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Original papers

New records and prevalence of metazoan parasites of fish in the southeastern Brazilian region

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ABSTRACT. *Hoplias* aff. *malabaricus* is abundant in the Mogi-Guaçu River. The aim of this study was to perform an inventory of the species of metazoan that parasite this species of fish taken from oxbow lakes of the Mogi-Guaçu River. The Mann-Whitney test was used to statistically analyze the possible influence of the sex of the host on the group with the highest parasite richness and the greatest abundance of parasites. Simpson's diversity index was used to determine parasite diversity among the zoological groups of parasites of *H.* aff. *malabaricus* with the highest index. A total of 78 specimens of *H.* aff. *malabaricus* were examined. Among the zoological groups of metazoans found, the phylum Nematoda had the greatest number of species. Among these, the larval stage of *Contracaecum* sp. was most abundant. The sex of the host had a significant effect, with parasites more abundant in female fish ($Z(U)=0.043$; $p<0.05$). The digenean *Parspina argentinensis*, the nematodes *Procamallanus* (*S.*) *iheringi*, *Rhabdochona acuminata* and *Hysterothylacium* sp. and copepods *Vaigamus* sp. and *Lernaea cyprinacea* have not previously been recorded as parasites of *H.* aff. *malabaricus*.

Keywords: freshwater fish, fish parasites, helminths, lake

Introduction

According to [1], total fish diversity is estimated at 27,977 species, with 4,500 continental fish species found in neotropical regions [2]. The Parana River basin, which is the second largest in South America and the fourth largest in the world, has approximately 350 recorded fish species [3]. The Mogi-Guaçu River is one of the most heavily studied among the rivers of the Upper Paraná River basin, having long been the subject of fish fauna studies, such as those by [4–14] and more by [15].

This intense research into the fish of the Mogi Guaçu River has resulted in several parasitological studies. The first studies of metazoan parasites of fish from this river were coordinated by the researcher Lauro Travassos and dates from the second decade of the twentieth century [16], with research subsequently continued by others [17–23]. Many of these studies were limited to qualitative approaches or focused on the zoonotic potential of larvae, such as those by *Eustrongylides ignotus* and *Contracaecum multipapillatum* [24].

With the aim of increasing knowledge of the parasitic fauna of fish from the Mogi-Guaçu River, metazoan parasites of the native fish species, *Hoplias malabaricus* were analyzed, focusing on infrapopulations.

