



Cestode and nematode larvae of hygienic-sanitary importance parasitizing *Balistes capriscus* Gmelin, 1789, collected from fish markets of the state of Rio de Janeiro, Brazil

Mayla Monique dos Santos LEITE¹ , Marcelo KNOFF^{2*} , Michelle Cristie Gonçalves da FONSECA² ,
Nilza Nunes FELIZARDO¹ , Delir Corrêa GOMES² , Sergio Carmona de SÃO CLEMENTE^{1†}

Abstract

Balistes capriscus is an appreciated fish species in the state of Rio de Janeiro, Brazil, due its excellent quality flesh. The constant presence of helminth larvae in the abdominal musculature, viscera and serosa of individuals of the species has been the subject of complaints among local fish traders because of economic losses due to their repugnant aspect. Considering their hygienic-sanitary importance and significance for collective health, the presence of helminth larvae was investigated in 44 individual fish of *B. capriscus* purchased from fish markets in the municipalities of Rio de Janeiro, Niterói, Campos dos Goytacazes and Cabo Frio, state of Rio de Janeiro, in 2017–2018. Cestode larvae, identified as Diphyllbothriidae gen. sp. and *Callitetrarhynchus gracilis*, were found parasitizing the abdominal cavity, abdominal musculature, mesentery and liver serosa. Nematode (Raphidascaridiidae) third-instar larvae, identified as *Hysterothylacium deardorffoverstreetorum* and *Raphidascaris* sp., were found parasitizing stomach, intestine, liver, spleen, liver serosa, mesentery and abdominal cavity. The highest parasitic indices were for *Raphidascaris* sp., with prevalence of 70.45%, mean intensity of 15.61, mean abundance of 11 and infection range of 1–76 specimens per host. Considerations of the zoonotic potential and hygienic-sanitary significance of these helminths are presented to increase food safety for consumers.

Keywords: grey triggerfish; Diphyllbothriidae; Trypanorhyncha; Ascaridoidea; fish sanitary inspection.

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

1 Introduction

The fish species *Balistes capriscus* Gmelin, 1789, or grey triggerfish, occurs in the Eastern Atlantic, from the Mediterranean to Moçamedes, Angola, and in the Western Atlantic, from Nova Scotia, Canada, to Argentina. Individuals reach around 40 cm in total length when adult and live in marine waters up to 50 m deep. The species feeds on benthic invertebrates, such as mollusks and crustaceans. It is consumed mostly fresh, smoked, and dried-salted, and is appreciated by consumers due to its excellent quality flesh (Bernardes et al., 2005; Froese & Pauly, 2017).

The maintenance of hygienic-sanitary conditions in fish markets of the municipalities of Rio de Janeiro, Niterói, Campos dos Goytacazes and Cabo Frio, state of Rio de Janeiro, Brazil, has been a concern for municipal health surveillance. For example, larvae of diphyllbothriid and trypanorhynch cestodes and anisakid nematodes have been found in the abdominal musculature and viscera serosa of *B. capriscus* at these fish markets, which cause a repugnant appearance resulting economic losses. The oriental cuisine and the consumption of raw fish, such as sushi and sashimi, have aroused a popular taste in western countries, increasing exposure to the risk of accidental infection by fish parasites (Broglia & Kapel, 2011).

Cestodes of the order Diphyllbothriidea Kuchta, Scholz, Brabec and Bray, 2008 (broad tapeworms), are the principal agents

