TRYPANORHYNCHA CESTODES PARASITES OF GUAIVIRA IMPORTANT IN SEAFOOD HYGIENE

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ABSTRACT

Fish belonging to *Oligoplites* genus are found along the Americas' Atlantic coast. Cestodes belonging to the Trypanorhyncha order, although not possessing zoonotic character, can provide repulsive aspect to seafood; studies have shown that these cestodes may lead antibody responses when ingested. This study aimed to search cestodes belonging to the Trypanorhyncha order with importance in fish inspection, infesting the commercial fish named "guaivira", which comprises three species: *Oligoplites saurus; O. palometa; O. saliens*; they were marketed in the cities of Niterói (Rio de Janeiro State) and Ubatuba (São Paulo State), Brazil. Necropsies were performed in 56 specimens of this fish and parasites' larvae were collected. Among the studied fish, 19 (33.9%) fish were infested by cestodes larvae belonging to the Trypanorhyncha order. In total, 47 plerocerci belonging to the *Pterobothrium crassicolle* species were collected, having the mesentery and serosa of the intestine as infection sites. This is the first record of *Pterobothrium crassicolle* infesting *O. saurus*. It was concluded that the *Oligoplites saurus* is an intermediate host of *Pterobothrium crassicolle*.

Keywords: fish inspection; *Oligoplites saurus; Pterobothrium crassicolle.*

CESTOIDES TRYPANORHYNCHA PARASITOS DE GUAIVIRA IMPORTANTES NA HIGIENE DO PESCADO

RESUMO

Os peixes pertencentes ao gênero *Oligoplites* são encontrados ao longo do litoral atlântico das Américas. Os cestoides da ordem Trypanorhyncha, apesar de não possuírem caráter zoonótico, podem conferir aspecto repugnante ao pescado; estudos demonstram que estes cestoides podem induzir respostas humorais quando ingeridos. O presente estudo objetivou pesquisar cestoides da ordem Trypanorhyncha com importância na inspeção do pescado infestando o peixe comercial guaivira, que compreende três espécies: *Oligoplites saurus, O. palometa, O. saliens,* comercializados nos municípios de Niterói, RJ e Ubatuba, SP. Foram necropsiados 56 espécimes deste peixe e as larvas de parasitos coletadas. Dos peixes estudados, 19 (33,9%) estavam parasitados com larvas de cestoides da ordem Trypanorhyncha. Foram coletados no total, 47 plerocercos da espécie *Pterobothrium crassicolle,* tendo como sítios de infecção o mesentério e serosa dos intestinos. Este é o primeiro registro de *P. crassicolle* infestando *O. saurus.* Concluiu-se que a espécie *O. saurus* é um hospedeiro intermediário de *Pterobothrium crassicolle.*

Palavras–chave: inspeção de pescado; *Oligoplites saurus; Pterobothrium crassicolle.*

Artigo Científico: Recebido em 14/04/2016 – Aprovado em 19/07/2016

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INTRODUCTION

Oligoplites genus includes many species of fish; three of them occur on the Brazilian coast and in the waters of the Western Atlantic and Eastern Pacific, i.e., *O. saliens, O. saurus,* and *O. palometa*, also known popularly as guaivira or tibiro. They are species inhabiting coastal waters of low salinity and are found mainly in bays; they feed on small fish and crustaceans (MENEZES and FIGUEIREDO, 1980). The Trypanorhyncha order houses great species diversity; the teleost fish are one of its intermediate hosts, and elasmobranch fish are its definitive hosts (CAMPBELL and BEVERIDGE, 1996). Although the cestodes in this order have no zoonotic character, some studies have shown their capacity to cause humoral responses in mices wich shows that they may cause the same responses in humans (MATTOS et al., 2013). In addition, these parasites are important in the seafood inspection by causing repugnant aspect when the infestation is massive; consequently, this fact causes the fish disposal. This study aimed to deepen the knowledge in relation to *Oligoplites* genus, regarding to parasitism by cestodes species with relevance for the fish inspection.

