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Three new species of monogeneans parasitic on Atlantic cutlassfish *Trichiurus lepturus* (Perciformes: Trichiuridae) from Southeastern Brazil

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ABSTRACT. Three new species of monogeneans parasitic on the gills and esophagus of *Trichiurus lepturus* (Linnaeus, 1758) in the Southern Brazilian coast are described herein. Between July 2006 and June 2007, 120 specimens of *T. lepturus* were acquired from local fishermen in Guanabara Bay (23°1'52"S, 43°11'56"W), Rio de Janeiro State, Brazil. *Pseudempleurosoma guanabarensis* sp. nov. (Dactylogyridae) presented 15% prevalence and mean intensity 1.8, and differs from all congeners by presenting larger body, largest rectangular dorsal bar, and larger MCO with muscular sheath in the base. *Encotyllabe souzalimae* sp. nov. (Capsalidae) showed 7.5% prevalence and mean intensity 1.3, differs from all congeners by the size and position of the testes, morphology of the MCO, and morphology and size of the anchors. *Octoplectanocotyla travassosi* sp. nov. (Plectanocotylidae) showed 8.3% prevalence and mean intensity 1.5, and differs from all congeners by presenting a third pair of smaller hooks between the outer (more robust) and inner pair (thinner and larger); number and shape of the genital spines and shape of the clamp sclerites.

Keywords: Monogenea, *Pseudempleurosoma*, *Encotyllabe*, *Octoplectanocotyla*.

Três espécies novas de monogenéticos parasitos do peixe-espada *Trichiurus lepturus* (Perciformes: Trichiuridae) no litoral sudeste do Brasil

RESUMO. Três novas species de monogenéticos parasitos das brânquias e do esôfago de *Trichiurus lepturus* (Linnaeus, 1758) do litoral sudeste do Brasil são descritas no presente trabalho. Entre Julho de 2006 e Junho de 2007, 120 espécimes de *T. lepturus* foram adquiridos de pescadores artesanais na Baía da Guanabara (23°1'52"S, 43°11'56"O), Estado do Rio de Janeiro, Brasil. *Pseudempleurosoma guanabarensis* sp. nov. (Dactylogyridae) apresentou prevalência de 15% e intensidade média de 1,8 e difere das espécies congenéricas por apresentar o corpo maior, barra dorsal maior e retangular, e órgão copulatório masculino com bainha muscular na base. *Encotyllabe souzalimae* sp. nov. (Capsalidae) com prevalência de 7,5% e intensidade média de 1,3 difere das outras espécies do gênero pelo tamanho e posição relativa dos testículos, morfologia do complexo copulatório masculino e tamanho e formato das âncoras. *Octoplectanocotyla travassosi* sp. nov. (Plectanocotylidae) com prevalência de 8,3% e intensidade média de 1,5 pode ser diferenciada das demais espécies do gênero, por apresentar um terceiro par de ganchos entre o par externo (mais robusto) e o par interno (mais fino e alongado) no haptor; número e formato dos espinhos genitais; e formato dos escleritos do grampo.

Palavras-chave: Monogenea, *Pseudempleurosoma*, *Encotyllabe*, *Octoplectanocotyla*.

Introduction

Monogenea is a relatively understudied group of parasites of marine fish from Neotropical region. Monogeneans are only approximately 14% of the total number of parasite-host associations recorded in marine fish from Neotropics, and are approximately 18% of the parasite-host associations in Brazilian fishes (LUQUE; POULIN, 2007). To date, there are 80 species of monogeneans recorded as parasites on marine fish from Brazil (COHEN; KOHN, 2008; KOHN; COHEN, 1998).

The cutlassfish *Trichiurus lepturus* (Linnaeus, 1758) is a widely distributed species, recorded among the latitudes 60°N and 45°S. This is a demersal-pelagic and piscivorous species, commercially important, and could be included among the six species with larger volume of world capture (BITTAR et al., 2008; MARTINS; HAIMOVICI, 2000; MARTINS et al., 2005).

