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The occurrence of *Austrodiplostomum compactum* (Lutz, 1928) (Digenea: Diplostomidae) metacercariae in the eyes of loricariid fish (Siluriformes: Osteichthyes: Loricariidae) from Brazil

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Abstract

The aim of this study was to report the occurrence of *Austrodiplostomum compactum* metacercariae in the eyes of 98 specimens of loricariid fish (*Hypostomus ancistroides*, *H. hermanni*, *H. iheringii*, *H. margaritifer*, *H. regani*, *H. strigaticeps*, *Hypostomus* sp. and *Megalancistrus parananus*) from the Chavantes reservoir (23°07′36″S and 49°37′35″W) located in the rio Paranapanema, upper Paraná river basin, municipality of Ipaussu, São Paulo State, Brazil. Fish were collected from October 2007 to February 2009 using nylon monofilament gill nets and transported to the field laboratory where they were euthanized and the eyes were taken and examined under a stereomicroscope. *Hypostomus ancistroides* and *M. parananus* were not infected by this diplostomid. *Hypostomus hermanni* and *H. margaritifer* were represented by only one specimen but both had a high intensity of *A. compactum* metacercarie (27 and 35, respectively). *Hypostomus strigaticeps* ($n = 45$) and *H. iheringii* ($n = 28$) were the most representative specimens and the prevalence, mean intensity of infection and mean abundance were 24.4%, 10.3 and 2.7, and 64.2%, 13.1 and 8.4, respectively. No correlation was observed between the intensity of infection and the standard length ($r = -0.223$; $P = 0.827$) and weight ($r = 0.03$; $P = 0.779$) of studied fish. Similarly, linear regression among these variables showed a poor correlation and indicated that the infection by *A. compactum* metacercariae occurs similarly in small and large fish specimens. A seasonal pattern of infection was not observed. *Hypostomus hermanni*, *H. iheringii*, *H. margaritifer* and *H. strigaticeps* were new hosts recorded for *A. compactum* metacercariae. A review of morphometric data of *A. compactum* metacercariae is presented.

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Introduction

Austrodiplostomum compactum (Lutz, 1928) (Niewiadomska, 2002a, b) is a digenetic trematode of the order Strigeoidea whose metacercariae (fig. 1) occur in the eyes of a wide variety of fish species (Ostrowski-Núñez, 1982; Pineda-López, 1985; Osorio-Sarabia *et al.*, 1987; Garcia *et al.*, 1993; Kohn *et al.*, 1995; Pavanelli *et al.*, 1997; Almeida, 1998; Silva-Souza, 1998; Martins *et al.*, 1999, 2002; Santos *et al.*, 2002; Paes *et al.*, 2003; Machado *et al.*, 2005; Novaes *et al.*, 2006; Yamada *et al.*, 2008; Zica *et al.*, 2009). The metacercariae of these digeneans can infect the vitreous humour (Scholtz *et al.*, 1995; Silva-Souza, 1998; Amato *et al.*, 2001) and, more rarely, the brains of the intermediate fish hosts (Ostrowski-Núñez, 1982; Silva-Souza, 1998; Amato *et al.*, 2001).

In Brazil, *A. compactum* metacercariae have been reported previously in fishes of the orders Characiformes, *Serrasalmus maculatus* (Kner, 1858) (Characidae), *Hoplias malabaricus* (Bloch, 1794) (Erythrinidae) and *Schizodon borellii* (Boulenger, 1900) (Anostomidae); Siluriformes, *Hypostomus regani* (Ihering, 1905) (Loricariidae) and *Auchenipterus osteomystax* (Miranda-Ribeiro, 1918) (Auchenipteridae); and Perciformes, *Cichla ocellaris* (Block & Schneider, 1801), *Cichla monoculus* (Spix & Agassiz, 1831), *Crenicichla britskii* (Kullander, 1982), *Cichlasoma paranaense* (Kullander, 1983), *Geophagus brasiliensis* (Quoy & Gaimard, 1824), *Satanoperca pappaterra* (Heckel, 1840) (Cichlidae) and *Plagioscion squamosissimus* (Heckel, 1840) (Scianidae) (see Machado *et al.*, 2005; Novaes *et al.*, 2006; Yamada *et al.*, 2008; Zica *et al.*, 2009).

