

Short notes

Parasitism by *Clinostomum marginatum* (Clinostomidae) in neotropical electric fish (Gymnotiformes) in the Brazilian Amazon

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ABSTRACT. Gymnotiformes are currently recognized as electric fish, the vast majority of which emit low power electrical discharges. *Clinostomum* trematodes have been the subject of numerous investigations in several host species due to the ability of metacercariae of this genus to infect the muscles, fins and internal organs of fish. The present study aimed to record, for the first time, the occurrence of parasitism by *Clinostomum marginatum* in Gymnotiformes from the natural environments of two tributaries of the Amazon River in the Brazilian Amazon. Two specimens of *Gymnotus coropinae*, ten specimens of *Brachyhyppopomus brevirostris*, two specimens of *Gymnorhamphichthys petiti* and one specimen of *Microsternarchus bilineatus* were captured. Infection with *C. marginatum* was identified in the form of 12 encysted metacercariae in the epidermis, with a prevalence of 0.1%, a mean infection intensity of 12/fish and an average abundance of 1.2. This is the first record of parasitism by the *C. marginatum* in South American Gymnotiformes of the *B. brevirostris* species.

Keywords: Digenea, continental fish, parasites of Amazon fish

Introduction

Parasitism by trematodes of the genus *Clinostomum* has been the subject of numerous investigations in several host species, due to the ability of metacercariae of this genus to infect the muscles, fins and internal organs of fish, and also because of their zoonotic potential [1–5]. During the life cycle in the fish, second intermediate host, metacercariae of *Clinostomum* spp. are the cause of the pathology known globally as „yellow-grub disease”, which affects many freshwater fish [6,7].

C. marginatum has already been reported parasitizing fish, amphibians, and reptiles during its metacercaria phase, and is usually found on the host body wall below the integumentary tissue, causing characteristic nodular swelling that can easily be

seen during physical examination. Adult forms are found in the oral cavity and in the esophagus of piscivorous birds, with records in the North and South American continents [8].

Streams of upland forests in the Amazon river basins host specialized communities of gymnotiform fish with small body sizes, which that seek refuge in submerged vegetation, root mats and litterfalls. New sampling and identification methodologies have enabled studies in unexplored areas, contributing to greater description of species of this group, with numbers growing gradually over the last 20 years [9]. This has supported more robust studies of the parasitic diversity that can affect these individuals and the consequences of the parasite-host relationship.

