

## MORPHOLOGICAL CHARACTERS AND HYGIENIC-SANITARY SIGNIFICANCE OF *Tentacularia coryphaenae* IN *Coryphaena hippurus* FROM BRAZIL

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### ABSTRACT

*Coryphaena hippurus*, or dolphinfish, is a popular species of fish pursued by sport fishing tournaments of the Yacht Club of Rio de Janeiro, with markets then in its own fish market, because of its desirable flesh. However, the presence of helminths, including in the musculature, results in commercial losses due their repugnant appearance. The aim of this investigation was to identify taxonomically these helminths using morphometrical and morphological data from analyses using optical and scanning electron microscopy, and to determine their hygienic-sanitary significance. Seven specimens of *C. hippurus* collected in January and November 2014 were necropsied and had their organs investigated. The helminths encountered included plerocercoids of *Tentacularia coryphaenae*, a trypanorhynch cestode. The seven specimens of *C. hippurus* were parasitized by a total of 204 plerocercoids, with infection sites being musculature, mesentery, stomach and liver serosa. Beyond the repugnance of these parasites, the presence of plerocercoids is worrisome because of the potential risk of allergic reactions by humans, thereby reinforcing the hygienic-sanitary significance of monitoring for these parasites.

**Key words:** Cestode; Trypanorhyncha; dolphinfish; fish hygiene; sport fishing.

## CARACTERES MORFOLÓGICOS E SIGNIFICÂNCIA HIGIÊNICO SANITÁRIA DE *Tentacularia coryphaenae* EM *Coryphaena hippurus* DO BRASIL

### RESUMO

*Coryphaena hippurus*, ou dourado é uma espécie popular de peixe pescada em torneios de pesca esportiva do Iate Clube do Rio de Janeiro, a qual é comercializada na sua própria peixaria, devido à apreciação de sua carne. Contudo a presença de helmintos, inclusive na musculatura, resulta em perdas comerciais devido ao seu aspecto repugnante. O objetivo desta investigação foi identificar taxonomicamente estes helmintos utilizando dados de análises morfológicas e morfológicas através das microscopias ótica e eletrônica de varredura e determinar sua significância higiênico-sanitária. Em janeiro e novembro de 2014, sete espécimes de *C. hippurus* foram necropsiados e seus órgãos investigados. Os helmintos encontrados foram identificados como plerocercoides de *Tentacularia coryphaenae*, um cestóide Trypanorhyncha. Os sete espécimes de *C. hippurus* estavam parasitados por um total de 204 plerocercoides, tendo como os sítios de infecção a musculatura, mesentério, estômago e serosa do fígado. Além da repugnância destes parasitos, a presença de plerocercoides é um fator preocupante porque há o risco de reações alérgicas em humanos, por isto salienta a sua significância higiênico-sanitária no monitoramento destes parasitos.

**Palavras-chave:** Cestóide; Trypanorhyncha; dourado; higiene do pescado; pesca esportiva.

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## INTRODUCTION

The Yacht Club of Rio de Janeiro (ICRJ), sponsors sport fishing tournaments during which several species of oceanic fish are captured. These fish include economically valuable species with desirable meat, and so they are not only considered trophies, but are also sent to ICRJ's own fish market. One of these species is the dolphinfish, *Coryphaena hippurus* Linnaeus, 1758. The species *C. hippurus* is cosmopolitan, which has a cosmopolitan distribution throughout, tropical and subtropical seas (GIBBS and COLLETTE, 1959; MENEZES and FIGUEIREDO, 1980). In Brazil, the dolphinfish is highly valued for food and is an important constituent of recreational and commercial fisheries (GIBBS and COLLETTE, 1959; DITTY *et al.*, 1994; PALOMINO *et al.*, 1998).

The maintenance of hygienic-sanitary conditions at the ICRJ fish market has been a concern. For example, larvae of trypanorhynch cestodes have been found in the musculature of dolphinfish at the ICRJ fish market, which usually produce a repugnant appearance resulting losses in sales. Cestodes of the order Trypanorhyncha possess worldwide distribution, and occur in marine animals of tropical and subtropical regions (PALM, 2004).

