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Anisakidae and Raphidascarididae larvae parasitizing *Selene setapinnis* (Mitchill, 1815) in the State of Rio de Janeiro, Brazil

Larvas Anisakidae e Raphidascarididae parasitos de *Selene setapinnis* (Mitchill, 1815) no Estado do Rio de Janeiro, Brasil

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Abstract

Between February and August, 2012, thirty specimens of Atlantic moonfish, *Selene setapinnis*, were purchased in local markets in Niterói, State of Rio de Janeiro, Brazil, with the aim of analyzing the presence of anisakid nematodes, establishing their rates of parasitism and infection sites, due to importance in the sanitary inspection. A total of sixty nematode larvae, belonging to at least two species were found: nine larvae of *Terranova* sp., Anisakidae, with prevalence (P) of 13.3%, mean intensity (MI) of 2.25, mean abundance (MA) of 0.30 and range of infection intensity (RI) from 1 to 6; and 51 larvae of *Hysterothylacium fortalezae*, Raphidascarididae, with P = 26.7%, MI = 6.40, MA = 1.70, and RI = 1-17. The infection sites for *Terranova* sp. were the mesentery and liver serosa; and for *H. fortalezae*, the infection sites were the mesentery, abdominal cavity and liver serosa. New morphological data from scanning electron microscopy, on the external structures of *H. fortalezae* (mainly at the posterior end), are presented. This is the first report of *H. fortalezae* parasitizing *S. setapinnis*.

Keywords: Anisakidae, Raphidascarididae, *Terranova* sp., *Hysterothylacium fortalezae*, *Selene setapinnis*.

Resumo

De Fevereiro a Agosto de 2012, trinta espécimes de peixe galo, *Selene setapinnis*, foram adquiridos de mercados locais em Niterói, Estado do Rio de Janeiro, Brasil, com o objetivo de analisá-los quanto à presença de nematoides anisaquídeos, estabelecendo seus índices parasitários e sítios de infecção, devido a sua importância na inspeção sanitária. Do total de sessenta larvas de nematoides, pelo menos duas espécies foram encontradas: nove larvas de *Terranova* sp., Anisakidae, com prevalência (P) de 13,3%, intensidade média (IM) de 2,25, abundância média (AM) de 0,30, e amplitude de variação da intensidade de infecção (AI) de 1 a 6; e 51 larvas de *Hysterothylacium fortalezae*, Raphidascarididae, com P = 26,7%, IM = 6,40, AM = 1,70, e AI = 1 a 17. Os sítios de infecção para *Terranova* sp. foram o mesentério e a serosa do fígado; e para *H. fortalezae*, os sítios de infecção foram o mesentério, cavidade abdominal e serosa do fígado. Novos dados morfológicos das estruturas externas, principalmente da extremidade posterior de *H. fortalezae*, são evidenciados por microscopia eletrônica de varredura. Este é o primeiro relato de *H. fortalezae* parasitando *S. setapinnis*.

Palavras-chave: Anisakidae, Raphidascarididae, *Terranova* sp., *Hysterothylacium fortalezae*, *Selene setapinnis*.

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Introduction

Selene setapinnis (Mitchill, 1815), the Atlantic moonfish, reaches about 40 cm in length. It forms shoals and lives near the ocean bottom; however, individuals of small and medium size are very common in the shallow waters of bays and estuaries. These fish feed on other smaller fish and crustaceans. They are distributed from Nova Scotia, Canada, to the shores of the Gulf of Mexico and South America, reaching as far as northern Argentina (MENEZES & FIGUEIREDO, 1980); they have commercial importance in Brazil, mainly in the southern and southeastern regions (BEGOSSI, 2011; CLAUZET et al., 2005; OCCHIALINI & SCHWINGEL, 2003).