There are a few records of monogenean parasites of *T. lepturus* from Brazil, and most species were not

identified to species level and are restricted to Rio de Janeiro. Silva et al. (2000a and b) reported four species of monogeneans: *Octoplectanocotyla trichiuri* Yamaguti, 1939, *Diplectanotrema* sp., *Encotyllabe* sp. and *Neobenedenia* sp. More recently, Carvalho and Luque (2009) recorded *Neobenedenia melleni* (MacCallum, 1927).

During a parasitological survey, numerous specimens of monogenean parasitic on cutlassfish from Guanabara Bay were collected in the Rio de Janeiro State, Brazil, and three new species were described in the present paper.

Material and methods

Between July 2006 and June 2007, 120 specimens of *T. lepturus* were acquired from local fishermen in Guanabara Bay (23°1'52"S, 43°11'56"W), Rio de Janeiro State, Brazil. Gills were removed and placed in vials containing 1:4000 formalin; the esophagus and stomach of the hosts were removed and observed in a stereoscopic microscope. Collected parasites were fixed and stored in 5% formalin. Some specimens were stained with Gomori's trichrome, and others were mounted in Gray and Wess's medium, as described in Boeger and Vianna (2006). Illustrations were done using a drawing tube mounted on an Olympus BX-51 phase contrast microscope. Measurements are in micrometers; means are followed by the range and the number of measured specimens (n) in parentheses. For the species of *Octoplectanocotyla*, the nomenclature of the clamp sclerites followed Llewellyn (1956). Prevalence and mean intensity of infections were according to Bush et al. (1997). Type specimens were deposited in the Helminthological Collection of the Institute Oswaldo Cruz (CHIOC), Rio de Janeiro State, Brazil.

Results and discussion

Monogenea van Beneden, 1858
Dactylogyridae Bychowsky, 1933.
Pseudempleurosoma Yamaguti, 1965
Pseudempleurosoma guanabarensis sp. nov. (Figure 1).

Description (based on 12 specimens). Body slender; ellipsoid; 2,098 (1,350 - 2,750; n = 12) long; greatest width 690 (425 - 850; n = 12) near midbody. Single pair of head glands extends to pharyngeal region. Two pairs of eye-spots. Oral aperture ventral; 119 (85 - 185; n = 12) from anterior end; pharynx globular; 120 (65 - 160; n = 12) long; 119 (35 - 160; n = 12) wide. Intestinal caeca united posterior to testis, lateral diverticula without hematin pigment. Short peduncle, haptor 93 (68 - 138; n = 11) long, 125 (70 - 185; n = 11) wide, not distinctly set off from body proper; two pairs of dissimilar anchors and 14 hooks. Dorsal anchors 52 (38 - 58; n = 11) length, connected by a single rectangular dorsal bar, 25 (23 - 28; n = 10) long, 13 (10 - 18; n = 10) wide. Ventral anchors 12 (10 - 14; n = 10) length, with two bars: one attached, 7 (5 - 10; n = 9) length; and a free irregular bar 11 (8 - 13; n = 9) length. Hooks 12 (8 - 15; n = 8) long, filamentous hook about 2/3 shank length. Genital atrium median, smooth, at 207 (170 - 290; n = 12) from anterior end. Sclerotized male copulatory organ (MCO), tubular, 59 (45 - 70; n = 10) long, with proximal muscular sheath, and seminal vesicle and prostatic reservoir at the base; accessory piece sclerotized, with irregular shape, at the base of MCO, 29 (18 - 40, n = 12) long. Testis post-ovarian, oval, 142 (90 - 235; n = 11) long, 119 (60 - 165) wide. Ovary turned back on itself, 140 (80 - 170; n = 10) long, 167 (75 - 200; n = 10) wide. Vagina simple, opening close to genital atrium. Vitellaria scattered throughout trunk, absent in region of reproductive organs. Vitelline ducts united anteriorly to ovary, at 476 (320 - 650; n = 12) from anterior end. Eggs 90 (75 - 100; n = 12) long, (40 - 75; n = 12) wide, without polar filament.

