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LARVAL ANISAKID NEMATODES OF THE FLOUNDER, *PARALICHTHYS ISOSCELES* JORDAN, 1890 (PISCES: TELEOSTEI) FROM BRAZIL

NEMÁTODOS ANISAKIDOS LARVARIOS DEL LENGUADO *PARALICHTHYS ISOSCELES* JORDAN, 1890 (PISCES: TELEOSTEI) EN BRASIL

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Abstract

Between October 2006 and March 2008, 60 specimens of *Paralichthys isosceles* were captured in the littoral of the State of Rio de Janeiro, Brazil, to be investigated for anisakid nematodes, due to their zoonotic relevance. Five species of larval anisakid were present, in a total amount of 1,900 worms. One thousand eighty hundred and twenty larvae of *Hysterothylacium* sp., 71 of *Raphidascaris* sp., four of *Terranova* sp., three of *Anisakis simplex* and two of *Contracaecum* sp. were recovered. Parasites were found infecting the abdominal cavity, mesentery, intestine, liver, stomach mucosa, stomach, ovaries, abdominal musculature, and serosas of the heart, spleen, stomach, kidneys and ovaries. *Hysterothylacium* sp. appeared with a prevalence (P) of 100%, mean intensity (MI) of 30.3, range of infection (RI) of 1 to 596 and mean abundance (MA) of 30.3; *Raphidascaris* sp. with P = 36.7%, MI = 3.2, RI = 1 to 29 and MA = 1.18; *Anisakis simplex* with P = 5%, intensity (I) = 1 and MA = 0.05; *Terranova* sp. with P = 5%, MI = 1.3, RI = 1 to 2 and MA = 0.07; *Contracaecum* sp. with P = 3.3% I = 1 and MA = 0.03. This is the first report of parasitological indexes and sites of infection related to larval *A. simplex*, *Contracaecum* sp., *Terranova* sp., *Hysterothylacium* sp. and *Raphidascaris* sp. in *Paralichthys isosceles*. Larvae are described and illustrated. *Paralichthys isosceles* is a new host record for larvae of *Anisakis simplex* and *Hysterothylacium* sp.

Key words: Anisakidae larvae – Brazil - flounder - *Paralichthys isosceles* - zoonotic potential.

Resumen

Desde Octubre hasta Marzo, 2006, 60 ejemplares de *Paralichthys isosceles* fueron colectados en el litoral del Estado do Rio de Janeiro, Brasil, con el objetivo de hacer un inventario de nematodos anisákidos considerando su importancia en las zoonosis. Cinco especies larvales son representadas con un total de 1.900 parásitos, siendo 1.850 muestras de *Hysterothylacium* sp., 71 de *Raphidascaris* sp., cuatro of *Terranova* sp., tres de *Anisakis simplex* y dos de *Contracaecum* sp. Los parásitos fueron encontrados en la cavidad abdominal, mesenterio, intestino, hígado, mucosa estomacal, estomago, ovarios, musculatura abdominal, y en las serosas del corazón, bazo, estomago, riñones y ovarios. Larvas de *Hysterothylacium* sp. aparecieron con una prevalencia (P) de 100%, intensidad media (MI) de 30,3, amplitud de intensidad (RI) de 1 a 596 y abundancia media (MA) de 32, *Raphidascaris* sp. con P = 39%, MI = 3,2, RI = 1 a 29 y MA= 1,2, *Anisakis simplex* con P = 5%, Intensidad (I) = 1 y MA= 0,06, *Terranova* sp. con P = 5%, (I) = 1 y MA= 0,05, *Contracaecum* sp. con P = 3,0%, I =1 y MA = 0,03. Este es el primero reporte de índices parasitarios y sitios de infección de larvas de *A. simplex*, *Contracaecum* sp. *Terranova* sp., *Hysterothylacium* sp. y *Raphidascaris* sp. en *Paralichthys isosceles* que es un nuevo registro de hospedero para larvas de *Anisakis simplex* y *Hysterothylacium* sp.

Palabras clave: larva de Anisakidae - lenguado - *Paralichthys isosceles* - importancia en zoonosis - Brasil.