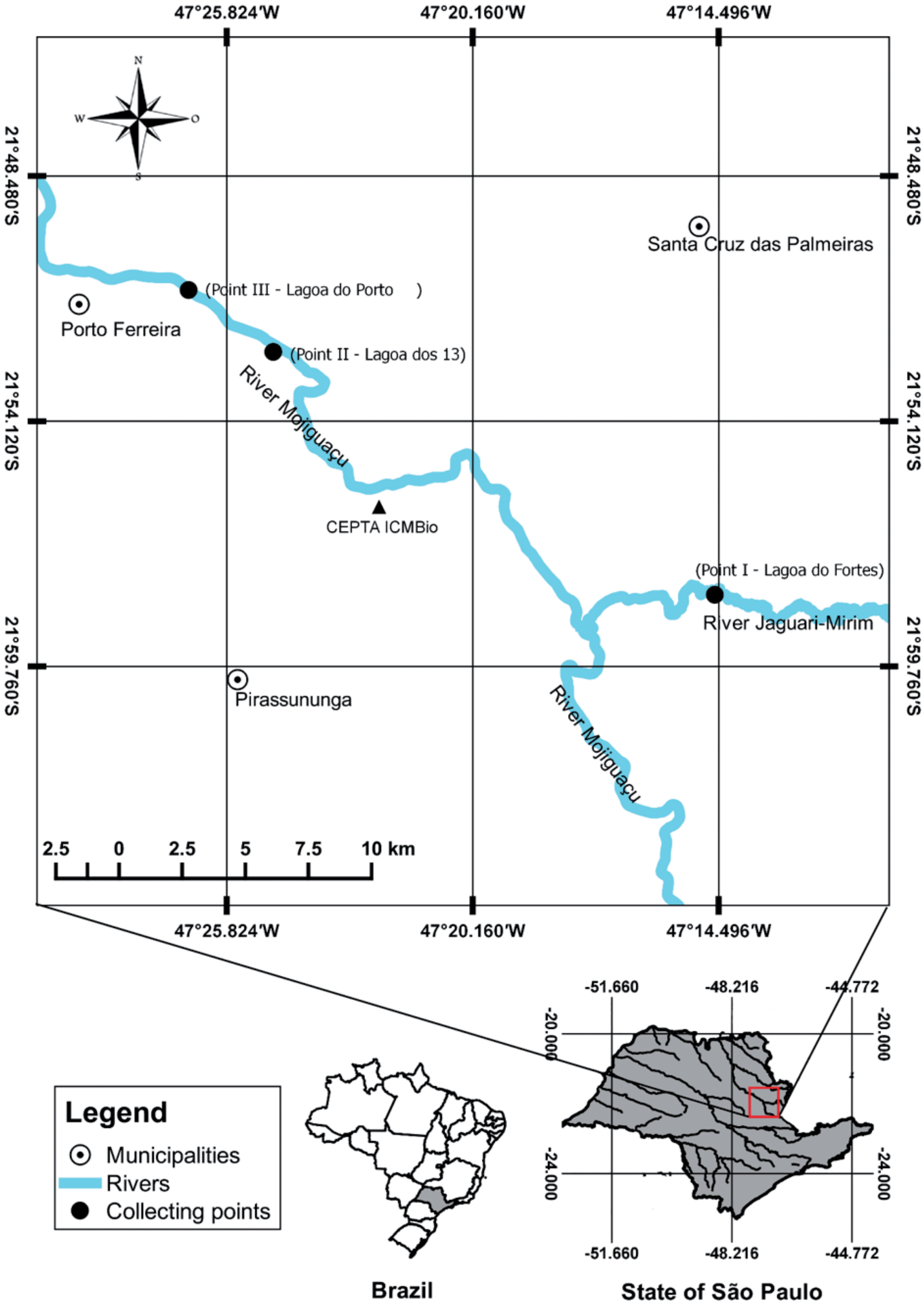

Figure 1. Map of Brazil, highlighting the Paraná River Basin, the Sub-basins of the Mogi-Guaçu River and collection points (Quantum GIS-1.5, 2012; Geosystec, 2008). Point I; Point II and Point III.

Table 1. Geographical position and number of sampled specimens of *H.* aff. *malabaricus* at captured in oxbow lakes of the Mogi-Guaçu River, were collected between February 2010 and June 2012

Location	Geographic Coordinates	(n=fish collected)
Mogi-Guaçu		
Point I- Fortes lakes	(47°14'35,2"W; 21°58'05,9"S)	(n=39)
Point II- 13 lakes	(47°24'41,2"W; 21°52'26,2"S)	(n=17)
Point III- Porto lakes	(47°26'42,2"W; 21°51'05,6"S)	(n=22)

Materials and Methods

The specimens of *H.* aff. *malabaricus* were captured in lakes of the Mogi-Guaçu River. Samples were collected between February 2010 and June 2012, and 78 fish from three different collection points were analyzed (Fig. 1). The sites were georeferenced (GPS) (Table 1).

The specimens of *H.* aff. *malabaricus* were captured with gillnets with different mesh sizes (30 and 35 mm). After collection, the fish were euthanized by spinal cord transection in the cervical region, and parasites were collected and fixed in accordance with [25]. Identification of nematode parasites was performed in accordance with [19] and [26–29]. Digeneans and Acanthocephala were identified in accordance with [29], crustaceans in accordance with [30] and the Monogenea were identified in accordance with [30–32].