of food-borne cestodosis. Ingestion of undercooked or raw fish (second intermediate host) infected with *Adenocephalus Nybelin*, 1931, *Diphyllbothrium* Cobbold, 1858, *Dibothriocephalus* Lühe, 1899 or *Diplogonoporus* Lönnberg, 1892, causes diphyllbothriosis or diplogonoporosis, respectively. Clinical symptoms of cestodosis caused by adult worms are usually mild or even asymptomatic, the most common clinical signs being abdominal discomfort or pain, constipation, diarrhea and weakness; only extremely rarely has anemia or vitamin B12 deficiency been reported (Waeschenbach et al., 2017). Parasitism of some Brazilian fish, including *B. capriscus*, *Genypterus brasiliensis* Regan, 1903, *Paralichthys isosceles* Jordan, 1890, and *Lophius gastrophysus* Miranda-Ribeiro, 1915, by diphyllbothriid larvae has been reported in papers on taxonomy, parasite ecology and hygienic-sanitary conditions (Alves et al., 2005; Knoff et al., 2008, 2011b; Felizardo et al., 2010). In South America, *Dibothriocephalus latus* (Linnaeus, 1758) Lühe, 1899 (= *Diphyllbothrium latus*), *Dib. dendriticum* (Nitzsch, 1824) and *Adenocephalus pacificus* (Nybelin, 1931) have been reported as parasitizing humans (Acha & Szyfres, 2003). Most cases of human diphyllbothriosis reported in Brazil are related to *Diphyllbothrium* sp. and *Dib. latus*, and were diagnosed based on the examination of eggs and proglottids. The patients experienced anamneses after the ingestion of raw, poorly-cooked or smoked fish meat, with

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¹Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, RJ, Brasil

²Instituto Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil

*Corresponding author: knoffm@ioc.fiocruz.br

†Deceased in August 2020

imported salmon being mainly responsible (Knoff et al., 2011a; Dias et al., 2016).

Trypanorhynch cestodes are parasites of marine fish and invertebrates, especially in tropical and subtropical regions. Adults inhabit the gastrointestinal tract of elasmobranchs while larvae occur in the celomic cavity, visceral serosa and musculature of cephalopod, crustacean and teleost intermediate hosts (Palm, 2004). Parasitism of Brazilian fish by trypanorhynch larvae has been reported in papers on taxonomy, parasite ecology and hygienic-sanitary conditions (São Clemente et al., 1997; Felizardo et al., 2010; Oliveira et al., 2019; Diniz et al., 2021). Trypanorhynch larvae acquire significance due to their repugnant aspect, especially when present as massive infections in musculature and organs, which can make commercialization infeasible due to sanitary inspection and/or consumer rejection, thus resulting in economic losses (Oliveira et al., 2019; Diniz et al., 2021). The allergenic potential of some of these cestode species has been studied in tests using murine models (Mattos et al., 2015).

Nematodes of the families Anisakidae and Raphidascarididae are commonly found in marine fish acting as intermediate hosts while definitive hosts can be aquatic mammals or fish-eating birds. Humans become accidental hosts by eating infected raw or undercooked fish (Anderson, 2000). Anisakid and raphidascaridid larvae have been reported parasitizing several marine fish species in Brazil (Knoff et al., 2007; Felizardo et al., 2009; Fontenelle et al., 2015; Fonseca et al., 2016; Diniz et al., 2021). Audicana & Kennedy (2008) reported the zoonotic potential of anisakid nematodes because they can cause allergic and/or anaphylactic reactions and gastrointestinal problems. The species highlighted as causing the most cases of anisakidosis are *Anisakis simplex* (Rudolphi, 1809) and *Pseudoterranova decipiens* (Krabbe, 1878). There was a reported case of human intestinal infection by the raphidascaridid *Hysterothylacium aduncum* (Rudolphi, 1802) (Yagi et al., 1996). An autochthonous case causing human anisakidosis in Brazil was reported by Cruz et al. (2010). The allergenic potentials of *H. deardorffoverestreetorum* (Knoff et al., 2012) and *C. multipapillatum* (Drasche, 1882) Baylis, 1920 have been showed using a murine model (Ribeiro et al., 2017; Fontenelle et al., 2018).

According to Brazilian legislation about fish and their derivative products, any fish with a repugnant appearance, as in any musculature possessing massive parasite infection, is considered improper for consumption (Brasil, 2017).

Larvae of cestodes and of anisakid and raphidascaridid nematodes parasitizing *B. capriscus* have been reported in studies concerning the composition and structure of parasite communities. These helminth larvae could be recognized as part of this community composition and species richness, suggesting the importance of their ecological traits and their significance for fish sanitary inspection (Alves et al., 2005; Dias et al., 2009).

The aim of this study was (i) to identify the species of endohelminths that parasitize *Balistes capriscus*, (ii) to describe their parasitic indices and (iii) to reinforce the importance of hygienic-sanitary surveillance of fish when affected by parasites with zoonotic potential. It is known that some endoparasites (larvae of diphyllbothriid trypanorhynch cestodes and anisakid

nematodes) are known for their zoonotic potential, that is, they can also cause diseases in humans. Furthermore, the repugnant appearance caused by these parasites can make their sale impossible, generating economic losses. Wich highlights the importance of the study.

