Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00407

Diphyllobothriidae cestode parasites of flounders *Paralichthys patagonicus* (Jordan, 1889) and *Xystreurys rasilis* (Jordan, 1891) collected off the coast of Rio de Janeiro, Brazil

Michelle Cristie Gonçalves da FONSECA[1], Nilza Nunes FELIZARDO[2], Delir Corrêa GOMES[1], Sergio Carmona de São CLEMENTE[2†], Marcelo KNOFF[1*]

Abstract

The present paper is a continuation of the survey about helminths parasitizing the flounders, *Paralichthys patagonicus* and *Xystreurys rasilis,* marine fish purchased from fish markets in the municipalities of Cabo Frio, Niterói, Rio de Janeiro, and Angra dos Reis, Rio de Janeiro State, Brazil. Specifically, this paper reports the results of an investigation concerning Diphyllobothriidae cestodes parasitizing these hosts. A total of 72 fish specimens were caught by professional fishermen off the coast of Rio de Janeiro State. The fish were measured and necropsied, and their organs were investigated for Diphyllobothriidae cestodes. A total of 12 flounders were parasitized by 23 specimens of Diphyllobothriidae gen. sp., infecting the stomach, intestinal serosa, abdominal cavity, and abdominal musculature. The taxonomic identification of these parasites was based on morphological and morphometric characteristics using bright-field microscopy. The specimens were identified as Diphyllobothriidae gen. sp., and their parasitic indices were presented. This is the first record of Diphyllobothriidae gen. sp. parasitizing the flounders *P. patagonicus* and *X. rasilis* in South America. The presence of these cestode plerocercoids is worrisome because of the potential risk of diphyllobothriasis, thereby reinforcing the hygienic-sanitary significance of monitoring these parasites.

Keywords: Diphyllobothriidae; *Paralichthys patagonicus*; *Xystreurys rasilis*; fish sanitary inspection; zoonotic potential.

Practical Application: Cestode larvae with the potential to cause zoonoses in humans.

1 INTRODUCTION

The flounder species *Paralichthys patagonicus* (Jordan, 1889) and *Xystreurys rasilis* (Jordan, 1891) (Paralichthyidae) represent important primary fishery resources in the coastal waters of Brazil (Figueiredo & Menezes, 2000). According to Cerqueira et al. (1997) and Massa et al. (2005), the flounder fishery is referred to as "fine fishing" due to its high commercial interest, meat quality, and market price, and because of the extensive sale on the domestic and foreign markets.

Oriental cuisine has awakened popular taste in Western countries, causing an increase in the consumption of raw fish, such as sushi and sashimi, resulting in increased exposure to the risk of accidental infection by fish parasites. Therefore, maintaining hygienic-sanitary conditions in fish markets has become a concern for municipal health surveillance, and in addition to ensuring that the fish are in good condition for consumption, it is necessary to avoid the economic losses caused by the disgusting appearance of fish parasitized by helminths (Broglia & Kapel, 2011).

Cestodes of the order Diphyllobothriidea (Kuchta, Scholz, Brabec, and Bray, 2008) are the principal agents of food-borne cestodosis. The ingestion of undercooked or raw fish infected with members of Diphyllobothriidae (Lühe, 1910), such as *Adenocephalus* (Nybelin, 1931), *Diphyllobothrium* (Cobbold, 1858), *Dibothriocephalus* (Lühe, 1899), or *Diplogonoporus* (Lönnberg, 1892), can cause the zoonosis known as diphyllobothriasis or diplogonoporosis, respectively (Waeschenbach et al., 2017). Reports of Diphyllobothriidae species parasitizing Brazilian fish, including another flounder Paralichthyidae, *Paralichthys isosceles* (Jordan, 1890), have been stated in papers on taxonomy, parasite ecology, and hygienic-sanitary conditions (Alves et al., 2005; Felizardo et al., 2010; Knoff et al., 2008, 2011b; Leite et al., 2022). In South America, *Dibothriocephalus latus* (Linnaeus, 1758) (Lühe, 1899) (= *Diphyllobothrium latum*), *Dibothriocephalus dendriticum* (Nitzsch, 1824), and *Adenocephalus pacificus* (Nybelin, 1931) have been reported as parasitizing humans (Acha & Szyfres, 2003; Dias et al., 2016; Hack et al., 2021; Knoff et al., 2011a; Sharma et al., 2018). Most cases of human diphyllobothriasis reported in Brazil were related to *Diphyllobothrium* sp. and *D. latus* and were diagnosed based on the examination of eggs and proglottids, with patient anamneses after the ingestion of raw, poorly cooked, or smoked fish meat (Dias et al., 2016; Knoff et al., 2011a).

