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Scientific Article

Biodiversity of the metazoan parasites of *Hoplias malabaricus* (Bloch, 1794) from the Jacaré-Pepira River, Tietê-Jacaré River Basin, São Paulo State, Brazil

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ABSTRACT

We recorded the parasitic fauna of *Hoplias malabaricus* in a Neotropical River from southeastern Brazil, the Jacaré-Pepira River, in the Tietê-Batalha River Basin. The objective of this study was to inventory the composition of the parasitic fauna of *H. malabaricus* collected, through ecological and biodiversity approaches. A total of 30 specimens of *H. malabaricus* were collected in 2018 using fishing nets. Parasites were prevalent in 96.6% of the hosts, with 710 parasites specimens collected, divided into 22 species from 4 different taxa (i.e., Monogenea, Digenea, Nematoda, and Acanthocephala). This was the first survey about the parasitic fauna of *H. malabaricus* in this river, where three species are new records for this host (i.e., *Gussevia* sp. 1, *Gussevia* sp. 2, and *Raphidascaris* sp.). All species found are new records for the Jacaré-Pepira River, which together with all the data obtained in the study, collaborates to increase knowledge and understanding of the global fish parasite biodiversity.

Keywords: Fish parasitology; Species inventory; Parasitic biodiversity; Parasitic ecology.

Biodiversidade dos metazoários parasitos de *Hoplias malabaricus* (Bloch, 1794) do Rio Jacaré-Pepira, Na Bacia Do Rio Tietê-Jacaré, Estado de São Paulo, Brasil

RESUMO

Aqui são apresentados os registros da fauna parasitária de *Hoplias malabaricus* em um rio neotropical do Sudeste do Brasil, o rio Jacaré-Pepira, localizado na bacia hidrográfica do Tietê-Jacaré. Trinta espécimes de *H. malabaricus* foram coletados em 2018 usando-se redes de espera. Parasitos foram prevalentes em 96,6% dos hospedeiros, com 710 espécimes coletados, divididos em 22 espécies de quatro diferentes taxas (monogenea, digenea, nematoda e acanthocephala). Esse foi o primeiro levantamento da fauna parasitária de *H. malabaricus* nesse rio, onde três espécies de parasitos são novos registros nesse hospedeiro (*Gussevia* sp. 1, *Gussevia* sp. 2 e *Raphidascaris* sp.). Além disso, todas as espécies de parasitos identificadas são novos registros para a localidade, que juntamente com todos os dados obtidos no estudo vão colaborar para melhor conhecimento e entendimento da biodiversidade global de parasitos de peixes.

Palavras-chave: parasitologia de peixes; inventário de espécies; biodiversidade de parasitos; ecologia de parasitos.

INTRODUCTION

Despite the large number of species of known organisms, it is not possible to know exactly how many are still alive and coexisting. Parasites are part of a group that often goes unnoticed when we talk about biodiversity conservation (Poulin and Morand, 2004). In fact, parasitism is one of the most successful ecological relationships once they have followed their hosts' evolution and because of the high number of parasites species that exist today (Poulin and Morand, 2004). However, the constant advance of urbanization and the increasing in environmental impacts as a result of negative anthropic activities can cause the extinction of species that have not even been described by the science yet (Carlson et al., 2017; Carlson et al., 2020; Dougherty et al., 2015).

In Brazil, the first records of studies on parasitic metazoans were about *Cercaria blanchardi*, the larval form of *Schistosoma mansoni*; from then on, several studies were recorded in the country. However, only a small part of them managed to associate the larvae with their adult stage, that is, most of the known parasites do not have their biological cycle elucidated (Pinto and Melo, 2015). Most trematode species need at least one intermediate host, usually annelids, amphibians, arthropods, echinoderms, molluscs, fish, among others. In these hosts, metacercariae, the infective forms that will be transmitted to the definitive hosts by ingestion of the second intermediate host, develops (Pinto and Melo, 2015).