Statistical analysis was performed using the SAS statistical software program [33]. The Mann-Whitney *U* test was used to assess the possible influence of the sex of the host on the group with the highest parasite richness and the most abundant parasite species. Spearman's rank correlation coefficient, r_s, was used to determine possible correlations between the total length of the host and the abundance of infection. Pearson correlation coefficient, *r*, was used to determine the possible correlation between the total length of the host and

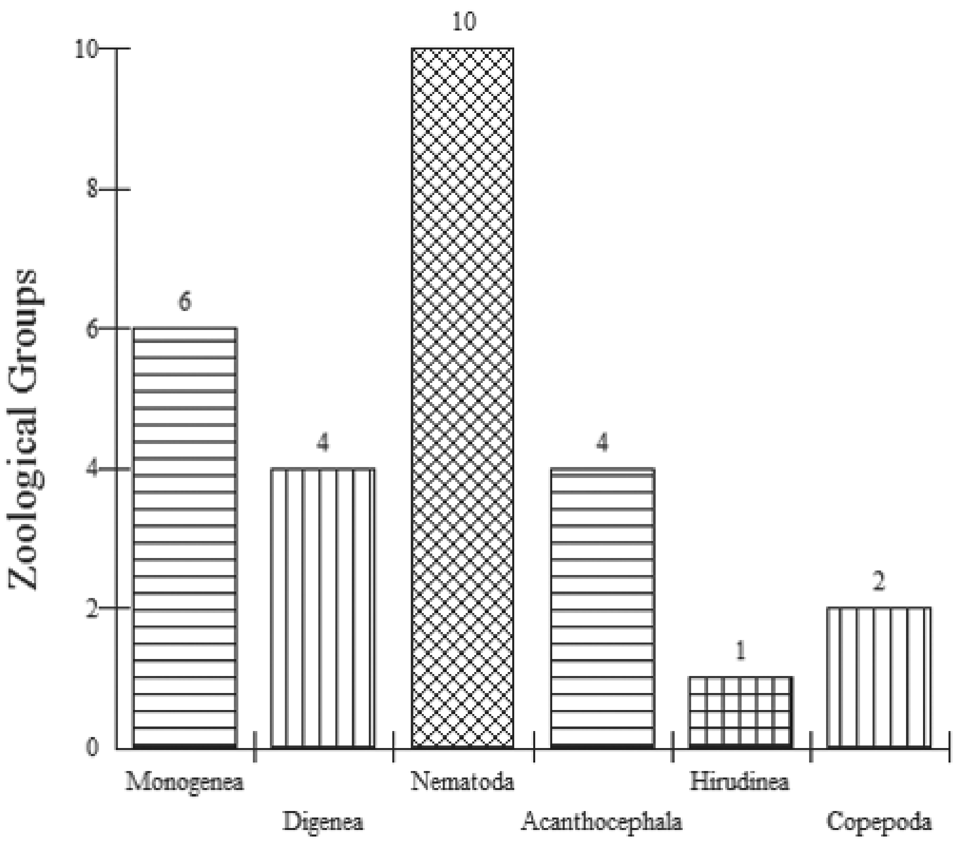

Figure 2. Richness of parasite species obtained from specimens of *Hoplias* aff. *malabaricus,* by zoological groups in oxbow lakes of the Mogi-Guaçu River, were collected between February 2010 and June 2012

marasite prevalence.

The Chi-squared test (χ^2) with Yates correction was used to analyze the influence of the host collection site on the prevalence of zoological groups and the most abundant species of parasite of *H.* aff. *malabaricus.* The tests cited were applied to species of parasites with a prevalence above 10%, according to the recommendations of [34], and a statistical significance level of $p \leq 0.05$ was adopted [35]. The parasitological terms used, such as prevalence and abundance, were used in accordance with [36].

Simpson's diversity index was used to determine diversity among the zoological groups of parasites of *H.* aff. *malabaricus* with the highest index, with this index calculated with the ESTIMATES 2.0 software program. The Simpson diversity index ranges from 0, low diversity, to almost 1, high diversity [37].

The relative condition factor (*Kn*) was calculated for the host species with the greatest parasitic diversity. The relative condition factor corresponds to the ratio between the weight observed (Wo) and the theoretically expected weight for a given length, i.e., *Kn*=Wo/We [38]. The constants a and b of the length-weight ratio were used to estimate the theoretically expected body weight values (We), using the formula *We*=a.Ls b, in which Ls corresponds to standard length.

Results

All the 78 fish examined were considered adults because of their developed gonads. A total of 27 *H.* aff. *malabaricus* were male and 51 were female, with a mean length of 26.55±6.86 cm and a mean weight of 248.47±67.87 g.

