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Anisakidae and Raphidascarididae nematode larvae of hygienic-sanitary importance parasitizing *Lobotes surinamensis* (Actinopterygii) obtained from fish markets in the State of Rio de Janeiro, Brazil

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Abstract

Lobotes surinamensis is an important fishery resource, occurring in tropical and subtropical marine regions throughout the world. Considering the hygienic-sanitary and zoonotic importance of Anisakidae and Raphidascarididae nematode larvae, 50 specimens of *L. surinamensis* were acquired from fish markets in the State of Rio de Janeiro from May 2023 to December 2024. The fish were analyzed, and 40 nematode larvae belonging to three species of the Anisakidae (2) and Raphidascarididae (1) families were found, parasitizing the abdominal cavity, mesentery, and intestine. The species identified were *Contracaecum* sp., *Terranova* sp., and *Hysterothylacium* sp., with prevalence of 26, 14, and 12%; mean intensity of 1.53, 1.28, and 1.83; and mean abundance of 0.40, 0.18, and 0.22, respectively. The taxonomic identification of these parasites was based on their morphological and morphometric characters, determined using bright-field and scanning electron microscopies. This is the first report of these nematode larvae parasitizing specimens of *L. surinamensis* and is an important indicator for establishing fish hygiene control. In addition to repulsiveness, the presence of these larvae is of concern due to the risk of allergic reactions, thus intensifying the hygienic-sanitary importance of monitoring these parasites to guarantee safe food for the consumer.

Keywords: *Lobotes surinamensis*; Anisakidae; Raphidascarididae; zoonotic potential; fish sanitary inspection.

Practical Application: Nematode larvae with the potential to cause anisakidosis in humans.

1 INTRODUCTION

Lobotes surinamensis (Bloch, 1790) has a wide geographic distribution, inhabiting tropical and subtropical marine regions throughout the world. Adults inhabit bays, estuaries, and the lower courses of large rivers (Froese & Pauly, 2025; Menezes & Figueiredo, 1980). In Brazil, its incidence and consumption have increased over the years, becoming an important fishing resource associated with artisanal fishing, being consumed by riverside communities and the population residing along the Brazilian coast. Due to its increasing incidence, the excess production started being sold in large fish markets, making it more appreciated by humans (Santos, 2023; Sazima et al., 2009; E. R. Silva et al., 2017).

The global distribution of marine fish parasites is of great relevance to the health of the populations and is responsible for economic losses by the fishing industry (Miguel et al., 2022). Brazilian legislation establishes that fish become unfit for consumption when there is massive muscular infestation by parasites. In addition, fishery and aquaculture products infected with endoparasites transmissible to humans cannot be used for raw consumption without first being subjected to freezing (Brasil, 2020).

Nematodes of the Anisakidae and Raphidascarididae families are commonly found in marine fish, which act as intermediate hosts, while the definitive hosts are normally aquatic mammals and piscivorous birds. Humans are accidental hosts resulting from the consumption of raw or undercooked infected fish (Anderson, 2000). Anisakid and raphidascaridid larvae have been reported parasitizing several marine fish species in Brazil, with emphasis on the hygienic-sanitary aspects (Diniz et al., 2022; Leite et al., 2021; Menezes et al., 2023; Miguel et al., 2022; A. M. Silva et al., 2017).

Audicana and Kennedy (2008) published information concerning the zoonotic potential of anisakid nematodes. Human infections by these nematodes have been reported in Europe, the Americas, and Korea, but mostly in Japan (Adams et al., 1997; Audicana et al., 2002; Chai et al., 2005; D'Amico et al., 2014). The first clinical case of anisakid nematodes in Brazil was reported by Cruz et al. (2010), where the presence of an Anisakis-like larva was observed in the stomach of an adult man.

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Considering the zoonotic and hygienic-sanitary aspects, the objective of the present study was to report the presence of third-stage anisakid and raphidascarid nematode larvae parasitizing *L. surinamensis*, acquired from municipal markets in the State of Rio de Janeiro. The larvae were detected by morphological and morphometric analyses using optical and scanning electron microscopies, calculating the parasitic indices and reporting the infection sites, in addition to highlighting the hygienic-sanitary importance for collective human health and providing baseline data for future investigations.

