

# First record in South America of *Didymosulcus palati* and *Didymosulcus philobranthiarca* (Digenea, Didymozoidae) with new hosts records and pathological alterations

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*Two species of Didymozoidae, Didymosulcus palati (Yamaguti 1970) and Didymosulcus philobranthiarca (Yamaguti 1970) were reported for the first time in South America, Atlantic Ocean, parasitizing three different tuna species from the coast of Rio de Janeiro, Brazil: Thunnus atlanticus (Lesson), Thunnus albacares (Bonnaterre) and Thunnus obesus (Lowe). Pairs of D. philobranthiarca were found on gill arches of T. albacares and T. obesus, in longitudinal rows of yellow cysts located inside grooves in the hard denticle palate (new site) of the three hosts species studied, and as disperse groups of cysts in the operculum (new site) and gill arches of T. atlanticus (new host record). D. palati occurred as disperse groups of encysted worm pairs in the gill arches of T. albacares and T. obesus and in gill arches and operculum of T. atlanticus (new host record). The pathological alterations induced by D. philobranthiarca in the palate of T. atlanticus are described for the first time. Original measurements and figures are presented.*

Key words: *Didymosulcus palati* - *Didymosulcus philobranthiarca* - Didymozoidae - *Thunnus* spp. - pathological alterations

Three species of Scombridae from the coast of Rio de Janeiro were found to be parasitized with two different Didymozoidae species. *Thunnus atlanticus* (Lesson) is an epipelagic and small tuna, restricted to the Western Atlantic Ocean, which is commonly known as blackfin tuna and as “albacorinha” in Brazil. *Thunnus albacares* (Bonnaterre) is found worldwide in tropical and subtropical seas, above and below the thermocline, but is absent from the Mediterranean Sea, and is known as yellowfin tuna and as “albacora de lage” in Brazil. *Thunnus obesus* (Lowe) an epipelagic and mesopelagic species present in oceanic waters, occurs from the surface to a depth of about 250 m, and is found worldwide in tropical and subtropical waters of the Atlantic, Indian and Pacific Oceans, but is absent from the Mediterranean Sea; the species is commonly known as big eye tuna and as “albacora bandolim” in Brazil. These tuna species feed on a wide variety of fishes, crustaceans and cephalopods.

Trematodes of the family Didymozoidae are parasites of marine fishes mainly belonging to the families Scombridae, Exocoetidae, Serranidae and Sphyraenidae, found predominantly in tropical and subtropical waters (Nikolaeva 1985). The occurrence of *Didymosulcus palati* (Yamaguti 1970) Pozdnyakov (1990) originally described in the Pacific Ocean, and of *Didymosulcus philobranthiarca* (Yamaguti 1970) Pozdnyakov (1990) found in the Pacific and Indian Oceans, is reported for the first time in Brazil, representing the first record of

this species in South America and in a new host. Pathological alterations induced in *T. atlanticus* by *D. philobranthiarca* are also described for the first time.

## MATERIALS AND METHODS

Forty five *T. atlanticus* (45-82 cm total body length; 1.3-6.0 kg), 35 *T. obesus* (42-80 cm total body length; 1.2-8.0 kg) and 38 *T. albacares* (38-80 cm total body length; 0.55-7.8 kg), were examined from January 2004 to April 2007. The fishes were obtained from the Atlantic Ocean, off Cabo Frio, state of Rio de Janeiro, Brazil (22°52'46"S, 42°01'07"W). Samples of palate containing encysted worms were fixed in formalin 10% for pathology. For identification, the parasites were released from dissected cysts and fixed in AFA (alcohol, formalin, acetic acid), stained in alcoholic-acid carmine, dehydrated in alcohol series, cleared in methyl salicylate and mounted in Canada balsam. Measurements are in micrometers, with the mean in parentheses followed by the number of specimens measured in brackets. For the pathological studies, fragments of the mucous palate and operculum of *T. atlanticus* containing cysts of *D. philobranthiarca* were removed, immediately fixed in 10% formalin and sent to the Laboratório de Patologia da Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil. The material was then routinely processed for paraffin embedding and stained with hematoxylin and eosin (HE). Ecological terminology follows Bush et al. (1997). Statistical significance level was evaluated at  $p \leq 0.05$ . Photographs were taken using Sony digital camera. Light micrographs were taken with a digital camera connected to Nikon Eclipse E 800 microscope. The studied specimens are deposited in the Helminthological Collection of Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

