.89 | 1.41±0.64 |
| Iguaçu | 325.38±37.98 | 15.40±4.67 | 1.26±0.63 | 57.75±24.13 | 1.45±0.34 |
| Upper Paraná | 622.30±126.81 | 15.40±4.19 | 1.52±0.80 | 41.14±23.40 | 2.75±1.50 |
| Middle Paraná | 468.12±47.37 | 34.33±6.48 | 3.17±1.30 | 9.85±3.40 | 0.42±0.08 |
| Lower Paraná | 415.29±79.69 | 54.60±13.93 | 3.74±1.63 | 11.8±5.80 | 0.56±0.09 |
| Upper Uruguay | 452.04±93.24 | 14.54±3.22 | 1.74±0.30 | 95.75±6.88 | 1.86±0.22 |
| Middle Uruguay | 780.90±70.22 | 22.07±5.80 | 3.12±1.96 | 22.67±15.43 | 0.66±0.11 |
| Lower Uruguay | 650.35±108.47 | 36.10±9.01 | 2.97±1.36 | 16.93±5.40 | 0.67±0.15 |
| Paraguay | 426.44±225.85 | 43.00±18.71 | 2.41±0.87 | 10.1±4.37 | 0.81±0.32 |
| **Sub-basin** | **Temperature (°C)** | **pH** | **Conductivity (μS.cm$^{-1}$)** | **DO (mg.L$^{-1}$)** | **Turbidity (NTU)** |
| Paranaíba | 24.35±1.81 | 7.21±0.19 | 41.82±4.32 | 6.26±0.97 | 8.64±3.38 |
| Grande | 24.10±2.8 | 7.20±0.33 | 38.45±7.07 | 7.16±1.16 | 11.58±6.70 |
| Tietê | 24.11±3.10 | 7.27±0.30 | 182.42±43.98 | 6.92±1.54 | 12.1±6.46 |
| Paranapanema | 23.01±3.16 | 7.25±0.27 | 55.10±6.56 | 7.91±0.96 | 24.2±11.41 |
| Iguaçu | 25.14±3.37 | 7.34±0.14 | 51.26±4.74 | 7.57±0.79 | 20.52±7.70 |
| Upper Paraná | 21.73±3.69 | 7.19±0.32 | 46.31±6.44 | 7.80±1.12 | 12.75±4.91 |
| Middle Paraná | 23.21±6.19 | 7.36±0.22 | 64.64±4.96 | 7.98±1.36 | 40.49±7.56 |
| Lower Paraná | 22.10±7.40 | 7.47±0.31 | 118.16±24.69 | 7.85±2.21 | 37.80±6.93 |
| Upper Uruguay | 17.71±1.77 | 6.86±0.13 | 32.26±2.13 | 8.68±0.46 | 14.51±2.12 |
| Middle Uruguay | 21.84±4.49 | 7.42±0.14 | 45.75±2.75 | 8.63±0.91 | 32.39±7.41 |
| Lower Uruguay | 22.22±6.75 | 7.44±0.21 | 59.24±20.58 | 8.69±1.22 | 31.30±5.68 |
| Paraguay | 26.40±3.87 | 6.75±0.24 | 67.84±15.33 | 5.17±1.28 | 27.40±13.22 |

**Table 4.** Spearman correlations between species richness and water quality variables. Bold = significant correlations.

| Variables | R2 | p | Variables | R2 | p |
|---|---|---|---|---|---|
| Total Nitrogen | 0.12 | 0.27 | Temperature | 0.27 | **0.01** |
| Total Phosphorus | 0.11 | 0.29 | pH | -0.15 | 0.15 |
| Chlorophyll | 0.28 | **0.00** | Conductivity | 0.08 | 0.48 |
| Depth | 0.13 | 0.23 | D.O. | -0.24 | **0.02** |
| Transparency | 0.02 | 0.87 | Turbidity | -0.07 | 0.53 |

Summer rains can carry nutrients and organic matter from the margins of aquatic environments resulting in increased food concentration and a reduction in competition for resources. The same tendency was observed for the rainy season in a study performed on a tropical lake in Mexico (Jiménez-Contreras et al. 2018). Richness may also be related to the sediment mixture caused by intense rains. This process provides a favorable condition for hatching of dormant stages (i.e., resting eggs), resulting in an increase in rotifer species richness.

Greater rotifer species richness was observed in the Lower Paraná sub-basin. Rotifers have low locomotion capacity and are carried by drifting through the central channel of the river and consequently the species richness increase towards downstream.

Barra Bonita Reservoir in the Tietê River sub-basin was the site with the greatest richness. Despite being a reservoir with a high degree of anthropogenic disturbance, including eutrophication (Tundisi et al. 2008), many studies have shown high biodiversity for other groups, which include rotifers (Matsumura-Tundisi & Tundisi 2005, Rocha et al. 2006). In the Barra Bonita Reservoir, Matsumura-Tundisi & Tundisi (2005) found 32 species of rotifers. However, in our work we found 22 species. The Spearman correlation suggested a positive relation between richness and chlorophyll levels, with Barra Bonita Reservoir demonstrating the highest values of observed chlorophyll. Presumably, this higher richness is a result of greater numbers of tolerant rotifer species (Allen et al. 1999).

The commonest species in the La Plata basin were *Keratella americana, K. cochlearis*, and *Hexarthra mira*. Others have reported the occurrence these species in the Uruguay (Di Persia & Neiff 1986), Paraguay (Frutos et al. 2006, Branco et al. 2018) and Upper Paraná Rivers (Bonetto & Wais 2006), indicating the wide distribution of these rotifers in the study area.

*Colurella* has been found in several inland waters (Arroyo-Castro et al. 2019, Tasevska et al. 2019, Wei et al. 2019). In the La Plata Basin we found two species of this genus: *Colurella adriatica* Ehrenberg, 1831 and *C. obtusa* (Gosse, 1886). *Colurella adriatica* originates in the Adriatic Sea and has been described as endemic (Ehrenberg 1831), but it is now widely distributed, including in Neotropical regions (Segers 2007). We found it in Foz do Areia Reservoir, in the Iguaçu sub-basin, Paraná State, which is its first record in Brazil.

*Enteroplea lacustris* is widely distributed in the Australasia, Neoarctic, Neotropical, Oriental, and Paleoarctic regions (Segers 2007). In Brazil, it occurs in Mato Grosso do Sul (Roche & Silva 2017) and Paraná States in the Paranapanema River basin (Dias et al. 2011, Roche & Silva 2017). We found it in Três Irmãos Reservoir, Tietê sub-basin, São Paulo State, near the Paraná River, indicating a gap in previous studies of this region.

For Argentina, Ferrando & Claps (2016) recorded 351 species of monogonont rotifers from lotic and lentic environments. Among the species they recorded, we found 43 (12.2%). Three other species of rotifers (*Gastropus hyptopus*, *Harringia rousseleti*, and *Lecane thienemanni*) found in our study are new records for Argentina. *Gastropus hyptopus* was found in the La Plata River Basin, in Rosario, Argentina. In Brazil, it had been registered in several regions (Serafim Jr. et al. 2003, Bonecker et al. 2005, Serafim-Júnior et al. 2010, Souza-Soares et al. 2011). *Harringia rousseleti* and *L. thienemanni* were recorded for the first time in Argentine reaches of the Paraná River, in the Bella Vista municipality. A new locality was found for *L. ludwigii*, which had been recorded in Corrientes Province (José de Paggi 1996); however, there is no previous record in the La Plata River estuary where we collected it.

We found a non-native species in the La Plata River basin, *Kellicottia bostoniensis* (Rousselet, 1908), which is native to North America (Edmondson 1959). For Argentina, José de Paggi (2002) first recorded the species in the Iguaçu River and Salto Grande Reservoir. We found the species in the La Plata River (Uruguay and Argentina reach), where there were no prior records of it. We thus extended the known distribution of *K. bostoniensis*. It is possible that its occurrence in the La Plata basin is related to aquaculture activities as has occurred in other regions (Coelho & Henry 2017). In many reservoirs of the La Plata basin, there are aquaculture activities, mainly with non-native fish species (Azevedo-Santos et al. 2011, Nobile et al. 2018). This rotifer may be introduced from cage aquaculture in upstream rivers (e.g., Grande and Paranapanema Rivers) and reached downstream areas where we captured it.

Seven Neotropical endemic species (sensu José de Paggi 1996) were found in the La Plata River basin. Their presence highlights the importance of preserving the condition of these ecosystems. However, anthropogenic stressors imperil many areas where these seven species occur. For example, in the Barra Bonita and Três Irmãos Reservoirs, where *Brachionus dolobratus, B. mirus,* and *L. proiecta* were captured, waters are polluted (Rodgher et al. 2005, Favaro et al. 2018). Similarly, eutrophic tributaries in the Grande River sub-basin (Melo et al. 2017) may affect endemic rotifer species. Another example is the occurrence of *L. amazonica* in the La Plata River; which also receives water from these polluted river basins. Conservation policies must be discussed for the entire La Plata system because of fluvial connectivity (Azevedo-Santos et al. 2019).

In conclusion, surveys covering wide spatial extents, such as in our study, are important for increasing the knowledge of species diversity and distribution. Our findings may contribute to future monitoring studies as well as management and conservation programs for the La Plata River basin. Finally, we recommend that future rotifer surveys should be concentrated in Paraguay and Uruguay River reaches because of the scarcity of data from them.

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## Author Contributions

Bárbara A. Martins: Contribution to data analysis, interpretation, Contribution to manuscript preparation.

Paula N. Coelho: Contribution to manuscript preparation, Contribution to critical revision, adding intellectual content.

Marcos G. Nogueira: Substantial contribution in the concept and design of the study, Contribution to data collection, Contribution to critical revision, adding intellectual content.

Gilmar Perbiche-Neves: Substantial contribution in the concept and design of the study, Contribution to data collection, Contribution to critical revision, adding intellectual content.

## Conflicts of Interest

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

## Ethics

The data collection was complied with the guidelines established by the ethics committees of Universidade Estadual de São Paulo.

## Data availability

The species were deposited in the Laboratory of Ecology and Continental Waters, Institute of Biosciences of Botucatu at the Universidade Estadual Paulista Júlio de Mesquita Filho (Unesp), Brazil. Lot 009, samplings 1 to 44.

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# Inventory

## An inventory of Ichthyofauna of the Pindaré River drainage, Mearim River basin, Northeastern Brazil

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***Abstract:*** In the present work, we conducted an extensive long-lasting inventory of the fishes, using different collection methodologies, covering almost the entire Pindaré River drainage, one of the principal tributaries of the Mearim River basin, an area included in the Amazônia Legal region, northeastern Brazil. We reported 101 species, just three of them being non-native, demonstrating that the composition of this studied fish community is majority composed of native species. We found a predominance of species of the orders Characiformes and Siluriformes, corroborating the pattern usually found for the Neotropical fish fauna. Similar to other studies, this inventory was mainly dominated by small characids, representing 21% of the species herein recorded. When comparing the present survey with other species lists published for this region (including the States of Maranhão and Piaui), we can conclude that the freshwater fish fauna of the State of Maranhão is probably still underestimated. We reported 41 more species, and one more species than Soares (2005, 2013) and Abreu et al. (2019) recorded for the entire Mearim River basin, respectively. We believe, however, that the number of species presented by Abreu et al. (2019) is overestimated. We compared our results with all other freshwater fish species inventories performed for the hydrological units Maranhão and Parnaíba *sensu* Hubbert & Renno (2006). With these comparisons, we concluded that our results evidenced that a high effort was put in the inventory here presented. The two works including more species recorded from coastal river basins of the hydrological units Maranhão and Parnaíba were the works published by Ramos et al. (2014) for the Parnaíba River basin, one of the main and larger river basin of Brazil, and the compiled data published by Castro & Dourado (2011) for the Mearim, Pindaré, Pericumã, and upper Turiaçu River drainages, including 146 and 109 species, respectively. Our survey recorded only 45 less species than Ramos et al. (2014), and eight less species than Castro & Dourado (2011). However, it is essential to emphasize that the number of species presented by Castro & Dourado (2011) is probably overestimated since they did not update and check the taxonomic status of the species of their compiled data. In several cases, they considered more than one name for the same species.
***Keywords:*** *Amazônia Legal; Endemic species; Ichthyology; Maranhão; Neotropical Region; Species list.*

## Inventário da ictiofauna da drenagem do rio Pindaré, bacia do rio Mearim, nordeste do Brasil

***Resumo:*** No presente trabalho nós conduzimos um inventário de peixes extensivo e de longa duração, utilizando diferentes métodos de coletas, e cobrindo a vasta maioria da drenagem do Rio Pindaré, um dos principais afluentes da bacia do Rio Mearim, uma área incluída na região da Amazônia Legal, nordeste do Brasil. Nós registramos 101 espécies, apenas três delas sendo exóticas, demonstrando que a composição dessa comunidade de peixes estudada é majoritariamente composta por espécies nativas. Nós encontramos uma predominância de espécies das ordens Characiformes e Siluriformes, corroborando com o padrão geralmente encontrado na fauna de peixes Neotropicais. De maneira similar a outros estudos, o presente inventário foi principalmente dominado por espécies de pequenos caracídeos, representando 21% das espécies aqui registradas. Quando comparamos o presente inventário com outros inventários realizados para a região (incluindo os Estados do Maranhão e Piauí), nós podemos concluir que a fauna de peixes de água doce do estado está provavelmente subestimada. Nós registramos 41 mais espécies, e uma espécie a mais do que Soares (2005, 2013) e Abreu et al. (2019) registraram para a bacia inteira do Rio Mearim, respectivamente.

Entretanto, nós acreditamos que o número de espécies apresentados por Abreu et al. (2019) está superestimado. Nós comparamos nossos resultados com todos os outros inventários de peixes de água doce realizados nas unidades hidrológicas Maranhão e Parnaíba *sensu* Hubbert & Renno (2006). Com essas comparações pudemos concluir que nosso resultado evidencia o grande esforço colocado no inventário aqui apresentado. Os dois trabalhos incluindo mais espécies registradas para bacias costeiras nas unidades hidrológicas Maranhão e Parnaíba foram os trabalhos publicados por Ramos et al. (2014) para a bacia do Rio Parnaíba, uma das principais e maiores bacias hidrográficas do Brasil, e a compilação de dados publicada por Castro & Dourado (2011) para as drenagens dos Rios Mearim, Pindaré, Pericumã e alto Turiaçu, incluindo 146 e 109 espécies, respectivamente. Nosso inventário registrou 45 espécies a menos do que o trabalho de Ramos et al. (2014), e oito espécies a menos do que Castro & Dourado (2011). Entretanto, é importante enfatizar que o número de espécies apresentadas por Castro & Dourado (2011) está provavelmente superestimado, pois eles não atualizaram nem checaram o status taxonômico das espécies de seus dados compilados, e em vários casos eles consideraram mais de um nome para a mesma espécie.

***Palavras-chave:*** *Amazônia Legal; Espécies endêmicas; Ictiologia; Lista de espécies; Maranhão; Região Neotropical.*

## Introduction

The South America is the continent with the richest ichthyofauna of the world, with currently estimates of more than 9,100 species, about 27% of all the fishes around the world (including freshwater fishes and nearshore marine waters) (Reis et al. 2016). The freshwater ichthyofauna is richer than the marine ichthyofauna in South America: about 5,160 are freshwater fishes, representing about 1/3 of all the freshwater fish species of the world, occurring in about 12% of the total continental surface area of the planet. New estimates, however, suggest that the diversity of freshwater ichthyofauna in the Neotropical region is still underestimated, and may be much greater, on the order of eight to nine thousand species (Albert & Reis 2011, Reis et al. 2016), a similar estimate already proposed by Schaefer (1998).

The Amazonia biome, which occurs in the Neotropical region, extends across all countries in northern South America (Martins & Oliveira 2011, Van Der Sleen & Albert 2018, Val 2019), comprising an area of more than eight million km$^2$ (Van Der Sleen & Albert 2018), with more than five million km$^2$ belonging to Brazil (Val 2019). The biome is covered with dense tropical rainforests, being a large and important center for freshwater fish diversity, having more than 3,000 species (Van Der Sleen & Albert 2018). This high diversity is distributed in several aquatic ecosystems, such as large rivers, lakes, streams, floating vegetation, and beaches (Santos & Ferreira 1999). In addition to the Amazon River basin, the Amazonia biome comprises other river basins and drainages (Van Der Sleen & Albert 2018, fig., 1), such as Orinoco River basin and Guiana shield basins, both located to the north of the biome region; and a series of coastal river basins and drainages in its eastern portion, after the mouth of the Amazon River, in the State of Pará, and in the west and center of the State of Maranhão; forming the Amazônia Legal area (Martins & Oliveira 2011, Van Der Sleen & Albert 2018).

The State of Maranhão stands out for presenting few studies related to its freshwater ichthyofauna, especially in the area of taxonomy, causing gaps and lack of information related to the taxonomy and systematics of the species and groups, species composition, geographical distribution and biogeography of the ichthyofauna from the State (Guimarães et al. 2018). Biodiversity estimates and inventories of freshwater fishes have been continually published in the last decades for water bodies occurring in the State of Maranhão (e.g. Garavello et al. 1998, Piorski 1998, Castro 2001, Castro et al. 2002, Piorski et al. 2003, Soares 2005, Piorski et al. 2007, Castro et al. 2010, Barros et al. 2011, Martins & Oliveira 2011, Sousa et al. 2011, Fraga et al. 2012, Almeida et al. 2013, Viana et al. 2014, Ribeiro et al. 201, Lima et al. 2015, Matavelli et al. 2015, Ramos et al. 201, Melo et al. 2016, Piorski et al. 2017, Brito et al. 2019, Lima et al. 2019, Teixeira et al. 2019). However, the published information regarding the diversity and composition of fishes from the Mearim River basin is limited to only one book published by Soares (2005) and reprinted by Soares (2013), focusing mainly in commercial importance and large size species, and a book chapter by Martins & Oliveira (2011). This book chapter compiled data based on Soares (2005), on a study about the fishing practice among indigenous groups performed by Piorski et al. (2003), and on non-published data, such as a project report and a graduate course completion work. In addition to these two works, Abreu et al. (2019) published a paper on the Historical biogeography of fishes from coastal basins of Maranhão State. Aiming to conduct their biogeographic analysis, they make a matrix of fish species occurring in coastal drainages of the State based on examination of material deposited in CPUFMA (Coleção de Peixes da Universidade Federal do Maranhão) and compiled data from published works (e.g., Reis et al. 2003, Soares 2005, Buckup et al. 2007, Lucinda et al. 2007, Soares et al. 2009, Mérona et al. 2010, Barros et al. 2011, Lima & Caires 2011, Claro-García & Shibatta 2013, Ramos et al. 201, Melo et al. 2016, Bartolette et al. 2017, Dagosta & de Pinna 2017, Piorski et al. 2017), listing 160 fish species for all the coastal river drainages of the State of Maranhão, and 100 species for the Mearim River basin. This recent paper, however, was not a taxonomic revision, neither a species inventory, thus many species had not their taxonomic status revised and updated (see Abreu et al. 2019: S2). A common fact among these three works is that none of them presented voucher numbers for their reported species (not designating testimony material for their identifications). Therefore, the present study aims to present an extensive long-lasting inventory, using different collection methodologies, of the fishes from the Pindaré River drainage, one of the main drainages of the Mearim River basin, a river basin included at the Amazonia Legal area in the State of Maranhão, northeastern Brazil. This inventory focused on the entire ichthyofauna occurring in Pindaré River drainage.

## Material and Methods

### *1. Study area*

This study was carried out in the rivers, streams, and lagoons of the Pindaré River drainage, Mearim River basin, located in the State of Maranhão, Northeastern Brazil (Figure 1). The Pindaré River rises in the Serra do Gurupi (5º 9 'S 46º 54' W), in elevations of approximately 300 meters (Silva et al. 2017). It travels about 575.59 km until it flows into the Mearim River in the vicinity of its mouth in São Marcos Bay (Silva et al. 2017). Its main tributaries are the rivers Buriticupu, Negro, Paragominas, Zutiua, Timbira, Água Preta, and Santa Rita (Silva et al. 2017). The Pindaré River and its tributaries have a simple hydrological regime, with two well-defined seasons: the maximum - full - water that runs from February to May and the minimum - drought or ebb - that last from August to November (IBGE 1997).

