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New host record and geographical distribution of Nematoda parasitizing *Hypophthalmus marginatus* Valenciennes (Siluriformes) from the Tocantins River, Brazil

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ABSTRACT. *Hypophthalmus marginatus* is among the most commercialized fish from the Amazon region, with fish fillets exported to Southeastern Brazil and other countries. In the present study, the *H. marginatus* analyzed were parasitized by third-stage larvae of *Hysterothylacium* sp. and *Anisakis* sp. These nematodes are being reported for the first time in *H. marginatus* collected from the Tocantins River, representing a new host and geographical records, and expanding knowledge of the hosts of these nematodes in the Neotropical Region. A brief description with original measurements is presented.

Keywords: nematode; Anisakidae; Rhaphidascarididae; *Hysterothylacium*; *Anisakis*.

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Introduction

The Tocantins River is the second largest river in Brazil, located in the Tocantins-Araguaia hydrographic region, and is a hotspot for fish biodiversity, in which several species are endemic to the region (Agência Nacional de Águas [ANA], 2019). However, despite this diversity, only a small number of fish species have been examined for helminths (Eiras, Takemoto, Pavanelli, & Adriano, 2011).

Hypophthalmus marginatus Valenciennes, commonly known as mapará, is a pelagic fish that is commercialized by slaughterhouses from the Amazon region, which export the fish fillets to Southeastern Brazil and some countries in Europe and North America, besides being among the 10 most commercialized fishes in the Amazon region (Cutrim & Batista, 2005; Costa, Oshiro, & Silva, 2010; Souza & Inhamuns, 2011). Despite the economic importance of *H. marginatus*, it has never been studied for helminths and, according to Velasco et al. (2015), the parasite fauna of *H. marginatus* may include parasite species that have not yet been described in the literature. Therefore, the aim of this study is to characterize the nematode fauna of *H. marginatus* from the Tocantins River, State of Maranhão, identifying species with zoonotic potential.

Material and methods

Collections for this study were authorized by the Biodiversity Authorization and Information System (SISBIO, number 61650-1), and was approved by the Commission of Ethics in the Use of Animals, protocol number 031/2019 from *Universidade Estadual do Maranhão* (UEMA). Between March 2018 and May 2019, 11 specimens of *H. marginatus* (standard length 33-38 cm; total weight 250- 511 g) obtained from the Tocantins River, State of Maranhão, Brazil, were examined for helminths (Figure 1). Fishes were acquired with the aid of local fisherman and taken to the *Núcleo de Estudos Morfofisiológicos Avançados* from the *Universidade Estadual da Região Tocantina do Maranhã*o, São Luís, Maranhão to be necropsied. Internal organs were fixed in 70% alcohol, immediately after the capture and sent to the *Laboratório de Helmintos Parasitos de Peixes*, *Fundação Oswaldo Cruz*, State of Rio de Janeiro, where helminths were collected with the aid of a stereoscopic microscope. Nematodes were cleared with lactophenol and observed using a Zeiss Axioscope 2 microscope with differential interference contrast (DIC), equipped with a camera

lucida and a Sony MPEG Movie EX DSC-S75 digital camera. All measurements are given in millimeters; range values are followed by means. The terminology related to parasite ecology is according to Bush, Lafferty, Lotz, and Shostak (1997). Specimens studied were deposited in the *Coleção Helmintológica do Instituto Oswaldo Cruz* (CHIOC) in Brazil.

