

Short notes

Host–parasite interaction between trematode, *Clinostomum marginatum* (Clinostomidae) and armoured catfish, *Pterygoplichthys pardalis* (Loricariidae) from Brazilian Amazon

Darlison Chagas de Souza¹, Luciclara Ferreira de Sousa², Tassio Alves Coelho¹, Lincoln Lima Corrêa²

¹Programa de Pós-graduação em Biodiversidade (PPGBEES), Instituto de Ciências e Tecnologia das Águas - ICTA, Universidade Federal do Oeste do Pará. Av. Mendonça Furtado, nº 2946, Fátima, CEP 68040-470, Santarém, PA, Brasil

²Instituto de Ciências e Tecnologia das Águas - ICTA, Universidade Federal do Oeste do Pará - UFOPA, Av. Mendonça Furtado, nº 2946, Fátima, CEP 68040-470, Santarém, Pará, Brasil

Corresponding Author: Lincoln L. Corrêa; e-mail: lincorre@gmail.com

ABSTRACT. Clinostomid trematodes are only widely studied due to the ability to infect their metacercariae, which can affect amphibians, fish, snakes and occasionally mammals, with occasional records in humans. The Loricariids constitute the most diverse family of neotropical fish, with more than 800 registered species. They present a large heterogeneity of colors and body forms that reflect its high degree of ecological specialization and importance on economic aspects such as ornamentation and food. In spite of these factors, studies involving the diversity of parasitic fauna that affect in these fish is still scarce. In this aspect, our study presents the first record of occurrence of the parasite *Clinostomum marginatum* in association with the Loricariid *Pterygoplichthys pardalis* in the Amazon region of Brazil. In September 2018, in the river mouth of Tapajós river, tributary of Amazon river, 32 specimens of *P. pardalis* and six specimens of *Aphanotorulus emarginatus* were captured. The parasitological studies revealed the occurrence of metacercariae of *C. marginatum* in association with *P. pardalis*, representing a prevalence of 12.5% with a mean abundance of 0.18 and a medium intensity of infection of 1.5. No *C. marginatum* parasitism was observed in *A. emarginatus*.

Keywords: metacercariae, Digenea, fish, Amazon

Introduction

Trematodes of genus *Clinostomum* have been the subject of numerous studies in a diverse group of hosts, being known as amphibian, fish, reptiles and occasionally mammalian parasites, with human parasitism occurring in Asia as zoonosis [1–5]. The metacercariae of this parasite have the ability to infect external locomotion structures, muscles and internal organs during the life cycle. In the infection of the secondary intermediate host, in freshwater fish *Clinostomum* spp. they are the cause of the "yellow-spot disease", such spots are the result of

encystation below the integumentary tissue, causing visible nodular swelling. The adult forms of this parasite can be found in the oral and esophageal cavity of piscivorous birds distributed in the South and North American continents [6,7].

Loricariidae has approximately 800 known species, being the largest family of neotropical fish, representing 25% of the diversity of Siluriformes, distributed in seven subfamilies: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae, Neoplecostominae and Otothyriinae. These fish are characterized by the presence of bony plaques lining their body and having a single pair of

