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Nematodes and acanthocephalans of hygienic-sanitary importance parasitizing *Hyporthodus niveatus* (Valenciennes, 1828) (Actinopterygii) collected from fish markets of the municipality of Niterói, RJ, Brazil

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Abstract

Hyporthodus niveatus is an appreciated and valuable commercial fish species in the municipality of Niterói, state of Rio de Janeiro, Brazil, due its excellent quality flesh. The constant presence of helminths 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 helminths was investigated in 20 individual fish of *H. niveatus* purchased from fish markets in the municipality of Niterói in 2021–2022. Nematodes, identified as third-instar larvae of *Contracaecum* sp., *Terranova* sp., *Hysterothylacium deardorffoverstreetorum* and *Raphidascaris* sp., were found parasitizing the intestine and abdominal cavity. Acanthocephalans, identified as juveniles of *Corynosoma australe*, were found parasitizing the intestine. The highest parasitic indices were for *H. deardorffoverstreetorum,* with prevalence of 30%, mean intensity of 1.5, mean abundance of 0.45 and infection ranges of 1–2. Considerations about the zoonotic potential and hygienic-sanitary significance of these parasites are presented in order to increase food safety for consumers.

Keywords: *Hyporthodus niveatus*; nematodes; acanthocephalans; zoonotic potential.

Practical Application: Nematodes and acanthocephalans with potential to cause zoonoses in humans.

1 Introduction

The fish *Hyporthodus niveatus* (Valenciennes, 1828), snowy grouper, occurs in the Western Atlantic Ocean from Canada to southern Brazil. It is a large demersal marine species, reaching 1.2 m in length and living on sandy bottoms from 30 to 525 m deep. It is a valuable commercial food fish, being very appreciated as food, with limited fishing pressure due to not forming shoals (Figueiredo & Menezes, 1980; Bernardes et al., 2005; Froese & Pauly, 2021).

The maintenance of hygienic-sanitary conditions in fish markets of the municipality of Niterói, state of Rio de Janeiro, Brazil, has been a concern for municipal health surveillance. For example, helminths have been found in the abdominal musculature and viscera serosa of *H. niveatus* at fish markets, which cause a repugnant appearance resulting in local economic losses. Furthermore, some kinds of helminth larvae, such as anisakid nematodes, can cause accidental human infections and allergy (Broglia & Kapel, 2011).

Nematodes of the families Anisakidae and Raphidascarididae can be transmitted to humans, with fish acting as intermediate, paratenic or definitive hosts (Anderson, 2000). Anisakid and raphidascaridid nematode 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., 2022; Leite et al., 2022; Miguel et al., 2022). Audicana & Kennedy (2008) reported on the zoonotic potential of anisakid nematodes. An autochthonous case causing human anisakidosis has also been reported for Brazil (Cruz et al., 2010). The allergenic potentials of anisakid and raphidascaridid larvae have been shown using a murine model (Ribeiro et al., 2017; Fontenelle et al., 2018).

Acanthocephalans, or thorny-headed worms, are endoparasitic helminths that can be found causing pathological conditions in marine and freshwater fish worldwide (Amin, 2013). Some cases of human acanthocephaliasis have been reported in association with the ingestion of raw fish, such as with species of *Corynosoma* (Polymorphidae) parasitizing Alaskan Eskimos (Schmidt, 1971) and in Japan (Acha & Szyfres, 2003; Fujita et al., 2016). Polymorphid acanthocephalans have been reported parasitizing marine fish species in Brazil (Santos et al., 2008; Eiras et al., 2016; Fonseca et al., 2019). Fonseca et al. (2019) have suggested studies to evaluate the zoonotic potential of acanthocephalan species.

