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Scientific Article

Contracaecum spp. (NEMATODA: ANISAKIDAE) AND *Eustrongylides* spp. (NEMATODA: DIOCTOPHYMATIDAE) NEMATODE LARVAE WITH ZOONOTIC POTENCIAL FOUND IN TWO FISH SPECIES FROM TRAMANDAÍ RIVER BASIN, SOUTHERN BRAZIL*

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ABSTRACT

Parasitic nematodes can infect both wild and cultivated fish species and may be the source of many diseases. The consumption of raw fish meat in many countries has been the responsible of nematode related zoonosis. Knowing the risk of consumption of contaminated fish by the local communities, the main goal of this paper is to quantify and report the presence of larvae with zoonotic potential of *Contracaecum* spp. and *Eustrongylides* spp. found in two exotic fish species from the Tramandaí River Basin in southern Brazil. The sample sites were two lagoons of the basin, the Itapeva lagoon and Palmital lagoon, sampled in the period of April 2018. Forty specimens of *Acestrorhynchus pantaneiro* and twenty specimens of *Trachelyopterus lucenai* were examined. A. pantaneiro presented a 58% and 4% prevalence and a mean intensity of 3.17 and 1 for *Contracaecum* spp. and *Eustrongylides* spp., respectively. Meanwhile *T. lucenai* presented a prevalence of 70% and 5% and a mean intensity of 1.92 and 1 of *Contracaecum* spp. larvae and *Eustrongylides* spp. larvae, respectively. Although larvae of *Contracaeucum* spp. are reported only in the literature in experimental infections, they were able to complete their biological cycle in some mammals with certain pathogeneses. However, larvae of *Eustrongylides* spp. infect humans, as reported in the literature, causing severe damage to the intestinal tract. Knowing that local populations may be at risk, we recommend that all prophylactic measures be taken in relation to fish consumption. This study also contributes to the knowledge concerning the parasitic fauna of the host species studied.

Key words: zoonosis; anisakidosis; eustrongylidiasis.

Contracaecum spp. (NEMATODA: ANISAKIDAE) E *Eustrongylides* spp. (NEMATODA: DIOCTOPHYMATIDAE) LARVAS DE NEMATÓDEOS COM POTENCIAL ZOONÓTICO ENCONTRADAS EM DUAS ESPÉCIES DE PEIXES DA BACIA DO RIO TRAMANDAÍ, SUL DO BRASIL

RESUMO

Nematódeos são parasitas quem podem infectar tanto espécies de peixes silvestres como cultivados, no entanto, em altas intensidades podem ser origem de várias doenças. O consumo de carne crua de peixe em diversos países tem sido responsável por zoonoses relacionadas com nematódeos. Sabendo do risco do consumo de pescado contaminado pelas populações locais da área de estudo, o objetivo desse trabalho foi quantificar e reportar o primeiro registro de larvas com potencial zoonótico dos gêneros *Contracaecum* e *Eustrongylides* em duas espécies de peixes exóticas na Bacia do Rio Tramandaí, sul do Brasil. As coletas dos peixes ocorreram em duas lagoas da bacia, Lagoa Itapeva e Lagoa Palmital no período de abril de 2018. Foram necropsiados 50 exemplares de *Acestrorhynchus pantaneiro* e 20 de *Trachelyopterus lucenai*. A. pantaneiro apresentou 58% de prevalência e 3,17 de intensidade média de *Contracaecum* spp. e 4% e 1 de *Eustrongylides* spp., *T. lucenai* apresentou 70% de prevalência e 1,92 de intensidade média de *Contracaecum* spp. e 5% e 1 de *Eustrongylides* spp. Embora larvas *Contracaeucum* spp. só sejam reportadas na literatura em infecções experimentais, mostraram completar seu ciclo biológico em alguns mamíferos com certa patogenia. No entanto, larvas de *Eustrongylides* spp. já foram reportadas na literatura infectando humanos, causando danos severos na parte intestinal. Sabendo disso as populações locais podem estar em risco, recomenda-se então que todas medidas profiláticas sejam tomadas quanto ao consumo de peixe. O trabalho também contribui para o conhecimento da fauna parasitária das espécies hospedeiras estudadas.