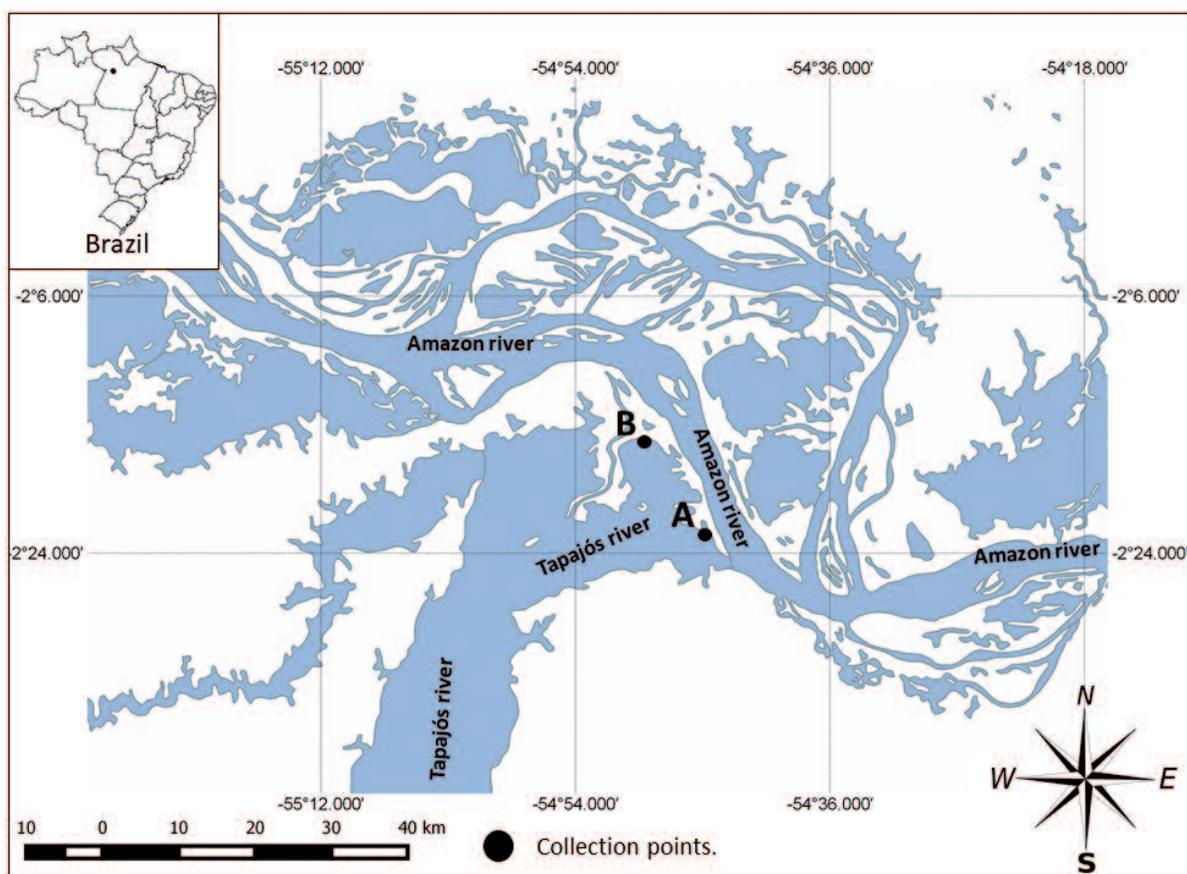


Figure 1. Loricariidae capture points. **A.** Igarapé dos Reis ($2^{\circ}20'50.16''\text{S}/54^{\circ}46'8.48''\text{O}$); **B.** Enseada Grande ($2^{\circ}15'38.22''\text{S}/54^{\circ}56'45.22''\text{O}$).

barbels. They have omnivorous habits, feeding on algae, small invertebrates and debris, and their distribution is across South America, Costa Rica and Panama. Furthermore, the heterogeneity of colors and body shapes presented by these fish reflect the high degree of ecological specialization as this clade can be found in different habitats, from stagnant low-oxygen waters to rapids [8–12].

Due to their varied shapes and colors, some species of Loricariidae are of commercial importance in aquaculture as ornamental fish [13,14]. Another important aspect of loricariids, especially species of higher body robustness such as *Pterygoplichthys* in the Brazilian Amazon region, is its use for human consumption, being widely marketed in traditional fairs and trades of the Amazonian cities located on the banks of rivers, or processed into flour directed to areas of tourist exploitation [15].

Pterygoplichthys pardalis is naturally distributed in the tropical region of South America, mainly in environments located on the Amazon River, but recent studies have demonstrated its range expansion, being registered in the United States,

Mexico, Serbia, Philippines, Vietnam, Singapore, Malaysia and Indonesia [16–17]. Thus, the present study registers for the first time the occurrence of *Clinostomum marginatum* parasite in association with loricariid *P. pardalis* in the Brazilian Amazon region.