A total of 1,713 parasite specimens were studied. These were from 27 species, with an Mean abundance of =33.5; ±27.8. The parasite species belonged to five taxonomic groups: the phylum Nematoda, the Platyhelminthes (Monogenea and Digenea), Acanthocephala, Annelida (Hirudinea) and Crustaceans (copepods). Among these taxonomic groups, the phylum Nematoda was the richest, with ten species. Of the other taxonomic groups, the number of species varied from one (Hirudinea) to six (Monogenea) (Fig. 2).

The infection site with the highest diversity of parasites was the intestine, where a total of 12 species were found (one species of Digenea, seven species of Nematoda and four species of Acanthocephala). Most species found were in the adult form, but some were identified in the larval

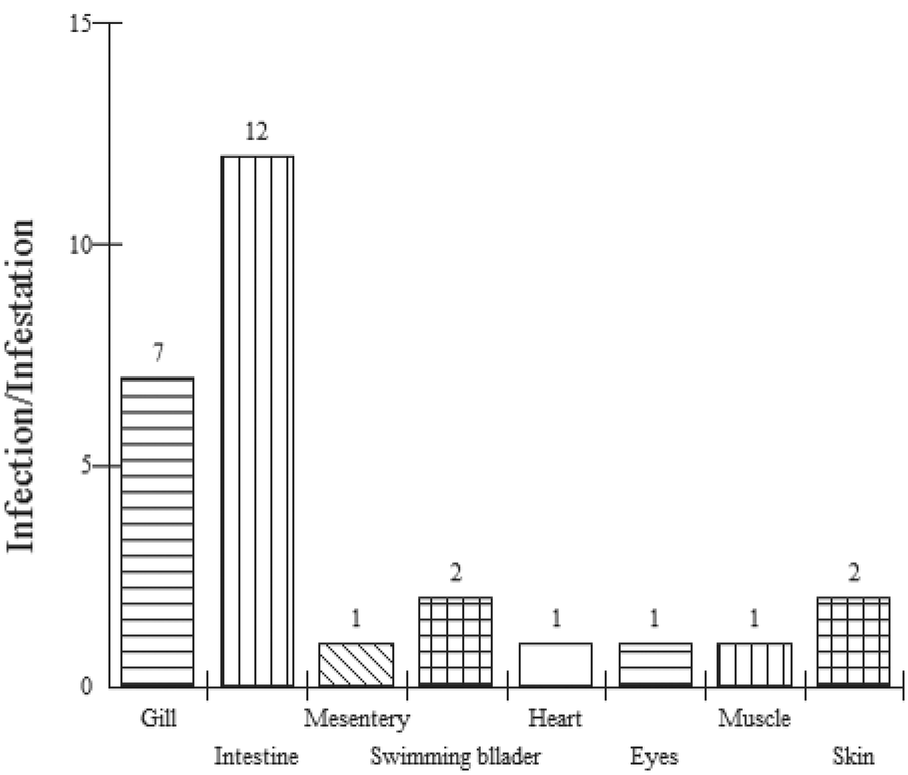

Figure 3. Richness of species of parasite from *Hoplias* aff. *malabaricus* by infection/infestation site in oxbow lakes of the Mogi-Guaçu River, were collected between February 2010 and June 2012

Table 2. Parasite taxon grouped according to host species of *H.* aff. *malabaricus* were captured in oxbow lakes of the Mogi-Guaçu River, were collected between February 2010 and June 2012. Asterisk: first report for this host; DN: deposit number; SI: site of infection; P%: prevalence; A: abundance; L: larva; G: gills; E: eyes; SB: swim bladder; H: heart; I: intestine; M: mesentery; Mu: muscles; Sk: skin.