2 Material and methods

Forty-four specimens of *Balistes capriscus* (20 – 51 cm total length), were purchased from fish markets of the municipalities of Rio de Janeiro (10), Niterói (10), Campos dos Goytacazes (10) and Cabo Frio (14), state of Rio de Janeiro, Brazil, between March 2017 and July 2018. Fish were transported on ice to the laboratory where they were identified according to Figueiredo & Menezes (2002). Necropsy was performed and the internal organs and musculature were examined. Found cestode plerocercoids and blastocysts were removed from the musculature, serosa and organs and transferred to Petri dishes containing distilled water. The plerocerci cysts were then 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 (ethanol, formalin, and acetic acid), stained with Langeron's carmine, dehydrated in an increasing ethanol series, clarified in beechwood creosote and preserved as whole mounts in Canada balsam. Some diphyllbothriid plerocercoids were separated for later analysis by scanning electronical microscopy (SEM). Found nematode larvae were placed in Petri dishes with 0.65% NaCl solution, fixed in AFA at 60 °C, preserved in 70% ethanol and later clarified with Amman's lactophenol (Knoff & Gomes, 2012). Taxonomic classification of cestodes and nematodes followed Caira & Jensen (2017) and De Ley & Blaxter (2002), respectively. Species identification followed Knoff et al. (2008, 2011a, b) for diphyllbothriid cestodes and Dollfus (1942), Carvajal & Rego (1985), São Clemente (1986), Palm (2004) and Menezes et al. (2018) for trypanorhynch cestodes followed, while identification of nematodes followed Felizardo et al. (2009) and Knoff et al. (2012). Cestode and nematode larvae were observed using an Olympus BX-41 brightfield microscope and measurements were made in millimeters (mm), with means provided in parentheses. Parasitic indices were calculated following Bush et al. (1997). Some cestode specimens were prepared for SEM as described by Torres et al. (2013). The samples, fixed in 70% ethanol, were dehydrated in an ethanol series (70% to 100% ethanol), CO₂ critical point dried, coated in gold, and then examined and photographed using a scanning electron microscope (Jeol JSM-6390LV), under 15 kV acceleration voltage. Representative specimens of each parasite species were deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil.

3 Results

Among the 44 analyzed specimens of *B. capriscus*, 4 (9.09%) were found parasitized by diphyllbothriid cestode plerocercoids (total 9), 8 (18.18%) by trypanorhynch cestode plerocerci (total 9) and 31 (70.45%) by third-instar (L₃; total 514) raphidascaridid nematode larvae. Some nematode larvae were alive and showed high motility. Figure 1 shows one of the

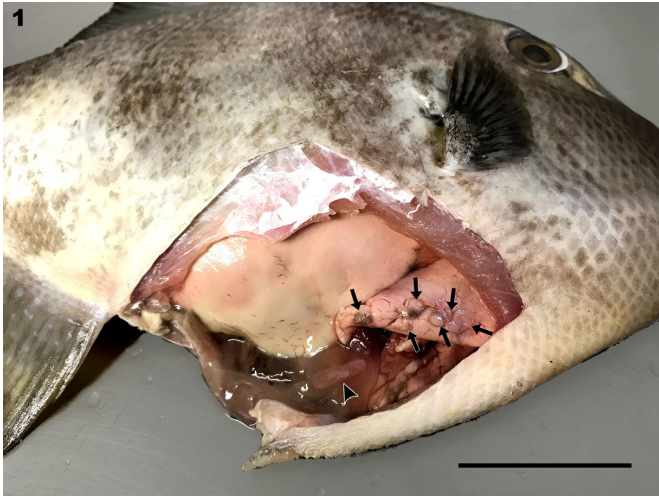


Figure 1. *Balistes capriscus* with abdominal cavity opened showing, visible to the naked eye, cestode larva (arrowhead) free in abdominal cavity and nematode larvae (arrows) on liver serosa. Scale bar: 5 cm.

collected fish, with the abdominal cavity opened, parasitized with one cestode blastocyst and many nematode third-instar larvae in liver serosa infection.

