# NEMATODE AND CESTODE LARVAE OF HYGIENIC-SANITARY IMPORTANCE IN *Lopholatilus villarii* (ACTINOPTERYGII) IN THE STATE OF RIO DE JANEIRO, BRAZIL

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**ABSTRACT**

The tile fish, *Lopholatilus villarii*, occurs from Brazil to Argentina where it is commercially important it because possesses highly appreciated meat. The aim of this study was to identify the larvae of anisakid and raphidascaridid nematodes and trypanorhynch cestodes in *L. villarii* purchased in fish markets of the municipality of Niterói, state of Rio de Janeiro, Brazil, and calculate their parasitological indices and present their sites of infection. From August 2015 to September 2016, 31 specimens of *L. villarii* were investigated. The fish were necropsied and their viscera and musculature analyzed. The helminths found were processed according to standard helminthological techniques for taxonomic identification. Of 31 fish analyzed, 28 (90.3%) were parasitized with larvae of anisakid and raphidascaridid nematodes, *Anisakis* sp., *Terranova* sp., *Pseudoterranova* sp. and *Hysterothylacium deardorffoverstreetorum* ($L_3$ and $L_4$) and plerocercus of the trypanorhynch cestode *Otobothrium* sp. Their parasitological indices were calculated. The helminths parasitized mainly the serosas of intestine, stomach and liver, and *H. deardorffoverstreetorum* was also parasitizing the abdominal musculature of one fish. This is the first report of anisakid and raphidascaridid nematodes and trypanorhynch cestodes in *L. villarii.* Hygienic-sanitary aspects were discussed.

**Key words**: Malacanthidae; Anisakidae; Raphidascarididae; Trypanorhyncha.

# LARVAS DE NEMATOIDES E CESTOIDES DE IMPORTÂNCIA HIGIÊNICO-SANITÁRIA EM *Lopholatilus villarii* (ACTINOPTERYGII) NO ESTADO DO RIO DE JANEIRO, BRASIL

**RESUMO**

O peixe batata, *Lopholatilus villarii,* ocorre do Brasil a Argentina e possui uma carne apreciada e comercialmente importante. O objetivo deste estudo foi identificar as larvas de nematoides Anisakidae e Raphidascarididae e cestoides Trypanorhyncha de *L. villarii* obtidos em mercados de pescado do município de Niterói, Estado do Rio de Janeiro, Brasil, calcular seus índices parasitários e apresentar os sítios de infecção. De agosto de 2015 a setembro de 2016, foram investigados 31 espécimes de *L. villarii*. Os peixes foram necropsiados e suas vísceras e musculatura e analisados. Os helmintos encontrados foram processados de acordo com as técnicas em helmintologia para permitir a identificação taxonômica. Dos 31 peixes analisados, 28 (90,3%) estavam parasitados com larvas de nematoides anisaquídeos e rafidascaridídeos: *Anisakis* sp., *Terranova* sp., *Pseudoterranova* sp. e *Hysterothylacium deardorffoverstreetorum* ($L_3$ e $L_4$) e plerocercos de cestoides Trypanorhyncha: *Otobothrium* sp: Seus índices parasitários foram calculados. Os helmintos estavam parasitando, principalmente, as serosas do intestino, estômago e fígado e *H. deardorffoverstreetorum* estava também na musculatura abdominal de um peixe. Este é o primeiro registro de nematoides anisaquídeos e rafidascaridídeos e cestoides Trypanorhyncha em *L. villarii.* Aspectos higiênico-sanitários foram discutidos.

**Palavras-chave:** Malacanthidae; Anisakidae; Raphidascarididae; Trypanorhyncha.

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## INTRODUCTION

The tile fish, *Lopholatilus villarii* Miranda-Ribeiro, 1915 (Malacanthidae) occurs in the Southwest Atlantic from Brazil to Argentina, and is known for its good tasting meat, which has commonly been marketed fraudulently as mottled grouper, *Mycteroperca rubra* Bioch, 1793, or Argentinian sandperch, *Pseudopercis semifasciata* Cuvier, 1829 (FIGUEIREDO and MENEZES 1980; LIMA and MESQUITA, 1996; PAIVA and ANDRADE-TUBINO, 1998; FROESE and PAULY, 2016).

The nematodes of the families Anisakidae and Raphidascarididae have been reported parasitizing several Brazilian marine fish in studies on classical and molecular taxonomy, ecology and hygiene and health, since the larvae migrate after the death of the hosts (FELIZARDO *et al*., 2009; DIAS *et al*., 2010,2011; KNOFF *et al*., 2012,2013; FONTENELLE *et al*., 2013,2015; RIBEIRO *et al*., 2014; PANTOJA *et al*., 2015; KURAIEM *et al*., 2016; FONSECA *et al*., 2016). Human infections with these nematodes have been reported in Europe (Netherlands, Spain, France, Italy, Belgium, Denmark, England and Germany), in the Americas (United States of America, Chile, Peru and Brazil), in Korea and, especially, in Japan, where more than 2,000 cases occur annually (ADAMS *et al*., 1997; AUDICANA *et al*., 2002; ACHA and SZYFRES, 2003; CHAI *et al*., 2005; CRUZ *et al*., 2010; D'AMICO *et al*., 2014).