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INTRODUCTION

The anisakid nematodes can be transmitted to man and fishes act as intermediate, paratenic or definitive hosts for these helminths (Anderson, 2000). Third stage larvae remains infective for over three years (Lopez Sabater & Lopez Sabater, 2000), and are considered in public health approaches, as a potential risk factor when ingested (Roepstorff *et al*., 1993).

Species of flounders included in Paralichthyidae occurring in the Brazilian coast and allocated under *Paralichthys* Girard 1858, represent one of the most outstanding fishery resources in Brazil (Figueiredo & Menezes, 2000), being largely utilized in the internal and external markets.

The present investigation aimed at the identification of larval anisakid worms recovered from specimens of *Paralichthys isosceles* Jordan, 1890 in the littoral of the State of Rio de Janeiro, Brazil, with data on taxonomy, parasitological indexes and infection sites.

MATERIAL AND METHODS

From October 2006 to March 2008, sixty specimens of *Paralichthys isosceles* Jordan, 1890, measuring 35.0±5.9 cm and weighting 625±25.2 g, were captured in the littoral of the State of Rio de Janeiro, Brazil (21º15'S-23º23'S, 40º29'W-44º28'W) to be further investigated for helminths. Fishes were maintained in isothermal boxes with ice and carried to the Laboratory of Helminth Parasites of Vertebrates, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro.

Identification of specimens was obtained in accordance with Figueiredo & Menezes (2000). Nematodes were collected, fixed, clarified and preserved according to Eiras *et al*. (2006). To better understand the structure of the lateral alae in specimens of *Hysterothylacium* sp. and *Raphidascaris* sp., transversal sections at the level of the esophagus were obtained by means of the techniques after Anderson (1958), and Esser (1986). The study of the buccal structures was made on the basis of the method of Moravec (1998). The adopted taxonomic classification of the Anisakidae follows Hartwich (1974) and larval identification was obtained after Rego *et al*. (1983), Smith (1984), Peter & Maillard (1988), Incorvaia & Díaz de Astarloa (1998), Timi *et al*. (2001) and Bicudo *et al*. (2005). Figures were made by means of a drawing tube connected to a Olympus BX41 brightfield microscope. Measurements ranges are in millimeters (mm) with means in parentheses (Table 1). Parasitological indexes related to prevalence (P), intensity (I), mean intensity (MI), range of infection (RI) and mean abundance (MA), are in accordance with Bush *et al*. (1997). Representative specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC). Comparison of species of *Anisakis*, *Contracaecum*, *Terranova*, *Hysterothylacium* and *Raphidascaris*, presently studied, was made with those previously reported by Rego *et al*. (1983) and Bicudo *et al*. (2005), also obtained from fishes of the Brazilian littoral and that are deposited in the CHIOC nos. 31945 a-b, 31946 a-b, 31947 a-b, 31948 a-b, 35351, 35352 and 35353.

RESULTS

The 60 investigated fishes were parasitized with anisakid larvae (prevalence = 100%), in a total amount of 1,900 free or encapsulated larvae. One thousand-eight hundred and twenty larvae represented L3 and L4 larval stages of *Hysterothylacium* sp., 71 of *Raphidascaris* sp., four of *Terranova* sp., three of *Anisakis simplex*, and two of *Contracaecum* sp. (Table 1). Parasitological indexes, infection sites and CHIOC deposit numbers are depicted in Table 2.

Anisakinae Railliet & Henry, 1912
Anisakis Dujardin, 1845
Anisakis simplex (Rudolphi, 1809) (Figs. 1 and 2)
Description based on three third-stage larvae: cuticle with thin transversal striation. Anterior extremity with a dorsal and two poorly developed ventro-lateral lips. Six cephalic papillae, one pair in the dorsal lip and a pair in each ventro-lateral lip. Boring tooth below the oral aperture, between the two ventro-lateral lips. Excretory pore opening beneath the boring tooth. Deirids inconspicuous. Ventriculus longer than large. Ventricular appendix and intestinal cecum absent. Two nearly spherical rectal glands. Tail conical, mucron present.