Palavras-chave: zoonoses; anisakidosis; eustrongylidiasis.

INTRODUCTION

Parasite nematodes can infect species of both wild and cultivated fish and can also occur in healthy fish, however, in high intensities they can be the source of fish illnesses (Yanong, 2002). Moreover, all fish species are capable of carry at least one species of parasite, which can be found in both larval and adult stages (Meneguetti et al., 2013). Fish meat has been demonstrated as a healthy alternative compared with red meat, due to its high protein content, fatty acids percentages and low-fat percentage (Prado and Capuano, 2006). However, the consumption of raw fish meat in many countries has been responsible for nematode related zoonoses (Audicana et al., 2002; de Souza et al., 2016). The increased popularity of Asian food in the west and the poor preparation of the fish makes the Brazilian population susceptible to contracting diseases (Masson and Pinto, 1998; Okumura et al., 1999; de Magalhães et al., 2016).

Many nematodes species are reported to be parasitizing fish, however, only some species can infect humans (Adams et al., 1997). In freshwater fish, among the nematode families that cause most zoonoses, the Anisakidae family (Abdallah et al., 2012; Santana et al., 2017) and Dioctophymatidae (Spalding and Forrester, 1993; Narr et al.; 1996; Eiras et al., 2018) stand out.

In the family Anisakidae, the three genera that cause most zoonoses is *Anisakis* (Audicana et al., 2002; dos Reis, Sardella and Luque, 2016; Cipriani et al., 2016), *Pseutoterranova* (Mcclelland, 2002; Arizono et al., 2011; Weitzel et al., 2015) and *Contracaecum* (Vidal-Martinez et al., 1994; Barros et al., 2004; De Souza et al., 2016). In the Dioctophymatidae family the main zoonotic genus is *Eustrongylides* (Xiong et al., 2009; Melo et al., 2016).

The *Eustrongylides* genus have biological cycles which start with releasing eggs in piscivorous birds feces, their definitive hosts (Barros et al., 2004, 2006; Tavares et al., 2017). The *Contracaecum* genus in addition to piscivorous birds has marine mammals as definitive hosts too, being able to begin its cycle by the feces of both. (Nadler et al., 2005; Mattiucci and Nascetti, 2007). Both genera have species that parasitize fresh water fish, however, *Contracaecum* genus are only found in the L3 larval stage (Klimpel and Palm, 2011). The same happens with *Eustrongylides* genus, which can be found encysted or in the L4 larval stage (Coyner et al., 2002; Meneguetti et al., 2013). In both nematode genera the fish operate like intermediary or paratenic hosts and when a human being consumes raw or poorly cooked fish, they become the paratenic host (Valles-Veja et al., 2017).

The human disease caused by *Eustrongylides* larvae is known as Eustrongylidiasis, although rare, it is characterized by the penetration of the larvae into the wall of the host intestines, which can cause severe pain (Walderhaug, 2007; Netto, 2009). Anisakidosis is the disease caused by infection of nematodes larvae belonging to the family Anisakidae (Buchmann and Mehrdana, 2016; Younis et al., 2017; Castellanos et al., 2018; Cavallero et al., 2018). In a freshwater environment, this disease can be caused by larvae of the genera *Contracaecum, Hysterothylacium* and rarely by *Anisakis*, which is mostly marine but can be transported to the freshwater environments by migratory and estuarine fishes. (Moravec, 1998; Porto et al., 2015; Santana et al., 2017). After ingestion, the larvae act similarly to the *Eustrongylides* genus and penetrate the intestine, causing ulcers and occasionally perforation (De la Rue et al., 2010; Zullo et al., 2010; Bao et al., 2017).

Regarding the analyzed hosts, *Acestrorhynchus pantaneiro* (Menezes, 1992) is a fish from the Characiformes order, popularly known as "peixe-cachorro" (dog fish) in Brazil. A benthopelagic predator with carnivorous habits (Meurer and Zaniboni, 2012). In Rio Grande do Sul state it occurs naturally in the Uruguai river basin, although, because of unknown reasons, it is considered an alien species in the Rio Tramandaí basin.