### *2. Sampling design*

The collection of samples was conducted at 28 collecting sites distributed within the boundaries of Pindaré River drainage, Mearim River Basin, comprising rivers, streams, lagoons, and lakes (Table 1, Figures 1, 2). The sampling design of this study based on the establishment of (1) fixed sites for seasonal collection (18 collection sites with 50 meters in size, dry and wet seasons, from 2011 to 2017), (2) five random expeditions performed between 2017 and 2020, during both wet and dry seasons, which covered almost the entire drainage.

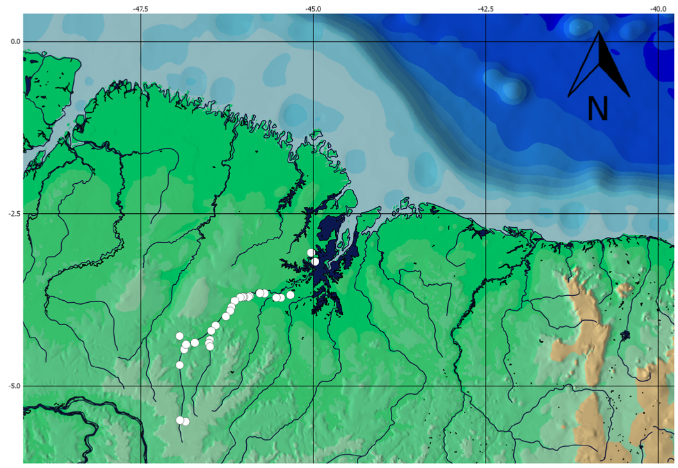


**Figure 1.** Collecting sites at Pindaré river drainage, Mearim river basin.

### *3. Collection and identification of specimens*

Fishes were collected with manual trail-net (2 m long × 1.8m high; mesh size, 2mm), cast nets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100 mm), and dip nets (mesh size 5 and 10 mm). The collected ichthyological material was euthanized in a buffered solution of ethyl-3-amino-benzoate-methanesulfonate (MS-222) at a concentration of 250 mg/l until completely ceasing opercular movements, according to animal welfare laws and guidelines (Close et al. 1996, 1997; Leary et al. 2013).

**Table 1.** Collecting sites within the Pindaré River drainage, Mearim River basin, Maranhão, Brazil.

| Site | Locality | Municipality | Coordinates |
|---|---|---|---|
| 1 | Igarapé Açaizal | Matinha - MA | 3° 3'50.59"S 45° 1'46.93"W |
| 2 | Lago de Viana | Viana-MA | 3°11'37.58"S 44°58'16.92"W |
| 3 | Bacia 814/815 | Santa Inês-MA | 3°40'49.99"S 45°19'50.85"W |
| 4 | Olho d'água dos Carneiros | Pindaré-Mirim-MA | 3°43'11.20"S 45°28'5.64"W |
| 5 | Rio Zutiua | Pindaré-Mirim-MA | 3°43'1.79"S 45°32'2.98"W |
| 6 | Igarapé Jundiá | Pindaré-Mirim-MA | 3°39'21.37"S 45°42'22.75"W |
| 7 | Lago do Lírio | Alto Alegre do Pindaré-MA | 3°39'12.22"S 45°46'25.06"W |
| 8 | Igarapé Timbira | Alto Alegre do Pindaré-MA | 3°41'43.77"S 45°55'13.80"W |
| 9 | Igarapé Mineirão | Alto Alegre do Pindaré-MA | 3°42'30.23"S 45°56'20.33"W |
| 10 | Igarapé Arapapá | Alto Alegre do Pindaré-MA | 3°42'26.91"S 46° 0'25.18"W |
| 11 | Igarapé Caititu | Alto Alegre do Pindaré-MA | 3°42'30.69"S 46° 1'19.53"W |
| 12 | Igarapé do Fausto | Alto Alegre do Pindaré-MA | 3°42'50.26"S 46° 3'29.61"W |
| 13 | Igarapé Igarapá | Alto Alegre do Pindaré-MA | 3°45'51.31"S 46° 8'15.45"W |
| 14 | Igarapé Jenipapo | Alto Alegre do Pindaré-MA | 3°51'20.24"S 46°11'9.56"W |
| 15 | Igarapé Araparizal | Alto Alegre do Pindaré-MA | 3°54'33.34"S 46°12'6.24"W |
| 16 | Igarapé Presa de Porco | Buriticupu-MA | 3°59'27.50"S 46°15'53.94"W |
| 17 | Pontilhão Km 353+900 | Buriticupu-MA | 4° 7'22.57"S 46°24'49.93"W |
| 18 | Rio Buritizinho | Buriticupu-MA | 4°11'53.71"S 46°28'41.00"W |
| 19 | Rio Buritizinho | Buriticupu-MA | 4°19'46.02"S 46°29'46.00"W |
| 20 | Rio Buritizinho | Buriticupu-MA | 4°22'52.02"S 46°30'35.00"W |
| 21 | Rio Buritizinho | Buriticupu-MA | 4°25'44.99"S 46°29'41.00"W |
| 22 | Rio dos Sonhos | Bom Jesus das Selvas-MA | 4°22'19.74"S 46°42'53.33"W |
| 23 | Rio Pindaré | Bom Jesus das Selvas - MA | 4°16'32.81"S 46°56'6.99"W |
| 24 | Rio Pindaré | Bom Jesus das Selvas-MA | 4°23'51.99"S 46°50'33.48"W |
| 25 | Rio Pindaré | Bom Jesus das Selvas-MA | 4°28'10.05"S 46°52'16.00"W |
| 26 | Rio Pindaré | Novo Bacabal - MA | 4°41'49.35"S 46°56'2.02"W |
| 27 | Rio Pindaré | Pindarezinho - MA | 5°29'50.86"S 46°55'52.01"W |
| 28 | Igarapé S/N | Buritirana - MA | 5°31'4.47"S 46°50'58.04"W |

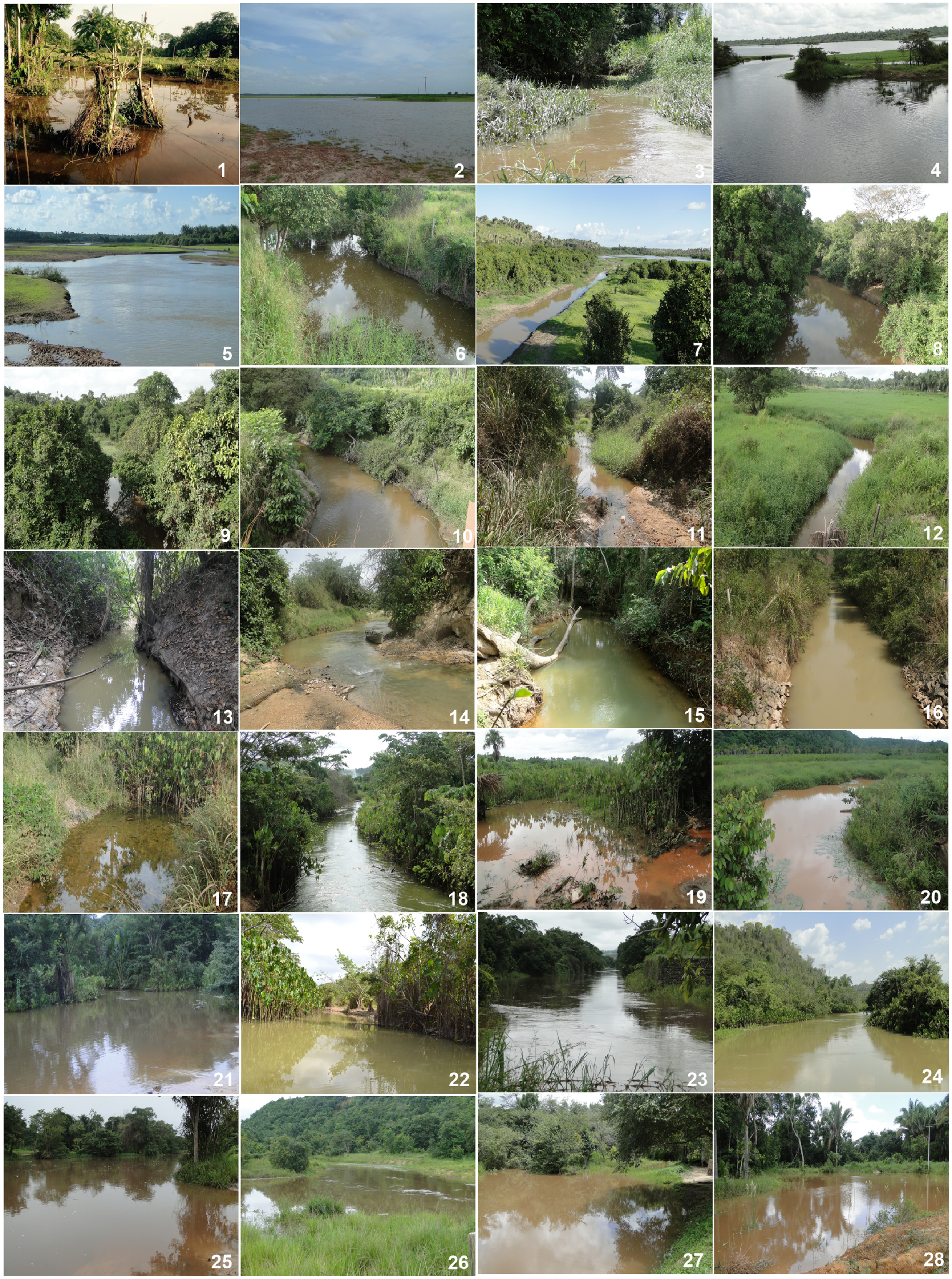


**Figure 2.** Sampled localities in the Pindaré river drainage. Numbers follow Table 1. Photographs by E.C. Guimarães and P.S. Brito.

Specimens selected for morphological analysis were fixed in formalin and left for ten days, after which they were preserved in 70% ethanol. Specimens selected for future molecular analysis were fixed and preserved in absolute ethanol. Sorting and identification of specimens were carried out at the Laboratório de Sistemática e Ecologia de Organismos Aquáticos of the Universidade Federal do Maranhão using specialized bibliography for each taxonomic group and consulting experts. The ichthyological material was deposited in the Coleção de Peixes da Universidade Federal do Maranhão (CPUFMA) and Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais of the Universidade Federal do Maranhão (CICCAA). The taxonomic classification, the names of species considered as valid, authors and years of species descriptions, and geographic distribution, were based on the compilations made by Fricke et al. (2020a, b), where the authors gather all the most recent classifications for each group of fish. The name of the collections and the acronyms can be consulted in Fricke & Eschmeyer (2020).

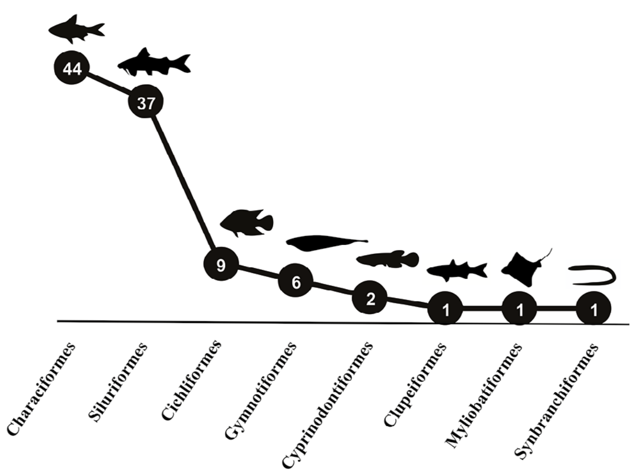


**Figure 3.** Ranking of richness by orders in the Pindaré river drainage, Mearim river basin.

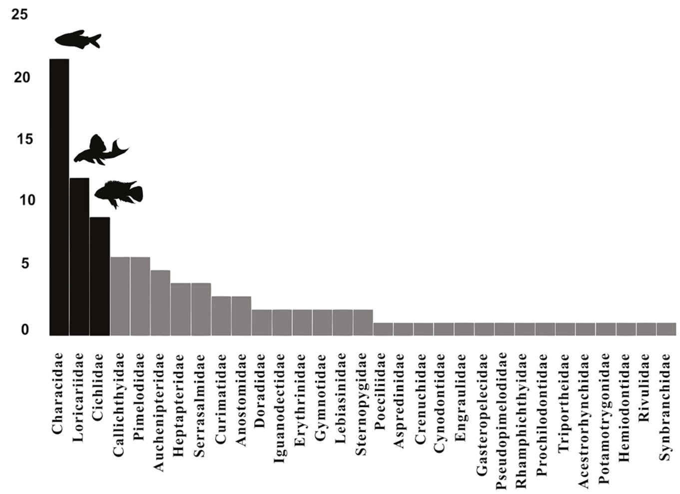


**Figure 4.** Ranking of richness by families in the Pindaré river drainage, Mearim river basin.

## Results

This fish survey resulted in 101 species representing eight orders and 32 fish families occurring in the Pindaré river drainage (Table 2, Table S1). Orders comprising the highest percentage of species richness were Characiformes 44 (43%), Siluriformes 37 (37%), Cichliformes 9 (9%), and Gymnotiformes 6 (6%), representing 96% of the total species richness. Cyprinodontiformes (*Anablepsoides* Huber 1992 and *Poecilia* Bloch & Schneider 1801), Clupeiformes (*Anchovia* Jordan & Evermann 1895), Myliobatiformes (*Potamotrygon* Garman, 1877) and Synbranchiformes (*Synbranchus* Bloch 1795) complete the list of Orders, with two (Cyprinodontiformes) and one species (other orders). The most species diverse family was Characidae 21 (21% of total species), followed by Loricariidae 12 (12%) and Cichlidae 9 (9%) (Table 2). From these 101 species herein recorded, just three are non-native, while the other 98 are native species.

**Table 2.** List of species collected at the Pindaré river dranaige, Mearim River basin.

| CLASS/ORDER/FAMILY/SPECIES | Popular Names |
|---|---|
| ELASMOBRANCHII | |
| MYLIOBATIFORMES | |
| **Potamotrygonidae** | |
| *Potamotrygon motoro* (Müller & Henle 1841) | Raia |
| ACTINOPTERYGII | |
| CLUPEIFORMES | |
| Engraulidae | |
| *Anchovia* sp. | Sardinha |
| CHARACIFORMES | |
| **Crenuchidae** | |
| *Characidium* cf. *zebra* | Canivete |
| **Erythrinidae** | |
| *Hoplias malabaricus* (Bloch 1794) | Traíra |
| *Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829) | Jejú |

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|---|---|
| **Cynodontidae** | |
| *Cynodon gibbus* (Spix & Agassiz 1829) | Cachorrinha |
| **Serrasalmidae** | |
| *Metynnis lippincottianus* (Cope 1870) | Dolár |
| *Myloplus rubripinnis* (Müller & Troschel 1844) | Pacu |
| *Pygocentrus nattereri* Kner 1858 | Piranha-vermelha |
| *Serrasalmus rhombeus* (Linnaeus 1766) | Piranha-preta |
| **Hemiodontidae** | |
| *Hemiodus* cf. *parnaguae* | Flecheira |
| **Anostomidae** | |
| *Leporinus* aff. *friderici* | Piau |
| *Megaleporinus macrocephalus* (Garavello & Britski, 1988) * | Piavuçu |
| *Schizodon dissimilis* (Garman 1890) ** | Piau-cabeça-gorda |
| **Curimatidae** | |
| *Curimata macrops* Eigenmann & Eigenmann, 1889** | Branquinha |
| *Psectrogaster rhomboides* Eigenmann & Eigenmann 1889 | Sagüiru |
| *Steindachnerina notonota* (Miranda Ribeiro 1937) | Biruba |
| **Prochilodontidae** | |
| *Prochilodus lacustris* Steindachner 1907** | Curimbatá |
| **Lebiasinidae** | |
| *Copella arnoldi* (Regan 1912) | Copella |
| *Nannostomus beckfordi* Günther 1872 | Peixe-lápis |
| **Triportheidae** | |
| *Triportheus signatus* (Garman 1890) | Voadeiras |
| **Gasteropelecidae** | |
| *Gasteropelecus sternicla* (Linnaeus 1758) | Peixe-Borboleta |
| **Iguanodectidae** | |
| *Bryconops* aff. *caudomaculatus* | João-duro |
| *Piabucus dentatus* (Koelreuter 1763) | - |
| **Acestrorhynchidae** | |
| *Acestrorhynchus falcatus* (Bloch 1794) | Peixe-cachorro |
| **Characidae** | |
| *Aphyocharax* sp. | Enfermerinha |
| *Astyanax* cf. *bimaculatus* | Piaba |
| *Brachychalcinus parnaibae* Reis 1989** | Piaba |
| *Charax awa Guimarães*, Brito, Ferreira & Ottoni 2018** | Cacunda |
| *Ctenobrycon* cf. *spilurus* | Piaba |
| *Hemigrammus* aff. *ocellifer* | Piaba |
| *Hemigrammus* cf. *rodwayi* | Piaba |
| *Hemigrammus* sp. | Piaba |
| *Hyphessobrycon caru* Guimarães, Brito, Feitosa, Carvalho-Costa & Ottoni** | Tetra, piaba |
| *Knodus victoriae* (Steindachner 1907) ** | Piaba |
| *Microschemobrycon* sp. | - |
| *Moenkhausia* cf. *intermedia* | Piaba |
| *Moenkhausia oligolepis* (Günther 1864) | Lambari-olho-de-fogo |
| *Phenacogaster* cf. *pectinata* | Piaba |

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|---|---|
| *Poptella compressa* (Günther 1864) | Piaba |
| *Pristella maxillaris* (Ulrey 1894) | Tetra Pristella |
| *Psellogrammus kennedyi* (*Eigenmann*, 1903) | Piaba |
| *Roeboides margareteae* Lucena 2003** | Saicanga |
| *Roeboides sazimai* Lucena 2007** | Saicanga |
| *Serrapinnus* sp. | Piaba |
| *Tetragonopterus argenteus* Cuvier 1816 | Matupiri |
| GYMNOTIFORMES | |
| **Gymnotidae** | |
| *Gymnotus carapo* Linnaeus 1758 | Tuvira, sarapó |
| *Electrophorus varii* de Santana, Wosiacki, Crampton, Sabaj, Dillman, Mendes-Júnior & Castro 2019. | Poraquê |
| **Rhamphichthyidae** | |
| *Rhamphichthys atlanticus* Triques 1999** | Tuvira, Ituí |
| **Hypopomidae** | |
| *Brachyhypopomus pinnicaudatus* (Hopkins, Comfort, Bastian & Bass 1990) | Tuvira, Ituí |
| **Sternopygidae** | |
| *Eigenmannia virescens* (Valenciennes 1836) | - |
| *Sternopygus macrurus* (Bloch & Schneider 1801) | Tuvira, Ituí |
| SILURIFORMES | |
| **Aspredinidae** | |
| *Pseudobunocephalus timbira* Leão, Carvalho, Reis & Wosiacki, 2019 | - |
| **Auchenipteridae** | |
| *Auchenipterichthys* sp. | - |
| *Ageneiosus ucayalensis* Castelnau 1855 | Mandubé |
| *Auchenipterus menezesi* Ferraris & Vari, 1999** | - |
| *Tatia intermedia* (Steindachner 1877) | Tatia |
| *Trachelyopterus galeatus* (Linnaeus 1766) | Mole, Cumbá |
| **Doradidae** | |
| *Hassar affinis* (Steindachner 1881) ** | Botinho |
| *Platydoras brachylecis* Piorski, Garavello, Arce H. & Sabaj Pérez 2008** | Platydoras |
| **Heptapteridae** | |
| *Imparfinis* sp. | - |
| *Mastiglanis asopos* Bockmann 1994 | Bagrinho |
| *Pimelodella parnahybae* Fowler 1941 ** | Mandi Chorão |
| *Rhamdia quelen* (Quoy & Gaimard 1824) | Bagre |
| **Pimelodidae** | |
| *Cheirocerus goeldii* (Steindachner, 1908) | - |
| *Hemisorubim platyrhynchos* (Valenciennes 1840) | Lírio |
| *Pimelodus blochii* Valenciennes 1840 | Mandi |
| *Pimelodus ornatus* Kner 1858 | Mandi |
| *Pseudoplatystoma punctifer* (Castelnau 1855) | Surubim |
| *Sorubim lima* (Bloch & Schneider 1801) | Surubi bico-de-pato |
| **Pseudopimelodidae** | |
| *Batrochoglanis villosus* (Eigenmann 1912) | - |
| **Callichthyidae** | |
| *Callichthys callichthys* (Linnaeus 1758) | Tamboatá |