Contracaecum Railliet & Henry, 1912
Contracaecum sp. (Figs. 3 and 4).
Description based on two third-stage larvae: cuticle with thin transversal striation, more evident in the posterior extremity of the body. Anterior extremity with a dorsal lip and two poorly developed ventro-lateral lips. Six cephalic papillae, one pair in the dorsal lip and a pair in each ventro-lateral lip. Boring tooth near the oral aperture, between the two ventro-lateral lips. Excretory pore opening beneath the boring tooth. Deirids inconspicuous. Ventriculus small and sub spherical. Ventricular appendix nearly twice longer than the intestinal cecum. Two almost spherical rectal glands. Tail conical, mucron absent.

Terranova Leiper & Atkinson, 1914
Terranova sp. (Figs. 5 and 6).
Description based on four third-stage larvae: cuticle with thin transversal striation more evident in the posterior extremity of the body. Anterior extremity with a dorsal and two poorly developed ventro-lateral lips. Six cephalic papillae, one pair in the dorsal lip and a pair in each ventro-lateral lip. Boring tooth below the oral aperture, between the two ventro-lateral lips. Excretory pore opening beneath the boring tooth. Deirids inconspicuous. Ventriculus longer than large. Ventricular appendix absent. Intestinal cecum twice the length of the ventriculus. Two nearly spherical rectal glands. Tail conical, mucron absent.

Raphidascaridinae Hartwich, 1954
Hysterothylacium Ward & Margath, 1917
Hysterothylacium sp. (Figs. 7-10).
Description based on 54 third-stage larvae; cuticle with lateral alae extending along the body with a wedge-shaped support, devoid of basal extension. Anterior extremity with a dorsal and two poorly developed ventro-lateral lips**.** Nine cephalic papillae, two pairs in the dorsal lip together with a large papilla and a pair in each ventro-lateral lip. Boring tooth absent. Excretory pore opening below the nerve ring. Ventriculus nearly spherical. Ventricular appendix twice longer than the esophagus. Intestinal cecum present. Four sub-spherical rectal glands. Tail conical, mucron present.

Description based on 50 four-stage larvae: morphological characteristics similar to those present in third-stage larvae were observed, except for the more developed lips and the presence of a caudal multispinous process responsible for the cactus-tail format in the former.

Raphidascaris Railliet & Henry, 1915
Raphidascaris sp. (Figs. 11-13)
Description based on ten third-stage larvae: cuticle smooth, devoid of striations. Lateral alae extending along the body. Poor developed lips. Ventral boring tooth present. Ventriculus larger than long. Ventricular appendix present. Intestinal cecum absent. Excretory pore below the nerve ring. Two rectal glands nearly spherical. Tail pointed with well defined transversal striations in the terminal portion.

DISCUSSION

Larvae of *Anisakis* recovered from *Paralichthys isosceles* were identified to *A. simplex*, taking into account the defined morphological characteristics of the species, in accordance with Timi *et al*. (2001). Some differences were related to the wider range observed in the length of the worms in comparison with those reported by Timi *et al*. (2001) in *Engraulis anchoita* Hubbs & Marini, 1935, *Anisakis* (type I) in *P. patagonicus* Jordan, 1889 by Incorvaia & Diaz de Astarloa (1998) in Argentina, *Anisakis* sp. in *Pomatomus saltatrix* (L.) by Rego *et al*. (1983), in *Trichiurus lepturus* L. by Barros & Amato (1993) and in *Prionotus punctatus* (Bloch, 1793) by Bicudo *et al*. (2005) captured in the Brazilian coast. Bicudo *et al*. (2005) affirmed that the measurements so far obtained were close to those reported for *A. simplex* by Smith (1983) in euphausiids, differing from them by the smaller proportion related to body and esophagus length.

Data on larvae of *Contracaecum* sp. presently studied are in accordance with those reported by Rego *et al*. (1983), when larvae were recovered from specimens of *Pomatomus saltatrix*, from *Engraulis anchoita* by Timi *et al*. (2001) and *Contracaecum* sp. (type B) from *P. patagonicus* and from *P. orbignyanus* (Valenciennes, 1839) by Incorvaia & Diaz de Astarloa (1998). Tavares and Luque (2006) listed *Contracaecum* sp. larvae parasitizing several teleosteans, including *P. isosceles* from the Brazilian littoral.

