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CESTODES OF THE FLOUNDER *PARALICHTHYS ISOSCELES* JORDAN, 1890 (OSTEICHTHYES - PARALICHTHYIDAE) FROM THE STATE OF RIO DE JANEIRO, BRAZIL

CESTODOS DEL LENGUADO *PARALICHTHYS ISOSCELES* JORDAN, 1890 (OSTEICHTHYES - PARALICHTHYIDAE) EN EL ESTADO DE RIO DE JANEIRO, BRASIL

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Abstract

From October 2006 to March 2008, sixty specimens of *Paralichthys isosceles*, were captured in the littoral of the State of Rio de Janeiro Brazil. The present study deals with the taxonomic identification of the cestodes parasitizing this fish species, as well with the analysis of parasitological indexes, sites of infection, taking into account the hygienic-sanitary importance of the host in Brazilian and foreign markets. Fifty-six (93.3%) out of the collected fishes were infected with at least one specimen of metacestodes. Diphyllobothriidea were represented by plerocercoids of two species: *Diphyllobothrium* sp. 1 (similar to *D. latum*) in stomach, stomach mucosa and mesentery and *Diphyllobothrium* sp. 2 (similar to *D. dendriticum*) in intestine, liver, ovary and abdominal cavity. Trypanorhyncha, were represented by *Grillotia carvajalregorum*, in stomach, intestine, liver, mesentery, abdominal cavity, abdominal and dorsal musculatures, *Nybelinia lingualis* in stomach, stomach mucosa, intestine, mesentery, spleen serosa, dorsal musculature, *Heteronybelinia nipponica* in intestine, kidney serosa, abdominal cavity and abdominal musculature, *Otobothrium* sp. in the stomach, intestine, liver, mesentery and abdominal cavity, *Callitetrarhynchus gracilis* and *Pterobothrium heteracanthum* in abdominal musculature, *P. crassicolle* in stomach serosa. Tetraphyllidea, were represented by *Scolex pleuronectis* in stomach and intestine. Remarks on the zoonotic potential of species included in Diphyllobothriidae and Trypanorhyncha and the role they play concerning sanitary inspections are presented. This is the first report of metacestodes parasitizing specimens of *P. isosceles.*

Key words: Brazil - Diphyllobothriidea - *Paralichthys isosceles* - parasitological indexes - Tetraphyllidea - Trypanorhyncha - zoonotic potential.

Resumen

De octubre de 2006 a marzo de 2008, sesenta ejemplares de lenguado *Paralichthys isosceles*, fueron capturados en el litoral del Estado de Rio de Janeiro, Brasil. El presente estudio trata de la identificación taxonómica de los cestodos que parasitan esta especie de pez, así como el análisis de los índices parasitológicos, los sitios de infección, teniendo en cuenta la importancia higiénico-sanitaria del hospedero en los mercados brasileños y extranjeros. Cincuenta y seis (93.3%) de los peces colectados estaban infectados con al menos un ejemplar de metacestodos. Diphyllobotriidea estuvo representados por plerocercoides de dos especies: *Diphyllobothrium* sp. 1 (similar a *D. latum*) en el estómago, la mucosa del estómago y en el mesenterio y *Diphyllobothrium* sp. 2 (similar a *D. dendriticum*) en el intestino, el hígado, el ovario y la cavidad abdominal. Trypanorhyncha estuvo representados por *Grillotia carvajalregorum*, en el estómago, intestino, hígado, ovario, mesenterio, la cavidad abdominal, musculatura abdominal y dorsal, *Nybelinia lingualis* en el estómago, la mucosa del estómago, el intestino, mesenterio, la serosa del bazo y la musculatura dorsal, *Heteronybelinia nipponica* en el intestino, la serosa del riñón, cavidad abdominal y la musculatura abdominal, *Otobothrium* sp. en el estómago, intestino, hígado, mesenterio y la cavidad abdominal. *Callitetrarhynchus gracilis* y *Pterobothrium heteracanthum* en la musculatura abdominal, *P. crassicole* en la serosa del estómago. Tetraphyllidea estuvo representados por *Scolex pleuronectis* en el estómago y el intestino. Observaciones sobre el potencial zoonótico de las especies incluidas en Diphyllobothriidae y Trypanorhyncha y el papel que desempeñan en materia de inspecciones sanitarias son presentadas. Este es el primer informe de metacestodos parasitando especímenes de *P. isosceles*.

Palabras clave: Brasil - Diphyllobothriidea - índices parasitarios - *Paralichthys isosceles* - potencial zoonótico - Tetraphyllidea - Trypanorhyncha.

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INTRODUCTION

This paper deals with the continuity of studies related to the helminth parasites of the flounder, *Paralichthys isosceles* Jordan, 1890, an important fish resource of commercial importance either in local or overseas markets (Felizardo *et al*., 2009 a, b). The present investigation aims at the identification of cestode species under Diphyllobothriidea, Trypanorhyncha and Tetraphyllidea that were found parasitizing this host as well as to supply data on taxonomy, parasitological indexes, sites of infection, taking into account their importance referring to public health and sanitary inspection.