Loricariid fish (family Loricariidae) have scarcely been examined for diplostomids. Amato *et al.* (2001) found diplostomid metacercariae of an undetermined species on the surface of, and within, the kidney ducts, on the surface of the liver and the peritoneum, and within the abdominal cavity and brain of *Loricariichthys anus* (Valenciennes, 1840) from Rio Grande do Sul State, Brazil.

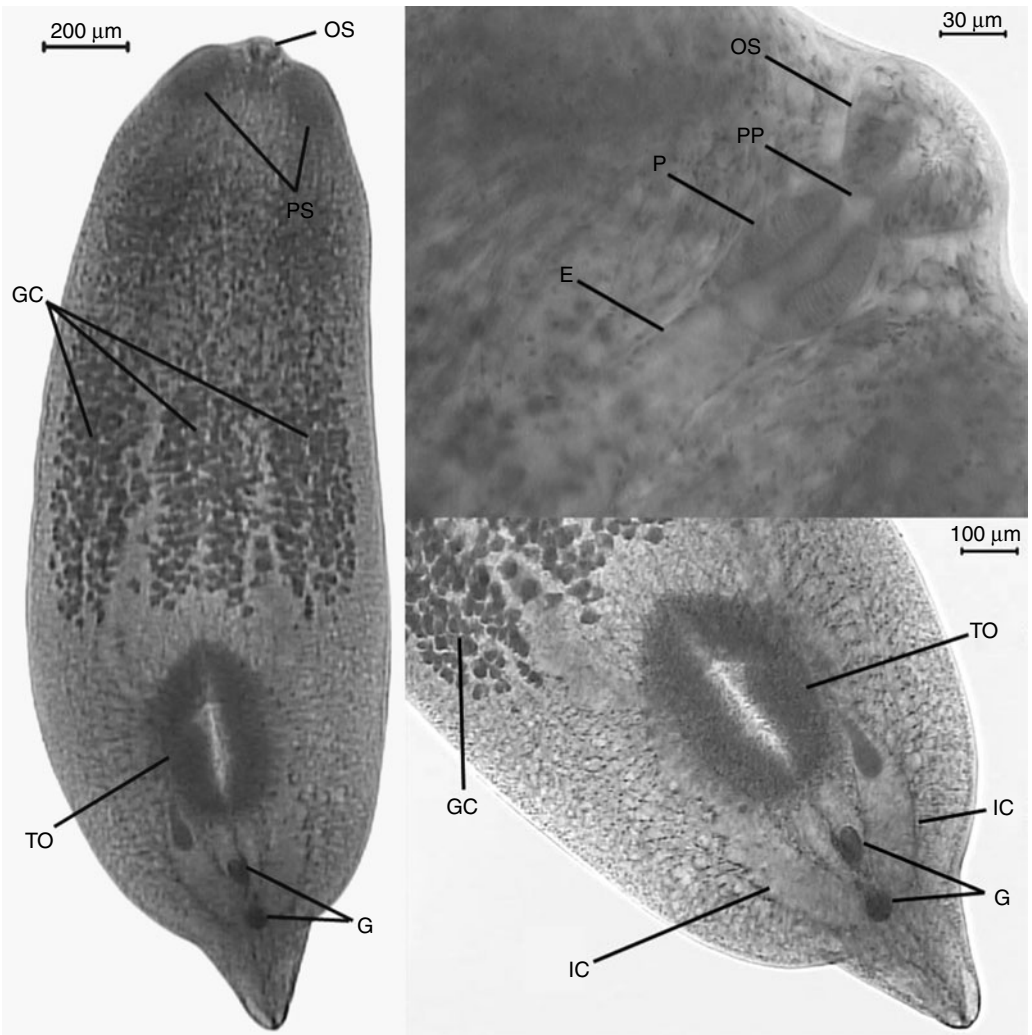

Fig. 1. Specimen of *Austrodiplostomum compactum* metacercaria (Digenea: Diplostomidae). E, oesophagus; G, gonads; GC, gland cells; IC, intestinal caeca; OS, oral sucker; P, pharynx; PP, prepharynx; PS, pseudo-suckers; TO, trybocitic organ.

Hypostomus regani was recently recorded as a host for *A. compactum* metcercariae (Yamada *et al.*, 2008; Zica *et al.*, 2009). However, no other studies of infection by these metacercariae in loricariid fish have been conducted.

