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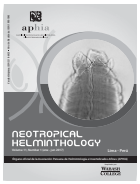


ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

# ANISAKIDAE AND RAPHIDASCARIDIDAE NEMATODES PARASITES OF TUNA (PERCIFORMES: SCOMBRIDAE) FROM STATE OF RIO DE JANEIRO, BRAZIL

# NEMATODOS ANISAKIDAE Y RAPHIDASCARIDIDAE PARÁSITOS DE ATUNES (PERCIFORMES: SCOMBRIDAE) DEL ESTADO DE RÍO DE JANEIRO, BRASIL

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## ABSTRACT

We purchased 276 tuna from the fish market, between January/2000 and December/2002 including 107 specimens of *Katsuwonus pelamis* (Linnaeus, 1758), 5 *Auxis thazard* (Lacepède, 1800), 92 *Thunnus albacares* (Bonnaterre, 1788), 30 *Thunnus atlanticus* (Lesson, 1830), 25 *Thunnus obesus* (Lowe, 1839), and 17 *Thunnus thynnus* (Linnaeus, 1758) caught offshore of Cabo Frio, State of Rio de Janeiro. Fifty six (20.28%) of fish were parasitized by at least one parasite. A total of 196 third-stage nematodes larvae were collected and identified as: *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878), *A. physeteris* Baylis 1923, *Contracaecum* sp. and *Raphidascaris* sp. This study is the first report in Brazil of new hosts records of *A. simplex* in *T. albacares*, *T. atlanticus*, and *T. obesus*, *A. physeteris* in *T. albacares*, *Contracaecum* sp. in *T. atlanticus* and *T. obesus*, and *Raphidascaris* sp. in *T. albacares* and *T. obesus*. Their parasite indices of prevalence, intensity/mean intensity, abundance/mean abundance and range of infection were presented. The nematodes were collected from up to five sites of infection, stomach, intestine, caecum, liver and abdominal cavity. *A. simplex* parasitizing *T. obesus* presented the higher level of prevalence (32%), mean intensity (4.6) and mean abundance (1.48); and *K. pelamis* presented the greater range of infection (1-17 parasites per fish) and number of sites of infection (stomach, intestine, caecum, liver and abdominal cavity). The importance of the presence of nematode larvae in these fish for public health was evaluated.

***Keywords***: Anisakid – *Auxis thazard* – *Katsuwonus pelamis* – Nematodes – Raphidascaridid – *Thunnus albacares* – *Thunnus atlanticus* – *Thunnus obesus* – *Thunnus thynnus*

## RESUMEN

Para el presente estudio, fueron adquiridos 276 atunes, entre enero de 2000 y diciembre de 2002, en mercados de peces, siendo 107 ejemplares de *Katsuwonus pelamis* (Linnaeus, 1758), 5 *Auxis thazard* (Lacepède, 1800), 92 *Thunnus albacares* (Bonnaterre, 1788), 30 *Thunnus atlanticus* (Lesson, 1830), 25 *Thunnus obesus* (Lowe, 1839), y 17 *Thunnus thynnus* (Linnaeus, 1758) pescados en el municipio de Cabo Frio, Estado de Rio de Janeiro, Brasil. Cincuenta y seis (20,28%) de los peces fueron parasitados por lo menos con una especie de parásito. Se colectaron un total de 196 nemátodos larvarios y fueron identificados como: *Anisakis* (Rudolphi, 1809, det. Krabbe, 1878), *A. physeteris* Baylis 1923, *Contracaecum* sp. y *Raphidascaris* sp. Este estudio es el primer registro en Brasil con nuevos registros para los hospederos de *A. simplex* en *T. albacares, T. atlanticus* y *T. obesus,* de *A. physeteris* en *T. albacares,* de *Contracaecum* sp. en *T. atlanticus* y *T. obesus* y de *Raphidascaris* sp. en *T. albacares* y *T. obesus*. Se presentaron sus índices parasitarios de prevalencia, intensidad/intensidad media, abundancia/abundancia media y el rango de la variación de la infección. Los nemátodos se colectaron en la mayoría de los cinco sitios de infección, estómago, intestino, ciego, hígado y cavidad abdominal. *A. simplex* parasitando *T. obesus* presentó el mayor nivel de prevalencia (32%), intensidad media (4,6) y abundancia media (1,48); y *K. pelamis* presentó el mayor rango de la variación de la infección (1-17 parásitos por pez) y el número de sitios de infección (estómago, intestino, ciego, hígado y cavidad abdominal). Se evaluó la importancia de la presencia de larvas de nematodos en estos peces para la salud pública.