1.1 Relevance of the work

The presence of third-stage larvae of anisakid and raphidascarid nematodes is relevant to food hygiene, due to the potential risk of allergic reactions and gastrointestinal problems. The presence of these nematode larvae is worrisome because of the potential risk of anisakidosis, thus reinforcing the importance of monitoring these parasites. Therefore, it is necessary to intensify inspections of fish-based foods and implement health education programs in order to guarantee safe food for the consumer.

2 MATERIALS AND METHODS

A total of 50 *L. surinamensis* specimens, 40–65 (52.14 ± 6.47) cm in total length and 1–7 (3.40 ± 1.46) kg, caught by professional fishermen, were purchased from fish markets in the municipality of Rio de Janeiro, State of Rio de Janeiro (RJ), Brazil, between May 2023 and December 2024. The fish were transported on ice to the Laboratório de Inspeção e Tecnologia de Pescados of the Faculdade de Medicina Veterinária of the Universidade Federal Fluminense, Niterói, RJ, Brazil, where they were necropsied and the internal organs and muscles examined. The fish were identified according to Menezes and Figueiredo (1980).

The nematode larvae were separated, placed in Petri dishes containing a 0.65% NaCl solution, fixed using hot AFA (60°C), preserved in 70° GL ethanol plus 5% glycerin, and then placed between a slide and coverslip, where they were clarified with Amann lactophenol to allow for the structural analysis (Knoff & Gomes, 2012). The taxonomic classifications of the anisakid and raphidascaridid nematodes were carried out according to De Ley and Blaxter (2002), and the species identifications according to Felizardo et al. (2009), Knoff et al. (2012), and Diniz et al. (2022).

The measurements were obtained by bright-field microscopy using an Olympus BX-41 microscope. The samples were analyzed by bright-field microscopy using a Zeiss Axiophot microscope equipped with a Nomarski differential interference contrast apparatus (DIC), and the images were obtained using a digital Canon camera (Power Shot A640) in the Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz (IOC), Fiocruz, RJ.

Some specimens were prepared for scanning electron microscopy (SEM) according to Lopes Torres et al. (2013). The samples preserved in ethanol were dehydrated in an ethanol series (70–100° GL), CO_2 critical point dried, coated in gold, and then examined and photographed using a SEM (Jeol JSM-6390LV) with an acceleration voltage of 10 kV. The measurements were reported in millimeters (mm), with the averages and pattern deviations in parentheses, unless otherwise indicated. The parasitic indices of prevalence, mean intensity, and mean abundance were calculated according to Bush et al. (1997). Representative specimens of each parasitic species were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz, Fiocruz (CHIOC), Rio de Janeiro, RJ, Brazil.

3 RESULTS

Of the 50 fish examined, 19 (38%) were infected, with a total of 40 third-instar nematode larvae parasitizing the abdominal cavity, mesentery, and intestine, belonging to three species of the Anisakidae (2) and Raphidascarididae (1) families. Most of the nematode larvae were alive and presented moderate motility. The taxonomic identification of each species found, and the description of their main morphological characteristics, are presented below. The morphometric data of the nematode larvae found are indicated in Table 1.

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

Table 1. Data of third-instar nematode larvae parasitizing *Lobotes surinamensis* collected from the State of Rio de Janeiro, Brazil.