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

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Some kinds of helminths parasitizing *H. niveatus*, such as digenetic trematodes, monogeneans, trypanorhynch cestodes and phillometrid nematodes, have been reported in studies concerning the composition and structure of parasite communities. These helminths could be recognized as part of community composition and species richness, suggesting the importance of their ecological traits and their significance for fish sanitary inspection (Faria & Silva 1934; Manter, 1947; Santos et al. 2000; Lima, 2004; Palm, 2004; Eiras et al., 2016; Alves et al., 2017; Moravec et al., 2020). However, there has been no reports of anisakid or raphidascaridid nematodes or acanthocephalans parasitizing *H. niveatus,* which motivated this survey of nematodes and acanthocephalans parasitizing the snowy grouper from the Brazilian coast.

Thus, the present investigation aimed to: (1) identify the species of third-stage anisakid and raphidascaridid nematode larvae and acanthocephalan juveniles parasitizing *H. niveatus* acquired in markets of the municipality of Niterói, state of Rio de Janeiro, by means of morphological and morphometric analyses using optical microscopy of specimens retrieved from the site of infection; (2) present their parasitic indexes and infection sites; and (3) demonstrate the hygienic-sanitary significance of these parasites to collective human health and provide baseline data for subsequent investigations.

2 Material and methods

Twenty specimens of *Hyporthodus niveatus* (42–67 cm total length), were purchased from fish markets of the municipality of Niterói, state of Rio de Janeiro, Brazil, between March 2021 and September 2022. Fish were transported on ice to the laboratory where they were identified according to Figueiredo & Menezes (1980). Necropsy was performed and internal organs and musculature were examined. Found helminths were placed in Petri dishes with 0.65% NaCl solution; nematode larvae were fixed in AFA (ethanol, formalin, and acetic acid) at 60 °C, preserved in 70% ethanol and later clarified with Amman's lactophenol; acanthocephalan specimens were fixed in cold AFA, preserved in 70% ethanol, stained in Langeron´s carmine, clarified in beechwood creosote, and preserved as whole mounts on Canada balsam, procedures according to Knoff & Gomes (2012). Taxonomic classification of nematodes followed De Ley & Blaxter (2002) and species identification relied on Felizardo et al. (2009) and Knoff et al. (2012). Taxonomic classification of acanthocephalans followed Amin (2013), while species identification relied on Pereira & Neves (1993), Sardella et al. (2005) and Fonseca et al. (2019). Nematode larvae and acantocephalan juveniles were observed using an Olympus BX-41 bright-field microscope and measurements were made in millimeters (mm), with means provided in parentheses. Samples were analyzed and images captured using a Canon Power Shot A640 digital camera coupled to a Zeiss Axiophot bright-field microscope using or not a Nomarski's differential interference contrast (DIC) apparatus. Parasitological indices of prevalence, mean intensity/intensity and mean abundance/abundance were calculated following Bush et al. (1997). Representative specimens of each parasite species were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil.

3 Results

Of the twenty investigated fish of *H. niveatus*, four contained a total of 16 live third-stage larvae (L3) of anisakid (3) and raphidascaridid (13) nematodes infecting the abdominal cavity, stomach and intestine. These include two species of the family Anisakidae with one specimen of *Contracaecum* sp. and two specimens of *Terranova* sp.; and two species of the family Raphidascarididae with nine specimens of *Hysterothylacium deardorffverstreetorum* and four specimens of *Raphidascaris* sp. Nematode larvae were alive and showed high motility. Three investigated fish contained a total of 98 juvenile polymorphid acanthocephalans (87 males and 11 females) infecting the intestine. The collected polymorphids were of the species *Corynosoma australe*, some of which were alive and exhibited limited motility. The specimens of nematodes and acanthocephalans were taxonomically identified as below.

Nematoda Potts, 1932, Cromadorea Inglis, 1983, Rhabdtida Chitwood, 1933, Ascaridomorpha De Ley and Blaxter, 2002, Ascaridoidea Baird, 1853, Anisakidae Railliet and Henry, 1912

Contracaecum Railliet and Henry, 1912

Contracaecum sp. Figure 1

One specimen of *Contracaecum* sp. was collected from the intestine of one individual of *H. niveatus*. Parasitic indices: prevalence 5%, intensity 1, abundance 0.05. Voucher specimen was deposited in CHIOC under the number 39373.