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|---|---|
| *Corydoras* aff. *splendens* | Coridora |
| *Corydoras* cf. *julii* | Coridora |
| *Corydoras vittatus* Nijssen 1971** | Coridora |
| *Hoplosternum littorale* (Hancock, 1828) | Tamboatá |
| *Megalechis thoracata* (Valenciennes 1840) | Tamboatá |
| **Loricariidae** | |
| *Ancistrus* sp. | Acari |
| *Farlowella* cf. *amazonum* | Acari-viola |
| *Hemiodontichthys acipenserinus* (Kner 1853) | - |
| *Hypoptopoma incognitum* Aquino & Schaefer 2010 | - |
| *Hypostomus* cf. *plecostomus* | Acari |
| *Loricaria* aff. *cataphracta* | rapa-canoa |
| *Loricariichthys* sp. | rapa-canoa |
| *Otocinclus* sp. | - |
| *Pterygoplichthys* sp. | Acari |
| *Pterygoplichthys parnaibae* (Weber, 1991) ** | Acari |
| *Rineloricaria* sp. | - |
| *Sturisoma* aff. *lyra* | Cascudo-Chicote |
| SYNBRANCHIFORMES | |
| **Synbranchidae** | |
| *Synbranchus marmoratus* Bloch 1795 | Muçum |
| CICHLIFORMES | |
| **Cichlidae** | |
| *Aequidens tetramerus* (Heckel 1840) | Acará |
| *Apistogramma* cf. *piauiensis* | Apistograma |
| *Cichla* sp.* | Tucunaré |
| *Cichlasoma zarskei* Ottoni 2011** | Acará |
| *Crenicichla brasiliensis* (Bloch 1792) | Jacundá |
| *Crenicichla* sp. | Jacundá |
| *Geophagus* cf. *parnaibae* | Acará |
| *Satanoperca jurupari* (Heckel 1840) | Acará |
| *Oreochromis* sp.* | Tilapia |
| CYPRINODONTIFORMES | |
| **Rivulidae** | |
| *Anablepsoides* cf. *vieirai* | killifishes |
| **Poeciliidae** | |
| *Poecilia sarrafae* Bragança & Costa 2011** | Guppy, barrigudinho |

* indicates exotic species. ** indicates endemic species to the hydrological units Maranhão and Parnaíba *sensu* Hubbert & Renno (2006)

## Discussion

In the present work, we conducted an extensive long-lasting inventory of fishes, using different collection methodologies, covering almost the entire studied drainage (Fig. 1 and Table 1). We found a predominance of species of the Orders Characiformes and Siluriformes, corroborating the pattern usually found for the Neotropical fish fauna (Lowe-McConnell 1999, Pelicice et al. 2005, Langeani et al. 2007; Vari et al. 2009, Polaz et al. 2014, Reis et al. 2016, Brito et al., 2019, Dagosta & de Pinna 2019). Similar to other studies, this inventory was mainly dominated by small characids (e.g. Ramos et al. 2014, Brito et al. 2019), probably due to their ability to obtain oxygen from upper layers of the water column, high trophic plasticity (Abelha et al. 2001), and exceptional species diversity in the Neotropical region (Dagosta & de Pinna 2019).

From these 101 species herein recorded, just three are non-native [*Megaleporinus macrocephalus* (Garavello & Britski, 1988), *Oreochromis* sp. and *Cichla* sp.] occurring in the middle-lower Pindaré River drainage region, while the other 98 are native species. This demonstrates that the composition of the studied fish community is majority composed of native species, with rare cases of introduced species.

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When comparing the present survey with other inventories published for the hydrological units Maranhão and Parnaíba *sensu* Hubbert & Renno (2006) (hereafter Mrn and Prn following the acronyms proposed by the same authors), we can conclude that the freshwater fish fauna of the State of Maranhão is probably still underestimated, as argued by Piorski (2010) and Guimarães et al. (2018). Were reported a total of 101 species to only one of the main drainages of the Mearim River basin, the Pindaré River drainage (Table 2), 41 more species, and one more species than Soares (2005, 2013) (60 species) and Abreu et al. (2019: S2) (100 species) recorded for the entire Mearim River basin, respectively. In addition, we reported only 59 less species than the number of species reported by Abreu et al. (2019: S2) (160 species) for all the coastal river systems of the State of Maranhão. We believe, however, that the number of species reported by Abreu et al. (2019) overestimated, and we will discuss it in detail below.

The two works including more species recorded from coastal river basins of the Mrn and Prn, were the works published by Ramos et al. (2014) for the Parnaíba River basin, the major coastal river basin of the Mrn and Prn, and the compiled data published by Castro & Dourado (2011) for the Mearim, Pindaré, Pericumã, and upper Turiaçu River drainages, including 146 and 109 species, respectively. The first one was an exhaustive inventory of one of the main and larger river basins of Brazil, and the largest of the hydrological units mentioned above (Mrn and Prn). The second one included compiled data from three distinguished river drainages: Mearim and Pindaré from the Mearim river system, the second major river system of the Mrn and Prn, and Turiaçu, one of the main river basins of the Mrn and Prn. Even so, our survey recorded only 45 less species than Ramos et al. (2014) and eight less species than Castro & Dourado (2011), which demonstrates the high effort put in the inventory here presented. It is important to emphasize that the number of species presented by Castro & Dourado (2011) is probably overestimated since they did not update and check the taxonomic status of the species of their compiled data. In several cases, they considered more than one name for the same species, for example, two names representing *Charax awa* Guimarães, Brito, Ferreira & Ottoni 2018 [*Charax gibbosus* (Linnaeus, 1758) and *Charax* sp.]; and four names representing *Acestrorhynchus* [*Acestrorhynchus falcatus* (Bloch, 1794), *Acestrorhynchus lacustris* (Lütken, 1875), *Acestrorhynchus heterolepis* (Cope, 1878), *Acestrorhynchus microlepis* (Schomburgk, 1841)]. This situation, however, was not restricted to only those two mentioned examples, but also to several other fish species.

Based on the examination of material deposited in CPUFMA, and based on the examination of material collected during our survey, an exhaustive study conducted over nine years of collections (2011 to 2020), we verified some inconsistencies in the 100 species listed for the Mearim River basin by Abreu et al. (2019: S2). Some species were misidentified, some genera had their diversity overestimated, and some species did not have their taxonomic status updated. Examining the material deposited in CPUFMA and CICCAA, we could notice that there were the following identification errors: 1- While conducting this inventory some specimens were provisionally identified as *Elachocharax pulcher* Myers, 1927, but after a detailed morphological inspection it was verified that, in fact, these specimens were *Hoplias malabaricus* (Bloch 1794) in the initial juvenile stage. 2 - A similar case occurred with some specimens of Gymnotiformes. While conducting this inventory, some specimens of Gymnotiformes presenting a paler coloration were provisionally identified as *Eigenmannia limbata* (Schreiner & Miranda Ribeiro, 1903). However, after a detailed morphological inspection, it was verified that these specimens were *Sternopygus macrurus* (Bloch & Schneider 1801), and this paler coloration occurred due to the fixation process of those specimens. 3 - *Poecilia branneri* Eigenmann, 1894 is, in fact, *Poecilia sarrafae* Bragança, Costa, 2011. 4- *Rhamphichthys rostratus* (Linnaeus, 1766) is *Rhamphichthys atlanticus* Triques, 1999. 5 - *Leporacanthicus galaxias* is *Pterygoplichthys* sp. 6 - *Nannostomus unifasciatus* Steindachner 1876 is *Nannostomus beckfordi* Günther 1872. 7- *Prochilodus brevis* Steindachner, 1875, is *Prochilodus lacustris* Steindachner, 1907. 8 - *Triportheus angulatus* (Spix, Agassiz, 1829) is *Triportheus signatus* (Garman, 1890). And 9 - And *Auchenipterus nuchalis* (Spix, Agassiz, 1829) is *Auchenipterus menezesi* Ferraris & Vari 1999 (Table 2).

Regarding the overestimation of the species diversity within some genera, we could notice that Abreu et al. (2019: S2) recorded the following species for the Mearim River basin: two species of *Geophagus* Heckel 1840, both species belonging to the *G. surinamensis* species group [*Geophagus parnaibae* Staeck, Schindler, 2006 and *Geophagus surinamensis* (Bloch, 1791)]; three species of *Crenicichla* Heckel 1840 [(*Crenicichla lepidota* Heckel, 1840, *Crenicichla marmorata* Pellegrin, 1904, and *Crenicichla brasiliensis* (Bloch 1792)]; two species of *Poecilia* (*Poecilia branneri* and *Poecilia sarrafae*); and three species of *Hemiodus* Müller 1842 [*Hemiodus microlepis* Kner, 1858, *Hemiodus parnaguae* Eigenmann, Henn, 1916, and *Hemiodus unimaculatus* (Bloch, 1794)]. However, based on the examination of the material collected during our exhaustive study (nine years of collections in the Pindaré River drainage) and examination of material from CPUFMA, it was reported the following number of species for those genera mentioned above: 1- Two species of the genus *Crenicichla* (*Crenicichla brasiliensis* and *Crenicihla* sp., this latter species only few specimens were collected from one single locality at the municipality of Pindaré-Mirim). 2- One species of *Geophagus* not identified at the species level (*G.* cf. *parnaibae*) since the need of a taxonomic study for this group along the coastal river basins of the State of Maranhão. 3 - One species of *Hemiodus* also not identified at the species level (*Hemiodus* cf. *parnaguae*) since the need of a taxonomic study for this group along the coastal river basins of the State of Maranhão. 4 - And one species of *Poecilia* (*P. sarrafae*), with its type locality in the Prn (Table 2). After this careful inspection and taxonomic update in the compiled data listed by Abreu et al. (2019: S2), we can conclude that the number of species for the Mearim River basin proposed by them was overestimated and included some taxonomically outdated data.

The Preguiças and Periá River basins, two very small coastal river basins when compared to the major coastal river basins of the Mrn and Prn, possess a total of 56 recorded species (Piorski et al. 2017, Brito et al. 2019, 2020). This number is about half of the number that we have reported for the Pindaré River basin. This fact, however, was already expected due to the small size of the two basins mentioned above. In relation to the Munin River basin, a medium-size coastal river basin from eastern Maranhão, we reported at least five times more species than the surveys performed by Matavelli et al. (2015) (13 species), including in addition to water bodies of the lower Munin, water bodies the other rivers system, such as the lower Parnaíba and other and smaller coastal river basins; and Ribeiro et al. (2014) (20 species) for an area of the upper Munin River basin.

When comparing our study with the surveys published by Barros et al. (2011) (69 species) and Nascimento et al. (2016) (64 species), both for the Itapecuru River basin, one of the main coastal river basins of the Mrn and Prn, we can notice that we report about a third more species than these both studies. Moreover, finally, we recorded 46 more species than the survey published by Melo et al. (2016) (65 species) for the lower portion of the Parnaíba River basin. All these numbers and facts pointed out above only emphasize the great sampling force that we carried out in our inventory, as well as the importance of the present work.

From the 98 native species herein reported, it was not possible to identify 32 taxa accurately at the species level (about 31%). The reason that did not allow us to identify these 32 taxa accurately at the species level were: The lack of taxonomic knowledge and information on fish species and groups occurring in the State of Maranhão (Guimarães et al. 2018). Some of these species may be part of species complex or groups taxonomically still poorly resolved (e.g., *Anablepsoides* cf. *vieirai*, *Apistogramma* cf. *piauiensis*, *Astyanax* cf. *bimaculatus*, *Characidium* cf. *zebra*, *Geophagus* cf. *parnaibae*, *Hemigrammus* aff. *ocellifer*, *Leporinus* aff. *friderici*, *Loricaria* aff. *cataphracta*, *Phenacogaster* cf. *pectinata*). In this case, we can take as example the species *Apistogramma piauiensis* Kullander 1980. This species was described based on three specimens, one subadult female (holotype) and two juveniles (paratypes), from the Parnaíba River basin, in the State of Piaui. Although it is very similar to *Apistogramma caetei* Kullander 1980, described based on four species (three types and one additional male) from the Apeu and Caeté River basins, in the eastern State of Pará (Kullander 1980). As the Pindaré River is located between the type locality of these two species, these species are very similar and difficult to differentiate from each other, and they were described based on few material and were never redescribed; it is difficult to know exactly which of them occur in the Pindaré River basin, or if the population is an undescribed species. Although some aquarium publications recorded *A. piauiensis* as the species occurring in the Pindaré River drainage (e.g., Link & Staeck 1995, Schindler 1998), we believe that a comprehensive taxonomic study should be carried out in populations of this genus along the coastal river basins of Maranhão, as well as, these two species should be redescribed. Another example is *Anablepsoides vieirai* Nielsen 2016. This species was described from one single locality in the Parnaíba River basin (Nielsen 2016). Despite this is the closest geographically species of the *Anablepsoides urophthalmus* species group to the Pindaré River basin, the general color pattern of the specimens collected in the Pindaré River drainage is quite different from the color pattern of the specimens from the type locality of the species. In addition, the type locality of *Anablepsoides urophthalmus* (Günther 1866) is from Belém, State of Pará (Fricke et al. 2020a), a location not so far from the studied drainage. Therefore, a taxonomic study among the populations occurring in the coastal river system from Belém (State of Pará) to the Parnaíba River basin is still necessary to let we know which of these two species occurs in the Pindaré River system, or if it is an undescribed species. A third example is *Geophagus parnaibae* Staeck & Schindler 2006, which was described from tributaries of the Parnaiba River basin, in the State of Maranhão (Staeck & Schindler 2006). Although our few collected specimens from the Pindaré River drainage morphologically resemble this species, there are aquarium publications that argue that the population of the Pindaré River drainage is, in fact, an undescribed species, known by aquarists as *Geophagus* sp. "Pindare" (e.g., Grad, 2004). In addition, several species of this genus are reported for the Tocantins River Basin, Lower Amazon, and coastal river basin of Guiana Shield (Fricke et al. 2020a). Therefore, this is another group that needs to be better taxonomically studied, mainly the populations of the coastal river systems from Belém (State of Pará) to the Parnaíba River basin.

Other species such as, *Astyanax bimaculatus* (Linnaeus 1758), *Characidium zebra* Eigenmann 1909, *Hemigrammus ocellifer* (Steindachner 1882), *Leporinus friderici* (Bloch 1794), *Loricaria cataphracta* Linnaeus 1758, *Phenacogaster pectinata* (Cope 1870) are already known to be species complexes based on several studies (e.g., Isbrucker, 1972, Lucena et al. 2010, Fricke et al. 2020a), needing comprehensive taxonomic studies to reveal the hidden diversity under these species names. Some taxa probably correspond to still undescribed species (e.g., *Ancistrus* sp., *Aphyocharax* sp., *Bryconops* aff. *caudomaculatus*, *Hemigrammus* sp., *Microschemobrycon* sp., *Loricariichthys* sp., *Serrapinnus* sp., and *Sturisoma* aff. *lyra*) that still need a more detailed taxonomic study to make sure that they are new to the science. Besides, some of these species had very few specimens collected (e.g. *Microschemobrycon* sp. and *Sturisoma* aff. *lyra*), which makes taxonomic studies difficult. It is important to emphasize that in the last two decades several new species occurring in the Mearim River basin have been described, what reinforces this possibility that these taxa could be still undescribed species (e.g., Ferraris & Vari 1999, Triques 1999, Lucena 2003, 2007, Piorski et al. 2008, Bragança & Costa 2011, Ottoni 2011; Guimarães et al. 2018, Guimarães et al. 2019, Leão et al. 2019, Santana et al. 2019). From the 98 native species herein recorded, 32 of them were not possible to be accurately identified at the species level. Thus, we will not address these species in our biogeographic comments. From the remaining 66 species, 25 (almost half of the species) did not occur in the Amazon River basin [*Auchenipterus menezesi* Ferraris & Vari, 1999, *Brachychalcinus parnaibae* Reis 1989, *Charax awa* Guimarães, Brito, Ferreira & Ottoni 2018, *Cichlasoma zarskei* Ottoni 2011, *Crenicichla brasiliensis* (Bloch 1792), *Corydoras vittatus* Nijssen 1971, *Curimata macrops* Eigenmann & Eigenmann, 1889, *Hassar affinis* (Steindachner 1881), *Hyphessobrycon caru* Guimarães, Brito, Feitosa, Carvalho-Costa & Ottoni 2019, *Knodus victoriae* (Steindachner 1907), *Myloplus rubripinnis* (Müller & Troschel 1844), *Pimelodella parnahybae* Fowler 1941, *Platydoras brachylecis* Piorski, Garavello, Arce H. & Sabaj Pérez 2008, *Psectrogaster rhomboides* Eigenmann & Eigenmann 1889, *Poecilia sarrafae* Bragança & Costa 2011, *Prochilodus lacustris* Steindachner 1907, *Pterygoplichthys parnaibae* (Weber, 1991), *Schizodon dissimilis* (Garman 1890), *Steindachnerina notonota* (Miranda Ribeiro 1937), *Triportheus signatus* (Garman 1890), *Rhamphichthys atlanticus* Triques 1999, *Roeboides margareteae* Lucena 2003, and *Roeboides sazimai* Lucena 2007]; 18 of them (*Auchenipterus menezesi*, *Brachychalcinus parnaibae*, *Charax awa*, *Cichlasoma zarskei*, *Corydoras vittatus*, *Curimata macrops*, *Hassar affinis*, *Hyphessobrycon caru*, *Knodus victoriae*, *Pimelodella parnahybae*, *Platydoras brachylecis*, *Poecilia sarrafae*, *Prochilodus lacustris*, *Pterygoplichthys parnaibae*, *Schizodon dissimilis*, *Rhamphichthys atlanticus*, *Roeboides margareteae*, *Roeboides sazimai*) being endemic to the Mrn and Prn (see Fricke et al. 2020a). All the remaining species herein reported (41 species) have their distribution known to the Amazon River basin (see Fricke et al. 2020a), showing a considerable biogeographic influence of the Amazon basin in the Pindaré River drainage.

According to Rosa et al. (2003), the fish fauna of the Maranhão-Piaui ecoregion, which includes the Prn and part of Mrn, was historically pointed out as poorly endemic. Otherwise, the low level of endemism recorded during the past decades would be related to few sampling effort on the whole region (Piorski 2010, Ramos et al. 2014, Guimarães et al. 2018). Several species in the Maranhão-Piaui rivers are known to occur along the Amazon basin (including coastal rivers in Suriname and the Guianas), a distribution pattern suggested by Barros et al. (2011), who observed a predominance of Amazonian species in the Itapecuru basin, as well as corroborated for some putative species by Guimarães et al. (2016, 2017a, b). Besides, this influence of the Amazon River basin in theses hydrological units (Mrn and Prn) was also advocated by Hubert & Renno (2006) and Dagosta & de Pinna (2017), in their biogeographic studies. However, these same authors also advocated the possibility of the coastal river basin of the State of Maranhão constituting one or more areas of endemism. Both papers, however, suggest that data related to the freshwater ichthyofauna from this region are too scarce to have a more conclusive hypothesis. However, a list of several species endemic to the Mrn and Prn, or also occurring just on neighboring areas, was provided Guimarães et al. (2018), reinforcing the hypothesis that the coastal river basins of the State of Maranhão could constitute one or more areas of endemism, as suggested as a possibility by Hubert & Renno (2006) and Dagosta & de Pinna (2017).

## Supplementary Material

The following online material is available for this article:
Table S1 – Examined material

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## Author Contributions

Erick C. Guimarães: 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.

Pâmella S. de Brito: 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.

Cléverson Storck Gonçalves: Substantial contribution in the concept and design of the study.