1.1 Relevance of the work

The presence of diphyllobothriid cestodes is important for food hygiene because parasitized fish are usually rejected by

Received: Oct. 7, 2024.
Accepted: Oct. 22, 2024.
[1]*Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil.*
[2]*Universidade Federal Fluminense, Faculdade de Veterinária, Niterói, RJ, Brazil.*
*Corresponding author: knoffm@ioc.fiocruz.br
[†]*In memorian.*
Conflict of interest: nothing to declare.
Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (308048/2013-8 and 150140/2018-5).

consumers due to their unpleasant appearance and the fact that they can cause zoonosis, which is known as diphyllobothriosis. Thus, increased intensity of inspection of fish-based foods, as well as the implementation of health education programs, is necessary. Application of hazard analysis and a critical control points plan at all points of the production chain is recommended to eliminate, prevent, or reduce risks and ensure a safe and quality product.

2 MATERIAL AND METHODS

A total of 72 flounders were acquired: 36 specimens of *P. patagonicus*, mean length 39.2 cm (28.5–59.0 cm) and mean weight 747.3 g (280.0–2,530.0 g), and 36 *X. rasilis*, mean length 29.7 cm (14.0–51.0 cm) and mean weight 371.3 g (25.0–1,440.0 g). The fish were obtained from small markets, selling only fish caught offshore in the municipalities of Cabo Frio (22°52'46" S, 42°01'07" W), Niterói (22°53'00" S, 43°06'13" W), Rio de Janeiro (22°54'13" S, 43°12"35" W), and Angra dos Reis (23°00'24" S, 44°19'05" W), state of Rio de Janeiro, Brazil. The fish were transported on ice to the laboratory where they were identified according to Figueiredo and Menezes (2000) and Nakamura et al. (1986). Necropsy was carried out, and the internal organs and musculature were examined. Any cestode plerocercoids found were removed from the sites of infection. Some of these helminths were fixed in cold AFA (ethanol, formalin, and acetic acid) and preserved in 70% ethanol. They were then stained with Langeron's carmine, dehydrated in an increasing ethanol series, clarified in beechwood creosote, and preserved as whole mounts in Canada balsam according to Knoff and Gomes (2012). The taxonomic classification of Diphyllobothriidae cestodes was according to Caira and Jensen (2017), and the species identification was according to Knoff et al. (2008, 2011a). The samples were analyzed using an Olympus BX-41 bright-field microscope, and the images were obtained using a Canon digital camera (Power Shot A400). Measurements were made in millimeters (mm), with means provided in parentheses, obtained using the bright-field microscope. The parasitic indices of prevalence (P), mean intensity (MI), mean abundance (MA), and range of infection (RI) were calculated according to Bush et al. (1997). Representative specimens were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz – CHIOC, Rio de Janeiro, RJ, Brazil.

3 RESULTS

The analysis of 72 necropsied specimens of flounders revealed 12 infected fish, with a total of 23 plerocercoids, of which 20 were parasitizing *P. patagonicus* and 3 parasitizing *X. rasilis*. Most of the cestode larvae were alive and showed great motility.