Larvae identified as *Terranova* sp., are similar to those described by Rego *et al*. (1983) from *P. saltatrix* in Brazil and by Timi *et al*. (2001) from *Engraulis anchoita* in Argentina and Uruguay. They differ from *Terranova* type HB in Carangidae hosts reported by Deardorff *et al*. (1982) since these larvae present bigger ventriculus and intestinal

cecum, from T*erranova* sp. by Rego *et al*. (1985) recovered from specimens of *Pagrus pagrus* L., by the absence of mucron, and from *Terranova* type B that present larger ventriculus and that were reported by Incorvaia & Díaz de Astarloa (1998) parasitizing specimens of *P. orbignyanus* and *P. patagonicus*. *Terranova* sp. larvae were referred in several marine teleosteans in Brazil by Eiras & Rego, (1987) and Tavares & Luque (2006).

Third-stage larvae of *Hysterothylacium* sp. found in specimens of *P. isosceles* resemble *Hysterothylacium* sp. no. 2 of Petter & Maillard (1988) from *Mullus surmuletus* (L.), *Muraena Helena* L. and *Trachinus araneus* Cuvier, 1825, in the Mediterranean sea, *Hysterothylacium* MD of Deardorff & Overstreet (1981) collected in *Scomberomorus maculatus* (Mitchill, 1815) from the Northern Gulf of Mexico, *Hysterothylacium* KB in *Mene maculata* (Bloch & Schneider, 1801), *Mulloidichthys auriflamma* (Forsskal, 1775), *Otolithes argenteus* Cuvier, 1830, *Pseudorhombus arsius* (Hamilton, 1822), *Sphyraena jello* Cuvier, 1829, *Sphyraena obtusata* Cuvier, 1829 and *Upeneus sulphureus* Cuvier, 1829 from Kuwait by Petter & Sey (1997). These larvae are also similar to those reported in Brazil, previously identified as *Hysterothylacium* MD in *Micropogonias furnieri* (Desmarest, 1823) by Pereira Jr. *et al*. (2004) and *Hysterothylacium* sp. in *P. punctatus* by Bicudo *et al*. (2005). Revising the samples that were deposited in the CHIOC by the latter authors, it became evident that the larval tooth is absent in the examined larvae and that the specimens present, in fact, a prominent lip papilla. Moreover, third-stage larvae recovered from *P. isosceles* present the lateral alae with the wedge-shaped support and conical tail with mucron, identical to the observed in *Hysterothylacium* MD of Deardorff & Overstreet (1981). These larvae differ from those of two species that occur in fishes of the Brazilian littoral: *H. fortalezae* (Klein, 1973) in which the support of the lateral alae is T-shaped and tail ends in an agglomerate of six spines and *H. reliquens* (Norris & Overstreet, 1975) in which the lateral alae and the multispinous structure in the tail are absent (Deardorff & Overstreet, 1980); besides, larvae of both species present larval tooth during the third-stage (Deardorff & Overstreet, 1981).

Fourth-stage larvae are similar to *Hysterothylacium* sp. no. 2 of Petter & Maillard (1988) collected in several teleosteans from the Mediterranean and to *Hysterothylacium* MD of Deardorff & Overstreet (1981) in *Mugil cephalus* L. from the Gulf of Mexico.

Third-stage larvae of *Raphidascaris* sp. studied now, are similar to those of Rego *et al*. (1983) in *Pomatomus saltatrix* L. from the Brazilian littoral and to *R. acus* (Bloch, 1772) in *Perca flavescens* (Mitchill, 1814) collected by Smith (1984) in Canada, by presenting lateral alae. Nevertheless, they differ from the larvae collected in *Prionotus punctatus* from the Brazilian coast by Bicudo *et al*. (2005) since these larvae are devoid of lateral alae. Tavares & Luque (2006) report the distribution of *Raphidascaris* sp. larvae found parasitizing 31 species of teleostean hosts in Brazil, reinforcing the importance of these occurrences.

Timi *et al*. (2001), affirm that morphometric differences observed in specimens of a same species may occur due to the individual allometric development and also in accordance with Koie (1993) that the spatial confinement the first intermediate hosts (crustaceans and mollusks) are submitted can interfere in the size of the larvae parasitizing the fishes.