Based on the previous report on the occurrence of *A. compactum* metacercariae in fish of the genus *Hypostomus* (Yamada *et al.*, 2008; Zica *et al.*, 2009), the aim of the present study was to investigate the occurrence of infection by *A. compactum* metacercariae in the eyes of loricariid fish from Chavantes reservoir (see fig. 2), in the upper Paraná river basin, in the municipality of Ipaussu, São Paulo State, Brazil, in order to determine whether the reports of Yamada *et al.* (2008) and Zica *et al.* (2009) are incidental cases or whether these metacercariae are commonly distributed among loriicarid fish.

Materials and methods

The study was carried out from October 2007 to February 2009 in the Chavantes reservoir (fig. 2), in the upper Paraná basin, municipality of Ipaussu, São Paulo State, Brazil. The Chavantes reservoir is located in the middle of the Paranapanema River, between the states of São Paulo and Paraná (Sampaio, 1944). The Chavantes hydroelectric power plant has an installed power capacity of approximately 414 MW. The dam is located at 480 m altitude, and its hydrographic basin includes large rivers such as the Paranapanema, Itararé and Verde. It is a reservoir of the accumulation basin type, with maximum depth of 70–90 m near the barrage, operational useful maximum quota of 474 m, total volume of $9.410 \times 10^6\ m^3$, an area of 27,500 km^2 and 400 km^2 water mirror in its operational maximum quota (Duke Energy, 2010).

Fish were collected using a gillnet of nylon monofilament installed 2 days/month in two sites (Site 1: 23°07′30.71″S and 49°37′37.31″W; Site 2: 23°07′59.23″S and 49°36′10″W) of the reservoir (fig. 2), from 17.00 hours until 07.00 hours the following day, totalling 14 h of exposure. The collected loricariid fish specimens were transported to the field laboratory where they were measured (cm) and weighed (g). All data were presented as mean ± standard deviation (minimum – maximum) values. Then the fish were euthanized and the eyes were taken and examined under a stereomicroscope. Metacercariae found in the vitreous humour were fixed in AFA (alcohol–formaldehyde–acetic acid) solution under slight pressure of a coverslip for 10 min, and then transferred to 70% alcohol for further processing.

Identification of *A. compactum* metacercariae was based on the morphological studies of Kohn *et al.* (1995) and Zica *et al.* (2009). The main characteristics of *A. compactum* metacercariae (fig. 1) were: foliaceous body, slightly concave in the ventral face; small conical segment in the posterior region; small subterminal oral sucker; two lateral pseudosuckers in the anterior region; oval pharynx; short oesophagus; intestinal caeca ending near the posterior region; oval tribocytic organ; gland cells occupying most of the anterior region, extending from the beginning of intestinal caeca in the anterior region to the tribocytic organ (Kohn *et al.*, 1995; Santos *et al.*, 2002; Paes *et al.*, 2003; Novaes *et al.*, 2006; Zica *et al.*, 2009) (fig. 1). Morphometric data of *A. compactum* metacercariae are summarized in table 1.

Fish voucher specimens were deposited at the Ichthyological Collection of Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura (NUP) of the Universidade Estadual de Maringá, municipality of Maringá, Paraná State, Brazil and at the Laboratório de Biologia e Genética de Peixes, Instituto de Biociências (LBP) da Universidade Estadual Paulista, municipality of Botucatu, São Paulo State, Brazil. Metacercariae of *A. compactum* were