Main characteristics observed in one L3. Body 8.12 long, 0.17 wide. Cuticle with thin transverse striations, more evident and higher at posterior end of body. Anterior extremity with dorsal lip and two poorly-developed ventrolateral lips. Larval tooth present; opening of excretory pore below flat tooth. Ventricle small, lateralized. Two subspherical rectal glands present. Tail conical, long, without mucron.

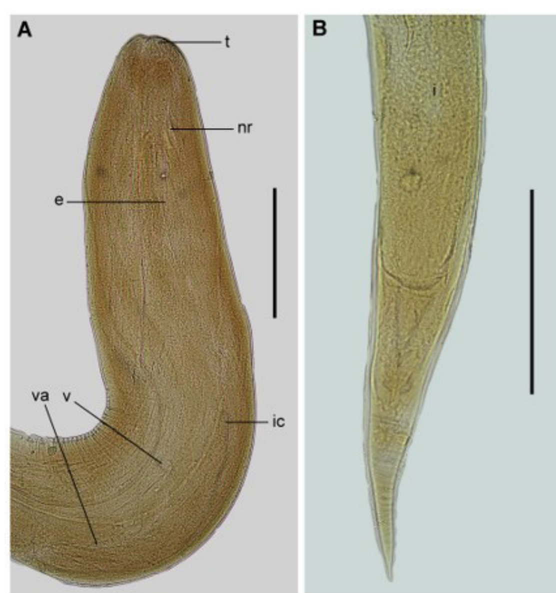

Figure 1. *Contracaecum* sp. third-instar larva collected from *Hyporthodus niveatus*. (A) Anterior portion; (B) Posterior portion. Larval tooth (t); nerve ring (nr); esophagus (e); intestine (i); intestinal cecum (ic); ventriculus (v); ventricular appendix (va). Scale bars: A and B = 0.20 mm.

Terranova Leiper and Atkinson, 1914

Terranova sp. Figure 2

Two specimens of *Terranova* sp. were collected from the intestine of two individuals of *H. niveatus*. Parasitic indices: prevalence 10%, mean intensity 1, mean abundance 0.1. Voucher specimens were deposited in CHIOC under the numbers 39374 and 39375.

Characteristics observed in two L3. Body 7.05–7.30 (7.17) long, 0.22–0.25 (0.23) wide. Cuticle with narrow transverse striations more evident at posterior end of body. Anterior extremity with one dorsal and two ventrolateral poorly-developed lips. Larval tooth present; opening of the excretory pores below flat tooth. Ventricle longer than wide. Ventricular appendage absent. Intestinal cecum twice the length of ventricle. Two subspherical rectal glands present. Tail conical, without mucron.

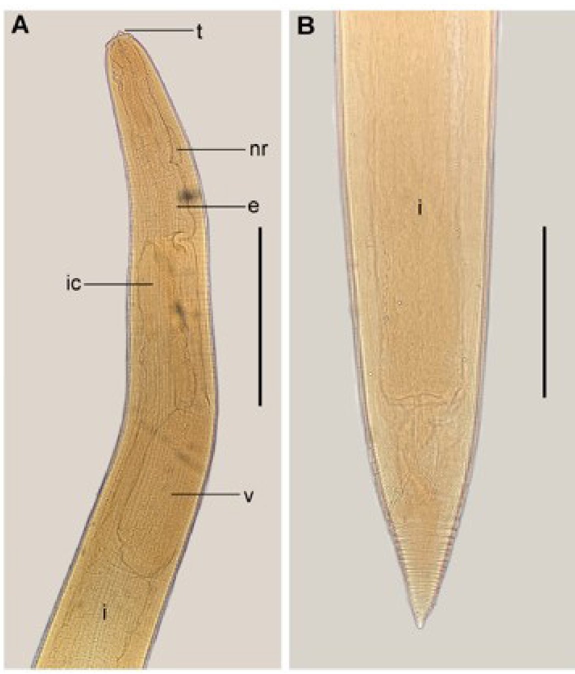

Figure 2. *Terranova* sp. third-instar larva collected from *Hyporthodus niveatus*. (A) Anterior portion; (B) Posterior portion. Larval tooth (t); nerve ring (nr); esophagus (e); intestine (i); intestinal cecum (ic); ventriculus (v). Scale bars: A = 0.40 mm and B = 0.20 mm.