There are no previous reports of larval cestodes included in Diphyllobothriidea, Trypanorhyncha and Rhinebothriidea (= Tetraphyllidea in part) infecting this host so far. Cestode species related to the two first orders referred above, are important when sanitary inspection and collective health are concerned. Specimens of *Diphyllobothrium* spp. are the agents of human diphyllobothriasis and are found in teleostean fishes, and the transmission occurs after the ingestion of raw, poor cooked or improperly frozen fish meat. *Diphyllobothrium latum*, *D. dendriticum* and *D. pacificum* are the species that have been referred in South America. Diphyllobothriidea plerocercoids are registered in teleostean hosts from the Brazilian coast (Pereira Jr., 1993; Alves *et al*., 2004, 2005; Pereira Jr. & Boeger, 2005; Knoff *et al*., 2008). In Brazil, several cases of human diphyllobothriasis were reported (Knoff *et al*., 2008), thus reinforcing the importance of investigations related to the presence of larval cestodes in the intermediate hosts and transmission patterns.

Larval stages namely plerocerci and plerocercoids of Trypanorhyncha that are found in several organs and in the musculature of teleosteans, the intermediate hosts, even without zoonotic potential are responsible for the repugnant aspect of the meat for the final consumer. Adult worms are recovered from the gastrointestinal system of elasmobranchs (Knoff *et al*., 2002); nevertheless, accidental human infections by larvae of Trypanorhyncha due to ingestion of raw fish meat have been reported (Bates, 1990). Two of the cases were related to specimens of *Hepatoxylon trichiuri* (Holten, 1802) Dollfus, 1942, that were found alive in the feces of humans in South Africa and Mozambique (Heinz, 1954; Fripp & Mason, 1983); besides, there is a register of a specimen of *Nybelinia surmenicola* Okada, 1929, adhered to the soft palate of a man in Japan (Kikuchi *et al*., 1981). In September, 2009, during the I Latin American Meeting on Helminthiasis, held in Teresópolis, Rio de Janeiro State, Brazil, there was a personal communication (Jorge Manuel Cárdenas-Callirgos), about the finding of a species of *Nybelinia* Poche, 1926 in the soft palate of a man in Lima, Peru. In the last decade, some reports have suggested that, possibly, species of Trypanorhyncha can provoke allergic reactions in humans (Rodero & Cuéllar, 1999; Vásquez-Lopez, 2001, 2002; Gomez-Morales *et al.*, 2008); this approach certainly will change the procedures adopted for the processing of fish meat taking into account helminth infections. Several results of studies related to the finding of trypanorhynch cestodes in fishes of the Brazilian littoral are already available (Cordeiro & Luque, 2004; São Clemente *et al*., 2004, 2007; Pereira Jr. & Boeger, 2005; Alves & Luque, 2006; Ferreira *et al*., 2006; Pinto *et al.*, 2006; Luque *et al*., 2008; Dias *et al*., 2009, 2010; Oliveira *et al.*, 2009; Porto *et al*., 2009).

Plerocercoid Tetraphyllidea larvae present different morphological types, difficulting their identification and for this reason, a collective name, *Scolex pleuronectis* Müeller, 1788 was proposed by Chambers *et al*. (2000), after maintaining the plerocercoids *in vitro*, and describing eleven different types of metacestodes, parasitizing fish species.

In the life-cycle of these cestodes, teleosteans and some marine mammals are the second intermediate hosts whereas elasmobranchs the definitive (Stunkard, 1977; Scholz *et al*., 1998; Agustí *et al*., 2005; Aznar *et al*., 2007). Recently, part of the Tetraphyllidea was reallocated in a new order, the Rhinebothriidea (Healy *et al.*, 2009). In Brazil, several teleostean fish species have been found infected with tetraphylidean metacestodes, mainly by those referred to as *S. pleuronectis* and *Scolex* sp. (Rego & Santos, 1983; Rego *et al*., 1983; Takemoto *et al*., 1996a, b; Knoff *et al*., 1997; Palm, 1997; Silva *et al*., 2000 a, b; Luque *et al.*, 2000, 2008; Luque & Alves 2001; Parraguassú *et al*., 2002; Alves *et al*., 2002a, b, 2003, 2004; Tavares *et al*., 2004; Alves & Luque, 2006).