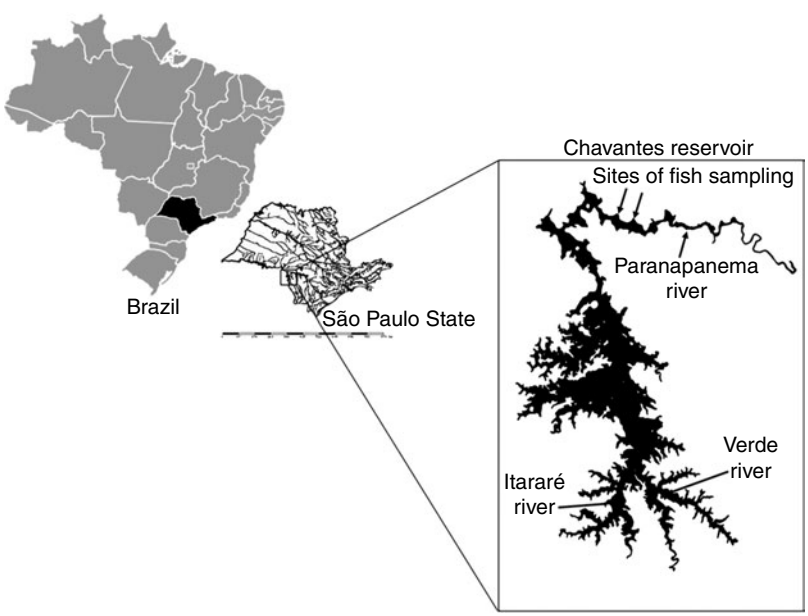

Fig. 2. Sites of loricariid fish (Siluriformes: Osteichthyes: Loricariidae) sampling in the Chavantes reservoir, in the upper Paraná river basin, municipality of Ipaussu, São Paulo State, Brazil.

Table 1. Morphometric data of *Austrodiplostomum compactum* (Digenea: Diplostomidae) metacercariae. Measurements are in micrometres; ranges are followed by means in parentheses.

	Kohn *et al.* (1995)	Santos *et al.* (2002)		Paes *et al.* (2003)	Novaes *et al.* (2006)	Zica *et al.* (2009)	Total variation for the species
Host	*Plagioscion squamosissimus*	*Plagioscion squamosissimus*	*Cichla ocellaris*	*Plagioscion squamosissimus*	*Geophagus brasiliensis*	*Hypostomus regani*	–
Number of specimens	14	20	20	–	5	10	–
Length	1470–2740 (2170)	880–1840 (1434)	960–2480 (1462)	1301–2376 (1911)	1584–1947 (1800)	1570–2281 (1988)	880–2740
Width	600–1180 (970)	400–792 (611)	560–960 (711)	482–854 (678)	537–706 (642)	543–864 (756)	400–1180
Length of conical segment (μm)	41–97 (77)	–	–	–	–	–	41–97
Oral sucker							
Length	41–97 (77)	44–90 (65)	40–98 (68)	51–87 (71)	45–83 (59)	69–102 (91)	40–102
Width	56–116 (79)	40–64 (52)	30–98 (56)	51–92 (73)	54–77 (68)	75–99 (84)	30–116
Lateral pseudosucker (1)							
Length	–	–	–	–	–	93–148 (132)	93–148
Width	–	–	–	–	–	68–157 (118)	65–146
Lateral pseudosucker (2)							
Length	–	–	–	–	–	78–168 (131)	78–168
Width	–	–	–	–	–	85–146 (119)	85–146
Prepharynx							
Length	–	2–10 (6)	4–20 (9)	–	–	–	2–20
Width	–	10–24 (17)	16–38 (23)	–	–	–	10–38
Pharynx							
Length	64–94 (83)	50–64 (62)	44–98 (68)	49–84 (69)	53–73 (61)	57–85 (73)	44–98
Width	45–79 (60)	40–60 (49)	38–78 (52)	45–74 (58)	50–64 (56)	57–80 (64)	38–80
Oesophagus							
Length	–	–	–	54–101 (72)	–	86–139 (111)	54–139
Width	–	–	–	29–53 (39)	–	–	29–53
Tribocytic organ							
Length	326–650 (507)	200–600 (285)	200–496 (308)	205–554 (401)	422–434 (428)	287–414 (373)	200–650
Width	251–500 (370)	160–232 (182)	120–320 (180)	127–347 (243)	220–319 (258)	178–310 (243)	127–500

deposited in the Coleção Helmintológica (CHIBB) of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista, municipality of Botucatu, São Paulo State, Brazil.

Mean intensity of infection and mean abundance were calculated according to Bush *et al.* (1997). The Mann–Whitney test was used to examine whether there are effects of sample site on the parasitism. The effect of seasonalilty on the parasitism was examined using pooled data among all host species. Seasons were compared with the Kruskall–Wallis test. Spearmann's correlation test was used to evaluate the correlation between the intensity of infection and the fish standard length and weight using the software SigmaStat 3.0 (Jandel Corporation, San Rafael, California, USA).