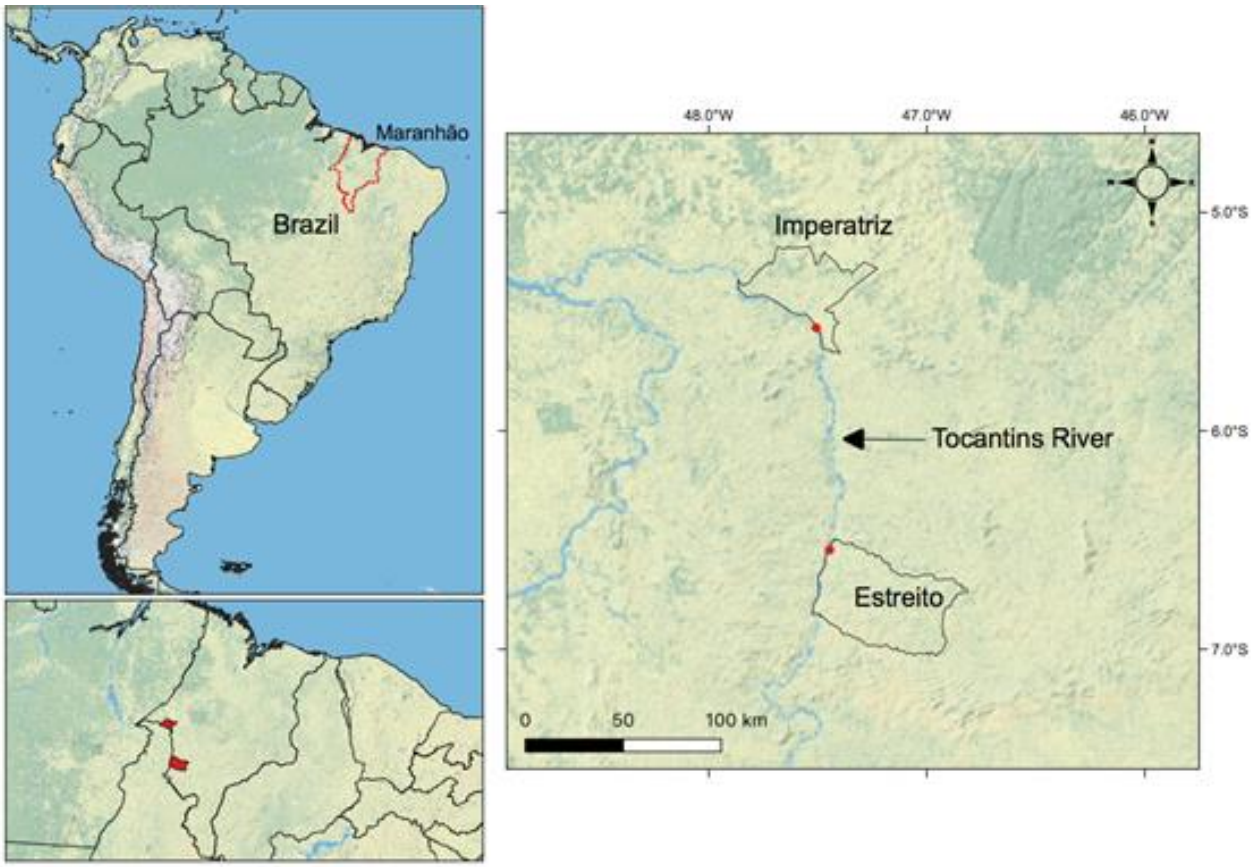

Figure 1. Middle Tocantins River showing the collection points, Maranhão, Brazil. In the municipality of Estreito, collection took place upstream of the Hydroelectric Plant; and in the city of Imperatriz, collection occurred in the village of Embiral, Maranhão.

Results

Eleven specimens of *H. marginatus* were examined, and a total of 1606 specimens of *Hysterothylacium* sp. and 21 specimens of *Anisakis* sp., all third-stage larvae, were recovered. Considering that *H. marginatus* represents a new host record for these species, the main measurements are given herein.

Raphidascarididae Hartwich, 1954
Subfamily Raphidascaridinae Hartwich, 1954
Hysterothylacium Ward & Margath, 1917
Hysterothylacium sp. third-stage larvae (Figure 2)
CHIOC number: 38996, 38997, 38999
Site of infection: Mesentery, lumen of intestine, lumen of stomach and liver.
Infection parameters: All 11 fish examined were parasitized with 4-321 nematodes, with a total of 1606 worms collected.

Description (based on 20 specimens): Cuticle smooth. Body 1.81-2.77 (2.17) long, 0.07- 0.10 (0.08) wide. Anterior end with ventral cephalic tooth. Nerve ring and excretory pore 0.100-0.125 (0.108) and 0.090-0.172 (0.121) from anterior extremity, respectively. Oesophagus muscular and narrow, 0.125-0.237 (0.205) long, followed by ventriculus 0.012-0.017 (0.016) long and 0.015-0.030 (0.024) wide. Intestinal caecum short, 0.012-0.025 (0.020) long, slightly exceeding ventriculus anteriorly. Ventricular appendix 0.685-1.062 (0.847) long. Tail conical, with rounded tip without mucron. Anus 0.037-0.075 (0.050) to posterior region.

