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NEW DATA ON THREE SPECIES OF *KOELLIKERIOIDES* (DIGENEA, DIDYMOZOIDAE) IN BRAZILIAN TUNA FISH

NUEVOS DATOS SOBRE TRES ESPECIES DE *KOELLIKERIOIDES* (DIGENEA, DIDYMOZOIDAE) EN ATÚN DE BRASIL

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Abstract

Four species of scombrid fishes (Perciformes) from the coastal zone of Rio de Janeiro, Brazil, were examined for helminth parasites. These are *Thunnus albacares* (Bonnaterre, 1788), *Thunnus atlanticus* (Lesson, 1831), *Thunnus obesus* (Lowe, 1839) and *Euthynnus alletteratus* (Rafinesque, 1810). Three species of the genus *Koellikerioides* Yamaguti, 1970: *K. apicalis* Yamaguti, 1970, *K. externogastricus* Yamaguti, 1970 and *K. intestinalis* Yamaguti, 1970, were recorded from the gill raker, the outer muscle layer of the stomach wall and the inner surface of the small intestine, respectively. Original measurements, parameters of infection and original figures are presented. *K. apicalis* and *K. intestinalis* showed the typical aggregated distribution pattern. Statistical analysis showed that there was a significant negative correlation between the total lengths of *E. alletteratus* and the prevalence of *K. intestinalis*. The findings of *K. apicalis* in *T. atlanticus* and of *K. intestinalis* in *T. atlanticus* and *E. alletteratus* represent new host records. All species are reported for the first time in the Southwest Atlantic Ocean.

Keywords: Fish parasites - *Koellikerioides apicalis* - *Koellikerioides externogastricus* - *Koellikerioides intestinalis* - Scombridae.

Resumen

Cuatro especies de peces escómbridos (Perciformes) de la zona costera de Río de Janeiro, Brasil, fueron examinados para estudiar sus helmintos parásitos: *Thunnus albacares* (Bonnaterre, 1788), *Thunnus atlanticus* (Lesson, 1831), *Thunnus obesus* (Lowe, 1839) y *Euthynnus alletteratus* (Rafinesque, 1810). Tres especies del género *Koellikerioides* Yamaguti, 1970: *K. apicalis* Yamaguti, 1970, *K. externogastricus* Yamaguti, 1970 y *K. intestinalis* Yamaguti, 1970, se registraron en las branquiespinas, en la capa muscular externa de la pared del estómago y en la superficie interna del intestino delgado, respectivamente. Se presentan las medidas originales, los parámetros de la infección y las ilustraciones originales. *K. apicalis* y *K. intestinalis* mostraron el patrón típico de distribución agregada. El análisis estadístico mostró que había una correlación negativa significativa entre la longitud total de *E. alletteratus* y la prevalencia de *K. intestinalis*. Los resultados de *K. apicalis* en *T. atlanticus* y de *K. intestinalis* en *T. atlanticus* y *E. alletteratus* representan nuevos registros de hospedadores. Todas las especies se reportan por primera vez en el Océano Atlántico Sudoccidental.

Palabras clave: Parásitos de peces-*Koellikerioides apicalis* - *Koellikerioides externogastricus* - *Koellikerioides intestinalis* - Scombridae.

INTRODUCTION

The Thunnini are unique among bony fishes in having counter-current heat exchange systems that allow them to retain metabolic heat, maintaining a warmer internal temperature than the surrounding water. Species of *Thunnus* are found worldwide and are known to migrate extensively (Collete & Nauem, 1993), except *Euthynnus alletteratus* (Rafinesque, 1810) that is less migratory than other tuna (Fischer, 1978). According Madhavi & Ram (2000) the high vagility and endothermy require high metabolic energy which is met through foraging on large quantities of food items comprising crustaceans, fish, mollusks and polychaetes that serve as intermediate and paratenic hosts for didymozoid and other helminth parasites. Didymozoids are parasites of marine teleosts, mainly scombrids and rarely are found in freshwater fishes. They represent a different group of parasites by the general morphology highly adapted to different habitats, not common to other digenean parasites.

In our studies with helminth parasites of Scombridae from Brazil, the most prevalent and abundant species belong to the Digenea family Didymozoidae Monticelli, 1888 (Justo & Kohn 2005, 2009, 2010; Kohn & Justo, 2008; Justo *et al.*, 2008, 2009) which agree with the results in different parts of the world (Ishii, 1935; Yamaguti, 1970; Lester *et al.* 1985; Nikolaeva, 1985; Pozdnyakov, 1996; Madhavi & Ram 2000; Munday *et al.*, 2003; Mladineo *et al.*, 2010). Brazil is the most important South American country for fishing of scombrids in the Southwest Atlantic Ocean, but very little is known about the parasites of these species.

The aim of this study is to contribute to the increase of the knowledge and expansion of the geographical distribution of didymozoid parasites of tuna in the area of the Southern Atlantic Ocean.

