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Helminth larvae of hygienic-sanitary importance parasitizing *Fistularia petimba* Lacepède, 1803, collected from fish markets of the municipality of Cabo Frio, RJ, Brazil

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Abstract

Fistularia petimba is a fish species that has gained growing interest due to its appreciation for sushi and sahimi in Japanese restaurants of the coastal cities of Cabo Frio and Armação de Búzios, state of Rio de Janeiro, Brazil. The constant presence of helminths in the viscera, serosa and adjacent musculature of this fish has been the subject of important 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 32 individual fish of F. petimba purchased from fish markets in the municipality of Cabo Frio in 2019. Cestode larvae, identified as Tentacularia coryphaenae, were found parasitizing the abdominal cavity and mesentery. Nematode larvae, identified as Hysterothylacium fortalezae and H. deardorffoverstreetorum, were found parasitizing stomach, intestine, liver, spleen, liver serosa, mesentery and abdominal cavity. Hysterothylacium fortalezae had the highest parasitic indices, with a prevalence of 43.75%, mean intensity of 8.07, mean abundance of 3.53 and range of infection of 3-25 specimens per host. Considerations of the zoonotic potential and hygienic-sanitary significance of these helminths are presented to increase food safety for consumers.

Keywords: red cornetfish; *Tentacularia coryphaenae*; *Hysterothylacium fortalezae*; *H. deardorffoverstreetorum*; fish sanitary inspection.

Practical Application: Helminth larvae with potential to cause disgust and anisakidosis in humans.

1 Introduction

The fish species *Fistularia petimba* Lacepède, 1803, or red cornetfish, inhabits coastal areas over soft bottoms, usually at depths greater than 10 meters, where it is benthopelagic and feeds on small fish and shrimps. It occurs in the Western Atlantic Ocean from Massachusetts, USA, to Argentina; in the Eastern Atlantic Ocean from Galicia, Spain, to Namibia; in the Mediterranean and Indo-Pacific from the Red Sea and East Africa to Hawaii, Japan and south to Victoria, Australia. Along the Brazilian coast, the species has been recorded among rocky reefs of the North, Southeast and South regions (Froese & Pauly, 2018; Marceniuk et al., 2019; Psomadakis et al., 2019).

The maintenance of hygienic-sanitary conditions in fish markets of the neighboring municipalities of Cabo Frio and Armação de Búzios in the state of Rio de Janeiro, Brazil, has been a concern for municipal health surveillance. Indeed, helminth larvae have been found in the abdominal musculature and viscera serosa of *F. petimba* at these fish markets, which cause a repugnant appearance resulting economic losses. 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). The worldwide distribution of marine fish parasites is of great importance to population health and is responsible for economic losses in the fishing industry. For these reasons, the relevance of parasite-related studies has increased. Brazilian legislation regarding fish and their derivative products indicates that any fish with a repugnant appearance, including those with musculature possessing a massive parasite infection, whether or not it may affect the health of consumers, is considered improper for consumption (Brasil, 2017).

While helminth parasitism of the red cornetfish has been reported throughout the coasts of India, Taiwan, Vietnam, Japan and Hawaii (Myers & Kuntz, 1967; Yamaguti, 1970; Parukhin, 1971; Dyer et al., 1988; Hasegawa et al., 1991; Bray & Cribb, 2003, Arthur & Te, 2006; Amin et al., 2019; Prasad, 2021), it has yet to be reported off Brazil.

The cestode order Trypanorhyncha comprises a great diversity of helminth species that parasitize fish and marine invertebrates, especially in tropical and subtropical regions. The adult trypanorhynchs inhabit the gastrointestinal tract of elasmobranchs, while larval forms can infect several species of

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teleosts, elasmobranchs, and marine invertebrates (Campbell & Beveridge, 1994; Palm, 2004). Parasitism of Brazilian fish by trypanorhynch larvae has been reported in several studies on taxonomy, parasite ecology and hygienic-sanitary conditions (São Clemente et al., 1997; Felizardo et al., 2010; Oliveira et al., 2019; Diniz et al., 2022; Leite et al., 2022). 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 (Silva et al., 2017; Oliveira et al., 2019; Diniz et al., 2022; Leite et al., 2022). Furthermore, studies using murine models indicated an allergenic potential for some of these cestode species (Mattos et al., 2015).