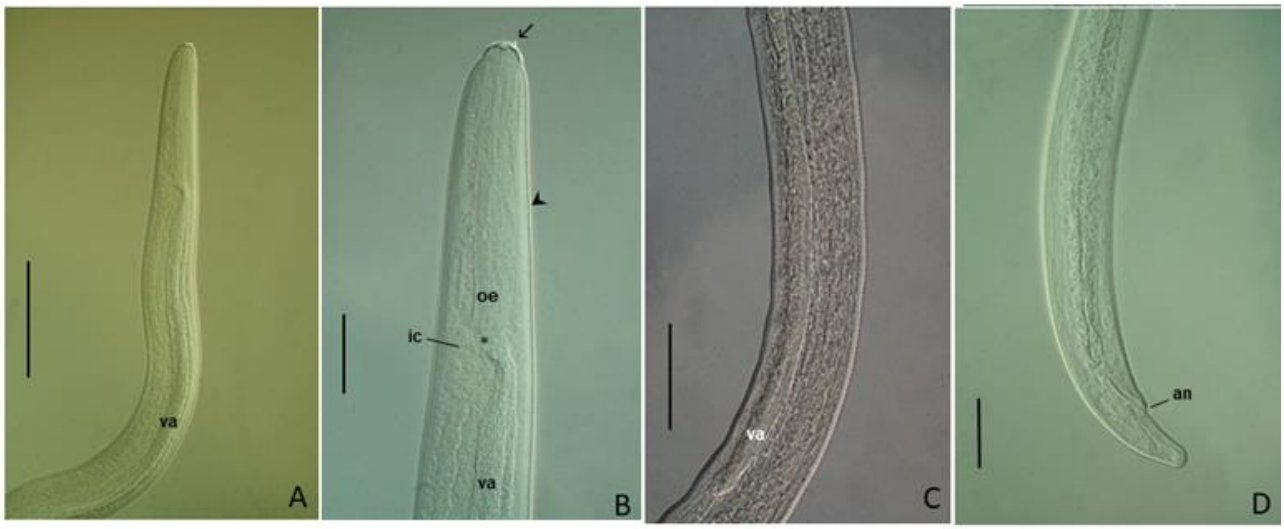

Figure 2. *Hysterothylacium* sp. third-stage larva. (A) Anterior region, general view. (va = ventricular appendix). Bar: 0.20 mm. (B) Detail of anterior region showing oesophagus (oe), cephalic tooth (thin arrow), excretory pore (arrowhead), ventriculus (asterisk), intestinal cecum (ic), ventricular appendix (va). Bar: 0.06 mm. (C) Detail of posterior region of ventricular appendix (va). Bar: 0.07 mm. (D) Detail of posterior region showing the anus (an). Bar: 0.05 mm.

Anisakidae Skrjabin & Karokhin, 1945
Subfamily Anisakinae Chabaud, 1965
Anisakis Dujardin, 1845
Anisakis sp. third-stage larvae (Figure 3)
CHIOC number: 38995, 38998
Site of infection: Mesentery and liver.
Infection parameters: Six out of 11 fish examined were parasitized with 1-7 nematodes, with a total of 21 worms collected.

Description (based on 3 specimens): Third-stage larvae encapsulated. Body length 9.32-13.87 (11.25), maximum body width 0.27-0.32 (0.30). Anterior end with a dorsal lip and two ventrolateral lips poorly developed; cephalic tooth present. Excretory pore opening on lip base, just posterior to cephalic tooth; Muscular oesophagus 0.74-1.10 (0.92) in length, ending in glandular ventriculus. Ventriculus 0.33-0.42 (0.37) long and 0.13-0.16 (0.15) wide. No caeca or diverticula present. Nerve ring 0.10-0.25 (0.17) from anterior end. Rectum short and oblique to anus. Tail conical with single terminal mucron. Anus 0.10-0.75 (0.41) from posterior end.