In Brazil, Cordeiro & Luque (2004) analyzed the ecology of the community of parasitic metazoans of *S. setapinnis* along the coast of the State of Rio de Janeiro where the quantitative dominance of endoparasitic species was registered. In these localities, anisakid and raphidascaridid larvae showed high prevalence values, represented by *Anisakis* sp., *Contracaecum* sp., *Terranova* sp., *Hysterothylacium* sp. and *Raphidascaris* sp. According to these authors, this suggests that *S. setapinnis* occupies an intermediate trophic level in marine habitats, as part of the diet of piscivorous birds and marine mammals.

In addition to nematode larvae, digenetic trematodes have also been reported parasitizing *S. setapinnis* in Brazil: by Amato (1982) in the State of Santa Catarina and Wallet & Kohn (1987) in the State of Rio de Janeiro. Dyer et al. (1985) also reported this in Puerto Rico.

The present study aimed to analyze the anisakid nematode larvae parasitizing *S. setapinnis* sold in markets in the municipality of Niterói, State of Rio de Janeiro, in order to establish their rates of parasitism, infection sites due to importance in the sanitary inspection.

Materials and Methods

Between February and August, 2012, thirty specimens of the fish species *S. setapinnis* (ranging in length from 37.0 to 46.0 cm and weight from 0.750 to 1.0 kg) were purchased in the municipal market of Niterói, State of Rio de Janeiro, Brazil. They were transported in cool boxes to the Fishery Technology and Inspection Laboratory, School of Veterinary Medicine, Universidade Federal Fluminense, Niterói, State of Rio de Janeiro, for analysis on the presence of nematodes. The fish species was identified in accordance with Menezes & Figueiredo (1980).

After the specimens had been necropsied and filleted, the nematode larvae that were found were placed in Petri dishes with 0.65% NaCl solution, fixed in hot AFA (alcohol, formalin and acetic acid) (60 °C), preserved in a solution of 70 °GL ethanol plus 5% glycerin and clarified with Amman's lactophenol, as described by Eiras et al. (2006).

The taxonomic classification of nematodes was made in accordance with Fagerholm (1991). The larvae were identified as described by Deardorff & Overstreet (1981), Timi et al. (2001), Felizardo et al. (2009a) and Knoff et al. (2012).

Images from bright-field microscopy using Nomarski's differential interference contrast (DIC) were obtained using a Canon digital camera (Power Shot A640) coupled to a Zeiss Axiophot microscope. For topographic characterization of the cuticular surface, sixteen third-stage larvae of *Hysterothylacium fortalezae* Klein, 1973, were analyzed using SEM. This material was processed as described by Lopes Torres et al. (2013). The samples were examined under a JEOL 5320 scanning electron microscope operating at an acceleration voltage of 15 kV.

To clarify morphological details, drawings and analyses under SEM were produced only using *H. fortalezae* specimens. Morphometric analyses were done from drawings made on an Olympus BX41 microscope coupled to a drawing tube. Measurements are shown in millimeters (mm) with the averages in parentheses, unless otherwise indicated. The parasitological indices of prevalence, mean intensity and mean abundance were obtained as described by Bush et al. (1997).

Representative specimens of *Terranova* sp. and *H. fortalezae* were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

Results

Among the thirty specimens of *S. setapinnis*, ten (33.3%) were infected by at least one species of nematode larvae. In total, sixty parasites were collected, belonging to the families Anisakidae and Raphidascarididae. Among the third-stage larvae (L_3) collected, nine were of *Terranova* sp. and 51 of *H. fortalezae*.

The parasitological indices of prevalence, mean intensity, mean abundance and range of infection, the infection sites and the CHIOC deposit number are shown in Table 1.

The morphometric and morphological data from the third-stage larvae of *Terranova* sp. and *H. fortalezae,* obtained from four and ten specimens, respectively, are shown in Table 2.

Anisakidae Railliet & Henry, 1912

Terranova Leiper & Atkinson, 1914

Terranova sp. (Figure 1)

Table 1. Prevalence (P), mean intensity (MI), mean abundance (MA), range of infection (RI), infection site (IS) and CHIOC deposit number of third-stage larvae collected from *Selene setapinnis* (January to August 2012) commercialized in the state of Rio de Janeiro, Brazil.

