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

Larvas Anisakidae e Raphidascariidae 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*, Raphidascariidae, 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, Raphidascariidae, *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 anisáquideos, estabelecendo seus índices parasitários e sítios de infecção, devido a sua importância na inspeção sanitária. Do total de sessenta larvas de nematoídes, 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*, Raphidascariidae, 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 liver serosa; e para *H. fortalezae*, as infecções foram o mesentério, cavidade abdominal e liver serosa. 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, Raphidascariidae, *Terranova* sp., *Hysterothylacium fortalezae*, *Selene setapinnis*.

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Introduction

Selene setapinnis (Mitchell, 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 raphidascarid larvae showed high prevalence values, represented by Anisakis sp., Contraucatea 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 in accordance with Menezes and 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 Deardoff & 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 Axiopt 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 López 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 Raphidascaridae. Among the third-stage larvae (L3) 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.

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 L3: 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)                  |
| Ventricle (L)    | 0.25-0.43 (0.33)                   | 0.055-0.060 (0.058)               |
| Ventricle (W)    | 0.13-0.16 (0.14)                   | 0.055-0.065 (0.060)               |
| Ventricle 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                  |

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

**Figure 1. Terranova sp. (L3) 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 (L3) 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.
Description of the main morphological features observed in L₃, 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 (Mitchell, 1815), Pseudoprius alepidotus Linnaeus, 1766, P. buriti 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 L₃ has approximately
In the present study, six to eight spiny 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 L₃ and from 5.0 mm to 13.5 mm for L₄. 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 L₁ to L₄ (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 *H. fortalezae* close to that of *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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