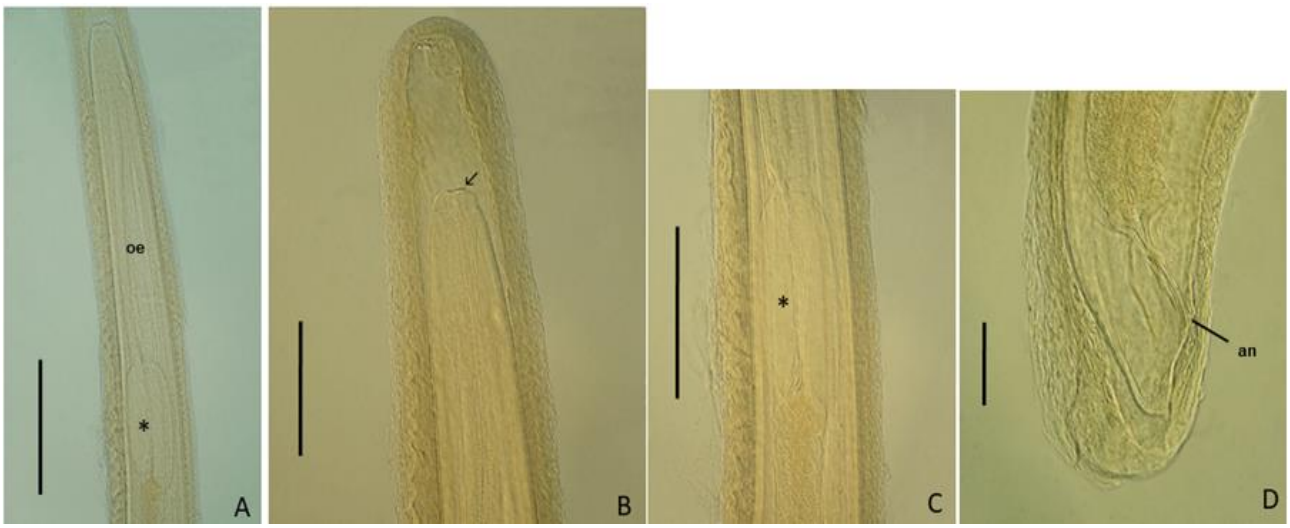

Figure 3. *Anisakis* sp. third-stage larva, encapsulated. (A) Anterior region, showing the oesophagus (oe) and the ventriculus (asterisk). Bar: 0.5 mm (B) Detail of anterior region showing the cephalic tooth (thin arrow). Bar: 0.1 mm. (C) Detail of ventriculus (asterisk). Bar: 0.3 mm. (D) Detail of posterior region showing the anus (an). Bar: 0.3 mm.

Discussion

According to Luque, Pereira, Alves, Oliva, & Timi (2017), South American fish parasites are insufficiently researched, and their study is important to understand their roles in ecosystems, and impact on pisciculture and fish-borne parasitic zoonoses, in addition to providing tools for conservation of biodiversity.

The superfamily Ascaridoidea Baird, 1853 includes two important families, Anisakidae and Raphidascarididae, which are composed of nematodes that complete their life cycles using intermediate or paratenic hosts in the aquatic environment (Anderson, 1992; Alves et al., 2020). Although some authors consider that the genus *Hysterothylacium* is included in the family Anisakidae Skrjabin & Karokhin, 1945, we agree with Li et al. (2018), who transferred this genus to the family Raphidascarididae Hartwich, 1954.

The presence of Anisakidae third-stage larvae in fishes may result in economic losses to the fishing industry due to reduced marketability of fishery products. Larvae of the genera *Anisakis* Dujardin, 1845, *Pseudoterranova* Krabbe, 1878, and *Contracaecum* Railliet and Henry, 1913 are mainly responsible for a fish-borne zoonosis known as anisakidosis and allergic reactions. Ingestion of raw, partially cooked, salted, marinated, or smoked fish or cephalopods infected with these larvae can provoke symptoms with a gastrointestinal or allergic nature, ranging from mild to severe clinical manifestations (Buchmann & Mehrdana, 2016; Mattiucci, Cipriani, Paoletti, Levsen, & Nascetti, 2017; Bao et al., 2019).

Although members of the genus *Hysterothylacium* are considered nonpathogenic for humans, some of these species have been associated with allergic symptomatology (Fernández-Caldas et al., 1998; Valero, Terrados, Díaz, Reguera, & Lozano, 2003), and there is a report of a case of human infection with a female *Hysterothylacium aduncum* in Japan (Yagi et al., 1996). Besides this, experimental fish infection with *Hysterothylacium* indicates that they can affect the host growth rate and health, with economic impacts (Balbuena, Karlsbakk, Kvenseth, Saksvik, & Nylund 2000; Karlsbakk, Otterlei, Høie, & Nylund, 2001). According to Rodrigues et al. (2015), massive infections with nematode larvae can increase the risk of parasite migration to the muscles or to the abdominal cavity, facilitating human exposure to larvae.