In recent years, studies on parasites of fish hosts have received greater attention due to their directly impact in commercialization capacity (Luque, 2004) and the possibility of being used as highly sensitive bioindicators of environmental contamination and changes in the biodiversity (D'Amelio and Gerasi, 1997; Lafferty 1997; Sures et al., 2017; Vidal-Martínez et al., 2009), since they are directly linked to the structuring of animal communities, acting as conductors of biodiversity (Hudson et al., 2006).

This study was carried out with the trahira fish, *Hoplias malabaricus* (Bloch, 1794), a species widespread in all hydrographic basins of South America, except in the transandine area and in the rivers of Patagonia (Fowler, 1950). Fish of this species can be found in most hydrographic courses in Brazilian inland waters, giving not only preference to lentic waters but also found in small and large rivers (Azevedo and Gomes, 1943). Despite not having morphological adaptations, the species has the ability to survive in environments with low oxygen rates, which explains its great dispersion (Barbieri, 1989). It is a predatory species of the "sit and wait" type, a solitary hunter of benthic habits, being found in shallow waters near submerged or marginal vegetation (Gião et al., 2020). As an adult, trahira feeds mainly on other fish, while juvenile individuals feed on plankton (Paiva, 1974).

There are several studies on the parasitic fauna of *H. malabaricus* collected from different locations in South America (Paraná River, Doce River, Tietê River, Paranapanema River, São Francisco River, Amazon Basin, and Marajó Island, among others), where four groups were identified: Monogenea Van Beneden, 1858; Digenea Carus, 1863; Nematoda Potts, 1932; and Hirudinea Linnaeus, 1758. However, in the Jacaré-Pepira River, there are no studies about the parasitic diversity of this fish host till present.

The objective of the study was to inventory the composition of the parasitic fauna of *H. malabaricus* collected in the Jacaré-Pepira River, Tietê-Jacaré Basin, state of São Paulo, Brazil, through ecological and biodiversity approaches.

MATERIALS AND METHODS

Study area

The Jacaré-Pepira River (Figure 1) has its source located in the municipality of São Pedro (22º32'55"S and 47º54'50"O), in the central portion of the state of São Paulo, and its mouth at 400 m altitude, on the Tietê River in the municipality of Ibitinga (21º45"28"S and 48º49'44"O) (Almeida et al., 1999; Barrella, 1989; Metzger et al., 1998). It has large wetlands in its floodplain, popularly known as "Pantaninho Paulista"; in addition to being one of the most conserved rivers in the basin, with great biodiversity, it is inserted in an Environmental Protection Area (EPA), Ibitinga (CBH-TJ, 2015). Its vegetation is predominantly the Brazilian Savanna, but it has some remaining areas of Seasonal Semideciduous Forest. It has a hot summer with dry winter, with average temperatures: maximum 32°C and minimum 5°C and an annual rainfall index of 1500–2000 mm (IPMET, 2017).

Fish sampling

The collections were made monthly at the same point of the Jacaré-Pepira River, until the number of 30 *H. malabaricus* specimens were reached.

