

# Metacercariae of *Austrodiplostomum* spp. (Digenea: Diplostomidae) infecting the eyes and brains of fish in Brazilian Amazon

*Metacercaria de Austrodiplostomum* spp. (Digenea: Diplostomidae) infectando olhos e cérebros de peixes na Amazônia Brasileira

Márcio Jonathan Soares Lima<sup>1</sup> , Renato Pastor Veiga<sup>1</sup> ,  
Luciclara Ferreira de Sousa<sup>1</sup> , Maxwell Barbosa de Santana<sup>1</sup> ,  
Marcos Sidney Brito Oliveira<sup>2</sup> , Marcos Tavares-Dias<sup>2</sup> , Lincoln Lima Corrêa<sup>1\*</sup> 

**ABSTRACT:** The aim of this paper was to report the occurrence of *Austrodiplostomum* spp. in the eyes and brain of *Acaronia nassa*, *Caquetaia spectabilis*, *Satanoperca acuticeps*, *Curimatella* sp. and *Crenicichla marmorata* in a lake of the Amazon River system in the state of Pará (Brazil). Of 49 fish examined, 10.2% were parasitized by *Austrodiplostomum* spp. metacercariae, and *A. nassa* was the host with higher number of metacercariae. In five examined fish species, a total of 51 metacercariae were collected, with 45 found in the eyes and 6 in the cranial vault. In the eyes, the metacercariae were free and active in the vitreous humor, but no opacification was observed. In the brain, the metacercariae were also free and active, and located mainly below the encephalon, on the cranial floor, at the height of the ophthalmic lobes and near the optic nerve. In laboratory observations, however, the infected fish did not exhibit any behavioral disorders, and this may be related to the low level of parasitism. This was the first report of this digenean for *C. spectabilis*, *S. acuticeps*, *Curimatella* sp., *C. marmorata* and *A. nassa*.

**KEYWORDS:** Amazon; Digenea; parasites; freshwater fish.

**RESUMO:** O objetivo deste trabalho foi relatar a ocorrência de *Austrodiplostomum* spp. nos olhos e cérebros de *Acaronia nassa*, *Caquetaia spectabilis*, *Satanoperca acuticeps*, *Curimatella* sp. e *Crenicichla marmorata* em um lago do rio Amazonas no estado do Pará (Brasil). Dos 49 peixes examinados, 10,2% foram parasitados por metacercárias de *Austrodiplostomum* spp., *A. nassa* sendo o hospedeiro com maior número de metacercárias. Em cinco espécies de peixes examinadas, foram coletadas 51 metacercárias, sendo 45 encontradas nos olhos e 6 na caixa craniana. Nos olhos, as metacercárias estavam livres e ativas no humor vítreo, mas nenhuma opacificação foi observada. No cérebro, as metacercárias também eram livres e ativas, localizadas principalmente abaixo do encéfalo, no assoalho do crânio, na altura dos lobos oftálmicos e próximo ao nervo óptico. Em observações laboratoriais, no entanto, o peixe infectado não apresentou distúrbios comportamentais, o que pode estar relacionado ao baixo nível de parasitismo. Este foi o primeiro relato de *Austrodiplostomum* spp. em *C. spectabilis*, *S. acuticeps*, *Curimatella* sp., *C. marmorata* e *A. nassa*.

**PALAVRAS-CHAVE:** Amazônia; Digenea; parasitas; peixes de água doce.

<sup>1</sup>Universidade Federal do Oeste do Pará – Santarém (PA), Brazil

<sup>2</sup>Embrapa Amapá – Macapá (AP), Brazil

\*Corresponding author: lincorre@gmail.com

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## INTRODUCTION

The Amazon River system is formed by various orders of rivers, lakes, and other geographic events. It contains around 20% of world's freshwater and a tremendous richness of fish, and its approximately 3,000 species (JUNK, 2013) make it one of the main resources for human food and the local economy. These fish are hosts for a variety of groups of Digenea from the Diplostomidae family, including *Austrodiplostomum* spp. LOCKE et al. (2010) have suggested that the identification of these metacercariae may not be based only on morphology, and the use of molecular markers is required. So, in this paper we considered the name *Austrodiplostomum* spp.

