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Hysterothylacium larvae (Nematoda, Anisakidae) in the freshwater mussel *Diplodon suavidicus* (Lea, 1856) (Mollusca, Unioniformes, Hyriidae) in Aripuanã River, Amazon, Brazil

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ABSTRACT

Larvae of *Hysterothylacium* use various invertebrates as intermediate hosts. Definite hosts include fish, birds, reptiles or marine mammals. This study describes the occurrence of *Hysterothylacium* (Nematoda, Anisakidae) larvae parasitizing the pericardic cavity of *Diplodon suavidicus* (Unioniformes, Hyriidae) specimens collected in the Amazon basin, Brazil. This is the first record of this nematode parasitizing freshwater bivalves in South America. The high prevalence, medium intensity and medium abundance suggest that *D. suavidicus* acts as intermediate host for *Hysterothylacium* species in that environment.

1. Introduction

Freshwater molluscs are relatively common in Amazonian rivers with clear and turbid waters (Haas, 1949). Among the bivalves, *Diplodon suavidicus* (Lea, 1856) has a wide distribution across the Amazon basin (Bonetto, 1967; Haas, 1932, 1969; Mansur and Valer, 1992; Pimpão and Mansur, 2009). Although there is a wide distribution of molluscs in Brazil, there are few records of Nematodas using these organisms as hosts (Thiengo et al., 2000).

The genus *Hysterothylacium* Ward and Margath (1917) belongs to the Anisakidae family, and it is frequently mistaken with the *Contracaecum* genus. While *Contracaecum* possesses an excretory pore next to the ventral interlabium, in *Hysterothylacium* this pore is located on the nerve ring region.

According to Luque et al. (2007) adult *Hysterothylacium* are found parasitizing fish. The larvae can be found in marine and freshwater fish as well as some invertebrates that, in this case, act as intermediate hosts. To date, there is no record of *Hysterothylacium* larvae parasitizing molluscs in Brazil.

In the present work, it is documented the occurrence of *Hysterothylacium* larvae in the pericardic cavity of *Diplodon suavidicus* specimens from Aripuanã River, tributary of the Madeira River, state of Amazonas, Brazil.

2. Material and methods

Individuals of *D. suavidicus* were manually collected from the Aripuanã river, an affluent on the right hand side margin of the Madeira river (between 05°58′23.4″S 60°12′37.4″W and 06°08′55.8″S 60°11′44.3″W). The collection was made during the dry season, between the 5th and 8th September, 2007. Part of the specimens was maintained for 24 h in bottles with water from the collection site and pure menthol crystals ($C_{10}H_{20}O$) for the relaxation of soft parts. Subsequently, all samples were fixed in 70% alcohol.

In the laboratory, the bivalves had their shells removed, allowing the visualization of the nematodes. They were removed with tweezers through a small cut on the mantle of the host, above the pericardic cavity. The number of parasites per host was recorded and all nematodes were fixed in 70% alcohol. The specimens were then analysed by light microscopy, where they were cleared and kept in lactic acid during the entire procedure. A drawing tube was attached to a light microscope in order to aid with the drawings. Measurements are given in millimeters (mm), followed by the mean and the range in parentheses. Bivalves and nematodes were deposited in the collection at the National Institute of Amazonian Research (Instituto Nacional de Pesquisas da Amazônia, INPA), Manaus, Brazil.

3. Results

Nematoda
Anisakidae
Hysterothylacium sp. (Fig. 1)