Results

Ninety-eight specimens of loricariid fish were collected. These include *Hypostomus ancistroides* (Ihering, 1911), *H. hermanni* (Ihering, 1905), *H. iheringii* (Regan, 1908), *H. margaritifer* (Regan, 1908), *H. regani*, *H. strigaticeps* (Regan, 1908), *Hypostomus* sp. and *Megalancistrus parananus* (Peters, 1881) (table 2).

Metacercariae of *A. compactum* were found in the eyes of 34 specimens of the studied fish (overall

Table 2. Data on infection by *Austrodiplostomum compactum* metacercariae in the eyes of loricariid fish sampled from the Chavantes reservoir, in the upper Paraná river basin, municipality of Ipaussu, São Paulo State, Brazil.

Fish species	Standard length (cm) Mean ± SD (range)	Weight (g) Mean ± SD (range)	IF/TF	Mean intensity of infection	Mean abundance	CHIBB**
Hypostomus ancistroides LBP 7581, NUP 6787*	12.9 ± 0.7 (10.4–17.3)	55.9 ± 6.4 (32.7–102.4)	0/10	–	–	–
Hypostomus hermanni† LBP 7582	9.4	36.2	1/1	27	27	4721
Hypostomus iheringii† LBP 7578, 7583	12.7 ± 0.5 (7.2–19.6)	72.7 ± 8.3 (10.4–218.6)	18/28	13.1 ± 3.2 (1–48)	8.4 ± 2.4 (0–48)	4722–4739
Hypostomus margaritifer† NUP 6785	19	203.2	1/1	35	35	4740
Hypostomus regani LBP 7580	18.2 ± 2.1 (14.2–21.4)	153.8 ± 44.1 (74.3–226.3)	1/3	10	3.3 ± 3.3 (0–10)	4741
Hypostomus strigaticeps† LBP 7577	13.7 ± 0.4 (7.8–20.1)	88.2 ± 7.7 (14.3–254)	11/45	10.3 ± 2.4 (2–25)	2.7 ± 0.9 (0–25)	4742–4752
Hypostomus sp. NUP 6784, 6787	12.3 ± 0.7 (10–14.6)	59.5 ± 11.3 (24.8–103.8)	2/5	17.5 ± 16.5 (1–34)	7 ± 6.8 (0–34)	4753–4754
Megalancistrus parananus LBP 7579	18.1 ± 2.4 (12.6–26.5)	234.2 ± 98.9 (57.1–610.9)	0/5	–	–	–

IF/TF, number of infected fish/total number of fish.
* Numbers in the first column are the accession numbers of voucher host species in the Ichthyological Collection of the Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura (NUP) of the Universidade Estadual de Maringá, Municipality of Maringá, Paraná State, Brazil and at the Laboratório de Biologia e Genética de Peixes, Instituto de Biociências (LBP) da Universidade Estadual Paulista, Municipality of Botucatu, São Paulo State, Brazil.
** Numbers in the last column are the accession numbers of voucher helminth species in the CHIBB (Coleção Helmintológica of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista, Municipality of Botucatu, São Paulo State, Brazil).
† New host record for *Austrodiplostomum compactum* metacercariae.

prevalence = 34.7%). Only *H. ancistroides* and *M. parananus* were not infected by this diplostomid. *Hypostomus hermanni* and *H. margaritifer* were represented by only one specimen each but both had a high intensity of infection by *A. compactum* metacercariae (27 and 35 metacercariae, respectively). Infection was detected in *H. regani* and *Hypostomus* sp. The former had one specimen parasitized with 10 metacercariae in the eyes and the latter had two specimens parasitized with one and 34 diplostomids (table 2).

Hypostomus strigaticeps ($n = 45$) and *H. iheringii* ($n = 28$) were the species with the highest numbers of fish sampled in the study. For these species, the prevalence of infection was 24.4% and 64.2%, respectively. *Hypostomus iheringii* presented with a slightly larger number of parasites. However, abundance was higher in *H. iheringii* (13.1) than in *H. strigaticeps* (10.3) (table 2).

There was no correlation between the intensity of infection and the fish standard length ($n = 98$; $r = -0.0223$; $P = 0.827$) or weight ($n = 98$; $r = 0.0286$; $P = 0.779$), and there was no effect of sample site on the parasitism ($P = 0.766$). Also, a seasonal pattern of infection was not observed ($P = 0.815$), although a slight tendency for an increase during autumn could be detected (fig. 3).

