



Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

METACERCARIAE OF *CLINOSTOMUM* SP. (TREMATODA: CLINOSTOMIDAE) INFESTING ORNAMENTAL CICHLIDS CULTIVATED IN THE PERUVIAN AMAZONIA

METACERCARIA DE *CLINOSTOMUM* SP. (TREMATODA: CLINOSTOMIDAE) INFESTANDO A CÍCLIDOS CULTIVADOS ORNAMENTALES EN LA AMAZONÍA PERUANA

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ABSTRACT

The ornamental fish trade is one of the most important economic activities for thousands of people in the Amazon region. Among fish that are exported from Peru, *Apistogramma* sp., *Cichlasoma amazonarum* (Kullander, 1983) and *Pterophyllum scalare* (Schultze, 1823) are highly demanded in the ornamental fish trade activities. To date, in Peru, studies concerning parasites that affect aquaculture activities are scarce. The present study is first to report the occurrence of *Clinostomum* sp. parasitizing three species of cichlids in the Peruvian Amazonia. Samples of *Apistogramma* sp., *C. amazonarum* and *P. scalare* were collected in a fish farming pond of the “Amazon Research Center for Ornamental Fishes (ARCOF) in Iquitos, Peru. Analyzed samples revealed the presence of *Clinostomum* sp. parasitizing the fins of the fish. As some species of cichlids are cultivated in the Peruvian Amazon for exportation as ornamental fish, the presence of this trematode could compromise the health and appearance of the fish and may cause mortalities, either, due to susceptibility to predation by piscivorous birds or due to injuries caused by the infection of parasites.

Key words: Aquaculture – cichlids – *Clinostomum* – ornamental fish – piscivorous bird

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RESUMEN

El comercio de peces ornamentales es una de las actividades económicas más importantes para miles de personas en la región amazónica. Entre los peces que se exportan desde Perú, *Apistogramma* sp., *Cichlasoma amazonarum* (Kullander, 1983) y *Pterophyllum scalare* (Schultze, 1823) son muy demandados en las actividades comerciales de peces ornamentales. A la fecha, en el Perú, los estudios referentes a los parásitos que afectan las actividades acuícolas son escasos, es así que el presente estudio registra por primera vez en la Amazonía peruana la ocurrencia de *Clinostomum* sp. parasitando tres especies de cíclidos. Se recolectaron muestras de *Apistogramma* sp., *C. amazonarum* y *P. scalare* en un estanque de piscicultura del “Centro de Investigación de Peces Ornamentales de la Amazonía (ARCOF)” en Iquitos, Perú. Las muestras analizadas revelaron la presencia de *Clinostomum* sp. parasitando las aletas de los peces. Como algunas especies de cíclidos se cultivan en la Amazonía peruana para su exportación como peces ornamentales, la presencia de este trematodo podría comprometer la salud de los peces y causar mortalidades, ya sea por susceptibilidad a la depredación por aves piscívoras o por lesiones causadas por la infección de parásitos.

Palabras claves: Acuicultura – Ave piscívora – cíclidos – *Clinostomum* – Pez ornamental

INTRODUCTION

The ornamental fish trade is one of the most important economic activities for thousands of people in the Amazon region (Moreau & Coomes, 2007). Nearly all Amazon fish exported from the natural environment are obtained by local fishermen, who transfer the fish to agents who, later on, transfer them to exporters so as to reach fish stores worldwide. It has been estimated that in this transport chain, 73% of the fish die, due to several causes (Olivier, 2001).

Cichlids are one of the major ornamental fish families, due to the large number of species and the demand for these fish for ornamental purposes. This demand is explained by the variety of shapes, extravagant colors and special behavior of the different species that are included in this family (Kullander & Silfvergrip, 1991; Garcia-Dávila *et al.*, 2021). According to Garcia-Dávila *et al.* (2021), in the Peruvian Amazonia, 37 species of cichlids are commercialized as ornamental fish. Between these species, *Apistogramma* sp., *Cichlasoma amazonarum* (Kullander, 1983) and *Pterophyllum scalare* (Schultze, 1823) are highly demanded for ornamental fish importers.

In the region of Loreto, Peru, most of the cichlids used in the ornamental trade are captured from natural environments while others are bred under captivity in artificial ponds (Garcia *et al.*, 2021).