	***Contracaecum* sp.**	***Terranova* sp.**	***Hysterothylacium* sp.**
Body (L)	4.9–8.0 (6.1 ± 0.89)	4.2–6.6 (4.8 ± 0,87)	3.8–7.3 (5.9 ± 1.28)
Body (W)	0.15–0.30 (0.22 ± 0.03)	0.20–0.24 (0.21 ± 0.01)	0.11–0.25 (0.20 ± 0.05)
Larval tooth	Present	Present	Absent
Excretory pore*	Opens beneath boring tooth	Opens beneath boring tooth	0.34–0.39 (0.36 ± 0.01)
Nerve ring*	0.10–0.22 (0.13 ± 0.03)	0.14–0.16 (0.15 ± 0.005)	0.15–0.40 (0.30 ± 0.09)
Esophagus (L)	0.55–0.85 (0.72 ± 0.07)	0.72–0.95 (0.84 ± 0.06)	0.51–1.0 (0.71 ± 0.17)
Esophagus (W)	0.01–0.09 (0.06 ± 0.01)	0.28–0.38 (0,33 ± 0.04)	0.06–0.15 (0.10 ± 0.03)
Ventriculus (L)	0.05–0.09 (0.05 ± 0.01)	0.17–0.22 (0.19 ± 0.01)	0.05–0.10 (0.08 ± 0.02)
Vap (L)	0.40–0.47 (0.44 ± 0.01)	Absent	0.45–0.55 (0.49 ± 0.03)
Intestinal cecum (L)	0.25–0.46 (0.34 ± 0.05)	0.35–0.50 (0.43 ± 0.05)	0.10–0.29 (0.21 ± 0.07)
Tail (L)	0.11–0.17 (0.13 ± 0.01)	0.18–0.24 (0.20 ± 0.02)	0.15–0.25 (0.20 ± 0.03)
Mucron (L)	Absent	Absent	0.01–0.06 (0.04 ± 0.01)

Measurements are in millimeters; means and standard deviations in parentheses. *Distance from anterior end; Vap: ventricular appendix; L = Length; W = Width.

Ascaridoidea Baird, 1853; and Anisakidae Railliet and Henry, 1912; *Contracaecum* Railliet & Henry, 1912; *Contracaecum* sp. Figures 1 and 2.

The main characteristics observed were based on 20 specimens (18 whole mounts and two by SEM): body—elongated and thin; cuticle with thin transverse striations, more evident at the posterior end of the body; anterior end with one dorsal lip and two poorly developed ventrolateral lips; six cephalic papillae are present, one pair on the dorsal lip and one pair on each ventrolateral lip; boring tooth near the oral aperture, between the ventrolateral lips; excretory pore opening beneath the boring tooth; the ventriculus is small and sub-spherical; the ventricular appendix is nearly twice the length of the intestinal caecum; four sub-spherical rectal glands are present; tail is conical and mucron absent.

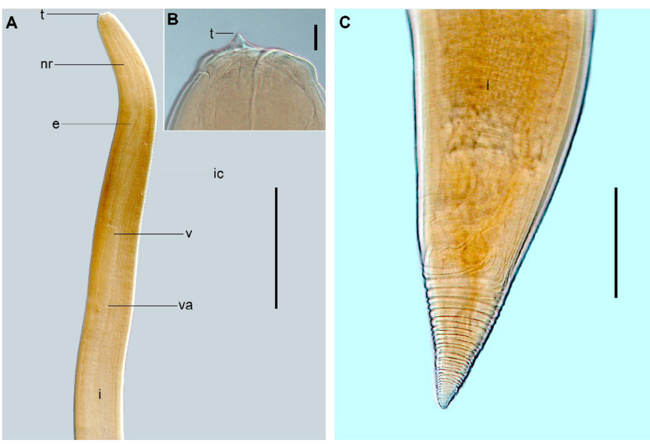

Figure 1. *Contracaecum* sp. third-instar larva obtained from *Lobotes surinamensis*. (**A**) Anterior portion; **(B)** Detail of larval tooth; **(C)** Posterior portion. Never ring (nr); larval tooth (t); esophagus (e); intestine (i); intestinal cecum (ic); ventriculus (v); ventricular appendix (va). Scale bars: A = 0.5 mm, B = 0.05 mm, and C = 0.1 mm.