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## RESULTS

*D. philobranthiarca* was found in longitudinal rows of yellow cysts (Fig. 1) located inside grooves in the hard denticle palate of the three hosts species studied and as disperse groups of cysts in the operculum and gill arches of *T. atlanticus* (new host record) and in gill arches of *T. obesus*. The cysts containing two identical specimens were covered with a thin, hard and transparent material. This parasite was detected in 17 of 35 *T. obesus* specimens examined, in 15 of 38 *T. albacares* specimens, and in 20 of 45 specimens of the new host *T. atlanticus* (Table). *D. palati* occurred as disperse groups of encysted worm pairs in the gill arches of *T. albacares* and *T. obesus* and in gill arches and operculum of *T. atlanticus* (new host record). This species was identified in 4 of 38 specimens of the type host *T. albacares*, in 8 of 35 *T. obesus* specimens, and in 5 out of 45 *T. atlanticus* specimens (Table).

Since the morphology of *D. philobranthiarca* and *D. palati* has been well described by Yamaguti (1970), we only present a brief description including the main findings.

*D. philobranthiarca* (Yamaguti 1970) Pozdnyakov, (1990) (Figs 1, 2)

Voucher specimens deposited: CHIOC n: 36934; 36935; 36936 a-c; 36937 a-c; 36938, 36939; 36940 a-b; 36941.

Measurements of 13 adult specimens: forebody 400-800 (590) [n = 9] by 60-100 (80) [n = 9] largest width; hindbody 2,000-4,700 (3,300) [n = 13] long by 400-900 (700) [n = 13] wide, comma-shaped, furrowed longitudinally with two distinct anterior lobes; mouth terminal, oral sucker pyriform 25-45 (35) [n = 6] long by 15-35 (22) [n = 6] wide; esophagus simple; caeca narrow in forebody, tubular in hindbody reaching to near posterior extremity. Testes sausage-shaped slightly curved, 500-800 (600) [n = 6] long by 50-140 (80) [n = 9] wide;



Fig. 1: cysts of *Didymosulcus philobranthiarca* (arrow) in the hard denticle palate of *Thunnus atlanticus*. Bar = 2.5 mm.

vas deferens running alongside metraterm in forebody and opening together, ventral to oral sucker. Ovary tubular divided into four branches, two anterior and two posterior. Seminal receptacle present. Vitelline gland with three main branches which bifurcates originating eight terminal branches. Uterine coils occupying most of available space of hindbody; eggs bean-shaped 15-17 (15) [n = 13] by 10-12 (10) [n = 13].



Fig. 2: photomicrography of *D. philobranthiarca*. Bar = 0.25 mm.

*D. palati* (Yamaguti 1970) Pozdnyakov (1990) (Fig. 3)

Voucher specimens deposited: CHIOC n: 36942; 36943 a-b; 36944; 36945; 36946 a-c; 36947; 36948; 36949 a-g.

Measurements based on 17 adult worms: forebody subcylindrical, flattened, 300-740 (460) [n = 15] long by 50-110 (70) [n = 15] wide attached to hindbody between two anterior lobes. Hindbody 1,400-2,700 (1,900) [n = 17] long by 500-900 (800) [n = 17] wide, with two anterior lobes and median ventral furrow and posterior extremity curved ventrally. Oral sucker terminal 25- 40 (31) [n = 12] long by 12-25 (21) [n = 12] wide; pharynx 15-22 (18) [n = 9] in diameter; esophagus narrow; caeca narrow in forebody and inflated in hindbody, ending near posterior extremity. Testes sausage-shaped, 350-480 (410) [n = 11] long by 50-130 (80) [n = 7] wide; vas deferens running alongside metraterm; genital pore ventrolateral to oral sucker. Ovary divided in two tubular main branches 30-50 wide, uniting together at the front end

TABLE

Prevalence, mean intensity and mean abundance of *Didymosulcus philobranthiarca* and *Didymosulcus palati* from three hosts

Host	<i>D. philobranthiarca</i>			<i>D. palati</i>		
	Prevalence (%)	Mean intensity	Mean abundance	Prevalence (%)	Mean intensity	Mean abundance
<i>T. atlanticus</i>	44.4 ± 15	254.4 ± 149.7	113.1 ± 73.9	13.3 ± 10	79.3.4 ± 109.9	11.5 ± 9.3
<i>T. albacares</i>	39.5 ± 16	419.5 ± 76.1	165.6 ± 121.8	10.5 ± 10	46.0 ± 49.4	4.8 ± 5.5
<i>T. obesus</i>	48.6 ± 17	143.7 ± 121.7	69.8 ± 61.1	22.9 ± 14	50.3 ± 30.2	11.5 ± 9.3