Nematodes of the families Anisakidae and Raphidascarididae, order Rhabdtida, are commonly found in marine fish serving as intermediate hosts, while definitive hosts can be fish-eating aquatic mammals and 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., 2022; Leite et al., 2022). Audicana & Kennedy (2008) reported the zoonotic potential of anisakid nematodes because they can cause allergic and gastrointestinal problems. The species highlighted as causing the most cases of anisakidosis are of the genera Anisakis and Pseudoterranova, namely A. simplex (Rudolphi, 1809) and P. decipiens (Krabbe, 1878). Yagi et al. (1996) reported a case of human intestinal infection by the raphidascaridid Hysterothylacium aduncum (Rudolphi, 1802). An autochthonous case causing human anisakidosis in Brazil was reported by Cruz et al. (2010). The allergenic potentials of *H*. deardorffoverestreetorum Knoff, Felizardo, Iñiguez, Maldonado Jr, Torres, Pinto and Gomes, 2012, and C. multipapillatum (Drasche, 1882) Baylis, 1920, have been demonstrated using a murine model (Ribeiro et al., 2017; Fontenelle et al., 2018).

The present investigation aimed to: (i) identify the species of plerocercoid trypanorhynch cestode larvae and raphidascaridid nematode larvae that parasitize *F. petimba*; (ii) report the parasitic indices for these species; and (iii) reinforce the importance of hygienic-sanitary surveillance of fish when infected by parasites that can cause diseases in humans and that can cause a repugnant appearance that makes their sale impossible, generating economic losses.

2 Material and methods

Thirty-two specimens of *F. petimba* (74-166 cm total length, 830-2,500 g weight), were purchased from fish markets of the municipality of Cabo Frio, state of Rio de Janeiro, Brazil, in January and September 2018. The fish were transported in isothermal boxes with ice to the laboratory for necropsy, where they were identified according to Figueiredo & Menezes (1980). Necropsy was performed and internal organs were transferred to Petri dishes containing 0.65% NaCl solution, for musculature examination using a negatoscope. Retrieved cestode larvae were placed in Petri dishes containing distilled water and maintained in a refrigerator for at least 24 h to permit

relaxation of scolices and extroversion of tentacles. The larvae were subsequently fixed in cold AFA (alcohol, formalin and acetic acid), stained with Langeron's carmine, differentiated in 0.5% hydrochloric acid-ethanol solution, dehydrated in a series of increasing ethanol concentrations (70-100 °GL), clarified with beach-wood creosote and whole mounted/preserved in Canada balsam, between slide and cover slip. Nematode larvae were recovered in Petri dishes containing 0.65% NaCl solution and then fixed with hot (60 °C) AFA, preserved in 70 °GL ethanol plus 5% glycerin and placed between slide and coverslip to be clarified with Aman's lactophenol to permit analysis of structures (Knoff & Gomes, 2012). The taxonomic classifications used for trypanorhynch cestodes and raphidascaridid nematodes were those of Beveridge et al. (2017) and De Ley & Blaxter (2002), respectively. Species identification followed Campbell & Beveridge (1994), Beveridge & Campbell (1996) and Palm (2004) for trypanorhynch cestodes and Felizardo et al. (2009), Knoff et al. (2012) and Fontenelle et al. (2015) for raphidascaridid nematodes. Specimens were examined with an Olympus BX-41 bright-field microscope and images were obtained using a Cannon Power Shot A640 digital camera coupled to a Zeiss Axiophot microscope. Measurements were obtained from specimens and are given in millimeters as range followed by mean in parenthesis. Parasitic indices were calculated according to Bush et al. (1997). Representative specimens of each parasite species were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Fiocruz, Rio de Janeiro, RJ, Brazil.