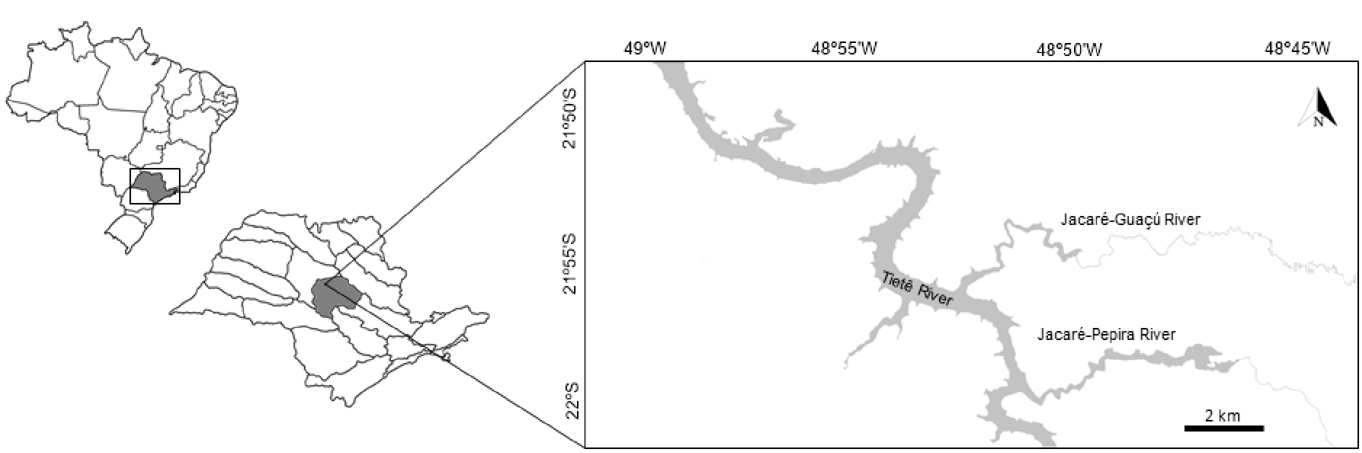

Figure 1. Location of the study area in Jacaré-Pepira River, Tietê-Jacaré sub-basin, state of São Paulo, Southeast Brazil.

For fish collection, we used fishing nets with meshes ranging from 2 to 10 cm, made of nylon. Collections were made with the support of local fishermen. After collection, fish were anesthetized with a clove oil (eugenol 150 mg/L) solution and then euthanized using medullary section (method authorized by the Comitê de Ética no Uso de Animais [CEUA] of the Universidade do Sagrado Coração, authorization nº 3353050417). All collection methodology was made under the authorization of the Chico Mendes Institute for Biodiversity Conservation (ICMBio), through the Biodiversity Information System (SISBIO) (authorization number: 40998-3).

Procedures for collecting, recording, processing, and identifying parasites

Collected fish were packed in individual plastic bags in the field to preserve the integrity of the parasitic fauna. The fish were then transported in a thermal box to the laboratory and kept refrigerated in a freezer until necropsy, where data about the individuals' weight (g), standard length (cm), total length (cm), and sex were recorded.

The collection, registration, and processing of endoparasites and ectoparasites was carried out according to the procedures indicated by Eiras et al. (2006).

Statistical analysis

The prevalence, mean intensity, and mean abundance of each component of the parasitic infracommunities were calculated according to the methodology by Bush et al. (1997). Parasitic diversity was determined for each infracommunity by the Brillouin index (HB) (Zar, 1999) and by the Shannon Diversity Index (H′). Margalef richness index (d) (Zar, 1999) and Pielou uniformity index (J′) were also calculated. The Mann-Whitney U test (Zar, 1999) was applied to determine the effect of the sexes in relation to the parasitic abundance of each species of parasite. The distribution pattern was obtained using the Green Index (GI), statistical test d, and the Dispersion Index (DI).

The sampling efficiency was verified by the observed species richness accumulation curve (sobs) together with the Bootstrap richness estimator, the most suitable for small samples. All the tests mentioned above were applied only to parasite species with a prevalence higher than 10%.

RESULTS

The 30 specimens of *H. malabaricus* necropsied and analyzed had an average standard length of 27±3.20 cm and an average weight of 426.04±127.86 g. Of this total, 17 were male and 13 were female, and only one fish specimen showed no infection/infestation by any species of parasite, totaling 96.6% of parasitized fish hosts.

A total of 710 parasite specimens were collected, divided into 4 taxa (i.e., Monogenea, Digenea, Nematoda, and Acanthocephala) and in 22 species (Table 1). The species with the highest prevalence was *Contracaecum* sp. (53.3%) and *Austrodiplostomum compactum* (Lutz, 1928) (4.26 parasites per analyzed fish), which was the most abundant parasite found. The highest value of medium intensity was found in the species *Raphidascaris* sp. (60 parasites per infected host).

Regarding community status, 19 species were classified as satellite species and 3 as a secondary species. All species with a prevalence higher than 10% obtained an aggregate distribution pattern according to the DI and GI (DI>1 and GI>0) (Table 2). Only the species *Gussevia* sp. 1 had their abundance influenced by the hots' sex [Z(U)=1.9252, p=0.0271].