**Palabras clave:** Anisakidos – *Auxis thazard* – *Katsuwonus pelamis* – Nematodos – Raphidascarididos – *Thunnus albacares* – *Thunnus atlanticus* – *Thunnus obesus* – *Thunnus thynnus*

## INTRODUCTION

The tuna fish, skipjack tuna *Katsuwonus pelamis* (Linnaeus, 1758); frigate tuna *Auxis thazard* (Lacepède, 1800); yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788); blackfin tuna *Thunnus atlanticus* (Lesson, 1830); bigeye tuna *Thunnus obesus* (Lowe, 1839), and northern Bluefin tuna *Thunnus thynnus* (Linnaeus, 1758) (Perciformes: Scombridae), usually living in surface water depth to over 500m (Figueiredo & Menezes, 2000).

Parasitic nematodes are important pathogens associated with human and animal health. Some inhabit the marine environment, where they are widespread in a variety of hosts. Adults are commonly found in the digestive tract of marine mammals. Larvae infect aquatic invertebrates and non-mammalian vertebrates as intermediate hosts (Klimpel & Palm, 2011).

Parasitic infections associated with consumption of fishery products have always been a concern for the public health and for economy. The main food-borne zoonoses associated with the consumption of fishery products are mainly attributable to trematodes, cestodes and nematodes. Among these parasites, the anisakid nematodes are the most important under the sanitary point of view, since they are capable to induce pathologies in humans such as anisakidosis, anisakiosis and pseudoterranovosis (Klimpel & Palm, 2011). In humans, these parasitoses can occur after the ingestion of raw, poor cooked/smoked or superficially salted fish meat containing infective larvae (Acha & Szyfres, 2003). These could be result of combination of two factors: direct action by the larvae of Anisakidae during tissue invasion; and interactions between the host immune system and the substances released by the parasite or through its presence. Studies including the possibility to result allergic processes in humans (Kagei & Isogaki, 1992; Ubeira *et al*., 2000; Oomori *et al*., 2008; Tejada *et al*., 2015).

The aims of the present study were to investigate the presence of the anisakid and raphidascaridid nematode larvae in *K. pelamis*, *A. thazard*, *T. albacares*, *T. atlanticus*, *T. obesus*, and *T. thynnus* from the State of Rio de Janeiro, Brazil, identifying them by morphological characters, stabilish the parasitary indices of prevalence, intensity, mean

intensity, abundance, mean abundance, range of infection, and infection sites; and evaluate the importance indetecting the presence of nematode larvae in these fish for public health.

## MATERIALS AND METHODS

A total of 276 tuna specimens, acquired between January/2000 and December/2002, i.e., 107 *K. pelamis*, (47-81 cm of fork length - FL and 1.8-12 kg of weigh - W), 5 *A. thazard* (45-53 cm - FL and 1.5-2.9 kg - W), 92 *T. albacares* (34-83 cm - FL and 1-5.5 kg - W), 30 *T. atlanticus* (32-55 cm - FL and 1-3.5 kg - W), 25 *T. obesus* (40-75 cm - FL and 1.6-8 kg - W), and 17 *T. thynnus* (50-80 cm - FL and 2,2-9,4 kg - W), were obtained in small markets selling fish caught from offshore of the municipality of Cabo Frio, State of Rio de Janeiro, Brazil. Fish were necropsied "*in loco*" and their viscera were maintained in isothermal boxes with ice and carried to the Laboratory of Helminth Parasites of Fish, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. The tuna species were identified in accordance with Figueiredo & Menezes (2000). Internal organs were examined, and nematode larvae found were placed in Petri dishes with 0.65% saline.