Materials and Methods

In September 2018, at the mouth of the Tapajós River, tributary of the Amazon River, in the locations called Enseada Grande and Igarapé dos Reis (Fig. 1), 32 specimens of armoured catfish, *Pterygoplichthys pardalis* (average weight 359.7 ± 98.6 g, mean standard length 33.4 ± 2.6 cm) were captured with the aid of gillnets and six specimens of *Aphanotorulus emarginatus* (mean weight 150.0 ± 21 g, mean standard length 27.2 ± 2.4 cm) (Fig. 2).

After the fish were caught, they were anesthetized and euthanized by the spinal transection method, weight (g) and total length (cm) were measured and then subjected to physical examination for parasitological collection. The

procedures for collecting trematode fixation followed that established by [18]. The identified cysts were removed *in loco*, the metacercariae fixed in alcohol and sent to the Universidade Federal do Oeste do Pará for screening and taxonomic identification procedures.

The collected parasites were quantified and stained by the Langeron Acid Carmine method [19], mounted on permanent slides with Canadian balm and morphologically identified according to [20,21]. The trematodes were examined by light microscopy at 100 to 400× magnification at the Laboratory of Microscopy and Sample Collection from the Universidade Federal do Oeste do 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 [22].

Results

Four specimens of *P. pardalis* presented infection with *C. marginatum* metacercariae, representing a prevalence of 12.5% with an average abundance of 0.18 and a mean intensity of infection of 1.5. Metacercariae were found on the eye cavity and ventral host fins (Fig. 3). Representatives of the

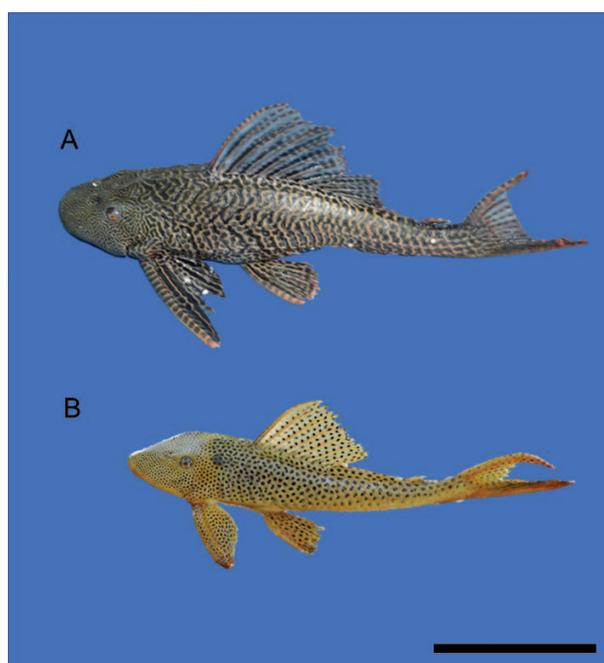


Figure 2. Armored catfish caught at Tapajós River Mouth. **A.** *Pterygoplichthys pardalis*; **B.** *Aphanotorulus emarginatus*. Scale bar = 10cm.

species *A. emarginatus* were not infected with *C. marginatum*. The cercariae morphometry presented variations when compared with existing studies (Table 1).