As adults these cestodes inhabit the gastrointestinal tract of elasmobranchs, while their larval forms are found in several species of teleost and elasmobranch fish, and a variety of marine invertebrates (CAMPBELL and BEVERIDGE, 1994). According to the Brazilian legislation that deals with fish and their derivative products, any fish with a repugnant appearance is considered improper for consumption, as in any musculature possessing massive parasite infection, whether or not it may affect the health of consumers (BRASIL, 2007). Furthermore, the presence of trypanorhynch larvae in fish is considered disconcerting since they can pose a risk of allergic reactions in humans (MATOS *et al.*, 2013).

The aims of this investigation were to: (1) identify the species of trypanorhynch cestodes parasitizing *C. hippurus* caught during sport fishing tournaments in the state of Rio de Janeiro by means of morphological and morphometric analyses using optical and scanning electron microscopy of specimens retrieved from the site of infection; (2) demonstrate the hygienic-sanitary significance of these parasites to collective human health; and (3) provide baseline data for subsequent investigations.

## METHODS

In January and November 2014, seven specimens of dolphinfish, *C. hippurus* (85-100 cm total length and 6.5-7 kg) caught by fishermen during ocean sport fishing of the coast of the municipality of Cabo Frio, state of Rio de Janeiro, Brazil were brought to ICRJ and sent to its fish market. Fish species were identified according to MENEZES and FIGUEIREDO (1980).

The fish were then necropsied and filleted, and their organs separated into plastic bags, labeled and transported on ice in isothermic boxes for examination in the Laboratory of Helminth Parasites of Vertebrates, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro. The three dolphinfish captured in January 2014 possessed plerocercoids of trypanorhynch cestodes parasitizing musculature, stomach, liver serosa and mesentery. The other four dolphinfish, caught in November 2014, had only their musculature examined, because their commercialization was prohibited by the massive presence of trypanorhynch plerocercoids.

In the laboratory, retrieved cestodes were placed in Petri dishes containing distilled water and maintained in a refrigerator for at least 24h to permit the relaxation of scolices and extroversion tentacles. Some of the larvae were fixed in cold AFA, stained with Langeron's carmine, differentiated in 0.5% hydrochloric acid-ethanol solution, dehydrated in a series of increasing alcohol concentrations and clarified with beach-wood creosote. Ten specimens were then mounted whole preserved in Canada balsam and measured, while the other specimens were preserved in 70°GL ethanol (KNOFF and GOMES, 2012).

The specimens were observed with an Olympus BX-41 bright-field microscope, and images were obtained using a Cannon Power Shot A640 digital camera coupled to Zeiss Axiophot microscope. Topographical descriptions of surface of scolices was performed using scanning electron microscopy (SEM). For SEM the cestodes were processed as described by LOPES TORRES *et al.* (2013), which involved, in short, fixation in ethanol 70°GL, dehydration in an acetone series, CO<sub>2</sub> critical-point drying and gold coating (20 nm deposition). Images were made using a JEOL LV 6940 SEM under 30kV acceleration voltage.

Taxonomic identification followed CAMPBELL and BEVERIDGE (1994), BEVERIDGE and

CAMPBELL (1996), PALM (2000, 2004) and KNOFF *et al.* (2004). Terminology for cestode larvae followed CHERVY (2002). Measurements are given in millimeters and as range followed by means in parenthesis. For confirmation of identification, specimens were compared with the plerocercoids of *T. coryphaenae* collected from individuals of *Genypterus brasiliensis* from Rio de Janeiro, RJ, Brazil (CHIOC n° 36420); *Scomberomorus cavala* from Rio de Janeiro, RJ, Brazil (CHIOC n° 35633); *Lophius gastrophysus* from Niterói, RJ, Brazil (CHIOC n° 36691); *Katsuwonus pelamis* from RJ, Brazil (CHIOC n° 36541 a-b), and Cabo Frio, RJ, Brazil (CHIOC n° 37150 a-b, 37151, 37152); and *Thunus albacares* from Cabo Frio, RJ, Brazil (CHIOC 37153).

Voucher specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), FIOCRUZ, Rio de Janeiro, RJ, Brazil.

## RESULTS

The three specimens of *C. hippurus* collected in January 2014 were parasitized by a total of 108 plerocercoids of trypanorhynch cestodes in the abdominal musculature, mesentery, stomach and liver serosa. The four hosts collected in November 2014 were parasitized by a total of 96 plerocercoids

of the same species. These fish were removed from commercialization and discarded by the ICRJ fish market due to the massive infections in the abdominal musculature and the severe hemorrhage caused by the large number of cestodes (Figure 1 a-c). All plerocercoids were found alive and demonstrated high motility.