Cestodes of the order Trypanorhyncha are of hygienic importance because of their repugnant aspect, particularly when teleostean fish present massive infections in their musculature and organs that may make commercialization infeasible due to sanitary inspection and/or rejected by the consumer (AMATO *et al*., 1990; SÃO CLEMENTE *et al*., 2004,2007; FELIZARDO *et al*., 2010). This order possesses a great diversity of species with global distributions. As adults, these worms inhabit the stomach and intestine of elasmobranch fish, while the larval forms can infect a large number of teleostean fish and marine invertebrates in tropical and subtropical regions, but rarely freshwater fish and other vertebrates (CAMPBELL and BEVERIDGE, 1994; PALM, 2004). These cestodes cause significant economic losses. Furthermore, accidental infections of humans by larval trypanorhynchs have been reported due to the ingestion of raw fish meat, and although they do not present zoonotic potential, recent research reports that these cestodes can cause allergic disorders in humans because immunological hypersensitivity has been demonstrated for some species in studies using a murine model (RODERO and CUÉLLAR, 1999; VÁZQUEZ-LÓPEZ *et al*., 2001, 2002; GÒMEZ-MORALES *et al*., 2008; MATTOS *et al*., 2013).

The aim of this study was to report, for the first time, larvae of anisakid and raphidascaridid nematodes and trypanorhynch cestodes as parasites of *L. villarii* marketed in the municipality of Niterói, RJ, Brazil. Parasitological indices were calculated and infection sites presented, with comments on the hygienic-sanitary significance of these parasites.

## METHODS

From August 2015 to September 2016, 31 specimens [total length 43-71 (52.7) cm; weight 0.970 – 4.810 (1.925) kg] were acquired from fish markets in the municipality of Niterói, state of Rio de Janeiro, Brazil. The fish were transported in an isothermal box to the laboratory for necropsy, where they were identified according to FIGUEIREDO and MENEZES (1980).

After necropsy, the internal organs were transferred to Petri dishes containing physiological solution with 0.65% NaCl. The nematode larvae found dead were fixed in AFA (70% alcohol - formaldehyde - acetic acid), while live larvae were fixed in hot AFA at 60 ºC, so they would die distended, preserved in a solution of 70 °GL ethanol plus 5% glycerin and clarified with Amman's lactophenol (KNOFF and GOMES, 2012). Taxonomic classification of nematodes was in accordance with PETTER and MAILLARD (1988); TIMI *et al.* (2001); FELIZARDO *et al.* (2009); KNOFF *et al.* (2012) and FONSECA *et al.* (2016).

Plerocerci of trypanorhynchs were transferred to distilled water and the cysts opened under a stereomicroscope with the aid of sharp needles to release the larvae, which were then refrigerated for at least 24h to permit the relaxation of scolices and tentacular extroversion. The larvae were then fixed in cold AFA, stained with Langeron´s carmine, clarified in beechwood creosote, and preserved as whole mounts on Canada balsam according to KNOFF and GOMES (2012). The classification of Trypanorhyncha followed PALM (2004), BEVERIDGE and JUSTINE (2007) and SCHAEFFNER and BEVERIDGE (2013).

Measurements were obtained by bright field microscopy using an Olympus BX 41 microscope. The samples were then analyzed by bright-field

microscopy with a Zeiss Axiophot microscope using Nomarski's differential interference contrast (DIC) apparatus, and images obtained with a Canon digital camera (Power Shot A640). Some specimens were prepared for scanning electron microscopy (SEM) as described by LOPES TORRES *et al*. (2013). The samples fixed in 70% ethanol were dehydrated in an ethanol series (70º to 100º GL), $CO_2$ critical point dried, coated in gold, and then examined and photographed using a SEM (Jeol JSM-6390LV), under 15 kV acceleration voltage. Measurements were shown in millimeters (mm) with averages in parentheses, unless otherwise indicated.

The parasitological indices of prevalence, intensity, mean intensity, abundance and mean abundance were obtained as described by BUSH *et al.* (1997). Representative specimens of the parasites were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

## RESULTS

From the 31 specimens of *L. villarii* analyzed, 28 (90.3%) were parasitized with a total of 226 individual nematode larvae of Anisakidae and Raphidascarididae, 225 of which were third instar larvae ($L_3$) [two *Anisakis* sp. (Figure 1 a-c), 14 *Terranova* sp. (Figures 2 a-c; 3 a-c), three *Pseudoterranova* sp. (Figures 4 a-c; 5 a-b) and 206 *Hysterothylacium deardorffoverstreetorum* Knoff, Felizardo, Iñiguez, Maldonado Jr, Torres, Pinto and Gomes, 2012 (Figures 6a-c; 7a-c)], and one fourth instar larvae ($L_4$) [*H. deardorffoverstreetorum* (Figures 8a-c; 9a-c)]. Additionally, 26 fish (83.9%) were parasitized with a total of 414 trypanorhynch cestode plerocerci of the family Otobothriidae, identified as *Otobothrium* sp. (Figures 10a-b; 11).

The morphological and morphometric data of the helminths found are shown in Tables 1, 2 and 3. The parasitological indices of prevalence, intensity/mean intensity and abundance/mean abundance, as well as the range of infection, sites of infection and the CHIOC deposit numbers, are shown in Table 4.

All larval specimens of *Anisakis sp.*, *Terranova sp.*, *Pseudoterranova sp.* and various *H. deardorffoverstreetorum* were free, and some of *H. deardorffoverstreetorum* were causing granulomas in serosas. One *Anisakis sp.*, *Pseudoterranova sp.*, and various *H. deardorffoverstreetorum* were live with high motility. The unique *H. deardorffoverstreetorum* L4 was found live and parasitizing the intestinal serosa. Only one fish possessed one *H. deardorffoverstreetorum* L3 parasitizing the abdominal musculature.

The trypanorhynch cestode *Otobothrium sp.* exhibited tiny plerocerci with spherical blastocysts. Only one fish was parasitized with a large number of cestodes (232), which were located in the intestine serosa where they were becoming visible and producing a repugnant appearance (Figure 12). After extroversion, most plerocerci were alive and exhibited moderate motility.