Trachelyopterus lucenai is a catfish from the Siluriformes order, commonly known as "penharol" or "porrudo" in Brazil, and it is one of the thirteen species that compose the *Trachelyopterus* genus (Bertoletti et al., 1995). Just like *A. pantaneiro*, in Rio Grande do Sul state, it is native of the Uruguai river basin (Bertoletti et al., 1992). In the Tramandai river basin it is considered an alien species and was reported by Schifino et al. (2004) and Artioli and Maia, (2016). Its feeding habit can be described as generalist/opportunistic and it feeds mainly on fish, insects and mollusks (Maia et al., 2013).

Knowing the risk of consumption by the local population the main goal of this paper is to quantify and report the presence of larvae with zoonotic potential of *Contracaecum* spp. and *Eustrongylides* spp. found in these two-alien species of the Tramandaí River Basin, southern Brazil.

MATERIAL AND METHODS

Both host fish species used in the present work were collected April of 2018, in two distinct lagoons of the Tramandai river basin complex (Figure 1), with the help of professional fishermen, under the SISBIO permanent gathering license: 24968-1 and ethical license: CEUA-02/2018. The first place was Lagoa Palmital (29°49'21.22"S, 50° 9'52.75"O), Osório city, north coast of Rio Grande do Sul. The next was Lagoa Itapeva (29°27'17.49"S, 49°51'42.50"O), district of Lagoa Azul, Arroio do Sal city, northern coast of Rio Grande do Sul state. The fish were captured with the help of two fishing methods: gill nets and long lines with liver baits. In both localities the two species were captured in different proportions.

After the capture, the fish were cooled and moved to the Laboratory of Ichthyology of UNISINOS, being properly measured, weighed and sorted by species before being frozen. For the nematode search, the fish were necropsied, having their organs, muscles and head separated. The organs were sorted in Petri dishes containing saline solution 0.85%. The musculature of the fish's body was cut in a "V" shape while the head was separated from the spine. All the structures were analyzed with the assistance of needles and tweezer under a stereomicroscope model AxioPlan 2 by Zeiss.

When found, the nematodes were counted, spread in a Petri dish and fixated in A.F.A (alcohol, formalin and acetic acid) at 65°C. Right after, they were catalogued, separated and preserved in plastic micro tubes with alcohol 70°GL. The processes of fixation, clarification and storage followed (Amato and Amato, 2010). The identification of the nematodes was realized following (Moravec, 1998). Representative specimens were deposited in

Helminth Collection of UNISINOS Laboratory of Ichthyology, represented by: *Contracaecum* spp. (larvae): UNICTIO 1-4-1b; UNICTIO 1-5-1; UNICTIO 1-5-1b; UNICTIO 1-13-1; UNICTIO 1-13-1b; UNICTIO 1-27-1; UNICTIO 1-27-1b; UNICTIO 1-27-1B; UNICTIO 1-30-1; UNICTIO 1-37-1; UNICTIO 1-45-1; *Eustrongylides* sp. (larvae) UNICTIO 1-11-1b; UNICTIO 1-45-1c; UNICTIO 2-3-1D.

The morphometry and the microphotographs were performed through a ZEISS microscope model AxioPlan 2 with ocular scale and camera connected to the image software AxioVision 4.8.2 (ZEISS, 2012). The quantitive descriptors (prevalence, mean intensity of infection and abundance) followed the reviewed formulas by Bush et al. (1997). The formulas are as follows:

Prevalence: (Total number of parasitized hosts/Total number of examined hosts) x100

Mean Intensity: Total number of collected parasites from a determined species/Number of hosts infected by the parasite

Mean Abundance: Total number of collected parasites from a determined species/Total number of examined hosts