	S. setapinnis					
	P (%)	**MI**	**MA**	**RI**	**IS**	**CHIOC**
Terranova sp.	13.3	2.25	0.30	1 - 6	M, LS	35851, 35852
H. fortalezae	26.7	6.40	1.70	1 - 17	M, LS, AC	35850

M = mesentery; LS = liver serosa; AC = abdominal cavity.

Description of the main morphological features observed in L_3: cuticle with crosscutting narrow striations most evident on the posterior body portion (Figure 1c); anterior end with a dorsal lip and two ventrolateral lips, all of them poorly developed (Figures 1a, 1b); a pair of cephalic papillae on the dorsal lip and a pair of cephalic papillae on each ventrolateral lip (Figure 1b); larval tooth below the mouth opening between the ventrolateral lips (Figure 1b); excretory portion below the larval tooth (Figure 1b); ventricle longer than wide; absent ventricular appendix (Figure 1a); intestinal cecum larger than the ventricle (Figure 1a); three spherical rectal glands; and conical tail and absent mucron (Figure 1c).

Raphidascarididae Hartwich, 1974

Hysterothylacium fortalezae (Klein, 1973) (Figures 2, 3, 4)

Table 2. Morphological and morphometric data of Anisakidade and Raphidascarididae third-stage larvae collected from *Selene setapinnis* (January to August 2012) commercialized in the state of Rio de Janeiro, Brazil.

	***Terranova* sp.**	***H. fortalezae***
Length	3.75-6.12 (4.81)	4.50-7.0 (6.05)
Width	0.17-0.21 (0.16)	0.14-0.22 (0.19)
Larval tooth	present	absent
Excretory pore*	opens beneath boring tooth	opens beneath nerve ring
Nerve ring**	0.15-0.21 (0.19)	0.16-0.24 (0.20)
Esophagus (L)	0.52-0.81 (0.67)	0.57-0.80 (0.70)
Ventriculus (L)	0.25-0.43 (0.33)	0.055-0.060 (0.058)
Ventriculus (W)	0.13-0.16 (0.14)	0.055-0.065 (0.060)
Ventricular appendix (L)	absent	0.45-0.60 (0.53)
Intestinal cecum (L)	0.45-0.76 (0.57)	0.15-0.21 (0.18)
Tail (L)	0.12-0.28 (0.17)	0.12-0.20 (0.17)
Mucron	absent	absent
Tuft of 6-8 spinous structures	absent	present
Spines (L)	-	5.0-7.5 (7.0) µm

*Inconspicuos in some specimens. **From anterior end; L = Length; W = Width. Measurements are in milimeters (mm) with means in parentheses, unless otherwise indicated.