3 Results

Fifty percent of the 32 specimens of *Fistularia petimba* were parasitized by helminth larvae. Two individual fish were parasitized by a single plerocercoid of the same trypanorhynch cestode species and 14 by 135 third-instar larval specimens of two nematode raphidascaridid species. The cestode plerocercoids were alive and some of the nematode larvae were alive and showed high motility. The specimens were taxonomically identified as below.

Platyhelminthes Minot, 1876, Rhabditophora Ehlers, 1985, Neodermata Ehlers, 1985, Cestoidea Rudolphi, 1808, Eucestoda Southwell, 1930, Trypanorhyncha Diesing, 1863, Trypanobatoida Beveridge, Haseli, Ivanov, Menoret and Schaeffner, 2017, Tentacularioidea Poche, 1926, Tentaculariidae Poche, 1926, *Tentacularia* Bosc, 1797.

3.1 Tentacularia coryphaenae Bosc, 1802. Figure 1A-1C and Figure 2A-2B

Two specimens of *T. coryphaenae* were collected from abdominal cavity and mesentery of two different individuals of *F. petimba*. The parasitic indices were: prevalence 6.25%, mean intensity 1, and mean abundance 0.06. The plerocercoids were deposited in CHIOC under the numbers 40016 and 40017.

Description of the main features are based on two whole mounted, measured, and uncompressed specimens. Scolex acraspedote, widest at level of anterior portion of pars bothrialis or velum. Pars bothrialis with elongated bothriae, occupying almost 80% of scolex, with sessile margins, 5.80-6.07 (5.94) long, 1.74-1.81 (1.77) wide. Pars vaginalis nearly 4.5 times smaller than

scolex, with sheaths straight on anterior two-thirds and sinuous close to bulbs, 1.20-1.74 (1.47) long. Pars bulbosa 1.05-1.17 (1.11) long, 0.54-0.73 (0.63) wide. Individual bulbs 1.06-1.20 (1.11) long, 0.17-0.22 (0.19) wide. Pars post-bulbosa well-developed. Velum 1.80-2.55 (2.17) long. Appendix 1.84-2.10 (1.97) long. Tegumentary structures present include porosities and filiform microtriches on scolex and hook-like microtriches along bothrial margins. Tentacles excluding hooks 0.06-0.10 (0.08) wide at basal region and 0.02-0.07 (0.05) wide at metabasal region. Basal hooks tridentiforme, 0.010-0.015 (0.012) long, implantation basal, 0.007-0.012 (0.009) wide. Basal armature of tentacles with bilateral symmetry of ascending hook rows with identical V-shaped patterns on bothrial and antibothrial surfaces and forming identical, inverted, unarmed V-shaped patterns on external and internal surfaces. Metabasal armature with rotational symmetry, arranged in quincunx. Metabasal hooks unciforme 0.012-0.032 (0.022) long, base 0.010-0.025 (0.021) wide. Hooks of same shape on both sides of tentacle.

Nematoda Potts, 1932, Cromadorea Inglis, 1983, Rhabditida Chitwood, 1933, Ascaridomorpha De Ley and Blaxter, 2002,

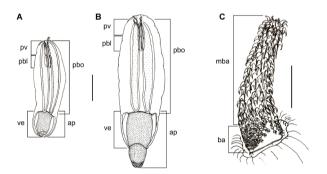


Figure 1. *Tentacularia coryphaenae* plerocercoids collected from *Fistularia petimba*. A-B. Entire worms, with retracted and extroverted appendix, respectively. C. Detail of external surface of extroverted tentacle: basal armature (ba), metabasal armature (mba). Pars bothrialis (pbo), pars vaginalis (pv), pars bulbosa (pbl), velum (ve), appendix (ap). Scale bars: A and B = 1 mm, C = 0.1 mm.