*Austrodiplostomum* spp. has been responsible for verminous cataracts, also known as diplostomosis, in freshwater fish. The metacercariae of these digeneans that infect the eyes of fish can be found in the retina, vitreous, aqueous, and/or crystalline humor, and also in the brains of hosts. The presence of these metacercariae at heightened levels of abundance can cause exophthalmos, displacement of the retina, opacity of the crystalline lens, blindness, and even the death of host fish (HECKMANN, 1992; BULLARD; OVESTREET, 2008; MONTEIRO et al., 2016; VITAL et al., 2016). The formation of cataracts in infected fish is more intense after the parasites complete their larval development and are ready to infect the definitive host, augmenting the susceptibility of intermediate host fish to predation (SANTOS et al., 2002; PINTO; MELO, 2013; CORRÊA et al., 2014). This is therefore an efficient transmission method for these digeneans (MOURITSEN; POULIN, 2003; VITAL et al., 2016).

*Austrodiplostomum* spp. occurs in fish in North and South America (BULLARD; OVESTREET, 2008). In Brazil, it has been registered infecting the eyeballs of *Geophagus brasiliensis* (NOVAES et al., 2006), *Geophagus surinamensis* (SANTOS et al., 2002), *Cichla ocellaris* (SANTOS et al., 2002), *Cichla* sp. (SANTOS et al., 2002), *Cichla monoculus* (MACHADO et al. 2005; VITAL et al. 2016), *Satanoperca papaterra* (MACHADO et al. 2005), *Hoplias malabaricus* (SANTOS et al., 2002; CORRÊA et al., 2014), *Hypostomus regani* (YAMADA et al., 2008; ZICA et al., 2009), *Schizodon borellii*, *Auchenipterus osteomystax*, *Serrasalmus maculatus* (YAMADA et al., 2008), and *Plagioscion squamosissimus* (KOHN et al. 1995; SANTOS et al., 2002; MACHADO et al., 2005; SANTOS et al., 2002; ALBUQUERQUE et al., 2017), all of which are fish from different ecosystems. Despite the wide distribution and the increasing number of cases of occurrence of these eye fluke infections in Brazilian fish, the possible impacts on wild fish species remain unknown or neglected. Furthermore, despite the large number of fish in the Brazilian Amazon, infection has been described in only nine species in the region (VITAL et al., 2016). The objective of the present study was therefore to report the occurrence of metacercariae of *Austrodiplostomum* spp. in the eyes and brain of five species of fish from a lake in the Amazon River system in the north of Brazil, and to evaluate the possible disorders in the behavior of infected hosts.