Hypostomus hermanni, *H. iheringii*, *H. margaritifer* and *H. strigaticeps* were new hosts recorded for *A. compactum* metacercariae.

Discussion

The present study reports the occurrence of *A. compactum* metacercariae in six loricariid fish species from the Chavantes reservoir, in the upper Paraná river basin, municipality of Ipaussu, with a record of four new hosts. The metacercariae of this diplostomid have low specificity since many intermediate fish hosts have already been found to be infected with *A. compactum* metacercariae in the eyes or brain, mainly in Brazil

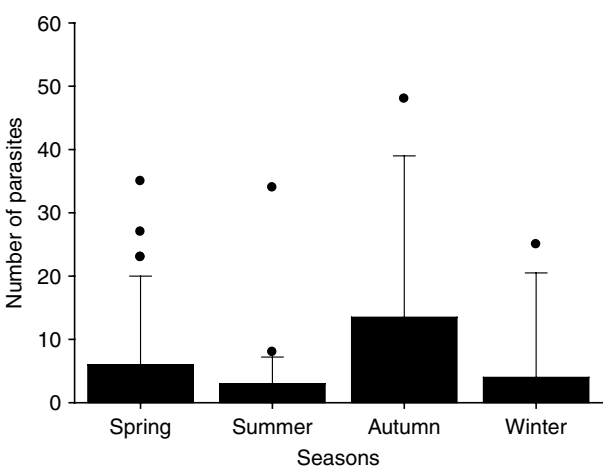

Fig. 3. Box plot of the number of *Austrodiplostomum compactum* metacercariae in the loricariid fish (Siluriformes: Osteichthyes: Loricariidae) sampled in the Chavantes reservoir, in the upper Paraná river basin, municipality of Ipaussu, São Paulo State, Brazil in different seasons. All median values were zero. The superior limit of the boxes represents the 75th percentile, error bars are 90th percentile and circles are outliers. No statistical differences were observed between the seasons ($P = 0.815$).

(Machado *et al.*, 2005; Novaes *et al.*, 2006; Yamada *et al.*, 2008; Zica *et al.*, 2009). However, the infection by *A. compactum* metacercariae is rarely observed in loricariid fish (Amato *et al.*, 2001; Yamada *et al.*, 2008; Zica *et al.*, 2009) and is restricted to Brazilian fishes.

Pojmanska & Chabros (1993) demonstrated that the prevalence of diplostomids was significantly lower in native fishes and higher for introduced species. In fact, it has been further demonstrated that the introduced species *P. squamosissimus* presents high infection levels by *A. compactum* metacercariae (Kohn *et al.*, 1995; Santos *et al.*, 2002; Paes *et al.*, 2003; Machado *et al.*, 2005). Similar data were observed by Machado *et al.* (2005) in several fishes from the floodplain of the upper Paraná river basin, Brazil. These authors hypothesized that this metacercaria species was introduced together with the hosts and is utilizing native fish from the region as alternative second intermediate hosts (Machado *et al.*, 2005). As highlighted by several authors (see Zica *et al.*, 2009 and references therein), progressively more native fishes have been reported as intermediate hosts for *A. compactum*, which appears to corroborate the hypothesis presented by Machado *et al.* (2005).

Eight loricariid fish species collected from two sites of the Chavantes reservoir were sampled in this study. Only two species (*H. ancistroides* and *M. parananus*) were not infected with *A. compactum* metacercariae. Zica *et al.* (2009) suggested that the infection by *A. compactum* metacercariae may be associated with environmental factors and biology of the hosts, as the first intermediate host is an aquatic snail and the loricariid specimens are usually bottom fish (Garavello & Garavello, 2004). The infection can occur because both (first and second intermediate hosts) use the same habitats, which increases the possibility of the encounter between host and parasite. In this way, the absence of infection in *H. ancistroides* and *M. parananus* may be related to some unknown ecological aspects of these hosts. In the case of *H. ancistroides*, it is known that this species lives preferentially under the marginal vegetation in the river (C.H. Zawadzki, pers. obs.) or under wood debris (Casatti, 2005), which could explain the absence of parasites in this species. Future studies will be conducted to clarify this.

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