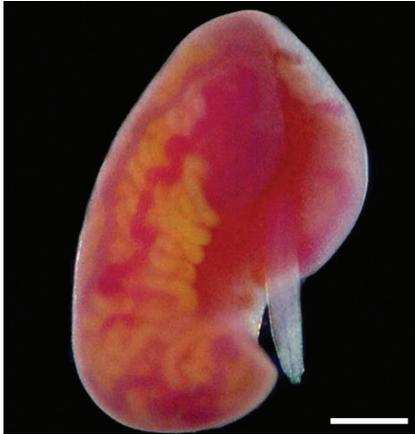


Fig. 3: photomicrography of *Didymosulcus palati*. Bar = 0.21 mm.

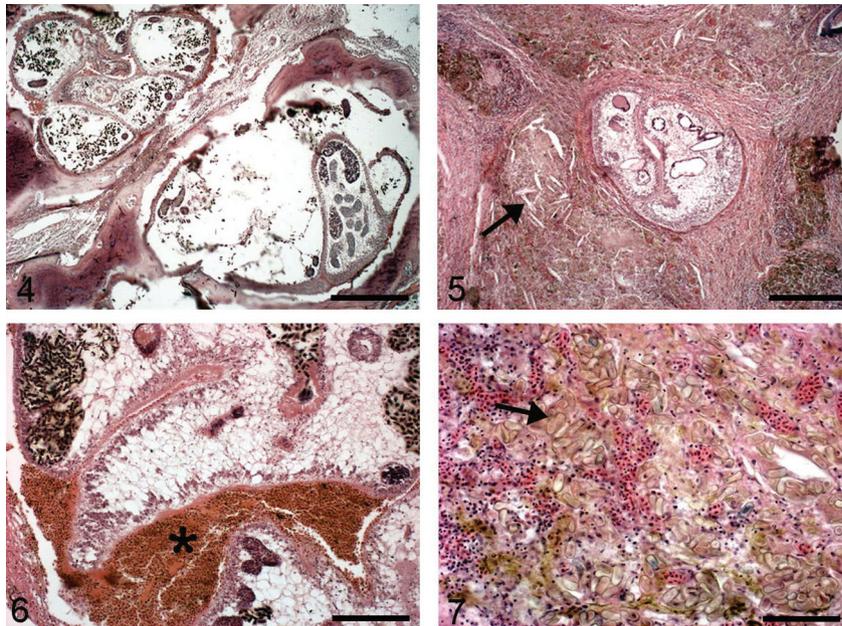
near the base of anterior lobes; one of the main branches may present a short side branch. Seminal receptacle and shell gland present. Four vitellarian tubules undivided, 20-50 (30) [n = 17] wide, extending along convex side of hindbody, dorsal to ovary, reaching to posterior end of hindbody, one branch terminating short of posterior extremity. Uterus occupying all available space of hindbody, egg reservoir oval, eggs bean-shaped 15-17 (15) [n = 17] long by 7-10 (10) [n = 17] wide.

*Pathological alterations induced by D. philobran-  
chiarca in palate of T. atlanticus* - Adult forms of *D. philobran-  
chiarca* encysted in pairs in the mucous of  
denticulate palate of *T. atlanticus* were surrounded by  
bone laminae (Fig. 4); red blood cells and egg masses

were frequently observed between the bone laminae and trematode tegument (Figs 6,7). The cysts were embed-  
ded in a highly vascularized fibrous connective tissue  
adjacent to bone laminae and surrounded by a connec-  
tive tissue capsule and red blood cells (Figs 4-6). This  
fibrous connective tissue was characterized by the pres-  
ence of a mixed inflammatory infiltrate, circular hemor-  
rhagic areas containing cholesterol clefts, and multiple  
egg masses surrounded by abundant brown-yellow pig-  
ment characteristic of melanomacrophage bodies and  
hemosiderin, observations suggesting parasitic migra-  
tion (Figs 4, 6). The eggs situated outside the parasites  
ranged from unstained to stained and contained acido-  
philic internal mass (Fig. 7).