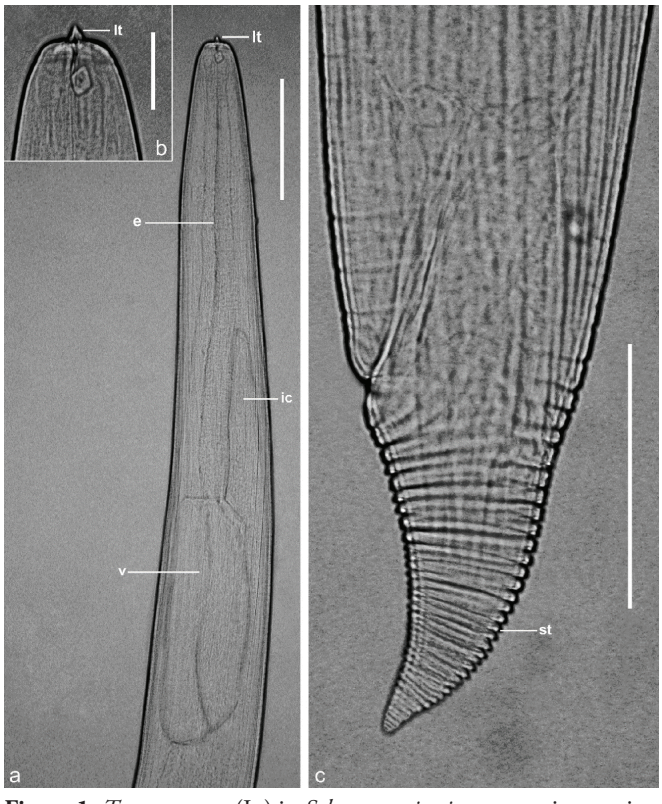

Figure 1. *Terranova* sp. (L_3) in *Selene setapinnis*: **a -** anterior portion showing larval tooth (lt), esophagus (e), ventricle (v) and intestinal cecum (ic); **b -** detail of larval tooth (lt); **c -** striated tail (st). Scale bars in **a =** 200 µm, **b** = 50 µm and **c** =100 µm.

Figure 2. *Hysterothylacium fortalezae* (L_3) in *Selene setapinnis*: **a -** anterior portion showing esophagus (e), ventricle (v), ventricular appendix (vap) and intestinal cecum (ic); **b -** posterior portion with tuft of eight spinous structures (ss). Scale bars in **a =** 200 µm and **b =** 50 µm.

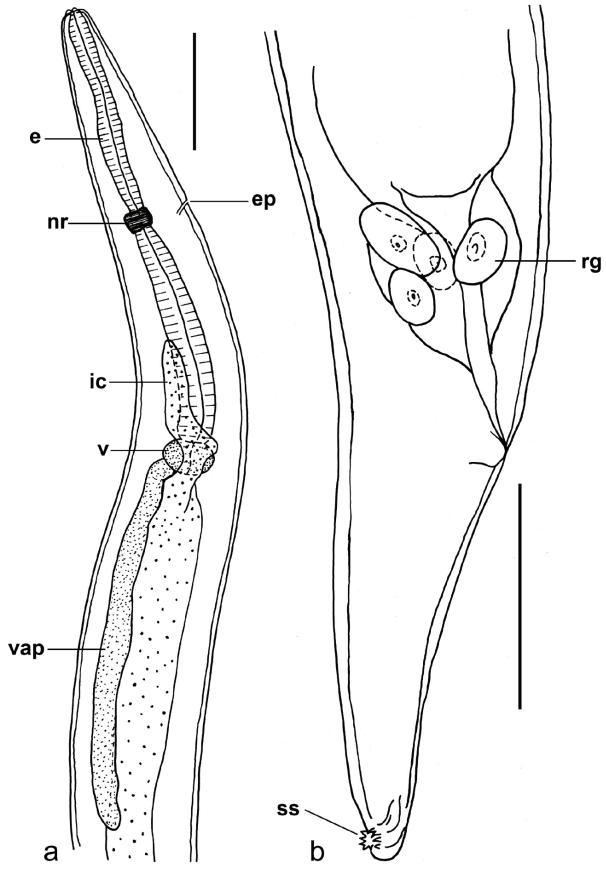

Figure 3. *Hysterothylacium fortalezae* (L_3) in *Selene setapinnis*: **a -** anterior portion showing esophagus (e), nerve ring (nr), excretory pore (ep), ventricle (v), ventricular appendix (vap) and intestinal cecum (ic); **b** - posterior portion with four rectal glands (rg) and tuft of eight spinous structures (ss). Scale bar in **a =** 200 µm and **b** = 100 µm.