Five species included in *Paralichthys*, were investigated for larval anisakid in the North and South America: *P. californicus* (Ayres, 1859) by Castillo-Sanches *et al*. (1998), *P. adspersus* (Steindachner, 1867) by Oliva *et al*. (1996), *P. patagonicus* and *P. orbignyanus* by Incorvaia & Díaz de Astarloa (1998) and *P. microps* (Günther, 1881) by Torres *et al*. (2000), when some parasitological indexes and infection sites were reported. In Chile, *Anisakis simplex* in specimens *of P. microps,* appeared with a prevalence 10% higher than the presently reported for *P. isosceles*. Moreover, former data refer to liver and ovaries found parasitized with *A. simplex.* The anisakid nematodes were present in all specimens of flounders from South and North Americas, with a higher mean abundance than the observed here and also occurring in the stomach and intestinal cecum. In Argentina, references to *Terranova* sp. are related to three species (Types A, B, B') in *P. patagonicus* and *P. orbignyanus* occurring with higher mean abundance when compared to the obtained in *P. isosceles*. Nevertheless, *Terranova* (Type A) appeared with a mean abundance of 0.08 similar to the values presently observed in despite of the difference related to other three infection sites reported now. For *Hysterothylacium* sp. parasitizing *P. isosceles* in Brazil, values referring to prevalence, mean intensity and mean abundance were higher

than those reported for other investigated flounders from South and North Americas. For *Raphidascaris* sp. recovered from *P. patagonicus* the mean abundance of the parasitism was of 4.8 when compared to 1.18 in *P. isosceles* also occurring in other four sites of infection, since previous reports only refer to the intestinal cecum.On the basis of the present results, the high infection levels, mainly considering the larvae of *Hysterothylacium* sp., as well as the infection sites, such as musculature and ovaries (Table 2) indicate a major importance, since the larvae, of zoonotic potential, when accidentally ingested can infect man, in accordance with data after Yagi *et al*. (1996) that referred to a case of human anisakidosis in Japan, caused by the ingestion of raw fish parasitized with larval *Hysterothylacium aduncum* (Rudolphi, 1802).

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Table 1. Morphological and morphometric data on third stage larval anisakid in *Paralichthys isosceles* from the littoral of the State of Rio de Janeiro, Brazil.