Nematodes were fixed in AFA (ethanol, formalin, and acetic acid) at 60 °C, preserved in 70 ºGL ethanol (Knoff & Gomes, 2012) and sent to Laboratory of Helminth Parasites of Vertebrates where the specimens were clarified with Amman's lactophenol. The taxonomic classification was in accondance with De Ley & Blaxter (2004) and larval identification was in accondance with Felizardo *et al*. (2009), and Petter & Maillard (1988). The specimens were observed by bright-field microscopy (Olympus BX-41). Parasitary therms used were according to Bush *et al*. (1997). Voucher specimens preserved in ethanol 70 °GL were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), FIOCRUZ, Rio de Janeiro, RJ, Brazil.

## RESULTS

Fifty six (20.28%) tuna were parasitized by, at least, one species of nematode parasite larvae. They were identified as belonging to Anisakidae Railliet & Henry, 1912, *Anisakis* Dujardin, 1809: *Anisakis simplex* (Fig. 1), *Anisakis physeteris* (Fig. 2), *Contracaecum* sp. (Fig. 3); Raphidascarididae Hartwich, 1954, *Raphidascaris* Railliet & Henry, 1915: *Raphidascaris* sp. (Fig. 4). The Anisakidae and Raphidascarididae specimens collected from tuna was represented by larval stages, most of them with high prevalence. A total of 276 third-stage nematode larvae ($L_3$) were collected. Parasitary indices of prevalence, intensity, mean intensity, abundance, mean abundance and range of infection, as well as the infection sites, and the CHIOC deposit numbers are the depicted in Table 1. The parasitism distribution found in these fish was: 27 in 107 *K. pelamis* (25%), 1 in 5 *A. thazard* (20%), 12 in 92 *T. albacares* (13%), 4 in 30 *T. atlanticus* (13%), 10 in 25 *T. obesus* (40%) and 2 in 17 *T. thynnus* (11%).

The species *A. simplex* collected of *T. obesus*, and *A. simplex* and *A. physeteris* collected of *A. thazard* occurred with higher parasitary indices of prevalence (32%, 20%, and 20%, respectively). The species *A. physeteris* and *Raphidascaris* sp. of *T. albacares* and *Contracaecum* sp. of *T. atlanticus* and *T. obesus* occurred with prevalence lower than 10%. The species *A. simplex* presented the highest values of mean intensity and mean abundance (8 and 0.94 respectively) in *T. thynnus*. *Anisakis simplex* was observed in the largest number of infection sites (stomach, intestine, liver, caecum and abdominal cavity) of *K. pelamis*.

Single infections occurred in 38 specimens, 30 parasitized with *A. simplex*, six with *A. physeteris* and two with *Raphidascaris* sp. Co-infections with two species were observed in 14 fish, i.e., 10 with *Anisakis simplex* and *A. physeteris*, two with *Anisakis simplex* and *Contracaecum* sp., and two with *Anisakis simplex* and *Raphidascaris* sp.

## DISCUSSION

The morphology and morphometry of Anisakidae *A. simplex*, *A. physeteris* and *Contracaecum* sp. $L_3$ in the present study was in accordance with the specimens reported by Petter & Maillard (1988) of fishes from western Mediterranean sea and by

