

Communication

[Comunicação]

**Cestodes Diphyllbothriidea parasitizing blackfin gosefish, *Lophius gastrophysus* Miranda-Ribeiro, 1915**

[*Cestoides Diphyllbothriidea* parasitos de peixe sapo-pescador, *Lophius gastrophysus* Miranda-Ribeiro, 1915]

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The *Lophius gastrophysus* (Miranda-Ribeiro, 1915) species come from the State of Rio de Janeiro, Brazil, to Argentina and are the only representing Lophiiformes fishes found in the Brazilian Southeast region (Figueiredo and Menezes, 1978). The species is highly valuable in Brazil, mainly for exportation purposes and due to its great acceptance in the international market and the increasing opportunities for exportation to Europe.

Diphyllbothriidea cestodes, mainly the *Diphyllbothrium* species, as is widely known, is very important for public health, since they are responsible for the accidental settling of human diphyllbothriasis. In the intermediate fish hosts, the diphyllbothriasis can cause disease and death in natural conditions and breeding (Rahkonen *et al.*, 1996). In humans this zoonosis can appear after they feed on raw fish meat, or poorly cooked or smoked, or submitted to inadequate freezing conditions that favors the survival of the *Diphyllbothrium* larvae (Scholz *et al.*, 2009). The classification of marine *Diphyllbothrium* species is somewhat confusing and the descriptions of *Diphyllbothrium* species of plerocercoids, whenever available, are incomplete (Andersen *et al.*, 1987) and thus, require detailed morphological and morphometric studies of these larval forms. *Diphyllbothrium latum* (Linnaeus, 1758), *D. dendriticum* (Nitzsch, 1824) and *D. pacificum* (Nybelin, 1931) are the species referred in South America, causing diphyllbothriasis (Knoff *et*

*al.*, 2008) and their plerocercoid larvae, when compared, to show morphological differences other than the maximum total length. Other relevant aspects may be taken into consideration, such as the morphological aspect of the tegument surface, the length of the microtrichies, the format of the scolex, the degree of its invagination, the histological arrangement of the muscular bundles, and even the infection site (Dick and Poole, 1985).

Regarding the blackfin gosefish, despite its commercial importance, there are few data on the helminths of this host, including those that can be harmful to health or cause economic losses, since to date there is only a single record of Trypanorhyncha cestodes infecting this fish species (São Clemente *et al.*, 2007).

The aim of this investigation was to identify the diphyllbothriid cestodes parasitizing specimens of *L. gastrophysus* in the State of Rio de Janeiro through the morphological and morphometric characterization with respective parasitic indexes related to prevalence, mean intensity, and mean abundance of infection.

From March 2000 to December 2005, 87 specimen of the blackfin gosefish *Lophius gastrophysus* were obtained from fishermen, markets, and fish exportation facilities in the municipalities of Cabo Frio (39 specimen), Niteroi (34 specimen), Duque de Caxias (six specimen), and Rio de Janeiro (eight specimen).

After being purchased, the specimen were transported in isothermal containers with ice to the Laboratório de Helminthos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Rio de Janeiro (LHPV/IOC/FIOCRUZ), to be investigated for helminths. The identification of fish was in accordance with Figueiredo and Menezes (1978). For recovery procedures, specimen were eviscerated; the organs and abdominal musculature were transferred to individual Petri dishes with a 0.65% NaCl solution to be examined under a stereoscope microscope. The filets, obtained after an incision from near the opercula to the insertion of the caudal fin, were observed through a candling table. Cestodes were processed for study in accordance with Eiras *et al.* (2006). A larva was observed under a bright field Olympus BX-41 microscope and measures were related to body and scolex length and width and length of the bothrial swelling. The movements of one of the worms that was recovered alive were recorded by a digital still camera Sony MVC-FD92 to permit the tracking of its displacement. The middle portion of the body was embedded in paraffin, processed for histological studies, cross-sectioned (7µm thick), stained with haematoxylin-eosin (HE), and observed under an Olympus BX-41 bright field microscope, for the analysis of internal structures of taxonomic interest. Another portion of the plerocercoid, with scolex and part of the body, was stained with Langeron's carmine and preserved as whole mount in Canada balsam. Photomicrographs were obtained in an Axiophot Zeiss bright field microscope. The taxonomic classification was based on Andersen and Gibson (1989) and Kuchta *et al.* (2008), and the parasitological indexes were according to Bush *et al.* (1997). A representative specimen of the cestode was deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ.