					Collection Points		
Parasites	**DN**	**SI**	**P%**	**A**	**PI**	**PII**	**PIII**
MONOGENEA							
Anacanthorus sp. nov. 1	Zuec pla 06	G	17.94	1.53	+	–	–
Anacanthorus sp. nov. 2	Zuec pla 07	G	8.97	0.43	–	+	–
Anacanthorus sp. nov. 3	Zuec pla 08	G	6.41	0.26	–	–	+
Dactylogyridae gen. sp.	Zuec pla 21	G	34.61*	2.69	–	+	+
Urocleidoides eremitus	Zuec pla 10	G	52.56	2.30	+	+	+
Urocleidoides cuiabai	Zuec pla 11	G	10.25	0.38	–	+	+
DIGENEA							
Austrodiplostomum compactum	Zuec pla 16	E	25.64	1.67	+	+	–
Diplostomum sp.	Zuec pla 18	SB	3.84	0.26	+	+	–
Ithyoclinostomum dimorphum	Zuec pla 19	H	14.10	0.32	+	+	+
Parspina argentinensis	Zuec pla 20	I	2.57*	0.19	+	–	–
NEMATODA							
Contracaecum sp. (L)	Zuec nm 03	M	75.64	3.34	+	+	+
Eustrongylides sp. (L)	Zuec nm 05	Mu	10.25	0.15	+	–	+
Goezia spinulosa	Zuec nm 06	I	5.12	0.12	–	+	–
Hysterothylacium sp. (L)	Zuec nm 07	SB	6.42*	0.26	–	–	+
Paraseuratum soaresi	Zuec nm 08	I	1.28	0.07	+	+	–
Procamallanus (*S.*) *amarali*	Zuec nm 09	I	2.28	0.05	+	–	+
Procamallanus (*S.*) *hilarii*	Zuec nm 10	I	39.74	0.49	–	+	+
Procamallanus (*S.*) *iheringi*	Zuec nm 11	I	20.52*	0.35	+	–	+
Procamallanus (*S.*) *innopinatus*	Zuec nm 12	I	15.38	0.31	+	+	–
Rhabdochona acuminata	Zuec nm 13	I	2.56*	0.05	–	+	+
ACANTHOCEPHALA							
Acanthocephala sp. (L)	Zuec aca 01	I	5.12	0.15	+	+	–
Neoechynorhynchus (*N.*)	Zuec aca 03	I	12.82	0.21	+	–	+
Quadrigyrus machadoi	Zuec aca 04	I	10.25	0.19	+	+	–
Quadrigyrus torquatus	Zuec aca 05	I	5.12	0.11	+	–	+
HIRUDINEA							
Glossiphonidae gen. sp.	Zuec cli 03	Sk	6.41	0.19	+	–	–
COPEPODA							
Vaigamus sp.	Zuec cru 474	G	16.67*	0.41	–	–	+
Lernaea cyprinacea	Zuec cru 475	Sk	6.41*	0.34	+	+	–

orm. The gills had the second highest number of parasites, being infected by six species of Monogenea and one of Copepoda (Fig. 3 and Table 2).

All the specimens of *H.* aff. *malabaricus* were infected by one or more species of parasite. Nematoda was the most prevalent taxonomic group, found in 64.1% of the specimens examined, followed by Monogenea (44.8%), Digenea (29.5%), Acanthocephala (15.4%), Copepods (19.2 %) and Hirudinea (6.4%). The prevalence rates of the species of parasite found ranged from 1.28% for *Paraseuratum soaresi* [28] to 75.64% for the larval form of *Contracaecum* sp. (Table 2).

The abundance of parasite significantly differ between points ($F_{(2,47)} = 23{,}904$; $P<0.001$) (Fig. 4).

Immature forms of Nematodes from the Anisakidae (*Contracaecum* sp.) family were the most abundant parasites in 59 of *H.* aff. *malabaricus* found in the three lakes studied.

Larvae and adults of Nematode group had the largest parasite richness, and the Mann-Whitney Test showed a significant difference between the sexes, with females of *H.* aff. *malabaricus* more parasitized than males (Z(*U*)=0.014; p<0.05). Among the Nematodes found in *H.* aff. *malabaricus*, the larval stage of *Contracaecum* sp. was most abundant, and there were significant differences between the sexes of the host, with female fish having greater abundance (Z(*U*)=0.043; p<0.05).

While the prevalence of Nematoda from the three ponds was greater, these differences were not significant when the collection points were compared [(χ^2=0.68); ($P_{1(a+b+c)}$ *p*=0.41; *U*=369.50), ($P_{2(a+b+c)}$ *p*=0.47; *U*=729.00) and ($P_{3(a+b+c)}$ *p*=0.62; *U*=217.30). The collection site of the hosts did not significantly influence the prevalence of *Contracaecum* sp., even though it was the species with the highest prevalence in the three lakes studied (χ^2=0.91, *p*=0.34).