## MATERIALS AND METHODS

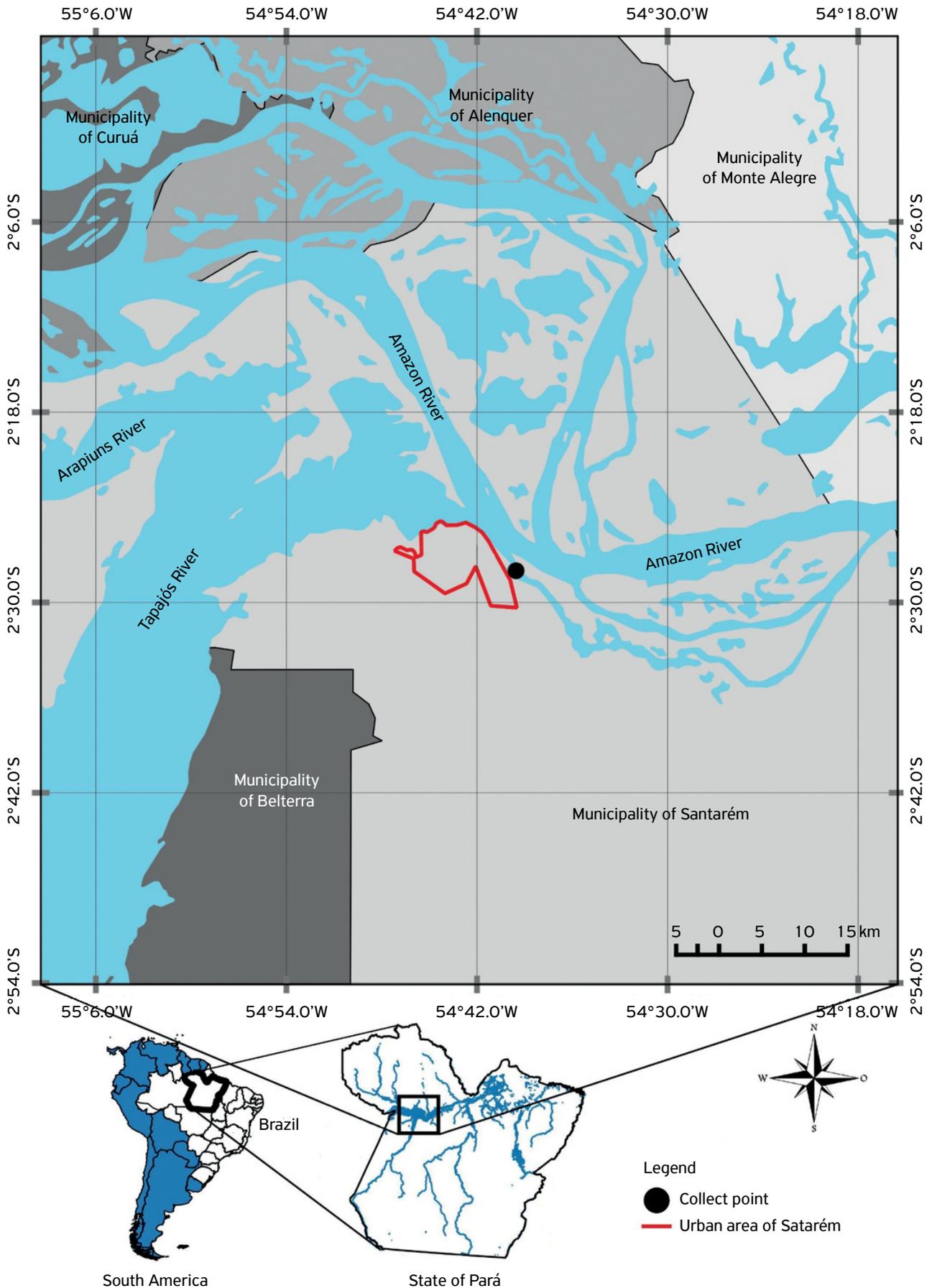
In October 2016, specimens of *Acaronia nassa* Heckel, 1840 (Cichlidae); *Caquetaia spectabilis* Steindachner, 1875 (Cichlidae); *Satanoperca acuticeps* Heckel, 1840 (Cichlidae); *Curimatella* sp. Eigenmann & Eigenmann, 1889 (Curimatidae) and *Crenicichla marmorata* Pellegrin, 1904 (Cichlidae) were collected in Lake Maicá in the state of Pará, Brazil (Fig. 1). This lake is located in the east of the municipal region of Santarém in the state of Pará, beginning in the Amazon River and extending as far as the Paran  do Ituqui River. It is connected to smaller lakes and contains various species of flora and fauna typical of the Amazon lowlands along its length.

Nets with differing mesh sizes were used for fish collection. These were placed at five points distributed along Lake Maic  (P1 02°28'25"S 054°39'23,7"W; P2 02°28'23,7"S 054°39'23,7"W; P3 02°28'24,4"S 054°39'16,4"W; P4 02°28'24,9"S 054°39'10,8"W, and P5 02°28'23,4"S 054°39'09,0"W) and inspected every four hours. Following capture, all the fish were transported in water tanks to the Multi-laboratory for the Production of Aquatic Organisms Laborat rio M ltiplo de Produ o de Organismos Aqu ticos (LAMPOA), where they were kept in aquariums with a capacity of 120 L and were treated for seven days with NaCl (100 g/m<sup>3</sup>), against possible cutaneous infection caused by capture and/or transport. Following identification, representative specimens of the parasites were deposited in the Zoological Collection of the Zoological Museum of UNICAMP, under voucher number ZUECPA 16.