Parasites are abundant in tropical climates, and are one of the major causes of death and disposal of ornamental fish, thus representing large economic losses. Because of the small size of these fish, they are easily affected by small numbers of parasites (Takemoto *et al.*, 2004).

Metacercariae of *Clinostomum* spp. have the ability to infect muscles, fins and internal organs of fish. This parasite is usually found on the host body wall below the integumentary tissue, causing characteristic nodular swelling that can be easily recognize during physical examination (Simsek *et al.*, 2018). In the life cycle of these parasites, the first intermediate hosts are species of freshwater snails which have been infected from eggs deposited in the water by definitive host fish-eating birds (Liao, 1993). Fish species act as second intermediate hosts, manifesting the pathology known as “yellow-grub disease” (Ramos-Alves *et al.*, 2001).

Thus, the present study registers for the first time in the Peruvian Amazonia the occurrence of *Clinostomum* sp. parasitizing ornamental cichlids collected from an artificial fish pond.

MATERIAL AND METHODS

In a fish farming pond of the “Amazon Research Center for Ornamental Fishes (ARCOF)” in Iquitos,

Peru (03° 46.433' S, 073° 15.688'W), specimens of “*Apistogramma tapiche*” dwarf cichlid *Apistogramma* sp. with 4.30 ± 0.88 cm standard length and 3.50 ± 0.68 g of total weight; “bujurqui” Amazon cichlid *C. amazonarum* with 4.28 ± 0.84 cm standard length and 3.60 ± 1.03 g of total weight and “pez ángel” wild Angelfish *P. scalare* with 7.20 ± 0.57 cm standard length and 5.80 ± 0.83 g of total weight (were observed with signs of erratic swimming: fish swam very close to the surface, showing difficulty in their movement). Specimens were collected and placed in plastic bags with water and oxygen for transportation to the “Laboratorio de Parasitología y Sanidad Acuicola” of the “Instituto de Investigaciones de la Amazonía Peruana” (IIAP), Loreto, Peru.

The surface of the body and fins were analyzed by using a stereomicroscope. Parasites observed in the fins were collected with dissecting needles and placed in Petri dishes for counting the number of individuals collected per infested fish. For fixation of the parasites, alcohol-formalin-acetic acid (AFA) was used for 24 h and then conserved in 70% ethanol. For taxonomic identification, it was necessary to stain the parasites with Langeron's alcoholic Carmin. Finally, stained parasites were observed under stereoscope and microscope. Parasitological indices (Prevalence, mean intensity and mean abundance) followed Bush *et al.* (1997). Parasites were deposited in the parasitological collection of the “Laboratorio de Parasitología y Sanidad Acuicola” from IIAP.

Ethical disclosures

This study was developed in accordance with the principles adopted by IIAP, and authorization from Ethics Committee of the IIAP was carried out.

RESULTS

Analyzed fish revealed the presence of metacercariae of *Clinostomum* sp. (Figs. 1 and 2) on the fins of infested fish species. The main characteristics observed in the parasites were: Body linguiform with slight strangulation at the level of ventral sucker. Oral sucker located in the center of a circular dilation of the body. Pharynx present with bifurcation of the intestinal cecum extending to the posterior extremity of the body. Large intestinal cecum with irregular contour; pre-equatorial acetabulum; testes located between the end of the second third and the upper region of the last third of the body, anterior and posterior testis with lobular triangular shape. Intratesticular and post-equatorial ovary. Intercaecal, pre-testicular and post-acetabular uterus. Vitelline consisting of small, post-acetabular, intra and extracaecal follicles.

According to site of infestation, for *Apistogramma* sp., 47% of the metacercariae were found on the dorsal fin; 39% in the anal fin; 10% on the caudal fin; 02% on the ventral fin and 02% on the pectoral fin. For *C. amazonarum*, 82% of the parasites were found on the dorsal fin; 11% on the pectoral fin; 05% on the caudal fin and 02% on the ventral fin. For *P. scalare*, 55% of the parasites were found on the dorsal fin; 20% on the caudal fin; 18% on the ventral fin and 07% on the pectoral fin (Fig. 3). Parasitological indices of *Clinostomum* sp. reported in the three-host species are showed in Table 1.