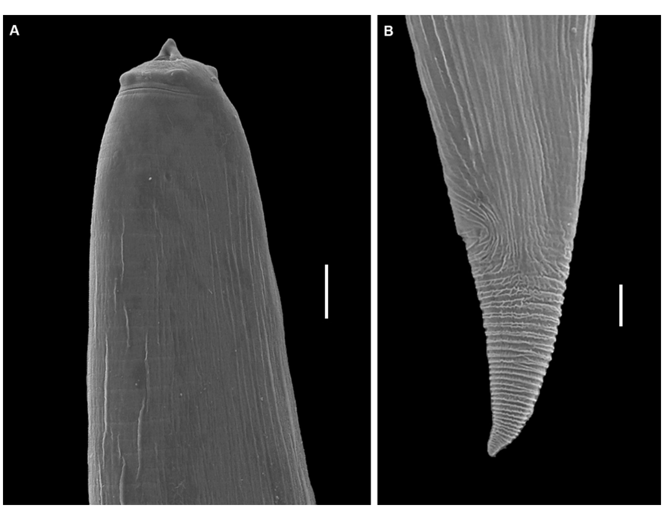

Figure 2. *Contracaecum* sp. third-instar larva obtained from *Lobotes surinamensis* by scanning electron microscopy. **(A)** Anterior portion, dorso-lateral view; **(B)** Posterior portion, lateral view. Scale bars: A and B = 20 µm.

The parasitic index of prevalence was 26%, mean intensity 1.53, and mean abundance 0.40. The voucher specimens were deposited in the CHIOC with the numbers 39697 and 39698.

Terranova Leiper & Atkinson, 1914, *Terranova* sp. Figures 3 and 4.

The main characteristics observed were based on nine specimens (seven whole mounts and two by SEM): body—elongated and thin; cuticle with thin transverse striations, more evident at the posterior end of the body; anterior end with one dorsal lip and two poorly developed ventrolateral lips; six cephalic papillae are present, one pair on the dorsal lip and one pair on each ventrolateral lip; boring tooth below the oral aperture, between the ventrolateral lips; excretory pore opening beneath the boring tooth; The ventriculus longer than wide; the ventricular appendix is absent; the intestinal caecum is twice the length of the ventriculus; four sub-spherical rectal glands are present; tail is conical and mucron absent.

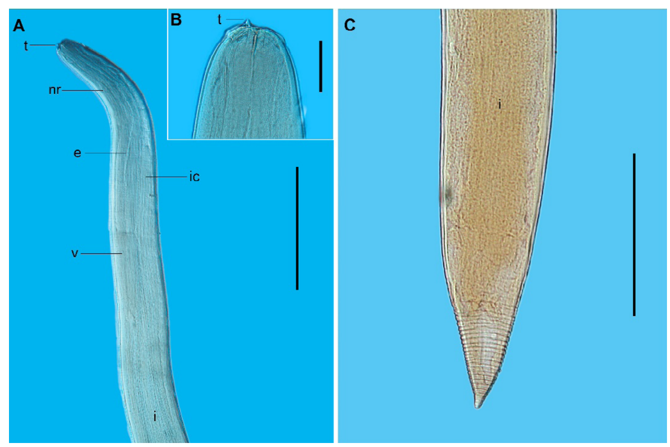

Figure 3. *Terranova* sp. third-instar larva obtained from *Lobotes surinamensis*. **(A)** Anterior portion; **(B)** Detail of larval tooth; **(C)** Posterior portion. Never ring (nr); larval tooth (t); esophagus (e); intestine (i); intestinal cecum (ic); ventriculus (v). Scale bars: A = 0.5 mm, B = 0.05 mm, and C = 0.2 mm.

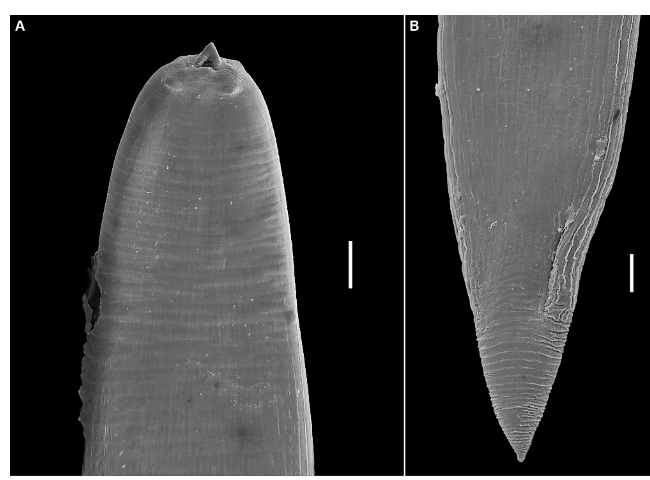

Figure 4. *Terranova* sp. third-instar larva obtained from *Lobotes surinamensis* by scanning electron microscopy. **(A)** Anterior portion, dorsal view; **(B)** posterior portion, lateral view. Scale: A and B = 20 µm.