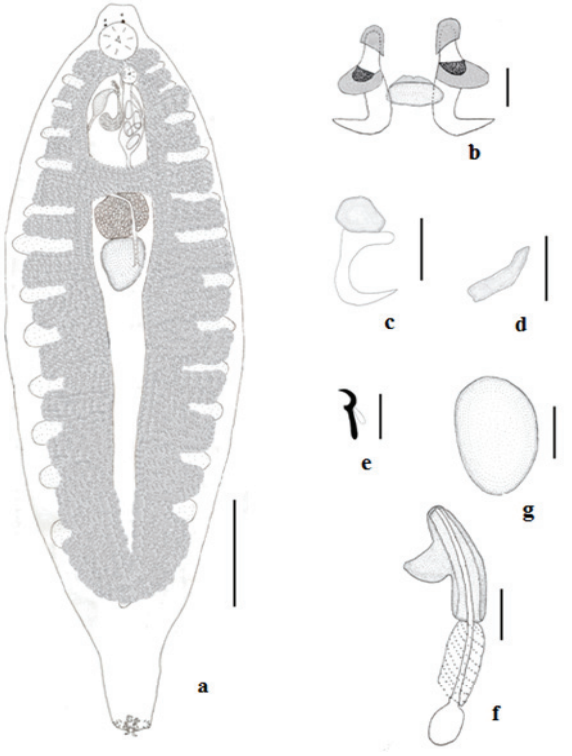

Figure 1. *Pseudempleurosoma guanabarensis* sp. nov. a) Holotype (dorsal view). b) dorsal anchor with ventral bar attached. c) ventral anchor. d) irregular ventral bar free. e) hook. f) male copulatory organ. g) egg. Scale-bars: Figure a = 300 µm; Figure b = 20 µm; Figure c-f = 10 µm; Figure g = 50 µm.

Type-host: *Trichiurus lepturus* (Linnaeus, 1758), Trichiuridae.

Site of infection: Esophagus.

Type-locality: Guanabara Bay (23°1'52"S, 43°11'56"W), Rio de Janeiro State, Brazil.

Prevalence: 15% (18 of 120 examined fish).

Mean intensity: 1.8 ± 1.3.

Type-specimens: Holotype CHIOC 37.389, Paratypes CHIOC 37.390 a-c, CHIOC 37.391

Specimens studied: Holotype and five paratypes of *Pseudempleurosoma gibsoni* Santos et al. (2001) (CHIOC 34.337a-e, 34.338).

Etymology: The specific name refers to the type-locality.

Remarks: The "diplectanotrema-group" (GERASEV et al., 1987) includes species parasitic on the esophagus and foregut of fishes. Earlier, this group was constituted by the following genera: *Diplectanotrema* Johnston et Tiegs, 1922, *Pseudempleurosoma* Yamaguti, 1965, *Neodiplectanotrema* Gerasev, Gaevskaia et Kovaleva, 1987, *Paradiplectanotrema* Gerasev, Gaevskaia et Kovaleva, 1987, *Pseudodiplectanotrema* Gerasev, Gaevskaia et Kovaleva, 1987, and *Metadiplectanotrema* Gerasev, Gaevskaia et Kovaleva, 1987 (GERASEV et al., 1987). Nevertheless, Santos et al. (2001) proposed *Metadiplectanotrema* as junior synonym of *Pseudempleurosoma*, and amended the diagnosis of this genus. According to these authors, the main characteristics of *Pseudempleurosoma* are: two pairs of dissimilar anchors, dorsal anchor large with unpaired median connecting bar, and ventral anchor smaller with two pairs of connecting bars associated with each anchor; caeca with numerous lateral diverticula, united posteriorly; copulatory organ tubular, slender, twisted or not; accessory piece present; genital pore median and ovary pretesticular (SANTOS et al. 2001). To date, four species of *Pseudempleurosoma* are known: *P. carangis* Yamaguti, 1965; *P. gibsoni* Santos et al. (2001); *P. caranxi* Gerasev, Gaevkaia et Kovaleva, 1987; and *P. myripristi* Gerasev, Gaevskaia et Kovaleva, 1987 (SANTOS et al., 2001).