MATERIAL AND METHODS

A total of 149 specimens of tuna fishes were examined between January of 2004 and April of 2007: 38 specimens of *Thunnus albacares* (Bonnaterre, 1788) (34 - 76 cm total body length; 0.550 - 7.8 kg), of which 15 (39.4%) were males and 23 (60.6%) were females; 45

Thunnus atlanticus (Lesson, 1831) (45 - 82 cm total body length; 1.3 - 6.0 kg) 16 (35.5%) males and 29 (64.5%) females; 35 *Thunnus obesus* (Lowe, 1839) (42 - 80 cm total body length; 1.2 - 8.0 kg) 21 (60%) males and 14 (40%) females and 31 *Euthynnus alletteratus* (Rafinesque, 1810) (31 - 80 cm total body length; 0.525 - 4.0 kg) 16 (51.6%) males and 15 (48.4%) females.

The fishes were obtained from 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). The parasites were released from dissected cysts and fixed with or without compression in AFA (alcohol 93%, formalin 5%, acetic acid 2%), stained in alcoholic-acid carmine, dehydrated in an 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, where applicable. Analysis of the parameters of infection, related to prevalence (P), mean intensity (MI), mean abundance (MA) and range of infection (RI) were based on Bush *et al.* (1997). Confidence intervals (95%) were calculated assuming a binominal distribution, using software Quantitative Parasitology 3.0 (Rózsa *et al.*, 2000). The quotient between variance and mean of 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 parasitism abundance. 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; 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). The tests were only applied to species that showed prevalence higher than 10% (Bush *et al.*, 1990). Statistical significance level $p \leq 0.05$ was adopted. Light micrographs were taken with a digital camera connected to a Nikon Eclipse E 800 microscope and confocal laser scanning light micrographs with a ZEISS LSM 510. Representative specimens were deposited in

the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

RESULTS

During a survey of the helminth parasites of scombrid fishes from Rio de Janeiro coast, three species of didymozoid described by Yamaguti (1970) in the genus *Koellikerioides* were recovered. The trematodes were found parasitizing four species of scombrid fishes: *T. albacares*, *T. atlanticus*, *T. obesus* and *E. alletteratus*.

Specimens of *Koellikerioides apicalis* Yamaguti, 1970 were found encysted in pairs on the gill raker from two (4.4%) out of 45 *T. atlanticus* (new host record), four (10.5%) out of 38 *T. albacares* and two (5.7%) out of 35 *T. obesus* examined. Specimens of *Koellikerioides intestinalis* Yamaguti, 1970 were easily observed in the wall and lengthwise on the inner surface of the small intestine from 16 (35.8%) out of 45 *T. atlanticus* (new host record), eight (23.7%) out of 38 *T. albacares*, five (14.3%) out of 35 *T. obesus* and six (19.4%) out of 31 *E. alletteratus* (new host record) examined. Specimens of *K. externogastricus* Yamaguti, 1970 were found encysted in the external wall of the stomach from one (2.6%) out of 38 *T. albacares* examined.

Brief descriptions with main measurements are presented, considering that the morphology of the studied species was already originally well described. Measurements of *K. apicalis* and *K. intestinalis* are presented in Tables 1 and 2 respectively and parameters of infection in Tables 3 and 4.

K. apicalis and *K. intestinalis* showed a typical aggregated distribution pattern (Table 5). Pearson's correlation coefficient demonstrates that the prevalence of *K. intestinalis* was negatively correlated with the total length of *E. alletteratus* ($r = -0.596$, $P = >0.001$) (Table 6). There was no correlation between the abundance of parasites and the total length of their hosts (Table 6). The mean abundance and prevalence of *K. intestinalis* parasitizing *T. albacares* were influenced by the sex ($Z = -2.70$, $P = 0.044$; $F = 0.006$) occurring only in females (Table 7).

Koellikerioides apicalis Yamaguti, 1970 (Figs. 1A-D, Table 1)

Hosts: *T. atlanticus* (new host record), *T. albacares* and *T. obesus*.

Site of infection: Encysted in pairs in gill raker.

Deposition of voucher specimens: CHIOC n: 37143-37147, 37148 a-b.

Cysts are easily observed in the gill raker and contain a pair of worms (Fig. 1A) with strong sexual dimorphism expressed by the male being smaller than the female and located in a special cavity in the female's body. Measurements of specimens from the three hosts are presented in Table 1.

Male (Fig. 1C): Brief description based on three compressed specimens. Body divided into two regions (anterior and posterior). Anterior region scoop-shaped, widest at oesophageal level.