The parasitic index of prevalence was 14%, mean intensity 1.28, and mean abundance 0.18. The voucher specimens were deposited in the CHIOC with the numbers 39699 and 39700.

Raphidascarididae Hartwich, 1954, *Hysterothylacium* Ward & Magath, 1917. *Hysterothylacium* sp. Figures 5 and 6.

The main characteristics observed were based on 11 specimens (eight whole mounts and three by SEM): body—elongated and thin; cuticle with lateral alae extending along the body with wedge-shaped support, devoid of basal extension; anterior end with one dorsal and two poorly developed ventrolateral lips; nine cephalic papillae were present, two pairs on the dorsal lip together with a large papilla and one pair on each ventrolateral lips; boring tooth is absent; excretory pore opening is beneath the nerve ring; the ventriculus is sub-spherical; The ventricular appendix is longer than the esophagus; intestinal cecum is present; four to six sub--spherical rectal glands are present; tail is conical with a mucron.

The parasitic index of prevalence was 12%, mean intensity 1.83, and mean abundance 0.22. The voucher specimen was deposited in the CHIOC with the number 39801.

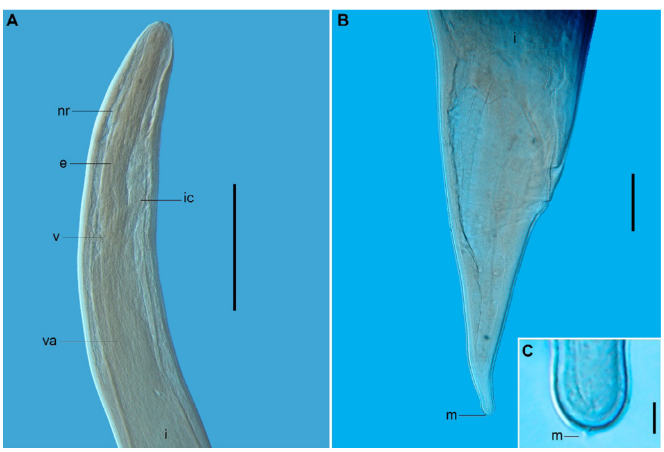

Figure 5. *Hysterothylacium* sp. third-instar larva obtained from *Lobotes surinamensis*. **(A)** Anterior portion; **(B)** Posterior portion; **(C)** Detail of mucron. Never ring (nr); esophagus (e); intestine (i); intestinal cecum (ic); ventriculus (v); ventricular appendix (va); mucron (m). Scale bars: A = 0.5 mm, B = 0.1 mm, and C = 0.025 mm.

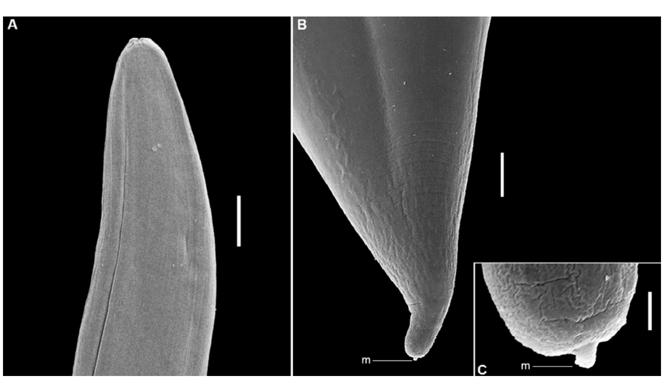

Figure 6. *Hysterothylacium* sp. third-instar larva obtained from *Lobotes surinamensis* by scanning electron microscopy. **(A)** Anterior portion, dorso-lateral view; **(B)** Posterior portion, dorso-lateral view, showing mucron (m); **(C)** Detail of mucron (m). Scale bars: A = 60 µm, B = 10 µm and C = 1.5 µm.