MATERIAL AND METHODS

From November 2014 to October 2015, 56 samples of 3 species of the *Oligoplites* genus (15 *of O. saurus*, 20 of *O. saliens*, and 21 of *O. palometa*) were acquired from Sepetiba Bay, Rio de Janeiro State, and from the county of Ubatuba, São Paulo State, Brazil. Fishes were packed with plastic bags and placed in cool boxes with ice to avoid their deterioration and ensure good conditions for collection of parasites. The tests were carried out in the Laboratory of Inspection and Fish Technology of Fluminense Federal University, located in Niterói City, Rio de Janeiro State, Brazil.

Each host had its species determined based on the key for identification of teleost marine fish in Southeastern Brazil, proposed by MENEZES and FIGUEIREDO (1980). The found cestodes blastocysts were collected, examined under a stereomicroscope, and disrupted with stylets for releasing the larvae, which were transferred to Petri dishes containing saline solution of 0.65% NaCl and kept in the refrigerator for 24 hours for tentacles' exposure. After this period, the larvae were fixed in alcohol, formaldehyde, and acetic acid (AFA) for 24 hours, and then they were stained with hydrochloric alcohol carmine of Langeron and clarified in Faia's creosote; later, they were mounted between slide and cover slip with Canada balsam, in accordance with the technique described by EIRAS et al. (2006). Studies of REGO (1987), CAMPBELL and BEVERIDGE (1996), and PALM (2004) were used to identify cestode Trypanorhyncha larvae. Scanning electron microscopy technique was used to observe structures and confirm the species of found parasite. Larvae were fixed in 70% ethanol and then dehydrated in a series of ethanol (70-100 °GL), dried by critical point of CO_2, coated in gold, examined, and photographed by scanning electron microscope (JEOL SM-25 SII) under 15kV accelerating voltage. The parasitic indexes of prevalence (P), mean intensity (MI), and mean abundance (MA) were analyzed in accordance with BUSH *et al.* (1997); representative parasite specimens were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC) (Helminthological Collection of the Oswaldo Cruz Institute), Fundação Oswaldo Cruz-Fiocruz (Oswaldo Cruz Foundation), Rio de Janeiro City, Rio de Janeiro State, Brazil, under numbers 38215, 38216.

The measures of the structures were obtained through a microscope Olympus BH2 equipped with millimetric ocular, which are in millimeters.

RESULTS

Among the 56 examined fish, 19 (33.9%) fish were parasitized by cestodes belonging to Trypanorhyncha order, *Pterobothrium crassicolle* species, contained in blastocysts, and 47 parasites were collected with the parasitic rates of Prevalence (P) = 71, 4%; mean intensity (MI)=2.05 and mean abundance (MA)=2.87 at *Oligoplites palometa*; and P=26,7%, MI = 1 and MA = 0,27 at *Oligoplites saurus,* and having the mesentery and serosa of the

intestine as infection sites. Among them, 43 parasites were collected from *O. palometa* and 4 collected from *O. saurus*; parasites were not found on studied specimens of *O. saliens*.

Table 1 shows the morphometric data of *Pterobothrium crassicolle,* collected in *Oligoplites saurus* and *Oligoplites palometa,* presenting their range of variation and the average between parentheses.

Table 1 - Morphometric data of *Pterobothrium crassicolle* collected on *Oligoplites saurus* and *Oligoplites palometa.*