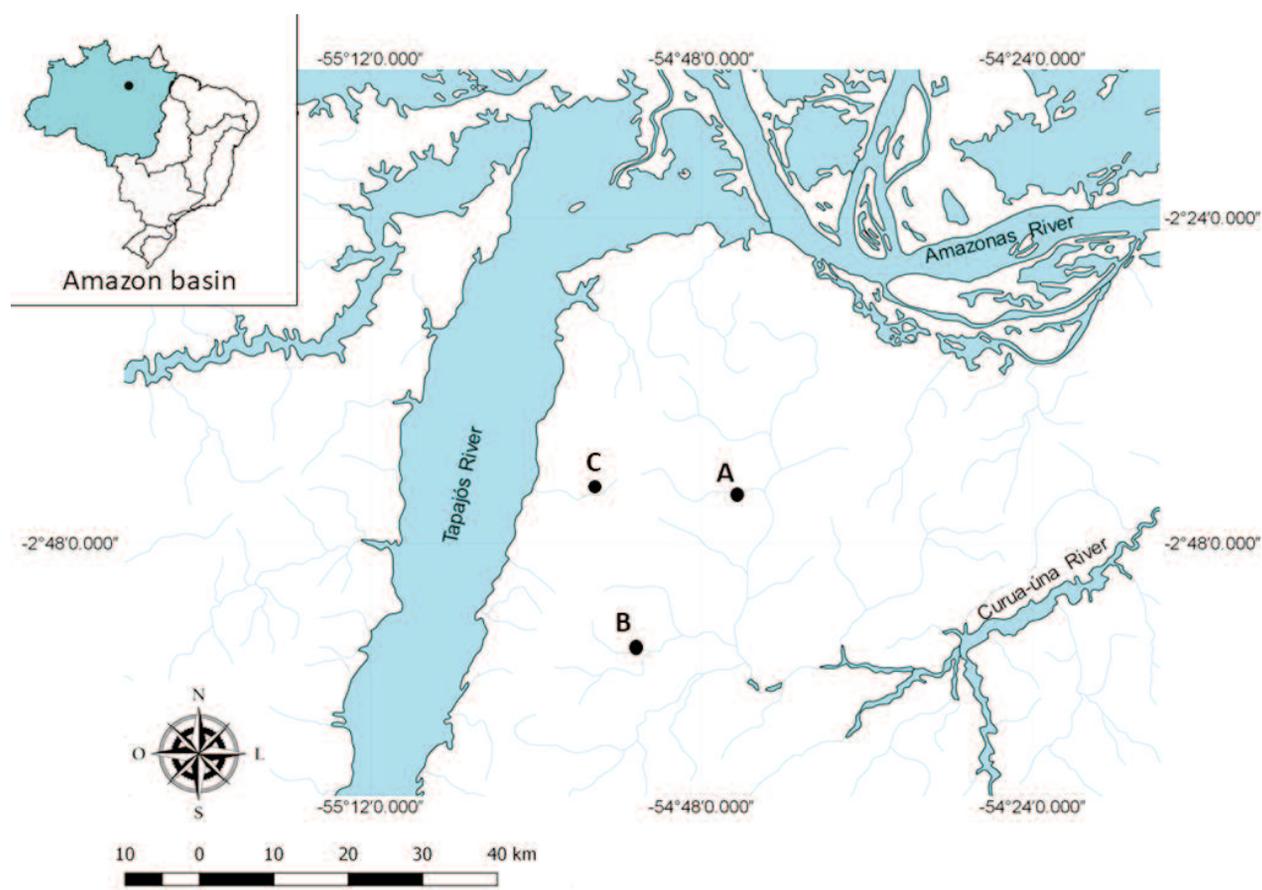


Figure 1. Geographic localization of collection points. A ($2^{\circ}44'15.10''\text{S}/54^{\circ}45'29.60''\text{O}$) and B ($2^{\circ}55'41.00''\text{S}/54^{\circ}52'44.50''\text{O}$) – Micro-basin of Curuá-Una River; C ($3^{\circ}6'16.90''\text{S}/55^{\circ}5'21.30''\text{O}$) – Tapajós River basin, tributaries of Amazon River.

In the monophyletic order of gymnotiform fish, five families, 30 genera and approximately 180 species are currently recognized, the vast majority of which emit low power electrical discharges [10]. This study aims to record the occurrence of parasitism by *C. marginatum* in Gymnotiformes natural to two tributaries of the Amazon River in the Brazilian Amazon.

Materials and Methods

In September 2017, 15 Gymnotiform specimens were captured at three sampling points, with points A and B located in the Curuá-Una River micro-basin, and point C in the Tapajós River Basin, both tributaries of the Amazon River, in the municipal districts of Mojuí dos Campos and Belterra in Western Pará State, Brazil (Fig. 1).

Two specimens of *Gymnotus coropinae* (mean length: 12.00 cm, mean weight: 3.05 g), ten specimens of *Brachyhypopomus brevirostris* (mean length: 17.34 cm, mean weight: 4.38 g), and two

specimens of *Gymnorhamphichthys petiti* (mean length: 11.50 cm, mean weight: 0.51 g) were captured, along with one specimen of *Microsternarchus bilineatus* (length: 9.40 cm, weight: 0.46 g) (Fig. 2).

The collected specimens were packed in oxygen-containing plastic bags and transported to the Universidade Federal do Oeste do Pará (the Federal University of Western Pará) for parasitological study procedures. In the laboratory environment, after being anesthetized and euthanized by the vertebral spine transection method, the total length (cm) and weight (g) was measured, and the specimens then underwent necropsy to collect endoparasites in accordance with [11], with the eyes, gills, skin and intestine examined with a stereomicroscope. The parasites found were quantified and prepared by Fair's ferric hematoxylin method, fixed slides were mounted in Canada balm, and the specimens were morphologically identified according to [12,13].

The trematodes were examined by light

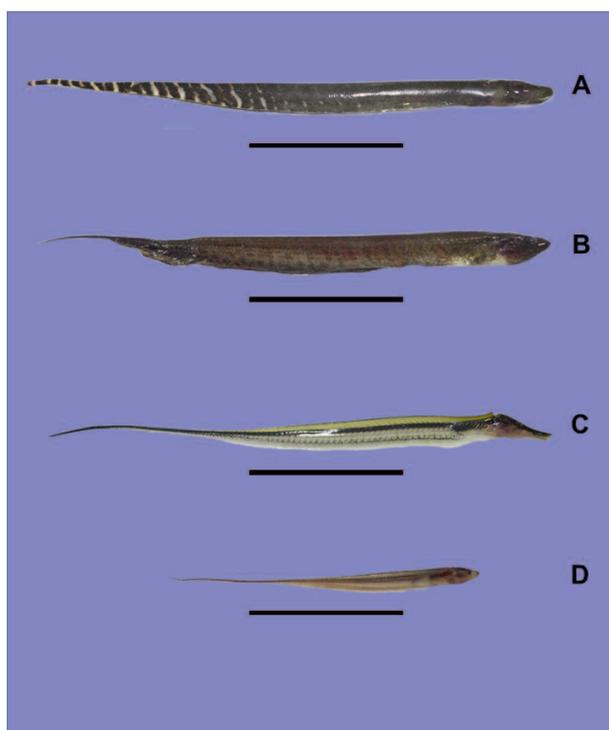


Figure 2. **A.** *G. coropinae*, **B.** *B. brevirostris*, **C.** *G. petiti*, **D.** *M. bilineatus*. Scale bar=5 cm

microscopy at 100 to 400× magnification at the Laboratory of Microscopy and Sample Collection of the Federal University of Western Pará-UFOPA. The parasites were photographed using a Zeiss Axioplan optical microscope with an Axiocam ERC 5s camera, and measurements of the organs of the reproductive system were taken with the aid of Blue

Zen edition 2 software. The ecological terms used were those proposed by [14].

Results

Only one specimen of *B. brevirostris* exhibited infection with *C. marginatum*, with 12 encapsulated metacercariae identified in the epidermis, corresponding to a prevalence of 0.1%, an average infection intensity of 12/fish and an average abundance of 1.2 (Fig. 3).

General description and measurements

Based on the morphometry of nine metacercariae. Linguiform body with slight strangulation at the level of ventral suction cup, oral collar present at the anterior extremity, oral sucker, shorter and with smaller diameter than the ventral ones, pharynx present with bifurcation of the intestinal cecum extending to the posterior extremity of the body, ventral sucker located in the anterior third of the body. 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, Cirrus pouch present on the right side of the anterior testis, ovary located in the inter-testicular space (Fig. 4). The specimens examined showed slight morphometric variations when compared to specimens from North and Central America (Table 1).

Table 1. Variations and morphometric comparison between *Clinostomum marginatum* (µm)

	<i>C. marginatum</i> Present study (n=9)	<i>C. marginatum</i> Caffara et al. [13]	<i>C. marginatum</i> Sereno-Uribe et al. [25]
Body length	5.684±472	5.402±672	3.300±363
Body width	1.789±140	1.329±173	730±620
Oral sucker (OS) length	298±38	312±104	183±18
OS width	363±33	290±106	196±15
Ventral sucker (VS) length	873±36	669±64	496±60
VS width	941±35	708±60	530±51
Distance between suckers	809±77	1243±142	539±84
Anterior testis (A) length	409±80	307±53	224±46
A width	635±88	389±77	247±41
Posterior testis (P) length	343±56	327±57	214±30
P width	691±118	405±56	280±36
Distance between testes	419±38	353±56	137±18

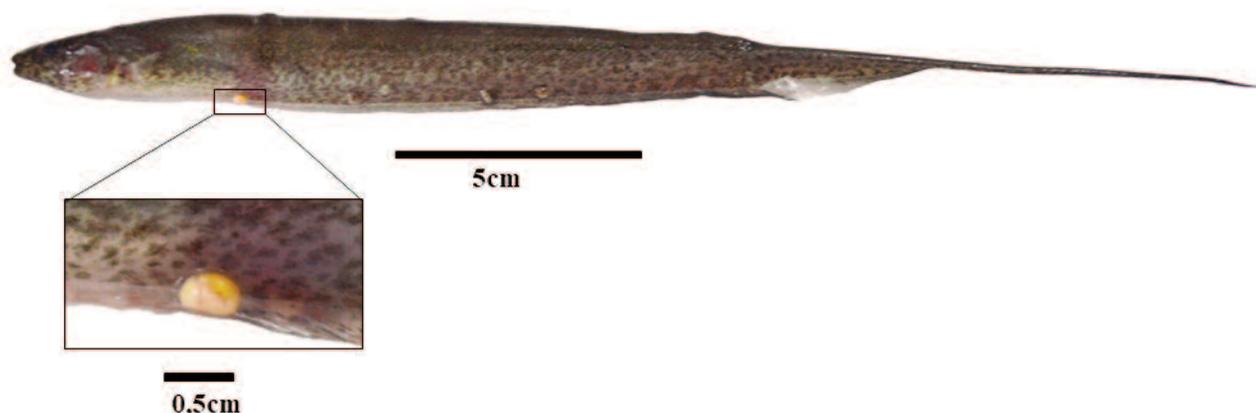


Figure 3. Metacercariae of *C. marginatum* parasitizing *B. brevirostris*

Taxonomic summary

Clinostomum marginatum (Rudolphi, 1819) (Fig. 4).

Host: *Brachyhyopomus brevirostris* (Steindachner, 1868) (family Hypopomidae)

Site of infection: Below the skin

Prevalence: 0.1%

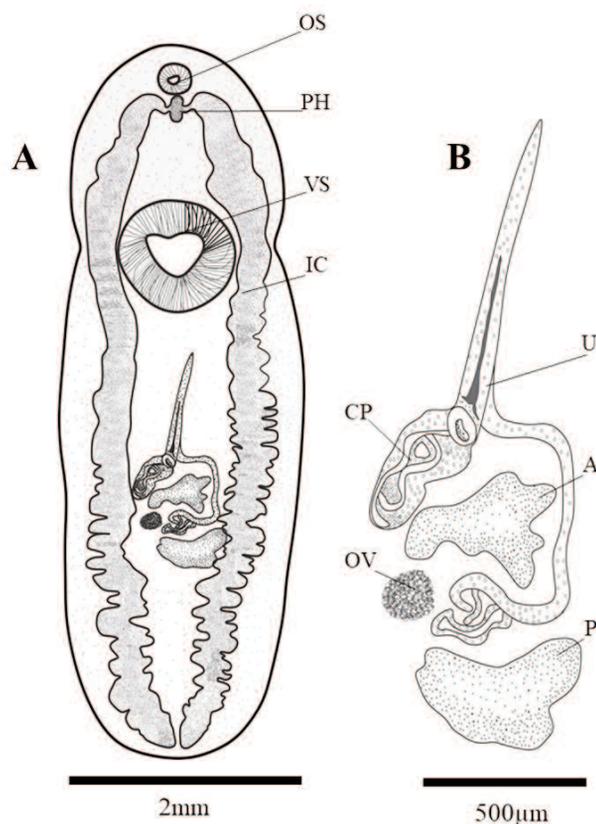


Figure 4. *C. marginatum* metacercaria. **A.** Parasite overview, OS – oral sucker, PH – pharynx, VS – ventral sucker, IC – intestinal cecum. **B.** Reproductive structures, U – uterine sac, CP - cirrus pouch, OV – ovary, A – anterior testis, P – posterior testis.

Discussion

According to [13] the width of the body, the organization of the genital complex and the distance between the sucker are characteristics that allow the differentiation of the *C. complanatum* and *C. marginatum* species, with the genital complex as the main identification criterion, reaffirming the validity of the *C. marginatum* species based on morphological features.

Parasitism by clinostomidae metacercariae has been reported in numerous fish in Brazil, such as *Cichla ocellaris* and *Crenicichla* sp. [15,16], *Semaprochilodus insigne* [17], *Hoplosternum littorale* [18], *Pterophyllum scalare* [6], *Geophagus brasiliensis* [19] and *Synbranchus marmoratus* [20]. However, sampling in an area with difficult logistical access has made it difficult to register new hosts. The present study therefore corroborates the findings of [21], which registered *Clinostomum* sp. parasitizing Gymnotiform fish.

These trematodes can have a varied range of intermediate hosts, and may affect various amphibian species [22] registered the occurrence of metacercariae in *Notophthalmus viridescens*, a species of North American Salamandridae, [23] identified parasitism in *Triturus carnifex*, a European Salamandridae, while [24] reported such an occurrence in *Rana catesbeian* and *R. chiricahuensi* in North America, which allows the exploration and diversification of new areas by these parasites.

According to [20] cysts were located in various regions of the body of *Gymnotus carapo*, as was recorded for fish species captured in other regions, corroborating the findings of the present study, where no variations were observed in terms of the

site of infection, with the parasites located along the body of the host at various positions below the skin.

Despite the efforts made in the last two decades to recognize the diversity of Gymnotiform fish, providing parasitologists with the possibility of studying new hosts, the knowledge of the parasitic diversity that can affect these vertebrates is still little known. This is therefore the first record of *C. marginatum* trematodes infecting South American Gymnotiformes of the species *B. brevirostris*.

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Compliance with ethical standards

The present study is in accordance with the principles of the Brazilian College of Animal Experimentation (COBEA), and authorized by the Animal Research Ethics Committee of the National Institute of Amazonian Research through certificate number 031/2016. The collections were authorized by the Biodiversity Authorization and Information System of the Brazilian government, under authorization number 54535.

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