The Margalef, Brillouin, and Shannon-Wiener indexes had mean values of, respectively, 0.88±0.52, 0.66±0.50, and 0.79±0.56, indicating high richness and diversity, and about the Pielou index, the mean values (0.83±0.18) indicate a high uniformity in the distribution of species in parasitic infracommunities.

Among the digenean species, only *Phyllodistomum* sp. and *Dendrorchis* sp. were found in adult form and the species *A. compactum*, *Sphincterodiplostomum musculosum* Dubois, 1936, Bucephalidae gen. sp., *Ithyoclinostomum* sp., *Posthodiplostomum* sp., Diplostomidae gen. sp., and *Clinostomum* sp. were in the metacercariae stage. The three species of nematodes found, i.e., *Contracaecum* sp., *Hysterothylacium* sp., and *Rhaphidascaris* sp., were in the L3 larval stage. The only species of Acanthocephala present was in the larval cystacanth stage and was not identified.

The observed species richness accumulation curve (Sobs) and the Bootstrap richness estimator did not show stability (Figure 2), indicating that the continuity in the sampling effort of the host fish can increase the parasite richness found.

DISCUSSION

The data obtained in the present study are the first records of the parasitic fauna of *H. malabaricus* for the Jacaré-Pepira River and have a relatively high diversity of species, mainly of ectoparasites of the Monogenea class. These higher rates for monogenean parasites are due to the habits of its larvae stages, which are found, most of the time, in lentic environments, which facilitates their transmission in hosts (Yamada et al., 2007), since *H. malabaricus* is also considered a species of environments with low current and abundant vegetation (Azevedo and Gomes, 1943; Gião et al., 2020).

The genus *Urocleidoides* was the one with the highest number of recorded species (*Urocleidoides cuiabai*, *Urocleidoides brasiliensis*, *Urocleidoides malabaricusi*, *Urocleidoides eremitus,* and *Urocleidoides naris*), the same number of species described by Gião et al (2020) in Rio Batalha, also belonging to the Upper Parana River Basin, and also by Ferreira et al. (2018) in six coastal river locations in the northeastern sector of the state of Pará (Eastern Amazon). According to Rosim et al. (2011), the presence of several species of the genus *Urocleidoides* can be interpreted by their high capacity for colonization and speciation in several individuals of different species and families.

Table 1. Prevalence, mean abundance, mean intensity, infestation/infection site, and community status of the parasitic fauna of *Hoplias malabaricus* (Block 1794), collected from the Jacaré-Pepira River, Tietê-Jacaré River Basin, state of São Paulo, Brazil.

Taxa	P (%)	MA	MI	IS	CS
Monogenea					
Gussevia sp. 1	23.3	2.9±1.3	12.7±4.1	G	SA
Gussevia sp. 2	6.6	0.2±0.1	4	G	SA
Urocleidoides cuiabai Rosim et al., 2011	3.3	0.1±0.1	5	G	SA
Urocleidoides brasiliensis Rosimet al., 2011	10	1.5±1.1	15±9	G	SA
Urocleidoides malabaricus Rosim et al., 2011	10	0.4±0.2	4±1	G	SA
Urocleidoides eremitus Kristsky, et al., 1986	3.3	0.03±0.03	1	G	SA
Urocleidoides naris Rosim et al., 2011	20	0.9±0.4	4.5±0.8	N	SA
Dactylogyridae gen. sp.	6.6	0.9±0.7	14.5±5.5	G	SA
Gyrodactylus trairae Boeger & Popazoglo 1995	10	0.1±0.08	1.3±0.1	BS/N	SA
Digenea					
Phyllodistomum sp.	13.3	0.2±0.1	1.7±0.1	UB/AC	SA
Austrodiplostomum compactum Lutz, 1928	50	4.2±1.1	8.5±1.5	E	SE
Sphincterodiplostomum musculosum Dubois, 1936	3.3	0.03±0.03	1	E	SA
Bucephalidae gen. sp.	3.3	0.4±0.4	12	BS	SA
Ithyoclinostomum sp.	3.3	0.2±0.2	6	ST/AC	SA
Posthodiplostomum sp.	13.3	0.3±0.2	2.5±1.1	ST/AC	SA
Diplostomidae gen. sp.	16.6	0.5±0.5	3.2±1.7	E	SA
Clinostomum sp.	3.3	0.03±0.03	1	BS	SA
Dendrorchis sp.	3.3	0.03±0.03	1	I	SA
Nematoda					
Contracaecum sp.	53.3	3.6±1.0	6.8±1.5	I/IC	SE
Hysterothylacium sp.	36.6	3.7±1.8	10.1±4.4	SB	SE
Raphidascaris sp.	3.3	2.0±2.0	60	I	SA
Acanthocephala					
Cistacanth	16.6	0.9±0.4	5.4±1.2	IC	SA