The fish were euthanized for parasitological analysis using the transection of the cervical cord method for parasitological analysis. The total length (cm) and weight (g) were measured, and the organs were examined to verify the occurrence of metacercariae of *Austrodiplostomum* spp. The metacercariae found were compressed between a slide and a cover slip, fixed in AFA (70% alcohol, formalin and acetic acid) and preserved in 70% alcohol. The metacercariae were subsequently stained with Carmine de Langeron, clarified with Methyl Salicylate, and mounted on permanent slides using Canada balsam (EIRAS et al., 2006). The taxonomic identification of the parasites was carried out in accordance with KOHN et al. (1995) and GIBSON et al. (2002). The morphometric analysis of the metacercariae was performed using an optical microscope (Zeiss Axioplan), the images were obtained with a digital camera (coupled Axiocam ERc 5s), and the measurements ( $\mu$ m) were performed using the Zen program. The ecological terms used (prevalence, mean intensity and abundance) were in accordance with the recommendations of BUSH et al. (1997).

## Compliance with Ethical Standards

Fish collection was authorized by IBAMA/ICMBio (no. 46202-3/2017). The collection and euthanasia procedures of the fish were approved by the Ethics Committee for Animal Research of the Universidade Federal do Oeste do Par  (West Par  Federal University) (no. 06001/2015 — CEUA/UFOPA).



**Figure 1.** Localization and georeferencing of collection points of fish species in a lake in eastern Amazon (Brazil).

## RESULTS

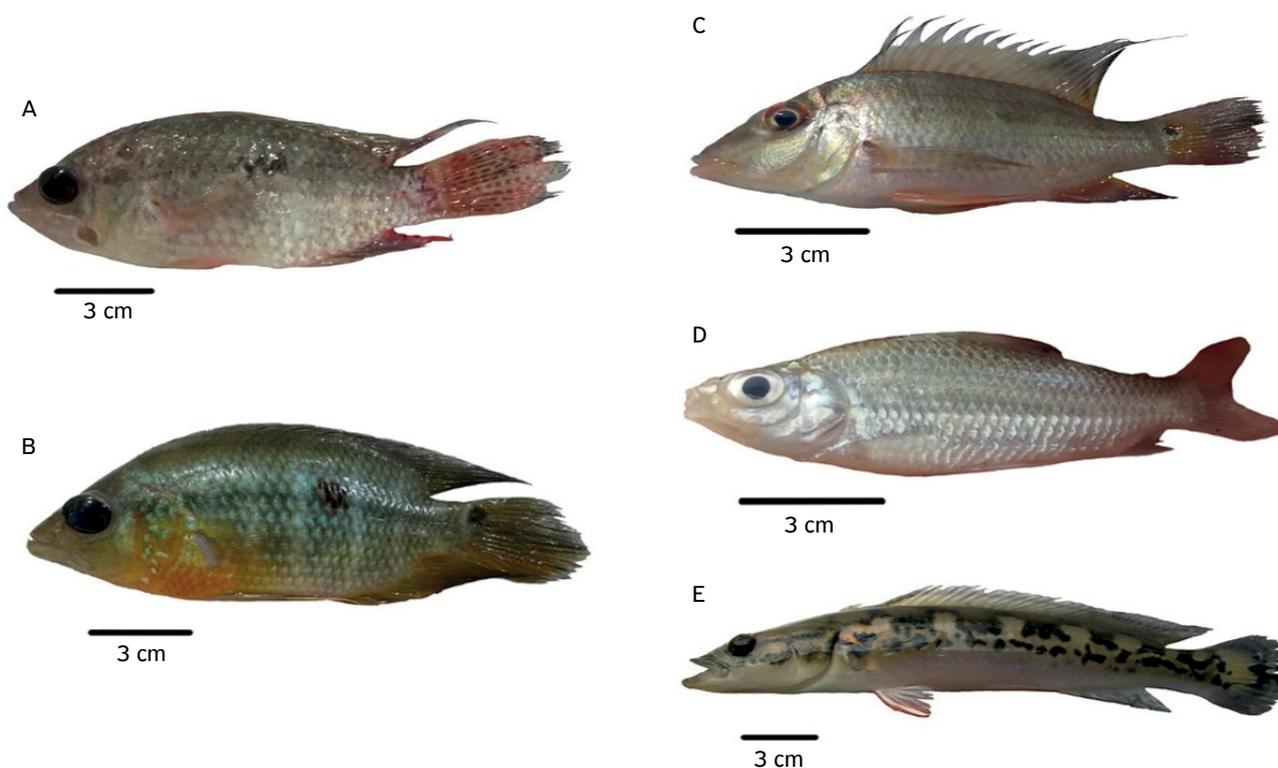
Of a total of 49 fish specimens from five species necropsied (Table 1 and Fig. 2), 10.2% were parasitized by metacercariae of *Austrodiplostomum* spp. A total of 51 metacercariae