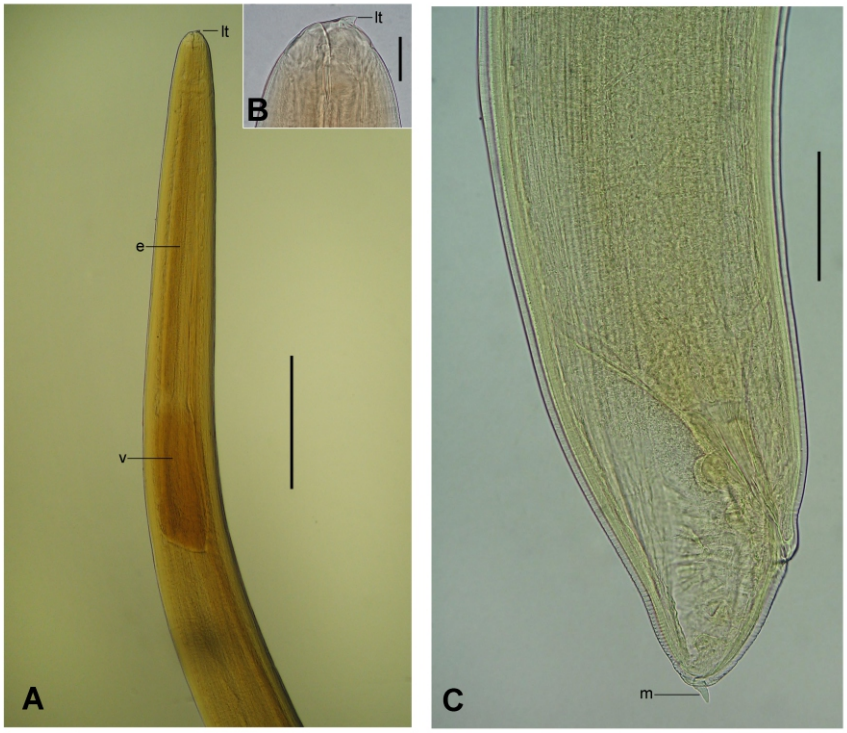


**Figure 1.** *Anisakis simplex* from *Auxis thazard*. A, anterior region, ventral view, showing larval tooth (lt), esophagus (e) and ventriculus (v); B, detail of larval tooth (lt), ventral view; C, detail of the tail showing mucron (m). Scale bars of A = 1000 μm, B = 50 μm and C = 100 μm.

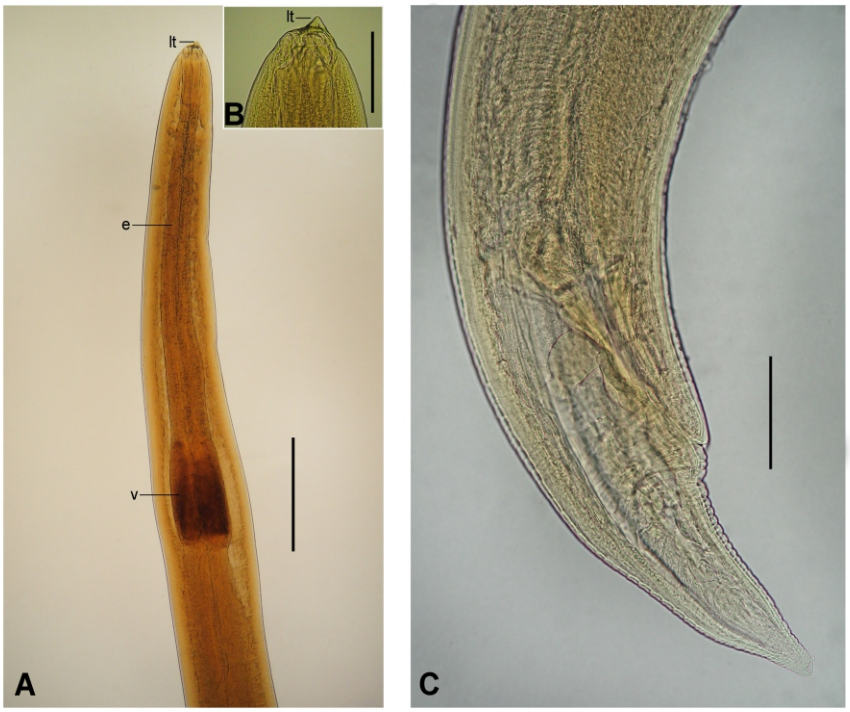


**Figure 2.** *Anisakis physeteris* from *Katsuwonus pelamis*. A, anterior region, ventral view, showing larval tooth (lt), esophagus (e) and ventriculus (v); B, detail of larval tooth (lt), ventral view; C, tail without mucron. Scale bars of A = 1000 μm, B = 200 μm and C = 100 μm.