In freshwater fishes from Brazil, *Hysterothylacium* sp. larvae have been reported in *Rhaphiodon vulpinus* Agassiz, 1829, *Salminus maxillosus* Valenciennes, 1850, *Galeocharax knerii* (Steindachner, 1879), *Hypophthalmus edentatus* Spix & Agassiz, 1829, *Loricariichthys* sp., *Plagioscion squamosissimus* (Heckel, 1840), and *Crenicichla lepidota* Heckel, 1840 from the Paraná River, State of Paraná (Moravec, Kohn, & Fernandes, 1993), in *Gymnotus carapo* Linnaeus, 1758 and *Leporinus friderici* (Bloch, 1794) from the upper Paraná River floodplain (Takemoto et al., 2009), in *Brachyplatystoma filamentosum* (Castelnau, 1855), *Brachyplatystoma rousseauxii* (Castelnau, 1855), and *Oxydoras niger* (Valenciennes, 1821) from Colares and Vigia Island, State of Pará (Rodrigues et al., 2015), in *Arapaima gigas* (Schinz, 1822) from fish farms from the State of Amazon (Andrade-Porto et al., 2015; Azevedo, Morey, & Malta, 2017), in *Hyphessobrycon eques* (Steindachner, 1882) from the Paranapanema River, State of São Paulo (Acosta & Silva, 2015), and in *Hoplias malabaricus* (Bloch, 1794) from the Jacaré-Pepira River and Jacaré-Guaçú River, State of São Paulo (Leite, Pedreira-Filho, Azevedo, & Abdallah, 2020). According to Moravec et al. (1993) these genera might be carried from marine to freshwater environments by some migratory fishes. In the present study, *Hysterothylacium* sp. third-stage larvae were found in all specimens of fish studied. No adult specimens were found in the studied fishes, showing that *H. marginatus* represents an intermediate or paratenic host for *Hysterothylacium* sp.

Among freshwater fishes from Brazil, *Anisakis* sp. larvae have been reported in *B. filamentosum*, *O. niger,* and *P. squamosissimus* from Colares and Vigia Island, State of Pará (Rodrigues et al., 2015), in *P. squamosissimus* from Marajó Bay, and the Tapajó River and Amazon River, State of Pará (Fontenelle et al. 2016; Souza, Eiras, Adriano, & Corrêa, 2020), in *Triportheus angulatus* (Spix & Agassiz, 1829) from Catalão Lake and the Solimões River, State of Amazon (Moreira, Oliveira, Morey, & Malta, 2017), and from *Serrasalmus altispinis* Merckx, Jégu and Santos, 2000 and *Pygocentrus nattereri* Kner, 1858 from the Solimões River, State of Amazon (Morey & Malta, 2018; Morais, Cárdenas, & Malta, 2019).

In the Tocantins River, despite its great ichthyological diversity, there are few records of Anisakidae larvae. Eiras, Pavanelli, Takemoto, and Nawa (2018) reported fish-borne nematode infections in humans in South America, with the majority of cases coming from marine fishes. *Contracaecum* sp. were recorded in *Cichla piquiti* Kullander and Ferreira, 2006 (Lacerda, Takemoto, Poulin, & Pavanelli, 2013) and in *Cichla kelberi* Kullander and Ferreira, 2006 and *C. piquiti* both from the Tocantins River (Yamada & Takemoto, 2013). Amato-Neto, Amato, and

Amato (2007) recorded a possible case of anisakiasis acquired by a group of fishermen who were traveling to Bananal Island, Tocantins, and ate raw fish. However, according to Eiras, Pavanelli, Yamaguchi, Takemoto, and Karling, (2015), this represents a case of gnathostomiasis and not anisakiasis.

In the current study, larvae of *Anisakis* sp. and *Hysterothylacium* sp. are reported for the first time in *H. marginatus*, representing a new host and geographical records. According to Eiras et al. (2018), the number of cases of infections in humans is underestimated and the population needs to be informed about the risk of certain eating habits, as well as which public health agencies should be more attentive to the creation of protocols that lead to correct diagnosis of parasitic diseases transmitted through the consumption of raw or improperly cooked fish.

Conclusion

The new host and geographic records for Ascaridoidea larvae presented in this study reinforce the importance of expanding the knowledge on the presence of third stage larvae of the family Anisakidae and Raphidascarididae in fish from the Tocantins River, given that the taxonomy of helminths of fishes from the Neotropical Region are the basis for understanding the biology and trophic relationships of these parasites.

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