



## ***Pterobothrium crassicolle* parasitizing *Paralichthys orbignyanus* (Osteichthyes, Paralichthyidae) in Brazil**

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### **ABSTRACT**

From September 2014 to November 2015, 30 *Paralichthys orbignyanus* specimens were obtained from Sepetiba Bay in the municipality of Rio de Janeiro, State of Rio de Janeiro, Brazil. The fishes were necropsied and cestode larvae were recovered from the abdominal cavity, mesentery, and serosas of the stomach, intestine, spleen, liver, kidney and gonads. Nineteen fish were parasitized by a total of 90 trypanorhynch plerocerci. Based on morphological characters, these were identified as *Pterobothrium crassicolle*. The parasitism indices for *P. crassicolle* on *P. orbignyanus*, were 63.3% for prevalence, 4.7 for mean intensity, 3 for mean abundance and 1 to 18 for range of infection. These parasites were studied due to their importance during fish sanitary inspection, if one considers the harm that the repulsive aspect of infected fish may cause to consumers. This is the first record of *P. crassicolle* plerocerci parasitizing *P. orbignyanus*.

**Key words:** *Paralichthys orbignyanus*, seafood safety, Trypanorhyncha, Brazil.

### **INTRODUCTION**

The flounder species *Paralichthys orbignyanus* (Valenciennes, 1839), lives in water that ranges from shallow to just over 20 m deep, and enters coastal lagoons from Rio de Janeiro, Brazil to Mar del Plata, Argentina. It feeds on pelagic and benthic species of crustaceans, reaches average length of 32

cm, and has both high commercial value and great ecological importance (Figueiredo and Menezes 2000).

The order Trypanorhyncha contains a great diversity of species, parasitizing marine fish and invertebrates. Adult worms live in the intestine of elasmobranchs fish, while the larval forms are found in the coelomic cavity, mesentery, visceral serosas and muscles of teleost fish, crustaceans and molluscs cephalopods (Campbell and Beveridge

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1994). Trypanorhynch cestodes in terms of fish sanitary inspection because of their repugnant appearance. According to the Brazilian Industrial and Sanitary Inspection of Products of Animal Origin Regulation, it is considered unsafe to eat the fish with massive muscle infestation by parasites, including those caused by trypanorhynch cestodes (Brasil 1952). It is common to encounter high rates of infection in the muscles, reinforcing the importance of conducting inspections for cestodes in a wide variety of teleost fish (Dollfus 1942, Amato et al. 1990, Pereira Jr 1993, São Clemente et al. 1995, 1997).

In some trypanorhynch species such as *Gymnorhynchus gigas* (Cuvier, 1817) Rudolphi, 1819 and *Molicola horridus* (Goodsir, 1841) plerocerci have antigenic components that can provoke anaphylatic episodes in humans (Rodero and Cuéllar 1999, Vázquez-López et al. 2002, Gómez-Morales et al. 2008). Using murine models, Mattos et al. (2013a, b) demonstrated by inoculation that crude extracts of *Pterobothrium heteracanthum* Diesing, 1850 and *P. crassicolle* are capable of inducing the production of specific IgE and IgG, so, inducing an allergic reaction in test animals.

In South America, there is one report of trypanorhynch cestode parasitizing the flounder *P. orbignyanus*; a *Grillotia* sp. plerocercus, in Argentina (Alarcos and Etchegoin 2010). In Brazil, species from this cestode order have been reported from other species of flounders, such as *P. isosceles* Jordan, 1890, *P. patagonicus* Jordan, 1889 and *Xystreuryx rasile* (Jordan, 1891), which have been found parasitized with plerocercoids of *Nybelinia lingualis* (Cuvier, 1817) Dollfus, 1927, *N. erythraea* Dollfus, 1960, *Heteronybelinia nipponica* (Yamaguti, 1952) Palm, 1999, and plerocerci of *Grillotia carvajalregorum* (Carvajal and Rego, 1983) Menoret and Yvanov, 2009, *Otobothrium* sp., *Pterobothrium crassicolle* Diesing, 1850, *P. heteracanthum* Diesing, 1850

and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (Felizardo et al. 2010, Fonseca et al. 2012).

The aim of this study was to identify the species of trypanorhynch parasitizing *P. orbignyanus*, collected from Sepetiba Bay, municipality of Rio de Janeiro, State of Rio de Janeiro, Brazil, their parasitism indices and infection sites, and to discuss the zoonotic importance of such infections.