G: gills; N: narine; BS: body surface; UB: urinary bladder; AC: abdominal cavity; E: eye; H: heart; ST: stomach; I: intestine; IC: intestinal cecum; SB: swimming bladder; SA: satellite; SE: secondary; P: Prevalence; MA: mean abundance; MI: mean intensity; IS: infestation/infection site; CS: community status.

Although the Monogenea class has great specificity in relation to its hosts, two species of the genus *Gussevia* were found and are new records for the host and, possibly, new species. This discovery reinforces the importance of studies in environments that have not yet been explored, as these represent a great opportunity for the occurrence of species that have not yet been described by science. In addition, *Gussevia* sp. 1 was the only species that had its abundance rates influenced by the sex of the hosts, where female fish was the most parasitized individuals by this species.

The hosts' physiological and biological factors may be related to the influence of sex on parasitic communities (Takemoto and Pavanelli, 2000). Lizama et al. (2005) reported considerable gender differences for the occurrence of *Tereancistrum curimba* (Lizama et al., 2005), found in *Prochilodus lineatus* (Valenciennes, 1836), where most of the parasitized hosts were females. The stress caused by the reproductive period can be one of the factors that led to the occurrence of this influence both in the study by Lizama et al. (2005) and in the present study, since during this period there may also be changes in the host's behavior, which facilitates parasitism (Takemoto and Pavanelli, 2000).

The high prevalence rate of larvae *Contracaecum* sp. follows the same patterns found in other studies with the same host (Fabio, 1982; Moravec et al., 1993; Moravec et al., 1997; Madi and Silva, 2005; Martins et al., 2005; Olivero-Verbel et al.,

Table 2. Dispersion index, Green Index, and statistical test d of the parasites of *Hoplias malabaricus* (Bloch, 1794), collected in the Jacaré-Pepira River, Tietê-Jacaré Basin, São Paulo state, Brazil, with prevalence higher than 10%.

Species	DI	d	GI
Gussevia sp. 1	18.5	25.2	18.4
Urocleidoides brasiliensis Rosim et al. (2011)	25.1	38.2	25.1
Urocleidoides malabaricusi Rosim et al. (2011)	4.2	15.6	4.2
Phyllodistomum sp.	1.9	10.7	1.9
Austrodiplostomum compactum Lutz, 1928	8.6	22.3	8.5
Diplostomidae gen. sp.	6.6	19.7	6.6
Contracaecum sp.	8.9	22.8	8.9
Cistacanth	5.7	18.3	5.7
Hysterothylacium sp.	26.7	39.3	26.6
Gyrodactylus trairae Boeger & Popazoglo 1995	1.4	9.0	1.3
Urocleidoides naris Rosim et al. (2011)	7.7	21.2	7.7
Posthodiplostomum sp.	4.0	15.2	3.9

DI: Dispersion index; GI: Green Index; d: statistical test “d”.
SI>1=aggregate distribution. d≥1.96=aggregate distribution. GI>0=aggregate distribution (the higher the value, the greater the degree of aggregation).

2006), and this occurs due the life cycle of these parasites, which use fish as secondary hosts or paratenic hosts (Moravec et al, 1993), with the feeding dynamics of *H. malabaricus*, a species considered piscivorous as an adult (Paiva, 1974). Their eating habits can lead to the accumulation of these parasites in the body of these hosts, justifying the high rates found.