Table 1. Parasitological indices of *Clinostomum* sp. reported in three species of cichlids. AF = analyzed fish, PF = parasitized fish, P% = prevalence, N = number of *C. marginatum* recorded in each host species, mI = mean intensity of infestation, mA = mean abundance of infestation.

Species of fichtlids	AF	PF	P%	N	mI	mA
<i>Apistogramma</i> sp.	20	16	80	222	13.87 ± 2.4	11.1 ± 1.8
<i>Cichlasoma amazonarum</i>	33	19	57.57	55	2.89 ± 0.62	1.66 ± 0.44
<i>Pterophyllum scalare</i>	5	4	80	44	11 ± 2.5	8.8 ± 1.9

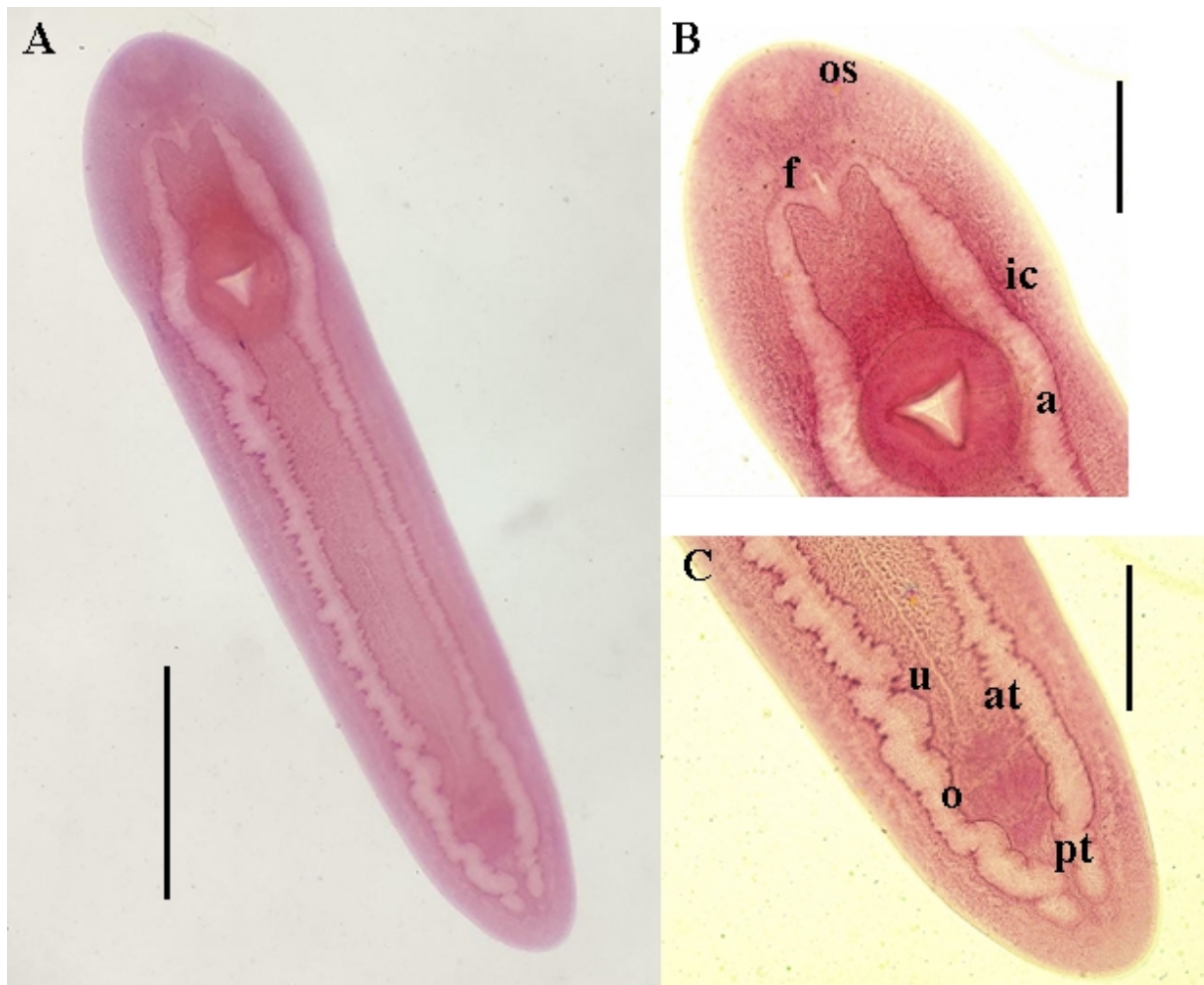


Figure 1. Metacercariae of *Clinostomum* sp. A. Ventral view showing the complete body. B. Anterior part of the body. C. Posterior part of the body. os = oral sucker, f = pharynx, ic = intestinal caecum, a = acetabulum, u = uterus, at = anterior testes, o = ovary, pt = posterior testes. Scale bar: A = 2 mm, B, C = 300 μ m.

DISCUSSION

Infections of *Clinostomum* spp. have been reported in different fish species. In Brazil: *Semaprochilodus insignis* (Jardine, 1841); *Cichla ocellaris* Bloch & Schneider, 1801; *Crenicichla* sp. (Castelo, 1984; Thatcher, 1991); *Hoplosternum littorale* (Hancock, 1828) (Sao Clemente *et al.*, 1998); *P. scalare* (Ramos-Alves *et al.*, 2001); *Synbranchus marmoratus* Bloch, 1795 (Isaac *et al.*, 2004); *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Paraguassú *et al.*, 2005); *Pygocentrus nattereri* Kner, 1858 (Morais *et al.*, 2011); *Colossoma macropomum* (Cuvier, 1816) (Murrieta-Morey & Malta, 2016);

Pterygoplichthys pardalis (Castelnau, 1855) (De Souza *et al.*, 2020a); *Gymnotus coropinae* Hoedeman, 1962, *Gymnorhamphichthys petiti* Géry & Vu, 1964, *Brachyhypopomus brevirostris* (Steindachner 1868) and *Microsternarchus bilineatus* Fernández-Yépez, 1968 (De Souza *et al.*, 2020b). In the present study, *Apistogramma* sp., *C. amazonarum* and *P. scalare* are cited for the first time as hosts of *Clinostomum* sp. metacercariae and therefore, we constitute the first study in the Peruvian Amazon related to infestations of trematodes in fish species cultivated.

The fact that the dorsal and anal fins were the most common locations of the parasites is probably due to low movements of those fins, therefore