Investigated fish specimen were parasitized with *Diphyllobothrium* sp. plerocercoids that were found in the intestinal serosa and abdominal cavity (Figure 1.1). One of the worms was recovered alive and showed intense activity when transferred to a Petri dish with a 0.65% NaCl solution (Figure 1.2), thus permitting the tracking of the specimen displacement (Figure 1.3 a-d). The prevalence of the parasitism, mean

intensity, and mean abundance of *Diphyllobothrium* sp. in the blackfin goosefish was 2.3%, 1, and 0.02, respectively.

Description of *Diphyllobothrium* sp., based on two specimen, with measurements, in one: body smooth, slightly rugose, 1.2cm long, 0.1cm wide. Scolex introverted, inconspicuous, under stereomicroscope, only observed under bright field microscopy, was 0.27mm long, 0.75mm wide (Figure 2.1), frontal glands present in the scolex, reaching up to 1/3 of the body. Bothrial swelling 0.20mm long. Microtriches hardly observed, 2.5-5 (3.75) µm (Figure 2.2). Transversal histological cross-sections in the middle region of the body showed a not so thick external epiderm, epidermic longitudinal musculature in a single layer, and parenchymal longitudinal musculature well developed in the anterior region, becoming less developed near the posterior portion (Figures 2.3-4).

Representative specimen was deposited in the CHIOC under no. 37182a (with both body extremities) and 37182b (HE histological sections of the middle of the body). This is the first report of *Diphyllobothrium* sp. plerocercoids infecting specimens of *L. gastrophysus*.

The observed morphological characteristics related to the small-sized; retracted scolex; the bothrial swellings; the inconspicuous microtriches; the frontal glands in the scolex, extending to 1/3 of the body; and the epidermic longitudinal musculature in a single layer are very similar to those described in *D. latum* by Andersen and Gibson (1989).

*Diphyllobothrium* plerocercoids parasitizing teleost fishes in Brazil are poorly known. Recently, Knoff *et al.* (2008), observed larval *Diphyllobothrium* cestodes in the cusk-eel, *Genypterus brasiliensis* Regan, 1903. The larvae observed so far were similar to *D. dendriticum*, with a pseudo-segmented rugose body, with conspicuous developing genital apparatus in most of the specimen, that were 4.0-2.29 (1.30) cm long, 0.13-0.29 (0.17) cm wide, scolex 0.065-0.13 (0.10) cm long and 0.067-0.16 (0.12) cm wide, bothrial swelling 0.04-0.10 (0.16) cm long, microtriches 7.5-11.25 (10) µm long, different from the presently observed material shared closest characteristics with *D. latum*.