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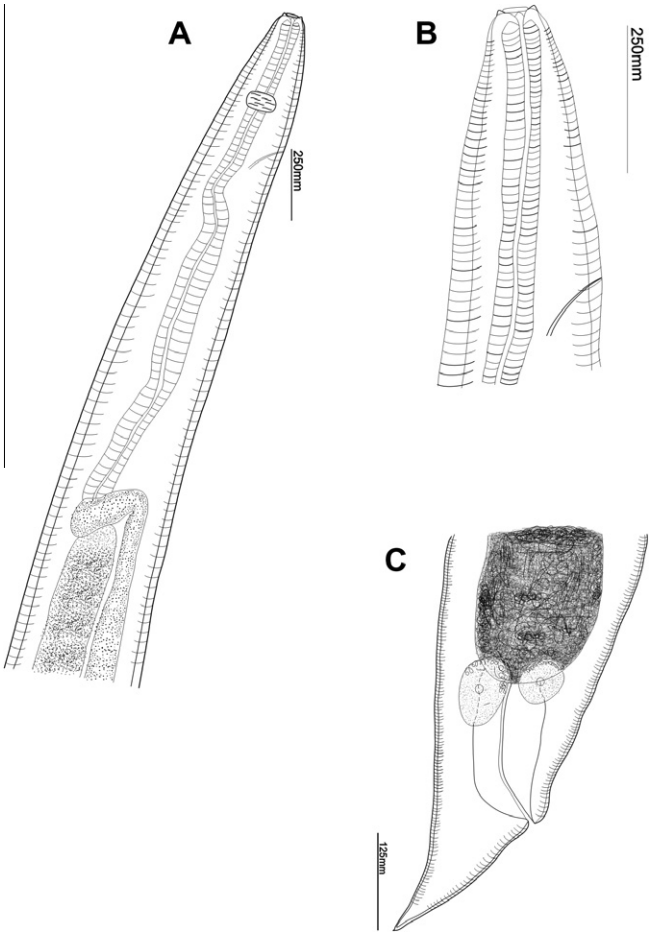

Fig. 1. *Hysterothylacium* sp. (A) Anterior portion; (B) details of anterior portion e (C) details of posterior region.

Description (based on eight specimens) Third larval stage, 19.9 (17.4–23.1) total length; 0.53 (0.45–0.62) maximum width. Cuticle transversally striated. No lateral alae. Larval teeth at the anterior extremity. Oesophagus 1.85 (1.45–2.78) long. Ventriculus with appendix, 2.09 (1.6–2.4) long; and 1.5 (0.91–2.0) large. Excretory pore anterior to the level of the nerve ring.

Host: *Diplodon suavidicus* (Lea, 1856) (Mollusca, Unioniformes, Hyriidae). Host examined: (based on 68 specimens). Hosts showed a mean length of 32.4 mm (varying between 22.45 and 44.7), (Fig. 2)

Prevalence and intensity: from the 68 molluscs collected, 56 were parasitized. The prevalence was 82%, with a mean intensity of 4.71 and mean abundance of 3.88. The amplitude of variation was between 1–16 individuals per host.

Site of infection: pericardic cavity

Diplodon suavidicus. INPA 1291; 1260; 1265; 1273; 129; 1300; 1306.

Hysterothylacium sp. INPA 1291; 1260; 1265; 1273; 129; 1300; 1306.

4. Discussion

The Anisakidae family shows a worldwide distribution and parasitizes all classes of vertebrates, including fish, mammals, birds and reptiles (Moravec, 1998). Their life cycle is still not clear for most species and many intermediate and definitive hosts are not known yet.

Some larvae can have a zoonotic character and reach men through the ingestion of raw or improperly cooked fish meat. Clinical signs depend on the site where the larva is deposited, but it generally causes abdominal pain and vomiting, as well as some allergic reactions (Fumarola et al., 2009; Valls et al., 2005). Nematodes of the *Hysterothylacium* genus reach sexual maturity inside the intestine of fish or marine mammals. Larvae of *Hysterothylacium* are found using a great variety of organisms as intermediate hosts (Jackson et al., 1997; Marcogliese, 1996; Bicudo et al., 2005; Navone et al., 1998).

This is the first report of *Hysterothylacium* larvae in Mollusca for the Amazon and Brazil. It is also the first record of a South American Hyriidae freshwater mussel as an Anisakidae intermediate host.

Thiengo et al. (2000) also recorded the presence of Anisakidae larva species in South American molluscs. However, these authors investigated the gastropod mollusc *Gundlachia radiata* (Guilding, 1828) and identified the larvae as belonging to the *Contracaecum* genus. Luque et al. (2007) recorded the presence of *Hysterothylacium* larvae in amphipods in New Zealand. However, the prevalence found by Thiengo et al. (2000) and Luque et al. (2007) were low compared to this study. From the 65 *Gundlachia radiata* specimens collected, only three were parasitized by *Contracaecum* larvae and with a maximum intensity of two larvae per host. From the amphipods collected by Luque et al. (2007), around one to 10% of the hosts were parasitized, depending on the sampling site, with one or two nematodes being found per host.