The prevalence of the five species of parasite correlated with the overall length of *H.* aff. *malabaricus*. For *Urocleidoides eremitus* [39], *Contracaecum* sp. and *Procamallanus (Spirocamallanus) hilarii*, the correlations were positive and for Dactylogyridae gen. sp., *A. compactum* and *Procamallanus* (*Spirocamallanus*) *iheringi* [40], the correlations were negative (Table 3).

The group of parasites with the greatest diversity in *H.* aff. *malabaricus* was the Nematodes (n=10; *S*=0.9065), followed by Monogenea (n=6; *S*=0.7928) and Digenea, and Acanthocephala (n=4; *S*=0.7667 for both). As previously described, among the nematodes, *Contracaecum* sp. was the most abundant (Table 2). Based on this data the relative condition factor (*Kn*) of specimens of *H.* aff. *malabaricus* infected and uninfected with *Contracaecum* sp., resulted in a *Kn*=1.073 and *Kn* of 1.129 respectively. The Mann-Whitney U test showed significant difference between the mean relative condition factor of infected specimens of *H.* aff. *malabaricus* compared with uninfected specimens ($n=78_{(A+B)}$; Z(*U*)=2.29; *p*=0.01).

Discussion

Although *H.* aff.. *malabaricus* is widely distributed, being found in all the river basins of South America [41] and is considered a complex species [42–45] until the beginning of the second decade of

Table 3. Spearman (r_s) and Pearson (r) coefficient correlation values evaluating the relationship between total length of species of *H.* aff. *malabaricus* and abundance and/or prevalence captured in oxbow lakes of the Mogi-Guaçu River, were collected between February 2010 and June 2012

Parasites	r_s	*P*	R	*P*
MONOGENEA				
Anacanthorus sp. 1	-0.162	0.111	-0.631	0.219
Dactylogyridae gen. sp.	-0.182	0.116	-0.932*	0.026
U. eremitus	0.401*	<0.001	0.950*	0.034
U. cuiabai	0.132	0.244	0.312	0.615
DIGENEA				
A. compactum	-0.457*	<0.001	-0.713*	0.015
I. dimorphum	0.123	0.294	0.307	0.615
NEMATODA				
Contracaecum sp. (larva)	0.392*	<0.001	0.868*	0.045
Eustrongylides sp. (larva)	0.059	0.520	0.682	0.320
P. (*S.*) *hilarii*	0.376*	<0.001	-0.091	0.762
P. (*S.*) *iheringi*	-0.420*	<0.001	0.813*	0.035
P.(*S.*) *innopinatus*	-0.207	0.071	0.267	0.658
ACANTHOCEPHALA				
Neoechinorhynchus (*N.*) *macronucleatus*	0.075	0.531	0.568	0.319
Quadrigyrus machadoi	-0.186	0.213	-0.681	0.210
COPEPODA				
Vaigamus sp.	0.041	0.310	0.629	0.225