### DISCUSSION

*Didymosulcus palati* was first identified by Yamaguti  
(1970) in hard denticle palate and partly in the tooth plate  
of gill arches of *T. albacares* (= *Neothunnus macropterus*)  
from Hawaii, Pacific Ocean. In the present study,  
this species was found encysted in gill arches of the  
three host species studied and in the operculum of  
*T. atlanticus* (new host record) from Brazil, Atlan-  
tic Ocean, representing the first record of this spe-  
cies in South America. Our specimens are similar to  
the original description, differing only in few mea-  
surements: forebody width ranging from 50-110 µm  
in our specimens and from 120-200 µm in type-speci-  
mens; oral sucker measuring 12-15 µm in our specimens  
and 21-93 µm in the type-material. A side branch of the  
ovary branches as described by Yamaguti (1970) was  
only observed in one out of the 17 worms examined.



Figs 4-7: histological sections of the palate of *Thunnus atlanticus* with *D. philobran-  
chiarca*. 4: pairs of parasites embedded in the bone tissue. HE. Bar = 0.20 mm; 5: encysted parasites surrounded by a connective tissue capsule and embedded in the highly vascularized fibrous connec-  
tive tissue adjacent to bone laminae showing mixed inflammatory infiltrate and circular hemorrhagic areas containing cholesterol clefts (arrow) HE. Bar = 0.22 mm; 6: *D. philobran-  
chiarca* surrounded by red blood cells (asterisk). HE. Bar = 0.11 mm; 7: eggs of *D. philobran-  
chiarca* (arrow) embedded in the fibrous connective tissue highly vascularized. These eggs were surrounded by abundant brown-yellow pigment, ranged from  
unstained to stained and contained acidophilic internal mass. HE. Bar = 0,06 mm.

*D. philobranthiarca* was originally described on gill arches of *T. albacares* (= *Neothunnus macropterus*) and *T. obesus* (= *Parathunnus sibi*) from Hawaii by Yamaguti (1970), in the genus *Didymocystis* and later transferred to *Didymosulcus* by Pozdnyakov (1990). This species was also reported in *T. obesus* and in *Thunnus alalunga* from Indian Ocean by Nikolaeva and Dubina (1985). In the present study, we found this species on the gill arches of *T. albacares* and *T. obesus* and in a new host, *T. atlanticus*, with the trematode parasitizing new sites, i.e., the palate of the three hosts species studied and the operculum of *T. atlanticus*.

The microscopic lesions associated with adult *D. philobranthiarca* trematodes and their eggs were severe and characterized by marked fibrosis, a chronic diffuse inflammatory process and hemorrhage. These results differed from those obtained by other investigators who reported that tissue infected with didymozoids usually presents little or even no tissue reaction or damage (Gibson et al. 1981, Karlsbakk 2001). Eiras and Rego (1987) and Pascual et al. (2006) also observed eggs masses, hemorrhages and lymphocyte infiltration in the connective tissue surrounding the parasitic nodules in the operculum of tuna fishes parasitized with didymozoids.

Kamegai (1971) reported the presence of a didymozoid species in the muscles of a marine fish in Japan and identified it as the adult worm of eggs found in human feces transmitted through fish consumption. According to this author, the presence of eggs of an unknown trematode species in human feces has been known in Japan since 1913. Chung and Cross (1975) found didymozoid-like eggs in stools samples of 41% of 158 school children from a Taiwan offshore island, which were probably acquired by the consumption of marine fish. Carney et al. (1987) and Cross et al. (1989) found the eggs of didymozoids, among other helminths, in human feces from the Philippines. Recently, Pascual et al. (2006) described numerous ribbon-like aggregations of eggs of a didymozoid embedded in the musculature of the Atlantic mackerel *Scomber scombrus* L. These authors stated that among the largest, most numerous, and most diversified assembly of muscle-invading marine parasites are the free or encysted stages of didymozoid digenean trematodes and anisakid nematodes.

Heavy infections can reduce the commercial value of tuna fish, mainly because of macroscopic alterations that make the flesh unappealing to eat (Lester 1979, 1980, Cribb & Williams 1992, Pascual et al. 2006). Thompson (2000) recorded emerging zoonoses and allergies associated with the presence of helminth parasites in fish muscle, emphasizing the economic and public health importance of instituting control measures for edible seafood during inspections. In this respect, the increasing consumption of raw or uncooked fish in Brazil associated with the occurrence of these parasites in tuna fish may represent a risk factor of human infection.

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