Cestoda Trypanorhyncha Diesing, 1863

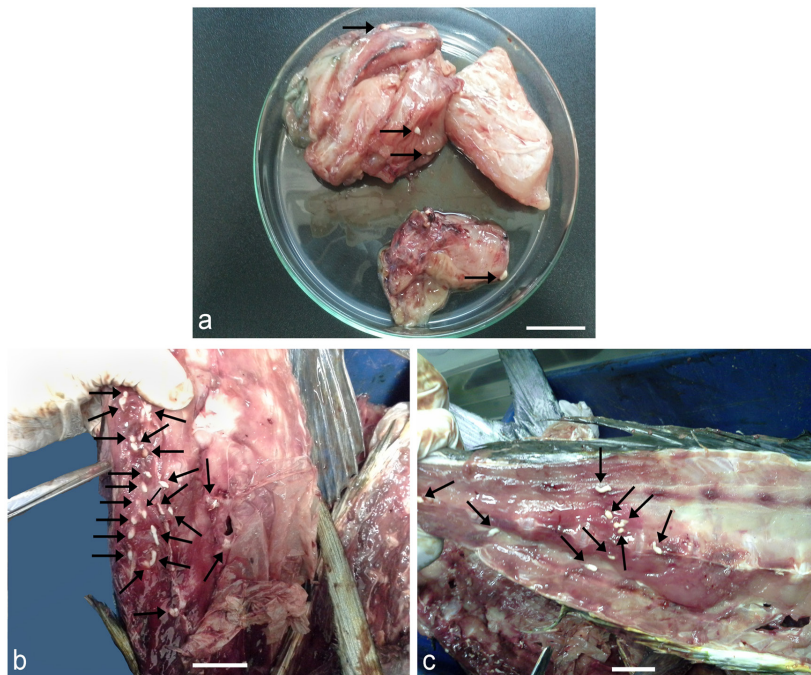
Tentaculariidae Poche, 1926

*Tentacularia* Bosc, 1797

*Tentacularia coryphaenae* Bosc, 1797

(Figures 2 a-b; 3 a-c)

Description: based on 10 mounted and measured, not compressed specimens, and 9 SEM specimens. Scolex acraspedote, 3.75-7.08 (4.80) (n=10) long with velum included, widest at the level of anterior portion of pars bothriialis or velum, 1.20-1.83 (1.46) (n=10) wide. Pars bothriialis with elongated bothriae, occupying almost 80% of the scolex, with sessile margins, 2.25-6.50 (3.83) (n=6) long, 1.20-1.65 (1.42) (n=6) wide. Pars vaginalis nearly 4.5 times smaller than scolex, with straight sheaths on its anterior two-thirds and sinuously close to the bulbs, 0.78-1.28 (0.98) (n=8) long. Pars bulbosa, 0.88-1.15 (1.02) (n=8) long, 0.20-0.53 (0.37) (n=8) wide. Individual bulbs, 0.75-1.08 (0.86) (n=27) long, 0.10-0.25 (0.15) (n=27) wide. Pars post-bulbosa well-developed. Velum, 0.68-1.75 (1.17) (n=10) long.



**Figure 1.** *Tentacularia coryphaenae* plerocercoid infection in *Coryphaena hippurus*. **a.** Fillets of the abdominal musculature of hosts collected in January 2014; arrows indicate the presence of the cestode parasites. **b-c.** Musculature of hosts collected in November 2014; arrows indicate the large number of cestodes causing severe hemorrhage. Bars a = 2.5 cm; b-c = 3 cm.

Appendix 0.98-6.15 (2.07) long. Tegumentary structures present include porosities and filiform microtriches on the scolex, and hook-like microtriches along the bothrial margins.