Pseudempleurosoma guanabarensis sp. nov. differs from all congeners by having larger body, rectangular dorsal bar, and MCO larger with muscular sheath on the base. Additionally, the new species differs from *P. carangis* by the lack of twisted MCO; from *P. gibsoni* by presenting sclerotized terminal accessory piece, muscular sheath at MCO base, and eggs without polar filament; from *P. caranxi*, by having larger dorsal bar, smaller ventral bars, larger MCO and eggs; and from *P. myripristi* by the absence of polar filaments in the eggs, presence of rectangular dorsal bar, and larger MCO.

Capsalidae Baird, 1853

Encotyllabe Diesing, 1850

Encotyllabe souzalimae sp. nov. (Figure 2).

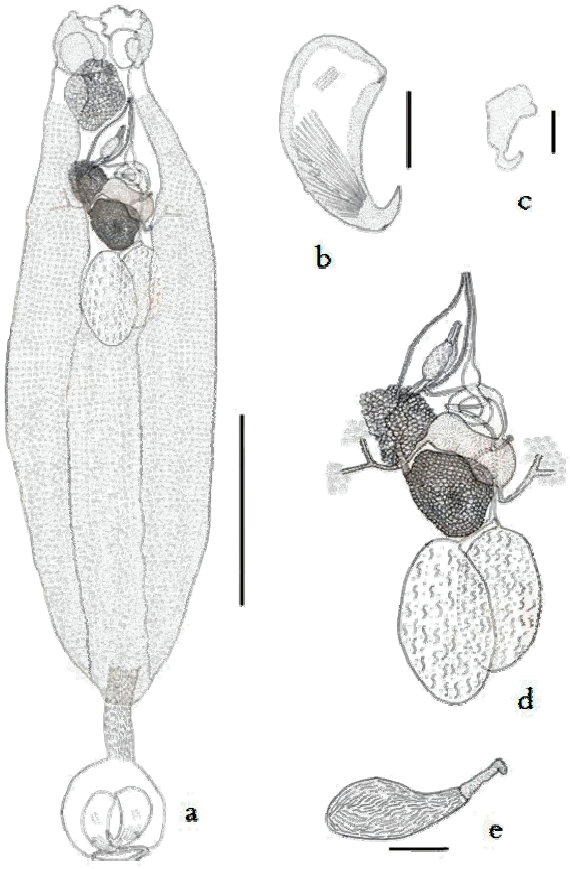

Figure 2. *Encotyllabe souzalimae* sp. nov. a) Holotype (ventral). b) larger anchor. c) smaller anchor. d) reproductive system. e) penis. Scale-bars: Figure a = 700 μm; Figure b and e = 100 μm; Figure c = 15 μm; Figure d = 350 μm.

Description (based on seven specimens). Total length 4,179 (2,775 - 4,950; n = 7); body prer length 3,339 (2,000 - 4,025; n = 7); maximum width (excluding infolds of lateral margins) 982 (750 - 1,250); haptor bell-shaped, 453 (380 - 580; n = 7) long, 450 (200 - 670; n = 7) wide. Haptor armed with pair of large broad anchors, pair of smaller anchors and 14 hooks; distal end of haptor involved by thin membrane. Large anchors with furrows in the external surface, and margins thickened on the internal surface; superficial root with rifts on the anterior extremity, and shank with small protuberance on posterior margin, 307 (260 - 360; n = 7) long, 155 (140 - 190; n = 6) wide. Small anchor, with unequal roots, enlarged base, curved shank, 30 (27.5 - 32.5; n = 7) long, 14 (12.5 - 17.5; n = 5) wide. Larger anchor length: smaller anchor length ratio 10:1 (9:1 - 12:1; n = 6). Hooks 13 (12 - 14; n = 6) length. Muscular Buccal organ, sucker-