#### MATERIALS AND METHODS

From September 2014 to November 2015, 30 specimens of the flounder *P. orbignyanus* were collected by professional fishermen in Sepetiba Bay, municipality of Rio de Janeiro, State of Rio de Janeiro, Brazil. All were 32-53 cm in length. The fish were transported in isothermal boxes to the Laboratory of Fish Inspection Technology of the Veterinary Medicine School of Fluminense Federal University, where they were necropsied and filleted. The fish species was identified according to Figueiredo and Menezes (2000).

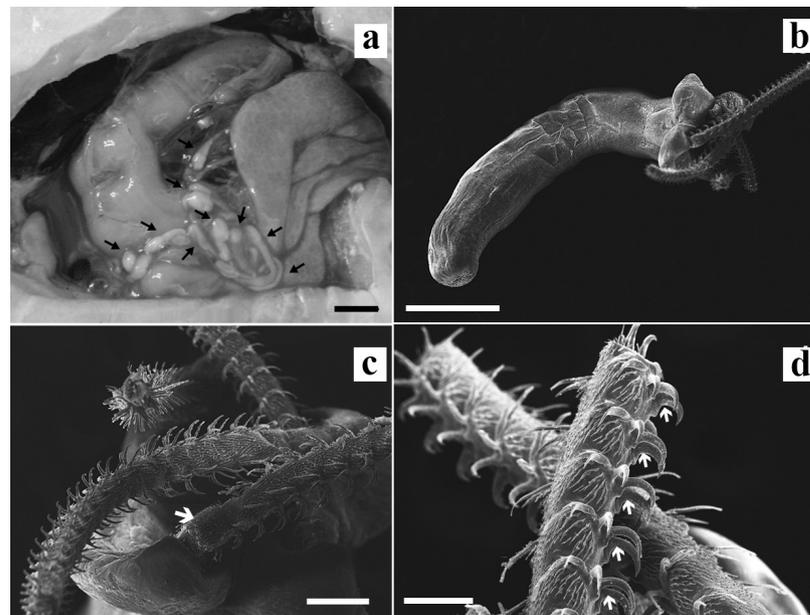
Trypanorhynch plerocerci were collected and transferred to a Petri dish with 0.65% NaCl solution, and opened with the aid of sharp needles under the stereomicroscope to release the larvae. These were then put in the refrigerator for at least 24h to allow the scolices to relax and to promote tentaculare version. Larvae were subsequently fixed in cold AFA, stained with Langeron's carmine, clarified in beechwood creosote, and preserved as whole mounts on Canada balsam, following Knoff and Gomes (2012). For morphologic studies, whole mounts were studied using a bright-field Olympus BH-2 microscope. For scanning electron microscope (SEM) studies, fixed samples were dehydrated in graded ethanol series (70-100°GL) for one hour each step, dried using critical-point CO<sub>2</sub>, coated with gold and analyzed with a JEOL 5320 scanning electron microscope operating at an acceleration voltage of 15 kV. Trypanorhyncha

classification was based on Palm (2004), and the species identification of collected plerocerci was based on São Clemente (1986), Rego (1987), Campbell and Beveridge (1996). Indices of parasitic prevalence, mean intensity and mean abundance were calculated according to Bush et al. (1997). Representative specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), FIOCRUZ, Rio de Janeiro, RJ, Brazil.

### RESULTS

From the 30 *P. orbignyanus* specimens from Sepetiba Bay, nineteen were parasitized by a total of 90 plerocerci of Trypanorhynch cestodes in the abdominal cavity, mesentery, and serosas of the stomach, intestine, spleen, liver, kidney and gonads. (Figure 1a). The specimens were taxonomical identified as belonging to the family Pterobothriidae Pintner, 1931, genus *Pterobothrium* Diesing, 1850,

species *P. crassicole* Diesing, 1850, based on morphological characters observed by bright-field and scanning electronic microscope (Figures 1b-d). Specimens from the current study had plerocerci each with blastocist. Elongate scolex, acraspedote; bothria, pyriform, on mobile pedicels, in cruciform arrangement; pedunculus scolecis subcylindrical, narrower than pars bothrialis; principal rows of metabasal armature form an alternating half-spiral of 5 large heteromorphous, hollow, hooks, with small interpolated hooks between principal rows on bothrial and antbothrial surfaces; distinctive basal armature and swelling present on internal and external surfaces of tentacle, macro hooks present on internal surface, asymmetrical basal swelling of tentacle present; hook files 1(1') widely separated, falciform; intercalary row (s) present proximal to each principal row; intercalary rows extend onto external surface to merge with band of hooks occupying midline of tentacle external



**Figure 1** - *Pterobothrium crassicole* plerocerci parasitizing *Paralichthys orbignyanus*: **a**. Plerocerci in the visceral serosas (arrows). **b-d**. Plerocercus by scanning electron microscopy. **b**. Entire worm. **c**. Distinctive basal armature with swelling on the external surface of tentacle (arrow). **d**. Falciformes macrohooks row on the internal face of tentacle (arrows). The scale bars in **a** = 0.5 cm, **b** = 1 mm, **c** = 200  $\mu$ m and **d** = 100  $\mu$ m.

surface; sheath shightly sinuous; bulbs elongate; pars postbulbosa present.