The taxonomic identification of the collected helminths follows.

Platyhelminthes Minot, 1876, Rhabditophora Ehlers, 1985, Neodermata Ehlers, 1985, Cestoidea Rudolphi, 1808, Eucestoda Southwell, 1930, Diphylobothriidea Kuchta, Scholz, Brabec & Bray, 2008, Diphylobothriidae Lühe, 1910

Diphylobothriidae gen. sp. Figure 2AB.

Main characteristics observed in six plerocercoids (three whole mounts and three by SEM) from nine collected. Body smooth, slightly rugose with conspicuous external segmentation; 6.75–11.25 (8.63) long by 1.02–1.92 (1.48) wide. Scolex with two distinct bothria, one dorsal and one ventral, unarmed.

Trypanorhyncha Diesing, 1863, Trypanoselachoidea Beveridge, Haseli, Ivanov, Menoret & Schaeffner, 2017, Lacistorhynchoidea

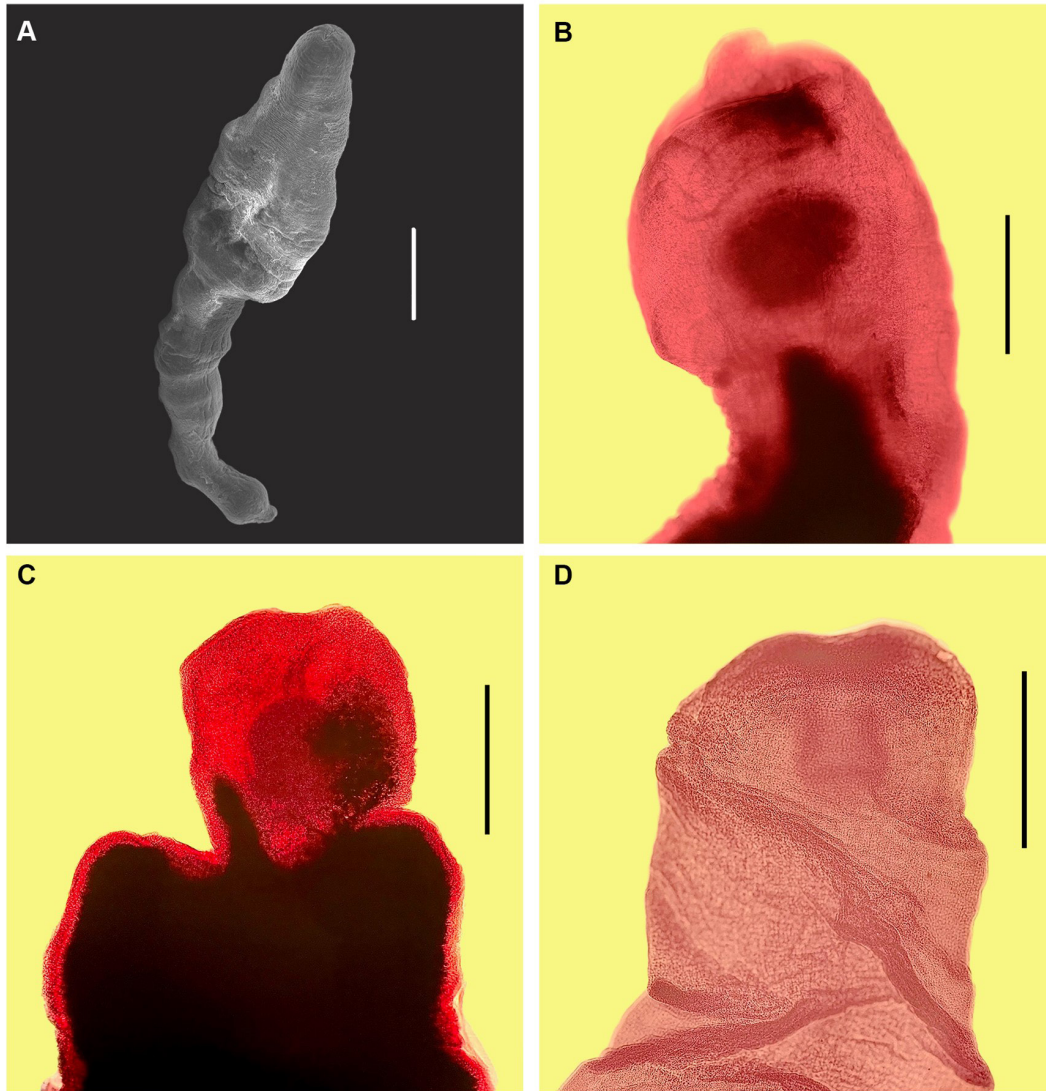


Figure 2. Plerocercoids of *Diphylobothriidae* gen. sp. collected from *Balistes capriscus*. A. Entire worm under SEM. B–D. Anterior extremity of plerocercoids. Scale bars: A = 1.0 mm; B–D = 0.4 mm.

Guiart, 1927, Lacistorhynchidae Guiart, 1927, *Callitetrarhynchus* Pintner, 1931

Callitetrarhynchus gracilis (Rudolphi, 1819) Pintner, 1931 (Figure 3AB)

Main characteristics observed in seven plerocerci (four whole mounts and three by SEM) from nine collected. Plerocerci with blastocyst. Scolex elongated, thin and acraspedote. Plerocerci with remarkable size range within this same host. Smaller specimens with scolex 1.10–1.50 (1.35) long by 0.12–0.15 (0.13) wide and appendix 0.12–1.22 (0.75) long by 0.10–0.15 (0.12) wide. Larger specimens with scolex 3.45–5.50 (4.77) long by 0.37–0.72 (0.60) wide and appendix 8.00–13.37 (11.00) long by 0.62–0.77 (0.67) wide. Two patteniform bothria with weakly notched posterior margins. Pars vaginalis long, tentacle sheaths regularly sinuous, less sinuous on pars bothrialis region. Bulbs elongated. Retractor muscles originate in anterior 1/3 of bulbs. Pars postbulbosa present, small, absent in some. Metabasal armature poeciloacanthous, atypical, heteromorphous; hooks