

Trypanorhynch cestodes parasitizing *Mugil liza* (Mugiliformes: Mugilidae) commercialized in the state of Rio de Janeiro, Brazil

Cestoides Trypanorhyncha parasitando *Mugil liza* (Mugiliformes: Mugilidae) comercializados no estado do Rio de Janeiro, Brasil

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Abstract

The aim of the present study was to identify, through morphological and morphometric analyses, the species of trypanorhynch cestodes found as plerocerci in the intestinal serosa of *Mugil liza* and to determine their parasitic indices. One hundred and fifty specimens of this mullet collected off the coast of the state of Rio de Janeiro were necropsied and the trypanorhynch cestodes found were fixed and preserved as whole mounts for morphological analysis. The plerocerci were identified as *Callitetrarhynchus gracilis* and *Pterobotrium crassicole*, both with a prevalence of 0.67%, an intensity of 1 and abundance of 0.0067, in single infections. This is the first record of a trypanorhynch cestode parasitizing *M. liza* in Brazil. Although the parasitic indexes were low, from a hygienic-sanitary perspective the plerocerci of these species are visible to the naked eye, and thus can disgust consumers and make marketing the fish unfeasible.

Keywords: *Pterobotrium crassicole*, *Callitetrarhynchus gracilis*, *Mugil liza*, Brazil.

Resumo

O presente estudo objetivou determinar taxonomicamente as espécies de plerocercos de cestoides Trypanorhyncha encontradas na serosa intestinal das tainhas *Mugil liza*, através do estudo morfológico e morfométrico e indicar seus índices parasitários. Foram coletados 150 espécimes desta espécie de tainha do litoral do estado do Rio de Janeiro. Após as necropsias, os cestoides Trypanorhyncha encontrados foram fixados e preservados em montagens permanentes para permitir a análise de suas estruturas morfológicas. Os plerocercos foram identificados como *Callitetrarhynchus gracilis* e *Pterobotrium crassicole* e ambas as espécies apresentaram prevalência de 0,67%, intensidade de 1 e abundância de 0,0067, em infecções únicas. Este é o primeiro registro de cestoides Trypanorhyncha parasitando *M. liza* no Brasil. Quanto ao aspecto higiênico-sanitário, vale ressaltar que, embora os índices parasitários registrados tenham sido baixos, os plerocercos dessas espécies estavam visíveis a olho nu, podendo causar repugnância ao consumidor e inviabilizar a comercialização do pescado.

Palavras-chave: *Pterobotrium crassicole*, *Callitetrarhynchus gracilis*, *Mugil liza*, Brasil.

Mullets are a group of great economic importance both for fishing and aquaculture (CROSETTI, 2016). In 2014, the global production of mullets was 728,546 tonne, of which 140,187 tonne (19.24%) were produced by aquaculture (FAO, 2016). The mullet *Mugil liza* Valenciennes, 1836, is widely distributed along the Atlantic coast of South America from Venezuela to Argentina

(MENEZES et al., 2010). The species is an important economic resource that supports many small communities through both fishing and aquaculture. *Mugil liza* is abundant in the western South Atlantic and has a great consumer market (REIS & D'INCAO, 2000; SILVA & ARAÚJO, 2000; KATSELIS et al., 2005; PINA & CHAVES, 2005). The state of Rio de Janeiro is the most important producer of *M. liza* in Southeast Brazil, producing more than 1,405 ton/year, mainly from artisanal catches (BRASIL, 2007).

Fish parasites are a major component of marine biodiversity, with cestodes playing a significant role in aquatic environments

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(ROHDE, 2005; KURIS et al., 2008). These parasites can be used as biological indicators for fish stock separation (PALM, 1999; MALEK, 2004; MACKENZIE et al., 2008), and have been used as an early warning system to monitor pollution and environmental degradation (THIELEN et al., 2004; MARCOGLIESE, 2005).

The order Trypanorhyncha Diesing, 1863, is a cosmopolitan group of marine cestodes with more than 270 recorded species, they are parasites of marine fish and invertebrates, especially in tropical and subtropical regions, with adult worms living in the intestine of an elasmobranch final host, while the larval forms are found in crustaceans and mollusc cephalopods, and musculature, coelomic cavity, mesentery and visceral serosa of teleosts (CAMPBELL & BEVERIDGE, 1994; KNOFF et al., 2002; PALM, 2004, 2010; PALM et al., 2009). Trypanorhynch cestodes, such as *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931, and *Pterobothrium crassicole* Diesing, 1850, are of hygienic-sanitary importance due to their repugnant aspect, this is particularly true when teleostean fish have massive infections in their musculature and organs, which can make commercialization infeasible due to sanitary inspection and/or rejection by the consumer, thus resulting in economic losses (KURAIEM et al., 2016; ZUCHINALLI et al., 2016).