were recovered, being 45 from the eyes and 6 from the brain. In *A. nassa* were recovered 18 metacercariae and *S. acuticeps* 15 metacercariae (Table 2).

In the host eyes, the metacercariae were free and active in the vitreous humor, but no sign of opacification was observed.

**Table 1.** Biometric parameters of fish infected by *Austrodiplostomum* spp. collected in a lake in eastern Amazon (Brazil).

Hosts	N	Weight (g)	Total length (cm)	Standard length (cm)
<i>Caquetaia spectabilis</i>	19	68.5 ± 59.8	16.0 ± 3.4	13.3 ± 2.9
<i>Satanoperca acuticeps</i>	4	46.4 ± 1.0	14.0 ± 1.0	11.5 ± 1.0
<i>Curimatella</i> sp.	19	6.5 ± 1.0	3.0 ± 1.0	-
<i>Crenicichla marmorata</i>	1	181.2	27.5	24.5
<i>Acaronia nassa</i>	6	77.0 ± 1.0	15.5 ± 1.0	12.0 ± 1.0



**Figure 2.** Species of host fishes infected by *Austrodiplostomum* spp. in a lake in eastern Amazon (Brazil). (A) *Acaronia nassa*; (B) *Caquetaia spectabilis*; (C) *Satanoperca acuticeps*; (D) *Curimatella* sp.; (E) *Crenicichla marmorata*.

**Table 2.** Levels of infection by *Austrodiplostomum* spp. in fish from a lake in eastern Amazon (Brazil).

Hosts	Levels of infection				Infection sites			
	FE/PF	P (%)	MI	MA	RE	LE	CV	TNP
<i>Caquetaia spectabilis</i>	19/1	5.3	1.0	0.05 ± 0.2	0	1	0	1
<i>Satanoperca acuticeps</i>	4/1	25.0	15.0	3.7 ± 7.5	8	5	2	15
<i>Curimatella</i> sp.	19/1	5.3	2.0	0.1 ± 0.4	2	0	0	2
<i>Crenicichla marmorata</i>	1/1	100	15.0	15.0 ± 0	5	7	3	15
<i>Acaronia nassa</i>	6/1	16.7	18.0	3.0 ± 7.3	7	10	1	18

P: prevalence; MI: mean intensity; MA: mean abundance; RE: right eye; LE: left eye; CV: cranial vault; FE: fish examined; PF: parasitized fish; TNP: total number of parasites.

