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First record of larvae of *Hysterothylacium* (Nematoda: Anisakidae) with zoonotic potential in the pirarucu *Arapaima gigas* (Osteichthyes: Arapaimidae) from South America

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Abstract

Third-stage larvae (L_3) of *Hysterothylacium* sp. were collected by the first time in juveniles of pirarucu *Arapaima gigas* farmed in the Rio Preto da Eva, Amazonas state. Ninety-eight (98) out of 100 examined fish showed to be parasitized. Five hundred and ninety larvae of *Hysterothylacium* sp. were collected from the intestines, stomach and pyloric caeca. The mean intensity of parasite indexes was 6.02 (±5.75) ranging from 1 to 40 larvae per host and the mean abundance was 5.9 (±5.76). The *A. gigas* is the new host record for larvae of *Hysterothylacium* sp. in Brazil, and this is the first record of larvae of *Hysterothylacium* (Nematoda: Anisakidae) with zoonotic potential in the pirarucu from South America.

Keywords: pirarucu, nematodes, intermediate host, Amazônia, zoonosis.

Primeiro registro de larvas de *Hysterothylacium* sp.(Nematoda: Anisakidae) com potencial zoonótico em pirarucu *Arapaima gigas* (Osteichthyes: Arapaimidae) na América do Sul

Resumo

Larvas de terceiro estágio (L3) de *Hysterothylacium* sp. foram coletadas pela primeira vez em juvenis de pirarucu *Arapaima gigas* cultivados no Rio Preto da Eva, Estado do Amazonas. Noventa e oito (98) dos 100 peixes examinados estavam parasitados. Quinhentos e noventa larvas de *Hysterothylacium*sp.foram coletados no intestino, estômago e cecos pilóricos. O índice parasitário de intensidade média foi de 6,02 (±5,75) variando de 1 a 40 larvas por hospedeiro e o de abundância média foi de 5,9 (±5,76). *A. Gigas* é um novo registro de hospedeiro para larvas de *Hysterothylacium* sp. no Brasil, também sendo, o primeiro registro de larvas de *Hysterothylacium* sp. com potencial zoonótico em pirarucu da América do Sul.

Palavras-chave: pirarucu, nematodas, hospedeiro intermediário, Amazônia, zoonose.

1. Introduction

Nematodes of the family Anisakidae parasitize fish, mammals, birds and reptiles (Moravec, 1998), and are agents of anisakiasis, a parasitic disease brought about by the consumption of raw fish infected with anisakid larvae. The first case of anisakiasis was reported in the Netherlands (Van Thiel et al., 1960). Thereafter, many cases have been reported in Japan, Korea, Netherlands and in Western Europe in regions where raw fish are consumed. In Korea, a removal of *Anisakis* Dujardin, 1845 larvae from the human oropharynx was reported as the first case in 1971 (Kim et al., 1971). Larvae of anisakid nematodes can be observed in the muscle or adhered to the internal organs of marine and freshwater fish. Most papers are limited to reports on their occurrence and/or description (Moravec, 1998).

Among the larval anisakid found in freshwater fishes of the Neotropical region are the genus *Contracaecum* Railliet & Henry, 1912; *Hysterothylacium* Ward & Magath, 1917; *Pseudoterranova* Railliet & Henry, 1912; *Raphidascaris* Railliet & Henry, 1915 and *Terranova* Leiper & Atkinson, 1914 (Vicente et al., 1985; Moravec, 1998; Vicente and

Pinto, 1999; Vidal-Martinez et al., 2001; Abdallah et al., 2005; Tavares et al., 2007; Luque et al., 2011).

Human infection is related to accidental ingestion of infectious larvae L_3 found in raw, under cooked, smoked and salted fish (Adams et al., 1997). Although the larvae do not achieve the maturity in humans, they can provoke severe symptoms when by invading the intestine wall and produce eosinophilic granuloma around the worms characterized by neutrophils infiltration (Oshima, 1972). However, the antigen exposition causes allergic reactions (Fernández et al., 2001).

Species of the genus *Hysterothylacium* are parasitic in both larval and adult stage and can be found parasitizing the visceral cavity, mesentery and intestine of marine and freshwater fishes (Khaleghzadeh-Ahangar et al., 2011). In natural conditions, they reach their maturity in the intestine of fish and mammals and have copepods, polychaeta and other invertebrates as intermediate hosts (Moravec, 1998).