The parasitism indices for *P. crassicolle* from *P. orbignyanus*, were 63.3% for prevalence, 4.7 for mean intensity, 3 for mean abundance and 1 to 18 for range of infection. Specimens were deposited in the CHIOC (accession numbers 38.441 and 38442). Some fish had a large number of cestodes, giving a repugnant aspect (Figure 1a). Following eversion, all plerocerci were alive and showed moderate motility.

#### REMARKS

Collected *P. crassicolle* specimens from the current study are in accordance with the morphological features of species appearing in previous redescriptions and revisions (São Clemente 1986, Rego 1987, Campbell and Beveridge 1996, Dias et al. 2011, Zuchinalli et al. 2016).

To confirm the species identification of specimens collected in the present study, additional comparisons were made with whole mounted voucher specimens of *P. crassicolle* deposited in the CHIOC: 32253 of *Bagre marinus*, 32254 of *Zungaro zungaro* and 37845 of *Gobioides broussonneti* from State of Pará; 33902 of *Micropogonias furnieri* from State of Rio Grande do Sul; 33622, 33689-90, 38215-16 of *Oligoplites palometa*, 35632 of *Scomberomorus cavala*, 37354 of *Paralichthys isosceles* and 37750-51 of *P. patagonicus* from State of Rio de Janeiro.

#### DISCUSSION

*Pterobothrium crassicolle* has been reported in several teleost fish worldwide (Bates 1990, Palm 2004, Palm and Bray 2014). In Brazil, there are a number of records of this species in teleosts (Porto et al. 2009, Felizardo et al. 2010, Silva Júnior 2010, Dias et al. 2011, Fonseca et al. 2012, Videira et al. 2013, Zuchinalli et al. 2016), but this is the first

record of *P. crassicolle* plerocercus parasitizing *P. orbignyanus*.

Comparing the parasitism indices of *P. crassicolle* of the present study with those found in other species of *Paralichthys* collected in the State of Rio de Janeiro, showed that in *P. isosceles* and *P. patagonicus* the parasitism indices were lower, with prevalences of 1.7% and 29.6%, intensity/mean intensities of 4 and 2.5, and abundance/mean abundance of 0.07 and 0.74, respectively (Felizardo et al. 2010, Fonseca et al. 2012). The intensity and the range of infection in the present study was higher than that reported in *P. isosceles* and *P. patagonicus* in the State of Rio de Janeiro (1 and 1-4 parasites per host) (Felizardo et al. 2010, Fonseca et al. 2012). *Pterobothrium crassicolle* parasitized *P. patagonicus* at the same infection sites reported in the present study, as well as being present in the abdominal musculature, while *P. isosceles* was found only in stomach serosa (Felizardo et al. 2010, Fonseca et al. 2012). According to Luque et al. (2004), some factors, such as host body size, feeding habits and habitat, are closely linked to parasitism indices, and this could have influenced these results, obtained from conspecific host species in the same ecoregion.

Comparison of our data with that from hosts from other fish families collected in Brazil, showed higher prevalences of *P. crassicolle* in the present study, in *Micropogonias furnieri* (66.7%) from State of Rio Grande do Sul (Pereira Jr and Boeger 2005), in *Plagioscion squamosissimus* (78.6%) from State of Amapá (Silva Júnior 2010), in *Gobioides broussonnetii* (80.0%) from State of Pará (Videira et al. 2013), this latter host had slightly higher prevalence than in the present study. Mean intensity data reported for *M. furnieri* (5.15) from State of Rio Grande do Sul (Pereira Jr and Boeger 2005) was similar to that of the present study. However, lower indices of mean intensity/intensity than those of the present study were reported for *G. broussonnetii* (1.04) from State

of Pará and *Oligoplites saurus* (1) from Sepetiba Bay, State of Rio de Janeiro (Videira et al. 2013, Zuchinalli et al. 2016).

In the present study prevalence and range of infection of *P. crassicole* in the flounder *P. orbignyanus* were high, even though it was not found infecting the musculature. This Trypanorhynch species gave a repugnant aspect to the fish, due to the large size of the blastocyst, especially when it was present in great numbers. Another hygienic-sanitary aspect relevant to public health is that the crude extracts of *P. heteracanthum* and *P. crassicolle* are capable of inducing an allergic reaction in humans.

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