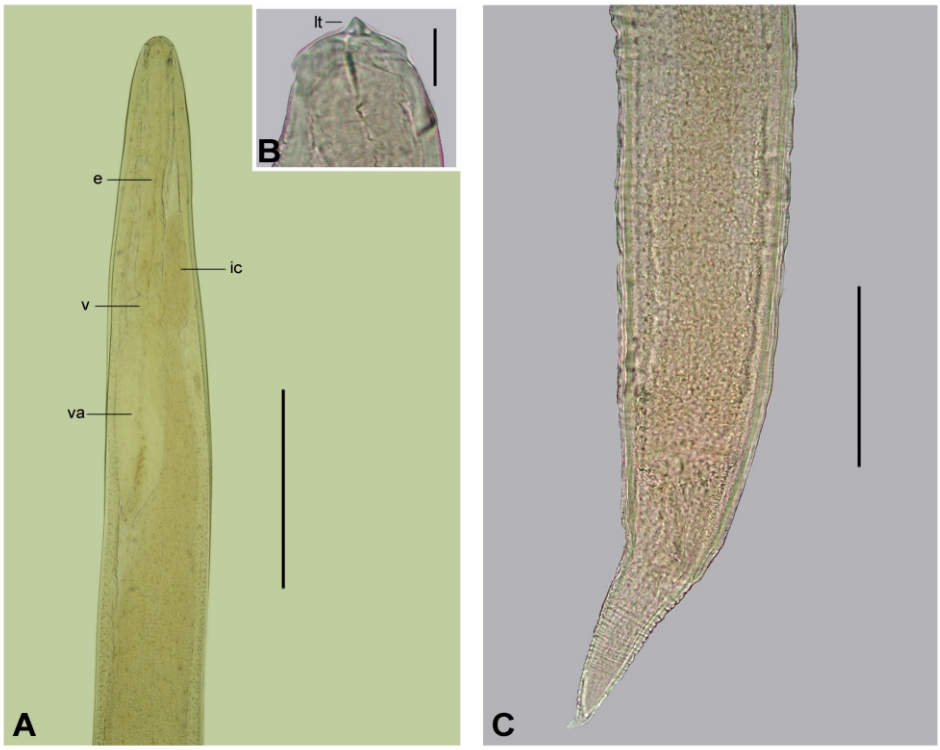


**Figure 3.** *Contracaecum* sp. from *Thunnus atlanticus*. A, anterior region, ventral view, showing esophagus (e), ventriculus (v), ventricular appendix (va) and intestinal cecum (ic); B, detail larval tooth (lt), lateral view; C, tail conical, transverse striation, mucron absent. Scale bars of A = 440 µm, B = 37.5 µm and C = 140 µm.