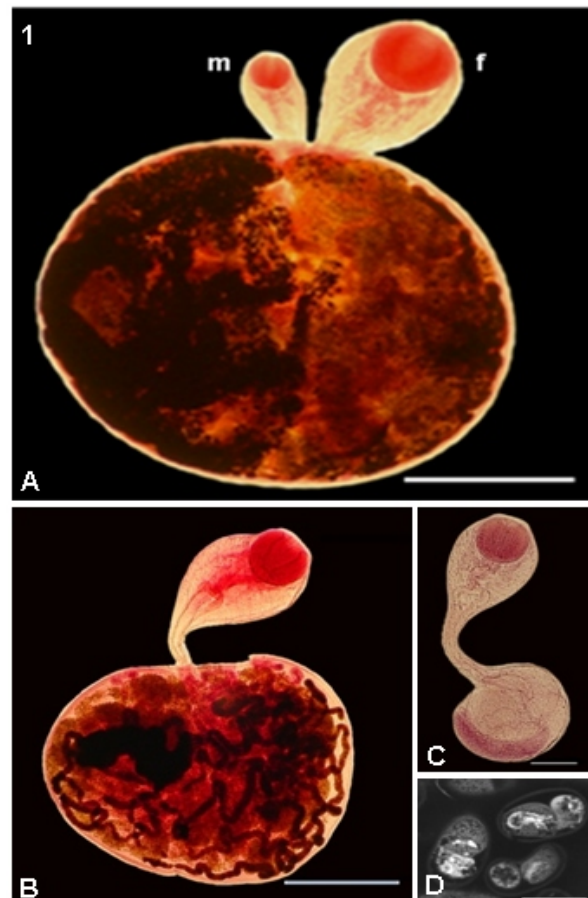


Figure 1. *Koellikerioides apicalis* Yamaguti, 1970: A: Light micrograph of male and female; B - Light micrograph of female; C - Light micrograph of male; D - Confocal scanning light micrograph of eggs. Scale bars= 500 µm (A, B); 200 µm (C); 10 µm (D).

Table 1.- Original measurements (μm) of females and males of *Koellikerioides apicalis* from *T. atlanticus* (new host record), *T. albacares* and *T. obesus*.

Hosts	<i>T. atlanticus</i> (new host)		<i>T. albacares</i>		<i>T. obesus</i>	
	♀	♂	♀	♂	♀	♂
Posterior region L	1200 – 1620 [n=2]	300 – 440 [n=2]	1320 – 2100 (1665) [n=4]	420 [n=1]	1300 [n=1]	-
Posterior region W	1640 – 2200 [n=2]	290 – 580 [n=2]	1700 – 2720 (2050) [n=4]	480 [n=1]	1500 [n=1]	-
Anterior region L	1000 – 1900 [n=2]	700 – 1600 [n=2]	1200 – 2000 (1625) [n=4]	860 [n=1]	1000 [n=1]	-
Anterior region W	460 – 640 [n=2]	200 – 360 [n=2]	460 – 720 (540) [n=4]	340 [n=1]	420 [n=1]	-
Oral sucker L	260 – 380 [n=2]	130 – 222 [n=2]	300 – 420 (365) [n=4]	200 [n=1]	240 [n=1]	-
Oral sucker W	280 – 400 [n=2]	125 – 217 [n=2]	310 – 440 (372) [n=4]	210 [n=1]	270 [n=1]	-
Pharynx L	50 – 122 [n=2]	30 [n=1]	115 [n=2]	-	-	-
Pharynx W	80 – 137 [n=2]	42 [n=1]	110 – 145 [n=2]	-	-	-
Oesophagus	125 – 250 [n=2]	100 – 125 [n=2]	175 – 310 (234) [n=4]	150 [n=1]	137 [n=1]	-
Ovarium W	40 – 60 [n=2]	-	40 – 62 (54) [n=4]	-	50 [n=1]	-
Vitelarium	40 – 65 [n=2]	-	40 – 62 (52) [n=4]	-	52 [n=1]	-
Eggs L	22 – 25 [n=10]	-	22 – 25 (23) [n=10]	-	22 – 25 [n=10]	-
Eggs W	15 [n=10]	-	15 (15) [n=10]	-	15 (15) [n=10]	-

L= lenght; W = width.

Posterior region rounded. Oral sucker well developed, globular, muscular, terminal; pharynx present; oesophagus surrounded by gland-cells, bifurcated into two caeca. Testis single, tubular; vas deferens opening ventral to oral sucker.

Female (Fig. 1B): Brief description of seven compressed specimens. Body divided into two regions (anterior and posterior). Anterior region of body scoop-shaped widest at level of oesophagus. Posterior region with a special cavity for male. Oral sucker terminal, muscular, globular, followed by pharynx; oesophagus surrounded by gland-cells, bifurcated into two caeca. Ovary long, bifurcated, with a total of four long winding terminal branches, ending in the middle of posterior region of body. Seminal receptacle oval, near anterior extremity of body's posterior region. Vitelline gland tubular,

ramified. Uterine coils occupying most of the available space in posterior region. Eggs bean-shaped, embryonated (Fig. 1D).

Remarks

Koellikerioides apicalis described by Yamaguti (1970) from the gill raker of *T. obesus* (= *Parathunnus sibi*) from Hawaii, Pacific Ocean, was reported by Pozdnyakov (1990, 1996) in *T. albacares*, *T. alalunga* (Bonnaterre, 1788) and *T. obesus* also from the Pacific Ocean. Mladineo & Tudor (2004) reported this species in the cartilaginous part of the gill arch and in the apical part of the pseudobranch of *Thunnus thynnus* (Linnaeus, 1758) from the Adriatic Sea. In the present study, *K. apicalis* was isolated from the gill raker of *T. obesus* (type-host), *T. albacares* and from a new host *T. atlanticus*. Morphology and

measurements of studied specimens agree with the original description except in the size of the anterior region of the male's body: 800-1600 long in our specimens compared to with 500-600 and 400-600 in Yamaguti's and Pozdnyakov's papers, respectively.

Koellikerioides externogastricus Yamaguti, 1970 (Fig. 2)

Host: *T. albacares*

Site of infection: Encysted in pairs in outer muscle layer of stomach wall.

Deposition of voucher specimens: CHIOC n: 37095 a-c.



Figure 2. *Koellikerioides externogastricus* Yamaguti, 1970. Light micrograph of male and female. Scale bar=2 mm.

Brief descriptions and main measurements based on compressed specimens of two males and three females. Cysts rounded and easily observed as yellowish prominences in outer muscle layer of stomach wall. Male smaller than female, placed in special cavity of female. Body divided into two regions (anterior and posterior) in both sexes. Male: Anterior region scoop-shaped 4600 – 5400 long by 220 – 360 wide at oesophageal level.

Posterior region reniform measuring, 900 long by 550 wide, enclosed in special hollow of female. Oral sucker muscular 102 – 120 long by 85 – 92 wide; pharynx 50 – 62 long by 35–50 wide; oesophagus bifurcated into two caeca. Testis single, cylindrical; vas deferens opening ventral to oral sucker.

Female: Anterior region of body scoop-shaped, 4400 – 5100 (4700) long by 320 – 550 (470) wide. Posterior region rounded, 5500 – 8300 (6930) long by 5200 – 9200 (6933) wide, with a special cavity to accommodate the male. Oral sucker muscular, 162 – 197 (180) long by 117 – 187 (152) wide, followed by pharynx 87 – 105 long [n=2] by 60 – 92 wide [n=2]; oesophagus narrow, sigmoid, bifurcated into caeca. Ovary long consisting of two long branches. Vitelline gland tubular, ramified, with several terminal branches. Uterine coils occupying most of the available space in posterior region. Metraterm well differentiated, opening ventral to the oral sucker. Eggs bean-shaped, embryonated, 20 long by 10 – 12 (10) wide [n=30].

Remarks

Koellikerioides externogastricus was found by Yamaguti (1970) encysted in the outer muscle layer of the stomach of *T. albacares* (= *Neothunnus macropterus*) and *T. obesus* from Hawaii, Pacific Ocean. It was later reported by Pozdnyakov (1990) from *T. alalunga*, *T. albacares* and *T. obesus* also in the Pacific Ocean. Our specimens were collected from the type-host *T. albacares*, in the same site of infection and the morphology and measurements agree with the original description.

Koellikerioides intestinalis Yamaguti, 1970 (Fig. 3, Table 2)

Hosts: *T. obesus*, *T. albacares*, *T. atlanticus* (new host record) and *E. alletteratus* (new host record).

Site of infection: Encysted in pairs on inner surface of small intestine.

Deposition of voucher specimens: CHIOC n: 37131 a-c, 37132 a-e.

Brief descriptions and main measurements (Table 2) based on compressed specimens of four males and eight females.

Table 2. Original measurements (μm) of females and males of *Koellikerioides intestinalis* from *T. atlanticus* (new host record), *E. alletteratus* (new host record), *T. albacares* and *T. obesus*.

Hosts	<i>T. atlanticus</i> (new host)		<i>E. alletteratus</i> (new host)		<i>T. albacares</i>		<i>T. obesus</i>	
	♀	♂	♀	♂	♀	♂	♀	♂
Posterior region L	610 – 800 (686) [n=5]	-	540 – 1000 (760) [n=5]	165 [n=1]	900 – 920 [n=2]	-	500 – 1600 (900) [n=8]	-
Posterior region W	1140 – 1640 (1338) [n=5]	-	990 – 1820 (1298) [n=5]	200 [n=1]	1680 – 1780 [n=2]	-	1000 – 2400 (1570) [n=8]	-
Anterior region L	600 – 695 (640) [n=3]	340 [n=1]	700 – 840 [n=2]	320 [n=1]	600 – 700 [n=2]	-	400 – 1400 (800) [n=5]	400 – 1400 (875) [n=4]
Anterior region W	105 – 225 (177) [n=3]	195 [n=1]	120 [n=1]	90 [n=1]	240 [n=1]	-	100 – 300 (180) [n=5]	100 – 320 (260) [n=4]
Oral sucker L	120 – 155 (140) [n=3]	95 [n=1]	90 – 150 (116) [n=4]	74 – 100 [n=2]	110 – 125 [n=2]	100 [n=1]	100 – 195 (149) [n=6]	75 – 120 (103) [n=4]
Oral sucker W	105 – 130 (118) [n=3]	75 [n=1]	80 – 125 (100) [n=4]	60 – 88 [n=2]	100 – 110 [n=2]	70 [n=1]	87 – 150 (122) [n=6]	50 – 110 (74) [n=4]
Pharynx L	-	-	36 [n=1]	20 [n=1]	-	-	60 – 95 (76) [n=5]	67 [n=1]
Pharynx W	-	-	28 [n=1]	24 [n=1]	-	-	55 – 90 (68) [n=5]	55 [n=1]
Eggs L	16 – 17 [n=10]	-	16 – 18 [n=10]	-	16 – 17 (16.5) [n=10]	-	15 – 18 (16) [n=10]	-
Eggs W	10 [n=10]	-	9 – 11 [n=10]	-	10 – 11 (10)	-	10 – 12 (11)	-

L= length; W = width.

Table 3. Prevalence (P%), mean intensity (MI), mean abundance (MA), range (R), Intensity (I) and confidence interval (CI) of *Koellikerioides apicalis* and *Koellikerioides externogastricus*.

Hosts	<i>K. apicalis</i>				<i>K. externogastricus</i>	
	P % (CI)	MI (CI)	MA(CI)	R	P % (CI)	I*
<i>E. alletteratus</i>	-	-	-	-	-	-
<i>T. albacares</i>	10.5 (3.68-24.71)	2.25 (2.00-2.50)	0.24 (0.05-0.50)	1-3	2.6 (0.06-13.81)	20
<i>T. atlanticus</i>	4.4 (0.80-15.20)	3.00 (2.00-4.00)	0.13 (0.00-0.40)	1-4	-	-
<i>T. obesus</i>	5.7 (1.03-19.53)	2.00	0.11 (0.00-0.29)	2	-	-

* One host parasitized.

Table 4. Prevalence (P%), mean intensity (MI), mean abundance (MA), range (R), Intensity (I) confidence interval (CI) of *Koellikerioides intestinalis*.

<i>K. intestinalis</i>					
Hosts	P % (CI)	MI (CI)	MA (CI)	R	
<i>E. alletteratus</i>	19.4 (7.45-37.48)	78.67 (40.67-170.67)	15.23 (4.77-42.71)	24-236	
<i>T. albacares</i>	23.7 (11.44-40.25)	52.44 (19.78-106.44)	12.42 (3.89-31.37)	8-196	
<i>T. atlanticus</i>	35.8 (23.76-53.46)	49.82 (32.00-68.71)	17.69 (10.00-29.07)	6-88	
<i>T. obesus</i>	14.3 (5.81-29.78)	23.20 (9.20-46.40)	3.31 (0.86-9.77)	6-66	

Table 5. Dispersion index (DI) and *d* test of *Koellikerioides intestinalis* and *Koellikerioides apicalis*

Hosts	<i>K.intestinalis</i>		<i>k. apicalis</i>	
	DI	<i>d</i>	DI	<i>d</i>
<i>E. alletteratus</i>	133.7	81.9*	-	-
<i>T. albacares</i>	123.9	87.2*	1.9	3.3*
<i>T. atlanticus</i>	59.7	63.2*	-	-
<i>T. obesus</i>	42.5	45.5*	-	-

(*) significant values.

Table 6. Values of Spearman's rank correlation coefficient (r_s) and Pearson's correlation coefficient (r) obtained in relations between total length of hosts, and abundance and prevalence of *Koellikerioides intestinalis*.

Hosts	r_s	<i>P</i>	r	<i>P</i>
<i>T. albacares</i>	0.136	0.416	0.058	0.731
<i>T. atlanticus</i>	-0.186	0.221	-0.114	0.739
<i>T. obesus</i>	0.006	0.973	-0.183	0.570
<i>E. alletteratus</i>	-0.137	0.464	-0.596	> 0.001*

P= level of significance, (*) significant values.**Table 7.** Normal approximation Z_c of Mann-Whitney test and Fisher's (*F*) test values used to evaluate possible relationships between the sex of hosts and abundance and prevalence of *Koellikerioides intestinalis*.

Hosts	Z_c	<i>P</i>	<i>F</i>
<i>T. albacares</i>	-2.70	0.044*	0.006*
<i>T. atlanticus</i>	-0.5	0.624	0.749
<i>T. obesus</i>	-1.96	0.235	0.134
<i>E. alletteratus</i>	-0.57	0.711	0.654

P= level of significance, (*) significant values.

Small cysts containing male and female worms were easily visible to the naked eye, located just under the mucosa of the small intestine. Male smaller than female, placed in special cavity of female. Body divided into two regions (anterior and posterior) in both sexes.

Male: Anterior region of body scoop-shaped wide at level of oesophagus. Posterior region rounded placed in special hollow of posterior region of

female, very difficult to see, due to large amount of eggs. Oral sucker terminal, followed by globular pharynx; oesophagus narrow, long, bifurcated into two caeca. Testis single, tubular, curved along convex margin of body's posterior region; vas deferens opening ventral to oral sucker.

Female: Anterior region of body scoop-shaped wide at oesophageal level. Posterior region oval

to rounded with a special cavity for male. Oral sucker terminal, muscular, globular, followed by muscular pharynx; oesophagus short. Ovary long, branched. Vitelline gland tubular, ramified. Seminal receptacle opening into genital junction. Uterine coils occupying most of the available space in posterior region. Eggs bean-shaped.

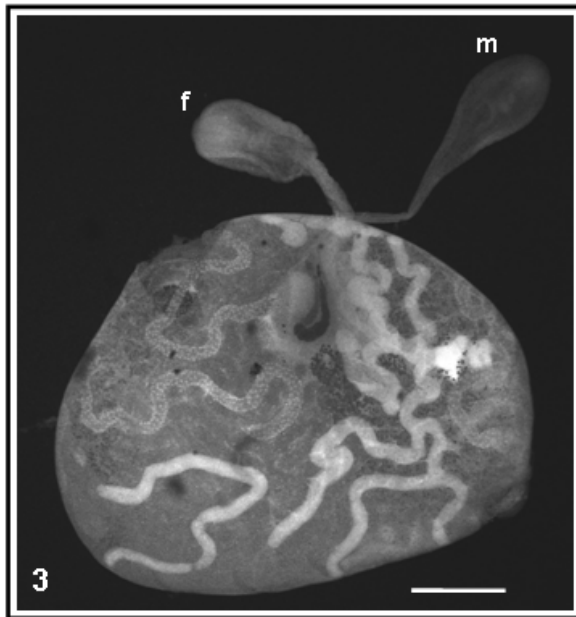


Figure 3. *Koellikerioides intestinalis* Yamaguti, 1970. Confocal scanning light micrograph of male (m) and female (f). Scale bar= 500 μ m.

Remarks

Koellikerioides intestinalis was described from the Pacific Ocean by Yamaguti (1970) in *T. obesus* and reported by Pozdnyakov (1990) in *T. albacares*, *T. alalunga* and *T. obesus*. In the Adriatic Sea this species was reported by Mladineo & Tudor (2004) from *T. thynnus*. Lately, Mladineo & Bočina (2009) published a paper on the type and ultrastructure of *K. intestinalis* and concluded that the ultrastructure of cysts does not differ in architecture or type of connective tissue fibers expressed at the parasitism site, being of host origin. Our specimens were found encysted lengthwise on the inner surface of the small intestine, the same site of infection as in the original description, parasitizing *T. albacares*, *T. obesus* and two new hosts: *T. atlanticus* and *E. alletteratus*. No significant differences between our specimens and those of the original description were observed.

DISCUSSION

The Digenea family Didymozoidae represents a very important group of trematodes parasites of tuna fish. In South America, mainly in the southern Atlantic Ocean, there is a small number of species reported, in contrast to well studied regions of the Pacific Ocean. The present study contributes to increase the knowledge and expansion of the geographical distribution of three species of *Koellikerioides*. Till now only two species of *Koellikerioides* had been reported in South America: one undetermined species referred by Tantaleán *et al.* (1992) from *Sarda chiliensis chiliensis* (Cuvier, 1832) in Peru and *Koellikerioides internogastricus* Yamaguti, 1970 reported by Justo *et al.* (2009) from *T. atlanticus*, *T. albacares* and *T. obesus* in Brazil.

Koellikerioides apicalis and *K. intestinalis* were reported by Mladineo & Tudor (2004) parasitizing the cage-reared northern bluefin tuna, *Thunnus thynnus thynnus* in the Adriatic-Sea showing high prevalence: 73.68 % and 57.89 %, respectively. In 2008, Mladineo *et al.* referred that the mean prevalence of these species collected in the same locality from the Atlantic bluefin tuna, *T. thynnus*, was 10.93% for *K. apicalis* and 54.64% for *K. intestinalis*.

Comparing with our results we conclude that the prevalence of *K. apicalis* (*T. albacares*: 10.5%, *T. atlanticus*: 4.4%, *T. obesus*: 5.7%) and of *K. intestinalis* (*E. alletteratus*: 19.4%, *T. albacares*: 21.1%, *T. atlanticus*: 35.6%, *T. obesus*: 14.3%) from Atlantic Ocean are smaller than the presented in the northern bluefin tuna and similar with the prevalence of *K. apicalis* from Atlantic bluefin tuna from the Adriatic.

Several papers about parasites of scombrid fishes in Brazil showed high prevalence for different species of didymozoids (Justo & Kohn, 2005, 2009, 2010; Alves & Luque, 2006; Kohn & Justo, 2008; Justo *et al.*, 2008, 2009), demonstrating wide geographical distribution for these trematodes.

It was not observed in our study, any correlation between the abundance of parasites and the total length of their hosts. Although, the prevalence of *K. intestinalis* was negatively correlated with the total length of *E. alletteratus*. As mentioned by Polyanski (1961), quantitative and qualitative changes in parasitism are expected as the fish

grows. Saad-Fares & Combes (1992) related that ontogenetical changes in the feeding behavior might have an influence on parasite prevalence and abundance in the host size classes.

In our results we observed that host sex does not influence the prevalence and intensity of infection. However, *K. intestinalis* showed a correlation in the abundance and prevalence with the sex host, as it was found only in the females of *T. albacares*. These correlations were surprising since differences in biological conditions of males and females of *T. albacares* are unknown. According to Luque *et al.* (1996) and Alves *et al.* (2002) the absence of correlations in parasite prevalence and abundance with the sex of the host fish is a widely documented pattern, and interpreted as a consequence of absence of sexual differences in some biological aspects of the fish. Our result could have been purely accidental in the studied sample and influenced by the higher number of females examined, making it necessary to study a larger number of individuals to confirm these results.

In this opportunity we report *K. apicalis*, *K. externogastricus* and *K. intestinalis*, for the first time in Brazil, representing the first record of these species in the southwest Atlantic Ocean and in new host records: *T. atlanticus* (*K. apicalis* and *K. intestinalis*) and *E. alletteratus* (*K. intestinalis*). These results increase the knowledge and geographic distribution of the family Didymozoidae in South America and indicate the importance of expanding the study to other species of tuna from the Atlantic Ocean.

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BIBLIOGRAPHIC REFERENCES

- Alves, DR. & Luque, JL. 2006. *Ecologia das comunidades parasitárias de cinco espécies de escombrídeos (Perciformes: Scombridae) do litoral do Estado do Rio de Janeiro, Brasil*. Revista Brasileira de Parasitologia Veterinária, vol. 15, pp. 167-181.
- Alves, DR, Luque, JL & Paraguassú, AR. 2002. *Community ecology of the metazoan parasites of Pink Cusk-eel, Genypterus brasiliensis (Osteichthyes: Ophidiidae), from the coastal zone of the State of Rio de Janeiro, Brazil*. Memórias do Instituto Oswaldo Cruz, vol. 97, pp. 683-689.
- Bush, AO, Aho, JM, Holmes, JC & Kennedy, CR. 1990. *Ecological versus phylogenetic determinants of helminths parasite community richness*. Evolutionary Ecology, vol. 4, pp. 1-20.
- Bush, AO, Lafferty, KD, Lotz, JM & Shostak, AW. 1997. *Parasitology meets ecology on its own terms: Margolis et al. revised*. Journal of Parasitology, vol. 83, pp. 575-583.
- Collette, BB & Nauen, CE. 1983. *FAO Species Catalogue. Vol. 2. Scombrids of the World. An annotated and illustrated catalogue of tuna, mackerels, bonitos and related species know to date*. FAO Fisheries Synopses, vol. 125, Rome, Italy, 137 p.
- Fischer, W. (Ed.). 1978. *FAO species identification sheets for fishery purposes. Western Central Atlantic (Fishing Area 31)*, vols. I-VII. FAO, Rome.
- Ishii, N. 1935. *Studies on the family Didymozoidae Monticelli, 1888*. Japanese Journal of Zoology, vol. 48, pp. 279-335.
- Justo, MCN, Tortelly, R, Menezes, RC & Kohn, A. 2008. *First record in South America of Didymosulcus palati and Didymosulcus philobranchiarca (Digenea, Didymozoidae) with new hosts records and pathological alterations*. Memórias do Instituto Oswaldo Cruz, vol. 103, pp. 207-210.

- Justo, MCN, Tortelly, R, Menezes, RC & Kohn, A. 2009. First record in South America of *Koellikerioides internogastricus* (*Digenea*, *Didymozoidae*) with new host record and pathological alterations. *Veterinary Parasitology*, vol. 161, pp. 158–161.
- Justo, MCN & Kohn, A. 2005. *Didymozoidae* (*Digenea*) parasites of *Scombridae* (*Actinopterygii*) from Rio de Janeiro coast, Brazil. *Revista Brasileira Zoociências*, vol. 7, pp. 333–338.
- Justo, MCN & Kohn, A. 2009. *Trematoda*, *Digenea*, *Didymozoidae*, *Coeliotrema thynni Yamaguti, 1938: First record in Brazil with three new host records*. *Check List*, vol.5, pp. 436–438.
- Justo, MCN & Kohn, A. 2010. *Trematoda*, *Digenea*, *Didymozoidae*, *Wedlia retrorbitalis (Yamaguti, 1970) and Wedlia submaxillaris (Yamaguti, 1970): First record in South America and the Atlantic Ocean*. *Check List*, vol. 6, pp. 387–389.
- Kohn, A & Justo, MCN. 2008. *Didymocystis lamotheargumedei n. sp. (Digenea: Didymozoidae) a parasite of three species of scombrid fishes*. *Revista Mexicana de Biodiversidad*, vol. 79, pp. 9S-14S.
- Lester, RJG, Barnes, A & Habib, G. 1985. *Parasites of Skipjack tuna, Katsuwonus pelamis. Fishery implications*. *Fish Bulletin*, v. 83, pp.343-356.
- Ludwig, JA & Reynolds, JF. 1988. *Statistical Ecology: A primer methods and computing*. Wiley-Interscience Publications, New York, 337 p.
- Luque, JL, Amato, JFR & Takemoto, RM. 1996. *Comparative analysis of the communities of metazoan parasites of Orthoprists ruber and Haemulon steindachneri (Osteichthyes: Haemulidae) from the southeastern Brazilian littoral: I. structure and influence of the size and sex of hosts*. *Revista Brasileira de Biologia*, vol. 56, pp. 279-292.
- Madhavi, R & Ham, BK. 2000. *Community structure of helminth parasites of the tuna, Euthynnus affinis, from the Visakhapatnam coast, Bay of Bengal*. *Journal of Helminthology*, vol. 74, pp. 337-342.
- Mladineo, I & Tudor, M. 2004. *Digenea of Adriatic cage-reared northern bluefin tuna (Thunnus thynnus thynnus)*. *Bulletin European Association of Fish Pathologist*, vol. 24, pp. 144-152.
- Mladineo, I & Bočina, I. 2009. *Type and ultrastructure of Didymocystis wedli and Koellikerioides intestinalis (Digenea, Didymozoidae) cysts in captive Atlantic bluefin tuna (Thunnus thynnus Linnaeus, 1758)*. *Journal of Applied Ichthyology*, vol. 25, pp. 762-765.
- Mladineo, I, Segvić, T & Petrić, M. 2010. *Do captive conditions favor shedding of parasites in the reared Atlantic bluefin tuna (Thunnus thynnus)?* *Parasitology International*, vol.60, pp. 25-33.
- Mladineo, I, Žilić, J & Čanković, M. 2008. *Health survey of Atlantic Bluefin Tuna, Thunnus thynnus (Linnaeus, 1758), reared in Adriatic cages from 2003 to 2006*. *Journal of the World Aquaculture Society*, vol. 39, pp. 281–289.
- Munday, BL, Sawada, Y, Cribb, T & Hayward, CJ. 2003. *Diseases of tuna, Thunnus spp. Journal of Fish Diseases*, vol. 26, pp. 187-206.
- Nikolaeva, VM. 1985. *Trematodes Didymozoidae fauna, distribution and biology. In: (William J.Hargis, Jr.). Parasitology and Pathology of Marine Organisms of the World Ocean*, NOAA Technical Report, NMFS, vol. 25, pp. 667-672.
- Polyanski, YI. 1961. *Ecology of parasites of marine fishes. In: (V.A. Dogiel G.K. Petrushevski & Y.I. Polyanski). Parasitology of Fishes. Oliver & Boyd, Edinburgh & London, p. 47.*

- Pozdnyakov, SE. 1990. *Helminths of Scombrid-like Fishes of the World's Oceans*. DVO ANSSSR, Vladivostok, 183 p. (In Russian).
- Pozdnyakov, SE. 1996. *Trematodes suborder Didymozoa*. Vladivostok: Tikhookeanskii Nauchno-Issledovatel'skii Rybokhozyaistvennyi Tsent, 319 p. (In Russian).
- Rósz, L., Reiczig, J. & Majoros, G. 2000. *Quantifying parasites in samples of hosts*. Journal of Parasitology, vol. 86, pp. 228–232.
- Saad-Fares, A & Combes, C. 1992. *Abundance/host size relationships in a fish trematode community*. Journal of Helminthology, vol. 66, pp. 187-192.
- Tantaleán, M, Sarmiento, BL & Huiza, FA. 1992. *Digeneos (Trematoda) del Perú*. Boletín de Lima, vol. 80, pp. 47-84.
- Yamaguti, S. 1970. *The digenetic trematodes of Hawaiian fishes*. Tokyo, Keigaku Publishers Co. 436 p.
- Zar, JH. 1996. *Biostatistical Analysis*. Upper Saddle River, New Jersey, Prentice-Hall, 662 p.

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