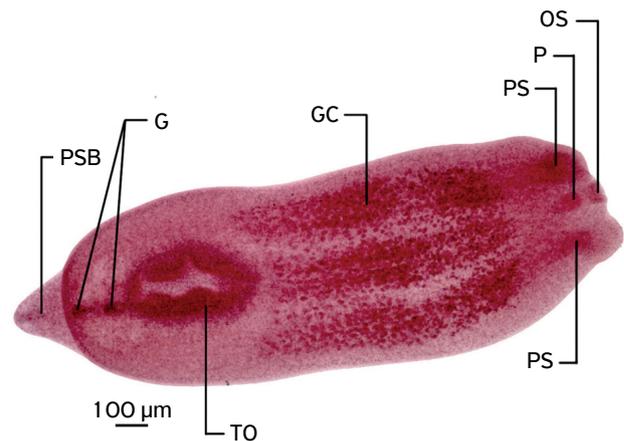
In the cerebral vault, the metacercariae were also free and active, and located mainly below the brain, on the cranial floor, at the height of the ophthalmic lobes, near the optic nerve. It was observed that the ophthalmic lobes, especially the veins that irrigate this region, were dilated and highly visible in the parasitized fish, a characteristic not observed in non-parasitized fish and/or those parasitized only in the eyes. However, in laboratory observations, the fish showed no behavioral changes.

*Austrodiplostomum* spp. exhibited a foliaceous body, slightly concave on the ventral surface, small conical segments in the posterior region, an oral suction cup located at the anterior extremity, pseudo-suckers located laterally to the oral suction cup, and an oval pharynx (Fig. 3). The morphometric measurements of the metacercariae were compared with those described in the literature (Table 3).

## DISCUSSION

Although metacercariae of *Austrodiplostomum* spp. have been found infecting the eyes, gills, swimming bladder, and musculature of several South American fish species due to their low parasitic specificity (ZICA et al., 2011) they infect the brains of hosts less frequently. In this study, we found lower intracranial abundance than in the eyes of infected fish. HECKMANN (1992) reported that infections of metacercariae of *Diplostomum mordax* in the cranial cavity of *Orestias agasii*, *O. olivaceus*, *O. luteus* and *Basilichthys bonariensis* induced compression of the neural tissue and migration of the metacercariae to the

brain, leading to hemorrhage; cell necrosis; inflammation; fibrosis and the rupture of nerve fibers. However, such alterations were not observed in fish in the present study infected by *Austrodiplostomum* spp., due to the low levels of parasitism, a finding corroborated by SIEGMUND et al. (1997) in *Basilichthys australis* infected by metacercariae of *Diplostomum mordax* and *Tylodelphys destructor*. CORRÊA et al. (2014) reported that *H. malabaricus*, with widespread infection by *Austrodiplostomum* spp. in the eyes and cranial cavity, presented alterations in swimming capacity such as imbalance and a lack of coordination. Host size, duration of transmission period, and host growth rate are important factors in brain disorders



OS: oral suckers; P: pharynx; PS: pseudo-suckers; GC: gland cells; TO: tribocytic organ; G: gonads; PSB: posterior segment of the body.

**Figure 3.** Metacercaria of *Austrodiplostomum* spp. from eyes and brain of fish species from a lake in eastern Amazon (Brazil).

**Table 3.** Comparison of morphometric ( $\mu\text{m}$ ) values of *Austrodiplostomum* spp. metacercariae in fish from a lake in eastern Amazon (Brazil) with studies carried out in Brazil.