Morphological description. The description was based on the morphometry of four metacercariae

Table 1. Morphometric data of cercariae of *Clinostomum marginatum* (Rudolphi, 1819)

Features (µm)	<i>C. marginatum</i> Present study	<i>C. marginatum</i> Caffara et al. [21]	<i>C. marginatum</i> Sereno-Uribe et al. [39]
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

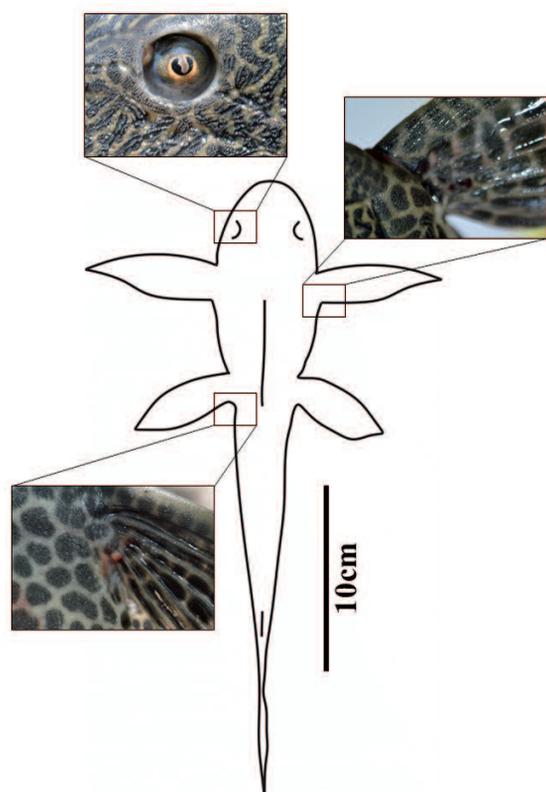


Figure 3. *Clinostomum marginatum* metacercaria sites in *Pterygoplichthys pardalis*

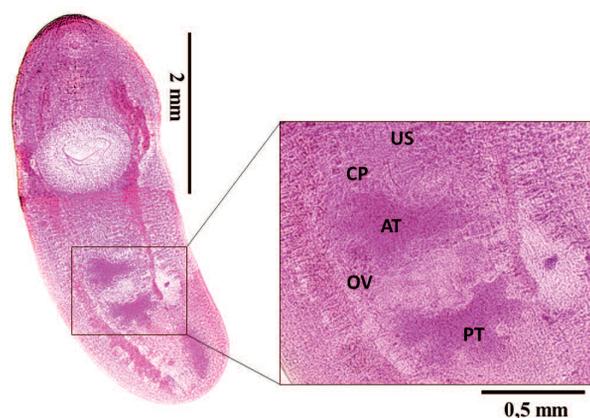


Figure 4. *Clinostomum marginatum* metacercaria. US - uterine sac, OV - ovary, CP - cirrus sac, AT - anterior testis, PT - posterior testis

(Fig. 4). Oral suckers smaller than ventral suckers, discrete oral collar at anterior extremity, predominantly linguiform body with slight C-shaped curvature, subtle attenuation at ventral sucker height. Pharyngeal present and bifurcated (Fig. 4A). Reproductive system structures located between the second and third thirds of the body.

Lobed testes, cirrus sac lateralized to the anterior testis, ovary located in the intertesticular space (Fig. 5B).

Taxonomic summary

Clinostomum marginatum (Rudolphi, 1819) (Fig. 4)
Host: *Pterygoplichthys pardalis* (Castelnau, 1855)
Site of infection: eye cavity and ventral fin region
Prevalence: 12.5%

Discussion

In Brazil, countless species of fish have been registered as hosts of *Clinostomum* metacercariae [6] recorded *C. marginatum* in cichlid *Pterophyllum scalare*, as well as [23] observed the presence of *Clinostomum* sp. in the fins of *Geophagus brasiliensis* in reservoirs and rivers of the state of Rio de Janeiro. Reported the occurrence of *C. detruncatum* parasitizing *Synbranchus marmoratus* in the Paraná River, state of Paraná [2]. In the rivers of the central Brazilian Amazon, [24] recorded *C. marginatum* in *Pygocentrus nattereri* from the Solimões River, [25] identified metacercariae in *Cichla ocellaris* and *Crenicichla* sp. [26] observed *C. marginatum* parasitism in *Colossoma macropomum* and [27] recorded the occurrence of metacercariae in *Semaprochilodus insigne* in the Negro and Amazon Rivers.