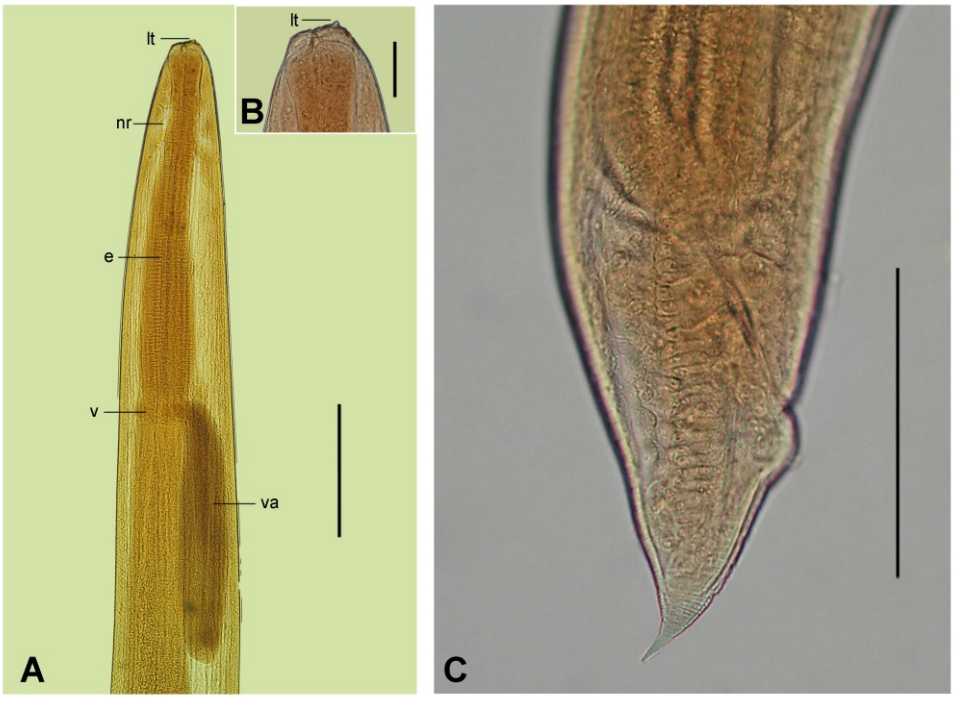


**Figure 4.** *Raphidascaris* sp. from *Thunnus obesus*. A, anterior region, ventral view, showing larval tooth (lt), nerve ring (nr), esophagus (e), ventriculus (v) and ventricular appendix (va); B, detail of larval tooth (lt), ventral view; C, tail pointed with well-defined striations, lateral view. Scale bars of A = 200 µm, B = 50 µm, and C = 400 µm.

**Table 1.** Prevalence (P), intensity/ mean intensity (I/MI), abundance/ mean abundance (A/MA), range of infection (RI), sites of infection (SI), and deposit number of Anisakidae and Raphidascarididae third-stage nematode larvae parasites of *Katsuwonus pelamis*, *Auxis thazard*, *Thunnus albacares*, *T. atlanticus*, *T. obesus*, and *T. thynnus* in CHIOC, from municipality of Cabo Frio, State of Rio de Janeiro, Brazil.

| | P (%) | I*/MI | A/MA | RI | SI | CHIOC |
|---|---|---|---|---|---|---|
| ***Katsuwonus pelamis*** | | | | | | |
| *Anisakis simplex* | 17.7 | 2.9 | 0.52 | 1-17 | S, I, L, C, AC | 36701, 36702, 36703, 36704 |
| *Anisakis physeteris* | 13.1 | 1.7 | 0.22 | 1-4 | S, L, C, AC | 36705, 36706, 36707, 36708 |
| ***Auxis thazard*** | | | | | | |
| *Anisakis simplex* | 20 | 2* | 0.40 | 1-2 | S | 36721 |
| *Anisakis physeteris* | 20 | 2* | 0.40 | 1-2 | S | 36722 |
| ***Thunnus albacares*** | | | | | | |
| *Anisakis simplex* | 11.9 | 3.7 | 0.44 | 1-10 | S, I, L, AC | 36709, 36710, 36711 |
| *Anisakis physeteris* | 1.1 | 2* | 0.02 | 1-2 | S | 38347 |
| *Raphidascaris* sp. | 1.1 | 1* | 0.01 | - | S | 36712 |
| ***Thunnus atlanticus*** | | | | | | |
| *Anisakis simplex* | 10 | 3.3 | 0.33 | 1-5 | S, I, C, AC | 36718, 36719 |
| *Contracaecum* sp. | 3.3 | 1* | 0.03 | - | AC | 38348 |
| ***Thunnus obesus*** | | | | | | |
| *Anisakis simplex* | 32 | 4.6 | 1.48 | 1-12 | S, I | 36713, 36714, 36715, 36716 |
| *Raphidascaris* sp. | 12 | 1 | 0.12 | - | S, I | 38349 |
| *Contracaecum* sp. | 4 | 1* | 0.04 | - | S | 36717 |
| ***Thunnus thynnus*** | | | | | | |
| *Anisakis simplex* | 11.7 | 8 | 0.94 | 1-11 | S | 36720 |

*Only the intensity is presented, because one specimen was parasitized.
AC = abdominal cavity, S = stomach, I = intestine, L = liver, C = caecum.