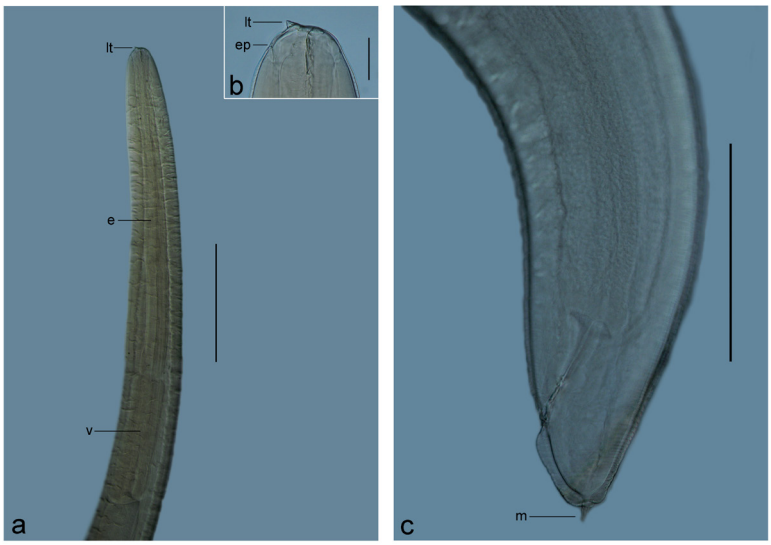


**Figure 1.** *Anisakis* sp. $L_3$ from *Lopholatilus villarii* observed by differential interference contrast, in lateral view. **a.** Anterior portion, showing larval tooth (lt), esophagus (e) and ventricle (v). **b.** Detail of larval tooth (lt) and excretory pore (ep). **c.** Posterior portion showing tail with terminal mucron (m). Bars a = 500µm; b = 50 µm; c = 100 µm.

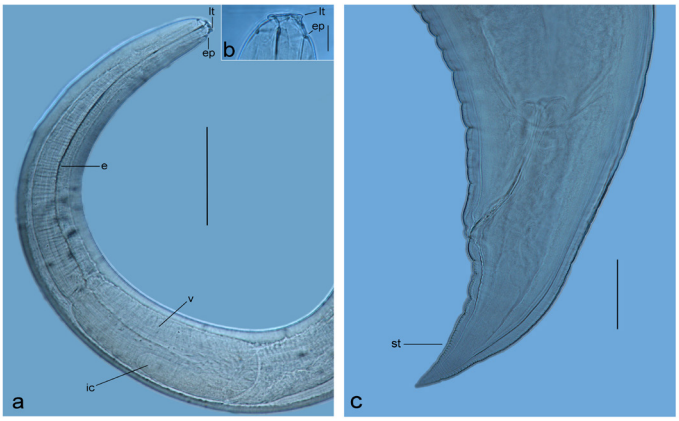


**Figure 2.** *Terranova* sp. $L_3$ from *Lopholatilus villarii* observed by differential interference contrast, in lateral view. **a.** Anterior portion, showing larval tooth (lt), excretory pore (ep), esophagus (e), ventricle (v) and intestinal cecum (ic). **b.** Detail of larval tooth (lt) and excretory pore (ep). **c.** Posterior portion showing striated tail (st). Bars a = 250 µm; b = 25 µm; c = 50 µm.

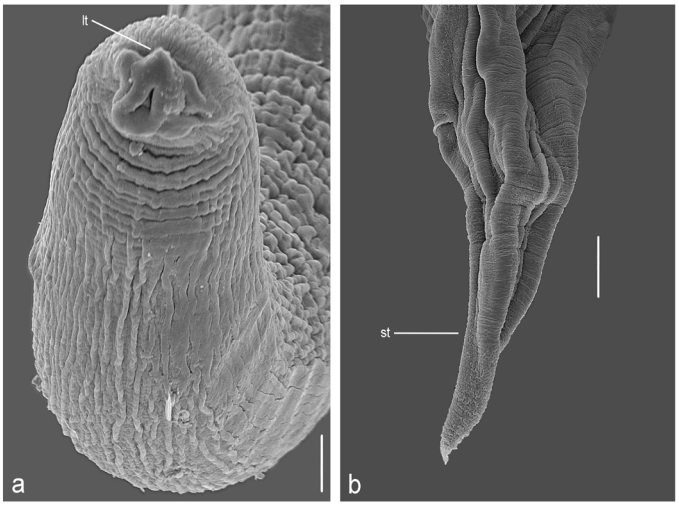


**Figure 3.** *Terranova* sp. $L_3$ from *Lopholatilus villarii* observed by scanning electron microscopy, in dorso-apical view. **a.** Anterior portion, showing larval tooth (lt). **b.** Posterior portion showing striated tail (st). Bars a and b = 20 µm.

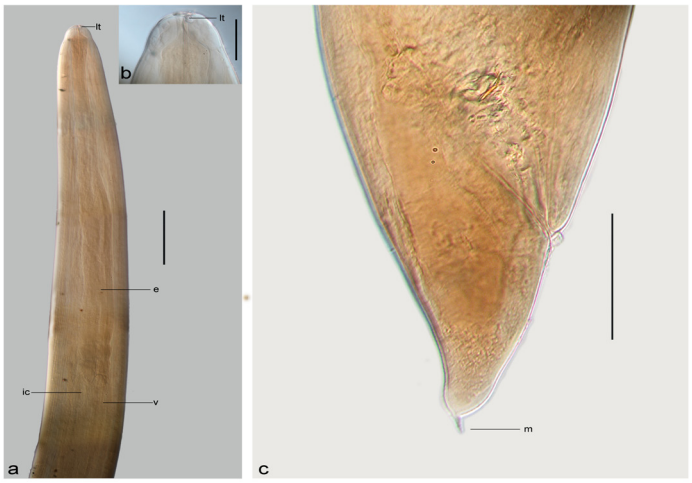


**Figure 4.** *Pseudoterranova* sp. $L_3$ from *Lopholatilus villarii* observed by differential interference contrast. **a.** Anterior portion, showing larval tooth (lt), esophagus (e), ventricle (v) and intestinal cecum (ic), in dorsal view. **b.** Detail of larval tooth (lt). **c.** Posterior portion showing tail with terminal mucron (m), in lateral view. Bars a = 200 µm; b = 50 µm; c = 100 µm.