Arapaima gigas (Schinz, 1822) (Osteichthyes: Arapaimidae) is the world's largest scale fish, may reach 3 m length, presents wide geographical distribution in the Amazonian region and occurs in the floodplain of the rivers Araguaia-Tocantins, Solimões-Amazonas and their effluents, Amazonas river in the Peruvian Andes, tributaries of the river systems Essequibo and Rupununi the Guiana (Imbiriba, 2001). It presents an excellent flesh, is commercialized fresh or sun-dried in fairs and markets (Imbiriba, 2001) and has been used for Japanese culinary as sushi, sashimi and ceviche (SEBRAE, 2009).

In Brazil, statistical data from FAO (2012) show *A. gigas* production of 10 ton with an estimated farm-gate of USD 130,000.00. Fingerlings production is low and represents one of the most important problems in *A. gigas* production. Its reproductive behavior and low female fecundity point out the *A. gigas* fingerlings production to be difficult and expensive (FAO, 2012). As the low production, the export volume is also prejudiced. Some assays of commercialization were made in international events in Europe and United States. The fillet of *A. gigas* is focused on the gourmet market, mainly restaurants where the prices range from US$ 20-25/kg in Europe and United States and USD 12-15/kg in South American cities (FAO, 2012).

Further epidemiological studies are required so as to show the prevalence and distribution of anisakid nematodes in Brazil (Cárdia and Bresciani, 2012). Eight nematode species were reported in *A. gigas*: *Camallanus tridentatus* (Drasche, 1884) (Camallanidae); *Capillostrongyloides arapaimae* Santos, Moravec & Venturieri, 2008 (Capillariidae) *Eustrongylides* sp. Jagerskiold, 1909 (Dioctophymatidae); *Gnathostoma gracilis* (Diesing, 1839) (Gnathostomidae); *Goezia spinulosa* (Diesing, 1839) (Anisakidae); *Nilonema senticosum* (Baylis, 1927) (Philometridae); *Terranova serrata* (Drasche, 1884) (Anisakidae) and *Rumai rumai* Travassos, 1960 (Philometridae) (Vicente et al., 1985; Moravec, 1998; Thatcher, 2006; Santos et al., 2008; Luque et al., 2011).

This study is the first report of *Hysterothylacium* sp. L_3 larvae parasites on juveniles of *A. gigas* farmed in the Amazonia and South America.

2. Material and Methods

A total of 100 fish (14.5±2.1 cm total length and 32.6±16.4 g weight) were captured in a fish farm in the Rio Preto da Eva, Amazonas state (2°41′55″S, 59°42′3″O). Fish and nematodes were processed according to Eiras et al. (2006). They were recovered from the intestine, stomach and the pyloric caecum were washed in 0.9% physiological saline, fixed in hot70% ethanol, clarified in phenol and examined using a Zeiss Axioscope 2 microscope equipped with a camera lucida. All measurements are in millimeters unless otherwise stated, and quoted as the ranges with means in parenthesis.

Nematode identification was based on morphology and morphometric parameters following Moravec (1998). Specimens of *Hysterothylacium* sp. larvae were deposited in the Helminthological Collection of Oswaldo Cruz Institute nº 35888CHIOC. (Wet material) and Invertebrate Collection of National Institute of Research of Amazonia nº 59 INPA. (Wet material). Parasitological descriptors were calculated according to Bush et al. (1997).

3. Results

Ninety eight percent of *A. gigas* were parasitized by L_3 larvae and the nematodes were identified as *Hysterothylacium* sp. (Figure 1). Three out of 98 infected fish presented ascitis, lesions and petechiae in the intestinal mucosa. One of them displayed a complete obstruction of the intestine. A total of 590 larvae of *Hysterothylacium* sp.were recovered from the intestine, stomach and pyloric caecum. The mean intensity was 6.02 (±5.75), and mean abundance 5.9 (±5.76) ranging from 1 to 40 larvae per host.

Nematoda Rudolphi, 1808

Ascaridoidea Railliet & Henry, 1915

Anisakidae Skrjabin & Karokhin, 1945

Raphidascaridinae Hartwich, 1954

*Hysterothylacium*s sp. third-stage larvae

Description (based on 10 specimens): Small, whitish nematodes. Length of body 2.140-2.875 (2.531±0.266), width 0.090-0.125 (0.107±0.013); cuticle smooth. Anterior end provided with ventral cephalic tooth 0.002 long. Length of anlagen of lips 0.015. Oesophagus narrow, 0.210-0.267 (0.225±0.092) long, provided by ventriculus 0.025-0.050 (0.032±0.011) long. Intestinal caecum very short, 0.050-0.160 (0.095±0.049) long, only slightly exceeding ventriculus anteriorly. Ventricular appendix 0.625-0.975 (0.852±0.135) long. Nerve ring and excretory pore 0.100-0.137 (0.119±0.015) and 0.110-0.132 (0.120±0.009) respectively, from anterior extremity. Tail conical, with rounded tip without mucron. Anus 0.062-0.100 (0.074±0.031) to posterior region (Figures 1 and 2).