The present study corroborates the findings of [28], who identified *Clinostomum* sp. parasitism in *P. pardalis* with metacercariae found in the caudal fin region with a prevalence of 1%, this record occurred in the region of the Paliza River in Mexico. Investigated the fauna of *P. pardalis* metazoan parasites in a tributary of the Amazon River system, but did not identify the occurrence of clinostomid parasites [29]. The present study is the second record of parasitic relationship involving these trematodes and Siluriformes, known as armoured catfish, in the Brazilian Amazon, but it is the first recorded occurrence in loriciariids, in this sense we corroborate the works of [30] that recorded the parasitism by *Clinostomum* sp. in *Hoplosternum littorale*.

Despite its natural distribution to the South American tropical region *P. pardalis* has been identified as invasive species in the North American, Asian and European continents [16] and [31–35]. According to [36], the introduction of exotic fish can threaten native biodiversity and the

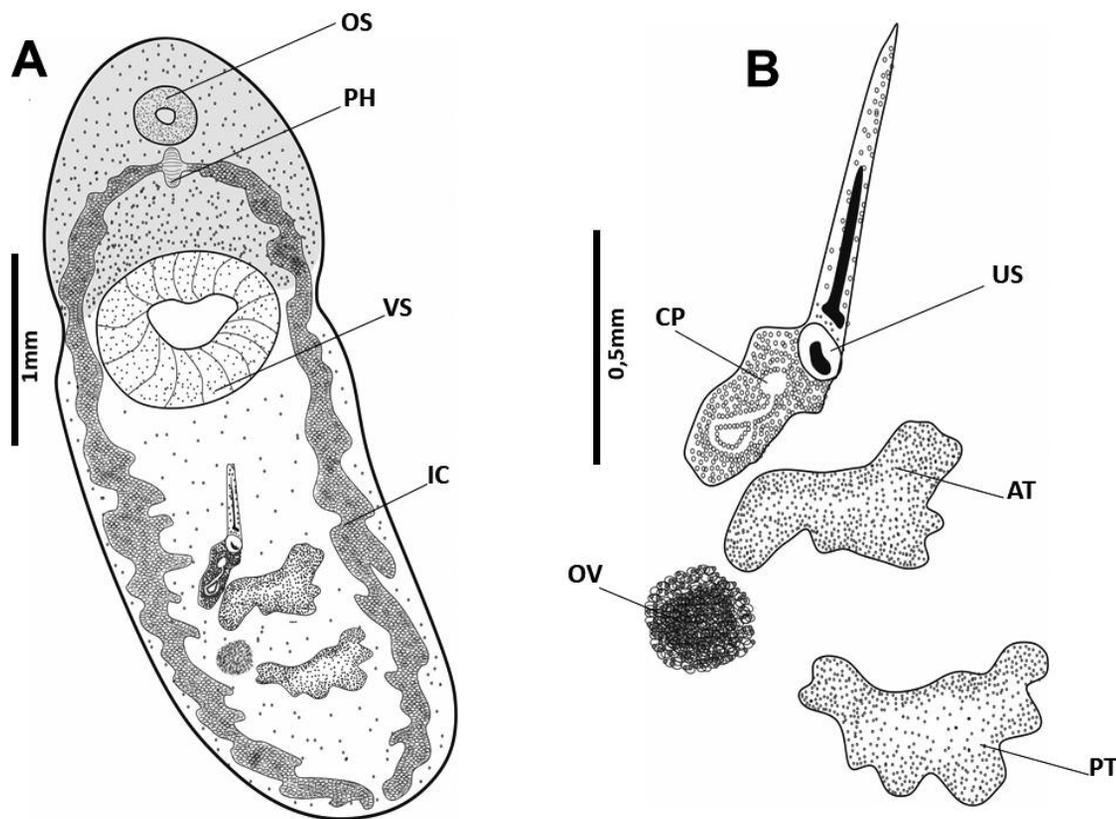


Figure 5. **A.** Metacercaria of *Clinostomum marginatum*, OS - oral sucker, PH - pharynx, VS - ventral sucker, IC - intestinal caecum. **B.** Reproductive structures, US - uterine sac, CP - cirrus sac, OV - ovary, AT - anterior testis, PT - posterior testis.

functions of aquatic communities globally. For [37] predation, competition and the spread of pathogens and parasites has been a matter of concern in recent decades, with special emphasis on the secondary host condition of a wide range of parasites that some fish may present, as it is known that parasites transmitted from exotic species can severely impact native species [38].