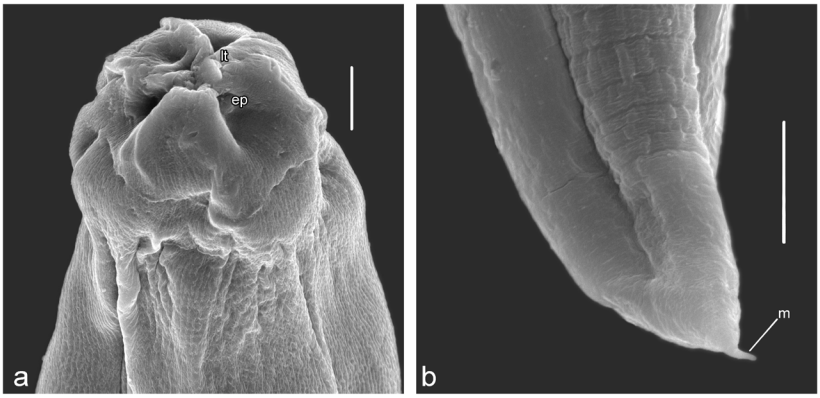


**Figure 5.** *Pseudoterranova* sp. $L_3$ from *Lopholatilus villarii* observed by scanning electron microscopy. **a.** Anterior portion, showing larval tooth (lt) and excretory pore (ep), in ventro-lateral view. **b.** Posterior portion showing tail with terminal mucron (m), in lateral view. Bars a and b = 10 µm.

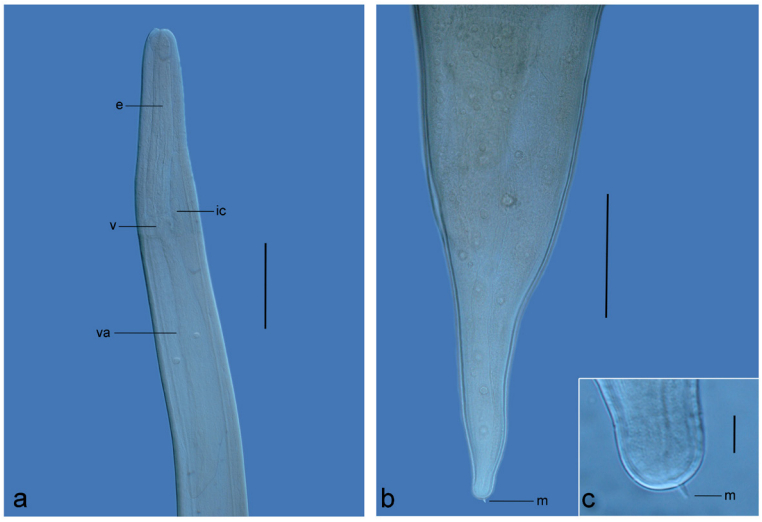


**Figure 6.** *Hysterothylacium deardorffoverstreetorum* $L_3$ from *Lopholatilus villarii* observed by differential interference contrast. **a.** Anterior portion, showing esophagus (e), ventricle (v) and ventricular appendix (va), in dorsal view. **b.** Posterior portion showing tail with characteristic mucron (m), in dorsal view. **c.** Detail of mucron (m). Bars a = 200 µm; b = 50 µm; c = 10 µm.

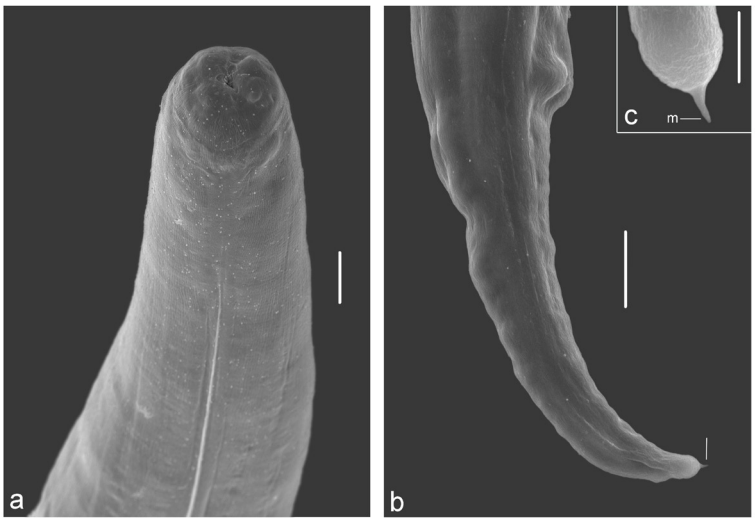


**Figure 7.** *Hysterothylacium deardorffoverstreetorum* $L_3$ from *Lopholatilus villarii* observed by scanning electron microscopy. **a.** Anterior portion, in lateral view. **b.** Posterior portion showing tail with characteristic mucron (m), in lateral view. **c.** Detail of mucron (m). Bars a and b = 20 µm; c = 5 µm.