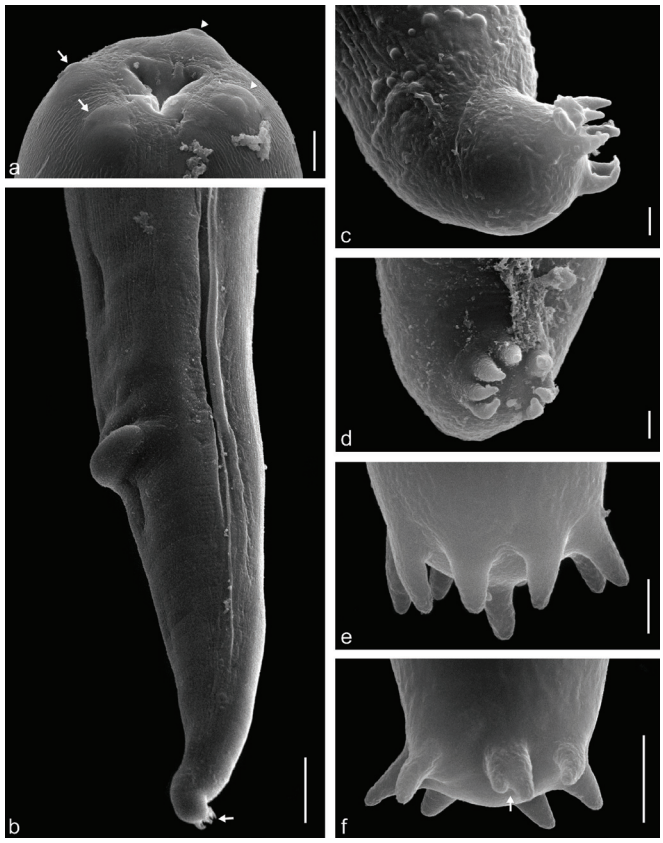

Figure 4. Scanning electron microscopy on *Hysterothylacium fortalezae* (L_3) in *Selene setapinnis*: **a -** anterior end showing the triangular opening mouth with one dorsal lip presenting two sets of papilla (arrow) and two lateroventral lips, each with a set of papillae (arrow head); **b -** posterior end showing the anus with projection and at the tip, in the dorsal direction, tuft of spinous structure (arrow); **c -** lateral view of tuft of spinous structure showing six spines; **d -** frontal view of tuft of spinous structure showing seven spines; **e -** tuft of spinous structure showing eight spines; **f -** tuft of spinous structure showing seven spines, one of them bifid, indicated by arrow. Scale bars in **a**, **c**, **d**, **e** and **f =** 5 µm, and **b =** 20 µm.

Description of the main morphological features observed in L_3, under bright-field and SEM microscopy: triangular mouth provided with one dorsal lip, with two sets of papillae, and two lateroventral lips, each one with a set of papillae (Figures 2a, 4a); a smooth cuticular covering without evident cuticular transverse striations with a slightly prominent lateral line along the body, but not conspicuous at the anterior end (Figures 4b); excretory pore is adjacent to the nerve ring located in the first third of the esophagus (Figure 3a); ventricle is slightly spherical, esophagus is slightly larger than the ventricular appendix; intestinal cecum present (Figures 2a, 3a); four oval rectal glands, anus provided with a projection; conical tail curved ventrally provided with a tuft of six to eight spinous structures, but seven are commonly found (Figures 3b, 4b, c, d, e, f).

Discussion and Conclusion

Species of *Terranova* genus have been reported on the Brazilian coastal waters. Most of them were collected from teleost fish, and have been identified only as *Terranova* sp., with few or even none details of morphological and morphometric features about them, avoiding an accurate specific identification and comparison with *Terranova* specimens collected in the present study (VICENTE et al., 1985; VICENTE & PINTO, 1999; TAVARES & LUQUE, 2006).

The morphology and morphometry of the *Terranova* sp. third-instar larvae were similar to those reported by Timi et al. (2001) in *Engraulis anchiota* Hubbs & Marini, 1935, from Argentina and Uruguay, and Felizardo et al. (2009a) in *Paralichthys isosceles* Jordan, 1890, from Brazil. Cordeiro & Luque (2004) found *Terranova* sp. in the mesentery of *S. setapinnis* with a prevalence of 21.3%. In this study, the prevalence was 13.3% but with more infection sites: both the mesentery and the liver serosa.