like, surrounded by incomplete frilled membrane 201 (150 - 230; n = 7) long, 170 (110 - 200; n = 7) wide (excluding membrane), complete sucker channel. Eversible pharynx, flask-shaped, glandular, anterior margin folded to form digital processes 293 (190 - 400; n = 7) long, 306 (220 - 450; n = 7) wide. Intestinal caeca branched, extending posteriorly to peduncle base, not confluent. Two pairs of eyespots at pharynx level. Testes paired, juxtaposed in the margins, pre-equatorial, oval and unequal in size; right testis larger, 348 (250 - 470; n = 6) long, 218 (150 - 250; n = 6) wide; left testis smaller, 300 (210 - 390; n = 6) long, 206 (150 - 250; n = 6) wide. Vas deferens joined posterior to ovary, winding anteriorly and dorsal to vitelline reservoir, ootype and penis; vas deferens expands within penis to form seminal vesicle and ejaculatory duct. Penis muscular, 265 (235 - 310; n = 5) long, 120 (110 - 140; n = 5) wide; divided in two areas: basal (pyriform and muscular), 174 (150 - 188; n = 5) long, 59 (50 - 65; n = 5) wide, and distal (elongated and sclerotized) with posterior extremity ending in right angle, 59 (50 - 63; n = 5) length. Prostatic reservoir present. Ovary pretesticular, oval, 217 (160 - 270; n = 7) long, 162 (120 - 200; n = 7) wide. Distance between right testis center and ovary center of 344 (270 - 400, n = 6), proportion between maximum values 1:1.4. Vitelline reservoir anterior to ovary, 244 (180 - 310; n = 7) long, 116 (80 - 150; n = 7) wide. Uterus extends parallel to posterior surface of the penis bulb. Conspicuous Mehlis' gland, surrounding ootype. Vitellaria extending from the level of penis to the base of peduncle. Vaginal aperture ventral to vitelline reservoir. Eggs not observed.

Type-host: *Trichiurus lepturus* (Linnaeus, 1758), Trichiuridae.

Site of infection: gills.

Type-locality: Guanabara Bay (23°1'52"S, 43°11'56"W), Rio de Janeiro State, Brazil.

Prevalence: 7.5% (9 of 120 examined fish).

Mean intensity: 1.33 ± 1.00.

Type-specimens: Holotype CHIOC 37.382; Paratypes CHIOC 37.383, 37.384, 37.385.

Etymology: This species is named in honor to Prof. Sueli de Souza Lima, for her contribution to study of helminth parasites of Brazilian vertebrates.

Remarks: Currently, the genus *Encotyllabe* Diesing, 1850 contains 16 nominal species parasitic on marine fish from several families in temperate, subtropical and tropical waters, and these species are: *E. nordmanni* Diesing, 1850 (type-species); *E. pagelli* Van Beneden et Hesse, 1864; *E. paronae* Monticelli, 1907; *E. vallei* Monticelli, 1907; *E. lintoni* Monticelli 1909; *E. pagrosomi* MacCallum, 1917; *E. spari* Yamaguti,1934; *E. pricei* Koratha, 1955; *E. lutjani* Tripathi, 1959; *E. chironemi* Robinson,1961; *E. embiotecae* Noble, 1966; *E. caranxi* Lebedev, 1967; *E. caballeroi* Velasquez, 1977; *E. fotedari* Gupta et Krishna, 1980; *E. kuwaitensis* Khalil et Abdul-Salam, 1988; and *E. xiamenensis* Li, Yan et Wang, 2004 (KHALIL; ABDUL-SALAM, 1988; LI et al., 2004; NOBLE; 1966; WILLIAMS; BEVERLEY-BURTON, 1989). Noble (1966) and Williams and Beverley-Burton (1989) considered *E. masu* Inshii et Sawada, 1938, *E. monticelli* Perez Vigueras, 1940, *E. pricei* Koratha, 1955 and *E. punctatai* Gupta et Krishna, 1980 as species inquirendae because these species were poorly described, based in only one or two specimens. Regarding the high variation of the morphometric characteristics, Khalil and Abdul-Salam (1988) selected as the most important characteristics to the diagnosis of *Encotyllabe* species the following features: body shape, relative size of several organs (pharynx, testes, ovary, vitelline reservoir, and penis), relative position of the testes, penis shape, extension of the vitellaria and size and shape of the anchors. Williams and Beverley-Burton (1989) stated that the detailed study of the anchor and hooks must be important to the diagnosis of *Encotyllabe* species.