Felipe Polivanov Ottoni: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

## Conflicts of Interest

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

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**Article**

# Diet of *Crossodactylus timbuhy* (Anura: Hylodidae) in the Reserva Biológica Augusto Ruschi, state of Espírito Santo, Brazil

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***Abstract:*** Anurans are predator and prey, playing an important role in ecosystem functioning. The diet composition is closely related to feeding strategy, and the information about prey items is useful to understand intra and interspecific interactions in trophic webs. Here we determined diet composition, feeding strategy, and relation between prey ingestion and body measures of *Crossodactylus timbuhy*, a recently described anuran species. We found 466 prey items from 20 prey categories in the stomach of 66 specimens (15 males and 51 females) of *C. timbuhy*. The diet consists mostly of Formicidae and Coleoptera, the items with the highest number, frequency of occurrence and prey importance. The diet composition was relatively similar to other species of *Crossodactylus*. Prey volume was positively related to frog size and weight, suggesting frogs may feed upon any prey they can swallow. Diet showed some variation between sexes. Despite females were larger and heavier than males, females had higher consumption of smaller prey, and ingested a larger number of prey categories. We suggest *C. timbuhy* has an invertebrate-opportunistic feeding habit. It is likely *C. timbuhy* uses a combination of 'sit-and-wait' and 'active search' strategies due to high consumption of both highly mobile and sedentary prey.
***Keywords:*** *Amphibia; Atlantic Forest; dietary preference; feeding ecology; predation.*

## Dieta de *Crossodactylus timbuhy* (Anura: Hylodidae) na Reserva Biológica Augusto Ruschi, Espírito Santo, Brasil

***Resumo:*** Os anuros são predadores e presas, desempenhando um importante papel no funcionamento dos ecossistemas. A composição da dieta está intimamente relacionada à estratégia de forrageamento das espécies e as informações sobre os itens consumidos são úteis para compreensão das interações intra e interespecíficas nas redes tróficas. O presente estudo objetivou determinar a composição da dieta, a estratégia de forrageamento e a relação entre a ingestão de presas e as medidas corporais de *Crossodactylus timbuhy*, uma espécie de anuro descrita recentemente. Foi analisado o conteúdo estomacal de 66 espécimes (15 machos e 51 fêmeas) de *C. timbuhy* e registrados 466 itens alimentares, distribuídos em 20 categorias de presas. A dieta consistiu principalmente de Formicidae e Coleoptera, as quais apresentaram maior número de itens consumidos, maior frequência de ocorrência e maior importância entre as presas registradas. A composição da dieta foi relativamente semelhante à de outras espécies do gênero *Crossodactylus*. O volume das presas foi positivamente relacionado com o tamanho e o peso dos espécimes, sugerindo que os indivíduos podem se alimentar de qualquer presa que eles possam engolir. A dieta apresentou variação entre os sexos. Apesar das fêmeas serem maiores e mais pesadas do que os machos, elas consumiram mais presas menores e ingeriram mais categorias de presas. Sugere-se que *C. timbuhy* tenha hábito alimentar invertebrado-oportunista. É provável que *C. timbuhy* apresente uma combinação de estratégias "senta-e-espera" e "forrageador ativo" devido ao alto consumo de presas altamente móveis e de presas sedentárias.
***Palavras-chave:*** *Amphibia; ecologia trófica; Mata Atlântica; preferência alimentar; predação.*

## Introduction

The Atlantic Forest comprises one of the biodiversity hotspots in the world (Myers et al. 2000). Despite its importance for conservation, the remaining forest still faces habitat loss and fragmentation (Ribeiro et al. 2011). The priorities for conservation are sites with high number of species, endemism, and threatened species. It is noteworthy that several key sites for conservation have new species discovered annually (Rossa-Feres et al. 2017). For example, the municipality of Santa Teresa, in southeastern Brazil, harbors more than 100 species of frogs, some of them only recently described (e.g. Ferreira et al. 2019).

Anurans play an important role in ecosystem functionality because they can act as predator and prey (Caldart et al. 2011, Hocking & Babbitt 2014). They are mainly opportunistic predators feeding mostly on small invertebrates (Solé & Rödder 2009, Cicort- Lucaciu et al. 2011, Ferreira et al. 2012). Some species have a narrow diet or even specialization on certain prey categories. For example, species of *Rhinella* and *Dendrobates* are mostly specialist on ants, beetles or termites (Solé et al. 2002, Ferreira & Teixeira 2012, Martínez et al. 2019). On the other hand, species of *Eleutherodactylus* and *Ceratophrys* have highly generalist diets, but with concentrated consumption on few prey categories (Duellman & Lizana 1994, Olson & Beard 2012). As prey, anurans contribute to the energy flow to higher trophic levels (Pough 1980). In addition to contributing to the knowledge of the natural history of species, the diet composition information is useful to understand intra and interspecific interactions in trophic webs, energy flow and ecosystem functioning.

The diet composition is closely related to feeding strategy (Toft 1980, Toft 1981, Huey & Pianka 1981, Perry & Pianka 1997). In general, sit-and-wait foragers are effective at capturing actively moving prey and have generalized feeding habits (Duellman & Trueb 1994). Contrarily, active foragers are effective at capturing sedentary prey and have specialized feeding habits (Huey & Pianka 1981, Toft 1981, Duellman & Trueb 1994). Some predators have more plasticity regarding feeding strategy by consuming both active and sedentary preys (Caldart et al. 2012). Also, most species can also adapt feeding strategy according to food availability (Huey & Pianka 1981, Menin et al. 2005).

The genus *Crossodactylus* Duméril and Bibron, 1841 belongs to the family Hylodidae and is composed of 14 species occurring in Brazil, Argentina and Paraguay (Frost 2020). Some species of *Crossodactylus* have small range distribution. For example, *Crossodactylus timbuhy* Pimenta, Cruz and Caramaschi, 2014 has been recorded only from Santa Teresa and Cachoeiro de Itapemirim municipalities, in the central region of Brazilian Atlantic Forest (Pimenta et al. 2014, Frost 2020). Diet studies suggests that ants are the main prey item of the species on the genus *Crossodactylus*, as shown for *C. gaudichaudii* Duméril & Bibron, 1841 (Almeida-Gomes et al. 2007), *C. trachystomus* (Reinhardt & Lütken, 1862) (previously *C. bokermanni*; Wachlevski et al. 2008), *C. schmidti* Gallardo, 1961 (Caldart et al. 2012) and *C. aeneus* Müller, 1924 (Jordão-Nogueira et al. 2006).

*Crossodactylus timbuhy* was recently described and there is still no information regarding its natural history, including its diet composition and feeding strategy. Here we analyzed the diet of one population of *C. timbuhy* in its type locality. We specifically aimed to determine the diet composition, the most important food items, and the feeding strategy. We also evaluated the relation between prey ingestion and body measures due to its relation to feeding strategy.

## Material and Methods

Fieldwork was carried out at Augusto Ruschi Biological Reserve (Reserva Biológica Augusto Ruschi; 19°45' and 20°00' S, 40°27' and 40°38' W), municipality of Santa Teresa, state of Espírito Santo, southeastern Brazil. This mountainous region is covered by montane and sub-montane vegetation (Rizzini 1979, Brasil 1983). Climate is classified as humid subtropical with temperate summer (Cfb) according to Köppen's classification (Alvares et al. 2014).

Sampling was performed along the Cachoeira trail (19°54' to 19°55' S, 40°33' W). Pitfall traps were originally designed to insect sample and also captured the anurans donated to us by the orthopteran researchers (see Acknowledgments; sampling permit ICMBio 37717-1). In total, 150 pitfall traps (buckets of 15 cm diameter and 15 cm height) were used in June 2013. Five buckets were placed in a line every 30 m along a 900 m transect on the forest floor and 200 m from a stream. Traps remained open for 48 hours. Buckets were filled with 70% ethanol for material preservation.

Adult frog specimens were weighed with a precision scale (± 0.01 g precision) and snout-vent length (SVL) was measured with calipers (± 0.1 mm precision). Upon removal of stomach content, sex of the specimens was also determined. Each prey item was identified at the lowest possible taxonomic level following Triplehorn & Johnson (2011) and Rafael et al. (2012). We distinguished the order Hymenoptera between Formicidae and Non-Formicidae categories. The larvae of different insect taxa were inserted into the category Insect larvae. The specimens were deposited in the Zoological Collection of the Museu de Biologia Professor Mello Leitão from the Atlantic Forest National Institute (Instituto Nacional da Mata Atlântica - INMA), Espírito Santo state, Brazil (MBML 8606-8633, 8635-8658, 8661-8674).

We counted the number of food items contained in each stomach, for each category of prey, and calculated the number of prey items ingested (N). The frequency of occurrence of each taxon relative to total of analyzed stomachs (F%) was also calculated. Length (L) and width (W) of each prey item were measured with calipers (± 0.1 mm precision) to calculate prey volume using the formula: $V = 4/3\pi * L/2 * (W/2)^2$ (Biavati et al. 2004). The percentage was also calculated for number and volume of prey. Index of relative importance of each taxon was based on: $Ix = (N\% + F\% + V\%)/3$ (according the modification proposed by Santos-Pereira et al. 2015). All these indices were calculated for both sexes together and for males and females separately.

The feeding strategy was calculated using the prey-specific abundance method and represented graphically (Amundsen et al. 1996). The prey-specific abundance was calculated as $P_i = (\sum S_i / \sum St_i) \times 100$, where $S_i$ is the stomach content comprising the number of prey *i* and $St_i$ is the total number of prey items in those stomachs with prey *i* (Amundsen et al. 1996). For graphical representation of the feeding strategy of *C. timbuhy*, the prey specific abundance ($P_i$) was plotted against the frequency of occurrence (F%) of each prey category, and graph interpretation followed Amundsen et al. (1996).

Data normality was determined by D'Agostino test (Ayres et al. 2007). The two-sample t-test was used to compare SVL and weight between males and females. We used Linear Regressions to evaluate the relation between SVL and weight; and Spearman Rank Correlation to assess the relation between frog size (SVL and weight) and the variables related to ingestion of prey (N and V).

Positive correlation between frog size and prey volume indicates frogs feed upon any prey that they can swallow (i.e. opportunistic feeder). Data were analyzed in Statistica (version 7.1) and R (R Core Team, 2014). The significance level was $P \leq 0.05$ (Zar 2010). The mean and standard deviation (SD) were provided.

## Results

### 1. Characterization of the frog sample

We evaluated the stomach content of 66 specimens of *C. timbuhy*, 15 males and 51 females. The sex ratio was 1:3.4 (male:female). Male SVL was 20.32 ± 1.63 mm and female SVL was 23.52 ± 1.29 mm. Male weight was 0.76 ± 0.15 g and female weight was 1.25 ± 0.20 g. Females were larger ($t = 7.91$; $P < 0.001$) and heavier ($t = 8.71$; $P < 0.001$) than males. There was positive relation between SVL and weight considering both sexes together ($F_{1,64} = 98.104$; $P < 0.001$; $R^2 = 0.599$) and only females ($F_{1,49} = 26.616$; $P < 0.001$; $R^2 = 0.338$), but not for males ($F_{1,13} = 2.478$; $P = 0.137$; $R^2 = 0.096$).

### 2. Diet composition

We identified 466 prey items from 20 categories, and all frogs had at least one prey item in their stomach content (Table 1). The mean number of prey items per stomach was 7.1 ± 5.9. The mean prey volume per stomach was 32.6 ± 63.5 mm³. Formicidae, Coleoptera and Insect larvae had the highest numerical proportion of prey items (N% = 31.5, 15.9 and 14.6, respectively). Formicidae and Coleoptera had the highest frequency of occurrence (F% = 63.6 both). Coleoptera had the highest volume of prey ingested (V% = 20.0). Formicidae was the most important prey item for *C. timbuhy*, followed by Coleoptera and Insecta larvae (*Ix* = 35.6, 33.2 and 27.6; Table 1).

### 3. Sexes differences on diet

Analyzing each sex separately, the mean number of prey items per stomach was 8.3 ± 8.0 for males, and 6.7 ± 5.2 for females. The mean prey volume per stomach was 16.8 ± 17.2 mm³ for males, and 37.3 ± 71.2 mm³ for females. Formicidae and Coleoptera had the highest numerical proportion of prey items for males (N% = 37.9 and 16.1, respectively);

**Table 1.** Prey items consumed by *Crossodactylus timbuhy* in the Augusto Ruschi Biological Reserve, Espírito Santo state, southeastern Brazil: number of prey items (N), frequency of occurrence (F%) in stomachs, volume of prey items (V), and relative importance of each prey (Ix). For number and volume of prey the percentage is also shown (in parentheses).

| Prey category | Male (N = 15) | | | | Female (N = 51) | | | | Population (N = 66) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N (%) | F% | V (%) | Ix | N (%) | F% | V(%) | Ix | N (%) | F% | V(%) | Ix |
| Arachnida | | | | | | | | | | | | |
| Acari | 3 (2.4) | 6.7 | 1.5 (0.6) | 3.2 | 7 (2.0) | 5.9 | 46.9 (3.3) | 3.7 | 10 (2.1) | 6.1 | 48.4 (2.9) | 3.7 |
| Araneae | 15 (12.1) | 66.7 | 15.8 (6.2) | 28.3 | 27 (7.9) | 39.2 | 78.0 (5.4) | 17.5 | 42 (9.0) | 45.6 | 93.8 (5.6) | 20.0 |
| Pseudoscorpionida | - | - | - | - | 1 (0.3) | 2.0 | 0.6 (0.0) | 0.8 | 1 (0.2) | 1.5 | 0.6 (0.0) | 0.6 |
| Ixodida | - | - | - | - | 2 (0.6) | 3.9 | 3.1 (2.1) | 2.2 | 2 (0.4) | 3.0 | 30.1 (1.8) | 1.7 |
| Chilopoda | 1 (0.8) | 6.7 | 3.9 (1.5) | 3.0 | 1 (0.3) | 2.0 | 0.3 (0.0) | 0.8 | 2 (0.4) | 3.0 | 4.2 (0.2) | 1.2 |
| Diplopoda | - | - | - | - | 1 (0.3) | 2.0 | 2.2 (0.2) | 0.8 | 1 (0.2) | 1.5 | 2.2 (0.1) | 0.6 |
| Insecta | | | | | | | | | | | | |
| Blattodea | - | - | - | - | 1 (0.3) | 2.0 | 1.3 (0.1) | 0.8 | 1 (0.2) | 1.5 | 1.3 (0.1) | 0.6 |
| Coleoptera | 20 (16.1) | 66.7 | 46.4 (18.3) | 33.7 | 54 (15.8) | 62.7 | 290.9 (20.3) | 32.9 | 74 (15.9) | 63.6 | 337.3 (20.0) | 33.2 |
| Dermaptera | - | - | - | - | 4 (1.2) | 7.8 | 9.3 (0.6) | 3.2 | 4 (0.9) | 6.1 | 9.3 (0.6) | 2.5 |
| Diptera | 4 (3.2) | 26.7 | 28.6 (11.3) | 13.7 | 29 (8.5) | 31.4 | 185.7 (13.0) | 17.6 | 33 (7.1) | 20.3 | 214.3 (12.7) | 16.5 |
| Hemiptera | 3 (2.4) | 13.3 | 2.0 (0.8) | 5.5 | 6 (1.8) | 11.8 | 88.5 (6.2) | 6.6 | 9 (1.9) | 12.1 | 90.5 (5.4) | 6.5 |
| Hymenoptera | | | | | | | | | | | | |
| Non-Formicidae | 12 (9.7) | 53.3 | 22.1 (8.7) | 23.9 | 36 (10.5) | 43.1 | 144.8 (10.1) | 21.3 | 48 (10.3) | 45.5 | 166.9 (9.9) | 21.9 |
| Formicidae | 47 (37.9) | 73.3 | 51.7 (20.4) | 43.9 | 100 (29.2) | 60.8 | 144.0 (10.0) | 33.4 | 147 (31.5) | 63.6 | 195.7 (11.6) | 35.6 |
| Isoptera | 2 (1.6) | 13.3 | 0.3 (0.1) | 5.0 | 2 (0.6) | 2.0 | 12.1 (0.8) | 1.1 | 4 (0.9) | 4.5 | 12.7 (0.7) | 2.0 |
| Insect larvae | 13 (10.5) | 53.3 | 20.2 (8.0) | 23.9 | 55 (16.1) | 51.0 | 261.5 (18.2) | 28.4 | 68 (14.6) | 51.5 | 281.6 (16.7) | 27.6 |
| Mantodea | - | - | - | - | 1 (0.3) | 2.0 | 3.9 (0.3) | 0.8 | 1 (0.2) | 1.5 | 3.9 (0.2) | 0.7 |
| Orthoptera | 1 (0.8) | 6.7 | 52.0 (20.5) | 9.3 | 3 (0.9) | 3.9 | 57.3 (4.0) | 2.9 | 4 (0.9) | 4.5 | 109.3 (6.5) | 4.0 |
| Trichoptera | - | - | - | - | 1 (0.3) | 2.0 | 1.1 (0.1) | 0.8 | 1 (0.2) | 1.5 | 1.1 (0.1) | 0.6 |
| Gastropoda | | | | | | | | | | | | |
| Pulmonata | 2 (1.6) | 13.3 | 3.9 (1.5) | 5.5 | 1 (0.3) | 2.0 | 0.9 (0.1) | 0.8 | 3 (0.6) | 4.5 | 4.8 (0.3) | 1.8 |
| Malacostraca | | | | | | | | | | | | |
| Isopoda | 1 (0.8) | 6.7 | 4.9 (1.9) | 3.1 | 10 (2.9) | 13.7 | 74.7 (5.2) | 7.3 | 11 (2.4) | 12.1 | 79.7 (4.7) | 6.4 |
| **Total number** | **124** | | | | **342** | | | | **466** | | | |
| **Total volume (mm³)** | | | **253.3** | | | | **1,433.9** | | | | **1,687.3** | |

Formicidae, Insect larvae and Coleoptera for females (N% = 29.2, 16.1 and 15.8, respectively; Table 1). Formicidae, Araneae and Coleoptera had the highest frequency of occurrence for males (F% = 73.3, 66.7 and 66.7, respectively); Coleoptera and Formicidae for females (F% = 62.7 and 60.8, respectively). Orthoptera and Formicidae had the highest volume in males (V% = 20.5 and 20.4, respectively); Coleoptera, Insect Larvae and Diptera in females (V% = 20.3, 18.2 and 13.0; Table 1). Formicidae and Coleoptera were the most important prey items for both males and females (male $Ix$ = 43.9 and 33.7, respectively; female $Ix$ = 33.4, and 32.9, respectively; Table 1).

Prey number was not related to frog SVL (rs = 0.032; P = 0.798; 66 pairs) or frog weight (rs = 0.000; P = 0.994; 66 pairs) for all population, even when analyze females (rs = 0.047; P = 0.743 and rs = 0.049; P = 0.728; 51 pairs) or males separately (rs = 0.362; P = 0.184 and rs = 0.407; P = 0.131; 15 pairs). Prey volume was positively related to frog SVL (rs = 0.274; P = 0.026; 66 pairs) and frog weight (rs = 0.323; P = 0.008; 68 pairs) for all population. For males, prey volume was related to weight (rs = 0.636; P = 0,011; 15 pairs), but not to SVL (rs = 0.438; P = 0.103; 15 pairs). For females, prey volume was not related to SVL (rs = 0.150; P = 0.292; 51 pairs) or weight (rs = 0.232; P = 0.100; 51 pairs).

### *4. Feeding strategy*

There was no dominant prey type on the diet of *C. timbuhy* due to the absence of any prey type in the upper right corner of the graph (Fig. 1). Most prey types were rare in the diet due to their positioning in the lower left corner of the graph. No between-phenotype component to the niche width nor within-phenotype component to any food type were observed. Thus, there is no specialization to any food type by individuals, although there is a tendency for diet generalization within the population of *C. timbuhy* (Fig. 1). *Crossodactylus timbuhy* is likely an invertebrate-opportunistic feeder.

## Discussion

### *1. Diet composition and Feeding strategy*

Our results corroborate other studies showing *Crossodactylu*s species feed mainly on Formicidae, Coleoptera and Insect larvae (João-Nogueira et al. 2006, Almeida-Gomes et al. 2007, Wachlevski et al. 2008, Caldart et al. 2012). Although the primary prey taxa consumed by *C. timbuhy* was similar to other congeners, the secondary items differed across species. Despite this, our data corroborated Caldart et al. (2012) who stated that the diet composition is relatively similar across *Crossodactylus* species. The diet similarities may reflect similar prey availability at the forest leaf litter and margins of forest streams, as proposed for Hylodidae species (Wachlevski et al. 2008).