The zoonotic potential of *Clinostomum* metacercariae has already been demonstrated in studies by [1] in Thailand and by [5] in fish from Slovakia and Ukraine. In the Amazon region the commercial demand for human consumption obeys the water regime of the Amazon River system having higher demand in summer, during the reduction of the river volume. Do not recommend the consumption of raw or undercooked meat from these fish, since human case records are located in countries that culturally use this animal protein without the cooking process [28].

In this study we registered a new host for the *C. marginatum* trematoid for the Amazon region, bringing a warning for better sanitary control of

Loricariidae species intended for ornamental exportation, as well as for the need for more robust studies in order to investigate possible seasonal relationships with infection rates of these fish.

Acknowledgements

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brasil (CAPES) for the grant scholarship awarded to Darlison C. Souza. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The study was approved by the Animal Experimentation Ethics Committee of the Federal University of Western Pará (CEUA No. 1020180045). It was also submitted to the genetic heritage and associated traditional national management system, as summarized below, and was registered with SisGen (A099911).

References

- [1] Tiewchaloern, S., Udomkijdech, 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.
- [2] 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 detrunctum* (Trematoda: Clinostomidae) metacercariae infecting *Synbranchus marmoratus*. *Journal of Parasitology* 102: 151-156. doi:10.1645/15-773
- [3] Aghlmandi F., Habibi F., Afraii M.A., Abdoli A., Shamsi, S. 2018. Infection with metacercaria of *Clinostomum complanatum* (Trematoda: Clinostomidae) in freshwater fishes from Southern Caspian Sea Basin. *Revue De Medecine Veterinaire* 169: 147-151.
- [4] Simsek E., Yildirim A., Yilmaz E., Inci A., Duzlu O., Onder Z., Pekmezci G. Z. 2018. Occurrence and molecular characterization of *Clinostomum complanatum* (Trematoda: Clinostomidae) in freshwater fishes caught from Turkey. *Parasitology Research* 117: 2117-2124. doi:10.1007/s00436-018-5898-3
- [5] Fedorčák J., Šmíga L., Kutsokon I., Kolarčík 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. doi:10.1111/jfd.13097
- [6] Ramos Alves D., Luque JI., Rodrigues Paraguassu A. 2001. Metacercárias de *Clinostomum marginatum* (Digenea: Clinostomidae) em acará-bandeira *Pterophyllum scalare* (Osteichthyes: Cichlidae) no estado do Rio de Janeiro, Brasil. *Parasitol al Día* 25: 70-72 (in Portuguese). doi:10.4067/S0716-07202001000100014
- [7] 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. doi:10.1590/S1519-69842005000300017
- [8] Covain R., Fisch-Muller S., Oliveira C., Mol J.H., Montoya-Burgos J. I., Dray S. 2016. Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Molecular Phylogenetic Evolutionary* 94: 492-517.
- [9] Nelson J.S., Grande T.C., Wilson M.V. 2016. *Fishes of the World*. John Wiley & Sons.
- [10] Baumgartner G., Pavanelli C.S., Baumgartner D., Bifi A.G., Debona T., Frana, V.F. 2012. In: *Peixes do baixo rio Iguazu. Siluriformes*. Maringá, Eduem:101-146.