Width of tentacles, without hooks, 0.07-0.09 (0.08) (n=35) at basal region and, 0.05-0.07 (0.06) (n=35), at metabasal region. Basal hooks tridentiforme 0.008-0.013 (0.009) (n=30) long, implantation basal, 0.006-0.012 (0.009) (n=30) wide. Basal armature of tentacles with bilateral symmetry of ascending hook rows with identical V-shaped patterns on bothrial and antibothrial surfaces, and forming an identical,

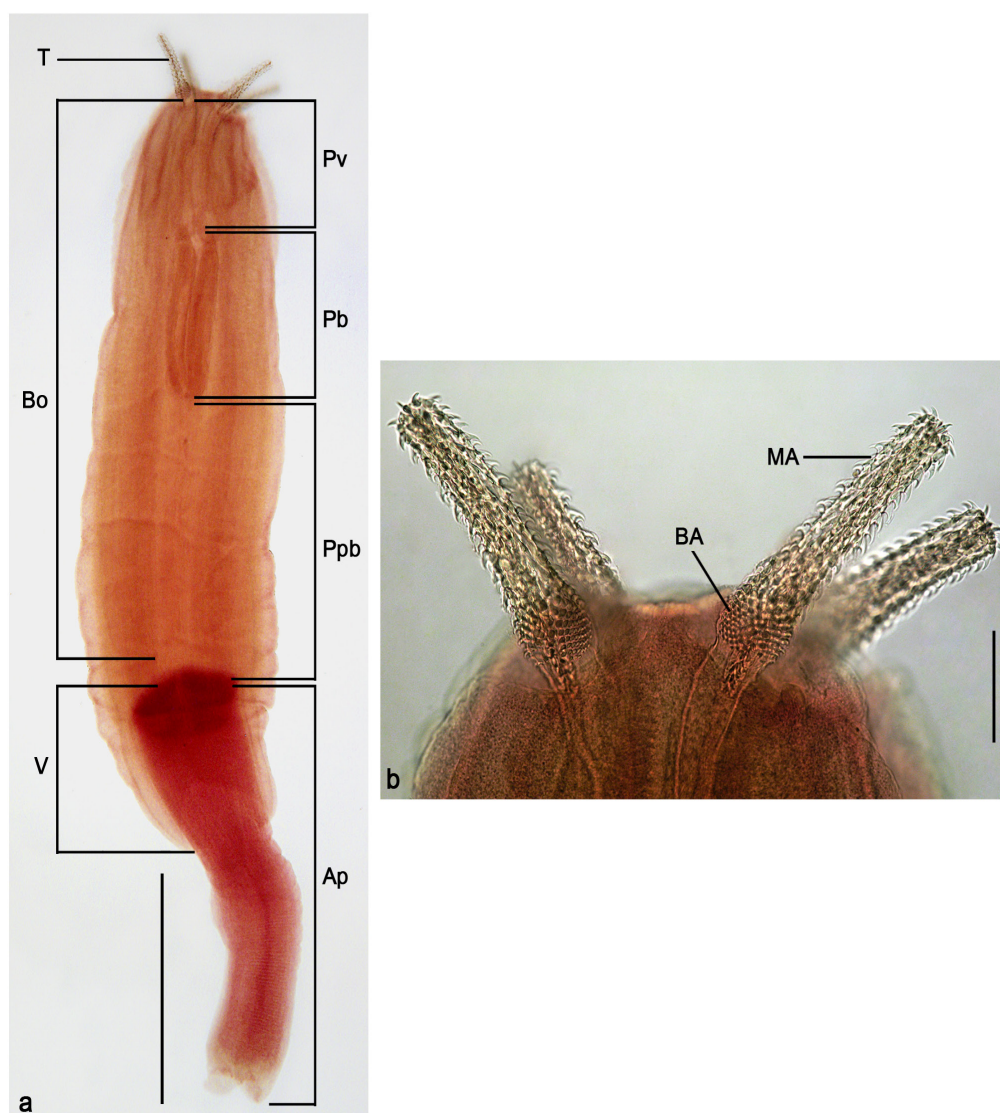
inverted, unarmed V-shaped pattern, on the external and internal surfaces. Metabasal armature with rotational symmetry, arranged in quincunx. Metabasal hooks unciforme 0.025-0.033 (0.027) long, base 0.020-0.030 (0.024) wide. Number of hooks/row 9-10. Hooks of same shape on both sides of tentacle.