Some trypanorhynch species, such as *Gymnorhynchus gigas* (Cuvier, 1817) Rudolphi, 1819, have larvae with antigenic components that are able to cause anaphylactic episodes (RODERO & CUELLAR, 1999). Vázquez-López et al. (2002) showed that proteins of *G. gigas* may alter motility and intestinal transit. By means of inoculation, Mattos et al. (2013) demonstrated that the molecules of the gross extracts of *Pterobothrium heteracanthum* Diesing, 1850, are capable of inducing the production of specific IgE and IgG, thus inducing an allergic reaction in murine models.

Several recent studies have aimed to elucidate the biology of species of *Callitetrarhynchus* and *Pterobothrium* that parasitize several species of fish along the Brazilian coast, with publications on taxonomy, parasite ecology, and hygienic-sanitary approaches (CORDEIRO & LUQUE, 2004; SÃO CLEMENTE et al., 2004, 2007; PEREIRA & BOEGER, 2005; ALVES & LUQUE, 2006; PINTO et al., 2006; LUQUE et al., 2008; DIAS et al., 2009, 2010, 2011; OLIVEIRA et al., 2009; PORTO et al., 2009; FELIZARDO et al., 2010; FONSECA et al., 2012; KURAIEM et al., 2016; ZUCHINALLI et al., 2016; MENEZES et al., 2018).

The present study represents a continuation of previous surveys of the metazoans and protozoans found in *M. liza* (= *M. platanus*) (OLIVEIRA et al., 1988; KNOFF & AMATO, 1991, 1992; KNOFF & SERRA-FREIRE, 1993; KNOFF et al., 1994, 1997; KNOFF & BOEGER, 1994). The aim was to identify species of trypanorhynch cestodes found in *M. liza* through the analysis of morphological and morphometric characters, and to determine and present their parasitic indices of prevalence, intensity and abundance.

A total of 150 specimens of *M. liza* measuring 35.5-59.5 cm (47.5 ± 16.97 cm) standard length, and weighing 600-3600 g (2100 ± 2121.32 g) that were caught between June 1984 and August 1988 by professional fishermen through artisanal fishing off the coast of the state the Rio de Janeiro, Brazil (22°54'13"S, 43°12'35"W) were analyzed. The hosts were carried in isothermal boxes to the Laboratório de Ictioparasitologia, Universidade Federal

Rural do Rio de Janeiro. The host specimens were identified in accordance with Menezes & Figueiredo (1985) and Froese & Pauly (2018). After necropsy, musculature was transferred to Petri dishes containing physiological solution with 0.65% NaCl. Cestode blastocysts were removed from the musculature for further investigation. Trypanorhynch plerocerci were transferred into distilled water and the cysts of plerocerci opened (under a stereomicroscope using sharp needles) to release the larvae, which were then refrigerated for at least 24 h to permit relaxation of scolices and tentacular extroversion. All larvae were fixed in cold AFA, stained with Langeron's carmine, dehydrated in an increasing alcoholic series, clarified in beechwood creosote and preserved as whole mounts in Canada balsam (AMATO et al., 1991). Taxonomic classification followed Cairá & Jensen (2017), while species identification followed Palm (2004). Photographs and measurements (in millimeters) were obtained from uncompressed specimens using bright field microscopy with an Olympus BX 41 microscope, unless otherwise indicated. Measurements are provided as means with ranges in parenthesis. Prevalence, intensity, and abundance indexes were calculated following Bush et al. (1997). Representative specimens of the parasites were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

One larval cestode was found on the intestine serosa of each of two of the 150 specimens of *M. liza* necropsied. The specimens are of different species, which are taxonomically identified as follows.

Trypanoselachoida *sensu* Beveridge, Haseli, Ivanov, Menoret & Schaeffner, 2017.

Lacistorhynchoidea Guiart, 1927

Lacistorhynchidae Guiart, 1927

Callitetrarhynchus Pintner, 1931

Callitetrarhynchus gracilis (Rudolphi, 1819) Pintner, 1931

(Figures 1 and 2)

Observed features of the specimen: plerocercus with blastocyst, appendix present. Scolex elongated, thin and acraspedote. Two pattelform bothria, notched posterior margin. Pars vaginalis long, tentacle sheaths regularly sinuous, less sinuous in the region of the pars bothrials. Bulbs elongated. Retractor muscles originate at the anterior 1/3 of bulbs. Pars postbulbosa absent. Principal rows of metabasal armature poeciloacanthous, heteromorphous, forming alternating half-spirals of eight principal hollow hooks, beginning on internal surface. Hooks 1(1') large and uncinat. Hooks 2(2') uncinat and largest. Hooks 3(3') falciform, large with large bases. Hooks 4(4') and 5(5') falciform. Hooks 6(6') spiniform, near external surface. Hook 7(7') elongate, s-shaped, larger than hook 8(8'). Hook 8(8') slender uncinat. A simple chainette ch (ch') is present.