MATERIAL AND METHODS

From October 2006 to March 2008, sixty specimens of *P. isosceles*, measuring 35.0±5.9 cm in length and weighing 625±25.2 g were obtained from professional fishermen. Fishes were captured in the littoral of the state of Rio de Janeiro, Brazil, latitudes 21°15'S-23°23'S, longitudes 40°29'W-44°28'W. After, they were carried in isothermal containers with ice to the Laboratorio de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro to be investigated for helminths. The identification of fishes is in accordance with Figueiredo & Menezes (2000). For recovery procedures, specimens were eviscerated; the organs and abdominal musculature were transferred to individual Petri dishes with a 0.65% NaCl solution to be examined under a stereoscope microscope. Whenever necessary, blastocysts were disrupted to permit the proper observation of the scolices. The filets, obtained after an incision from near the opercula to the insertion of the caudal fin, were observed by means of a negatoscope. Cestodes were recovered, fixed, clarified and preserved, according to Eiras *et al*. (2006). Classification of Diphyllobothiidea is based on Kuchta *et al*. (2008); that of Trypanorhyncha on Dollfus (1942, 1960), Carvajal & Rego (1983, 1985), Palm (1997,1999, 2004), in accordance to the taxonomy proposed by Campbell & Beveridge (1994); the classification of Tetraphyllidea follows Euzet (1994) whereas the cestodes presently considered under Rhinebothriidea were referred to after Healy *et al*. (2009). The terminology applied to larvae and microtriches was that indicated by Chervy (2002, 2009), respectively. Indexes of parasitism referred here follow Bush *et al*. (1997). For the study of plerocercoids, five micrometers thick sections were stained with haematoxylin-eosin (HE). Morphological comparison of the samples presently studied was made with specimens deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), where representative specimens herein considered were also included. Measurements were obtained in a brightfield microscope Olympus BX 41, and are in millimeters (mm), unless otherwise indicated. Means are followed by the range in parenthesis. The number of measured larvae is also indicated in parenthesis (n). Descriptions, measurements and drawings are restricted to the poor known specimens to add data to further identifications. Drawing was made with the aid of a drawing tube, and micrographs were made with a digital camera in a brightfield microscope Zeiss Axiophot. For scanning electron microscopy (SEM) procedures, samples were dehydrated in an alcoholic series (50-100ºGL), critical point dried with CO_2, coated with gold and electron micrographied under a JEOL SM25SII and Zeiss 962 microscope submitted to 30 KVolts. For parasitic indexes, abridgments are: P = prevalence, I = intensity, MI= mean intensity, RI = range of infection, A = abundance, MA = mean abundance. For infection sites, abridgments are: AC = abdominal cavity, S = stomach, L = liver, I = intestine, M = mesentery, SM = stomach mucosa, AM = abdominal musculature, DM = dorsal musculature, O = ovary, SpS = spleen serosa, StS = stomach serosa, OS = ovary serosa, KS = kidney serosa.

RESULTS

An amount of 1,205 larval cestodes were recovered from the different infection sites. These larvae were identified as described bellow:

Diphyllobothriidea Kuchta, Scholz,
Brabec & Bray, 2008
Diphyllobothriidae Lühe,1910
Diphyllobothrium Cobbold, 1858
Diphyllobothrium sp. 1
(Fig. 1)

Parasitological indexes: P = 6%, MI = 2.5, RI = 1-6, MA = 0.16; infection sites: S, M, M.; total of collected specimens: 10 plerocercoids; deposited specimens: CHIOC 37335a-c and 37336a-b.

Description (n=4): Plerocercoids with retracted scolex and hardly observed under light microscope, anterior extremity with a dorso-ventral swelling due to the retraction of the scolex, bothridial grooves present in the scolex reaching up 1/3 of the

body length. Body surface wrinkled, not segmented and without genital primordium. Body 1.45-2.40 (1.80) long, 0.45-0.57 (0.53) wide. Scolex 0.35-0.58 (0.46) long, 0.44-0.57 (0.51) wide. Bothridial groove 0.10-0.18 (0.14) long. There is a single layer of fibers on the epidermic longitudinal musculature. Microtriches 2-3.5 (2.3) µm long, inconspicuous under light microscopy and only observed in cross sections.

Remarks: Specimens of the sample here analyzed, are most alike to plerocercoids of those related to *D. latum*, that present microtriches 2 µm long, similar to the observed by Andersen *et al*. (1987) and Andersen & Gibson (1989), studying plerocercoids of several fish species from Europe and North America.

Diphyllobothrium sp. 2
(Fig. 2)

Parasitological indexes: P = 10%, MI = 1.6, RI = 1 – 2, MA = 0.13; infection sites: AC, I, L, O; total of collected specimens: 8 plerocercoids; deposited specimens: CHIOC 37337, 37338a-c and 37339a-c; examined samples: CHIOC 36873, 36874, 36875 and 36876.

Description (n=4): Plerocercoids with the scolex partially retracted, frontal glands present only at the scolex region, body surface wrinkled, segmented, and with genital primordium present. Body 10.40 - 24 (17.20) long 1.22 - 2 (1.61) wide. Scolex (0.97) long, (1.22) wide. Bothridial groove (0.10) long. There are two layers of fibers in the longitudinal epidermic musculature; parenchymal longitudinal musculature well developed. Microtriches conspicuous under light microscopy, 7.5 –15 µm (11.2 µm) long, and in cross sections.

Remarks: The specimens presently recovered are very similar to those collected in specimens of *Genypterus brasiliensis* Regan, 1903, from the Brazilian littoral by Knoff *et al*. (2008), reporting to plerocercoids of *Diphyllobothrium* sp., with microtriches 7.5 – 11.25 µm (10 µm) long, probably belonging to *D. dendriticum*, and that also are in agreement with the description of plerocercoids reported by Andersen *et al*. (1987) and Andersen & Gibson (1989). The parasitological indexes indicated by Knoff *et al*. (2008) in *G. brasiliensis* (P = 24.3 %, MI= 1.66, RI = 1 – 7, MA = 0.40), are different from those presently obtained, except for the similar mean intensity. Considering that the infection sites reported by Knoff *et al*. (2008) were related to the abdominal cavity, intestine serosa and the musculature near to the cloaca, two other sites, the liver and the ovary are now included. These differences may be due to the ecological habits of these two fish species.

This is the first occurrence of *Diphyllobothrium* sp. plerocercoids parasitizing specimens of *P. isosceles*.