In relation to community status, species are divided into central, secondary, or satellites, which are predictions of the hypothesis that lead to the distribution of species within an environment. The central species are those that are abundant locally and common (Bush et al., 1997), but that were not registered in the present work. The secondary ones (*A. compactum*, *Contracaecum* sp., and *Hysterothylacium* sp.) have intermediate values, when compared to the extremes (central and satellite) (Stock and Holmes, 1988), presenting a high dispersion power (Kennedy, 2001), and the satellites species are locally uncommon and tend to appear in small quantities, being considered rare (Bush et al., 1997). In the work, 19 species presented themselves as satellite species.

The reasons that may have led to the classification of the species *A. compactum*, *Contracaecum* sp., and *Hysterothylacium* sp. as secondary can be related to the habits of life and feeding of the fish hosts, already mentioned, since the infection by *A. compactum* occurs actively, when free cercariae natants penetrate the skin of the host, and the infection by *Contracaecum* sp. and *Hysterothylacium* sp. occur by eating smaller infected fish.

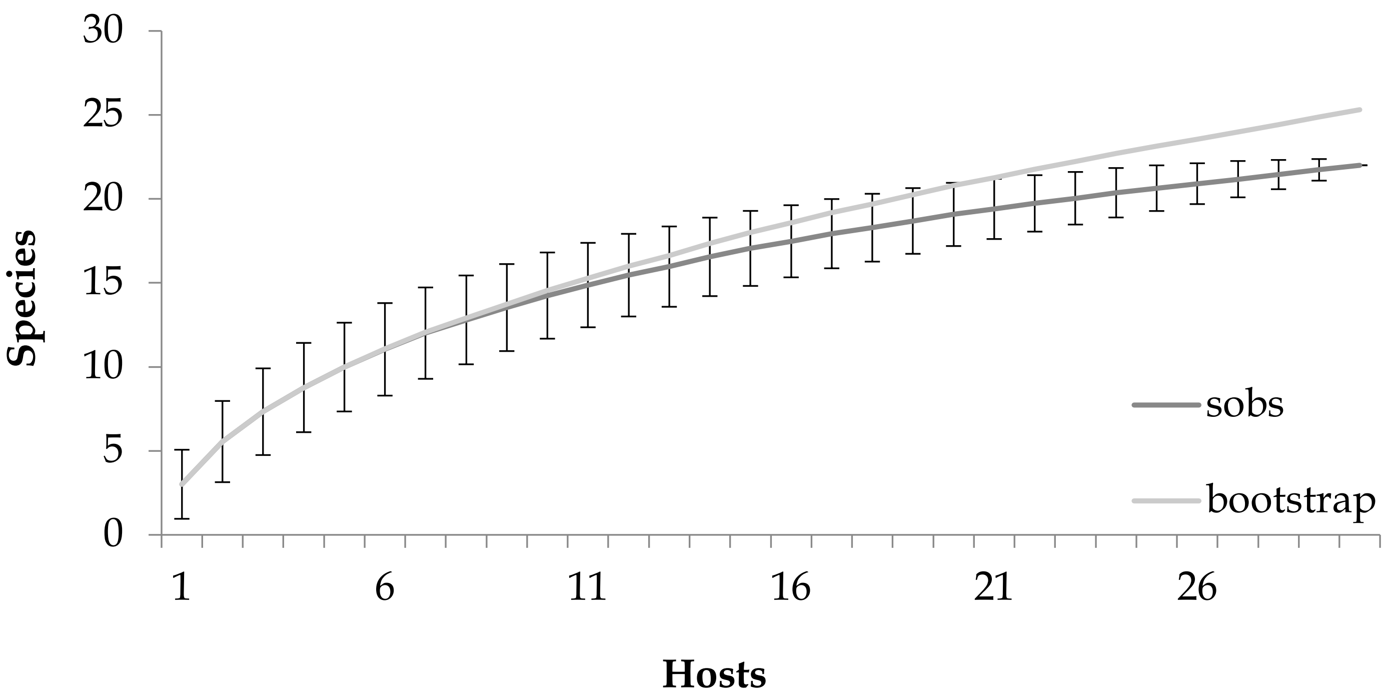

Figure 2. Richness accumulation of observed species (sobs) and Bootstrap richness estimator of the parasitic fauna of *Hoplias* malabaricus (Bloch, 1794), collected from the Jacaré-Pepira River, Tietê-Jacaré Basin, São Paulo state, Brazil.