The wide spectrum of prey taxa consumed by *C. timbuhy* suggests that this species has an opportunistic feeding habit. It is noteworthy mentioning, however, the high consumption of Formicidae, and that only worker ants were in the stomach content of *C. timbuhy.* The consumption of high abundance of worker ants can be a consequence of opportunistic feeding habit and prey availability, associated with the poor nutritional value of this item, compared to queens for example, that have more protein and fat content (Pianka & Parker 1975, Nielsen et al. 1985). Thus, may be advantageous to consume high number of workers to obtain the necessary energy supply, while avoiding the energy expenditure required to actively locate other types of prey with higher nutritional value (optimal foraging theory; Charnov 1976).

The consumption of both highly mobile insects (e.g. Formicidae) and sedentary ones (e.g. Insect larvae) indicated a combined use of both 'sit-and-wait' and 'active search' strategies (Huey & Pianka 1981). Although we have not evaluated prey availability, other studies indicated that the diet of *Crossodactylus* species reflects the availability of prey in the environment (e.g. Wachlevski et al. 2008, Caldart et al. 2012).

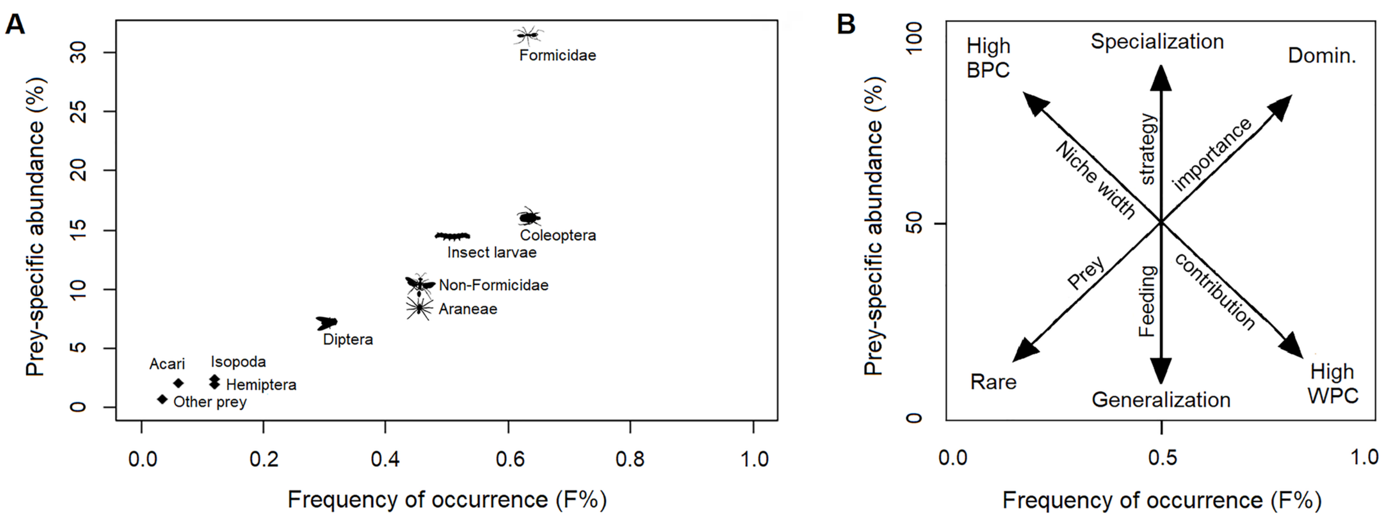


**Figure 1.** Feeding strategy of *Crossodactylus timbuhy* in the Augusto Ruschi Biological Reserve, Espírito Santo state, southeastern Brazil, according to prey-specific abundance (%) and frequency of occurrence (F%) of each prey category (A), and the diagram for feeding strategy interpretation considering the prey importance (rare to dominant), the niche width contribution (BPC = between-phenotype component; WPC = within-phenotype component) and the feeding strategy (B; based on Amundsen et al. 1996). For better visualization of Figure 1A, the y-axis was not shown in its total length.

Because social arthropods comprise approximately 70% of animal biomass in tropical forest (Hölldobler & Wilson 1990), it is not surprising that ants are important prey also for *C. trachystomus* (Wachlevski et al. 2008), *C. gaudichaudii* (Almeida-Gomes et al. 2007), *C. schmidti* (Caldart et al. 2012), and other anuran species such as *Hylodes phyllodes* Heyer & Cocroft, 1986 (Hylodidae; Almeida-Gomes et al. 2008), *Amazophrynella minuta* (Melin, 1941) (previously *Dendrophryniscus minutus*; Bufonidae; Toft 1980), *Rhinella crucifer* (Wied-Neuwied, 1821) (Bufonidae; Ferreira & Teixeira 2009) and *Leptodactylus natalensis* Lutz, 1930 (Leptodactylidae; Ferreira et al. 2007).

The SVL and weight of *C. timbuhy* were not related to the number of prey in the stomach, but was positively related to prey volume. Because *Crossodactylus* species have mouth width proportional to SVL and weight (Jordão-Nogueira et al. 2006), it is not surprising that larger and heavier individuals can feed upon more voluminous prey. Contrarily, SVL of *C. schmidti* and *C. trachystomus* have no relation to prey volume (respectively Caldart et al. 2012, Wachlevski et al. 2014). However, the size of the frog is generally a limiting factor in the selection of preys (Toft 1980, Borges et al. 2019).

### 2. *Sexes differences on diet and sample*

Diet showed some variation regarding number, frequency, and volume between males and females in the study area. Males were less opportunistic than females (13 prey categories), although males consume preys related to their weight, which can potentially allow the consumption of a wider range of prey. Despite females were larger and heavier than males, females had higher consumption of smaller prey and ingested a larger number of prey categories (n = 20). This higher tendency to opportunistic feeding by females may be related to the different energetic requirement between sexes (Duellman & Trueb 1994). For example, the maturation of large sex cells requires that females reduce energy expenditure during foraging (e.g. maintaining the consumption of Formicidae) and target consumption on more energetic and small prey (e.g. insect larvae). However, it is possible that the differences observed may also be related, at least in part, to the analysis of a smaller number of male samples (15 males and 51 females).

The difference on habitat use may also be related to diet variation between sexes and sex ratio. The sex ratio of *C. timbuhy* is the most dissimilar across studied congener species. Interestingly, females had three times more individuals than males in our sample. We suggest that males of *C. timbuhy* may had been calling near the stream and thus had fallen less than females into pitfall traps in the forest (i.e. > 15 m from the stream's edge). The differences on sampling methods across studies on diet of *Crossodactylus* may play a role in such difference between males and females. Caldart et al. (2012) used pitfall traps about 1 m from the water's edge and capture 58 males and 36 females. Wachlevski et al. (2008) used active leaf-litter sampling in 2x2m quadrats at a stream bank and found 59 males and 39 females. According to Wachlevski et al. (2008), males of *C. trachystomus* are territorial and remain near stream margins. Contrarily to males, females may have preference for locations farther from stream's edge outside the reproductive period. Thus, the possible difference on habitat use between sexes may also influence the access to food resources due to the availability of prey in each environment.

## Conclusion

*Crossodactylus timbuhy* is likely an invertebrate-opportunistic predator combining the use of both 'sit-and-wait' and 'active search' strategies. The species feeds upon similar prey to its congeners corroborating that diet composition is relatively similar across the genus. However, dietary studies of other species are still needed to determine whether prey preferences are conservative across the genus. The composition of the diet of *C. timbuhy* showed some variations between sexes. Females had higher tendency to opportunistic feeding, suggesting plasticity regarding diet and feeding strategy. We suggest that diet may be influenced by different energetic requirement between sexes and by the association between habitat preferences and prey availability, as well as sex ratio is influenced by habitat use x habitat sampled, but these patterns should be further investigated on future studies with *Crossodactylus* species.

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## Author Contributions

Ana Carolina Srbek-Araujo: Concept of the study, data analysis and interpretation, manuscript preparation.

Rodrigo Barbosa Ferreira: Concept of the study, data analysis and interpretation, manuscript preparation.

Elaine Costa Campinhos: Data analysis and interpretation, manuscript preparation.

Marcio Marques Mageski: Data analysis and interpretation, manuscript preparation.

## Conflicts of Interest

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

## Ethics

All the research meets the ethical guidelines, including adherence to the Brazilian legal requirements. The manuscript has been submitted solely to Biota Neotropica and is not published, in press, or submitted elsewhere.

## Data availability

Our original data are fully described in the manuscript.

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**Article**

# Anatomy of male and female reproductive organs of stink bugs pests (Pentatomidae: Heteroptera) from soybean and rice crops

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***Abstract:*** Pentatomidae comprises a diverse group of stink bugs widely distributed in the Neotropical region. Many species are phytophagous and cause injuries to plants, and can thus be defined as agricultural pests. In this study, the anatomy of the female and male reproductive tracts of three important agricultural pests in Colombia is described: *Piezodorus guildinii* Westwood, 1837 and *Chinavia ubica* Rolston 1983, found on soybeans, and *Oebalus insularis* Stål, 1872, found in rice crops. For that, light microscopy techniques were used. The anatomy of the reproductive tract of sexually mature males of the three species studied consisted of a pair of testes, *vas deferens*, seminal vesicles, ejaculatory bulb, an ejaculatory duct that opens into an aedeagus, and paired accessory glands. The reproductive tract of females consisted of a pair of ovaries, each with seven telotrophic-meroistic ovarioles, a pair of lateral oviducts, common oviduct, spermatheca, and a genital chamber. Telotrophic ovarioles were comprised of terminal filament, tropharium, vitellarium, and pedicel. Differences in size, color, and position of structures along the reproductive tract were observed between the species examined. Reproductive biology of insects provides informative characters for behavioral and evolutionary studies, as well as useful data for pest control strategies.
***Keywords:*** *reproductive morphology, Heteroptera, testes, ovaries.*

## Anatomia dos órgãos reprodutivos em machos e fêmeas de percevejos praga (Pentatomidae: Heteroptera) das culturas de soja e arroz

***Resumo:*** Pentatomidae compreende um diverso grupo de percevejos amplamente distribuídos na região Neotropical. Muitas espécies são fitófagas e causam algum tipo de injúria em plantas, podendo se configurar como pragas agrícolas. Neste trabalho, descrevemos a anatomia do trato reprodutor de machos e fêmeas em três importantes pragas agrícolas da Colômbia: *Piezodorus guildinii* (Westwood 1837) e *Chinavia ubica* (Rolston 1983) na cultura de soja e *Oebalus insularis* (Stål 1872) na cultura do arroz. Para isto, foram utilizadas técnicas de microscopia de luz. A anatomia do trato reprodutor em machos sexualmente maduros nas três espécies consistiu de um par testículos, *vasos deferentes*, vesículas seminais, bulbo ejaculatório, um ducto ejaculatório que se abre em um aedeagus e glândulas acessórias pareadas. O trato reprodutor das fêmeas consistiu de um par de ovários, cada um com sete ovaríolos telotróficos-meroísticos, um par de ovidutos laterais, uma espermateca e a câmara genital. Os ovaríolos telotróficos possuíam quatro diferentes regiões: filamento terminal, germário, vitelário e pedicelo. As diferenças entre as espécies foram observadas no tamanho, cor e posição das estruturas ao logo do trato reprodutivo. A biologia reprodutiva de insetos gera caracteres que fornecem informações para trabalhos envolvendo evolução, comportamento e estratégias de controle de pragas.
***Palavras-chave:*** morfologia reprodutiva, Heteroptera, testículos, ovários.

## Introduction

Stink bugs (Pentatomidae) comprise the fourth largest Heteroptera family, with approximately 4,700 species described (Grazia et al. 2015). Most pentatomids are phytophagous and generalists, feeding on sap and other plant-produced substances (Grazia et al. 2012). Pentatomids have piercing sucking mouthparts, and most of them are phytophagous, including several species that are severe pests of agricultural crops. However, some species, particularly in the subfamily Asopinae, are predatory and may be considered beneficial. Phytophagous species feed on a wide range of food resources and have an extensive global distribution, which leads many of them to be defined as important agricultural pests (Panizzi et al. 2000, Grazia et al. 2012, Li et al. 2017, Lucini et al. 2020).

In the Neotropical region, pentatomids are one of the major insect groups that cause damage to different crops (McPherson & MacPherson 2000, Panizzi et al. 2000, Smaniotto & Panizzi 2015). In crops such as soybeans and rice, these stink bugs can cause physiological changes in plants, affecting their development, flowering, fruiting, and therefore, crop performance and grain and seed quality (McPherson & McPherson 2000, Panizzi et al. 2000, Karban & Agrawal 2002, Possebom et al. 2020).

The stink bug *Piezodorus guildinii* (Westwood 1837) is widely distributed in the Neotropical region and is one of the most harmful pests to soybean crops in the Americas (Panizzi & Slansky 1985). If the attack strikes during grain development and pod filling, major losses might occur (Galileo & Heinrichs 1978). *Chinavia ubica* Rolston, 1983 is a minor pest of soybean crop (Silva et al. 2015); however, *Chinavia* Orian, 1965 - senior synonym of *Acrosternum* Fieber, 1860 – is polyphagous and causes damage to several crops, such as vegetables and fruits (Panizzi et al. 2000). The stink bug *Oebalus insularis* Stål, 1872 is a harmful pest to rice crops and is very common in Colombia, Central America, and the Caribbean. This species is responsible for significant economic losses in these regions due to its ability to suck grains during development, thus affecting grain amount and quality, reducing production by more than 50% (Gutiérrez et al. 1985, Rodriguez et al. 2006).

The comparative morphology of the reproductive tract in male and female stink bugs has already been described in several Pentatomidae species, and males generally exhibit a pair of testes, deferent ducts, ejaculatory bulb complex, paired accessory glands, and an ejaculatory duct (Pendergrast 1956, Adams 2001, Lemos et al. 2001, Candan et al. 2010, 2015, Araújo et al. 2011, 2020, Özyurt et al. 2013a, 2013b, 2014, 2015, Cremonez et al. 2017). On the other hand, females exhibit a pair of ovaries connected to the lateral oviducts by the pedicels of ovarioles, a common oviduct, and a spermatheca (Lemos et al. 2005, Candan et al. 2010, 2014, 2015, Cremonez et al. 2017). Insect ovaries and ovarioles can be classified into two types according to the presence (or absence) of nurse cells. In panoistic ovaries, nurse cells are absent, usually observed in more basal groups. In meroistic ovaries, the nurse cells are associated with the development of eggs. There are two types of meroistic ovaries, the polytrophic ovariole ones, in which oocytes are closely associated with the nurse cells, and the telotrophic ones, typical of Hemiptera and some Coleoptera, in which the nurse cells are at the apex of the ovary, in the germinal region (Bonhag 1958). These studies indicate that there are interspecific differences in reproductive anatomy regarding size or number of structures, the absence of any of them, and their color or position along the reproductive tract. Hence, the anatomy of these insects provides important characters for systematics and expands knowledge on the reproductive biology of the group.

The anatomy of both male and female reproductive tracts of three important pest insects was described in the present study: *P. guildinii* and *C. ubica*, found on soybeans, and *O. insularis*, found in rice crops, in order to provide knowledge on their reproductive biology and new data to be used in the systematics of these important agricultural pests.

## Materials and Methods

### 1. *Insects*

Ten individuals of each sex of the following species were actively collected with the aid of entomological nets from crops located in the municipality of Armero-Guayabal, Tolima, Colombia (5°09'72"N 74°54'19"O): *Oebalus insularis* Stål, 1872 on rice, *Chinavia ubica* Rolston, 1983 and *Piezodorus guildinii* Westwood, 1837 on soybeans (Fig. 1). For species identification, the original descriptions (Sailer, 1944; Rolston, 1983) and specific taxonomic keys for this group were used (Schwertner & Grazia 2007; Grazia et al. 2015).

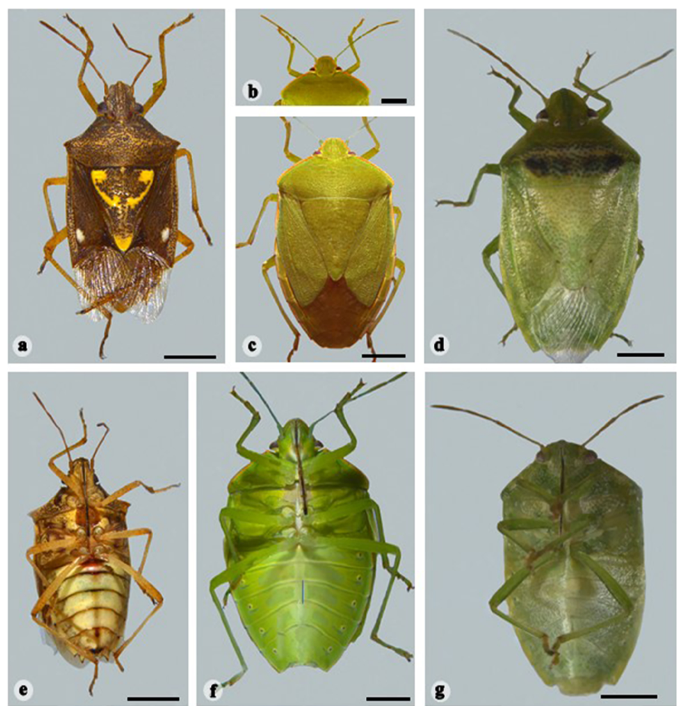


**Figure 1.** A-D. *Habitus* of dorsal and ventral areas of *Oebalus insularis* (A and E), *Chinavia ubica* (B-C and F), and *Piezodorus guildinii* adults (D and G). Bars: 2 mm.

### 2. *Light microscopy*

Insects were cryoanesthetized at -5 $^{0}$C for 10 min and their reproductive tracts were dissected in 0.1 M sodium chloride. For anatomical analysis, the reproductive tracts, freshly fixed in 2.5% glutaraldehyde buffered with sodium cacodylate, 0.1 M, pH 7.2 for a few minutes, were placed on histological slides with drops of the same buffer. Their reproductive tracts were photographed unstained using a Leica M205 C light microscope and a Leica MC 170 HD digital camera.

All measurements were performed using Image Pro-Plus (Media Cybernetcs), and lengths were expressed as the mean value of ten dissected individuals from each species analyzed. Species measurements were compared using ANOVA and Tukey's test. Data were analyzed using Generalized Linear Models (GLM) in the R software (R Core Team 2016).

## Results

The male reproductive tract of the three studied species consists of a pair of testes, a pair of *vas deferens*, a pair of ectodermal sacs, a complex ejaculatory bulb, accessory glands, and ejaculatory duct (Figures 2a, 3a, 4a). Each testis is comprised of testicular follicles, which are usually enclosed in a common sheath. Six testicular follicles were observed in *O. insularis* (Figure 2b). The testes and deferent ducts are externally covered by a red-pigmented peritoneal sheath (Figures 2a-d, 3a-b, 4a-c). In *P. guildinii*, the basal region of the deferent ducts exhibited a yellowish peritoneal sheath (Figures 3a, 3c).

Testis length ($F_{3;45}$ = 7.08; P = 0.001), testis width ($F_{3;45}$ = 19.65; P = 0.003), and *vas deferens* length ($F_{3;45}$ = 13.65; P = 0.002) varied significantly between the sampled species (Table 1 and Figures 2a, 3a-b, 4a-b). The *vas deferens* did not exhibit an enlarged and differentiated region along its length to be distinguished as a seminal vesicle (Figures 2a, 3a, 4a).