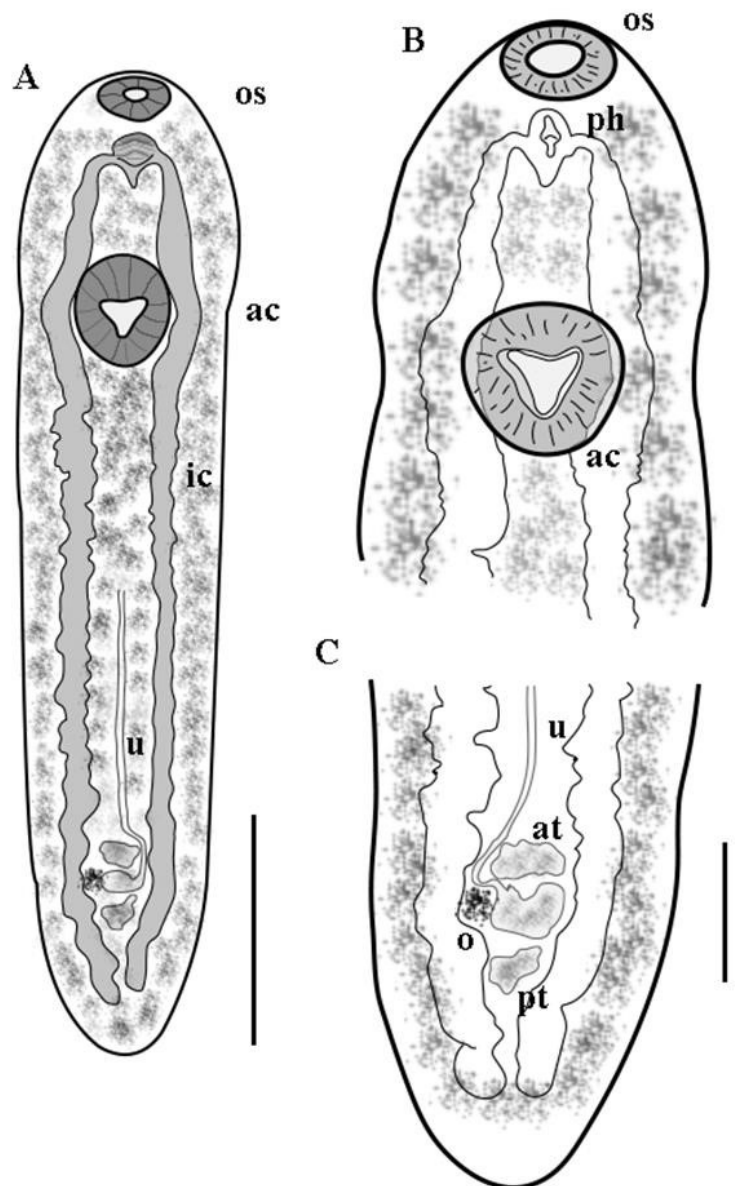


Figure 2. Esquematic design of metacercariae of *Clinostomum* sp. A. Ventral view showing the complete body. B. Anterior part of the body. C. Posterior part of the body. os = oral sucker, ph = pharynx, ic = intestinal caecum, a = acetabulum, u = uterus, at = anterior testes, o = ovary, pt = posterior testes. Scale bar: A = 2 mm, B, C = 300 μ m.

facilitating the contact with the invasive cercariae. On the other hand, the low value found for the infection in the caudal fin, as compared with the other fins, is likely to be due to the more intense movements of the caudal fin; therefore, making difficult the penetration by the cercariae into the body of *Apistogramma* sp. Similar report are present by Eiras *et al.* (1999) where infected loracarids present more metacercariae of *C.*

marginatum attached into dorsal and anal fins compared with the caudal fin that presented low levels of infection.

Metacercariae of trematodes may change the behavior of their second intermediate fish host, enhancing the chance to be eaten by the final host (Szidat, 1969; Simsak *et al.*, 2018). As an adaptive strategy, *Clinostomum* spp. are able to infect the