Figures 1-2. Anterior extremity of plerocercoids. Fig.1. *Diphyllobothrium* sp. 1. Scale bar = 200 µm. Fig. 2. *Diphyllobothrium* sp. 2. Scale bar = 500 µm.

Trypanorhyncha Diesing, 1863,

Homeacanthoidea Dollfus, 1942, Tentaculariidae

Poche, 1926

Nybelinia Poche, 1926
Nybelinia lingualis (Cuvier, 1817) Dollfus,1927

Parasitological indexes: P = 57 %, MI = 12.5, RI = 1-28, MA = 7.1; infection sites: S, SM, I, SpS, AM, DM; total of collected specimens: 427 plerocercoids; deposited specimens: CHIOC 37340, 37341, 37342, 37343a-c and 37344a-b; examined samples: CHIOC 32568, 34397, 34398, 34399a-d.

Remarks: The morphology of the collected specimens are in accordance with that described for larval specimens referred by São Clemente & Gomes (1989) in the elasmobranchs *Mustelus canis* (Mitchill, 1815) and *M. schmitti* Springer,

1939, and for the oncotaxy of adults reported by Gomes *et al*. (2005) in *Isurus oxyrinchus* Rafinesque, 1810; hosts were captured in the Brazilian coast. The present findings are in accordance with the statement of Palm (1999) affirming that *Nybelinia lingualis* has a wide geographical distribution and low host specificity. The larvae of *N. lingualis* collected now, were found in several infection sites and among those the abdominal and dorsal musculature, and inducing an intense infection in the stomach mucosa, with macroscopic hemorrhagic lesions.

Paralichthys isosceles is a new host record for *N. lingualis*.

Heteronybelinia Palm, 1999.
Heteronybelinia nipponica (Yamaguti, 1952) Palm, 1999

Synonym: *Nybelinia rougetcampanae* Dollfus, 1960; *Heteronybelinia rougetcampanae* (Dollfus, 1960) Palm, 1999.

Parasitological indexes: P = 35%, I = 4.2, RI = 1-26, MA = 1.5; sites of infection: I, AC, KS, AM; total of collected specimens: 77 plerocercoids; deposited specimens: CHIOC 37345, 37346, 37347 and 37348; examined samples: CHIOC 32567a, 34500, 34502, 36424.

Remarks: Most of the specimens presently collected are very similar, concerning length, to those of *H. nipponica* recovered from specimens *Genypterus brasiliensis* Regan, 1903 by São Clemente *et al.* (2004), and remarkably smaller than the plerocercoids found in specimens of *Sphyrna zygaena* (Linnaeus, 1758) by Knoff *et al.* (2004); both hosts were captured in the Brazilian littoral. Morphometric variation in the size of the scolex and tentacular hooks in this species had already been observed by Palm & Walter **(**2000), after considering *H. rougetcampanae* synonymous of *H. nipponica*; the species were said to be distinct on the basis of the different sizes of the related specimens. In the samples collected now, spiniform microtriches distributed on the bothrial edges were observed, as reported for plerocercoids parasitizing specimens of *Menticirrhus americanus* (Linnaeus, 1758) and *Umbrina canosai* Berg, 1895 by Pereira Jr. & Boeger (2005), and referred to as "cuticular spines". These structures were observed in the adult of *H. nipponica*, recovered from a specimen of *Sphyrna lewini* (Griffith & Smith, 1834) by São Clemente & Gomes (1992) and in the plerocercoid of *H. nipponica* from *Carcharhinus signatus* (Poey, 1868) by Knoff *et al.* (2004), all from the Brazilian coast. Nevertheless, sensory fossette with microtriches, as reported by Pereira Jr. & Boeger (2005) were not observed so far. The oncotaxy of plerocercoids recovered from specimens of *P. isosceles* is in accordance with the description of *H. nipponica*, since present the typical basal tentacular armature billhooks, with metabasal just above the billhooks without uncinate hooks and small bulbs, different from those observed in specimens of *H. yamaguti* (Dollfus, 1960) Palm, 1999, that show, after the last row of "billhooks", uncinate hooks and very enlarged bulbs (Knoff *et al*., 2004; Palm, 2004).

Paralichthys isosceles is a new host record for *H. nipponica.*

Otobothrioidea Dollfus, 1942, Otobothriidae Dollfus, 1942.
Otobothrium Linton, 1890
Otobothrium sp.

Parasitological indexes: P = 15 %, MI = 1.9, RI = 1-3, MA = 0.3; sites of infection: AC, I, M, S, L; total of collected specimens: 17 plerocerci; deposited specimens: CHIOC 37349, 37350, 37351a-b and 37352; examined samples: CHIOC 36428, 36429, 36430 and 36482.

Description (n=3): Scolex 250-300 µm (274 µm) long, 162-207 µm (178 µm) wide (without the appendix). Bothrium 157-187 µm (176 µm) long, (179 µm) wide. Pars vaginalis 132-195 µm (156 µm) long, bulbs 75-92 µm (80µm) long, 37-50 µm (42 µm) wide, pars bulbosa 82-100 µm (89 µm) long, 97-145 µm (114 µm) wide, velum (30 µm) long and appendix 50-62 µm (56µm) long.