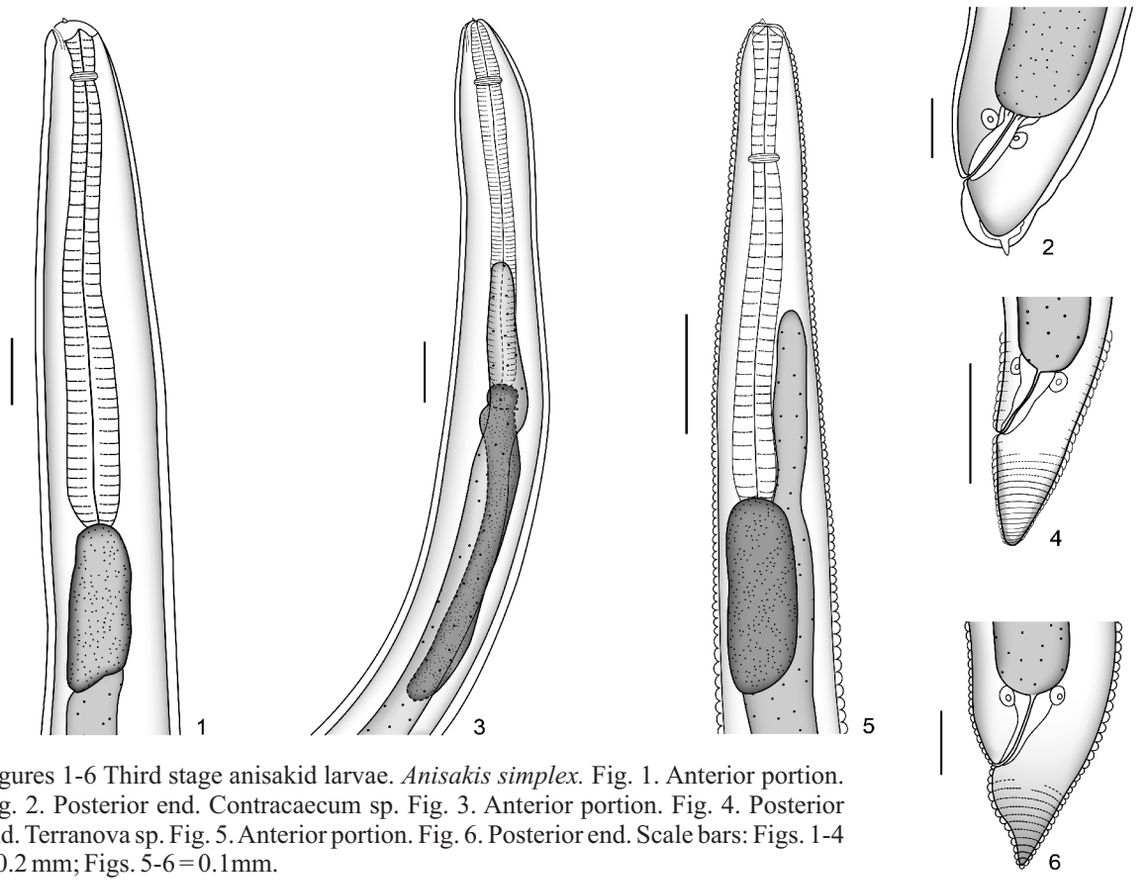
	Anisakis simplex	***Contracaecum* sp.**	***Terranova* sp.**	***Hysterothylacium* sp.**	***Raphidascaris* sp.**
Length	15.3 – 16.0 (15.6)	4.88 – 5.06 (4.97)	5.63 – 7.75 (6.13)	3.62 – 16.7 (10.1)	6.85 – 11.5 (9.17)
Width	0..35 – 0.37 (0.36)	0..9 – 0.22 (0.20)	0.16 – 0.18 (0.17)	0.11– 0.40 (0.25)	0.28 – 0.31 (29.5)
Larval tooth	present	present	present	absent	present
Escretory pore*	opens beneath	opens beneath	opens beneath	0.25 – 0.46 (0.36)	0.27 – 0.42 (0.36)
	boring tooth	boring tooth	boring tooth		
Nerve ring*	0.26 – 0.28 (0.27)	0.14 – 0.18 (0.16)	0.11– 0.22 (0.17)	0.12 – 0.46 (0.29)	0.12 – 0.28 (0.20)
Esophagus	1.53 –1.62 (1.57)	0.64 – 0.70 (0.67)	0.78 – 1.20 (0.99)	0.23 – 1.16 (0.69)	0.57 – 0.93 (0.85)
	0.55 – 0.60 (0.55)	0.03 – 0.09 (0.06)	0.36 – 0.41 (0.39)	0.04 – 0.19 (0.12)	0.05 – 0.10 (0.06)
Ventriculus	x	x	x	x	x
	0.22 – 0.25 (0.22)	0.04 – 0.10 (0.07)	0.15 – 0.17 (0.16)	0.05 – 0.15 (0.10)	0.08 – 0.15 (0.12)
Vap	absent	0.42 – 0.50 (0.46)	absent	0.35 – 1.37 (0.86)	0.28 – 0.80 (0.48)
Intestinal cecum	absent	0.28 – 0.33 (0.30)	0.35 – 0.36 (0.35)	0.05 – 0.32 (0.18)	absent
Tail	0.07 – 0.08 (0.08)	0.14 – 0.17 (0.15)	0.23 – 0.25 (0.24)	0.10 – 0.32 (0.20)	0.13 – 0.34 (0.25)
Mucron	0.02 – 0.03 (0.02)	absent	absent	0.03 – 0.08 (0.05)	absent