* significant Spearman and Pearson correlations occurred

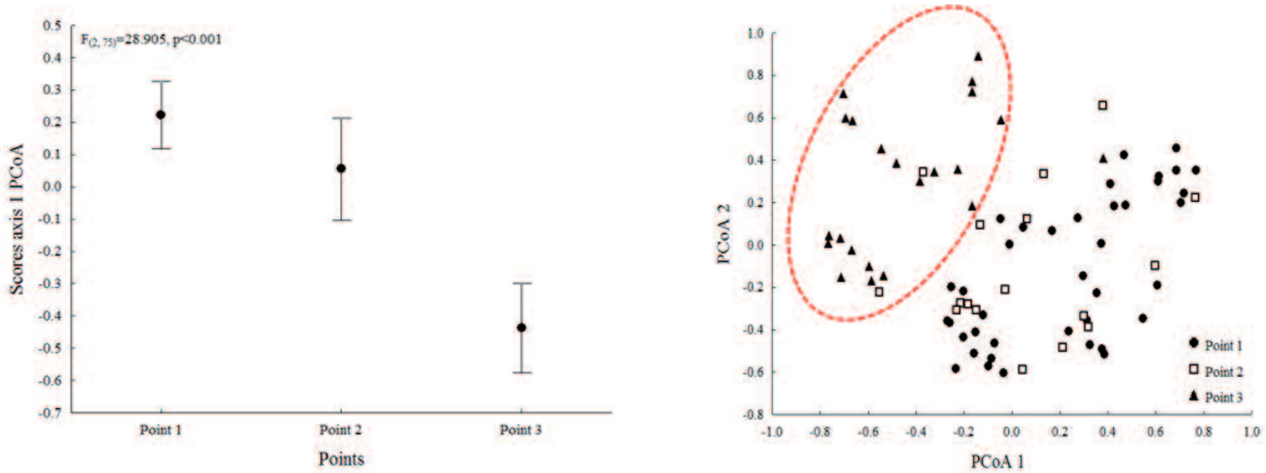

Figure 4. Representation of the mean abundance of parasites per sample points (Point 1, point 2 and point 3). Bars represent confidence interval of 95%.

this century the diversity of Monogenea described for this fish was restricted to *Urocleidoides eremitus* [39] found in fish from the Amazon region, and *Gyrodactylus trairae* [46], found in fish examined in the state of Rio de Janeiro [39,47].

According to a study by [32], which examined specimens of *H.* aff. *malabaricus* from different regions of Brazil and described *Urocleidoides malabaricusi*, *Urocleidoides naris*, *Urocleidoides cuiabai*, *Urocleidoides brasiliensis* and species of Monogenea have been recorded that could not be included in any known genre, giving them the status of Dactylogyridae gen. sp., considerably expanding knowledge of the diversity of Monogenea in this host. In this study the occurrence of *U. eremitus* and *U. cuiabai* and Dactylogyridae gen. sp. infecting *H.* aff. *malabaricus,* corroborated the work of [32].

The results of a study by [32] revealed the extent to which the diversity of Monogenean parasites of *H.* aff. *malabaricus* remains underestimated, and how such diversity may be increased by studies focused on the different regions of occurrence of this species/species complex. [47], in studies of *H.* aff. *malabaricus* in the Upper Paraná River, described for the first time the presence of Monogenea of the genus *Anacanthorus* infecting the gills of hosts from the *Hoplias* genus. In the present study in oxbow lakes of the Mogi-Guaçu River, the presence of three species of the genus *Anacanthorus* infecting the gills of *H.* aff. *malabaricus* were identified, corroborating the results obtained by [48] who found the *Anacanthorus* genus in two lakes in Pirassununga, although the results of the two studies differ in terms of prevalence.

Some species of Digenea have a low degree of host specificity. Metacercariae of *Austrodiplostomum compactum*, parasitic forms found in the eye and cranial box of fish, were recorded for the first time in the Paraná River Basin in *Plagioscion squamosissimus* in the Itaipu Hydroelectric Power Plant reservoir by [49]. A prevalence of 95% and 397 parasites infecting one fish [50]. Because of this high prevalence, this trematode species was probably introduced along with its definitive host [49]. In the present study prevalence was much lower, but this parasite is ecologically important, as it lives in the eyes of fish, impairing vision and changing behavior, making them susceptible to predators. This change in behavior was first described in *H.* aff. *malabaricus* from the São Francisco River by [51]. This behavior was not observed in *H.* aff. *malabaricus* in the present study.

In the present study only two young specimens of *H.* aff. *malabaricus* parasitized by *Parspina argentinensis* in the intestine were found. According to [16], who first described a list of fish and their parasites, collected between 1927 and 1985, from the bed of the Mogi-Guaçu River, the only parasites of *H.* aff. *malabaricus* were immature forms of *Amplicaecum* sp. (1983); *Procamallanus (Spirocamallanus) iheringi* (1927) and *Procamallanus (Spirocamallanus) inopinatus* (1946). As can be seen in the present study, *H. malabaricus* has a much greater parasitical diversity than that described by [16], in particular with regard to nematodes and Monogeneas. This is probably due to the greater number of fish specimens collected and to the fact that the fish are from oxbow lakes of the Mogi-Guaçu River.