Remarks: Plerocerci of *Otobothrium* sp. have been reported in specimens of *Balistes vetula* (Linnaeus, 1758) from the Brazilian coast by São Clemente *et al*. (1995), in the musculature, with no reference to parasitological indexes. Alves *et al*. (2005) although registering a prevalence of 20%, did not report to infection sites. Plerocerci of *O. cysticum* (Mayer, 1842) Dollfus, 1942 were found in specimens of *Scomberomorus maculatus* (Mitchill, 1815) and *Sphyraena guachancho* Cuvier, 1829

captured in the Brazilian northeast littoral (Palm, 1997), with prevalence of 18.8% and 25%, respectively, in the body cavity, and in specimens of *G. brasiliensis* by São Clemente *et al*. (2004) with prevalence of 17.6%, in the mesentery and celomatic cavity. During observation of specimens recovered from *G. brasiliensis*, the presence of a lateral bothrial pit and bill-hooks stippled in the basal armature were observed; these structures are exclusive to *O. propecysticum* Dollfus, 1969, and were referred by Beveridge & Justine (2007), in the revision of the genus, when considered *O. cysticum* as *species inquirenda*, taking into account the fact that the tentacular armature and mature specimens are unknown. Thus, specimens recovered from *G. brasiliensis* have to be referred to as *O. propecysticum*. In the plerocercum of *Otobotrium* sp., from *B. vetula*, cited by Alves *et al*. (2005), the presence of the bothrial pit and extroverted tentacles could not be observed; for this reason, the analysis of the oncotaxy and further identification were not attained. As there is no indication of deposit referring to the material collected by Palm (1997), the referred samples were unobserved. The plerocerci from *P. isosceles* presented inconspicuous tentacular hooks, bothridial structures and bothridial pits and thus, were not specifically identified.

Paralichthys isosceles is a new host record for *Otobothrium* sp.

Pterobothriidae Pintner, 1931, *Pterobothrium* (Diesing, 1850).
Pterobothrium heteracanthum Diesing, 1850

Parasitological indexes: P = 1.7%, I = 1, A = 0.017; site of infection: AM; total of collected specimens: 1 plerocercoid; deposited specimens: CHIOC37353; examined samples: CHIOC 31.925 a-b, 33798 a-d, 33799, 33800a-c, 34020a-b.

Remarks: The analysis of the studied specimen agrees with the description of *P. heteracanthum* presented by Campbell & Beveridge (1996). Pterobothriidae Pintner, 1931 was extensively revised by these authors that have examined specimens of *P. heteracanthum*, recovered from *Micropogonias furnieri* (Desmarest, 1823) captured in the Brazilian coast, referred by São Clemente (1986), detailing the oncotaxy of the species. The samples presently collected were also compared to those recovered from sciaenid hosts, deposited in the CHIOC (Pereira Jr. & Boeger, 2005), confirming the specific identification.

Paralichthys isosceles is a new host record for this cestode.

Pterobothrium crassicolle Diesing, 1850

Parasitological indexes: P = 1.7%, I = 4, A = 0.067; site of infection: StS; total of collected specimens: 4 plerocerci; deposited specimens: CHIOC 37354a-d; examined samples: CHIOC 31926a-b, 33622, 33689, 33690 and 33902a-b.

Remarks: *Pterobothrium crassicolle* has been registered in several marine and freshwater fishes in Brazil (Porto *et al*., 2009). The morphology of this cestode species recovered from *P. isosceles* agrees with the description of *P. crassicolle* (São Clemente, 1986; Rego, 1987; Campbell & Beveridge, 1996). The morphological comparison was made with specimens collected in *M. furnieri* by São Clemente (1986) and by Pereira Jr. & Boeger (2005), in *Oligoplites palometa* (Cuvier, 1832) by Takemoto *et al*. (1996b), thus confirming the specific identification.
Paralichthys isosceles is a new host record for *P. crassicolle.*

Grillotiidae Dollfus, 1969
Grillotia Guiart, 1927
Grillotia carvajalregorum (Carvajal & Rego, 1983) Menoret & Ivanov, 2009
(Figs.3-6)

Synonymy: *Progrillotia dollfusi* Carvajal & Rego 1983.
Parasitological indexes: P = 73 %, MI = 11.7, RI =1-56, MA = 8.6; sites of infection: AC, S, StM, I, L, AM, DM; total of collected specimens: 514 plerocerci; deposited specimens: CHIOC 37363a-e; examined samples: CHIOC 32018a (holotype), 32018b-d (paratypes), 33719-33735, 36372, 36373, 36431, 36432, 36519a-b, 36674, 36684 and 36698.

Remarks: Carvajal & Rego (1983) described the species *P. dollfusi* on the basis of plerocerci. Pereira Jr. & Boeger (2005) redescribed the peculiar armature of the basal region, that presents five hooks encircling the tentacle, anterior to the first semicircular row of principal hooks. Menoret & Ivanov (2009) re-named the species as *Grillotia*

carvajalregorum, after the finding of adult specimens recovered from *Squatina guggenheim* Marini, 1936, in Argentina. The plerocerci collected now were compared to those deposited in the CHIOC obtained in the Brazilian coast and presenting the same pattern referring to oncotaxy and microtriches, as observed by Pereira Jr. & Boeger (2005) e Menoret & Ivanov (2009).