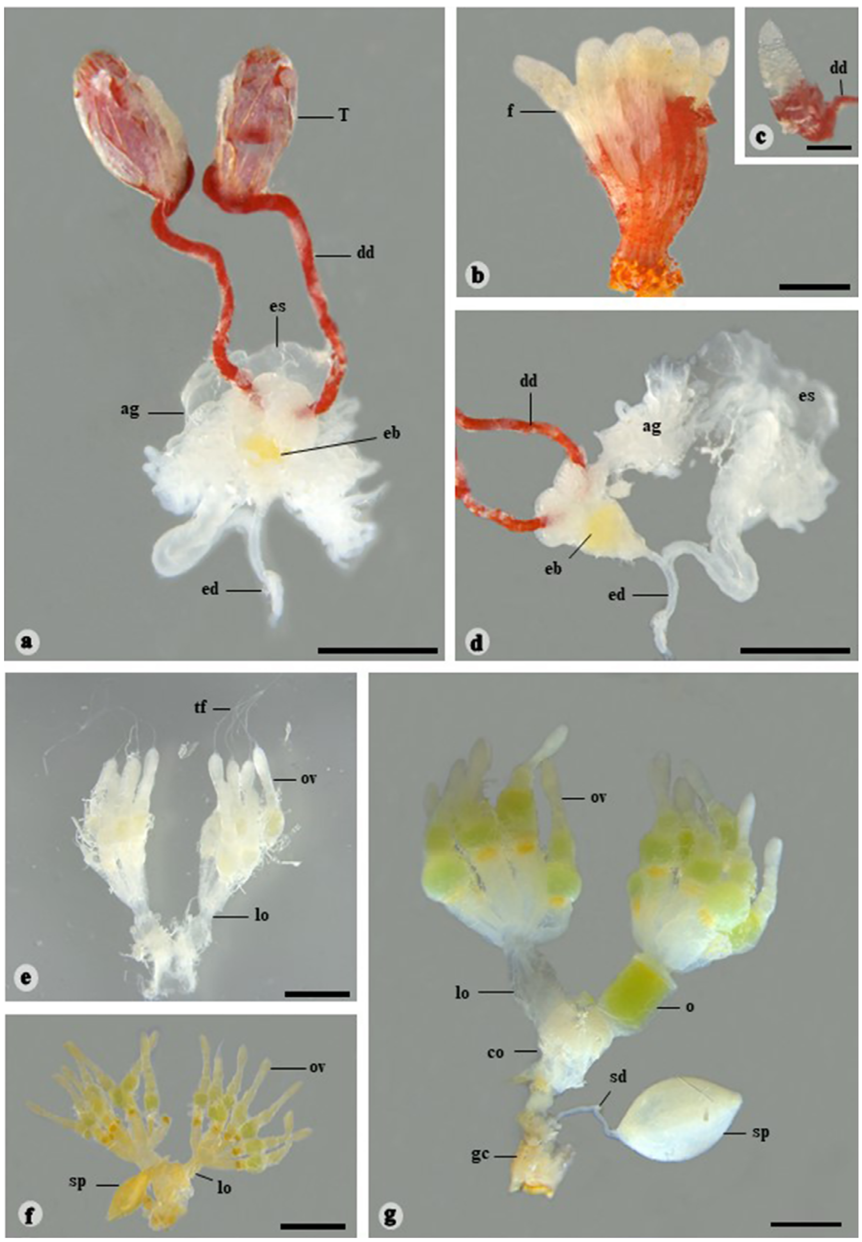


**Figure 2.** Photomicrograph of the anatomy of the male and female reproductive tract of *Oebalus insularis*. A. Male reproductive tract showing the testis (T), deferent ducts (dd), ectodermal sac (es), ejaculatory bulb (eb), accessory glands (ag), and ejaculatory duct (ed). B. Testis exhibiting six testicular follicles. C. Detail of a single testicular follicle. D. Region of the reproductive tract showing deferent ducts, accessory glands, ectodermal sac, ejaculatory bulb, and ejaculatory duct. E-G. Female reproductive tract with ovarioles (ov), terminal filaments (tf), lateral oviducts (lo), common oviduct (co), spermatheca (sp), spermathecal duct (sd), oocytes (o), and genital chamber (gc). Bars = A-C and D-G = 1 mm; C = 2 mm.

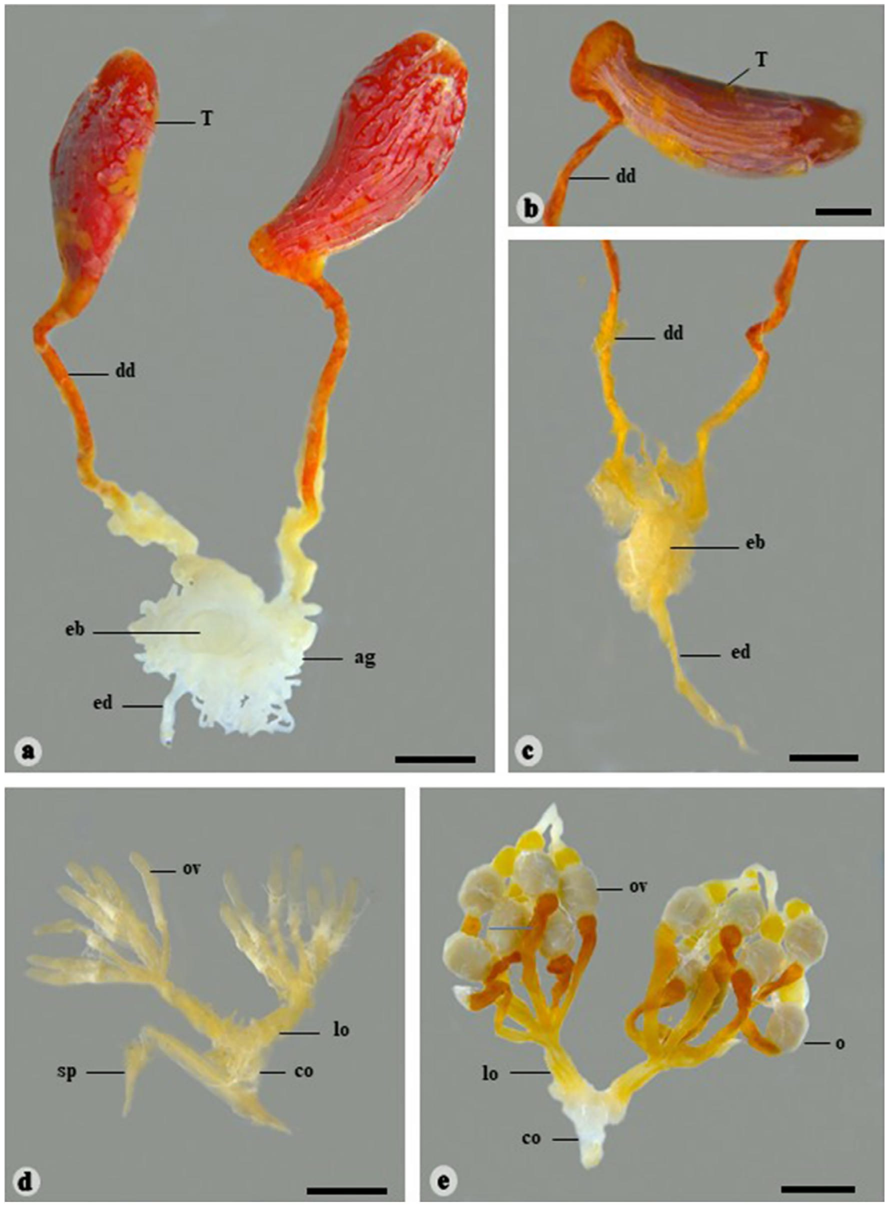


**Figure 3.** Photomicrograph of the anatomy of the male and female reproductive tracts of *Chinavia ubica*. A. Male reproductive tract showing the testis (T), deferent ducts (dd), ejaculatory bulb (eb), accessory glands (ag), and ejaculatory duct (ed). B. One testis. C. Region of the reproductive tract showing deferent ducts, ejaculatory bulb, and ejaculatory duct. D-E. Female reproductive tract showing ovarioles (ov), lateral oviducts (lo), common oviduct (co), spermatheca (sp), and oocytes (o). Bars = A-C = 1 mm; D-E = 2 mm.

Tubular accessory glands were observed in all three species studied (Figures 2a, 3a, 4a). In *O. insularis*, tubular accessory glands and ectodermal sac open in a single duct that converges into the ejaculatory duct near the proximal portion of the ejaculatory bulb (Figure 2d). This species has a yellow pear-shaped ejaculatory bulb (Figures 2a, 2d). In *C. ubica*, tubular accessory glands open at the base of the deferent ducts, immediately above the ejaculatory bulb, which was ovoid and transparent in this species (Figures 3c-d). In *P. guildinii*, tubular accessory glands and the ectodermal sac open in the region of a greenish oval-shaped ejaculatory bulb (Figure 4a-b). The ejaculatory duct of this species was long and folded (Figures 4a, 4c).

The female reproductive tract of the three studied species is formed by a pair of ovaries, each one with seven telotrophic-meroistic ovarioles connected by a pair of lateral oviducts, a spermatheca, a common oviduct, and a vagina that opens into the genital chamber (Figures 2e, 3e, 4c). The telotrophic-meroistic ovarioles developed synchronously and exhibited four different regions: terminal filament, tropharium, vitellarium, and pedicel (Figures 2e-h).

*O. insularis* oocytes had greenish coloration and a well-developed spermatheca connecting to the common oviduct through a spermathecal duct (Figures 2e-h). Oocytes are white in *C. ubica* (Figure 3e) and yellowish in *P. guildinii*, with an elongated spermatheca and a brown spermathecal duct (Figures 4d-e). In all three species, female adults exhibited structural changes in the reproductive tract during oogenesis (Figures 2e-h, 3e, 4c-d).

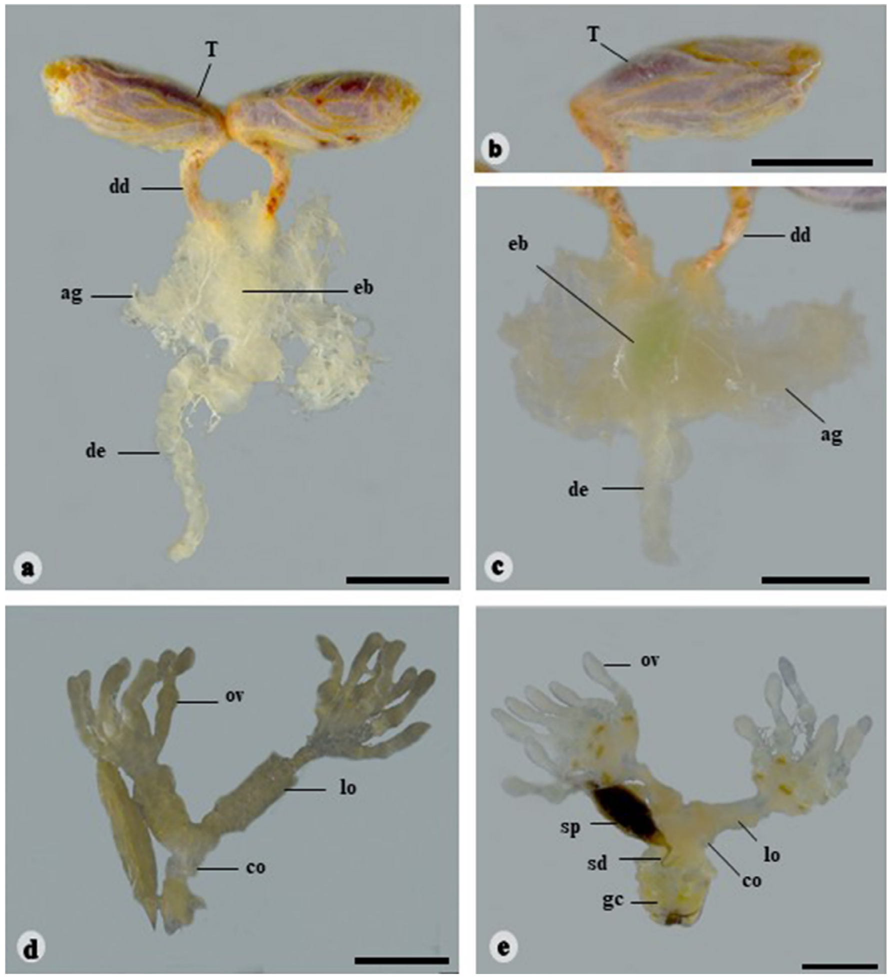


**Figure 4.** Photomicrograph of the anatomy of the male and female reproductive tracts of *Piezodorus guildinii*. A. Male reproductive tract showing the testis (T), deferent ducts (dd), ejaculatory bulb (eb), accessory glands (ag), and ejaculatory duct (ed). B. Testis. C. Region of the reproductive tract showing deferent ducts, accessory glands, ejaculatory bulb, and ejaculatory duct. D-E. Female reproductive tract showing ovarioles (ov), lateral oviducts (lo), common oviduct (co), spermatheca (sp), and genital chamber (gc). Bars = 1 mm.

**Table 1.** Averages measurements of total body length and width (mm) of testis, length of *vas deferens* and ratio of length of vas deferens to body in males of *Oebalus insularis*, *Chinavia ubica* and *Piezodorus guildinii* (n = 10).

| Species | Total body length (mm) | Testis length (mm) | Testis width (mm) | *Vas deferens* length (mm) | *Vas deferens* / Total width (mm) |
|---|---|---|---|---|---|
| *Oebalus insularis* Stal, 1872 | 7,4 ± 0,311 | 1.05 ± 0,187 | 0.56 ± 0,034 | 1,86 ± 0,222 | 0,25 |
| *Chinavia ubica* Rolston, 1983 | 13,4 ± 0,287 | 3.03 ± 0,293 | 1.36 ± 0,067 | 4,72± 0,125 | 0,35 |
| *Piezodorus guildinii* Westwood, 1837 | 8,9 ± 0,075 | 1.48 ± 0,122 | 0.67 ± 0,124 | 0,89± 0,328 | 0,1 |

## Discussion

The anatomy of the male reproductive tract maintains a general pattern in Pentatomidae, with some differences in size, shape, color, and location of structures (Pendergrast 1956, Adams 2001, Lemos et al. 2005, Rodrigues-Agna et al. 2008, Esquivel et al. 2009, Kaur & Patial 2012, 2016, Özyurt et al. 2013a, 2014, 2015, Jyoti et al. 2015, Araújo et al. 2020).

Among Pentatomidae, the number of testicular follicles can vary from three, e.g. in *Aeliomorpha lineaticollis* Westwood, 1837 (Kaur & Patial 2016), to seven, e.g. in *Apodiphus amygdali* Germar, 1817 (Ozyurt et al. 2014) and *Eurydema ventralis* Kolenati, 1846 (Ozyurt et al. 2015). Six testicular follicles were observed in *Oebalus insularis* Stål, 1872, as well as in *Nezara viridula* Linnaeus, 1758 (Esquivel et al. 2009), *Halys dentatus* Fabricius, 1775 (Jyoti et al. 2015), and *Podisus nigrispinus* Dallas, 1851 (Lemos et al. 2005) – ranging from 4 to 6 follicles. Furthermore, the presence of four follicles in *Oebalus ypsilongriseus* De Geer, 1773 (Araújo et al. 2020) demonstrates that differences concerning the number of follicles occur even within the same genus, and therefore, does not seem to be a potential character for the identification at taxonomic levels above genus.

Of nineteen revised Pentatomidae species, including the three studied here, seventeen exhibited a peritoneal sheath covering the testes and part of deferent ducts with a red tinge (Pendergrast 1956, Lemos et al. 2005, Kaur & Patial 2012, Ozyurt et al. 2013a, 2014, 2015, Jyoti et al. 2015, Araújo et al. 2020), whereas only two species – *N. viridula* (Esquivel et al. 2009) and *Thyanta perditor* Fabricius, 1794 (Araújo et al. 2020) – exhibited a yellow-orange peritoneal sheath. The basal region of deferent ducts with a yellowish color in *P. guildinii* is one of the major anatomical differences between the studied species. The color of the peritoneal sheath does not follow a pattern among Hemiptera (Gomes et al. 2013). However, the sheath is transparent in the studied aquatic Heteroptera families (Castanhole et al. 2008, 2010; Munhoz et al. 2020), as occurs in the subfamily Triatominae (Alevi et al. 2014). Lipophorin is one of the major insect lipoproteins (Van Hoof et al. 2005), particularly involved in lipid transport from absorption or synthesis to storage and uptake, such as the fat body, ovary, and testis (Jung & Yun 2007). Differences in diet and metabolism may be related to differences in pigment color of the peritoneal sheath of the reproductive tract.

Elongated testes have also been described for the pentatomids *Tropicoris punctipes* Stål, 1876, *Erthesina fullo* Thurnberg, 1783 (Kaur & Patial 2012), and *T. perditor* (Araújo et al. 2020). Among Pentatomidae, species can have elongated-ovoid (Esquivel et al. 2009, Kaur & Patial 2012, Ozyurt et al. 2013a, Araújo et al. 2020), cylindrical (Ozyurt et al. 2014), and kidney-shaped testes (Jyoti et al. 2015). The shape of testicles highly varies among Pentatomidae. However, this characteristic is conserved at the species level and may be a character of taxonomic potential.

In most Pentatomidae, spermatozoa migrate from the testes after spermatogenesis and are stored along the deferent ducts (Kaur & Patial 2012, 2016, Araújo et al. 2020), which was observed in the three studied species. The presence of a seminal vesicle has been described in some pentatomid species, *N. virdula* Pendergrast, 1956 and *Brachymena cincta* Fieber, 1861 (Abbasi 1973), *Dolycoris indicus* Stål, 1876 (Santos et al. 2003), and *E. ventralis* (Ozyurt et al 2015). Testis follicles with gem cells at different stages of spermatogenesis suggest that the species studied here have continuous spermatozoa production, which may allow multiple mating during adulthood, as reported in other Hemiptera (Moreira et al. 2008).

The anatomy of female reproductive tracts of *O. insularis*, *C. ubica*, and *P. guildinii* is similar to what has already been described in other Heteroptera (Kumar 1962, Pericart 1972, Lis 2003, Lemos et al. 2005, Ozyurt et al. 2013b). In mature female adults, oocyte development was observed probably due to vitellogenin availability in the hemolymph (Raikhel & Dhadialla 1992, Nijhout 1998, Fortes et al. 2011). The development pattern of ovarioles is different among Hemiptera species; however, it is synchronous in the three species studied here, as well as in *Porphyrophora polonica* Linnaeus, 1858 (Szklarzewicz 1998), *Steingelia gorodetskia* Nasonov, 1908 (Koteja et al. 2003), and in *Graphosoma lineatum* Linnaeus, 1758 (Ozyurt et al. 2013b); on the other hand, it is asynchronous in *Gossyparia spuria* Modeer, 1778 (Szklarzewicz 1998) and *Dactylopius coccus* Costa, 1835 (Ramírez-Cruz et al. 2008).

The units forming Heteroptera ovarioles generally consist of a terminal filament, tropharium, vitellarium, and pedicel (Davey 1958, 1959, Davey & Webster 1967, Ma & Ramaswamy 1987, Nijhout 1998, Szklarzewicz 1998, Lemos et al. 2005, Jahnke et al. 2006, Ozyurt 2013b). However, the number of ovarioles can vary even intra-specifically in response to reproductive strategy, seasonality, or resource availability (Wellings et al. 1980, Tschinkel 1987, Stewart et al. 1991). Seven ovarioles were observed In *O. insularis*, *C. ubica*, and *P. guildinii* while this number varies from 8 to 14 in most Heteroptera (Grozeva & Kuznetsova 1992, Bunig 1994, Lalitha et al. 1997, Lis 2003, Jahnke et al. 2006, Ogorzalek 2007, 2009, Ozyrt et al. 2013b). However, approximately 100 ovarioles have been observed in *Palaecoccus fuscipennis* (Szklarzewicz et al. 2005), 300 have been observed in *P. polonica* (Szklarzewicz 1998), and 400 have been observed in *Dysmicoccus coccus* (Ramírez-Cruz et al. 2008).

Females of *P. guildinii* lay an egg mass that ranges from 3 to 37 eggs (Panizzi et al 2000), similar to that observed in figures 4d-e from the present study, which indicates an approximate load of 42 eggs. Zachrisson et al (2014) demonstrated that *O. insularis* can deposit masses containing 17 to 24 eggs, yet figures 2e-g in the present study show a laying potential of up to 56 eggs in a short period. For this reason, the number of eggs deposited may depend primarily on the type of host plant, food quality, and defensive behavior against natural enemies (Panizzi et al 2000, Panizzi & Silva 2012, Zachrisson et al 2014).