Morphometrics provided in Table 1.

Parasitic indexes: prevalence 0.67%; intensity 1; abundance 0.0067.

Specimen deposited in CHIOC under the number 39063.

Grillotiidae Dollfus, 1969 and Pterobothriidae Pintner, 1931 clade *sensu* Beveridge, Haseli, Ivanov, Menoret & Schaeffner, 2017
Pterobothrium Diesing, 1850

Pterobothrium crassicole Diesing, 1850 (Figures 3 and 4)

Observed features of the specimen: plerocercus with blastocyst. Scolex elongate, acraspedote. Four pyriform bothria on mobile

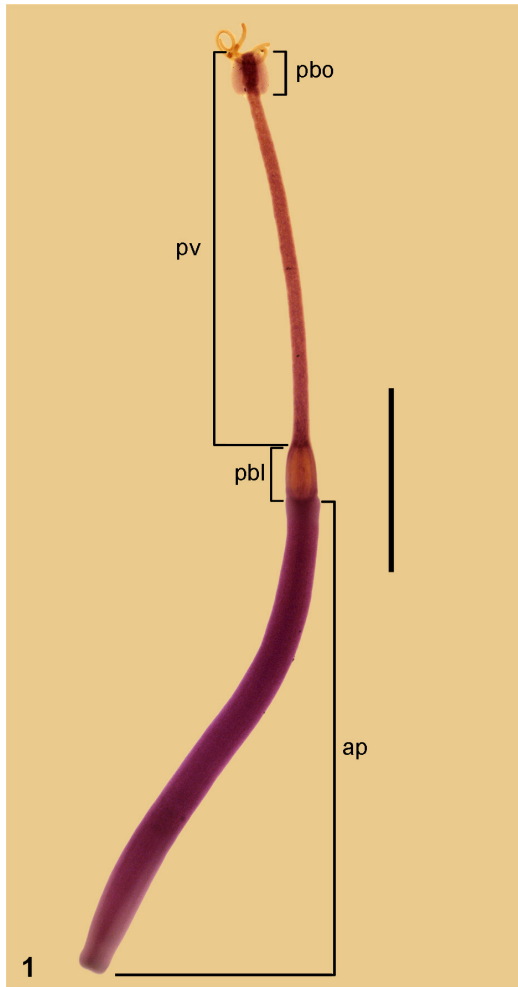


Figure 1. *Callitetrarhynchus gracilis* from *Mugil liza*. Entire plerocercus, pars bothrialis (pbo), pars vaginalis (pv), pars bulbosa (pbl) and appendix (ap). Scale bar = 1 mm.

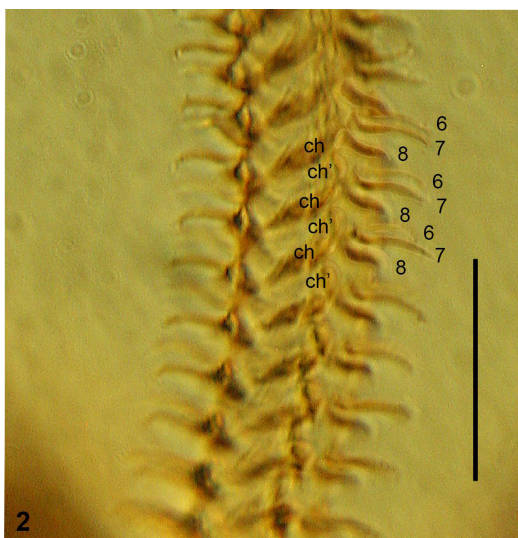


Figure 2. *Callitetrarhynchus gracilis* plerocercus from *Mugil liza*. Detail of the external surface hooks arrangement of the metabasal armature tentacle, showing the 6, 7 and 8 hooks and chainette (ch and ch'). Scale bar = 50 µm.

Table 1. Morphometric data for *Callitetrarhynchus gracilis* and *Pterobotrium crassicole* plerocerci collected from *Mugil liza* off the coast of the state of Rio de Janeiro, Brazil.