Taxonomic summary

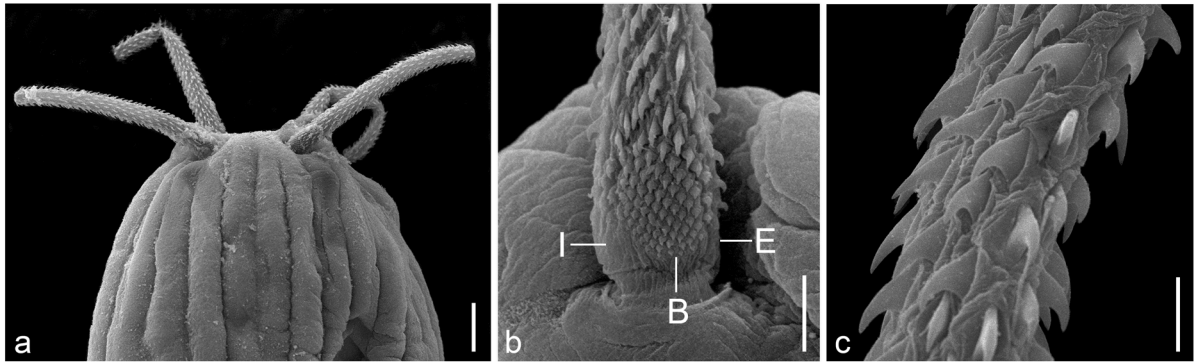
Collected specimens: 204 plerocercoids.

Specimens deposited: plerocercoids CHIOC n<sup>o</sup> 37998 a-e. Host: *C. hippurus*.

Locality: latitude 21°23' S, longitude 41°45' W, Cabo Frio, RJ, Brazil.



**Figure 2.** *Tentacularia coryphaenae* plerocercoid in *Coryphaena hippurus* under bright-field microscopy. **a.** Entire worm, showing tentacles (T), pars bothrialis (Bo), pars vaginalis (Pv), pars bulbosa (Pb), pars post-bulbosa (Ppb), velum (V), and appendix (Ap). **b.** Detail of extroverted tentacles, indicating the regions of basal armature (BA) and metabasal armature (MA), on the bothrial surface. Bars a = 1000  $\mu$ m; b = 100  $\mu$ m.



**Figure 3.** *Tentacularia coryphaenae* plerocercoid in *Coryphaena hippurus* under scanning electron microscopy. **a.** Detail of anterior region of scolex, showing tentacles and bothrial region. **b.** Detail of basal region in bilateral symmetry, with internal (I) and external surfaces (E) showing an unarmed inverted V-shaped pattern, and the bothrial surface (B) showing ascending rows of hooks (trident-like) in a V-shaped pattern beginning in the metabasal region. **c.** Detail of the metabasal region with uncinuate hooks with rotational symmetry and a quincunxial arrangement on the bothrial surface. Bars a = 200  $\mu\text{m}$ ; b = 50  $\mu\text{m}$ ; c = 20  $\mu\text{m}$ .

## DISCUSSION

The specimens of *T. coryphaenae* collected in the present study are in accordance with the descriptions of DOLLFUS (1942), KNOFF *et al.* (2004) and PALM (2004). The patterns of basal and metabasal hooks of the tentacular armature agree with those observed by BEVERIDGE and CAMPBELL (1996), PALM (2000) and KNOFF *et al.* (2004). In the present study, the presence of tegumentary ultrastructures of the scolex, such as porosities, filiform microtriche, and hook-like microtriches along the bothrial margins, were also observed by BILQEES and KHURSHID (1988), PALM (1995, 2000, 2004) and KNOFF *et al.* (2004).

In Brazil, *T. coryphaenae* has been reported parasitizing a variety of marine teleost hosts, including the dolphinfish and elasmobranch fishes. All these teleosts were collected from the coastal waters of the state of Rio de Janeiro. SILVA and SÃO CLEMENTE (2001) examined 596 fillets from 41 individuals of *C. hippurus* (size range of 65-148 cm total length) from the same area and found *T. coryphaenae* present in 25 fillets; 1 infected fillet from the 65-106 cm size class, and 24 infected fillets from the 107-148 cm size class.

A total of 56 parasites were found, for a prevalence of 0.47% and an intensity of infection of 1 parasite per fillet in the smaller size class, and a prevalence of 6.25% and an intensity of infection of 2.29 parasites per fillet in the larger size class; however, the authors did not provide information about the number of parasitized fish and the mean intensity of infection. Moreover, SÃO CLEMENTE (personal information)

did not observe massive infections by this species in the musculature.