- [11] Reis R.E., Pereira E.H.L., Armbruster J.W. 2006. Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys*. *Zoological Journal of the Linnean Society* 147: 277-299.
- [12] Armbruster J.W. 2004. Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. *Zoological Journal of the Linnean Society* 141: 1-80.
- [13] Emiroğlu Ö., Ekmekçi F.G., Aksu S., Başkurt S., Atalay M.A., Tarkan, A.S. 2016. Introduction and establishment of tropical ornamental fish, *Pterygoplichthys* spp. (Actinopterygii: Siluriformes: Loricariidae) in hot springs: aquarium trade as a potential risk for biodiversity in Turkey. *Acta Ichthyologica et Piscatoria* 46: doi:10.3750/AIP2016.46.4.07
- [14] Neves M.D.S., Couto M.V.S., Sousa N.C., Santos R.F.B., Tavares-Dias M., Fujimoto, R.Y. 2016. Transport stress in ornamental amazonian armoured catfishes *Cochliodon* sp. (L145) and *Hypostomus* sp. (L28) (Loricariidae). *Boletim do Instituto de Pesca* 42: 749-758. doi:10.20950/1678-2305.2016v42n4p749
- [15] Moroni F.T., Ortega A.C., Moroni R.B., Mayag B., Jesus R.S.D., Lessi E. 2015. Limitations in decision context for selection of amazonian armoured catfish acari-bod (*Pterygoplichthys pardalis*) as candidate species for aquaculture. *International Journal of Fisheries and Aquaculture* 7: 142-150. doi: 10.5897/IJFA15.0480
- [16] Wakida-Kusunoki A.T., Ruiz-Carus R., Amador-del-Angel E. 2007. Amazon sailfin catfish, *Pterygoplichthys pardalis* (Castelnau, 1855) (Loricariidae), another exotic species established in Southeastern México. *Southwestern Naturalist* 52: 141-144. doi:10.1894/0038-4909(2007)52[141:ASCPJC]2.0.CO;2
- [17] Hossain M., Vadas R., Ruiz-Carus R., Galib S.M. 2018. Amazon sailfin catfish *Pterygoplichthys pardalis* (Loricariidae) in Bangladesh: a critical review of its invasive threat to native and endemic aquatic species. *Fishes* 3: 14. doi:10.3390/fishes3010014
- [18] Eiras J.D.C., Takemoto R.M., Pavanelli G.C. 2006. Métodos de estudo e técnicas laboratoriais em parasitologia de peixes. 2nd ed. Maringá, Eduem.
- [19] Amato J.F.R., Boeger W.A., Amato, S.B. 1991. Protocolos para laboratório, coleta e processamento de parasitos do pescado. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro, Imprensa Universitária (in Portuguese).
- [20] Gibson D.I., Jones A., Bray R.A. 2002. Keys to the Trematoda. Vol. 1. 1st ed. London, CABI Publishing.
- [21] 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* 884-891. doi:10.1645/GE-2781.1
- [22] 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. doi:10.2307/3284227
- [23] Paraguassú A.R., Alves D.R., Luque J.L. 2005. Metazoários parasitos do acará *Geophagus brasiliensis* (Quoy; Gaimard, 1824) (Osteichthyes: Cichlidae) do reservatório de Lajes, Estado do Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinária* 14: 35-39.
- [24] Morais A.M., Varella A.M.B., Fernandes B.M., Malta, J.C.D.O. 2011. *Clinostomum marginatum* (Braun, 1899) and *Austrodiplostomum compactum* (Lutz, 1928) metacercariae with zoonotic potential of *Pygocentrus nattereri* (Kner, 1858) (Characiformes: Serrasalminidae) from Central Amazon, Brazil. *Neotropical Helminthology* 5: 8-14.
- [25] Thatcher V.E. 1981. Patologia de peixes da Amazônia Brasileira, 1. Aspectos gerais. *Acta Amazonica* 11:125-140. doi:10.1590/1809-43921981111125