hollow, spirals of 8 principal hooks in ascending half, beginning on internal surface. Hooks 1(1') large and uncinat; hooks 2(2') uncinat and long; hooks 3(3') falciform, large and with large bases; hooks 4(4') and 5(5') falciform; hooks 6(6') spiniform and located near external surface; satellite hook 7(7') is larger than hook 8(8'), both with slender uncinat shape. A simple chainette is present.

Nematoda Potts, 1932, Cromadorea Inglis, 1983, Rhabdida Chitwood, 1933, Ascaridomorpha De Ley & Blaxter, 2002, Ascaridoidea Baird, 1853, Raphidascardidae Hartwich, 1954

Hysterothylacium Ward & Magath, 1917

Hysterothylacium deardorffoverstreetorum Knoff, Felizardo, Iñiguez, Maldonado Jr, Torres, Pinto & Gomes, 2012. Figure 4A-C.

Main characteristics observed in five L₃ of 30 collected. Body 5.10–10.47 (7.65) long by 0.20–0.35 (0.27) wide. Cuticle with lateral alae extending along body with wedge-shaped support, devoid of basal extension. Anterior end with one dorsal and two poorly-developed ventrolateral lips. Nine cephalic papillae present, two pairs on dorsal lip together with a large papilla and one pair on each ventrolateral lip. Boring tooth absent. Excretory pore opening beneath nerve ring. Ventriculus sub-spherical. Ventricular appendix longer than esophagus. Intestinal cecum present. Four to six sub-spherical rectal glands present. Tail conical with mucron.

Raphidascaris Railliet & Henry, 1915

Raphidascaris sp. Figures 5A-C.

Main characteristics observed in five L₃ of 484 collected. Body 5.62–9.00 (7.63) long by 0.27–0.37 (0.39) wide. Cuticle with thin transverse striations, more evident on posterior end. Anterior end with one dorsal and two poorly-developed ventrolateral lips. Boring tooth near oral aperture, between ventrolateral lips. Excretory pore opening beneath nerve ring.



Figure 3. *Callitetrarhynchus gracilis*. A. Entire plerocercus (large specimen). B. Entire plerocercus (small specimen). Scale bars: A = 1.0 mm and B = 0.4 mm.