Encotyllabe souzalimae sp. nov. differs from *E. lintoni* for possessing testes juxtaposed in the margins, morphological differences in the penis and in the larger and smaller anchor; from *E. pagrosomi*, for having unequal testes, and by the relationship between length and width of the testes; from *E. spari*, by having testes unequal and juxtaposed in the margins, and by having shape differences in the penis; from *E. pricei*, by presence of complete sucker channel; from *E. lutjani* by having larger testes juxtaposed in the margins, larger pharynx, smaller haptor and anchor, and larger proportion among the anchors.

The new species can be separated from *E. embiotecae* for having testes juxtaposed in the margins, and differences in the shape of the larger and smaller anchor; from *E. caranxi* by the body shape, vitellaria distribution, and by differences in the shape of the larger anchor and the penis; from *E. fotedari* by the relative position of the testes and by the penis shape; and from *E. kuwaitensis* by the body shape, by having unequal testes and by the shape of the smaller anchor and penis. *Encotyllabe souzalimae* sp. nov. is closely related to *E. caballeroi* and *E. chironemi* due to vitellaria distribution and unequal testes. The new species differs from *E. caballeroi* and *E. chironemi* by having larger right testis, penis with distal sclerotized piece forming an

angle of 90°, larger anchors, with furrows in the surface; superficial root, with a rift on the anterior extremity, posterior margin of the shank with a small protuberance, and smaller anchor with unequal roots. Finally, *E. souzalimae* sp. nov. can be separated from *E. xiamenensis* by the smaller size, and morphological differences in the anchors.

Plectanocotylidae Poche,1926

Octoplectanocotyla Yamaguti, 1937

Octoplectanocotyla travassosi sp. nov. (Figure 3).

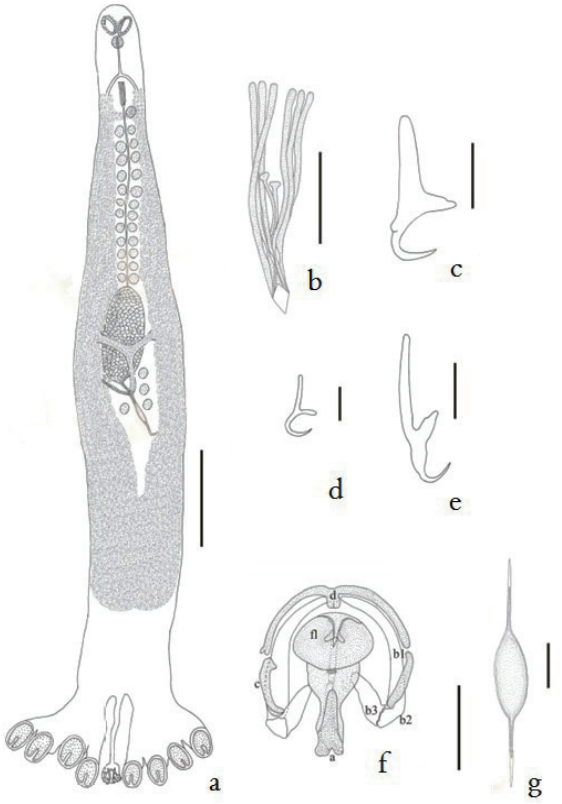

Figure 3. *Octoplectanocotyla travassosi* sp. nov. a) Holotype (ventral). b) male copulatory organ. c) outer hook. d) intermediate hook. e) inner hook. f) clamp with the sclerites a, b1, b2, b3, c, d; fl = fair-lead. g) egg. Scale-bars: Figure a = 160 µm; Figure b, d, g = 25 µm; Figure c = 5 µm; Figure f = 80 µm; Figure g = 40 µm.