Raphidascarididae Hartwich, 1954

Hysterothylacium Ward and Magath, 1917

Hysterothylacium deardorffoverstreetorum Figure 3

Nine specimens of *H. deardorffoverstreetorum* were collected from the abdominal cavity and intestine of six individuals of *H. niveatus*. Parasitic indices: prevalence 30%, mean intensity 1.5, mean abundance 0.45, range of infection 1–2. Voucher specimens were deposited in CHIOC under the numbers 39376 and 39377.

Main characteristics observed in seven L3. Body 6.05–13.75 (9.78) long, 0.25–0.50 (0.33) 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 single 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 sub-spherical rectal glands present. Tail conical, with mucron.

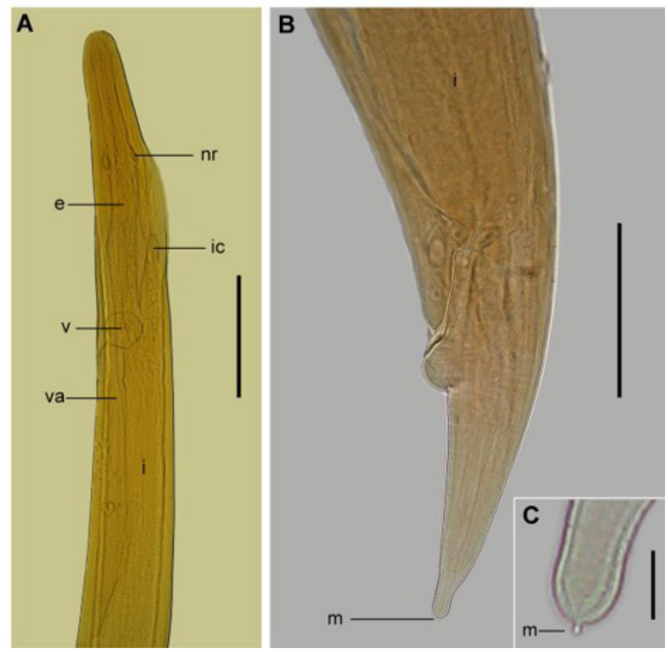

Figure 3. *Hysterothylacium deardorffoverstreetorum* third-instar larva collected from *Hyporthodus niveatus*. (A) Anterior portion; (B) Posterior portion. Nerve ring (nr); esophagus (e); intestine (i); intestinal cecum (ic); mucron (m); ventricular appendix (va); ventriculus (v). Scale bars: A = 0.40 mm, B = 0.20 mm, and C = 0.025 mm.

Raphidascaris Railliet and Henry, 1915

Raphidascaris sp. Figure 4

Four specimens of *Raphidascaris* sp. were collected from the intestine of one individual of *H. niveatus*. Parasitic indices: prevalence 5%, intensity 4, abundance 0.2. Voucher specimens were deposited in CHIOC under the number 39378.

Main characteristics observed in four L3. Body 3.18–4.05 (3.68) long, 0.12–0.17 (0.15) 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. Esophagus elongated with enlarged posterior end. Ventriculus wider than long. Ventricular appendix present. Intestinal cecum absent. Tail with pointed end with defined transverse striations.