In this study, the *H. fortalezae* larvae collected were concordant with the morphological characteristics cited by Deardorff & Overstreet (1981), which they reported in *Scomberomorus maculatus* (Mitchill, 1815), *Peprilus alepidotus* Linnaeus, 1766, *P. burti* Fowler, 1944, and *Anchoa hepsetus* (Linnaeus, 1758), originating from the Gulf of Mexico. One of the main features of this species is the presence of a tuft of spinous structures in the tail; and L_3 has approximately

six of these structures (DEARDORFF & OVERSTREET, 1981). In the present study, six to eight spinous structures were noted and were consistent with the previous description, but there was a difference in the larvae size with measurements from 1.7 mm to 3.5 mm for L_3 and from 5.0 mm to 13.5 mm for L_4. This shows that morphometric changes can occur in the parasite, in relation to its host and its habitat, as previously stated by Timi et al. (2001), and intraspecific variations can be caused by different fixing methods or by geographic variations and effects related the hosts as suggested by Hurst (1984), and must be related to the marine ecoregions of the world (SPALDING et al., 2007). In the present study, the observed total length of the larvae was 4.5 mm to 7.0 mm. Some bifurcated spines were noted, suggesting that, in the ripening process and molting of the larvae, these structures grow in number from the preexisting spines and that the larvae may have been at an intermediate stage in the process of changing from L_3 to L_4 (Figure 4f).

The first records of the genus *Hysterothylacium* and the species *H. fortalezae* in Brazil were made by Klein (1973), along the coast of State of Ceará, in a study on adult parasites of the stomach and intestine of scombrid fish. Guimarães & Cristofaro (1974) found this species in the intestine of *Harengula clupeola* (Cuvier, 1829) along the coast of the State of Bahia. In the present study, no adult specimens were found, thus indicating that the fish collected were serving as an intermediate host for this nematode.

Cavalcanti et al. (2012) reported the presence of 28 specimens of *Contracaecum fortalezae* (= *H. fortalezae*) in the gill chambers and gonads of fifteen samples of *Scomberomorus brasiliensis* Collette, Russo & Zavala-Camin, 1978, collected on the coast of the State of Rio Grande do Norte, Brazil. They also recorded a specimen of *Hysterothylacium* sp. parasitizing the intestine of *Trichiurus lepturus* Linnaeus, 1758. Cordeiro & Luque (2004) found that the prevalence of *Hysterothylacium* sp. in *S. setapinnis* was 7.8% and that the mesentery was the infection site. This differed from the infection sites found in the present study, which consisted not only of the mesentery but also of the liver serosa and abdominal cavity. For comparison, the morphology and morphometry of the specimens deposited by Cordeiro & Luque (2004) in CHIOC were analyzed. The samples that they classified as *Hysterothylacium* sp. were identified as *H. fortalezae*, because they showed morphology identical to the larvae collected now, with six to seven spines on the tail. The present study is the first report of this species parasitizing *S. setapinnis*.

Hysterothylacium fortalezae larvae have been reported on other hosts in the North Atlantic Ocean. Santana-Piñeros et al. (2012) found this type of larva in the intestine, mesentery, stomach and rectum of *Symphurus plagiusa* (Linnaeus, 1766) on the coast of Campeche, Mexico. Adult specimens of *H. fortalezae* were collected from the intestine of *Oligoplites saurus* (Bloch & Schneider, 1801) and *Scomberomorus maculatus* (Mitchill, 1815) originating from the Bay of Chetumal, Mexico, by Aguirre-Macedo et al. (2007).

Hysterothylacium reliquens (Norris & Overstreet, 1975) is another species of the genus *Hysterothylacium* that has been recorded in fish on the northeastern coast of Brazil. Its morphology is very close to that of *H. fortalezae*, but differs from the specimens found in the present study mainly in relation to the tail morphology, which has a multiple spiny structure with numerous tiny spines (DEARDORFF & OVERSTREET, 1980, 1981).

The genus *Hysterothylacium* has been mentioned as important for health surveillance studies (CAVALCANTI et al., 2012; FELIZARDO et al., 2009a, 2009b; FONTENELLE et al., 2013; YAGI et al., 1996). Continuation of studies on this parasitism is of sanitary importance from a public health point of view, in order to implement and reinforce hygiene standards and fish quality control.

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