Description (based on 10 adult specimens). Body elongated, tapering anteriorly and posteriorly before junction with haptor, 3,835 (2,480 - 5,625; n = 10) length, 411 (250 - 520; n = 10) width at the level of the ovary. Haptor with two defined lobes, each with four clamps, 158 (130 - 235; n = 6) long, 137 (120 - 200; n = 6) wide; sclerite c elongated, sclerite b3 associated with fair-lead. Median terminal lappet between two lobes, 328 (280 - 380; n = 5) length, bearing three pairs of hooks: outer pair robust, erected thumb one or two small protuberances on external curvature, 50 (45 - 55; n = 10) long, 7 (5 - 10; n = 10) wide; medial pair smaller, 10 (9 - 11; n = 6) long, 2 (2 - 4; n = 6) wide; inner pair, 62 (50 - 75; n = 10) long, 5 (5; n = 10) wide. Two buccal organs, 62 (53 - 65; n = 10) long, 51 (38 - 63; n = 10) wide. Pharynx 40 (25 - 63; n = 10) long, 46 (35 - 68; n = 10) wide; esophagus bifurcate anterior to genital pore; intestinal caeca extending back to junction of haptor, not joining posteriorly. Male copulatory organ with eight genital spines: six long and thin spines, 60 (46 - 71; n = 10) length; two short spines, more robust with thickened point, 29 (22 - 41; n = 8) length. Testes rounded, 38 (35 - 39; n = 7) in diameter, intercecal, 25-29 in number, distributed in two longitudinal rows, from bifurcation of esophagus to anterior portion of ovary; a few postovarian testes. Ovary folded upon itself. Genito-intestinal canal short, receptacle seminal and vagina absent. Eggs fusiforms, 160 (125 - 180; n = 6) long, 59 (45 - 68; n = 6) wide; polar filaments, 106 (98 - 113; n = 6) length.

Type-host: *Trichiurus lepturus* (Linnaeus, 1758), Trichiuridae.

Site of infection: gills.

Type-locality: Guanabara Bay (23°1'52"S, 43°11'56"W), Rio de Janeiro State, Brazil.

Prevalence: 8.3% (10 of 120 examined fish).

Mean intensity: 1.52 ± 1.10.

Type-specimens: Holotype CHIOC 37.386; Paratypes CHIOC 37.387 a-b, 37.388 a-b.

Etymology: This species is named in honor to Prof. Lauro Pereira Travassos, in recognition to his great contribution to helminthology.

Remarks: *Octoplectanocotyla* Yamaguti, 1937 includes two species *O. trichiuri* Yamaguti, 1937, recorded for the first time parasitizing gills of *Trichiurus japonicus* Temminck et Schlegel, 1844 from Japan Sea (YAMAGUTI, 1937), and the gills of *T. savala* Cuvier, 1829 and *T. muticus* Gray, 1831 from India (TRIPATHI, 1959); and *O. aphanopi* Pascoe, 1987 from gills of *Aphanopus carbo* Lowe, 1839 collected in the Northeast Atlantic Ocean (PASCOE, 1987).

Diagnosis of *Octoplectanocotyla* species is based on: number, size and shape variation of the genital spines, number and position of the testes, and the number, shape of the hooks and clamps (PASCOE, 1987). Llewellyn (1956) mentioned that *Plectanocotyle* species have two pairs of hooks on the lappet, but in several specimens a third pair of anchor is also present. The possibility of the third pair of hooks be vestige of the larval hooks was tested with observations in 15 specimens of *Plectanocotyle gurnardi* (van Beneden et Hesse, 1863), which did not reveal correlation between the size of the specimens and the presence of the third pair of hooks, evidencing

the stability of this taxonomic character among the plectanocotylids (LLEWELLYN, 1956).

Octoplectanocotyla travassosi sp. nov. presents the third pair of smaller hooks between the outer and the inner pairs. This characteristic was not recorded for the other two known species of *Octoplectanocotyla*. Also, *O. travassosi* sp. nov. differs from *O. trichiuri* by presenting eight genital spines (six of them larger and two smaller), by differences in the shape of the inner and outer pairs of hooks, in the testes distribution, by the larger number of testes, and by the larger sclerite c, as well as the largest association of the sclerite b3 with the fair-lead. *Octoplectanocotyla travassosi* sp. nov. differs from *O. aphanopi* by having eight smaller genital spines, with different shape. Moreover, the outer and inner pairs of hooks are larger and thinner, with different shape. The new species also presents eggs with smaller polar filaments, and larger sclerite c.

Conclusion

Three new species of monogeneans parasitic on marine fish *Trichiuris lepturus* were described: *Pseudempleurosoma guanabarensis* sp. nov., *Encotyllabe souzalimae* sp. nov. and *Octoplectanocotyla travassosi* sp. nov.

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