*Tentacularia coryphaenae* has been reported from the mesentery of *Genypterus brasiliensis* with a prevalence of 1.35 and an intensity of 1 specimen per individual (SÃO CLEMENTE *et al.*, 2004); in *Katsuwonus pelamis* L., 1758, with a prevalence of 92.9% and mean intensity of 36.3 and a range of infection of 1-110 plerocercoids per fish in the abdominal and body musculature (AMATO *et al.*, 1990), and in another case with a prevalence of 66.7%, mean intensity of 5.8 and range of infection of 1-17 parasites in the musculature (ALVES and LUQUE, 2006); in *Lophius gastrophysus* Miranda-Ribeiro, 1915, with a prevalence of 2.3% and an intensity of 1 parasite in the musculature (SÃO CLEMENTE *et al.*, 2007); in *Scomberomorus cavalla* (Cuvier, 1829), with a prevalence of 3% and an intensity of 2 parasites in the mesentery (DIAS *et al.*, 2011).

Among elasmobranchs, *T. coryphaenae* has been reported parasitizing species off the coast of a variety of different Brazilian states, including being found in the stomach and spiral valve of a *Carcharinus longimanus* Poey, 1861, in Recife, state of Pernambuco, without information about the number of parasites collected (REGO, 1977), and from the spiral valve of *Prionace glauca* (L., 1758), *C. longimanus* and *C. obscurus* (Lesueur, 1818) collected in the state of Santa Catarina with prevalences of 16.7%, 50% and 100% and mean intensities of 0.2, 0.5 and 12 parasites per fish, respectively (KNOFF *et al.*, 2002, 2004). Comparing the data of the present study with data for teleost species of commercial importance studied

along the Brazilian coast, the specimens of *C. hippurus* studied herein (the previous report of *C. hippurus* did not make mention of a massive infection) and *K. pelamis* had highest indices of parasitism. The finding may be due to the influence of different physico-chemical factors and biological interactions experienced by the fish parasite communities of the different environments.

The species *T. coryphaenae* is known to exhibit low host specificity during its final and intermediate hosts. It is distributed worldwide in tropical and subtropical seas, and inhabit widely migrating scombroids and large pelagic predatory fish as second intermediate hosts. Adults of *T. coryphaenae* have been recorded from 11 different final host species, and is known from over 80 species of smaller and larger pelagic or oceanic fish and squids (PALM, 2004).

The findings of the present study corroborate previous reports and CHIOC examined material that the dolphinfish *C. equiselis* and *C. hippurus*, and tunas *K. pelamis* and *T. albacares*, as well as other pelagic and cosmopolitan migratory teleosts known are intermediate hosts in Brazil and throughout the world, and reinforce the hypothesis that these fish are acting as bridges for transfer of *T. coryphaenae* throughout marine food webs and among oceans (PALM *et al.*, 2007).

*Hepatoxylon trichiuri* (Holten, 1802) Bosc, 1811, is another trypanorhynch species that parasitizes *C. hippurus* in Brazil (SÃO CLEMENTE *et al.*, 2001), with a prevalence of 4.9% and a mean intensity of 1 parasite per fish in the liver and stomach serosas, however, none of the fish of the present study were parasitized with this species.

AMATO *et al.* (1990) suggested the removal of abdominal musculature as method for exporting *K. pelamis* free of these trypanorhynch metacestodes, however, this procedure does not seem viable in the present study, because, of widespread distribution of large numbers of metacestodes in muscular fascia. This observation was also reported for another trypanorhynch species, *Myxonybelinia* sp., a parasite of *Lophius gastrophysus* Miranda-Ribeiro, 1915, collected off the coast of the state of Rio de Janeiro (SÃO CLEMENTE *et al.*, 2007). The present study represents the first report of *T. coryphaenae* causing severe hemorrhage in *C. hippurus*, which has never even been referred to in previous studies (SILVA and SÃO CLEMENTE, 2001).

## CONCLUSIONS

The present study found *T. coryphaenae* plerocercoids to have significant hygienic-sanitary importance for *C. hippurus*, mainly because of its massive presence in the musculature and the resulting severe hemorrhage. These findings indicate that commerce with this fish species is impracticable in the ICRJ fishmarket due to the repugnant appearance of parasitized individuals.

The possibility of causing hypersensitivity reactions in humans illustrates the importance of inspecting fish for these trypanorhynch parasites. Furthermore, the results of this study suggest that in the future immunological studies, with a murine model, into these plerocercoids will be subject are needed for evaluating the risk of causing allergic reactions in humans.

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