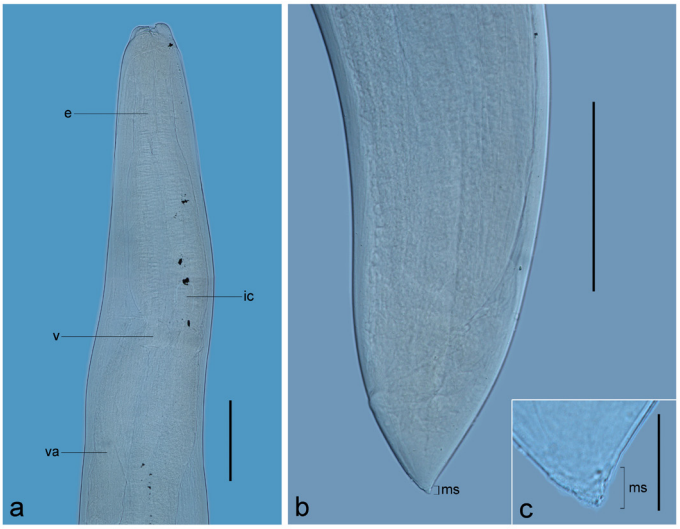


**Figure 8.** *Hysterothylacium deardorffoverstreetorum* $L_4$ from *Lopholatilus villarii* observed by differential interference contrast. **a.** Anterior portion, showing esophagus (e), ventricle (v), ventricular appendix (va), and intestinal cecum (ic), in lateral view. **b.** Posterior portion showing characteristic multi-spinuos (ms) tail, in lateral view. **c.** Detail of multi-spinous tail (ms). Bars a and b = 200 µm; c = 25 µm.

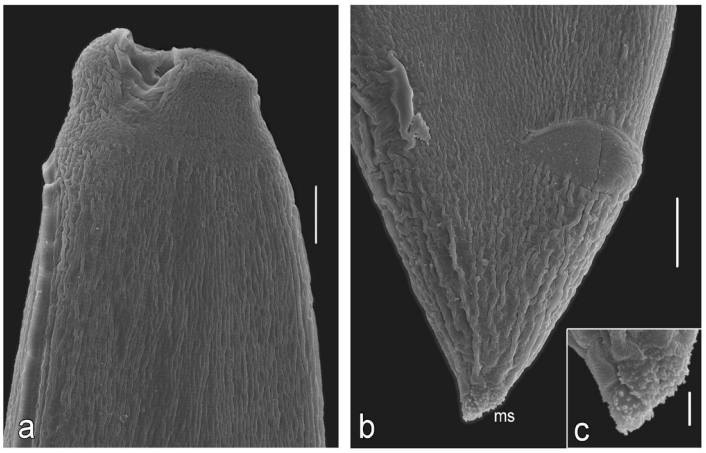


**Figure 9.** *Hysterothylacium deardorffoverstreetorum* $L_4$ from *Lopholatilus villarii* observed by scanning electron microscopy. **a.** Anterior portion, in ventro-lateral view. **b.** Posterior portion showing characteristic multi-spinuos (ms) tail, in ventro-lateral view. **c.** Detail of multi-spinous tail. Bars a and b = 20 µm; c = 5 µm.

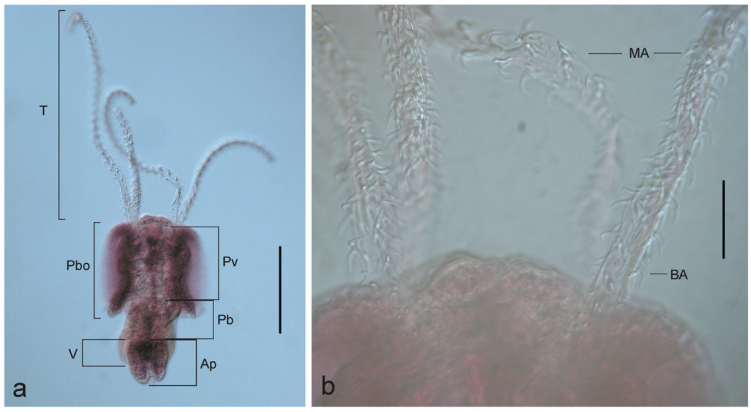


**Figure 10.** *Otobothrium* sp. plerocercus from *Lopholatilus villarii* observed by differential interference contrast. **a.** Entire worm, showing tentacles (T), pars bothrialis (Pbo), pars vaginalis (Pv), pars bulbosa (Pb), velum (V) and appendix (Ap), lateral view. **b.** Detail of extroverted tentacle, indicating basal armature (BA) and metabasal armature (MA) regions, external surface. Bars a = 100; b = 20 µm.

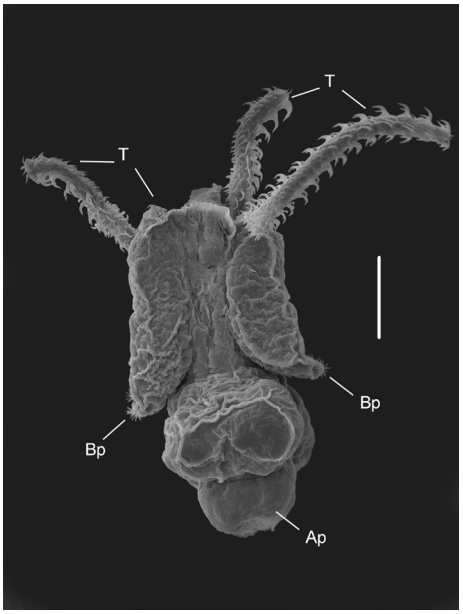


**Figure 11.** *Otobothrium* sp. plerocercus from *Lopholatilus villarii* by observed by scanning electron microscopy. Entire worm, showing tentacles (T), bothrial pits (Bp) and appendix (Ap), lateral view. Bar = 50 μm.