The taxonomic identification of the collected helminths was as follows:

Platyhelminthes (Minot, 1876), Rhabditophora (Ehlers, 1985), Neodermata (Ehlers, 1985), Cestoidea (Rudolphi, 1808), Eucestoda (Southwell, 1930), Diphyllobothriidea (Kuchta, Scholz, Brabec & Bray, 2008), Diphyllobothriidae (Lühe, 1910), and Diphyllobothriidae gen. sp. (Figures 1 and 2).

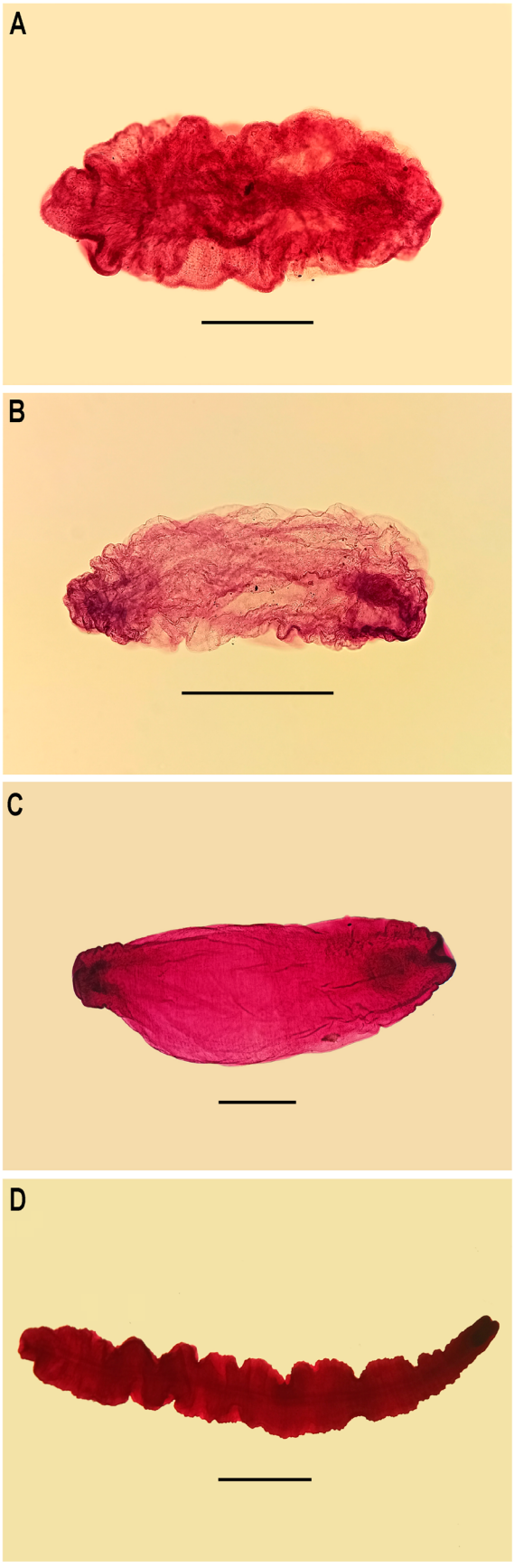

Figure 1. Plerocercoids of Diphyllobothriidae gen. sp. collected from *P. patagonicus*, entire worms. Scale bars: (A–C) 200 µm; (D) 2,000 µm.