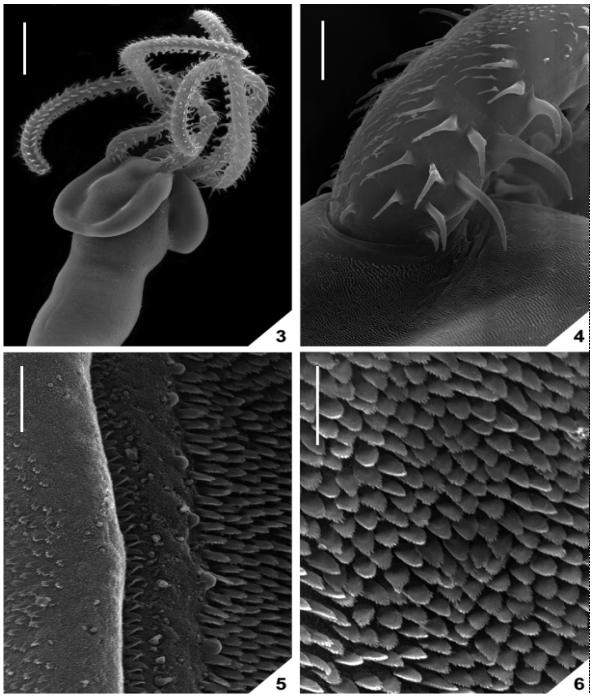
In the present study *G. carvajalregorum* appeared with the highest parasitological indexes when compared to the other species, except for the mean intensity. High parasitological indexes have been referred to other hosts from the Brazilian littoral, as occur in *Cynoscion guatucupa* (Cuvier, 1830) P = 90.48%, MI = 2683.46, MA = 2427,90, *Cynoscion jamaicensis* (Vaillant & Bocourt, 1883) P = 81.67%, MI = 433.45, MA = 353.98; *Macrodon ancylodon* (Bloch & Schneider, 1801) P = 96.67%; MI = 792.21 MA = 765.8 by Pereira Jr. & Boeger (2005); in *Pseudopercis numida* Miranda-Ribeiro, 1903 and *P. semifasciata* (Cuvier, 1829) P= 71% and 65.2%, MI= 2.8 and 4.7, respectively by Luque *et al.* (2008). The infection sites cited by those authors were the mesentery and celomatic cavity; presently, in specimens of *P. isosceles*, besides these sites, parasites occurred in the abdominal and dorsal musculatures. Adults of this cestode reported from *S. guggenheim* in Argentina, also appeared with a high prevalence (83%) in the six investigated hosts (Menoret & Ivanov, 2009). This is a new host record for *G. carvajalregorum*.

Poecilacanthoidea Dollfus, 1942,
Lacistorhynchidae Guiart, 1927,
Callitetrarhynchus Pintner, 1931
Callitetrarhynchus gracilis (Rudolphi, 1819)
Pintner, 1931

Parasitological indexes: P = 3.33%, I = 1, MA = 0.033; site of infection: AM; total of collected specimens: 2 plerocercerci; deposited specimens: CHIOC 37355 and 37356; examined samples: CHIOC 31924a-b, 32496, 32564, 33618, 33686, 33687, 33904a-b, 34327, 33328, 34303, 34308, 34509, 35005, 36374, 36433, 36532 and 36669.

Remarks: The morphology of the two specimens of *Callitetrarhynchus gracilis* recovered from *P. isosceles* is in agreement with previous descriptions by Dollfus (1942), Carvajal & Rego (1985) and Palm (2004). This species has been reported from several Brazilian teleosteans and elasmobranchs (Luque *et al*., 2000, 2008; Silva *et al*., 2000a, b; Luque & Alves, 2001; Knoff *et al*., 2002; Cordeiro & Luque, 2004; São Clemente *et al*., 2004, 2007; Pereira Jr. & Boeger, 2005; Alves & Luque, 2006; Ferreira *et al*., 2006; Pinto *et al.*, 2006; Dias *et al*., 2009). The finding of this species of Poecilacanthoidea in *P. isosceles* confirming previous reports, and also its low host specificity, since it has been collected from various infection sites of teleosteans.

Paralichthys isosceles is a new host record for *C. gracilis*.



Figures 3-6. Plerocercus of *Grillotia carvajalregorum*, scanning electron micrographs.
Fig. 3. Scolex, lateral view. **Fig. 4.** Hooks of basal armature, bothrial surface. **Fig. 5.** Distal bothrial surface, near bothrial groove. **Fig. 6.** Microtriches on distal bothrial surface. Scale bars: Fig. 3 = 200 µm; Fig. 4 = 30 µm; Fig. 5 = 5 µm; Fig. 6 = 4 µm.

Tetraphyllidea Carus, 1863, *Scolex* Müeller, 1788
Scolex pleuronectis Müeller, 1788
(Fig. 7)

Parasitological indexes: P = 40 %, MI = 9, RI = 1-70, A = 3.6; total of collected specimens: 216 plerocercoids; sites of infection: S, I; deposited

specimens: CHIOC 37357a-b, 37358, 37359, 37360, 37361 and 37362; examined samples: *Scolex pleuronectis* CHIOC 31952, 32672, 32673, 32674, 34304, 34309a-b, 34331, 34501, 34502, 34525, 34573, 34709a-b, 36245, 36547, 36554, 36414, 36679, 36689, 37155, 37157 and 37158; *Scolex* sp. CHIOC 33331, 33332 a-b, 33333 a-b, 33334 a-c, 33335, 33619, 33688, 36483, 36533, 36540 and 36210.