	<i>Callitetrarhynchus gracilis</i>	<i>Pterobotrium crassicole</i>
Scolex (L)	2.46	5.17
Appendix (L)	2.82	—
Appendix (W)	0.22	—
Pars bothrialis (L)	0.22	0.72
Pars bothrialis (W)	0.15	1.17
Pars vaginalis (L)	2.12	3.37
Pars vaginalis (W)	0.12	0.37
Pars bulbosa (L)	0.34	1.35
Pars bulbosa (W)	0.15	0.60
Bulbs (L)	0.29 - 0.31 (0.30)	1.13 - 1.33 (1.22)
Bulbs (W)	0.07 - 0.08 (0.07)	0.23 - 0.26 (0.25)
Pars postbulbosa	—	0.62
Tentacles (L)	0.10 - 0.75 (0.32)	0.77 - 1.65 (1.09)

Measurements are in millimeters, means in parentheses. L= length; W= width.



Figure 3. *Pterobotrium crassicole* from *Mugil liza*. Entire plerocercus, pars bothrialis (pbo), pars vaginalis (pv), pars bulbosa (pbl) and pars postbulbosa (ppb). Scale bar = 1 mm.



Figure 4. *Pterobothrium crassicole* plerocercus from *Mugil liza*. Detail of the tentacles, shown external surface intercalary microhooks to principal hooks (arrow), and swelling present on the distinctive basal armature tentacle (arrowhead). Scale bar = 250 μ m.

pedicels in a cruciform arrangement. Pedunculus scolecis subcylindrical, narrower than pars bothrials. Principal rows of metabasal armature forming alternating half-spirals of five large heteromorphous, hollow hooks; small interpolated hooks between principal rows on bothrial and antibothrial surfaces. Distinctive basal armature and swelling present on internal and external tentacle surface, macrohooks present on internal surface; asymmetrical basal swelling of tentacle present. Hooks 1(1') widely separated, falciform in proximal region, becoming smaller, stout and uncinat distally. Hooks 2(2') falciform, decreasing in size in distal metabasal region, heel gradually enlarging, toe gradually disappears. Hooks 3(3') falciform with short base and heel, gradually decreasing in size distally. Hooks 4(4') and 5(5') of proximal 12 rows digitiform, hooks 4(4') become falciform at row 13, hooks 5(5') remain digitiform along the entire file, markedly reducing in length in the apical region, heel and toe absent, falcate with pointed tips. Intercalary rows present proximal to each principal row; intercalary rows extend onto external surface to merge with band of hooks occupying midline of external surface of tentacle. Tentacle sheaths sinuous. Bulbs elongate. Pars postbulbosa present.

Morphometrics provided in Table 1.

Parasitic indexes: prevalence 0.67%; intensity 1; abundance 0.0067.

The specimen was deposited in CHIOC under the number 39062.

The morphology and morphometry described for *C. gracilis* in the present study is in accordance with the redescription of Carvajal & Rego (1985), São Clemente (1986), Palm (2004), and Menezes et al. (2018). Plerocerci of *C. gracilis*, like that found in the present study, have been reported parasitizing teleost fish in other countries and regions of the world such as Japan, Australia, Indonesia, Phillipines, Sri Lanka, India, Pakistan, Arabian Gulf, Egypt, Mozambique, Madagascar, South Africa, Namibia, Nigeria, Guinea, Senegal, Mauritania, Morocco, France, Italy, Atlantic and Pacific Coasts of the USA, including Hawaii, Gulf of Mexico, Bermuda, Cuba, Venezuela, Peru and Panama

(PALM, 1997, 2004; PALM & BRAY, 2014), and have been reported to exhibit a characteristic size range within the different hosts, which is in agreement with the suggestion of Palm (1997, 2004) that *C. gracilis* participates in a life-cycle with a four-host trophical web. The specimen of *C. gracilis* reported in the present study is similar to large plerocerci parasitizing some teleost fish from Brazil and other countries reported by Palm (1997, 2004) and Menezes et al. (2018).

The morphology and morphometry of the *P. crassicole* specimen of the present study is in accordance with description of São Clemente (1986) for *Pterobothrium* sp. (= *P. crassicole*); the redescription of Rego (1987), Campbell & Beveridge (1996), and Palm (2004); and the recent report of Zuchinalli et al. (2016).

The recovery of the trypanorhynchs *C. gracilis* and *P. crassicole* from specimens of *M. liza* in the present study represent the first time these parasite species have been documented infecting Brazilian mullets. There has only been one previously unidentified trypanorhynch species reported for *Mugil curema* Valenciennes, 1836, from local markets on Itamaracá Island and at Itapissuma, Northeast Brazil (PALM, 1997). Furthermore, these two species have been reported parasitizing Brazilian teleost and elasmobranch marine fishes (FONSECA et al., 2012; FELIZARDO et al., 2018), being now also reported to the Mugilidae family.

From a hygienic-sanitary perspective, although the parasitic indexes for the two recovered species were low, their plerocerci are visible to the naked eye, which can disgust consumers and make marketing the fish unfeasible.

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