Figure 1. *Hysterothylacium* sp. third stage larva observed by differential interference contrast (DIC). (a). Anterior end provided with ventral cephalic tooth (arrow) and excretory pore (arrow head) in the region of nerve ring (bar 0.035 mm); (b). Anterior region showing the excretory pore (EP) and the intestinal caecum (IC) (bar=0.04 mm); (c). Detail of intestinal caecum and ventricular appendix (VA) (bar=0.04 mm); (d). Posterior region showing conical tail and anus (arrow) (bar=0.625 mm).

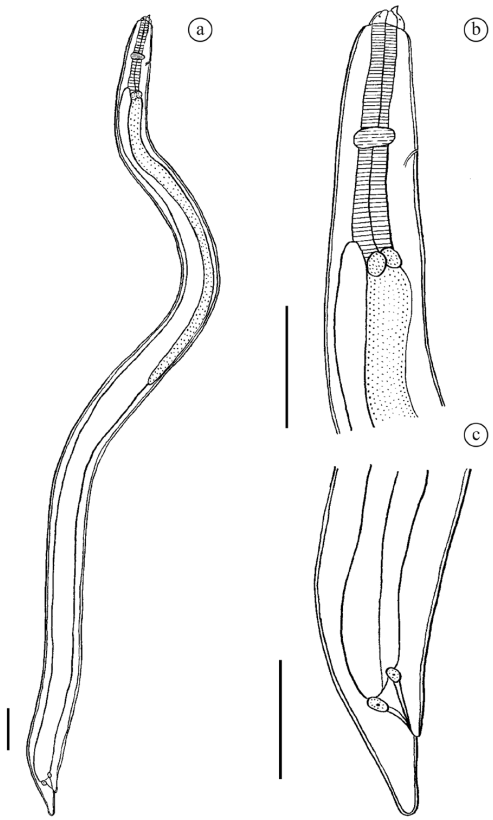

Figure 2. *Hysterothylacium* sp. third stage larva. (a). General view; (b). Anterior end; (c). Tail. Bar=0.1 mm.

4. Discussion

Most registers of *Hysterothylacium* sp. larvae are from marine fish, but these larvae might be carried from the marine environment into freshwaters by some migratory fishes. In Brazil, there are few records of *Hysterothylacium* sp. originating from freshwater environments. Martins et al. (2000) reported *Hysterothylacium* sp. and *Thynnascaris* sp. in *Plagioscions squamosissimus* (Heckel, 1840) (Sciaenidae) from Volta Grande Reservoir, Minas Gerais, Brazil; Moravec et al. (1993) reported *Hysterothylacium* sp. from different hosts from Paraná River, Brazil; Takemoto et al. (2009) reported this anisakid from *Gymnotus carapo* Linnaeus, 1758 (Gymnotidae) and *Leporinus friderici* (Bloch, 1794) **(**Anostomidae) from upper Paraná River floodplain, Brazil. However, this genus is being referred to for the first time in a fish from the Amazonian Region.

Moravec et al. (1993) reported *Hysterothylacium* sp. larvae from eight fish species of the Paraná River, Brazil. The morphology and measurements of the present material are similar to those described by them. Although many conspecific larvae present a mucron in the tail tip, the present nematode and that described by Moravec et al. (1993) do not bear this structure (Table 1). *Hysterothylacium* sp. larvae were also reported from the bivalve *Diplodon suavidicus* (Lea, 1856) (Mollusca, Unioniformes, Hyriidae) in Aripuanã River, Amazon, Brazil. Although these larvae were recovered from the same locality, the larvae found in *D. suavidicus* show to be larger-sized; while in the present study the larvae present an average total length of 2.531mm, those studied by Lopes et al. (2011) have an average total length of 19.9mm (Table 1).