Acanthocephala Rudolphi, 1802, Palaeacanthocephala Meyer, 1931, Polymorphida Petrochenko, 1956, Polymorphidae Meyer, 1931

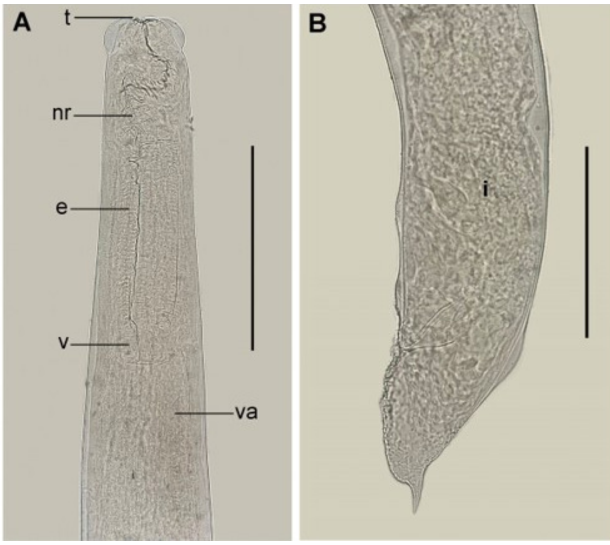

Figure 4. *Raphidascaris* sp. third-instar larva collected from *Hyporthodus niveatus*. (A) Anterior portion; (B) Posterior portion. Larval tooth (t); nerve ring (nr); esophagus (e); intestine (i); ventriculus (v); ventricular appendix (va). Scale bars: A = 0.40 mm, and B = 0.20 mm.

Corynosoma Lühe, 1904

Corynosoma australe Johnston, 1937. Figures 5 and 6

Ninety-eight specimens of *Corynosoma australe* were collected from the intestine of three individuals of *H. niveatus*. Parasitic indices: prevalence 15%, mean intensity 32.7, mean abundance 4.9, range of infection 1–62. Voucher specimens were deposited in CHIOC under the numbers 39781 and 39782.

Main characteristics observed in 14 juveniles (6 males and 8 females measured). Body pyriform, anterior region dilated, proboscis cylindrical. Body 1.37–1.87 (1.62) long, 0.47–0.72 (0.60) wide (males); 2.28–3.98 (2.98) long, 0.58–1.58 (0.95) wide (females). Proboscis 0.30–0.60 (0.52) long, 0.12–0.20 (0.16) wide (males); 0.48–0.88 (0.68) long, 0.10–0.28 (0.18) wide (females); wider at posterior end, armed with 18 longitudinal rows of 12–14 hooks each, 9–11 anterior hooks with well-developed posteriorly directed roots, and three small basal hooks with small anteriorly directed roots. Neck short, wider at posterior end. Anterior portion of trunk swollen and flattened in form of a bulb. Proboscis receptacle double-walled. Tegumental spines covering bulb dorsally at fore-trunk, extending to ventral surface at posterior end. Pair of moderately short, lobe-like leminisci reaching half the length of proboscis receptacle. In some female specimens, genital and tegumental spines are contiguous in the ventral region, but clearly distinguishable. Genital opening surrounded by triangular genital spines that are larger than tegumental spines. Male genital apparatus: testes rounded, contiguous; six claviform cement glands, followed by elongate Saefftigen's pouch; elongate seminal vesicle; and bursa copulatrix. Female genital apparatus: ovarian balls present; uterine bell elongate; uterus straight; muscular vagina divided into funnel, sphincter, and bulb. Male genital spines show a distinctive radial distribution pattern with 29 spines around bursa copulatrix opening. Female genital spines located on ventral side, smaller than those of males.