*From anterior end; Vap - ventricular appendix

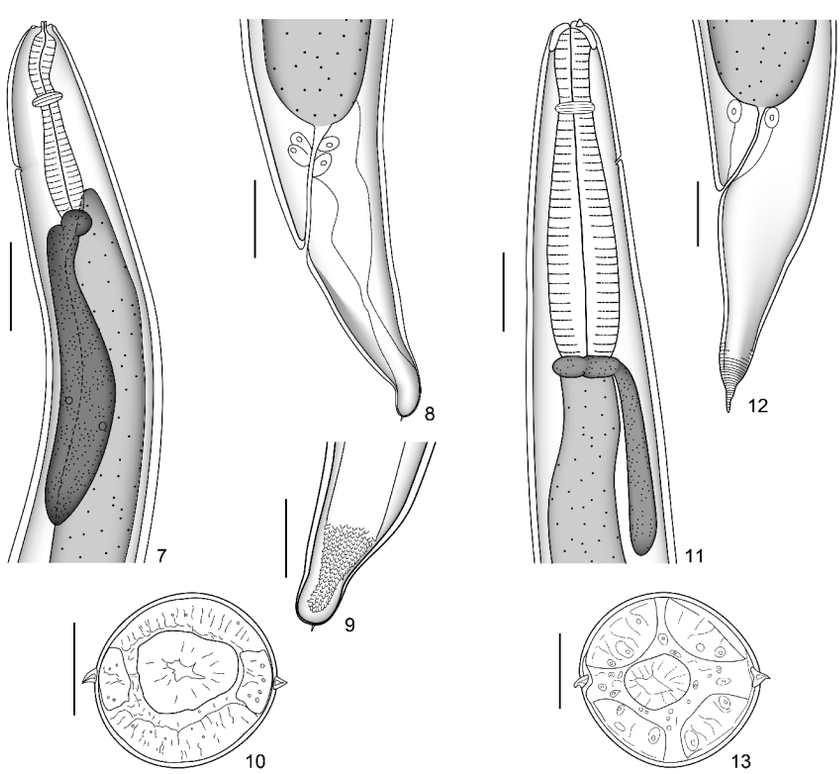
Table 2. Prevalence (P), intensity (I), mean intensity (MI), range of infection (RI), mean abundance (MA), infection sites (IS) and deposit numbers in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) of larval anisakids parasitizing specimens of *Paralichthys isosceles* from the littoral of the State of Rio de Janeiro, Brazil.

	P (%)	**I* / MI**	**RI**	**MA**	**IS**	**CHIOC No.**
Hysterothylacium sp.	100	30.3	1-596	30.3	AC, AM, IN, HS, KS, LS, M, O, OS, S, SM, SS	35642
Raphidascaris sp.	36.7	3.2	1-29	1.18	AC, IN, L, S, STS	35643
Anisakis simplex	5	1*	-	0.05	AC, IN, STS	35644
Terranova sp.	5	1.3	1-2	0.07	L, IN, M, SM	35646
Contracaecum sp.	3.3	1*	-	0.03	IN	35645

AC-Abdominal cavity; AM-abdominal musculature; IN-intestine; HS- heart serosa; KS- kidney serosa; L-liver; LS-liver serosa; M-mesentery; O-ovary; OS-ovary serosa; S-stomach; SM-stomach mucosa; SS-spleen serosa; STS-stomach serosa



Figures 1-6 Third stage anisakid larvae. *Anisakis simplex.* Fig. 1. Anterior portion. Fig. 2. Posterior end. Contracaecum sp. Fig. 3. Anterior portion. Fig. 4. Posterior end. Terranova sp. Fig. 5. Anterior portion. Fig. 6. Posterior end. Scale bars: Figs. 1-4 = 0.2 mm; Figs. 5-6 = 0.1mm.



Figures 7-13. Third stage anisakid larvae. *Hysterothylacium* sp. Fig. 7. Anterior portion. Fig. 8. Posterior end. Fig. 9 Posterior end of a third-stage larva, containing a four-stage molt, with a caudal multi-spinous structure. Fig. 10. Cross section at the middle of esophagus, lateral alae with a wedge-shaped support. *Raphidascaris* sp**.** Fig. 11. Anterior portion. Fig. 12. Posterior end. Fig. 13. Cross section at the middle of esophagus. Scale bars: Figs. 7-8, 11-12 = 0.2 mm; Fig. 9 = 0.025 mm; Fig. 10 = 0.1 mm; Fig. 13 = 0.05 mm.

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