In this study, we found 82% prevalence and intensity varying between 1 and 16 parasites per host. When comparing our study with the ones above, it is possible to affirm that *D. suavidicus* is acting as an intermediate host for this parasite in that ecosystem.

While a great quantity of larvae was found in the pericardic cavity of the host (maximum of 16 larvae), there was no necrosis or obstruction of the individual inside the valves. Although morphologically similar to the *H. cenotae* larva, the larvae found in *D. suavidicus* are greater in size; while *H. cenotae* has an average total length of 5.34 mm, the one in question shows a total length of 19.0 mm.

For the Neotropical region, there are only two known adult species of *Hysterothylacium* parasites of freshwater fish; *H. rhamdiae* collected in Argentina (Brizzola and Tanzola, 1995) and *H. cenotae* in Mexico, (Moravec et al., 1997), but none for the Amazonian region.

There is large numbers of record of *Hysterothylacium* larvae parasitizing freshwater and marine fish in Brazil (Felizardo et al., 2009; Moravec et al., 1993; Tavares et al., 2004; Luque et al., 2008) however; there is none of larvae or adults of *Hysterothylacium* in fish from the Amazonian region (Thatcher, 2006). This suggests that in that region, the final host of *Hysterothylacium* could be a fish not yet studied or even another final host such as aquatic mammals or reptiles.

From the record of larvae of *Hysterothylacium* species in *D. suavidicus* and lack of information regarding this region, complementary studies are necessary to identify the parasite species, understand its cycle and recognise its final hosts.

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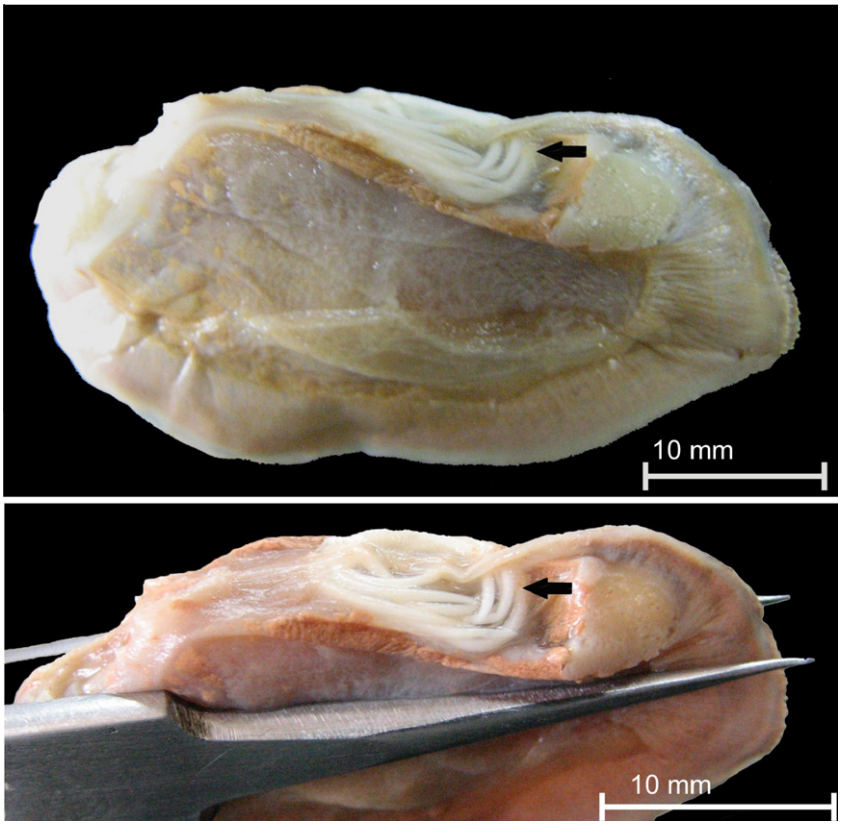

Fig. 2. Location of nematodes found in *Diplodon suavidicus.*

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