Measured portions	***Oligoplites palometa***	***Oligoplites saurus***
Pars botrialis (C)	600 – 630 (616,6)	595 – 623 (611)
Pars botrialis (L)	923 – 1120 (1007,6)	912 – 1107 (1039,7)
Pars vaginalis (C)	3225 – 4000 (3675)	3215 – 3987 (3567,3)
Pars bulbosa (C)	1008 – 1176 (1082)	1005 – 1164 (1083)
Pars bulbosa (L)	953 – 984 (967)	950 – 978 (963,7)
Pars post – bulbosa	224 – 315 (274,6)	218 – 309 (259)
Basal hooks of principal row		
1, 1′.	56 – 61 (58,3)	53 – 60 (56,3)
2, 2′.	52 – 55 (53,3)	51 – 54 (52,3)
3, 3′.	28 – 30 (29)	26 – 29 (27,7)
4, 4′.	40 – 45 (42,3)	39 – 43 (41)
5, 5′.	14 – 17 (15,3)	13 – 17 (15)
Metabasal hooks of principal row		
1, 1′.		
2, 2′.	44 – 48 (46)	43 – 49 (46)
3, 3′.	46 – 50 (47,3)	46 – 51 (48,3)
4, 4′.	60 – 63 (61,3)	59 – 62 (60,3)
5, 5′.	70 – 74 (72)	69 – 75 (72)
	74 – 78 (75,6)	72 – 77 (74,7)

L= length; W= width

The main morphological characteristics observed in 3 plerocerci derived from *O. palometa* and *O. saurus* were: plerocercus with blastocyst with caudal extension (Fig. 1), that shows four pyriform bothrios (Fig. 2), thin scolex, prebulbar organ absence; Bulbs elongate. Armature heterocanthous with band, hooks heteromorphous, hollow. Distinctive basal armature and swelling present. Metabasal armature consists of alternating half-spiral rows of 5 principal hooks each. Rows begin on internal face. Band of microhooks occupies space between terminations of principal rows on external face. Principal hooks 1(1') widely separated, falciform in proximal region, hooks 1(1') becoming smaller, stout and uncinate distally. Hooks 2(2') falciform, decreasing in size in distal metabasal region. Hooks 3(3') falcate with

short base and heel, gradually decreasing in size distally. Hooks 4(4') and 5(5') of proximal 12 rows digitiform, hooks 4(4') become falciform at row 13, hooks 5(5') remain digitiform along entire file but markedly reduced in length in distal region of tentacle. Principal rows in metabasal region separated by 2 - 4 rows of intercalary hooks (Fig. 3). Number of intercalary rows, hooks per intercalary row and hook size decrease toward tip of tentacle. In proximal metabasal region 4 intercalary rows present, reduced to 3 rows in distal region. External face of tentacle armed with band of spiniform microhooks. Basal armature distinctive. Internal face with 3 rows of hooks spiraling to bothrial face, opposite 2 rows spiraling to antibothrial surface. External surface armed with compact patch 11 - 12 rows of closely spaced hooks; proximally first 3 rows of hooks bent spiniform; distally rows 4 - 12 changing from spiniform hooks, to sickle-shaped hooks, as row proceed from sides to external surface; most distal hooks of this group are uncinate.

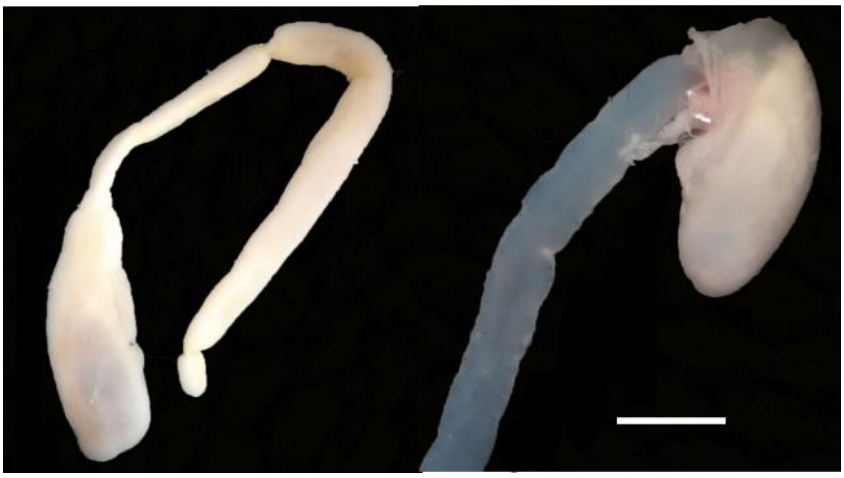

Figure 1. Plerocercoid with blastocyst with caudal extension of *Pterobothrium crassicolle* collected on *Oligoplites saurus.* Bar = 0, 3 cm.

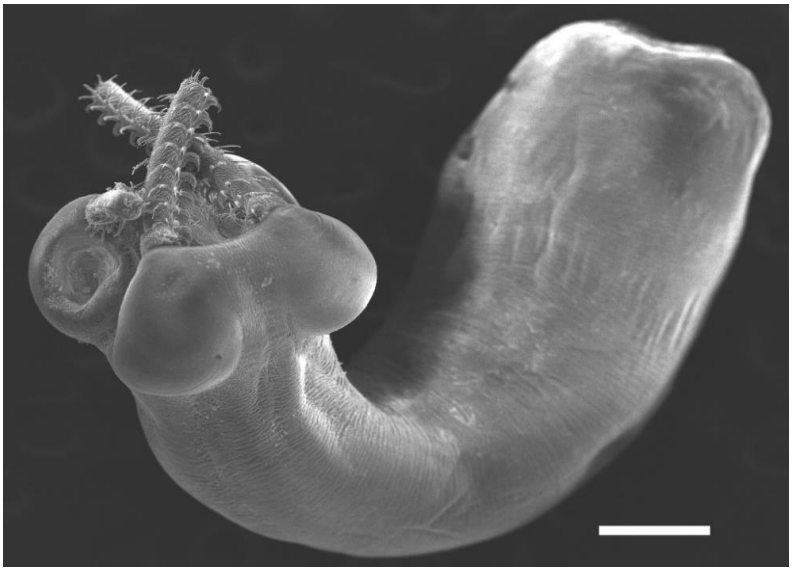

Figure 2. Pterobothrium crassicolle in scanning microscopy. Bar = 500 µm.

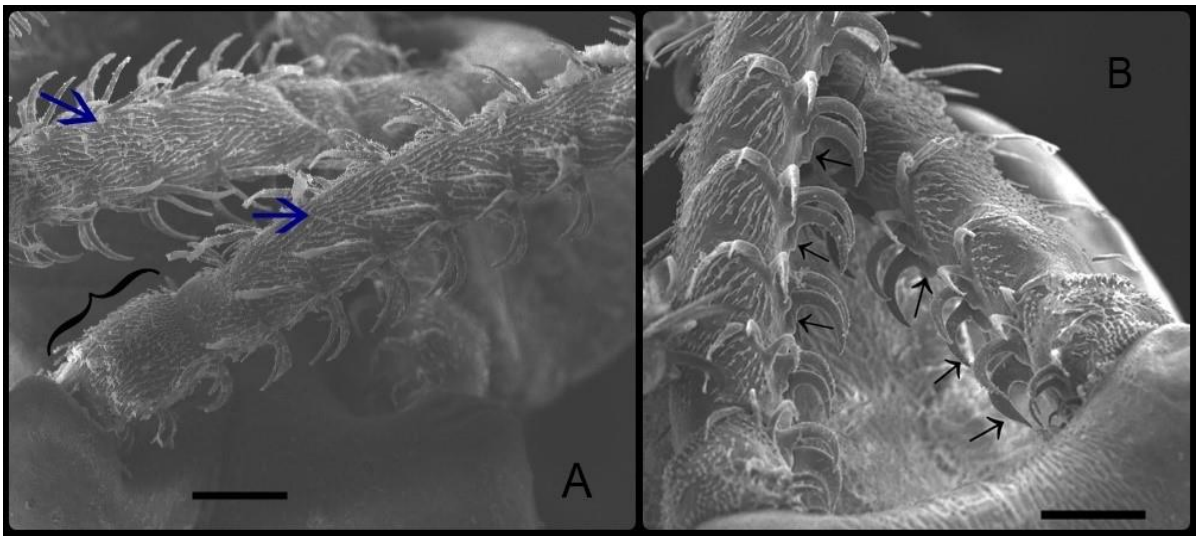

Figure 3. Pterobothrium crassicolle in scanning microscopy. A) blue arrows indicate groups of intercalary microhooks to principals hooks on external face; black key indicate distinctive basal armature and swelling present (Bar = 200µm); B) black arrows indicate alternating half-spiral rows of 5 principal hooks each, that begins on internal face (Bar = 100µm).