Most registers of *Hysterothylacium* sp. larvae in Brazil are found in fish captured from the littoral of the state of Rio de Janeiro, Southeastern Brazil. Tavares and Luque (2006) related 44 fish species from Rio de Janeiro as hosts. However, *Hysterothylacium* sp. larvae were also reported from the intestine, intestinal caecum, visceral cavity, liver and spleen of *Micropogonias furnieri* (Desmarest, 1823) in the state of Rio Grande do Sul, Southern Brazil (Pereira et al., 2004), mesentery of *Gymnotus* spp. from

Table 1. Mean values (mm) of L_3 *Hysterothylacium* sp. larvae parasite of *Rhaphiodon vulpinus* (Moravec et al. 1993) from Paraná river, *Diplodon suavidicus* (Mollusca, Unioniformes, Hyriidae) (Lopes et al., 2011) from Aripuanã river, Amazonas and the present material.

Characters	*R.vulpinus* Moravec et al. (1993)	*D. suavidicus* Lopes et al. (2011)	*A. gigas* present study
Total lenght	*	17.4-23.1(19.9)	2.140-2.875 (2.531)
Width	*	0.450-0.62 (0.530)	0.090-0.125 (0.107)
Oesophagus[L]	0.246-0.270	1.450-2.780 (1.850)	0.210-0.267 (0.225)
Ventriculus[L]	0.015-0.018	1.600-2.400 (2.090)[b]	0.025-0.050 (0.032)
Ventricular appendix[L]	0.857-0.966	*	0.625-0.975 (0.852)
Nerve ring[a]	0.129-0.147	*	0.100-0.137 (0.119)
Excretory pore[a]	0.144-0.171	[c]	0.110-0.132 (0.120)
Intestinal caecum[L]	0.024-0.033	*	0.050-0.160 (0.095)
Tail[L]	0.051-0.063	*	0.062-0.100 (0.074)

[L]length. [a]distance from anterior end. [b]it comprises the ventriculus with ventricular appendix. [c]excretory pore anterior to the level of the nerve ring. *No data registered.

Baía River, state of Mato Grosso do Sul, Central Brazil (Isaac et al., 2004) and in *Scomberomorus brasiliensis* Collette, Russo and Zavala-Camin, 1978 and *Trichiurus lepturus* Linnaeus, 1758 from the state of Rio Grande do Norte, Northeastern Brazil (Cavalcanti et al., 2012), showing its wide distribution throughout Brazil.

Although a prevalence of 98% was found in the present study, the mean intensity was similar to that found in *Macrodon ancylodon* (Bloch and Schneider, 1801) from the coastal zone of the state of Rio de Janeiro (Sabas and Luque, 2003). High prevalence (97.5%) was also reported in *Prionotus punctatus* (Bloch, 1793) from the municipality of Angra dos Reis, state of Rio de Janeiro (Bicudo et al., 2005) and the mean intensity and abundance were higher than those observed in this study. Conversely, Knoff et al. (2007) have related 27% prevalence of anisakids *Anisakis* sp., *A. physeteris* Baylis, 1923, *A. simplex* (Rudolphi, 1809), *Pseudoterranova* sp., *P. decipiens* (Krabbe, 1878), *Hysterothylacium* sp., *Raphidascaris* sp., *Contracaecum* sp. and *Terranova* sp. in *Genypterus brasiliensis* Regan, 1903 (Ophidiidae) examined from the marketed fish in the municipalities of Niterói and Rio de Janeiro state of Rio de Janeiro.

Similar lesions reported in this study in the intestinal mucosa were observed in *Paralichthys isosceles* Jordan, 1891 in which a total of *Hysterothylacium* sp. L_3 larvae showed 100% prevalence and mean intensity of 30.3 parasites per host (Felizardo et al., 2009).

Lopes et al. (2011) documented the occurrence of *Hysterothylacium* larvae in the pericardic cavity of specimens of *D. suavidicus* (Lea, 1856) (Mollusca, Hyriidae) from Aripuanã River, state of Amazonas, Brazil, but there was no record of this larvae parasitizing fishes in the Amazon until now. In Brazil, despite the recovery of anisakid larvae from different hosts in marine and freshwater fishes, there are no reports relative to human anisakiasis. However, with the globalization of food habits and the increasing consumption of raw fish, epidemiological studies are needed to show fish parasitism by anisakid nematodes, especially in the Amazonian region. *Arapaima gigas* is parasitized by three species of anisakid nematodes and two of them acts as definitive host *G. spinulosa* and *T. serrata. A. gigas* is probably intermediate or parathenic host of *Hysterothylacium* sp. Although these larvae are not common in humans, their zoonotic potential should be highlighted.

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