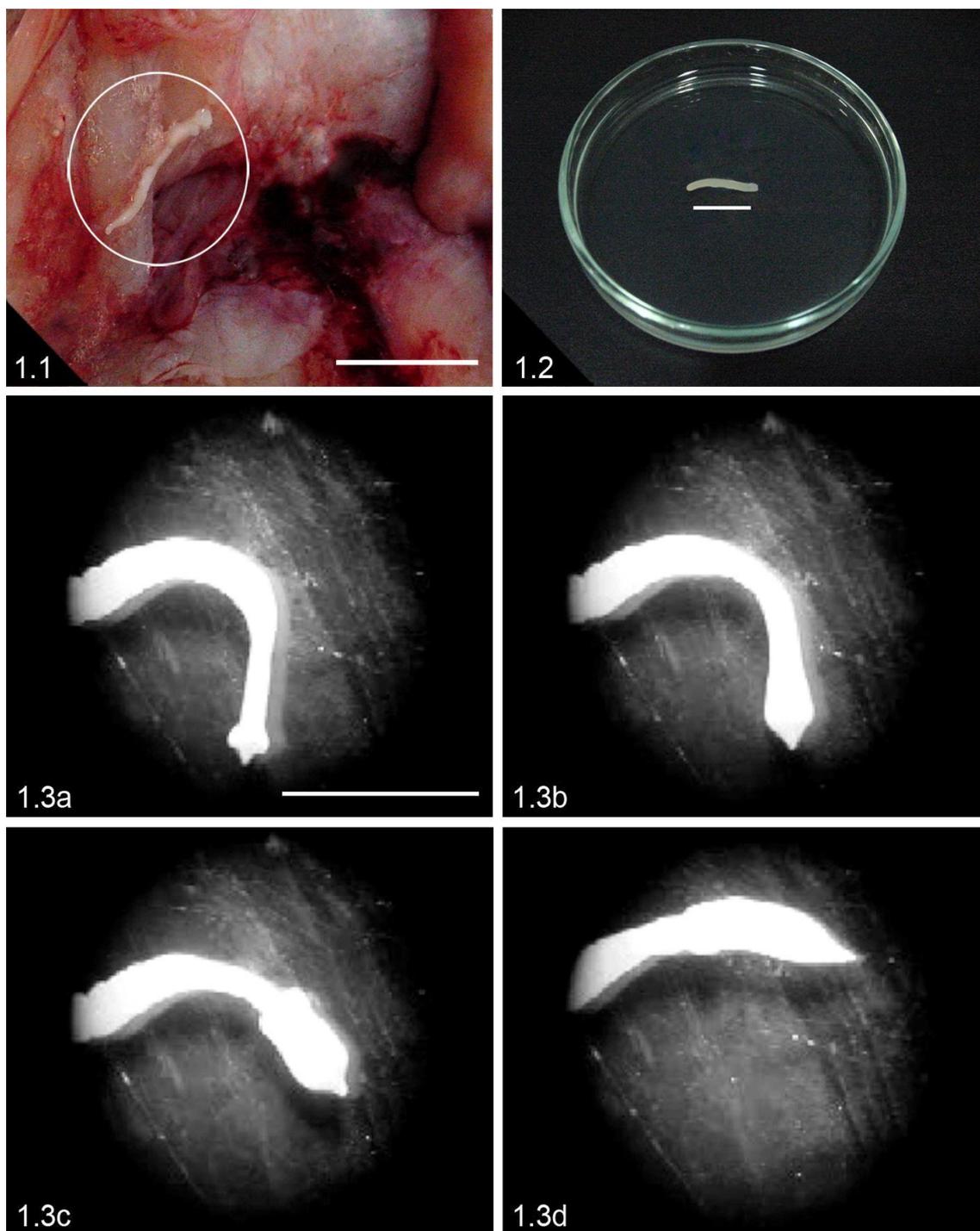


Figure 1. Plerocercoid of *Diphylobothrium* sp. 1: Abdominal cavity with plerocercoid, bar = 1cm. 2: Petri dish with 0.65% NaCl solution with the plerocercoid after the collect, bar = 1cm. 3: Sequence a-d permitting the tracking of plerocercoid displacment in a Petri dish with 0.65% NaCl solution, bar = 1cm.