Description (n=6): Plerocercoids 405-4000 µm (1810 µm) long, 90-310 µm (197 µm) wide. Retractile apical myzorhynchus (invaginated), 55-265 µm (150 µm) long, 70-325 µm (164 µm) wide. Four bothridia (inverted) divided into numerous loculi by a single longitudinal septum and several transverse septa, contained in pouches with 80-365 um (214 µm) long, 5-200 µm (125 µm) wide. Botridial stalks present.

Remarks: The morphology and morphometrics of specimens of *S. pleuronectis* recovered from *P. isosceles* present similarities with the scolices of adults of *Echeneibothrium* Van Beneden, 1850, presenting scolex with apical retractile myzorhynchus anf four pedunculate bothrids divided into transversal septa along with a single longitudinal septum, that are characteristic of this genus. Chambers *et al.* (2000) remark that in several species, the morphology related to the scolex of larval tetraphylids can be different in the adults. Euzet (1994) includes the species of this genus in Echeneibothriinae de Beauchamp, 1905. The morphology of the plerocercoids described now, is similar to that reported by Chambers *et al*. (2000) for the metacestode Type 5 (fig3a, p. 291) presenting the four evaginated bothridia divided into numerous loculi by a single longitudinal septum and several transverse septa, and also the so called "apical sucker" (= apical myzorhynchus). Authors suggest that these larvae could be included in Rhinebothriinae Euzet, 1953, in one of the genera: *Rhinebothrium* Linton 1889, *Caulobothrium* Baer, 1948 or *Rhabdotobothrium* Euzet, 1953, although adult cestodes classified in these genera does not present the "apical sucker", perhaps due to the degeneration of this structure during the development to the adult stage. Based on the morphology and morphometrics of the specimens here studied, their similarity with the species of *Echeneibothrium* is considered. Healy *et al*. (2009) refer that this genus of Echeneibothriinae should be included in the new order Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood, 2009 (synonym of Tetraphyllidea Carus, 1863 *in part*), with other five genera of Echeneibothriinae, considering the bothridia with stalks as observed in species of *Echeneibothrium*. Comparison of larval *Scolex* spp. deposited in the CHIOC, and recovered from fishes captured in the littoral of Rio de Janeiro, with the specimens studied here, similarities were observed in relation to the plerocercoids of *Scolex* sp. (*Scolex* sp.2), from *Mugil platanus*, by the presence of four pedunculate bothrids with stalks, divided into 12 transversal extroverted loculi with apical myzorhynchus as well as to the plerocercoids *S. pleuronectis* recovered from *Priacanthus arenatus* Cuvier, 1829; *Katsuwonus pelamis* (L.); *Tylosurus acus* (Lacepède, 1803); *Pseudopercis numida* Miranda-Ribeiro, 1903 and *P. semifasciata*

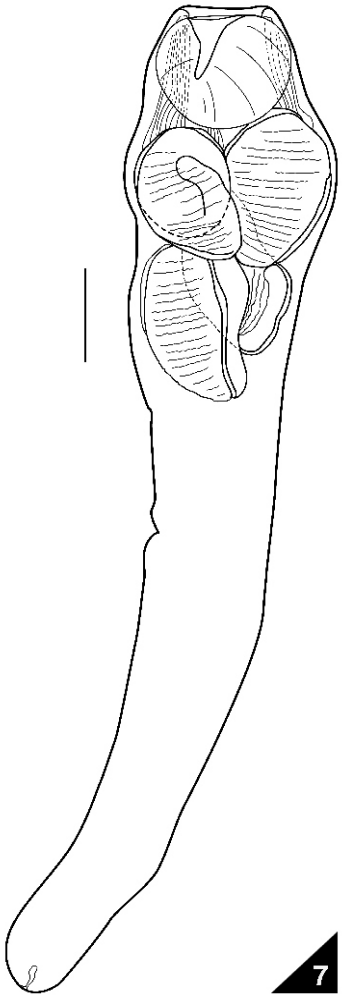


Figure 7. Plerocercoid of *Scolex pleuronectis*. Scale bar = 200 µm.

(Cuvier, 1829), that present bothria with retracted stalks inside the scolex.

Other plerocercoids deposited in the CHIOC as *Scolex* sp. and *S. pleuronectis* were also observed; considering that the samples were devoid of evaginated penduculate stalked bothria in the scolex, they are to be reconsidered in other genera apart from Rhinobothriidea sensu Healy *et al.* (2009). Those metacestodes are deposited and identified as *Scolex* sp. from *Mugil platanus* Günther, 1880 (*Scolex* sp.1), from *Orthopristis ruber* (Cuvier, 1830), *Haemulon steindachneri* (Jordan & Gilbert, 1882), *Oligoplites saliens* (Bloch, 1793) and as *S. pleuronectis* from *Pomatomus saltatrix* (Linnaeus, 1766), *M. furnieri*, *Trichiurus lepturus* Linnaeus, 1758, *Caranx latus* Agassiz, 1831, *Pagrus pagrus* (Linnaeus, 1758.), *G. brasiliensis*, *Paralonchurus brasiliensis* (Steindachner, 1875), *Scomber japonicus* Houttuyn, 1782, *Balistes capriscus* Gmelin, 1789, *Euthynnus alleteratus* (Rafinesque, 1810), *Sarda sarda* (Bloch, 1793), *S. brasiliensis*, *Katsuwonus pelamis* (Linnaeus, 1758), and *Anchoa tricolor* (Spix & Agassiz, 1829) (CHIOC 36258, some hooks were observed in the specimen of this sample).