Esophagus elongated with enlarged posterior end. Ventriculus wider than long. Ventricular appendix present. Intestinal cecum absent. Tail with pointed end with defined transverse striations.

Parasitic indices, infection ranges, infection site(s) and CHIOC deposit number for the collected cestodes and nematodes are shown in Table 1.

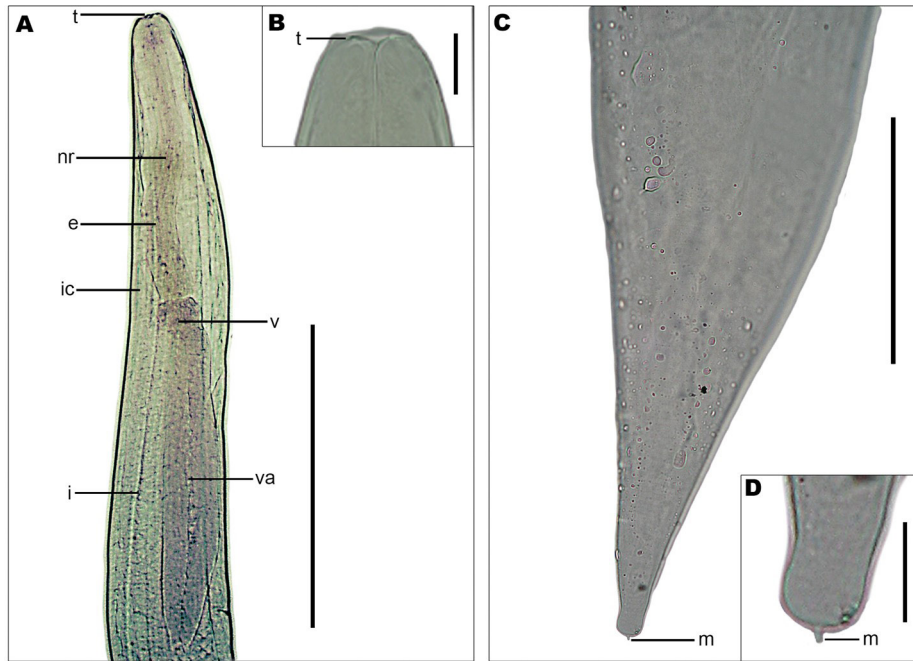


Figure 4. *Hysterothylacium deardorfffoverstreetorum* third-instar raphidascaridiid nematode larvae collected from *Balistes capriscus*. A. Anterior portion. B. Detail of cephalic end. C. Posterior portion. D. Detail of mucron. Larval tooth (t); nerve ring (nr); esophagus (e); intestinal cecum (ic); ventriculus (v); ventricular appendix (va); mucron (m). Scale bars: A = 0.60 mm, B and D = 0.025 mm and C = 0.20 mm.