**Table 1.** Morphological and morphometric data of third instar anisakid larvae collected from *Lopholatilus villarii* marketed in the municipality of Niterói, state of Rio de Janeiro, Brazil.

| | *Anisakis* sp. (n = 1) | *Terranova* sp. (n = 9) | *Pseudoterranova* sp. (n = 1) |
|---|---|---|---|
| Body (L) | 23.63 | 6.63-10.44 (8.73) | 11.5 |
| Body (W) | 0.38 | 0.20-0.33 (0.29) | 0.25 |
| Larval tooth | 0.013 | 0.010-0.015 (0.012) | 0.012 |
| Excretory pore [a] | below the larval tooth | below the larval tooth | below the larval tooth |
| Nerve ring [b] | 0.30 | 0.19-0.25 (0.21) | 0.36 |
| Esophagus (L) | 1.75 | 0.92-1.30 (1.08) | 1.33 |
| Ventriculus (L) | 0.70 | 0.31-1.35 (0.62) | 0.60 |
| Ventriculus (W) | 0.20 | 0.10- 0.25 (0,20) | 0.11 |
| Ventricular appendix | Absent | Absent | Absent |
| Intestinal cecum | Absent | 0.33-0.60 (0.46) | 0.59 |
| Tail (L) | 0.10 | 0.12-0.17 (0.15) | 0.15 |
| Mucron (L) | 0.025 | Absent | 0.012 |

[a] Inconspicuous in some specimens. [b] Distance to the anterior end. L = length. W = width. n = number of measured specimens.

**Table 2.** Morphological and morphometric data of third and fourth instar larvae of *Hysterothylacium deardorffoverstreetorum* collected from *Lopholatilus villarii* marketed in the municipality of Niterói, state of Rio de Janeiro, Brazil.

| | *Hysterothylacium deardorffoverstreetorum* $L_3$ (n= 45) | *Hysterothylacium deardorffoverstreetorum* $L_4$ (n=1) |
|---|---|---|
| Body (L) | 1.63-9.25 (4.44) | 10.2 |
| Body (W) | 0.08-0.25 (0.14) | 0.45 |
| Larval tooth | Absent | Absent |
| Excretory pore[a] | 0.15-0.54 (0.37) | - |
| Nerve ring[b] | 0.15-0.38 (0,27) | 0.21 |
| Esophagus (L) | 0.15-0.82 (0.41) | 0.93 |
| Ventriculus (L) | 0.03-0.11 (0.05) | 0.09 |
| Ventriculus (W) | 0.03-0.10 (0.07) | 0.13 |
| Ventricular appendix | 0.14-0.83 (0.48) | 0.73 |
| Intestinal caecum | 0.03-0.18 (0.08) | 0.18 |
| Tail (L) | 0.09-0.30 (0.14) | 0.15 |
| Mucron (L) | 0.002-0.004 (0.003) | Absent |

[a] Inconspicuous in some specimens. [b] Distance to the anterior end. L = Length. W = width. n = number of measured specimens.

**Table 3.** Morphological and morphometric data of *Otobothrium* sp. plerocercus collected from *Lopholatilus villarii* marketed in the municipality of Niterói, state of Rio de Janeiro, Brazil.

| | *Otobothrium* sp. (n=22) |
|---|---|
| Scolex (L) | 198-303 (249) |
| Scolex[a] (W) | 105-175 (144) |
| Appendix (L ) | 33-70 (54) |
| Pbo (L) | 88-195 (165) |
| Pbo (W) | 113-168 (137) |
| Pv (L) | 125-213 (16) |
| Pb (L) | 63-110 (80) |
| Ppb | Absent |
| Velum | Present |
| Bulbs (L) | 48-88 (69) |
| Bulbs (W) | 30-63 (46) |
| Tentacle (L) | 25-375 (169) |
| Tentacle (WBI) | 4-15 (11) |
| Tentacle (WBS) | 9-17 (13) |
| Tentacle (WM) | 7-12 (10) |
| Tentacle (WA) | 5-10 (9) |
| Pbo end to pb beginning[b] | 0-58 (22) |
| Bulb ratio[c] | 1 : 1.4 |
| Scolex ratio[d] | 2.2 : 2.4 : 1 |

L = Length. W = Width. Pars bothrialis = pbo. Pars vaginalis = pv. Pars bulbosa = pb. Pars post-bulbosa = ppb. [a]Maximum width at level of pb. [b]Distance. [c]W : L. [d] pbo : pv : pb. WBI = width at basal insertion. WBS = width at basal swelling. WM = width at metabasal. WA = width at apical. Measurements are presented in micrometers.

**Table 4.** Parasitological indices of prevalence (P), intensity/mean intensity (I/MI), abundance/ mean abundance (A/MA), range of infection (RI) and sites of infection (SI), and numbers of deposit in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) of the anisakid and raphidascaridid nematode larvae and *Otobothrium* sp. plerocercus cestode from *Lopholatilus villarii* marketed in the municipality of Niterói, state of Rio de Janeiro, Brazil.

| | P (%) | I/MI | A/MA | RI | SI | CHIOC |
|---|---|---|---|---|---|---|
| **Nematoda** | | | | | | |
| *Anisakis* sp. | 3.2 | 2* | 0.06* | - | IS | 38366 |
| *Terranova* sp. | 22.6 | 2 | 0.45 | 1-4 | IS, SS | 38364, 38365, 38367 |
| *Pseudoterranova* sp. | 6.4 | 1.5 | 0.09 | 1-2 | IS | 38368 |
| *Hysterothylacium deardorffoverstreetorum* | 87.1 | 7.7 | 6.68 | 1-28 | IS, SS, LS, AM | 38369, 38370 |
| **Cestoda** | | | | | | |
| *Otobothrium* sp. | 83.9 | 15.9 | 13.35 | 1-232 | IS, SS, LS | 38469, 38470, 38471a-c |

* Only the intensity is shown, because only one host was parasitized. IS = intestinal serosa. SS = stomach serosa. LS = liver serosa. AM = abdominal musculature.