High prevalence indexes of parasitism by this cestode have been already reported by Silva *et al.* (2000b) in *T. lepturus* (P = 96.3 %), Alves *et al.* (2002a) in *G. brasiliensis* (P = 87.3 %), Alves *et al.* (2003) in *S. japonicus* (P = 30 %), Alves *et al.* (2004) in *Urophycis brasiliensis* (Kaup, 1858) (P = 36 %) and Luque *et al.* (2008) in *Pseudopercis numida* (P = 43.6 %) and *P. semifasciata* (P = 53 %), from the littoral of Rio de Janeiro, thus confirming the present findings. Lower prevalence indexes were reported by Knoff *et al.* (1997) (P = 4.7 %, *Scolex* sp.1, and 0.7 %, *Scolex* sp.2) in *Mugil platanus*, Luque *et al.* (2000) (P = 3.6 %) in *C. latus*, Paraguassú *et al.* (2002) in *P. pagrus* (P = 5.5 %) and Alves *et al.* (2005) in *B. capriscus* (P = 10.7%). Previous data related to plerocercoids morphologically similar to those of the present study, namely *Scolex* sp.2 in *Mugil platanus,* and *S. pleuronectis* in *P. numida* and *P. semifasciata* were reported by Knoff *et al.* (1997) and Luque *et al.* (2008).

Paralichthys isosceles is a new host record for *Scolex pleuronectis.*

DISCUSSION

Considering that data on cestodes infecting specimens of *P. isosceles* are unavailable, results of the present investigation were compared to those related to other species of *Paralichthys* from other localities. Oliva *et al*. (1996) reported to adults of Pseudophyllidea (= Diphyllobotriidea?) of an unidentified genus, parasitizing the intestine of *P. adpersus* (Steindachner, 1867), in the North of Chile (P = 4.5 %, MA= 0.07) what differs from the results here obtained, when plerocercoids of *Diphyllobothrium* sp.1 and *Diphyllobothrium* sp.2 were recovered. Chilean authors have also referred to metacestodes of Trypanorhyncha, a plerocercoid of *Nybelinia surmenicola* Okada, 1929 and one plerocercum of *Lacistorhynchus dollfusi* Beveridge & Sakanari, 1987, whereas during the present investigation, other seven different species were recovered. The tetraphyllid *S. pleuronectis* was referred in the intestine (P = 5.7 % and MA= 0.29), in the same infection site here considered, but with remarkably higher parasitological indexes.

Castilho-Sánchez *et al*. (1998) reported to tetraphylid cestodes parasitizing specimens of *Paralichthys californicus* (Ayres, 1859), from Bahía de Todos Santos, Estero de Punta Banda, Bahía de San Quintín, Baja California and Pacific ocean in Mexico. Cestodes were found infecting the stomach, intestine and ceca, with prevalence ranging from 1 to 6, depending on the collection area; higher parasitic indexes were similar to those presently observed although with plerocercoids morphologically different (four sessile bothridia).

Considering ichthyo-sanitary approaches, the presence of Trypanorhyncha metacestodes in the musculature is harmless to humans only affecting the hosts besides the repugnant aspect conferred to the meat, often rejected by consumers and thus impeding its commercialization after the sanitary inspection (São Clemente *et al*., 2004). Recent studies dealing with medium sized or huge Trypanorhyncha plerocerci as *Molicola horridus* (Goodsir, 1841) Dollfus, 1942 and *Gymnorhynchus gigas* Robinson, 1959, have demonstrated that their ingestion can trigger allergenic processes in humans (Gòmez-Morales *et al*., 2008). The presence of medium sized Trypanorhyncha plerocerci as those of *P. crassicolle*, *P. heteracanthum* an *C. gracilis*, found

parasitizing the flounders presently investigated, even appearing with low parasitological indexes, must be of concern, mainly considering the species infecting the musculature (Dias *et al.*, 2009, 2010). Considering the occurrence of metacestodes in the musculature of fish specimens, as those studied now, it is suggested that the affected areas should be removed in order to permit the commercialization of the meat, in agreement with data after Amato *et al.* (1990) and São Clemente *et al.* (2004).

The finding of *Diphyllobothrium* spp. plerocercoids in the musculature of specimens of the flounder, indicates the risk of human contamination (Knoff *et al.* 2008), thus emphasizing the importance of alerting the sanitary inspection services and of adopting proper procedures for the control of this zoonosis. In order to avoid the spreading of helminth infections, individual efforts have to be made aiming at the change of acquired alimentary habits, mainly those related to the ingestion of raw or poor cooked fish, thus increasing the possibility of human infections (McCarthy & Moore, 2000; Knoff *et al.*, 2008).

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