The aggregate distribution pattern highly found in parasitic communities occurs mainly due to interference from climatic factors in the environment, genetic differences, differences in the level of infection due to the host, and behavioral and physiological heterogeneity (Zuben, 1997; Shaw and Dobson, 1995). Zuben (1997) highlighted that this aggregate distribution pattern favors community stability, which minimizes the occurrence of intraspecific interactions between parasites.

Parasitic communities showed high species richness (d=0.88), diversity (HB=0.66), and equitability (J′=0.83), when compared to the values found by Gião et al. (2020) (d=0.71, HB =0.52, J′=0.55), who carried out a survey of the parasitic metazoans of the same host in the Batalha River, state of São Paulo, with a number of specimens of *H. malabaricus* analyzed almost three times higher than the number of analyzed fish in the present study, and with 29 different species of parasites, 7 species more than those found in the present work. These high values, especially of equitability, found in the present study demonstrate that despite the number of species found being smaller compared to that found by Gião et al. (2020), they are more equitably distributed. *Hysterothylacium* sp. and *Raphidascaris* sp., registered high rates of mean abundance and intensity, once the only record made so far on *Hysterothylacium* sp. in this host was carried out by Leite et al. (2021), where the presence of this parasite was reported in the same region of the present study. About *Raphidascaris* sp., this is the first record for this host.

The large number of digenetic species can be justified by the fact that the fish act as both intermediate, harboring the larval phases, and definitive hosts, with the adult stage of the parasite (Takemoto et al. 2009), and also by the feeding habit of *H. malabaricus*. About *Clinostomum* sp. metacercariae, they can cause a pathogenesis known as “yellow spot disease,” where pigmented cells are concentrated around the cyst (Dias et al., 2003). Metacercariae from the Diplostomidae family, which are found parasitizing the structures responsible for the fish’s vision, cause a disease called “helminth cataract” or diplostomiasis, which in extreme cases can lead to loss of the animal’s vision (Martins et al., 1999).

The richness accumulation curve of the observed species and the richness estimator did not reach the asymptote, following the same patterns found in studies carried out by Gião et al. (2020), Pelegrini et al. (2018), and Leite et al. (2018) with other hosts, and with *H. malabaricus* in the same region where the Jacaré-Pepira River is inserted. This may be an indicator that even with a larger number of hosts analyzed and with new studies such as that of Lehun et al. (2020), who identified and classified 315 parasite taxa, of which 201 were identified at species level, the biodiversity of the parasitic communities in rivers of the Upper Paraná River basin is not yet fully known, which intensifies the importance of studies on the characterization of ichthyofauna for that region.

The parasitic fauna of *H. malabaricus* from the Jacaré-Pepira River proved to be well diversified and with the occurrence of greater numbers of individuals of ectoparasitic species, possible new species (*Gussevia* sp. 1 and *Gussevia* sp. 2), and new records for the host (*Gussevia* sp. 1, *Gussevia* sp. 2, and *Raphidascaris* sp.). These discoveries can assist in the study and understanding of the geographic distribution of these species. All species found are new records for the Jacaré-Pepira River, which, together with all the data obtained in the study, collaborates to increase knowledge and understanding of global biodiversity.

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CONFLICT OF INTERESTS

Nothing to declare.

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AUTHORS’ CONTRIBUTION

Bueno, R.M.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, writing – original draft. Leite, L.A.R.: writing – review & editing. Pelegrini, L.S.: writing – review & editing. Abdallah, V.D.: funding acquisition, resources, supervision, validation. Azevedo, R.K.:funding acquisition, resources, supervision, validation

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