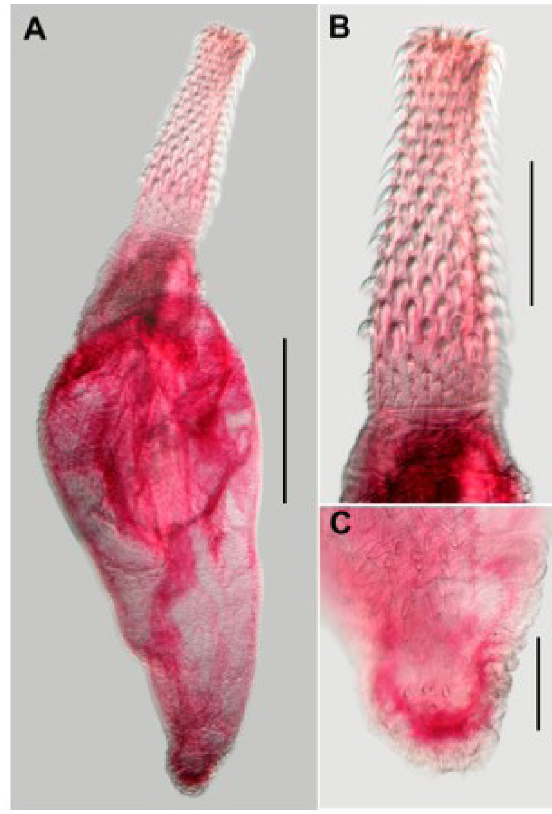

Figure 5. *Corynosoma australe* juvenile male collected from *Hyporthodus niveatus*, under DIC. (A) Entire worm in lateral view; (B) Detail of proboscis in lateral view; (C) Posterior end in lateral view. Scale bars: A= 0.40 mm, B = 0.20 mm and C = 0.10 mm.

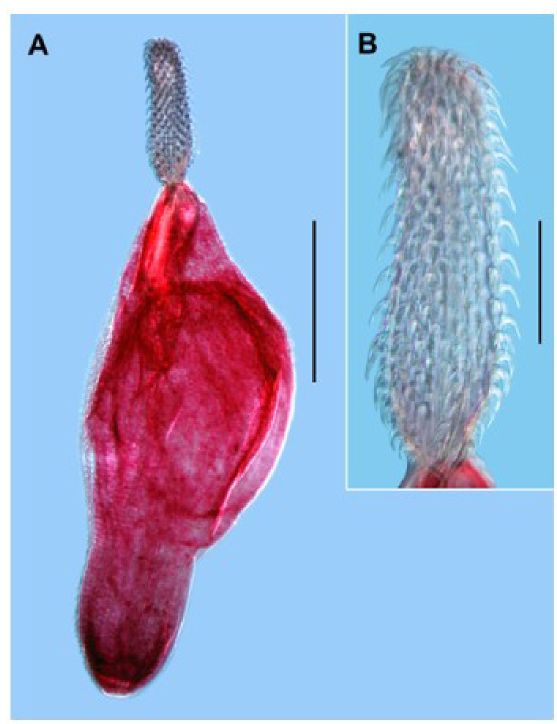

Figure 6. *Corynosoma australe* juvenile female collected from *Hyporthodus niveatus*, under DIC. (A) Entire worm in lateral view; (B) Detail of proboscis in lateral view. Scale bars: A= 1.0 mm and B = 0.25 mm.

4 Discussion

The nematode specimens found in the present study were identified as third-stage larvae of species of the family Anisakidae, *Contracecum* sp. and *Terranova* sp., and of the family Raphidascarididae, *H. deardorffoverestreetorum* and *Raphidascaris* sp. They were in accordance with morphological and morphometric species redescriptions (Felizardo et al., 2009; Knoff et al., 2012) of specimens collected from other fish species in the same region of the Brazilian coast.

Various species of fish of the Brazilian coast have been reported parasitized by *Contracecum* sp., *Terranova* sp., *H. deardorffoverestreetorum* and *Raphidascaris* sp. larvae, mainly collected from off the coast of the state of Rio de Janeiro (Eiras et al., 2016; Fonseca et al., 2016; Diniz et al., 2022; Leite et al., 2022; Miguel et al., 2022). The only nematode species previously reported parasitizing *H. nivetaus* was *Philometra* sp. (Dracunculoidea, Philometridae), from off the coast of Florida, USA (Moravec et al., 2020). Therefore, the present report represents the first record of larvae of anisakid and raphidascaridid nematodes parasitizing *H. niveatus*.

The acanthocephalan specimens found in the present study were identified as juveniles of *C. australe*, Polymorphidae. The material was in accordance with morphological and morphometric redescriptions of the species (Pereira & Neves, 1993; Knoff et al., 2001; Sardella et al. 2005; Fonseca et al., 2019) collected from Brazilian and Argentinean marine fish hosts.