The spermatheca is a structure typically present in the reproductive tract of female heteropterans, and is responsible for storing and maintaining viable spermatozoa until oocyte fertilization (Pendergrast 1957, Singh 1968, Kumar 1969a, 1969b; Ahmad & McPherson 1998, Rider & Chapin 1991). Interspecific variations in size, spermathecal shape, and number of associated glands are important characters for the systematics of this group (Kumar 1971, Rider & Chapin 1991, Ahmad & McPherson 1998, Candan et al. 2015). The spermathecas in the three species studied have different shapes and coloration, similar to *O. insularis*, in which this organ is quite developed with an evident spermathecal duct.

According to Panizzi & Silva (2012) the longevity of females from several stink bug species, including *P. guildinii* and *C. ubica*, is sharply reduced according to the reproductive behavior associated with sexual activity and copulation. This finding shows how data on reproductive morphophysiology can provide important information for strategies aiming at population control of pest insects.

Reproductive parameters of pentatomids can be affected by growth-regulating insecticides (Castro et al. 2012) via chemical communication, e.g. pheromones (McBrien & Millar 1999), and substrate vibrations (Laumann et al. 2018). These control alternatives should be monitored and analyzed through possible morphological changes in the reproductive tracts of female and male stink bugs.

The reproductive tract anatomy of female and male *O. insularis*, *C. ubica* and *P. guildinii* stink bugs in this study were described as the major characters that differentiate these species, as is the size of the testes and deferent ducts, and insertion sites of the accessory glands. The morphology of the reproductive tract generates important characters that can contribute to elucidate reproductive strategies as useful tools in agricultural pest management plans.

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## Authors' Contributions

Vinícius Albano Araújo: 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.

Tito Bacca: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to critical revision, adding intellectual content.

Lucimar Gomes Dias: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to critical revision, adding intellectual content.

## Conflict of interest

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

## Data availability

All data are presented in the results section.

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# Population density of *Trichoderma* fungi in natural environments and agrosystems of a Cerrado area

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***Abstract:*** Soil microorganisms present a great diversity, involving taxonomically distinct groups that play a role in the decomposition of organic matter, nutrient cycling, soil aggregation, among others. In this diversity, the fungi of the genus *Trichoderma* have been successful plant pathogen biocontrol agents, as plant growth promoters and as inducers of plant resistance to diseases. In addition, they are important in the sustainability of natural ecosystems. Aiming to verify the population density of *Trichoderma* fungi in natural environments and agroecosystems, in Cerrado area, samples of soils and roots from native vegetation and agroecological production system were collected in the Federal District, Brazil. The collection points were randomly selected, and each soil or root sample was individually wrapped. The soil adhered to the roots was removed for evaluations. Serial sample dilutions and number of Colony Forming Units (CFUs) of *Trichoderma* isolates were performed. The results showed that the number of CFU varied depending on the plant and location evaluated. The replacement of native vegetation by organic farming systems did not result in a significant reduction in this number.
***Keywords:*** soil microflora; biocontrol agent; microbial population.

## Densidade populacional de fungos do gênero *Trichoderma* em ambientais naturais e agrossistemas de uma área de Cerrado

***Resumo:*** Os microrganismos de solo apresentam uma grande diversidade, envolvendo grupos taxonomicamente distintos que desempenham papel na decomposição da matéria orgânica, ciclagem de nutrientes, agregação dos solos, dentre outros. Nesta diversidade, os fungos do gênero *Trichoderma* tem apresentado sucesso como agentes de biocontrole de fitopatógenos, como promotores de crescimento de plantas e, ainda, como indutores de resistência de plantas a doenças. Além disso, são importantes na sustentabilidade dos ecossistemas naturais. Com o objetivo de verificar a densidade populacional de fungos do gênero *Trichoderma* em ambientes naturais e agroecossistemas, em área de Cerrado, amostras de solos e raízes oriundas de vegetação nativa e de sistema de produção agroecológica foram coletadas na região do Distrito Federal, Brasil. Os pontos de coleta foram selecionados aleatoriamente, e cada amostra de solo ou raiz foi acondicionada individualmente. O solo aderido às raízes foi removido para as avaliações. Foram realizadas diluições seriadas das amostras e contagem do número de Unidades Formadoras de Colônias (UFCs) de isolados de *Trichoderma*. Os resultados mostraram que o número de UFC variou dependendo da planta e da localidade avaliada. A substituição da vegetação nativa por sistemas de cultivo orgânicos não resultou em importante redução neste número.
***Palavras-chave:*** microflora do solo; agente de biocontrole; população microbiana.

## Introduction

Agroecology is an important instrument for the sustainability of small-scale agricultural activities or family farming, mainly due to the low dependence on external inputs from the recommended production systems (Aquino & Assis 2007). Several studies have shown the importance of this farm model in maintaining soil quality and biological activity, in contrast to conventional agriculture (Crowder et al. 2010). In this sense, agricultural practices adopted in agroecological systems are considered strategic to reduce the impact of agricultural expansion on biodiversity in the edaphic environment (Hole et al. 2005). By prioritizing the use of inputs produced on the property, this production model emphasizes the interrelation of the chemical, physical and biological components of the agroecosystem, promoting the conservation of biodiversity, which is important in soil formation (Vandermer 1995). A challenge for scaling up agroecology lies in translating agroecological principles into practical strategies for soil, water and biodiversity management to increase yield and resilience (Nicholls & Altieri 2018).

According to Altieri & Nicholls (2000), it is the interactions between the various biotic components of the agroecosystem that will contribute to biological pest control, nutrient recycling, water conservation, soil conservation and / or regeneration, and increased agricultural productivity in a sustainable way. In this regard, microorganisms have played a major role in the sustainability of agrosystems. Some of the beneficial microorganisms often used in agriculture worldwide include the genera *Bacillus, Azospirillum, Trichoderma, Rhizobium, Mycorrhizae, Pseudomonas, Streptomyces* and many other groups (Gupta 2012). As an example, *Trichoderma* strains have been successfully used as biological control agents of various plant pathogens, being one of the most studied and known microorganisms in the world (Verma et al. 2007). But initially, this biopesticide activity was considered as the only benefit to be considered. Subsequently, it was demonstrated that species of this genus could also be used as biofertilizers, biostimulants, among others (Lorito et al. 2010, Woo & Peppe 2018), being used as inoculant in several agricultural crops.

Therefore, any change that may cause a loss in environmental diversity, influenced by agricultural use or the absence / presence of rainfall, for example, may modify biological diversity in the edaphic environment (Lançoni et al. 2013). Several methods have been used as indicators of changes in the soil microbial community. The isolation, cultivation and evaluation of microbial density in samples collected in this environment is the most widely used, due to its ease of execution (Antoniolli et al. 2010), although techniques based on the use of molecular markers may be more conclusive about the different groups of microorganisms. organisms and their ecology. These include the latest, based on extraction of microbial DNA directly from the soil (McPherson et al. 2018). However, there is little information on surveys and evaluation of the effects of environmental factors on the composition of beneficial fungal populations and plant pathogen antagonists in Brazilian soils.

*Trichoderma* fungi are often found in soil and organic matter in free-living form, adapt to different ecological conditions and colonize a multitude of substrates, as well as capable of more intimate associations with plant root systems. (Harman et al. 2004). As a constituent of the rhizospheric microbiota, *Trichoderma* acts on the translocation of minerals, solubilization and availability of nutrients to plants and the production of plant hormones. increase in productivity is related to the ability to colonize roots, while its action as a biocontroller has been attributed to the mechanisms of antibiosis, hyperparasitism, induction of resistance, favoring the plant in tolerance to biotic and abiotic stresses, solubilization and nutrient sequestration. in addition to inactivation of pathogen-linked pathogen enzymes. However, the functional variability between isolates of the same species in relation to their biocontrol and plant growth promotion activities is a well-proven fact (Martínez et al. 2013, Munir et al. 2014). Given the agricultural importance of *Trichoderma*, this work was conducted to verify the population density of this fungus in natural ecosystems and agroecosystems (organic production) of the Cerrado biome.

## Material and Methods

### *1. Collection of soil samples*

Soil samples from native vegetation and agroecological production system were collected from healthy vegetables in the Cerrado area, in four localities of the Federal District, always in the morning. In each area five subsamples of non-rhizospheric soils (NRhzS) were collected, containing 200g each, at random points, with a distance of 5 cm from the cultivated species (in ridges between ridges) and 0-10 cm deep, making up a sample composed of 1 kg. Similar procedure was adopted in natural vegetation areas, except for the absence of furrows. Roots and root fragments of the plant species were also collected for each rhizospheric soil (RhzS) collection point. Soil and root samples were individually wrapped and, from these, the attached soil was removed (Ethur et al. 2008). The collection sites were: Rajadinha Rural Nucleus II, Planaltina region, in the cultivation of pumpkin (*Cucurbita* sp.), eggplant (*Solanum melongena* L.), kale (*Brassica oleracea* L. var. *acephala* DC.), cassava (*Manihot esculenta* Crantz), Mexican Sunflower (*Tithonia* sp.), maize (*Zea mays* L.), bell pepper (*Capsicum annuum* L.), okra (*Abelmoschus esculentus* L. Moench), cabbage (*Brassica oleracea* var. *capitata* L) and tomato (*Lycopersicum esculentum* Mill.) and native plants *Cestrum* sp. (Solanaceae), *Cyathea* sp. (Cyatheaceae), *Miconia elegans* (Melastomataceae), *Tibouchina* sp. (Melastomataceae); Taguatinga Rural Nucleus, Taguatinga region, for eggplant, coffee (*Coffea arabica* L.), persimmon (*Diospyrus kaki* L.), Mexican Sunflower, maize, mucuna (*Mucuna pruriens* L. DC), tomato, pod (*Phaseolus vulgaris* L) and native crops *Cyathea* sp., *Cordiera* sp. (Rubiaceae) and *Trichilia pallida* (Meliaceae); Lamarão Rural Nucleus, Paranoá region, for eggplant, chives (*Allium schoenoprasum* L.), cabbage, spinach (*Spinacia oleracea* L.), cassava, Mexican Sunflower, maize, parsley (*Petroselinum crispum* Mill. Nym.) and native *crops Cordiera macrophylla, Miconia elegans, Zanthoxylum rhoifolium* (Rutaceae); Boa Esperança Rural Center, Ceilândia region, in the cultivation of eggplant, coffee, kale, cassava, Mexican Sunflower, maize, mucuna, pepper (*Capsicum* sp.), cabbage and tomato. In this place there was no native ecosystem area.

### *2. Fungus isolation*

For fungal isolation, 10 g of each soil sample was placed in Erlenmeyer, suspended in 90 mL of sterile water and stirred at 180 rpm at 25 ° C for 40 minutes. After the suspensions, the samples were diluted and 100 μL of each concentration were distributed in Petri dishes containing semi-selective Martin medium as described by Mello et al. (2007). For each sample four repetitions were performed.

*3. Morphological identification of fungi*

The plates were incubated at 25 ° C in B.O.D. (Biochemical Oxygen Demand) for two days in the dark and for 5-7 days with 12-hour photoperiod exposure. The cultures were evaluated daily until the appearance of a typical *Trichoderma* colony and considered as a colony forming unit (CFU). Slides were made for examination of morphological characteristics under the optical microscope and identification of the fungus at a generic level. The colonies confirmed as belonging to the genus were transferred to purified potato dextrose agar (PDA) medium, used for monosporic cultivation and stored at 4ºC.

*4. Statistical analysis*

First, it was verified whether the plant types (explanatory variable), native and cultivated, differed statistically as to the number of isolates of *Trichoderma* (response variable). This analysis was developed under the focus of Generalized Linear Models (GLMs), assigning Poisson distribution to the response variable, a natural choice for variables of this type (discrete counting). When necessary, the heterogeneity factor present in the data (overdispersion) was corrected via the Quasiverossimilitude method. Following this same logic, another model was adjusted to compare the locations within each culture. To obtain the variance analysis table in Poisson distribution GLMs, were used likelihood ratio (LR), which follows approximately Chi-Square distribution, this procedure is known as ANODEV. The analyzes were developed with the R free statistical language program. The adopted significance level was 5% (McCullagh & Nelder 1989).

## Results

From the soil samples from the four properties, 530 isolates of *Trichoderma* were obtained, 361 from agroecosystems and 169 from natural ecosystems.

*1. Analysis of results by location*

*1.1. Rajadinha Rural Center II - Property I*

Vegetation type interfered with the number of *Trichoderma* isolates obtained (LR = 12.4790, df = 1, p-value = 0.0004116), regardless of soil type (NRhzS or RhzS). Native species presented, on average, a larger number of colonies than cultivated ones (Figure 1A).

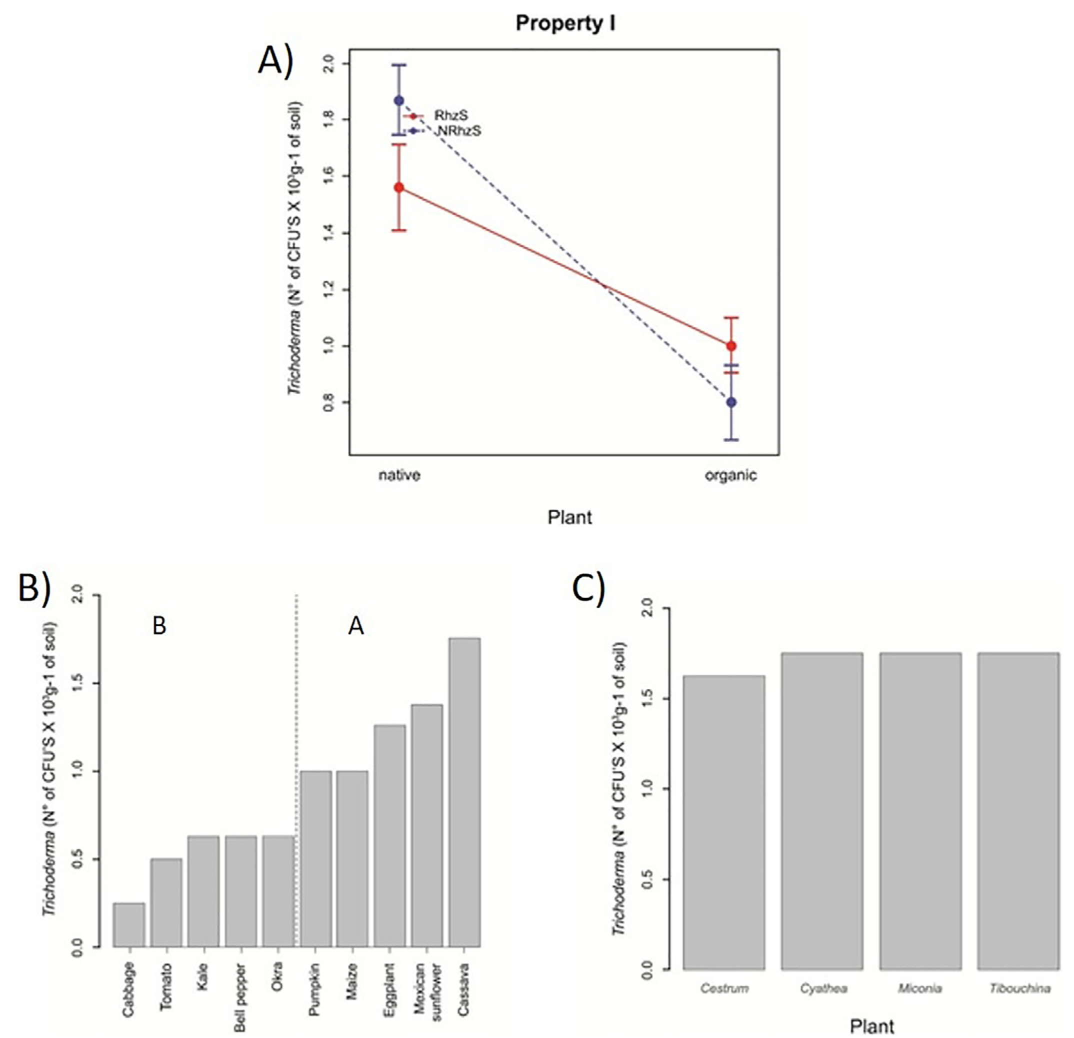


**Figure 1. A)** Average number of *Trichoderma* colony forming units (CFU) + standard deviation according to soil and plant type for property I. **B)** Average number of *Trichoderma* CFU obtained from rhizosphere of cultivated species. **C)** Average number of *Trichoderma* CFU obtained from rhizosphere of native species.

Regarding the cultivated species, significant differences were observed (LR = 17,119, df = 9, p-value = 0.04688), detecting two groups: cassava, Mexican Sunflower, eggplant, maize and pumpkin, with the highest number of CFU of *Trichoderma*; cabbage, tomato, kale, bell pepper and okra with the lowest number of CFU (Figure 1B). On the other hand, native species presented, on average, the same number of CFU (Figure 1C), not differing from each other.

*1.2. Taguatinga Rural Center - Property II*

Both soil sample type (NRhzS and RhzS) and vegetation type significantly influenced the average number of *Trichoderma* CFU (LR soil = 15.96, df = 1, p-value <0.0001; LR plant = 5.1549, df = 1, p-value = 0.02318). There was, on average, more CFU of this fungus in NRhzS than in RhzS, both for native and cultivated species (contrast = 0.39, standard error = 0.18, p-value = 0.0267), and the latter species had lower numbers. average of recovered isolates, in terms of CFU in relation to native vegetation (contrast = 0.74, standard error = 0.18, p-value <0.0001) - (Figure 2A).

Among the cultivated species there was significant difference (LR = 28.409, df = 9, p-value <0.0001). Two groups can be established: the first, presenting the highest average number of *Trichoderma* CFU composed of mucuna, pod and persimmon, coffee and Mexican Sunflower and the second group, with less CFU, containing kale, maize, eggplant, tomato and cassava (Figure 2B). Among native species, there were no significant differences in obtaining *Trichoderma* colonies (Figure 2C).

*1.3. Lamarão Rural Center - Property III*

The soil sample type (NRhzS and RhzS) had no effect on the mean number of CFU, which, however, suffered vegetation type interference (RV = 10.062, df = 1, p-value = 0.001514). Native species presented, on average, more CFU of *Trichoderma* than species of organic cultivation (Figure 3A).