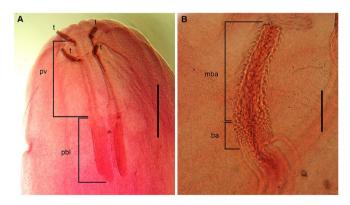


Figure 2. *Tentacularia coryphaenae* plerocercoid collected from *Fistularia petimba*. A. Detail of anterior region of scolex showing tentacular apparatus. B. Detail of external surface of extroverted tentacle: basal armature (ba), metabasal armature (mba). Tentacles (t), pars vaginalis (pv), pars bulbosa (pbl). Scale bars: A = 1 mm, B = 0.1 mm.

Ascaridoidea Baird, 1853, Raphidascarididae Hartwich, 1954, *Hysterothylacium* Ward and Magath, 1917.

3.2 Hysterothylacium fortalezae (Klein, 1973). Figure 3A-3C

One-hundred and thirteen third-instar (L_3) larvae were collected from stomach, intestine, mesentery and stomach serosa of 14 individuals of *F. petimba*, The parasitic indices were: prevalence 43.75%, mean intensity 8.07, mean abundance 3.53 and range of infection of 3-25 parasites per host. Voucher specimens were deposited in CHIOC under the numbers 38768; 38769; 38770.

Description of the main features are based on seven measured third-instar larvae specimens. Body with smooth cuticular covering without evident cuticular transverse striations but with slightly prominent lateral line along body, but not conspicuous at anterior end, 5.00-8.60 (6.13) long, 0.14-0.30 (0.20) wide. Mouth triangular with one dorsal lip with two sets of papillae, and two lateroventral lips each with a set of papillae. Nerve ring 0.10-0.40 (0.20) from anterior end. Excretory pore adjacent to nerve ring located in first third of esophagus. Esophagus 0.62-1.20 (0.83) long, 0.04-0.10 (0.07) wide. Ventriculus slightly spherical, 0.05-0.08 (0.06) long, 0.05-0.09 (0.07) wide. Ventricular appendix present, 0.45-0.91 (0.67) long. Intestinal cecum present, 0.13-0.31 (0.23) long. Four oval rectal glands present, anus provided with a projection. Tail conical, curved ventrally, 0.11-0.20 (0.15) long, provided with tuft of 6-8 spinous structures.

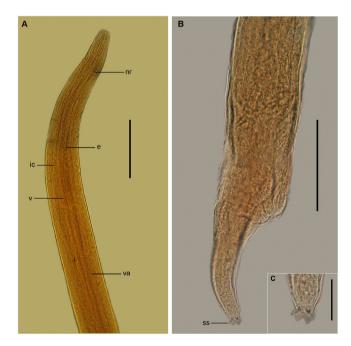


Figure 3. *Hysterothylacium fortalezae* third-instar larvae collected from *Fistularia petimba*. A. Anterior portion. B. Posterior portion. C. Detail of tuft of spinous structures. Nerve ring (nr), esophagus (e), intestine (i), intestinal cecum (ic), ventriculus (v), ventricular appendix (va), tuft of spinous structures (SS). Scale bars: A = 0.2 mm, B = 0.1 mm, C = 0.025 mm.

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

Twenty-two third-instar (L_3) larvae collected from stomach, intestine and stomach serosa of five individuals of *F. petimba*. The parasitic indices were: prevalence 15.63%, mean intensity 4.40, mean abundance 0.69 and range of infection 2-8 parasites per host. Voucher specimens were deposited in the CHIOC under the number 38767.

Description of the main features are based on six measured L_3 specimens. Body with cuticle and lateral wing extending along body, 6.50-8.70 (7.70) long, 0.16-0.28 (0.23) wide. Anterior extremity trilabial with one dorsal lip and two poorly-developed ventrolateral lips. Nine cephalic papillae, two pairs on dorsal lip together with a large papilla, and one pair on each ventro-lateral lip. Boring tooth present. Esophagus, 0.65-1.10 (0.88) long, 0.06-0.12 (0.08) wide. Excretory pore opening below nerve ring, sometimes inconspicuous. Nerve ring 0.27-0.38 (0.33) from anterior end. Ventriculus nearly spherical, 0.05-0.10 (0.06) long, 0.07-0.12 (0.08) wide. Ventricular appendix 0.36-0.64 (0.53) long. Intestinal cecum present, 0.10-0.20 (0.15) long. Four subspherical rectal glands present. Tail conical, 0.18-0.20 (0.19) long, mucron present.