Felizardo *et al*. (2009) of *Paralichthys isosceles* Jordan, 1890 from the coast of Rio de Janeiro, Brazil. These larvae have been reported from various brazilian teleost fish (Knoff *et al*., 2007, 2013; Felizardo *et al.,* 2009; Fontenelle *et al*., 2013; Ribeiro *et al*., 2014; Fonseca *et al*., 2016).

The specimens of *Raphidascaris* sp. larvae collected in the present study exhibited morphology consistent with the specimens from Rio de Janeiro, Brazil by Felizardo *et al*. (2009).

Mattiucci *et al*. (2002) investigating frigate tuna and northem bluefin tuna from Atlantic Coast of Brazil, found *A. typica* parasitizing *A. thazard* and *T. thynnus* reported *A. typica* from both hosts with prevalence of 100%. Comparing with the present study *A. thazard* was parasitized with *A. simplex* and *A. physeteris*, and *T. thynnus* with *A. simplex*. The prevalences were lower in all species (20%, 20%, and 11.7%, respectively).

Alves & Luque (2006) reported *K. pelamis* parasitized with *Anisakis* sp. 1 and *Anisakis* sp. 2 with prevalence (P), mean intensity (MI) and mean abundande (MA) of 40%, 2.67, 1.06 and 13.3%, 5.50, 0.73 respectively, comparing with the hosts of the present study it was parasitized with *A. simplex* and *A. physeteris* with P = 17.7%, MI = 2.9, MA = 0.52 and P = 13.1%, MI = 1.7 and MA = 0.22, respectively. In Alves & Luque (2006) the site of infection was the mesentery, differently in the present study the sites of infections of *A. simplex* were stomach, intestine, caecum, liver, and abdominal cavity and of *A. physeteris* were stomach, caecum, liver, and abdominal cavity.

Cardoso *et al*. (2006) analyzed the parasitism of 79 marine teleosten fish of the State of Rio de Janeiro from one *K. pelamis* reporting the presence of a species of Anisakid not identified. In the present study, was identified the presence of *A. simplex* and *A. physeteris.*

Iñiguez *et al*. (2009) reported *A. thazard* from Brazilian coast parasitized with *A. physeteris* P = 22.2% and *A. typica* P = 77.8%, comparing with the present study *A. physeteris* was very similar and was found a different species *A. simplex* P = 20%.

This study is limited to research due some papers had no complete information about parasitary indices and sites of infection.

The presence of larval stages of nematodes indicates the potential of teleostean fish species as an intermediate hosts in life cycles of parasites, trophically transmitted in the marine environment (Klimpel & Palm, 2011; Knoff *et al*., 2013). The present study corroborates this statement, since anisakid larval stages were recovered.

It is necessary to emphasize the higienic-sanitary importance of *Anisakis* species found on the present study, because this larvae causing anisakiasis worldwide (Klimpel & Palm, 2011). There is only one reported case of human "anisakidosis" in Brazil that was identified as *Anisakis*-like (Cruz *et al*., 2010). However, in the present study anisakid nematode larvae were not collected from musculature, even in this case it must be taken into account they where found alive and with great motility, cause that they are liable to migrate to the other eatable organs and musculature, so available to infect humans.

Considering the high number of anisakidosis cases worldwide, more education programs at all levels are necessaries focusing on consumption only of fish that was previously frozen or properly cooked, therefore, it is recommended that fish should be gutted on board the boat (Knoff *et al*., 2007, 2013; D'amico *et al*., 2014; Fonseca *et al*., 2016).

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