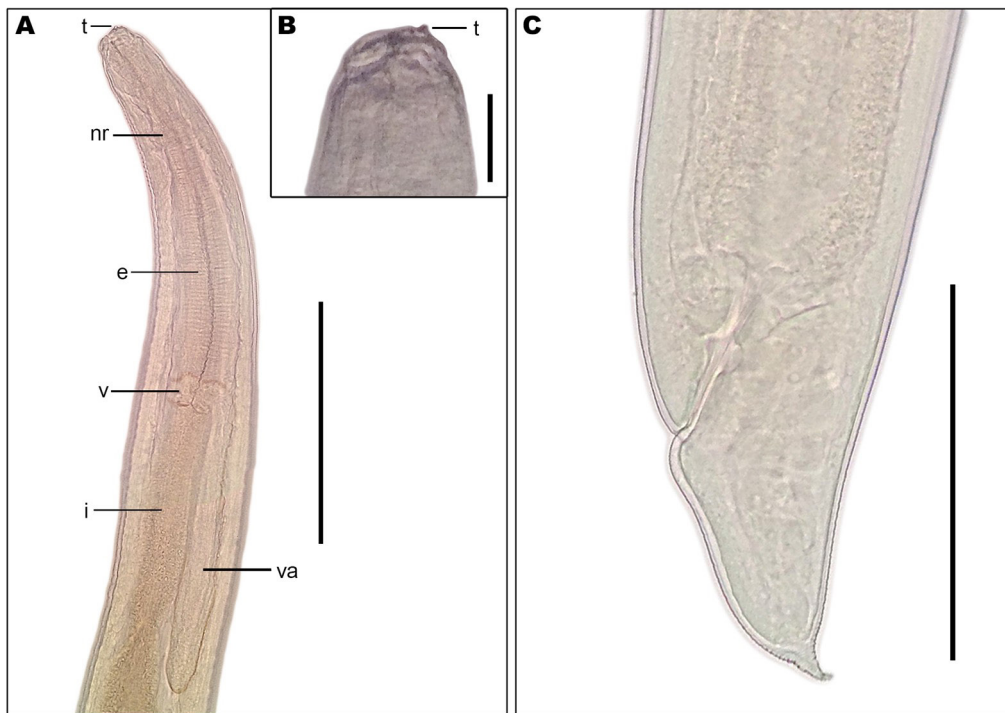


Figure 5. *Raphidascaris* sp. third-instar raphidascaridiid nematode larvae. A. Anterior portion. B. Detail of cephalic end. C. Posterior portion. Larval tooth (t); nerve ring (nr); esophagus (e); ventriculus (v); ventricular appendix (va); intestine (i). Scale bar: A = 0.40 mm, B = 0.025 mm and C = 0.20 mm.

Table 1. Prevalence (P), intensity/mean intensity (MI), mean abundance (MA), range of infection (RI), infection site(s) (IS) and CHIOC deposit number for diphylobothriid and trypanorhynch cestode larvae and third-stage raphidascaiid nematode larvae collected from *Balistes capriscus* marketed in the municipality of Niterói, state of Rio de Janeiro, between February and May 2019.

Helminths	P%	MI	MA	RI	IS	CHIOC
Cestodes						
Diphylobothriidae gen. sp.	9.09	2.25	0.20	1-5	AC	39579, 39580
<i>Callitetrarhynchus gracilis</i>	18.18	1.12	0.20	1-2	AC, AM, M, LS	39581, 39582, 39583, 39584
Nematodes						
<i>Hysterothylacium deardorffoverstreetorum</i>	11.36	6	0.68	1-21	AC, M, LS, L	39101
<i>Raphidascaris</i> sp.	70.45	15.61	11	1-76	AC, SP, L, LS, M, S, I	39102

AC = abdominal cavity, AM = abdominal musculature, I = intestine, L = liver, LS = liver serosa, M = mesentery, S = stomach and SP = spleen.

4 Discussion

Alves et al. (2005) previously reported larval diphylobothriid and trypanorhynch cestodes and larval anisakid and raphidascaiid nematodes infecting *B. capriscus* in Brazil from the same Warm Temperate Southwestern Atlantic province. They found the diphylobothriid (= pseudophyllidean not identified); the trypanorhynch *Nybelynia* sp. and *Callitetrarhynchus* sp.; the anisakids *Contracaecum* sp. and *Terranova* sp.; and the raphidascaiid *Raphidascaris* sp., from off the coast of Rio de Janeiro, Brazil. Luque & Tavares (2006) presented details of the systematics, biology and importance for collective health of anisakid and raphidascaiid larvae of marine boney fish collected from the state of Rio de Janeiro, where they listed the anisakids and raphidascaiid reported by Alves et al. (2005). In comparison to Alves et al. (2005), the present study did not observe larvae of *Nybelynia* sp., *Contracaecum* sp. or *Terranova* sp., but found *H. deardorffoverstreetorum*, showing some differences in the composition of these groups of larval helminths in this host. Interestingly, the parasitic indices reported by Alves et al. (2005) for diphylobothriid gen. sp., *Callitetrarhynchus* sp. and *Raphidascaris* sp., with prevalences of 1.5%, 16.7%, and 7.5% and mean abundances of 0.01, 1.1, and 0.1, respectively, indicate that the nematode *Raphidascaris* sp. had higher prevalence and mean abundance in the present study. Features observed among and within ecoregions are known to influence fish parasite communities and explain differences in species assemblages of parasites of teleost fish, as observed by Diniz et al. (2021). This difference in *B. capriscus* from off the state of Rio de Janeiro, Brazil, between the present and previous studies in the same ecoregion, appears to indicate that this fish species has variability in the trophic web that determines variability in the richness and abundance of larval helminths.

