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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

Expansion of host range for *Clinostomum Marginatum* (Rudolphi, 1819) (Digenea: Clinostomidae) in the Brazilian Amazon

Expansión del rango de hospedadores para *Clinostomum Marginatum* (Rudolphi, 1819) (Digenea: Clinostomidae) en la Amazonía Brasileña

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Running Head: host range expansion of *Clinostomum marginatum*

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ABSTRACT

Parasites of the genus *Clinostomum* Leidy, 1856, are widely distributed across all continents and can infect vertebrates from various groups. In fish, they are known as causative agents of "white spot disease," which, in some cases, may lead to host mortality. Reports of new hosts for these trematodes have been steadily increasing. In this context, the present study documents new host *Acestrorhynchus falcirostris* (Cuvier, 1819) for the species *Clinostomum marginatum* (Rudolphi, 1819) and contributes to understanding its distribution in the Brazilian Amazon.

Keywords: Amazon – endoparasites – fish – Trematodes – white spot disease

RESUMEN

Los parásitos del género *Clinostomum* Leidy, 1856, se encuentran ampliamente distribuidos en todos los continentes y pueden infectar a vertebrados de diversos grupos. En peces, se conocen como agentes causales de la enfermedad de la mancha blanca, que, en algunos casos, puede provocar la mortalidad del hospedador. Los informes de nuevos hospedadores para estos trematodos han aumentado constantemente. En este contexto, el presente estudio

documenta el nuevo hospedador *Acestrorhynchus falcirostris* (Cuvier, 119) para la especie *Clinostomum marginatum* (Rudolphi, 1819) y contribuye a comprender su distribución en la Amazonia brasileña.

Palabras clave: Amazonia – endoparasitos – enfermedad de la mancha blanca – peces – Trematodos

INTRODUCTION

The genus *Clinostomum* Leidy, 1857 (Digenea: Clinostomidae) has a cosmopolitan distribution, occurring in estuarine and freshwater systems worldwide (Tavares-Dias *et al*., 2021). These trematodes have a complex life cycle, with piscivorous birds serving as definitive hosts. Their eggs are released into aquatic environments, where they hatch into miracidia. Upon contact with mollusks of the genera *Lymnaea* Lamarck, 1799, *Radix* Montfort, 1810 (Gastropoda: Lymnaeidae), *Bulinus* O. F. Müller, 1781, *Biomphalaria* Preston, 1910, *Planorbella* Haldeman, 1843 and *Helisoma* Swainson, 1840 (Gastropoda: Planorbidae) they develop into sporocysts (Dias *et al*., 2005; Pinto *et al.*, 2015; Tavares-Dias *et al*., 2021). Fish act as intermediate hosts, being parasitized in the metacercarial stage. In some cases, the migration of these larvae into muscle tissues can severely affect the host, potentially leading to mortality (Calhoun *et al*., 2020).

Currently, 22 valid species of the genus *Clinostomum* have been recognized based on morphological and molecular data. Among these species, only four have been recorded in South America associated with Brazilian hosts: *Clinostomum complanatum* (Rudolphi, 1809), *Clinostomum detruncatum* Braun, 1899, *Clinostomum heluans* Braun, 1899, and *Clinostomum marginatum* (Rudolphi, 1819) (Montes *et al*., 2021; Tavares-Dias *et al*., 2021). The latter species, *C. marginatum*, has been recorded infecting fish, amphibians, reptiles, and mammals, including humans, with infections in fish primarily occurring in the tegument, where it causes easily

detectable localized swelling, while also frequently affecting the gills and viscera (Tavares-Dias *et al.,* 2021).

Despite recent advances in studying the metacercariae of this genus, particularly regarding taxonomy and distribution, research on fish diversity associated with these trematodes remains limited. It is estimated that only 5% of fish diversity in South American countries has been examined for parasitological studies (Choudhury *et al*., 2016). In Brazil, which harbors approximately 3,500 freshwater fish species, only around 2% have been analyzed for *Clinostomum* metacercariae (Tavares-Dias *et al.*, 2021; Froese & Pauly, 2025).

In Brazilian river basins, 28 fish species have been reported as hosts for this parasite, but new host records continue to emerge (Tavares-Dias *et al.*, 2021). Among the diverse fish species in these ecosystems, members of the genus *Acestrorhynchus* Eigenmann & Kennedy, 1903 stand out as potential hosts. This genus is the only representative of the family Acestrorhynchidae and comprises 15 valid species of Neotropical fish classified based on morphological, osteological, and molecular analyses. These species are endemic to South America with records in different hydrographic basins and restricted to freshwater environments (Timana-Mendoza *et al*., 2025; Garcia *et al*., 2025).

Among these 15 species, *Acestrorhynchus falcirostris* (Cuvier, 1819) is a migratory fish that inhabits lentic environments, including lakes and river margins in white, black, and clearwater systems. It is an opportunistic predator with a diverse diet, primarily feeding on small crustaceans and fish (Toledo-Piza, 2007; Brito *et al*., 2025). However, despite its abundance across various Amazonian regions, studies on its role as a parasite host remain limited. Given this gap in knowledge, this study aims to document a new host for *C. marginatum* and contribute to understanding its distribution in the Brazilian Amazon.

MATERIALS AND METHODS

Collection site

During environmental studies conducted in the Tapajós River basin, Pará State, Brazil, in December 2022, five specimens of *A. falcirostris* (Fig. 1) were captured using gill nets. A physical examination of the specimens revealed cutaneous elevations along their bodies. The freshly caught fish were subsequently sent to the Laboratório de Ecologia e Comportamento Animal (LECAn) at the Universidade Federal do Oeste do Pará for the parasitological analysis.

Preparation and identification of the parasites

In the laboratory, the identified cystic forms were carefully extracted using surgical instruments. A portion of the specimens were preserved in 70% ethyl alcohol for conservation. Ten specimens were excysted and pressed, then preserved and fixed in Rhalie Harri solution. Subsequently, the metacercariae were stained using the hydrochloric carmine method and slide-mounted with Canada balsam (Pinto *et al*., 2015, Calhoun *et al*., 2020). The metacercariae were examined using light microscopy at magnifications of 100 to 400 × at LECAN. The parasites were photographed with a Zeiss Axioplan optical microscope with the aid of an Axiocam ERc 5s camera, and measurements of the reproductive system organs were taken using Blue Zen 3.7 software. Taxonomic identification followed the criteria outlined by Caffara *et al.* (2011), and the parameters of prevalence and intensity of infection were calculated according to Bush *et al.* (1997) using the software Quantitative Parasitology 3.0 (Reiczigel *et al*., 2019). Two specimens were deposited in the Parasitological Collection at LECAN under access numbers: UFOPA-P(Tre)004 and UFOPA-P(Tre)005.

Scanning Electron Microscopy

External morphology was evaluated by scanning electron microscopy (SEM) previously fixed in ethyl alcohol (70%), transferred to glutaraldehyde solution (2.5%) in 0.15 M phosphate buffer (pH 7.3), and then subsequently fixed in osmium tetroxide (1%) in the same buffer, for two hours. The samples were dehydrated an increasing sequence of ethyl alcohol solutions, washed

in a solution of distilled water and filtered water (1:1). Dehydration was carried out with an increasing sequence of ethyl alcohol solutions, and drying was carried out by means of a critical point in CPD 020 (Balzer Union), with liquid CO_2. The samples were placed on double-sided tape in Stub and covered with a gold-palladium jet, for visualization in SEM Leo Stereoscan S-440.

Ethics aspects: This study was approved by the Animal Use Committee of the Universidade Federal do Amapá (authorization # 011/2021). The licenses for the collection of fishes used in the present study were granted by the Secretaria de Estado de Meio Ambiente e Sustentabilidade do Estado do Pará (SEMAS/PA) (authorization # 4757/2021).

RESULTS

Taxonomic summary

Class Trematoda Rudolphi, 1808

Subclass Digenea Carus, 1863

Family Clinostomidae Lühe, 1901

Genus *Clinostomum* Leidy, 1856

***Clinostomum marginatum* (Rudolphi, 1819**) (Fig. 2)

Host: *Acestrorhynchus falcirostris* (Cuvier, 1819).

Infection site: oral cavity, lower palate, tongue and integument.

Locality: Foz do Rio Tapajós, Bacia do Tapajós, Amazonia, Brazil (2° 22' 16.20" S, 54° 45' 38.24" W).

Prevalence: 60%, three of five specimens.

General description and measurements

Based on the morphometry of ten 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.

Among the *A. falcirostris* specimens examined, three individuals were infected with metacercariae, corresponding to a prevalence of 60%, with a mean intensity of 7.3 (IC = 3.6-11.9) and mean abundance of 4.4 (IC = 2.2-7.3) parasites. The parasitological examination revealed the presence of metacercariae adhered to the lower palate of the oral cavity, at the base of the tongue, as well as subcutaneous cysts in the mid-body region near the fins (Fig. 3).

In scanning electron microscopy (SEM), the parasites exhibited a generally tongue-shaped body with slight striations along the sides and a cavitary flexion at the level of the testes. The oral sucker was terminal with small rugosities, while the ventral sucker had raised, rough borders with irregular striae. The dermis was rough, with a punctate appearance and no visible alignment pattern. Longitudinal grooves were observed laterally on the dorsal side at the level of the pharynx.

Using light microscopy and hydrochloric carmine staining, we were able to observe that the oral sucker was structurally smaller than the ventral sucker, with a bifurcated pharynx and an intestinal tract extending to the posterior portion of the body. The testes were visible with a lobular, foliate appearance, located in the middle portion of the third body segment. The ovary was located in the intertesticular region, while the cirrus sac was found in the second third of the body, superior to the anterior testis. Based on these observations, the internal and external

morphology of the metacercariae was consistent with *C. marginatum*. The morphometric measurements of the internal and external structures of this parasite are presented in Table 1.

DISCUSSION

In Brazilian ecosystems, numerous fish species have already been recorded as infected by metacercariae of *Clinostomum* parasites. Among the known hosts are *Brachyhypopomus brevirostris* (Steindachner, 1868) (De Souza *et al*., 2020a), *Pterygoplichthys pardalis* (Castelnau, 1855*)* (De Souza *et al*., 2020b), *Pterophyllum scalare* (Lichtenstein, 1823) (Ramos-Alves *et al*., 2001), *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Paraguassú & Alves, 2005), *Synbranchus marmoratus* Bloch, 1795 (Acosta *et al*., 2016), *Pygocentrus nattereri* Kner, 1858 (Morais *et al*., 2011), *Cichla ocellaris* Bloch & Schneider, 1801 and *Crenicichla sp.* Heckel, 1840 (Thatcher, 1981), *Colossoma macropomum* (Cuvier, 1818) (Morey & Malta, 2016), and *Semaprochilodus insigne* (Jardine, 1841) (Castelo, 1984). These records highlight the diversity of hosts and the widespread distribution of *Clinostomum* across various Brazilian river basins.

In this study, we confirm the findings of De Souza *et al.* (2020b), where both hosts were sourced from the confluence of the Tapajós and Amazonas rivers in the municipality of Santarém, Pará state. De Souza *et al.* (2020a) had previously documented the occurrence of *C. marginatum* in hosts from tributary systems of the Tapajós River in the eastern Brazilian Amazon. Our study also corroborates the findings of Carvalho *et al.* (2003) and Duarte *et al.* (2022), who recorded infection by *Clinostomum* metacercariae in association with *A. lacustris* in the upper Paraná River and the middle São Francisco River in the state of Minas Gerais, Brazil, respectively. However, broad studies identifying potential hosts for this parasite are still limited, and occurrence records often arise from secondary data from studies on other parasitic organisms.

Although there are no recorded cases of human infection in the Brazilian Amazon, the zoonotic potential of this parasite has been confirmed, with the primary records originating from

the Asian and European continents (Tiewchaloern *et al*., 1999; Fedorčák *et al*., 2019). According to Mahdy *et al.* (2024), the disease caused by *Clinostomum* metacercariae in freshwater fish represents a globally significant threat, especially to human health. Furthermore, another important consideration is the economic losses this infection can cause in the aquaculture industry. High parasite infections can lead to slow growth, erratic behavior, and even death of the hosts (Sutili *et al.,* 2014).

The occurrence of trematodes in unusual infection sites has been demonstrated in the studies of Souza *et al*. (2018). However, for *C. marginatum*, this is the first record of metacercariae presence in the oral cavity of the host, corroborating Tavares-Dias (2019). Given that this occurrence is erratic in nature, there is still limited information on the possible outcomes of the parasite-host interaction at these infection sites. It is worth noting that infections in the oral cavity by this parasite are related to its adult form, which occurs in piscivorous birds in North and South America (Ramos-Alves *et al*., 2001; Silva-Souza & Ludwig, 2005).

With the increasing number of records of *Clinostomum* spp. in new hosts, further studies are needed, particularly those investigating the impact of these parasites on fish maintained in commercial farming systems in the Amazon. Integrative studies utilizing molecular tools are also necessary to clarify the phylogenetic relationships among parasites from different geographical regions.

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Investigation: DCS, RBS, ALD, TAC

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Project administration: DCS, RBS, ALD, TAC

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Software: DCS, RBS, ALD, TAC

Supervision: TAC

Validation: DCS, RBS, ALD, TAC

Visualization: DCS, RBS, ALD, TAC

Writing – original draft: DCS, RBS, TAC

Writing – review & editing: DCS, RBS, ALD, TAC

BIBLIOGRAPHIC REFERENCES

Acosta, A.A., Caffara, M., Fioravanti, M.L., Utsunomia, R., Zago, A.C., Franceschini, L., & da Silva, R.J. (2016). Morphological and molecular characterization of *Clinostomum detruncatum* (Trematoda: Clinostomidae) metacercariae infecting *Synbranchus marmoratus*. *Journal of Parasitology*, *102*, 151–156.

Brito, B. C., Peleja, J.R., Melo, S., de Freitas Goch, Y.G., & Viana, A.P. (2025). Relationship of mercury bioaccumulation with seasonality and feeding habits of fish species caught upstream and downstream of the Curuá-Una hydroelectric dam in the Brazilian Amazon. *Ecotoxicology*, *34*, 38–51.

Bush, A.O., Lafferty, K.D., Lotz, J.M., & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, *83*, 575.

Caffara, M., Locke, S.A., Gustinelli, A., Marcogliese, D.J., & Fioravanti, M.L. (2011). Morphological and molecular differentiation of *Clinostomum complanatum* and *Clinostomum marginatum* (Digenea: Clinostomidae) metacercariae and adults. *Journal of Parasitology*, *97*, 884–891.

Calhoun, D.M., Leslie, K.L., Riepe, T.B., Achatz, T.J., McDevitt-Galles, T., Tkach, V.V., & Johnson, P.T.J. (2020). Patterns of *Clinostomum marginatum* infection in fishes and amphibians: integration of field, genetic, and experimental approaches. *Journal of Helminthology*, *94*, e44.

Carvalho, S.D., Guidelli, G.M., Takemoto, R.M., & Pavanelli, G.C. (2003). Aspectos ecológicos da fauna endoparasitária de *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes, Acestrorhynchidae) da planície de inundação do alto rio Paraná, Brasil. *Acta Scientiarum Biological Sciences*, *25*, 479–483.

Castelo, F.P. (1984). Ocorrência de cistos de *Clinostomum marginatum* Rudolphi 1819 “Yellow Spot Disease” filé de jaraqui (*Semaprochilodus insignis* Schomburgk, 1814). *Journal of Chemical Information and Modeling*, *13*, 325–326.

Choudhury, A., Aguirre-Macedo, M. L., Curran, S. S., de Núñez-Ostrowski, M., Overstreet, R.M., Pérez-Ponce de León, G., & Santos, C.P. (2026). *Trematode Diversity in Freshwater Fishes of the Globe II: New World. Systematic Parasitology, 93*, 271–282.

De Souza, D. C., Nogueira, A., & Corrêa, L. L. (2020a). Parasitism by *Clinostomum marginatum* (Clinostomidae) in neotropical electric fish (Gymnotiformes) in the Brazilian Amazon. Annals of Parasitology, *66*, 101-106.

De Souza, D.C., Ferreira de Sousa, L., Coêlho, T.A., & Corrêa, L.L. (2020b). Host-parasite interaction between trematode, *Clinostomum marginatum* (Clinostomidae) and armoured catfish, *Pterygoplichthys pardalis* (Loricariidae) from Brazilian Amazon. *Annals of Parasitology*, *66*, 243–249.

Dias, E.J.R., Vrcibradic, D., & Rocha, C.F.D. (2005). Endoparasites infecting two species of whiptail lizard (*Cnemidophorus abaetensis* and *C. ocellifer*; Teiidae) in a 'restinga' habitat of north-eastern Brazil. *Herpetological Journal*, *15*, 133–137.

Duarte, R., Santos-Clapp, M.D.D. & Brasil-Sato, M.D.C. (2022). Metazoan endoparasites of *Acestrorhynchus lacustris* (Actinopterygii: Acestrorhynchidae) from lagoons bordering the upper and middle São Francisco river basin, Brazil. *Revista Brasileira de Parasitologia Veterinária*, *31*, e000422.

Fedorčák, J., Šmiga, Ľ., Kutsokon, I., Kolarčik, V., Koščová, L., Oros, M. & Koščo, J. (2019). Parasitic infection of *Cobitis elongatoides* Băcescu & Mayer, 1969 by zoonotic metacercariae *Clinostomum complanatum* (Rudolphi, 1814). *Journal of Fish Diseases*, 42, 1677–1685.

Froese R., & Pauly, D. (2025). *FishBase*. version (04/2025). World Wide Web electronic publication. www. fishbase.org

Garcia, T.D., Strictar, L., Fugi, R., & Vidotto-Magnoni, A.P. (2025). Does size matter? Exploring the influence of body size on predator–prey relationships, hunting mode and prey characteristics in Neotropical fishes. *Ecology of Freshwater Fish*, 34, e12803.

306 Mahdy, O.A., Abdel-Maogood, S.Z., Abdelsalam, M. & Salem, M.A. (2024). A multidisciplinary
307 study on *Clinostomum* infections in Nile tilapia: micro-morphology, oxidative stress, immunology,
308 and histopathology. *BMC Veterinary Research*, 20, 60.
309 Montes, M.M., Barneche, J., Pagano, L., Ferrari, W., Martorelli, S.R. & De León, G.P.P. (2021).
310 Molecular data reveal hidden diversity of the genus *Clinostomum* (Digenea, Clinostomidae) in
311 Argentina, with the description of a new species from *Ardea cocoi* (Ardeidae). *Parasitology*
312 *Research*, 120, 2779–2791.
313 Morais, A., Varella, A., Fernandes, B. & Malta, J. (2011). *Clinostomum marginatum* (Braun,
314 1899) and *Austrodiplostomum compactum* (Lutz, 1928) metacercariae with zoonotic potential
315 of *Pygocentrus nattereri* (Kner, 1858) (Characiformes: Serrasalmidae) from Central Amazon,
316 Brazil. *Neotropical Helminthology*, 5, 8–15.
317 Morey, G.A.M. & Malta, J.C.O. (2016). Metazoários parasitas das narinas do tambaqui
318 *Colossoma macropomum* (Cuvier, 1818) (Characiformes: Characidae) coletadas em lagos de
319 várzea da Amazônia Central, Brasil. *Folia Amazônica*, 25, 71–76.
320 Paraguassú, A.R., Alves, D.R. & Luque, J.L. (2005). Metazoários parasitos do acará *Geophagus*
321 *brasiliensis* (Quoy & Gaimard, 1824) (Osteichthyes: Cichlidae) do reservatório de Lajes, Estado
322 do Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinária*, 14, 35–39.
323 Pinto, H.A., Caffara, M., Fioravanti, M.L. & Melo, A.L. (2015). Experimental and molecular study
324 of cercariae of *Clinostomum* sp. (Trematoda: Clinostomidae) from *Biomphalaria* spp. (Mollusca:
325 Planorbidae) in Brazil. *Journal of Parasitology*, *101*, 108–113.
326 Ramos-Alves, D., Luque, J.L., & Rodrigues-Paraguassú, A. (2001). Metacercárias de
327 *Clinostomum marginatum* (Digenea: Clinostomidae) em acará-bandeira *Pterophyllum*
328 *scalare* (Osteichthyes: Cichlidae) no estado do Rio de Janeiro, Brasil. *Parasitología al Día*, *25*,
329 70–72.
330 Reiczigel, J., Marozzi, M., Fábián, I., & Rózsa, L. (2019). Biostatistics for parasitologists – a
331 primer to quantitative parasitology. *Trends in Parasitology*, *35*, 277–281.

Silva-Souza, A.T., & Ludwig, G. (2005). Parasitism of *Cichlasoma paranaense* Kullander, 1983 and *Gymnotus carapo* Linnaeus, 1814 by *Clinostomum complanatum* (Rudolphi, 1814) metacercariae in the Taquari river. *Brazilian Journal of Biology*, *65*, 513–519.

Souza, D.C., Correa, L.L., & Tavares-Dias, M. (2018). *Ithyoclinostomum dimorphum* Diesing, 1850 (Digenea, Clinostomidae) in *Hoplias malabaricus* (Erythrinidae) with the first report of infection of the eyes. *Helminthologia*, *55*, 343-349.

Sutili, F.J., Gressler, L.T. & Pelegrini, L.F.V.D. (2014). *Clinostomum complanatum* (Trematoda, Digenea): a parasite of birds and fishes with zoonotic potential in southern Brazil. A review. *Revista Brasileira de Higiene e Sanidade Animal*, 8, 99–114.

Thatcher, V.E. (1981). Patologia de peixes da Amazônia Brasileira, 1. Aspectos gerais. *Acta Amazonica*, *11*, 125-140.

Tavares-Dias, M., Borges, W. F., Santos, G. G., & de Oliveira, M. S. B. (2019). Parasites in gills of Aequidens tetramerus, cichlid from the lower Jari River, a tributary of the Amazon River, northern Brazil. *Boletim do Instituto de Pesca*, *45*, e.485.

Tavares-Dias, M., Silva, L.M.A., & Florentino, A.C. (2021). Metacercariae of *Clinostomum* Leidy, 1856 (Digenea: Clinostomidae) infecting freshwater fishes throughout Brazil: infection patterns, parasite–host interactions, and geographic distribution. *Studies on Neotropical Fauna and Environment*, *58*, 116–129.

Tiewchaloern, S., Udomkijdecha, S., Suvouttho, S., Chunchamsri, K., & Waikagul, J. (1999). *Clinostomum* trematode from human eye. *Southeast Asian Journal of Tropical Medicine and Public Health*, 30, 382–384.

Timana-Mendoza, C., Reyes-Calderón, A., Venail, P., Britzke, R., Santa-Maria, M.C., Araújo-Flores, J.M., Silman, M., & Fernandez, L.E. (2025). Hydrological connectivity enhances fish biodiversity in Amazonian mining ponds: insights from eDNA and traditional sampling. *Molecular Ecology*, *34*, e17784.

Toledo-Piza, M. (2007). Phylogenetic relationships among *Acestrorhynchus* species (Ostariophysi: Characiformes: Acestrorhynchidae). *Zoological Journal of the Linnean Society*, *151*, 691-757.

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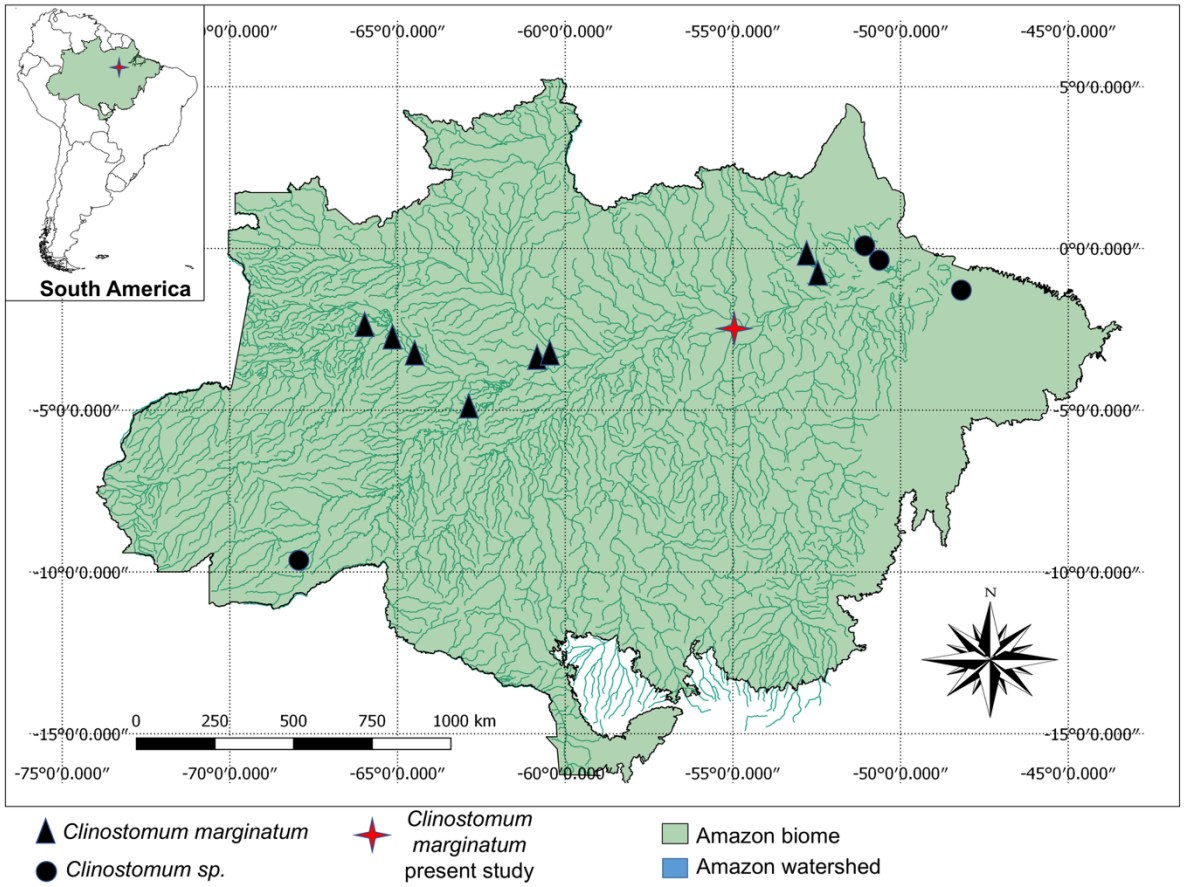

Figure 1. Map showing occurrence records of *Clinostomum* spp. in Brazil.

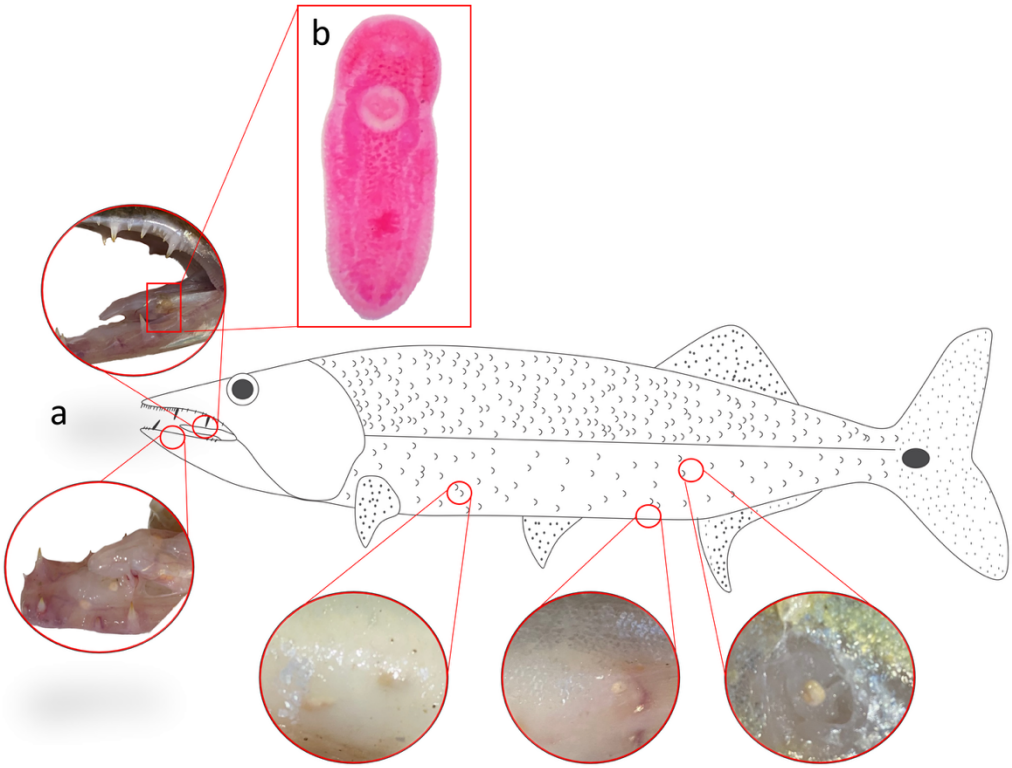

Figure 2. a – Infection sites in *Acestrorhynchus falcirostris* by *Clinostomum marginatum*. b – *C. marginatum* stained with hydrochloric carmine.

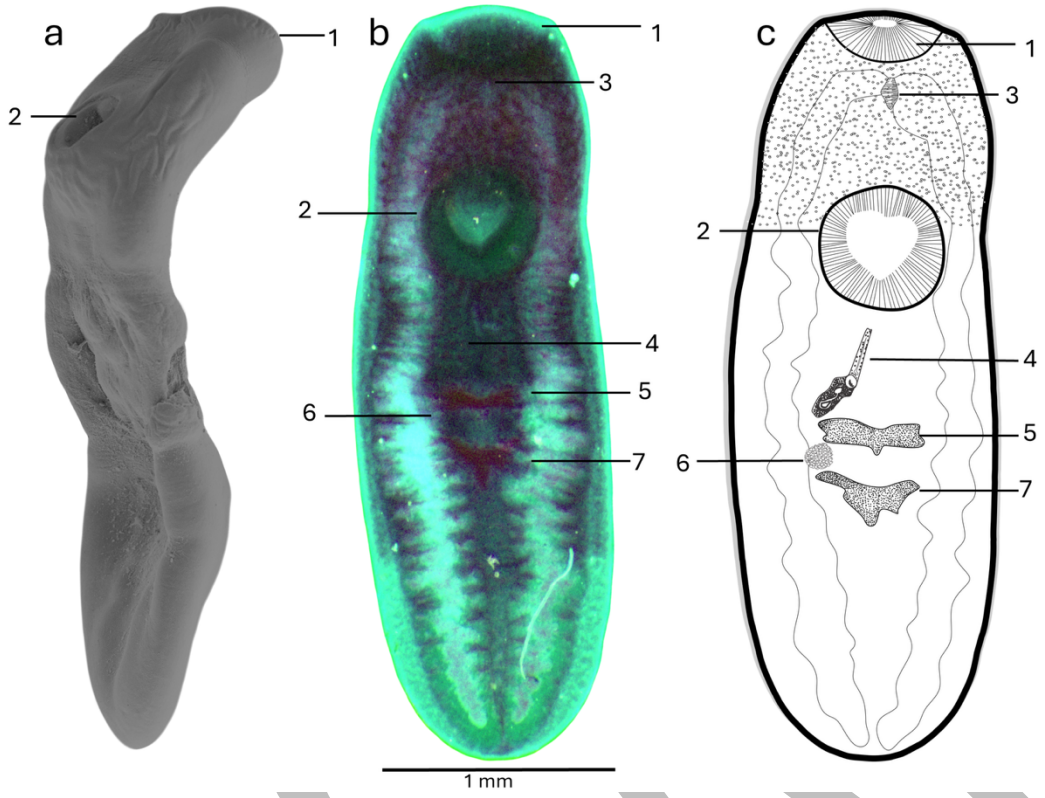

Figure 3. a – External morphology of *Clinostomum marginatum* metacercaria under scanning electron microscopy. b – Internal morphology of *C. marginatum* metacercaria. c – Schematic drawing of the internal and external structures of *C. marginatum* metacercaria. Numbers: 1 - Oral sucker; 2 - Ventral sucker; 3 - Pharynx; 5 - Anterior testis; 6 - Ovary; 7 - Posterior testis.

410 **Table 1.** Morphometric values for different records of *Clinostomum marginatum*. The values are

411 expressed in milimeters with respectively standard deviation.

Measures (μm)	*Clinostomum marginatum* Present study	*Clinostomum marginatum* Caffara *et al.* (2011)	*Clinostomum marginatum* Sereno-Uribe *et al.* (2013)
Body length	3.820±354	5.402±672	3.300±363
Body width	1.506±160	1.329±173	730±620
Oral sucker (OS) length	237±10	312±104	183±18
OS width	344±22	290±106	196±15
Ventral sucker (VS) length	695±31	669±64	496±60
VS width	906±31	708±60	530±51
Distance between suckers	578±85	1243±142	539±84
Anterior testis (AT) length	271±5	307±53	224±46
AT width	475±68	389±77	247±41
Posterior testis (PT) length	254±38	327±57	214±30
PT width	462±30	405±56	280±36
Distance between testes	578±85	353±56	137±18

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