4 Discussion

The morphology of the trypanorhynch cestode specimens collected here are in accordance with the description of Dollfus (1942) and redescriptions of Beveridge & Campbell (1996), Knoff et al. (2004) and Palm (2004) for the species *T. coryphaenae*.

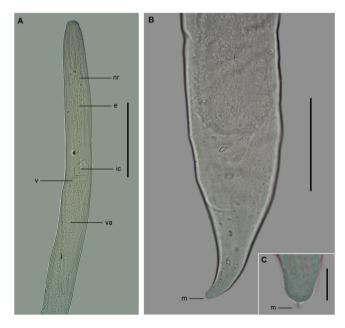


Figure 4. *Hysterothylacium deardorffoverstreetorum* third-instar larvae collected from *Fistularia petimba*. A. Anterior portion. B Posterior portion. C. Detail of mucron. Nerve ring (nr), esophagus (e), intestinal cecum (ic), intestine (i), ventriculus (v), ventricular appendix (va), mucron (m). Scale bars: A = 0.20 mm, B = 0.10 mm, C = 0.025 mm.

The plerocercoids of this cestode species have been reported worldwide parasitizing more than 60 marine teleost fish species and the adults in 11 host shark species (Palm et al., 2007). Tentacularia coryphaenae has been reported parasitizing several marine teleost and elasmobranch hosts in Brazil (Rego, 1977; Knoff et al., 2002, 2004; Silva et al., 2017). The species has been reported parasitizing some species of teleost fish in the state of Rio de Janeiro, and the presence of these worms in the meat and/ or viscera of fish immediately causes repugnance to merchants and consumers. Amato et al. (1990) reported plerocercoids of T. coryphaena parasitizing abdominal and body musculature of *Katsuwonus pelamis* L., 1758 with high parasitic indices of 92.9% prevalence and 36.3 mean intensity. Silva & São Clemente (2001) examined fillets of Coryphaena hippurus L., 1758, belonging to two fish size class, and found low indices of prevalence (0.47-6.25%) and intensity/mean intensity of infection (1-2.29). São Clemente et al. (2004) studied parasitism of Genypterus brasiliensis Regan, 1903, and reported T. coryphaenae with 1.35% prevalence and an intensity of 1 in the mesentery. Alves & Luque (2006) reported the species in the musculature of K. pelamis with high parasitic indices of 66.7% prevalence and 5.8 mean intensity. São Clemente et al. (2007) reported plerocercoids of T. corvphaenae in the musculature of Lophius gastrophysus Miranda-Ribeiro, 1915, with 2.3% prevalence and 1 of intensity. Lastly, Dias et al. (2011) reported this larvae in the mesentery of Scomberomorus cavala (Cuvier, 1829), with 3% prevalence and 2 of intensity. The present study represents the first report of T. coryphaenae in F. petimba in the world. Comparing the data of the present study with those of previous studies of teleost species of commercial importance done along the coast of the state of Rio de Janeiro reveals that the F. petimba specimens of the present study have low indices of parasitism and that these parasites were found in the abdominal cavity and mesentery, parts of fish which are not typical components of fish-based dishes for human consumption.

Recently, Silva et al. (2017) reported seven individuals of *C. hippurus*, acquired from sport fishing tournaments in the state of Rio de Janeiro, with massive infections of *T. coryphaenae* in the abdominal musculature (mean of 34 specimens per fish) and severe hemorrhage as a result. Even though the present study only collected a small number of *T. coryphaenae*, in terms of hygienic or sanitary considerations the simple presence of this worm in fish visible to the naked eye can cause disgust to the consumer and make fish marketing unfeasible, as reported by São Clemente et al. (2004), Dias et al. (2011) and Silva et al. (2017).