Some species of fish from Brazilian coast have been reported parasitized by *C. australe*, mainly collected from off the coast of the state of Rio de Janeiro (Eiras et al., 2016; Fonseca et al., 2019). However, no acanthocephalans have been reported parasitizing *H. niveatus*, and so this is the first record of an acanthocephalan parasitizing this species of serranid fish.

The zoonotic potential, hygienic-sanitary significance and collective health importance of the third-instar larvae of the anisakid and raphidascaridid nematodes of the present study must be emphasized. Although these larvae were found in the abdominal cavity, stomach and intestine of host fish, the risk of ingesting these parasites can not be ruled out since the larvae were found alive and, thus, with the capacity for post-mortem migration to musculature, leading to the possibility of human infection, as emphasized by Fonseca et al. (2019). The highest prevalence recorded in the present study was for *H. deardorffoverstreetorum* and the lowest for *Contracaecum* sp. Larvae of *Contracaecum* spp. are known to have zoonotic potential and can even confer risk to the consumer when at low prevalences, since the ingestion of a single larva, living or dead, can trigger allergic reactions and/or disorders in the involved organs (Audicana & Kennedy, 2008). Larvae of the genus *Hysterothylacium* should be given consideration as they have been reported to cause gastrointestinal discomfort in humans (Yagi et al., 1996). In addition, the allergenic potential of *H. deardorffoverestreetorum* and *C. multipapillatum*, collected from other Brazilian marine fish hosts, was demonstrated using a murine model (Ribeiro et al., 2017; Fontenelle et al., 2018). Preventive measures can reduce risks to consumer health, such as the evisceration of fish immediately after capture to reduce the risk of migration by anisakid and raphidascaridid larvae to host musculature, as has been suggested (Diniz et al., 2022; Leite et al., 2022; Miguel et al., 2022).

Parasitization of *H. niveatus* by the acanthocephalan species *C. australe* recorded in the present study indicates that this fish is positioned at an intermediate trophic level of the marine food web where it acts as paratenic host, as has been reported for other South American marine fish species (Eiras et al. 2016, Fonseca et al., 2019), while marine mammals and birds are final hosts (Hernández-Orts et al., 2017).

Corynosoma australe was found alive in the present study, which reinforces the importance of hygienic-sanitary practices because some species of these genera are involved with zoonoses. This fact was reported by Fujita et al. (2016), who also commented that such infections are closely associated with eating uncooked food and are mostly reported from Japan because of the traditional food culture there.

5 Conclusions

The presence of live third-stage larvae of anisakid and raphidascaridid nematodes in *H. niveatus* is an important indication for fish hygiene due to their zoonotic importance. Even though these nematodes have not been found in the musculature of fish, the edible part, they accomplish post-mortem migration from the viscera to the musculature, and can be accidentally ingested and infect humans. Furthermore, these nematode larvae are worrisome because of the potential risk of allergic reactions and anisakidosis for humans.

The specimens of the polymorphid acanthocephalan *Corynosoma australe* of the present study were not found in the musculature, but they can migrate to it via inadequate fish cleaning, with the rupture of intestine and stomach walls, and remain available for ingestion, potentially infecting consumers. As suggested by FAO/WHO (Food and Agriculture Organization of the United Nations, 2014) and Fujita et al. (2016), treatments of heating or freezing are desired for the prevention of parasite infections by these species, as is the usual case for other food-borne parasites. As suggested by Fonseca et al. (2019), further studies should be conducted to evaluate the zoonotic potential of this polymorphid species.

Hazard Analysis and a Critical Control Points Plan should be applied at all points of the production chain in order to eliminate, prevent or reduce risks, and ensure a safe and quality product, as proposed by Diniz et al. (2022), Leite et al. (2022) and Miguel et al. (2022). This reinforces the hygienic-sanitary significance of monitoring for the parasites of this study, as well as intensification of fish-based food inspections and the implementation of health education programs.

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