- [26] Murrieta-Morey G.A., Malta J.C. 2016. Metazoários parasitas das narinas do tambaqui *Colossoma macropomum* (Cuvier, 1818) (Characiformes: Characidae) coletadas em Lagos de Várzea da Amazônia Central, Brasil. *Folia Amazonica* 25: 71-76 (in Portuguese).
- [27] Castelo F.P. 1984. Ocorrência de cistos de *Clinostomum marginatum* Rudolphi, 1819 „yellow spot disease” em filé de Jaraqui (*Semaprochilodus insignis* Schomburgk, 1814). *Acta Amazonica* 14: 325-326. (in Portuguese). doi:10.1017/CBO9781107415324.004
- [28] Rodríguez-Santiago M A., García-Prieto L., Mendoza-Garfias B., González-Solís D., Grano-Maldonado M.I. 2016. Parasites of two coexisting invasive sailfin catfishes (Siluriformes: Loricariidae) in a tropical region of Mexico. *Neotropical Ichthyology* 14: doi:10.1590/1982-0224-20160021
- [29] Cardoso A.C.F., Oliveira M.S.B., Neves L.R., Tavares-Dias M. 2017. Metazoan fauna parasitizing *Peckoltia braueri* and *Pterygoplichthys pardalis* (Loricariidae) catfishes from the northeastern Brazilian Amazon. *Acta Amazonica* 47: 147-154. doi:10.1590/1809-4392201603232
- [30] São Clemente S.C., Matos E., Tortelly R., Lima F.C. 1998. Histopatologia do parasitismo por metacercárias de *Clinostomum* sp. em tamoata, *Hoplosternum littorale* (Hancock, 1828). *Parasitologia al Dia* 22: 38-40 (in Portuguese). doi:10.4067/S0716-07201998000100007
- [31] Fuller P.L., Nico L.G., Williams J. D. 1999. Non-indigenous fishes introduced into inland waters of the United States. American Fisheries Society. Special Publication No. 27 Bethesda, MD, American Fisheries Society.
- [32] Chavez J.M., De la Paz R.M., Manohar S.K., Pagulayan R.C., Vi J.R.C. 2006. New Philippine record of south american sailfin catfishes (Pisces: Loricariidae). *Zootaxa* 1109: 57-68. doi:10.11646/zootaxa.1109.1.6
- [33] Liang S.H., Chuang L.C., Chang M.H. 2006. The pet trade as a source of invasive fish in Taiwan. *Taiwania* 51: 93-98.
- [34] Capps K. 2008. The impacts of sailfish catfish (Siluriformes: Loricariidae) on invaded freshwater ecosystems. In: *Proceedings of Annual Meeting of American Ichthyologist and Herpetologist Society*: 23-28.
- [35] Piazzini S., Lori E., Favill L., Cianfanelli S., Vanni S., Manganelli G. 2010. A tropical fish community in thermal waters of southern Tuscany. *Biological Invasions* 12: 2959-2965. doi:10.1007/s10530-010-9695-x
- [36] Cucherousset J., Olden J.D. 2011. Ecological impacts of nonnative freshwater fishes. *Fisheries* 36: 215-230. doi:10.1080/03632415.2011.574578
- [37] Torchin M.E., Lafferty K.D., Dobson A.P., McKenzie V.J., Kuris A.M. 2003. Introduced species and their missing parasites. *Nature* 421: 628-630. doi:10.1038/nature01346
- [38] Poulin R., Paterson R.A., Townsend C.R., Tompkins D.M., Kelly D.W. 2011. Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology* 56: 676-688. doi:10.1111/j.1365-2427.2010.02425.x
- [39] Sereno-Urbe A.L., Pinacho-Pinacho C.D., García-Varela M., de León G.P.P. 2013. Using mitochondrial and ribosomal DNA sequences to test the taxonomic validity of *Clinostomum complanatum* Rudolphi, 1814 in fish-eating birds and freshwater fishes in Mexico, with the description of a new species. *Parasitology Research* 112: 2855-2870. doi:10.1007/s00436-013-3457-5

Received 11 February 2020

Accepted 06 May 2020