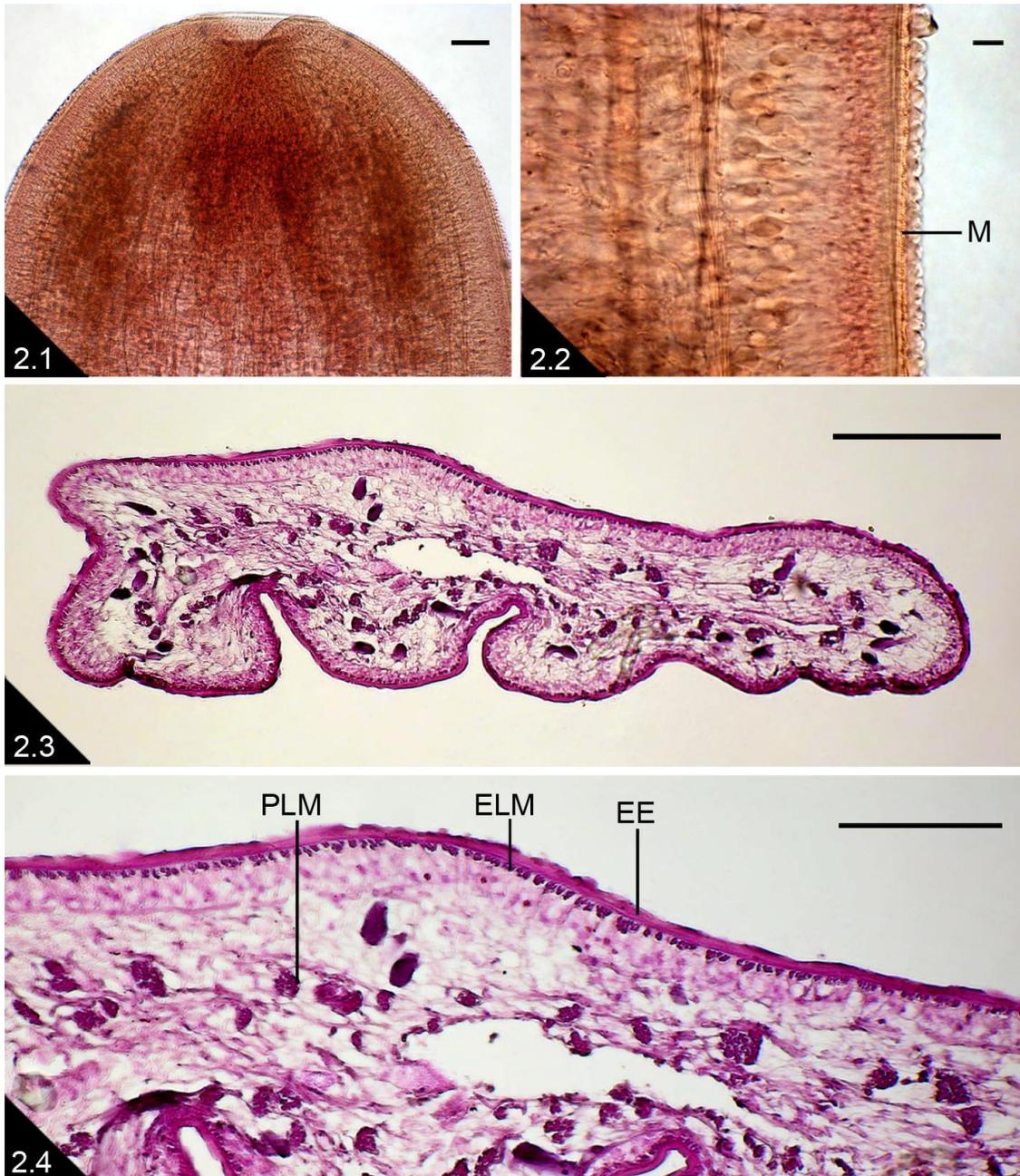


Figure 2. Plerocercoid of *Diphylobothrium* sp. 1: Detail of anterior extremity showing introverted scolex, bar = 100 $\mu$ m. 2: Detail of tegument indicating the microtriches (M), bar = 20 $\mu$ m. 3: Transversal histological cross-section in the middle region, bar = 200 $\mu$ m. 4: Detail of 2.3 showing external epiderm (EE), epidermic longitudinal musculature (ELM), and parenchymal longitudinal musculature (PLM) well developed, bar = 200 $\mu$ m.

Cases of human infection with diphylobothriid cestodes of the *Diphylobothrium* genus, mainly *D. latum*, have been reported in Brazil, with the statement that the transmission of diphylobothriasis has occurred after the

ingestion of raw, poorly cooked or smoked fish meat (Knoff et al., 2008; Mezzari and Wiebbelling, 2008). It reinforces the importance of the study of larval cestodes in Brazilian fishes, since in the present investigation, the

plerocercoid larva was found alive in the host, thus permitting to observe the movement and extroversion of the scolex within the bothrial region, that became evident and pointed, Figure 1.3 a-d, making it very similar to the plerocercoid of *D. latum* that is shown in Andersen *et al.* (1987). The parasitological indexes obtained on that fish were very low, and the sites of infection that were found in musculature indicate zoonotic potential. Chai *et al.* (2005) reported that besides Japanese food, in several other countries the ingestion of raw fish is common, permitting that live plerocercoids infect humans. According to McCarthy and Moore (2000), the change of alimentary habits is

to be considered a major risk factor, since it promotes the appearance and increase of zoonotic infections due to helminths. Thus, *Diphyllobothrium* sp. larvae parasitizing specimens of *L. gastrophysus* in Brazil can be harmful whenever fishes are inadequately ingested by consumers. Prevention methods related to human diphyllbothriasis already considered by Knoff *et al.* (2008) are priority and must be reported not only to the customers but also to fishermen and merchants.

Keywords: cestodes Diphyllbothriidea, *Lophius gastrophysus*

## RESUMO

Entre os meses de março de 2000 e dezembro de 2005, 87 espécimes de *L. gastrophysus* foram obtidos de supermercados e empresas de exportação localizadas nos municípios de Cabo Frio, Niteroi, Duque de Caxias e Rio de Janeiro. Os peixes foram necropsiados, filetados e seus órgãos investigados para presença de helmintos. Dois espécimes (2,3%) estavam parasitados por plerocercoides de *Diphyllobothrium* Cobbold, 1858 na cavidade abdominal e serosa do intestino, com intensidade média de um parasito por peixe e abundância média de 0,02, mostrando baixo potencial infectivo. Este é o primeiro registro de plerocercoides de *Diphyllobothrium* sp. no peixe sapo-pescador.

Palavras-chave: cestoides Diphyllbothriidea, *Lophius gastrophysus*

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