Dias et al. (2009) reported that the trypanorhynch found in *B. capriscus* was collected from the liver and mesentery, while the present study found them in these sites as well as in the abdominal cavity and abdominal musculature.

The specimens of *C. gracilis* of the present study consisted of two size classes of specimens, small and large plerocerci, occurring in the same host. Such small and large plerocerci have also been reported parasitizing some teleost fish from Brazil and other countries (Palm 1997, 2004; Menezes et al., 2018), and were reported showing a remarkable size range within different hosts. Thus, as suggested by Palm (1997, 2004), *C. gracilis* must be participating in a trophic chain of a four-host life cycle.

Regarding the zoonotic potential and hygienic-sanitary significance of the helminths found in the present study, plerocercoids of Diphylobothriidae gen. sp. can be involved in an important zoonosis (diphylobothriosis). Plerocerci of *C. gracilis* were visible to the naked eye, even when not found alive and at low prevalence, with up to two parasites per host; sometimes they were also found in the musculature, thus giving the consumer a repugnant aspect. In a study with a murine model, Mattos et al. (2015) warned about the ingestion of fish infected with species of trypanorhynch metacestodes as a possible cause of allergy in humans and that future studies should analyze this peculiarity. Furthermore, the hygienic-sanitary significance and collective health importance of the third-instar larvae of the raphidascaiid nematodes of the present study must be emphasized. Although these larvae were found in the serosa of the viscera and in the abdominal cavity of host fish, the risk of ingesting these parasites can not be ruled out since most of the larvae were found alive and, thus, with the capacity for post-mortem migration to the musculature, leading to the possibility of human infection. The highest prevalence recorded in the present study was for *Raphidascaris* sp. and the lowest for diphylobothriid gen. sp. and *H. deardorffoverstreetorum*. However, even the larvae of *Hysterothylacium* should be given consideration as they have been reported to cause gastrointestinal discomfort in humans (Yagi et al., 1996). The allergenic potential of *H. deardorffoverstreetorum* collected from other Brazilian marine fish hosts, was recently demonstrated using a murine model (Ribeiro et al., 2017). Preventive measures can reduce risks to consumer health, such as the evisceration of fish immediately after capture to reduce the risk of migration by raphidascaiid larvae to host musculature, as suggested by Diniz et al. (2021).

5 Conclusions

The presence of cestode plerocercoids and plerocerci and third-instar nematode larvae is worrisome because of the potential risk of diphylobothriosis, allergic reactions and “anisakidosis” for humans, thereby reinforcing the hygienic-sanitary significance of monitoring these parasites.

Intensification of fish-based food inspections and the implementation of health education programs are needed. 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 and quality product, as proposed by Diniz et al. (2021).

The occurrence of plerocercoids of the diphyllobotriid, plerocerci of the trypanorhynch and larvae of the raphidascaridiid nematode (*Raphidascaris* sp. with high parasitic indices) in the state of Rio de Janeiro, reinforce the results of previous studies on the parasite fauna of *B. capricus* indicating that the presence of these larvae might suggest an intermediate level for *B. capricus* in the marine trophic web.

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ERRATUM: Cestode and nematode larvae of hygienic-sanitary importance parasitizing *Balistes capriscus* Gmelin, 1789, collected from fish markets of the state of Rio de Janeiro, Brazil

Mayla Monique dos Santos LEITE¹ , Marcelo KNOFF^{2*} , Michelle Cristie Gonçalves da FONSECA² ,
Nilza Nunes FELIZARDO¹ , Delir Corrêa GOMES² , Sergio Carmona de SÃO CLEMENTE¹ 

Due to copyediting error the article “Cestode and nematode larvae of hygienic-sanitary importance parasitizing *Balistes capriscus* Gmelin, 1789, collected from fish markets of the state of Rio de Janeiro, Brazil” (DOI <https://doi.org/10.1590/fst.81521>), published in Food Science and Technology, 42, e81521, 2022, was published with an error.

On pages 1-8, where the text reads:

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The publisher apologizes for the errors.

¹Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, RJ, Brasil

²Instituto Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil