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Histopathology and autoecology of *Didymocylindrus simplex* (Digenea: Didymozoidae), parasite of *Katsuwonus pelamis* (Scombridae) in the Southwestern Atlantic Ocean, off South America

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ABSTRACT. Pathological alterations and autoecology of *Didymocylindrus simplex* in *Katsuwonus pelamis* (Linnaeus, 1758) in the Southwestern Atlantic Ocean off of South America are presented. This is the first record of the species in the region; the specimens are described. The pathologic changes observed most often were infiltration of lymphocytes and slight fibrosis of the connective tissue surrounding the parasite. The typical aggregated distribution pattern was observed for *Didymocylindrus simplex* Ishii, 1935. Statistical analysis demonstrated no correlation between the abundance and prevalence of parasites with the total length of the host. The sex of the host does not influence the prevalence and intensity of infection.

KEY WORDS. Didymozoids; histopathology; parasites of fish; Trematoda.

Digenean trematodes of the family Didymozoidae Monticelli, 1888, parasitizing marine fishes, predominantly Scombridae are widely documented in the literature. However, most publications correspond to taxonomic studies and only a few investigate the pathological reactions in the host and report data on the parasitological parameters of the species.

Continuing our studies of helminth parasites of scombrid fishes off the coast of Rio de Janeiro, Brazil, Southwestern Atlantic Ocean, *Katsuwonus pelamis* (Linnaeus, 1758) was found parasitized by *Didymocylindrus simplex* Ishii, 1935.

Katsuwonus pelamis is a species of great importance in Brazil because of the large fish canning industry. It is an epipelagic, oceanic species with adults distributed roughly within the 15°C isotherm, whereas larvae are mostly restricted to waters with surface temperatures of at least 25°C. Food items of this species include fishes, crustaceans and molluscs and cannibalism is common (Colette & Nauen 1983). Others studies about the helminthofauna of *K. pelamis* from the Brazilian coast were published by Kohn & Justo (2006, 2008) and Justo & Kohn (2005, 2012a, b).

This study describes, for the first time, the pathological alterations induced by *D. simplex* on *K. pelamis*. It also provides new geographical distribution records for didymozoid parasites of scombrid fish in South America, Southwestern Atlantic Ocean. The specimens collected are described.

MATERIAL AND METHODS

Sixty-one specimens of *K. pelamis* (26-73 cm in total body length and 1-8 kg in weight), including 30 males (49.2%) and 31 females (50.8%), were examined for helminths between January, 2004 and April, 2011.

Fishes were obtained by local fishermen from the coastal zone of the State of Rio de Janeiro, off Cabo Frio, Brazil (22°52'46"S, 42°01'07"W). Parasites were released from their cysts and fixed under cover glass pressure in AFA (alcohol 93%, formalin 5%, acetic acid 2%), stained in alcoholic-acid carmine, dehydrated in an alcohol series, cleared in methyl salicilate and mounted in Canada balsam. Analysis of the parameters of infection prevalence (%), mean intensity and range of infection were based on Bush *et al.* (1997) and the confidence intervals (CI) were calculated assuming a binominal distribution, using the software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000).

The quotient between variance and mean parasite abundance (index of dispersion) was used to determine distribution patterns and was tested by the *d* statistical index (Ludwig & Reynolds 1988).

The Spearman's rank correlation coefficient (r_s) was used to determine possible host-length correlations with parasite abundance. The Pearson's coefficient of correlation (r) was used to determine possible correlations between the host's total

length and prevalence of parasites, with angular transformation of prevalence values; the Mann-Whitney's U Test with Z_c normal approximation, to determine host sex effect on infection/infestation abundance of each parasite species (Zar 1996). A statistical significance level of $p \leqslant 0.05$ was adopted. Measurements are in micrometres, unless otherwise stated, with the mean in parentheses followed by the number of specimens measured in brackets, where applicable. Light micrographs were taken with a digital camera connected to a Nikon Eclipse E 800 microscope. Representative specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

For the histopathological analysis, gills of *K. pelamis* were removed, fixed in 10% formalin, decalcified with EDTA (tetrasodium ethylenediaminotetracetate) for three days to one month and prepared through a routine technique of dehydration in ethanol and then impregnation and embedding in paraffin. Sections of 5-10 µm were made with microtome. The sections were stained with hematoxylin and eosin (H&E) for general visualization of the affected tissues and organs (Michalany 1980).

TAXONOMY

Didymocylindrus simplex (Ishii, 1935), Yamaguti, 1970

Figs 1-3, 6-7

Description of studied specimens (measurements based on 10 adult specimens): Cylindrical yellow cysts, readily visible in gills (Figs 1 and 2) containing two hermaphroditic individuals similar in shape and size. Body divided into two distinct regions: anterior region elongate, widened in esophageal region, attached at ventral part of posterior region of body, which is larger, cylindrical and rounded at both ends (Fig. 3). Anterior region measures 400-900 (600) [n = 8] long by 80-130 (110) [n = 8] wide. Oral sucker terminal, 17-25 (19) [n = 9] long by 18-26 (20) [n = 9] wide, followed by muscular pharynx 22-37 (30) [n = 9] long by 22-35 (28) [n = 9] wide. Ventral sucker absent. Oesophagus varying in length according to state of contraction. Caeca narrow anteriorly, extending to posterior region of body. Posterior region, 1.4-3.1 (2.1) [10] mm long by 0.4-0.8 (0.6) [10] mm wide. Testes two, elongate, usually unequal in length, 185-315 (263) [n = 3] x 75-100 (92) [n = 3], at anterior margin of posterior region of body. Vas deferens extends anteriorly alongside well-developed metraterm. Genital pore ventrolateral to oral sucker. Ovary tubular, single, unbranched, measures 50-120 (80) [n = 8] wide. Vitelline gland tubular, single, unbranched, measuring 50-95 (73) [n = 3] wide. Uterus forming extensive coils occupying all available space in posterior region of body. Egg reservoir not observed. Eggs small, bean-shaped, embryonated, 12-15 (14) [n = 30] long by 7-10 (9) [n = 30] wide.

Host: *Katsuwonus pelamis*, skipjack tuna.

Locality: Off Cabo Frio (22°52'46"S, 42°01'07"W), Rio de Janeiro, Brazil, Southwestern Atlantic Ocean.

Site: Encysted in pairs in the epidermis of gill lamellae under the basal membrane.

Specimens deposited: Voucher CHIOC # 37081 a-g, 37082, 37083 a-b, 37809.

Prevalence: 39.3% (CI = 27.0-52.6).

Mean intensity: 15.0 (CI = 11.1-21.8).

Range of infection: 2-56.

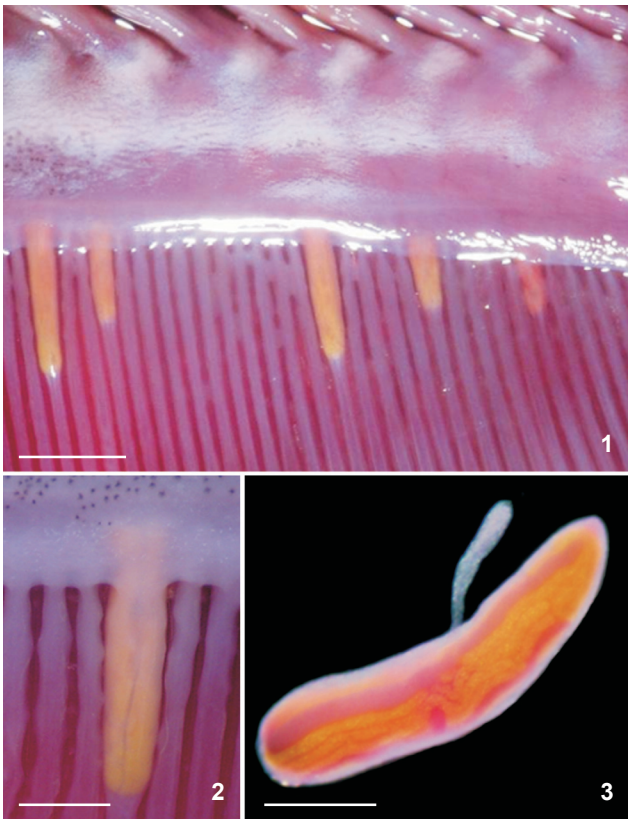
Remarks: *Didymocylindrus* Ishii, 1935 is presently represented by nine species: *D. filiformis* Ishii, 1935 (type species), *D. apharyngi* (Job, 1961), *D. fusiforme* (Ishii, 1935), *D. gasterale* (Abdul-Salam and Sreelatha, 1995), *D. koti* (Yamaguti, 1938), *D. operculare* (Madhavi, 1982), *D. simplex* (Ishii, 1935), *D. singularis* (Job, 1966), and *D. sphyraenae* (Yamaguti, 1959). Among them, only *D. fusiforme* (= *Didymoproblema fusiforme*) has been reported in the Southwest Atlantic (Justo & Kohn 2005). *Didymocylindrus simplex* was originally described by Ishii (1935) from the gills of *K. pelamis* from Japan and redescribed from the same host from Hawaii by Yamaguti (1970). Lester *et al.* (1985) reported it from *K. pelamis* collected in different areas of the Pacific Ocean and from Porto Rico, North Atlantic Ocean. Pozdnyakov (1996) provided original measurements and figures of this parasite from *K. pelamis* and *Australuzza novazelandica* (*sic*).

Didymocylindrus simplex were found parasitizing the epidermis of gill lamellae under the basal membrane of 24 of 61 specimens (39.3%) of *K. pelamis* with one to 28 cysts containing two similar hermaphroditic parasites with a total of 360 specimens. *Didymocylindrus simplex* showed a typical aggregated distribution pattern (DI = 21.2, d = 33.5). The Pearson's and Spearman's coefficients demonstrated no correlation between the abundance and prevalence of parasites and the total length of *K. pelamis* ($r = -0.075$, $p = 0.626$; $r_s = -0,047$, $p = 0.763$). The mean abundance and prevalence were not influenced by the sex of the hosts ($Z = -1.71$, $p = 0.087$).

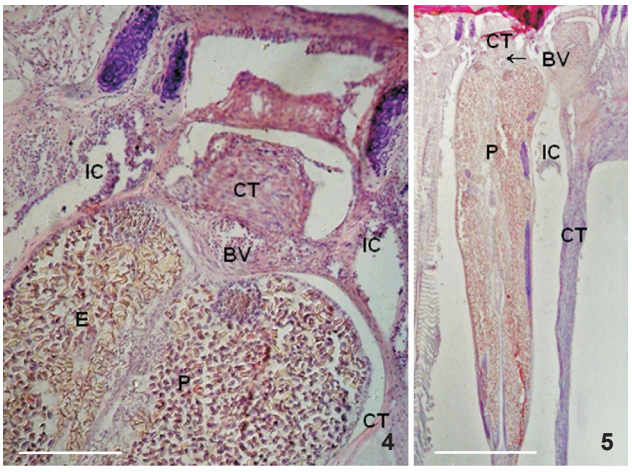
Histopathological analysis demonstrated that *D. simplex* infected the space between the basement membrane of the epithelium of the gill and the efferent artery of the primary gill lamellae (Fig. 4). A slight fibrosis of connective tissue surrounding the parasite was verified, formed by a few layers of collagenous tissue and fibroblasts and a slight inflammatory infiltrate with lymphocytes and granulocytic cells such as monocytes and neutrophils. Adjacent to the gill arch, in the place where the encystment of the parasite occurs, a fibrotic reaction with some blood vessels was observed, between the gill arch and the parasite. Besides that, a slight inflammatory infiltrate was observed adjacent to the fibrotic reaction (Figs 4 and 5).

DISCUSSION

Katsuwonus pelamis from the coast of Rio de Janeiro were highly parasitized by *D. simplex*. The specimens of this Digenea are described for the first time from off the Atlantic coast of



Figures 1-3. Photomicrographies of *Didymocylindrus simplex*: (1) gill lamellae of *Katsuwonus pelamis* infected with *D. simplex*; (2) cyst of *D. simplex* with two worms; (3) total. Scale bars 1 = 3 mm, 2-3 = 1 mm.



Figures 4-5. Histological sections of *Didymocylindrus simplex* in gill arch and lamellae of *Katsuwonus pelamis*. Scale bars: 4 = 200 µm, 5 = 500 µm. (BV) Blood vessel, (CT) connective tissue, (E) eggs, (IC) inflammatory cells, (P) parasite. Hematoxylin and eosin.

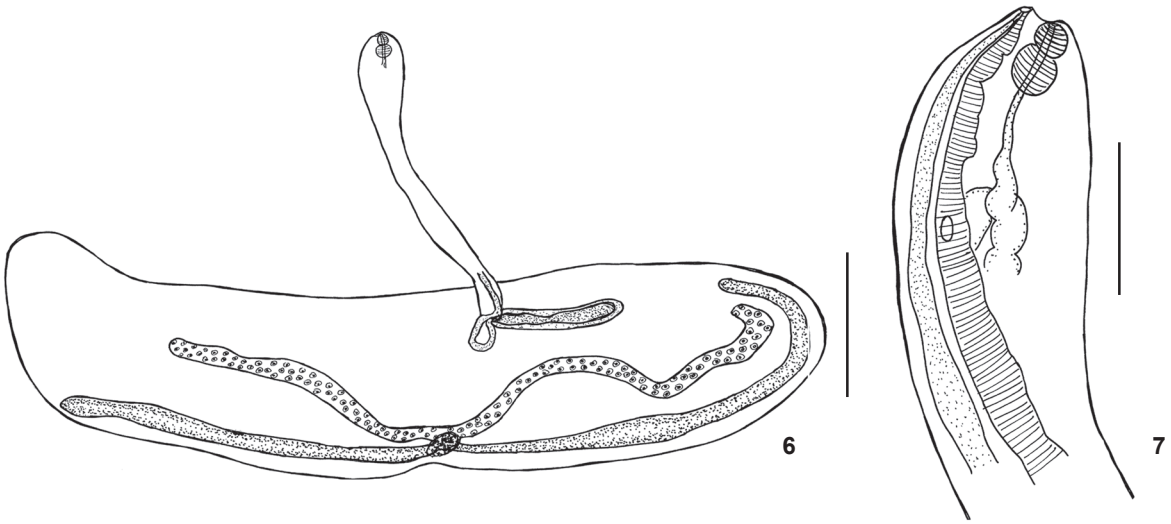
South America and histopathological studies are presented. No correlation was observed between the abundance and prevalence of the parasites and the total length of the hosts. According to Luque & Alves (2001), correlations between total length of the host and prevalence and abundance of the parasite is a pattern widely observed in marine fishes off the coast of Rio de Janeiro. However, this pattern cannot be generalized because in many host-parasite systems the correlations are positive but weak and non-significant (Saad-Fares & Combes 1992, Poulin 2000).

In our results we observed that the sex of the host does not influence the prevalence and intensity of infection. According to Luque *et al.* (1996), the absence of correlations in parasite prevalence and abundance with the sex of the fish-host is a widely documented pattern, and interpreted as a consequence of the absence of sexual differences in some biological aspects of the fish.