The identified sites of infection were the mesentery and the serosa of the intestine. Evaluating the prevalence and mean intensity in each studied host, it was found that the prevalence (P=71.4%) of *Pterobothrium crassicolle* on *O. palometa* was much higher than that one observed by TAKEMOTO *et al.* (1996) with P=2.4%, while the MI=2.04 was slightly higher than the one reported by TAKEMOTO *et al.* (1996), in which, MI=1.5.

DISCUSSION

The *Pterobothrium crassicolle* morphology observed in this study is consistent with the species redescription performed by REGO (1987), based on six specimens of CHIOC, which had the following average measures: (1) *pars botridialis* (0.480 x 0.912 mm); (2) *pars vaginalis* (2.52 mm); (3) *pars bulbous* (1.08 mm); (4) *pars post bulbous* (0.840 mm); (5) sickle metabasal hooks (the longest with 0.09 to 0.105 mm; the lowest with 0.07 to 0.08 mm); (6) internal metabasal hooks (0.04 to 0.06 mm); and (7) lateral metabasal hooks (0.055 to 0.09 mm). The *Pterobothrium crassicolle* morphology observed in this study is also consistent with the study of CAMPBELL and BEVERIDGE (1996), which performed revision of the family Pterobothriidae. Previous study by TAKEMOTO *et al.* (1996) with specimens of *O. saurus, O. palometa,* and *O. saliens* coming from Sepetiba Bay, Rio de Janeiro State, has identified the parasitism by larval stages of *Pterobothrium crassicolle* only on *O. palometa* specimens. LUQUE and POULIN (2004) have listed the parasitism by helminths in 50 species of Brazilian fish, including *O. saliens*, *O. palometa,* and *O. saurus;* just *O. palometa* was listed as host of *Pterobothrium crassicolle*. In both studies, *Scolex pleuronectis* and *Callitetrarhynchus gracilis* species of cestodes are described as parasites of these species, but they were not found in this study.

In previous studies, data were not found in relation to parasitism on *O. saurus*, in which, P=26.6% and MI=1; thus, comparisons were not able to be done.

This study, therefore, makes the first report of *Oligoplites saurus* as an intermediate host of *Pterobothrium crassicolle*.

CONCLUSIONS

In the study of guaiviras' parasitism, Oligoplites saurus, Oligoplites saliens, Oligoplites palometa marketed in the states of Rio de Janeiro and São Paulo, cestode larvae of Pterobothrium crassicolle were found at Oligoplites saurus and Oligoplites palometa, registering Oligoplites saurus as an intermediate host for the species for the first time.

Although *Pterobothrium crassicolle* has not a recognized zoonotic potential, besides the repulsive aspect they can cause, the possibility of leading to hypersensitivity reactions on humans demonstrates the importance of these parasites in fish inspection.

On the basis of the results and on the potential risk to consumer health, it is suggested attention during the sanitary inspection process by the authorities and also during the quality control by fisheries industries for the presence of these parasites in fishes for human consumption.

ACKNOWLEDGEMENTS

Thanks to Mr. Torres, Eduardo José Lopes, Romero Lascasas Porto Helminthology Laboratory, Department of Microbiology, Immunology, and Parasitology, School of Medical Sciences, State University of Rio de Janeiro (UERJ), Rio de Janeiro City, Rio de Janeiro State, Brazil, for conducting the scanning microscopy.

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