The results of the present study reported and analyzed infections/infestations quantitative and ecological by species of Nematoda, Monogenea, Digenea, Acanthocephala Copepoda and Hirudinea in *H.* aff. *malabaricus*. According to [52] some nematodes parasites of fish have low degrees of host specificity. The *Procamallanus (Spirocamallanus) inopinatus* nematode, for example, has been recorded in 51 fish species in Brazil. Thus, parasites which have host specificity, such as Monogenea [53] or require intermediate hosts, such as Digenea [54] may be more difficult to introduce/dispersion in new ecosystems.

Parasite species from various zoological groups were found in *Hoplias* aff. *malabaricus*. However, a study by [16] reported the occurrence of the Nematode *Amplicaecum* sp., *P.* (*S.*) *iheringi* and *P.* (*S.*) *inopinatus* in fish from the Mogi-Guaçu River.

Of the zoological groups of parasites found infecting *H.* aff. *malabaricus* in the present study. Among the species of nematode found in *H.* aff. *malabaricus*, *Contracaecum* sp. was the most abundant species. A similar result was reported in a study by [55], where the authors attributed this finding to the predation of smaller fish, resulting in cumulative infection of *H.* aff. *malabaricus*. Contrastingly [55] reported a prevalence of 14% for *Contracaecum* sp., less than the 75.64% found in the present study.

A study by [56] of 61 *H.* aff. *malabaricus* in oxbow lakes of the Mogi-Guaçu River, found a high abundance of *Contracaecum* of 90.4%, and that males were more infected than females. In the present study, the abundance of the nematode group, *Contracaecum* sp. was, however, significantly higher in female *H.* aff. *malabaricus,* in contrast to that described by [56]. This fact may be explained by the fact that female fish expend more energy during the breeding period, feeding more and becoming more susceptible, according to [57].

When the calculation of the relative condition factor (*Kn*) of *H.* aff. *malabaricus* infected and uninfected with *Contracaecum* sp. was analyzed, it could be seen that infection significantly influences the condition of the body of the fish, a finding which differs from results obtained by [56] where physical improvements were observed in *H. malabaricus* from oxbow lakes of the Mogi-Guaçu River that were uninfected with *Contracaecum* sp.

The only reports of traira from the Brazilian Amazon are of fish parasitized by Acanthocephalan of the species *Grasilisentis variabilis* and *Quadrigyrus brasiliensis* [30]. First reported traira parasitized by Acanthocephalan of the species in the city of Pirassununga in the state of Sao Paulo in southeastern Brazil [40]. First reported the species *Q. machadoi* parasitizing traira in São Paulo, and in addition to the species mentioned above, *H. malabaricus* infected with *Q. torquatus*, *N. (N.) macronucleatus*, *N. (N.) paraguayensis*. The present study, found only the larvae of *N. (N.) macronucleatus*, *Q. machadoi* and *Q. torquatus* parasites of *H.* aff. *malabaricus*. These were frequently found in Lake I of the Mogi-Guaçu River [21].

Of the Copepods recognized in Brazil, *Lernaea cyprinacea* (Linnaeus, 1758), was introduced a few years ago, with the start of cultivation of the common carp *Cyprinus carpio*. Although this parasite may have been introduced, there is a possibility that it was a parasite of native fish of the [58]. Describes the first case in rivers of Porto Alegre [58], this study supports this hypothesis and describes the first report of *H.* aff. *malabaricus* collected in oxbow lakes of the Mogi-Guaçu River in Points I and II. Differences in the diversity of parasites found in different species of fish have been mainly related to the environment and trophic levels [59].

Therefore, in the present study, these oxbow lakes are characterized by the presence of a wide variety of habitats and fish species, acting as a natural nursery, confirmed by the existence of the wide parasite diversity in *H.* aff. *malabaricus*. The present study recorded the digenean *P. argentinensis*, the nematodes *P. iheringi, R. acuminata* and *Hysterothylacium* sp. and the copepods *Vaigamus* sp. and *L. cyprinacea* all of which were previously unrecorded as parasites of *H.* aff. *malabaricus*.

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Authorized by ICMBio - No 27447-2/2010-2011, and procedures for fish collection and euthanasia were approved by the ethics committee of UNICAMP-CEUA - No 2090-1. Following

identification representative specimens of the parasites were deposited in the Zoological Collection of the Zoology Museum of UNICAMP.

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