According to Feist & Longhaw (2008), the gill is a primary target organ and pathogenicity usually depends on the intensity of infection where the presence of large number of parasites or cysts compromises respiratory functions. Most studies of histopathology of fishes associated with parasitism by didymozoids in gills arches, operculum, dermi and palate have demonstrated that the general components of the host defense responses are basically similar in all hosts, being restricted to the affected area, and do not appear to harm the fish (Lester 1980, Eiras & Rego 1987, Abdul-Salam & Sreelatha 1992, Pereira 1992a,b, 1994, Cruz-Lacierda *et al.* 2001, Mladineo 2006, Di Maio & Mladineo 2008, Justo *et al.* 2008, Tudkaew *et al.* 2008).

In this study, no gross histopathological alterations were found in the gills of *K. pelamis* parasitized by *D. simplex*; we observed that the parasite occupies a space between the basement membrane of the epithelium and the efferent artery and causes a typical chronic inflammatory reaction in the host, characterized by cellular infiltration (leucocytes) and connective tissue fibrosis. These results are consistent with those of Perera (1992a, b) and Mladineo (2006), who observed that the first response in the development of the parasitic cyst is the organization of a connective tissue envelope that firmly surrounds the cyst in different species of didymozoids. The envelope consists of few layers of collagenous tissue, with abundant fibrocytes and fibroblasts on the periphery, indicating the continuous activity of the process. For these authors, the absence of a strong inflammatory response was reflected in the absence of gross pathological signs on the gills. This suggests that didymozoids do not elicit a strong cellular response in the gills, but only the demarcation of the cyst by a proportionally thick capsule containing an abundance of connective elements in the invaded tissue.

Histopathological studies by Marino *et al.* (2003), with the cysts of *Unitubulotestis sardae* (MacCallum, 1916), showed fusion of secondary lamellae in the regions near the cyst and severe damage in the tissue where many cysts were present. The damage consisted of an infiltration of lymphocytes, plasmacells and eosinophil granulocytes, as well as the loss of



Figures 6-7. *Didymocylindrus simplex*: (6) total; (7) anterior extremity. Scale bars: 6 = 0.5 mm, 7 = 0.1 mm.

function of large areas of the respiratory mucosa. For Lester (1980) and Perera (1992a, b), neither of the didymozoids secreted a cyst wall and the only species found so far within a true cyst is the highly modified *Kollikeria filicollis* (Rudolphi, 1819), which was enclosed in a multi-layered wall of fibrous tissue with sheets of fibers and blood capillaries penetrating between the coils, so that the whole surface of the worm was in intimate contact with its host. On the other hand, in a study with didymozoid parasites, Perera (1994) noted that they appeared to be surrounded by a true capsule.

According to Williams (1959), in *K. filicollis* the capsules are a reaction of the host tissue against secretions from some of the subcuticular gland cells of the female worm, and the inner layer of the capsule is of parasitic origin, derived from discharge products of subcuticular gland cells.

On the other hand, Abdul-Salam & Sreelatha (1992) reported that the cyst of *Gonapodasmius epinepheli* is made up of a capsule composed predominantly by connective tissue of host origin. They found that the capsule was formed by three layers, which was not observed in this study. Lester (1980) also observed that light microscope studies on host defense response to didymozoids have indicated a typical chronic inflammatory reaction characterized by cellular infiltration and extensive fibrosis.

Perera (1992a) observed that the response of the host consisted mainly of a stretching of the lateral epithelium and the formation of a layer of columnar epithelial cells, and that this layer was observed above the basement membrane of the mature capsules. The formation of this layer may be due to a reaction of the host tissue to the parasite, and this type of epithelium was not observed in this study.

The results of the present study indicate that *D. simplex* does not pose risks to the hosts, since the changes observed in it were restricted only to the region where the parasite was inserted, and this is perhaps a reflection of the low degree of intensity of the parasite in the fish.

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