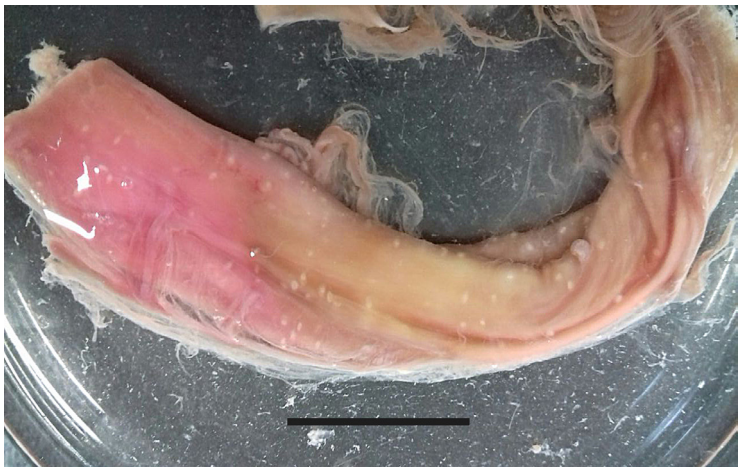

**Figure 12.** *Otobothrium* sp. plerocerci with tiny spherical blastocysts (indicated by arrows) in large numbers attached to the intestinal serosa of *Lopholatilus villarii*. Bar = 2 cm.

*Remarks*

The specimens of *Anisakis* sp. ($L_3$) exhibited morphological and morphometric features similar to those of the larval specimens of *Anisakis simplex* (Rudolphi, 1809) (=*Anisakis* sp.) reported by FELIZARDO *et al*. (2009), *Anisakis* type I by SAAD *et al.* (2012), *Anisakis* sp. by FONTENELLE *et al*. (2013) and KURAIEM *et al.* (2016) and *A. typica* (Diesing, 1860) by FONSECA *et al.* (2016) all from teleostean hosts collected from the state of Rio de Janeiro, Brazil.

The specimens of *Terranova* sp. ($L_3$) possessed larval tooth, conic and striated tail, absent mucron, elongated ventriculus (less than 7 times longer than wide) and intestinal cecum, all diagnostic characteristics of the genus. When compared to the specimens of other reports, one morphological difference was observed; the length of the intestinal cecum is approximately half the length of the ventriculus, while those *Terranova* sp. specimens from *Engraulis anchiota* Hubbs & Marini, 1935, from Argentina and Uruguay (TIMI *et al.*, 2001) and *Paralichthys isosceles* Jordan, 1890, *P. patagonicus* Jordan, 1889, *Xystreurys rasile* (Jordan, 1891) and *Cynoscion guatucupa* (Cuvier, 1830) from state of Rio de Janeiro, Brazil, had an intestinal cecum that was always almost twice the length of the ventriculus (FELIZARDO *et al.*, 2009; FONTENELLE *et al*., 2013; FONSECA *et al*., 2016).

*Pseudoterranova* sp. ($L_3$) specimens possessed a dorsal lip bearing a pair of double papillae, two ventrolateral lips, a tiny boring tooth between the ventrolateral lips, an excretory pore at the base of the ventrolateral lips, a long ventriculus, absent ventricular appendix, intestinal cecum and a tail with a mucron, which were in agreement with the description of *P. decipiens* (Krabbe, 1878) ($L_3$) collected from *Gadus morhua* L., 1758 fillets marketed in Nova Scotia, Canada and Brazil, *Thrysites atun* (Euphrasen, 1791) from New Zealand and *Trigla lucerna* L., 1758, from the Mediterranean Sea, France (MCCLELLAND, 1980; HURST, 1984; PETTER and MAILLARD, 1988; MAFRA *et al*., 2015). Morphometrically, specimens of the present study had a body size approximately three times smaller than those reported by the aforementioned authors.

The specimens of *H. deardorffoverstreetorum* ($L_3$) found in *L. villarii* exhibited similar morphological and morphometric features to the specimens from *P. isosceles, P. patagonicus, X. rasile*, *C. guatucupa*, *Chaetodipterus faber* (Broussonet, 1782), *Trachinotus carolinus* (Linnaeus, 1766), and *Priacanthus arenatus* Cuvier, 1829, all hosts collected from the state of Rio de Janeiro (KNOFF *et al.* 2012; FONTENELLE *et al*. 2013; RIBEIRO *et al.* 2014; KURAIEM *et al*. 2016; FONSECA *et al.* 2016).

The majority of *H. deardorffoverstreetorum* were collected from the interior of small granulomas, as was also observed by FELIZARDO *et al*. (2009) and KURAIEM *et al.* (2016).

The *H. deardorffoverstreetorum* ($L_4$) specimen found in the present study is in agreement with the morphology and morphometry of *Hysterothylacium* sp. n° 2 and *H. deardorffoverstreetorum* ($L_4$) reported by PETTER and MAILLARD (1988) and FELIZARDO *et al*. (2009), respectively. These larvae possesses morphology distinguishable from that of $L_3$, including having well-developed lips, posterior end with a caudal multi-spinous structure and mucron absent.

The specimens of *Otobothrium* sp. are in accordance with the morphological features of the genus presented in previous descriptions and revisions (PALM, 2004; BEVERIDGE and JUSTINE, 2007; SCHAEFFNER and BEVERIDGE, 2013). There are 11 valid species of *Otobothrium*: *O. alexanderi* Palm, 2004; *O. australe* Palm, 2004; *O. carcharidis* (Shipley & Hornell, 1906) Pintner, 1913; *O. crenacolle* Linton, 1890; *O. curtum* (Linton, 1909) Dollfus, 1942; *O. insigne* Linton, 1905; *O. minutum* Subhapradha, 1955; *O. mugilis* Hiscock, 1954; *O. parvum* Beveridge & Justine, 2007; *O. penetrans* Linton, 1907; and *O. propecysticum* Dollfus, 1969 (BEVERIDGE and JUSTINE, 2007; SCHAEFFNER and BEVERIDGE, 2013). The specimens of *Otobothrium* sp. are morphometricaly similar to four species, *O. curtum*, *O. minutum, O. parvum* and *O. propecysticum*, in possessing an extremely short scolex, but they differ from *O. curtum* in the presence of bothrial pits, and from *O. minutum* by the presence of little or no space between the end of the pars bothrialis and the beginning of the pars bulbosa (PALM, 2004; BEVERIDGE and JUSTINE, 2007).