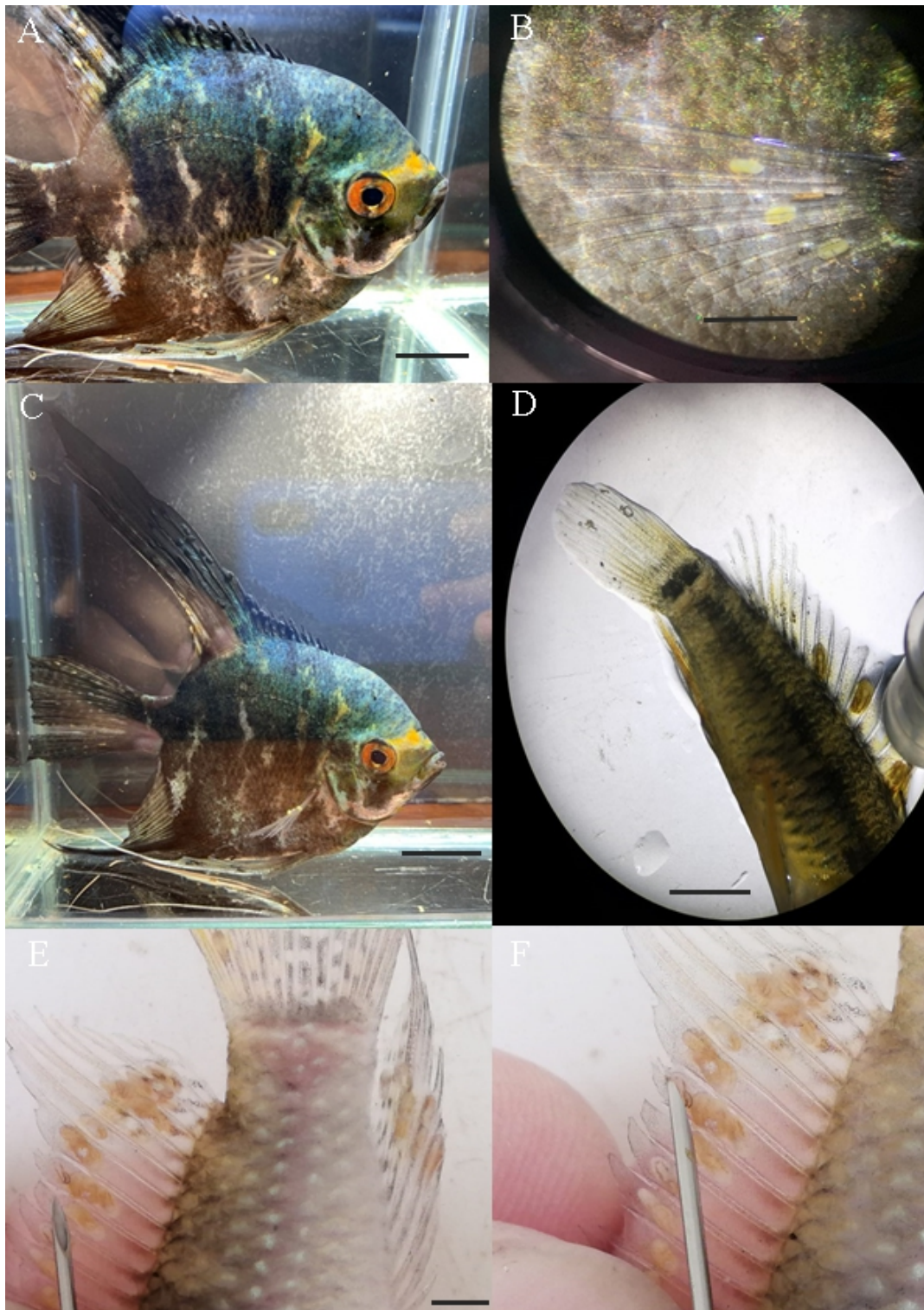


Figure 3. Metacercariae of *Clinostomum* sp. parasitizing the fins of ornamental cichlids. A. Lateral view of *Pterophyllum scalare* showing metacercariae in dorsal and pectoral fins. B. Pectoral fin of *P. scalare* with metacercariae. C. Lateral view of complete body of *P. scalare* with metacercariae on dorsal and pectoral fins. D. Dorsal fin of *Cichlassoma amazonarum* with trematodes. E. Anal fin of *Apistogramma* sp. with encysted metacercariae. F. Dorsal fin of *Apistogramma* sp. with encysted metacercariae. Scale bar A, C, D= 5 mm; B, E, F = 3 mm.

fins of the fish, causing difficulty for swimming and therefore be more susceptible to predation by piscivorous birds (Eiras *et al.*, 1999; Simsak *et al.*, 2018). Additionally, in infected fish, metacercariae induce to present slight degeneration of the muscle fibers and the destruction of the fat cells of the fins, making the movements and the ability to response to stimulus slower than in non-infected specimens (Eiras *et al.*, 1999). In the present study, the location of the metacercaria on the fins of cichlids may be a strategy to induce to erratic swimming, susceptibility to swimming closer to the surface of the water and to cause other disturbs for running away from their predators.

According to Lo *et al.* (1982), when a large amount of cercariae of *Clinostomum* spp. penetrate the fish body at the same time they may cause irritation and lesions to fish tissue, which may induce mortality of small fish. Furthermore, specimens may become weak and grew more slowly than healthy fish, dying during transportation (Lo *et al.*, 1982). As “Apistograma tapiche” *Apistogramma* sp., *C. amazonarum* and *P. scalare* are cultivated in the Peruvian Amazon for exportation as ornamental fish, the presence of *C. marginatum* could compromise the health of the fish and to cause mortalities, either due to susceptibility to predation by piscivorous birds or due to injuries caused by the infection of parasites. In that way, to prevent also economic losses, prophylactic measures should be taken into consideration: avoid the presence of aquatic snails and piscivorous birds near the breeding ponds.

Molecular studies will be necessary to be able to identify at the species level the metacercariae of *Clinostomum* collected from the analyzed cichlids. This, in order to guarantee the correct taxonomic identification based not only on morphological characters, but also based on the genetic sequence of the parasites.

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