4 DISCUSSION

In the present study, the third-instar nematode larvae specimens were identified as *Contracaecum* sp. and *Terranova* sp. (Anisakidae), and *Hysterothylacium* sp. (Raphidascarididae), which are in agreement with the morphological and morphometric descriptions of third-instar larvae found parasitizing other marine fish obtained on the Brazilian coast (Diniz et al., 2022; Felizardo et al., 2009; Fonseca et al., 2016; Knoff et al., 2012; Menezes et al., 2023). The larvae of *Hysterothylacium* sp. recorded in this study are similar to the larvae of *Hysterothylacium deardorffoverstreetorum* reported by Knoff et al. (2012) on the coast of the State of Rio de Janeiro.

Other groups of parasites obtained from the same hosts were reported as the digenetic trematode metacercary *Cardiocephaloides medioconiger* in South Carolina, USA (Buron et al., 2023), the copepod *Acusicola iamarinoi* in the State of Pará (Couto et al., 2023), and the trypanorhynch cestodes *Callitetrarhynchus gracilis* and *C. speciosus* from the coast of the State of Rio de Janeiro (Diniz et al., 2025). The nematode species previously reported parasitizing *L. surinamensis* were adults of *Philometra lobotidis* (Philometridae) and *Ichthyascaris* sp. (Raphidascarididae), both collected from the coast of Southwest Java, Indonesia (Moravec et al., 2012, 2022). Therefore, the present study represents the first report of nematode larvae of the genera *Terranova*, *Contracaecum,* and *Hysterothylacium* parasitizing *L. surinamensis*.

Although the anisakid and raphidascaridid larvae of the present study were only found in the abdominal cavity, mesentery, and intestine of the host fish, their presence in the serosa of the organs should not be underestimated, since the larvae were alive and are able to migrate post mortem to the musculature, leading to the possibility of human infection, as suggested by Dias et al. (2010). In addition, the presence of these larvae gives the fish a repugnant appearance, and their commercialization may be prevented after a health inspection, generating economic losses.

The main nematodes causing disease in humans belong to the genera *Anisakis*, *Pseudoterranova,* and *Contracaecum* (Acha & Szyfres, 2003; Adams et al., 1997). However, in addition to these helminths, Audicana and Kennedy (2008) suggested that other anisakid nematodes be studied to elucidate their zoonotic aspects.

Due to the increase in its incidence, the excess production of *L. surinamensis* has been sold at large fish markets, and it has therefore become more appreciated for human consumption (Sazima et al., 2009; E. R. Silva et al., 2017), which could increase the risk of accidental exposure to fish parasites.

The highest prevalence recorded in the present study was for *Contracaecum* sp., followed by *Terranova* sp., and the lowest for *Hysterothylacium* sp. However, even the *Hysterothylacium* larvae should be considered, since there are reports that they can cause gastrointestinal problems in humans (Yagi et al., 1996). The allergenic potential of *H. deardorffoverstreetorum* obtained from other Brazilian marine fish hosts was recently demonstrated using a murine model (Ribeiro et al., 2017). Fontenelle et al. (2018) demonstrated that larval antigens of *C. multipapillatum* are potentially capable of inducing allergic sensitization in mice, which may have direct implications for the possible induction of allergies in workers involved in the preparation and handling of fish for consumption.

5 CONCLUSIONS

The presence of live third-stage larvae of anisakid and raphidascarid nematodes in *L. surinamensis* is of concern, due to the potential risk of allergic reactions and gastrointestinal problems, thus reinforcing the importance of monitoring these parasites. Preventive measures can reduce risks to consumer health, such as eviscerating fish immediately after capture to reduce the risk of the migration of anisakid and raphidascarid larvae to the host muscles, as suggested by Diniz et al. (2022), thus ensuring safe food for the consumer.

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