## DISCUSSION

The parasitological indices of the anisakid and raphidascaridid nematodes were compared with those for other teleostean species from along the coast of the state of Rio de Janeiro reported since 2001.

The specimens of *Anisakis* sp. had indices similar to those reported for *A. physeteris* (Baylis,

1923), *A. simplex* and *Anisakis* sp. from *Genypterus brasiliensis* Regan, 1903 (KNOFF *et al*., 2007); *A. simplex* from *P. isosceles* (FELIZARDO *et al.,* 2009) and from *Lophius gastrophysus* Miranda-Ribeiro, 1915 (SAAD *et al*., 2012); *Anisakis* sp. from *C. guatucupa* (FONTENELLE *et al.,* 2013); and *A. typica* from *P. patagonicus* (FONSECA *et al*., 2016). Its indices were slightly lower than those for *Anisakis* type I from *L. gastrophysus* (SAAD *et al*., 2012); *Anisakis* sp. from *P. arenatus* (KURAIEM *et al*., 2016); *A. typica* from *Trichuris lepturus* (Linnaeus, 1758) (BORGES *et al*., 2012) and *X. rasile* (FONSECA *et al*., 2016).

The indices for the specimens of *Terranova* sp. were higher than those for specimens from *G. brasiliensis* (KNOFF *et al*., 2007), *P. isosceles* (FELIZARDO *et al*., 2009), *L. gastrophysus* (SAAD *et al*., 2012), *C. guatucupa* and *Selene setapinis* (Mitchill, 1815) (FONTENELLE *et al*., 2013, 2015) and from *X. rasile* (FONSECA *et al*., 2016); and were lower than those from *P. patagonicus* (FONSECA *et al*., 2016).

The indices for the specimens of *Pseudoterranova* sp. were similar to those of specimens from *Caranx hippos* (Linnaeus, 1766) (LUQUE and ALVES, 2001), *G. brasiliensis* (ALVES *et al*., 2002, KNOFF *et al*., 2007) and *Tylosurus acus* (Lacépède, 1803) (TAVARES *et al*., 2004), and to those of *P. decipiens* from *G. brasiliensis* (KNOFF *et al*., 2007); and were lower than those from *Caranx latus* Agassiz, 1831 (LUQUE and ALVES, 2001).

The indices for the specimens of *H. deardorffoverstreetorum* were much higher than those for *Hysterothylacium* sp. from *G. brasiliensis* (KNOFF *et al*., 2007). Additionally, the range of infection was more than one and half to four times higher than those of *Hysterothylacium* sp. from *Trachinotus carolinus* (Linnaeus, 1766) and *Chaetodipterus faber* (Broussonet, 1782) (RIBEIRO *et al*., 2014) and *T. lepturus* (BORGES *et al*., 2012); and as high as those *H. deardorffovestreetorum* from *P. isosceles* (FELIZARDO *et al*., 2009), *C. guatucupa* (FONTENELLE *et al*., 2013), *P. arenatus* (KURAIEM *et al*., 2016), *P. patagonicus* and *X. rasile* (FONSECA *et al*., 2016).

In Brazil, specimens of *Otobothrium* sp. have been reported from various teleostean fish, including *Balistes vetula* (Linnaeus, 1758) (SÃO CLEMENTE *et al*., 1995; ALVES *et al*., 2005); *G. brasiliensis* (SÃO CLEMENTE *et al.,* 2004); and *P. isosceles* (FELIZARDO *et al*., 2010); and as *O. cysticum* (=*Otobothrium* sp.) in mesentery of *Scomberomorus maculatus* (Mitchill, 1815) and *Sphyraena guachancho* (Cuvier, 1829) (PALM, 1997). In the present study, *Otobothrium* sp. was not found occurring with other trypanorhynch species.

Species of *Otobothrium* have been reported, and are now well known, for their ability to invade and inhabit fish meat, thus causing economic losses in teleostean fish with commercial importance (PALM *et al*., 1994). PALM *et al.* (2000) reported a massive infection by *O. cysticum* (=*Otobothrium* sp.) plerocerci in the musculature of *Peprilus burti* Fowler, 1944 and *P. alepidotus* (Linnaeus, 1766) in the Gulf of Mexico, where the species has varied in prevalence from 20% to 100% annually since 1970, with a range of infection of 1-3500 plerocerci per fish. In the present study, this trypanorhynch cestode was not found in the musculature.

## CONCLUSIONS

This is the first record of anisakid and raphidascaridid nematodes and trypanorhynch cestodes in *L. villarii.*

The presence of the larvae of these nematodes in organ serosas should not be underestimated, since they are capable of migrating from the time of capture to commercialization, which can take a few days. This was the case for the unique *H. deardorffoverstreetorum* larva found in the abdominal musculature in the present study, which would be sufficient to cause an accidental human infection, through the ingestion of improperly processed meat of this fish.

In addition, antigens from these nematodes can be released into fish meat and cause allergic disorders. It is suggested that additional research be undertaken to evaluate the knowledge used for diagnosis of not only nematodes but also trypanorhynch cestodes. This research should address the potential risk of these helminths to humans and potential for control and prevention of the parasitic diseases they cause.

Although trypanorhynch cestodes have not been found in musculature, their massive presence in the intestinal serosa of the one fish collected produced a repugnant appearance, which leads to loss of the commercial value or complete rejection of the fish by the consumer. Thus, it is suggested that fish viscera be removed when marketed.

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