Parasite measures	Lake Maicá, Pará state (Present study - n = 5)	Paraná River, Paraná state (YAMADA et al. 2008 - n = 6)	Lake Catalão, Amazonas state (ALBUQUERQUE et al. 2017 - n = 15)
Total length	1800 (1584 - 1947)	1037 (680 - 1190)	1783.5 (148.2 - 2024.4)
Overall width	642 (537 - 709)	568 (310 - 1010)	662.2 (589.1 - 782.4)
Length of oral suction cup	59 (45 - 83)	46 (19.2 - 60)	60.5 (44.8 - 72.4)
Width of oral suction cup	68 (54 - 77)	48.8 (28.8 - 60)	70.1 (47.6 - 81.7)
Length of pharynx	61 (53 - 73)	60 (36 - 96)	62.9 (46.9 - 79.4)
Width of pharynx	56 (50 - 64)	40 (14.4 - 55.2)	57 (41.3 - 69)
Length of right pseudo-suckers	99.4 (71.6 - 124.8)	-	103.8 (74.1 - 127.4)
Width of right pseudo-suckers	95.4 (69.6 - 190.2)	-	80.6 (59.5 - 95.1)
Length of left pseudo-suckers	93.8 (71.3 - 125.3)	-	99.5 (83.6 - 121.7)
Width of left pseudo-suckers	83.4 (63.6 - 116.4)	-	83.1 (63.7 - 105.6)
Length of tribocytic organ	428 (422 - 434)	236 (91.2 - 324)	344.9 (230.3 - 425.2)
Width of tribocytic organ	258 (220 - 319)	152 (81.6 - 228)	181.1 (122.2 - 238.2)
Acetabulum length	17.7 (4.8 - 29.6)	16.3 (9.6 - 28.8)	-
Acetabulum width	16.9 (5.6 - 32.6)	16.4 (9.6 - 33.6)	-

(SANDLAND; GOATER, 2001) and in the behavior of infected fish. Such brain and visual changes expose infected fish to predators such as aquatic piscivorous birds (BULLARD; OVESTREET, 2008), definitive hosts of this parasite.

Species of *Curimatella* are detritivorous fish that consume algae, debris, and associated microorganisms. *Acaronia nassa*, *C. espectabilis* and *S. acuticeps* are omnivorous fish that feed on aquatic invertebrates, algae, insects, and fish. *Crenichla marmorata* is a carnivorous fish that feeds on aquatic invertebrates and fish (SANTOS et al. 2004; FROESE; PAULY, 2017). *Biomphalaria straminea* and *Biomphalaria glabrata* snails were identified to be intermediate hosts of *A. compactum* in a lake in the southeast of Brazil (PINTO; MELO, 2013). In the lake studied in the present study, the presence of snails of the genus *Pomacea*, possible intermediate hosts of *Austrodiplostomum* spp. in the environment, was observed. *Austrodiplostomum* spp. adults inhabit the digestive tract of piscivorous birds such as *Phalacrocorax brasilianus* (PINTO; MELO, 2013; MONTEIRO et al., 2016), which has a large presence in the area of the present study, together with *Ardea alba*, snails, and fish, are believed to maintain the biological cycle of *A. compactum* in the region of the Amazonian ecosystem investigated here.

In *C. espectabilis*, *S. acuticeps*, *Curimatella* sp., *C. marmorata*, and *A. nassa*, there were low levels of infections by the metacercariae of *Austrodiplostomum* spp. Similar results were reported for *Hypostomus hermanni*, *Hypostomus iheringii* and *Hypostomus* sp. with *A. compactum* infection in the eyes (ZICA et al., 2011). However, high levels of *A. compactum* infections have been reported for *P. squamosissimus* from Paraná River, in the São Paulo state (SANTOS et al., 2002) and Catalão Lake, in the state of Amazonas (ALBUQUERQUE et al., 2017).

The measurements of *Austrodiplostomum* spp. from this study were similar to those described by ALBUQUERQUE et al. (2017) for *P. squamosissimus* from the western Amazon

region. PINTO; MELO (2013) found that the morphometric measurements of *A. compactum* metacercariae obtained from experimentally infected *Cyprinus carpio* were similar to those from other fish. However, they were different from the measurements reported by YAMADA et al. (2008) for *S. maculatus*, *H. regani*, *A. osteomystax*, and *S. borelli* from the Paraná River Basin (Brasil).

## CONCLUSIONS

Due to the low specificity of *Austrodiplostomum* spp., it has been found in several hosts of different watersheds. In this study, we extended the record of its geographic distribution to the eastern Amazon region, as well as added *C. espectabilis*, *S. acuticeps*, *Curimatella* sp., *C. marmorata*, and *A. nassa* as secondary hosts of *Austrodiplostomum* spp., increasing to 14 the number of hosts for this digenean in the Brazilian Amazon.

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