There was a significant difference for at least two cultivated species regarding the average number of *Trichoderma* CFU (LR = 23,291, gl = 9, p-value = 0.005574). Mexican Sunflower and eggplant

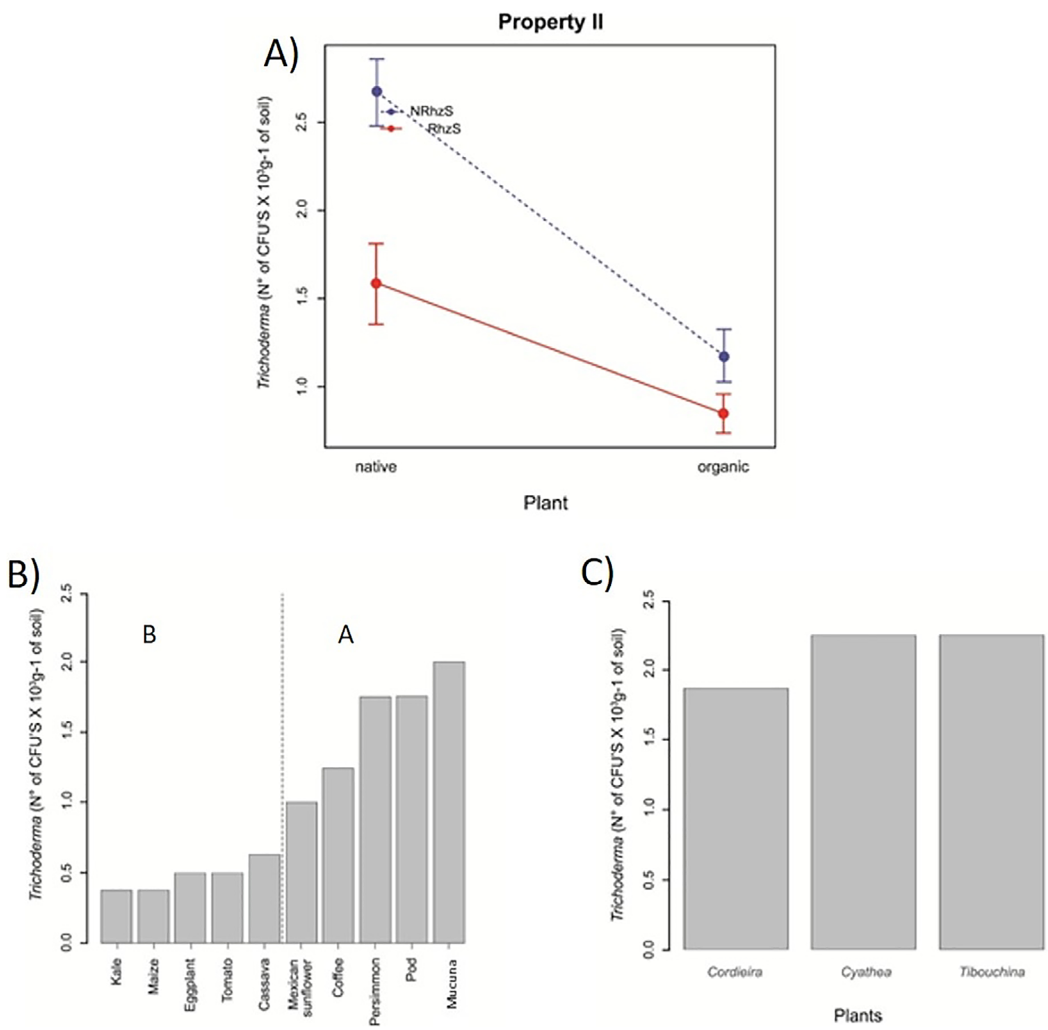


**Figure 2. A)** Average number of *Trichoderma* CFU + standard deviation according to soil and plant type for property II. **B)** Average number of *Trichoderma* CFU among cultivated species. **C)** Average number of *Trichoderma* CFU in native species.

presented more CFU than spinach, pumpkin, parsley and tomato (Figure 3B). Regarding native species, there were also differences in the number of CFU (LR = 10,312, df = 2, p-value = 0.005765): *C. macrophylla* had, on average, more CFU than *Z. rhoifolium* and *M. elegans* - (Figure 3C).

*1.4. Boa Esperança Rural Center - Property IV*

For this property, only isolates from cultivated species were analyzed. As for the type of soil sample, there was no statistical difference regarding the number of CFU (LR = 0.13437, df = 1, p-value = 0.7139) - (Figure 4A).

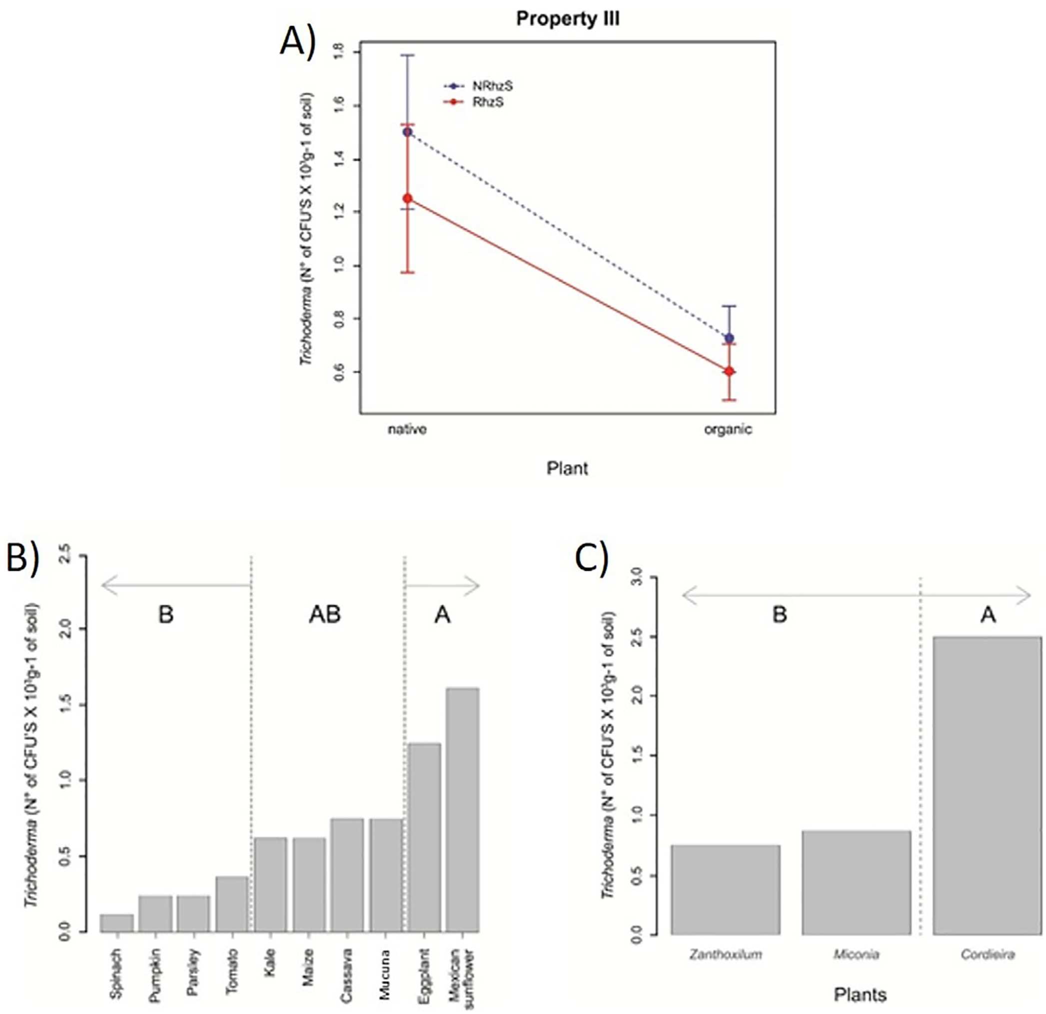


**Figure 3. A).** Average number of *Trichoderma* CFU + standard deviation according to soil and plant type for property III. **B)** Average number of *Trichoderma* CFU among cultivated species. **C)** Average number of *Trichoderma* CFU in native species

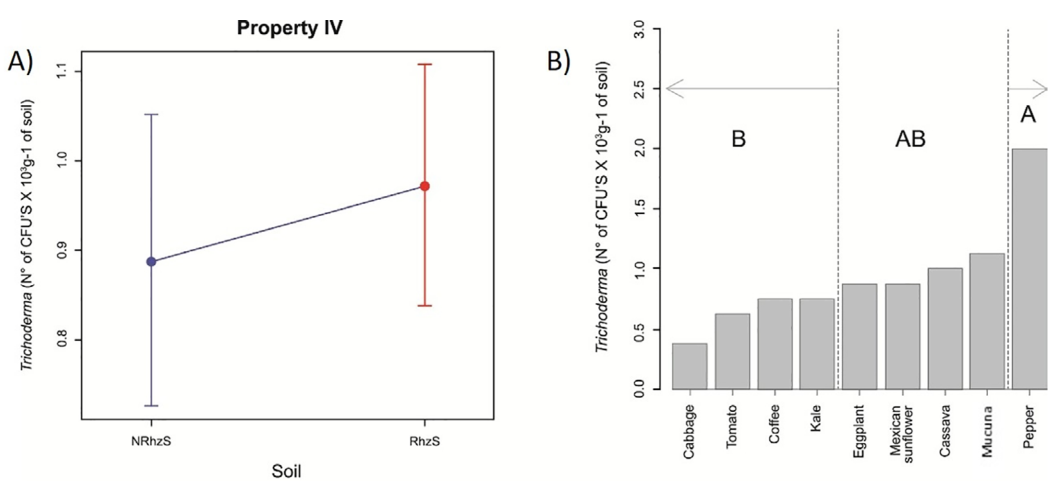


**Figure 4. A)** *Trichoderma* CFU mean number + standard deviation according to soil type for property IV. **B)** Average number of *Trichoderma* CFU among cultivated species.

In this case the average number of CFU in samples from ground cultivated with pepper was higher than in the cases of cabbage, tomato, coffee and kale (Figure 4B).

The locations for each crop were compared for the number of *Trichoderma* CFU obtained from eggplant, cassava, kale, Mexican Sunflower and tomato, species found concurrently in the four studied properties, since maize and mucuna were present in three of the four properties. With eggplant, the lowest average number of *Trichoderma* CFU was found for property II (LR = 10,059, df = 3, p-value = 0.01807). In the case of cassava, this number was higher in property I (LR = 9.5157, df = 3, p-value = 0.02317) and, with kale (LR = 1.2136, df = 3, p-value = 0.7498), Mexican Sunflower (LR = 3.1093, df = 3, p-value = 0.3751) and tomato (LR = 0.73695, df = 3, p-value = 0.8645) there was no significant difference in relation to the sample collection sites (Figure 5).

For some species (maize and mucuna), the fungus *Trichoderma* was recovered only at three sites (Figure 6). In this case, there was no significant difference between sites with corn crop (LR = 4.1822, df = 2, p-value = 0.1235) and, with mucuna, a higher number of CFUs were recovered in property II (LR = 12.552, df = 2, p-value = 0.001881).

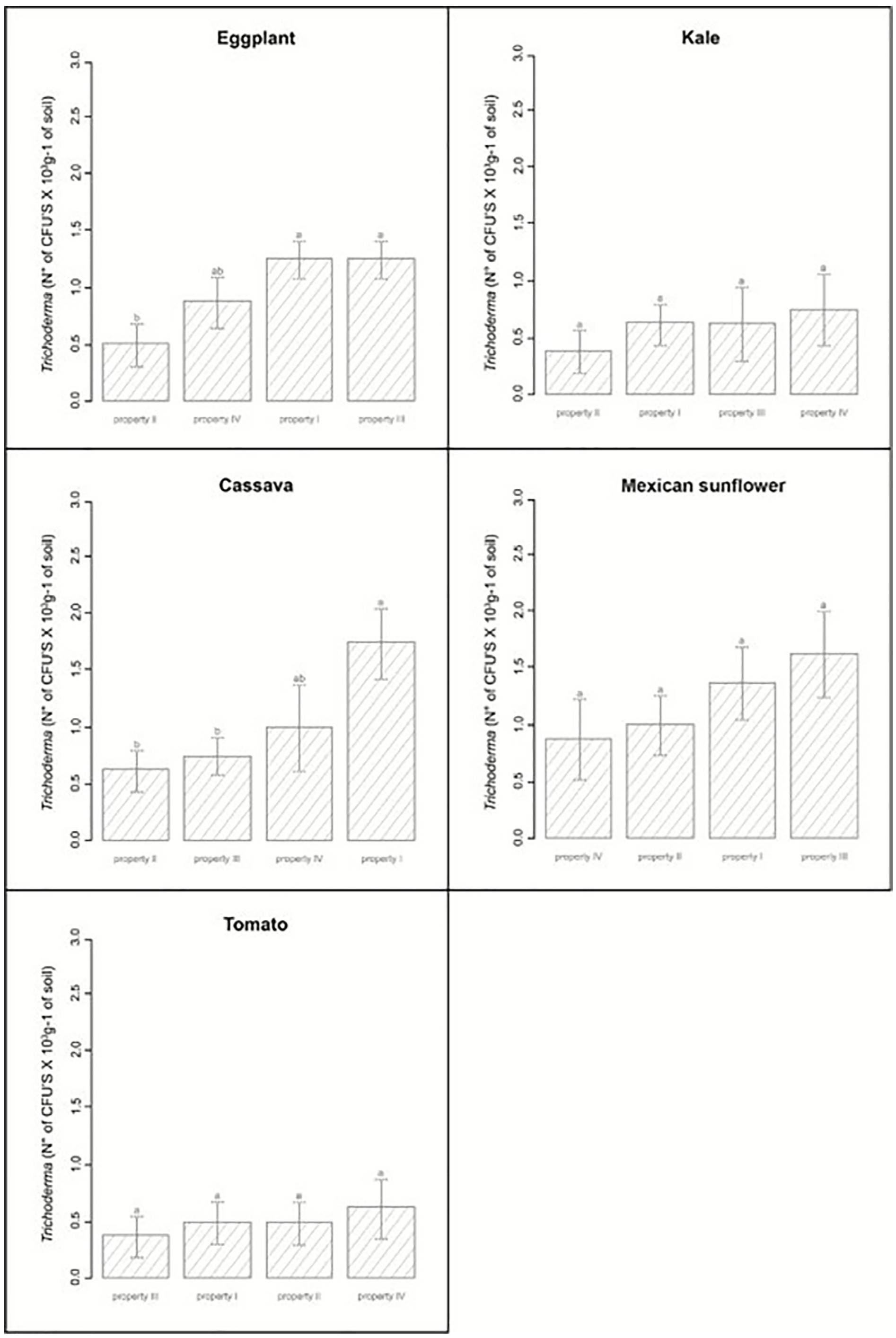


**Figure 5.** Average number of *Trichoderma* CFU + standard deviation observed at the four sites.

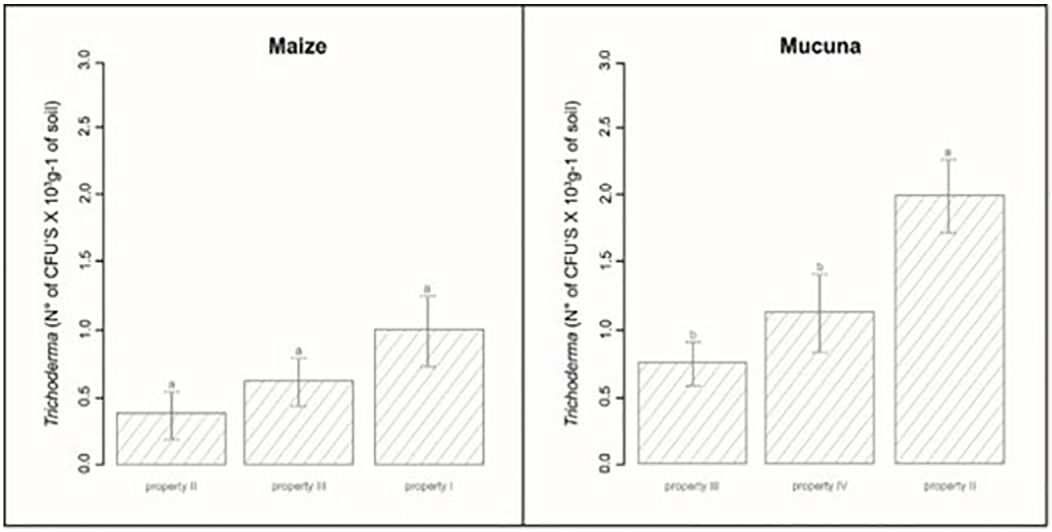


**Figure 6.** Average number of *Trichoderma* CFU + standard deviation observed at the three sites.

## Discussion

Only in property II the number of *Trichoderma* CFUs was higher in NRhzS than in RhzS (Figure 2A), probably due to the incorporation of crop residues or other organic matter in the soil, since in the other properties there were no significant differences in this number (Figures 1A, 3A and 4A). Moreover, according to Ethur et al. (2008), *Trichoderma* species settle better in soils when they contain vegetable remains and other forms of organic matter. Although in these soils there is greater interaction between microorganisms and plants (Mohammadi et al., 2011), it is also in them that the microbial flora suffers the most competition pressure (Dantas et al. 2009). Kredics et al. (2018) complement that various biotic and abiotic factors affect diversity populations of microbial communities in agroecosystems, including plant species and their growth stage, total microbial competition, pesticide or fertilizer application, as well as geographic region. However, only a few studies address the population, abundance and diversity of the genus *Trichoderma* in specific fields or agroecosystems. From the results obtained in this work, there is no need to remove parts of plant roots to obtain a representative number of *Trichoderma* isolates, since the number of *Trichoderma* isolates recovered from root-attached soil samples was similar. to non-rhizospheric soil samples. In areas cultivated with tomato and cucumber in conventional system, Ethur et al. (2008) obtained similar results. It is worth mentioning that, in studies of antagonistic potential, Jash & Pan (2007) found no differences in antagonism against *Rhizoctonia solani* when testing *Trichoderma* isolates from RhzS and NRhzS.

The areas of native vegetation, located around the crop, suffer a reduced anthropic effect, so the soil supposedly represents the ecological conditions of environmental stability because it is not influenced by disturbances of preparation and application of inputs, unlike cultivated areas, even treating it. itself from organic production. Probably, this fact explains the higher number of CFUs found in native vegetation areas in the three evaluated sites (Figures 1C, 2C and 3C), compared to those of cultivated area (Figures 1B, 2B and 3B). Brouwer & Riezebos (1998) has mentioned that in forest soils, nutrient losses from the ecosystem are lower. This provides better soil cover, higher organic matter content and greater floristic diversity, determining factors for larger soil settlement in number and microbial diversity (Ramos et al. 2012). According to Lourente et al. (2011), the diversity of native vegetation species (quantity and quality) implies the continuous deposition of organic substrates with varied composition, favoring the microbial mass content, but the substitution of native vegetation by cultivation systems may cause important changes in the attributes. soil chemicals in the first year of implementation. There are several reports that disturbances caused by land use and crops may also result in decreased microbial biodiversity (Bending et al. 2004, Mendes et al. 2012). Louzada et al. (2009), who studied the antagonistic action of *Trichoderma* isolates from various regions of Brazil against plant pathogens *Sclerotinia sclerotiorum* and *Fusarium solani*, mention studies by other authors showing that isolates from native areas would result in a higher percentage of potentially active *Trichoderma* isolates against plant pathogens in *in vitro* tests, with a mycelial growth inhibition of around 80%. However, in this work, the substitution of native vegetation by organic cultivation systems did not cause a significant reduction in the number of *Trichoderma* CFUs, confirming postulations made by different authors about the advantages of the organic cultivation system regarding the preservation of soil microflora and stabilization of agroecosystems.

Therefore, the results presented show the effect on the number of CFU of *Trichoderma*, depending on the culture and location evaluated (Figures 5 and 6). These properties, although located in Cerrado areas, have soils probably subjected to different treatments, which would have interfered with the number of recovered *Trichoderma* colonies. According to Frazão et al. (2010), soil microbial community is generally influenced by variations in soil temperature, water content and aeration, aggregate disruption, decreased soil cover, nutrient availability and organic substrates. Studies by Saravanakumar et al. (2016) from samples of coastal regions showed that the biodiversity of *Trichoderma* spp. was influenced by temperature, redox potential and pH. In the set of results, it was found a variation between *Trichoderma* population levels in the various organic crops evaluated, due to the different factors mentioned, although no correlation was studied or made regarding soil types and characteristics, only with the cropping systems.

Regarding diversity, molecular characterization work of *Trichoderma* isolates from target properties should be performed to verify the prevalent species in the soil of organic crops and native vegetation, because the methodology used for sample dilution and calculation of forming units of colonies (CFUs) do not allow the distinction of an introduced strain of *Trichoderma* populations residing in the investigated environment (Kredics et al. 2018). According to Louzada et al. (2009), there are no data in the literature reporting the loss of diversity of *Trichoderma* spp. with the continuous agricultural use of soils or even the possible relationship of such interferences with the reduction of the frequency of antagonism to pathogens. *Trichoderma* spp. are highly successful settlers in their habitats and are able to overcome adversity related to environmental variations around the world (Schuster & Schmoll 2010). Studies of this nature coupled with the knowledge of the real distribution and population dynamics of this fungal genus and its associations with different plant species and soils are crucial to ensure the efficiency and safety of the use of these microorganisms, especially in the biocontrol and promotion of plant growth.

Given the above and based on the results observed in this work, it is concluded that the types of crop and native vegetation influenced the distribution of the population of *Trichoderma* fungi in soils of organic farming system. On the other hand, the substitution of native vegetation by organic cultivation systems did not result in a significant reduction in the number of *Trichoderma* CFUs.

## Author Contributions

João Batista Tavares da Silva: substantial contribution in the concept and design of the study; contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision and adding intelectual content.

José Eustáquio Menezes: contribution to data collection, contribution to data analysis and interpretation and contribution to manuscript preparation.

Eder Marques: contribution to data analysis and interpretation and contribution to manuscript preparation.

Joseane Padilha da Silva: contribution to data analysis and interpretation.

Sueli Corrêa Marques de Mello: 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 and 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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