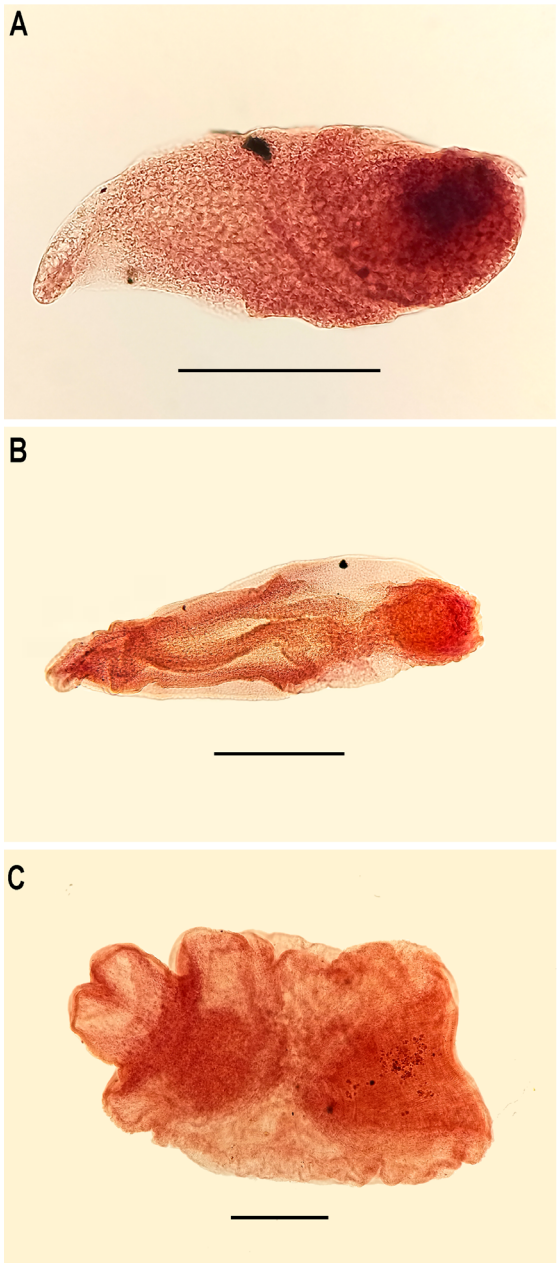

Figure 2. Plerocercoids of Diphyllobothriidae gen. sp. collected from *X. rasilis*, entire worms. Scale bars: (A and B) 200 µm, (C) 400 µm.

The main characteristics observed in the four plerocercoids found in *P. patagonicus* and the three found in *X. rasilis* were as follows: smooth body, slightly rugose with conspicuous external segmentation; 0.78–11.39 (3.56) long × 0.36–1.51 (0.70) wide (*P. patagonicus*) and 0.48–0.85 (0.72) long × 0.20–0.48 (0.31) wide (*X. rasilis*), and scolex with two distinct bothria, one dorsal and one ventral, unarmed.

- Host: *P. patagonicus.*
- Parasitic indices: *P* = 25%, *MI* = 2.22, *MA* = 0.55, *RI* = 1–4.
- Infection sites: Stomach, intestinal serosa, abdominal cavity, and abdominal musculature.
- Collected specimens: 20.
- Deposited specimen numbers: CHIOC 37824, 37825, and 37826.
- Host: *X. rasilis.*
- Parasitic indices: *P* = 8.33%, *MI* = 1.50, *MA* = 0.08.
- Infection sites: Stomach, intestinal serosa, and abdominal musculature.
- Collected specimens: 3.
- Deposited specimen number: CHIOC 37827.

This is the first record of Diphyllobothriidae gen. sp. parasitizing the flounders *P. patagonicus* and *X. rasilis* in South America.

4 DISCUSSION

The morphologies of the plerocercoid cestode specimens were identified by similarity with members of the family Diphyllobothriidae found in the present study and identified as Diphyllobothriidae gen. sp. Taking into account the methodologies adopted for the identification of diphyllobothriid species larvae in previous reports published in Brazil, in comparison with the present study, it is suggested that, in most of the cases, the identification should be referred to as Diphyllobothriidae gen. sp. (Caira & Jensen, 2017; Knoff et al., 2008, 2011a).

Previous studies carried out involving helminth parasites in flounders were recorded as follows: larval anisakid nematodes, cestodes; juvenile didymozoids; Trypanorhyncha cestodes; parasite communities; integrative taxonomy of Anisakidae and Raphidascarididae; parasitical evidence of stocks of flounders at small-end geographical scales on South American Atlantic coasts; acanthocephalan parasites; hemiurid and lecithasterid digenean trematodes and camallanid and cucullanid nematodes; and genetic and morphological characterization of a new species of the genus *Hysterothylacium* in *P. patagonicus*, *P. isosceles,* and *X. rasilis* (Alarcos & Timi, 2012; Alarcos et al., 2016; Felizardo et al., 2009, 2010, 2011; Fonseca et al., 2012, 2016, 2019, 2022; Knoff et al., 2012). In this context, parasites were found in flounder congeners, suggesting an association with the host-parasite, considering the growing scope of this cestode, and reporting a new record for parasitizing *P. patagonicus* and *X. rasilis.*

Morphometrically, the diphyllobothriids reported in the present study were smaller than those collected from *P. isosceles* off the coast of the state of Rio de Janeiro, Brazil (Felizardo et al., 2010).

Comparisons of the parasitic indices of the diphyllobothriiid specimens of the present study with those collected from specimens in *P. isosceles* reported by Felizardo et al. (2010) revealed that these authors found less prevalence (6% and 10%) and mean abundance (0.16 and 0.13) for *Diphyllobothrium* sp. 1 and *Diphyllobothrium* sp. 2, respectively, with a mean intensity of 1.6 for *Diphyllobothrium* sp. 2 and the highest mean intensity (2.5) for *Diphyllobothrium* sp. 1. These differences in parasitic indices could be related to the greater number of collection locations in the present study, where the hosts were collected in more than two municipalities in the state of Rio de Janeiro

(Rio de Janeiro and Angra dos Reis) and also in the municipalities of Cabo Frio and Niterói. The differences could also be due to various environmental and seasonal factors intrinsic to the collections themselves.

A comparison of the sites of infection in the present study with those of the diphyllobothriid specimens reported by Felizardo et al. (2010) in *P. isosceles* (abdominal cavity, intestine, liver, and ovary) showed that different sites of infection were recorded.

Regarding the zoonotic potential and hygienic-sanitary significance of the helminths found in the present study, diphyllobothriid plerocercoids can be involved in diphyllobothriasis (Waeschenbach et al., 2017). In Brazil, reports of diphyllobothriids indicate that the principal route of transmission is the ingestion of raw, poorly cooked, or smoked fish meat and incriminate imported salmon as the major factor responsible for human infection. Patients reported the ingestion of salmon and other native fish, and it is known that certain species of Brazilian fish captured in hinterland waters, but mainly in littoral waters, have been used in the preparation of exotic food (Eduardo et al., 2005; Knoff et al., 2011a). Furthermore, the presence of these cestodes has been reported in some fish species commercialized in Brazil (Leite et al., 2022), indicating the need to study other teleosteans that occur in Brazil, as suggested by Knoff et al. (2008, 2011b). Hence, salmon should not be considered the only agent of this zoonosis.

5 CONCLUSIONS

The presence of cestode plerocercoids is worrisome because of the potential risk of diphyllobothriasis, thereby reinforcing the hygienic-sanitary significance of monitoring these parasites.

The intensification of fish-based food inspections and the implementation of health educational programs are required. Hazard Analysis and a Critical Control Points plan should be applied at all points of the production chain to eliminate, prevent, or reduce risks, and ensure a safe product of high quality, as proposed by Leite et al. (2022) and Menezes et al. (2023).

ACKNOWLEDGEMENTS

The authors are grateful to Ricardo Baptista Schmidt of the Núcleo de Atividades de Extensão, IOC/Fiocruz, for processing the figures. This study was supported by fellowships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) S.C.S.C. (308048/2013-8) and M.C.G.F. (150140/2018-5).

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