The importance of trypanorhynch cestodes, such as *T. coryphaenae* examined in this work, comes from their repugnancy to fish consumers. In general, reports indicate that the presence of these parasites, especially when in large numbers, often requires fish to be discharged, either in processing facilities or during inspection procedures, resulting economic losses (Dollfus, 1942; Campbell & Beveridge, 1994; Palm, 2004; Silva et al., 2017). According to Dollfus (1942), trypanorhynch cysts are not transmissible to homeothermic vertebrates and the re-encapsulation of post-larvae does not occur in this group of hosts. However, studies have demonstrated that extracts from some trypanorhynch species can cause immune responses in mice, indicating the possibility of allergic reactions in humans

(Rodero & Cuéllar, 1999; Vázquez-López et al., 2001, 2002; Gómez-Morales et al., 2008; Mattos et al., 2015).

One of the nematode species found in the present study was identified as H. fortalezae, the specimens of which are in accordance with the description of third-stage larvae (L₂) specimens made by Fontenelle et al. (2015). The specimens found here were also compared with the H. fortalezae L, specimens deposited by Cordeiro & Luque (2004) and by Fontenelle et al. (2015) in CHIOC, to assist in species identification. Reports of adults and larvae of *H. fortalezae* from along the Brazilian coast list several fish hosts, such as *Harengula clupeola* (Cuvier, 1829), Scomberomorus brasiliensis Trichiurus lepturus L., 1758 and Selene setapinnis (Mitchill, 1815) (Fontenelle et al., 2015), but with lower prevalence than for the larvae found in the present study (43.75%). The infection sites found here were stomach, intestine and stomach serosa. Hysterothylacium fortalezae larvae have been reported parasitizing other hosts in the North Atlantic Ocean, such as the intestine, mesentery, stomach and rectum of Symphurus plagiusa (L., 1766) off the coast of Campeche, Mexico (Santana-Piñeros et al., 2012). Therefore, the present finding represents the first report of this species parasitizing *F*. petimba in the world.

The other nematode species found in the present study, identified as *H. deardorffoverstreetorum*, was in accordance with the morphological and morphometric species description of Knoff et al. (2012) for larvae parasitizing *P. isosceles* from the same region of the Brazilian coast.

Various species of fish from Brazilian coast, other than *F. petimba*, have been reported parasitized by *H. deardorffoverstreetorum* larvae, mainly collected from off the coast of the state of Rio de Janeiro (Diniz et al., 2022). The present report represents the first record of larvae of this nematode parasitizing *F. petimba*.

Regarding the zoonotic potential and hygienic-sanitary significance of the helminths found in the present study, the plerocercoids of the trypanorhynch cestode T. coryphaenae were visible to the naked eye. These plerocercoids have sometimes have been found in musculature of other fish species from the Brazilian coast, and give the consumer a repugnant aspect (Silva et al., 2017). In a study with a murine model, Mattos et al. (2015) warned about ingesting fish infected with larvae of trypanorhynch species as they may cause an allergic reaction in humans. Furthermore, the hygienic-sanitary significance and collective health importance of the third-instar larvae of the raphidascaridid nematodes of the present study must be emphasized. Although, these larvae were found in the intestine, stomach, mesentery, stomach serosa and abdominal cavity of the host, their ingestion can not be ruled out since most larvae were found alive and, thus, with the capacity for post-mortem migration to musculature, leading to the possibility of human infection. Additionally, *Hysterothylacium* spp. larvae should be given consideration because those of one species have been reported to cause gastrointestinal discomfort in humans (Yagi et al., 1996), in addition to the allergenic potential recently demonstrated using a murine model for H. deardorffoverestreetorum collected from other Brazilian marine fish hosts (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 raphidascaridid larvae to host musculature, as suggested by Diniz et al. (2022).

5 Conclusion

The presence of cestode plerocercoids and third-instar nematode larvae is worrisome because of the potential risk of allergic reactions and anisakidosis for humans, thereby reinforcing the hygienic-sanitary significance of monitoring for 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. (2022).

The occurrence of trypanorhynch cestode and raphidascaridiid nematode larvae parasitizing *F. petimba* off the coast of the state of Rio de Janeiro found here suggests an intermediate level in the marine trophic web for this host.

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