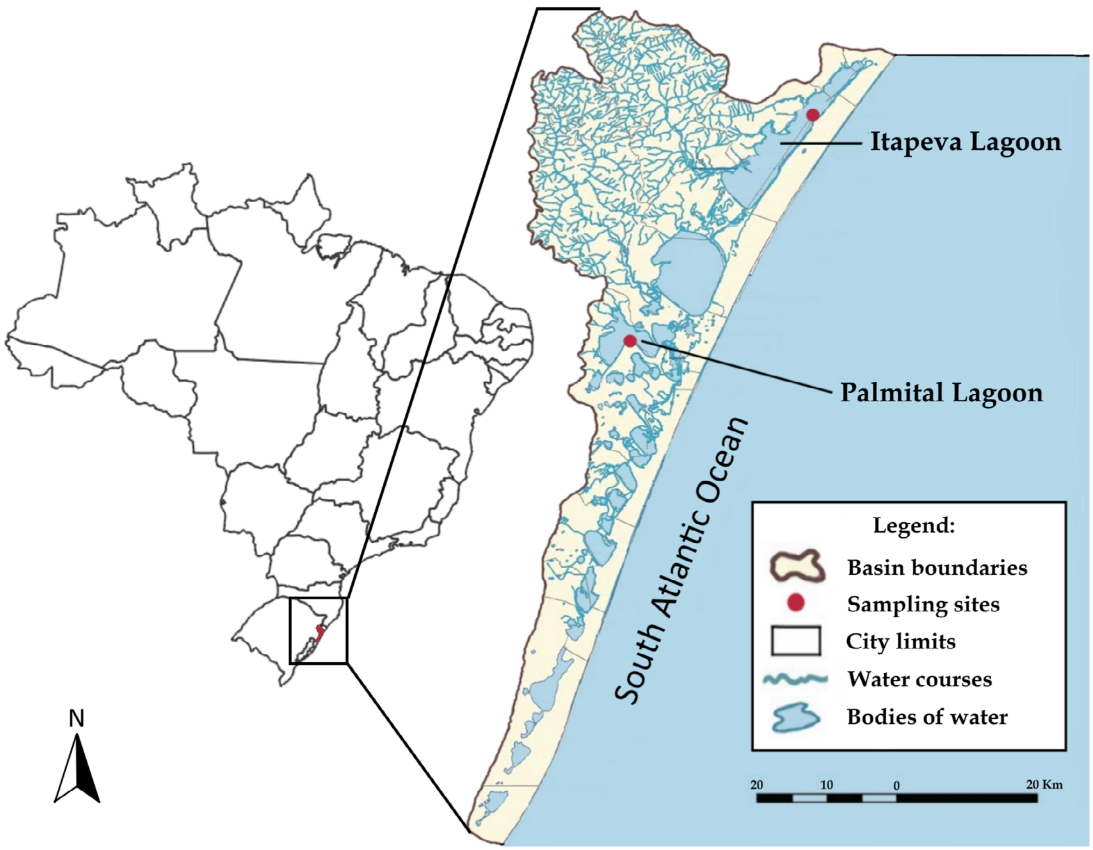

Figure 1. Sampling sites, Tramandaí river basin, north coast of Rio Grande do Sul. Source: author.

RESULTS

Of 70 fish necropsied, 50 of them were individuals of *Acestrorhynchus pantaneiro* (8 females, 42 males) (Figure 5), of which 29 were infected by larvae of the *Contracaecum* genus and 2 by larvae of *Eustrongylides* sp., and of the 20 samples of *Trachelyopterus lucenai* (11 females, 9 males), 14 of them were infected by 27 larvae of the *Contracaecum* genus (Figure 2 and 3) and one by larva from the *Eustrongylides* genus (Figure 3). The parasitological descriptors can be visualized below in (Table 1).

Table 1. Parasitological descriptors for the two analyzed host species

Host	Nematode	P(%)	MII Nematode/Fish	P Nematode/Fish	Infection Site
Acestrorhynchus pantaneiro	*Eustrongylides* spp.	4%	1	0.04	Musculature and intestines
	Contracaecum spp.	58%	3.17	1.84	Pyloric cecum, intestines, liver, mesentery and gonads
Trachelyopterus lucenai	*Eustrongylides* spp.	5%	1	0.05	Musculature
	Contracaecum spp.	70%	1.92	1.35	Mesentary, stomach, intestines and liver

(P= Prevalence; MA= Mean Abundance and MII= Mean Intensity of Infection)

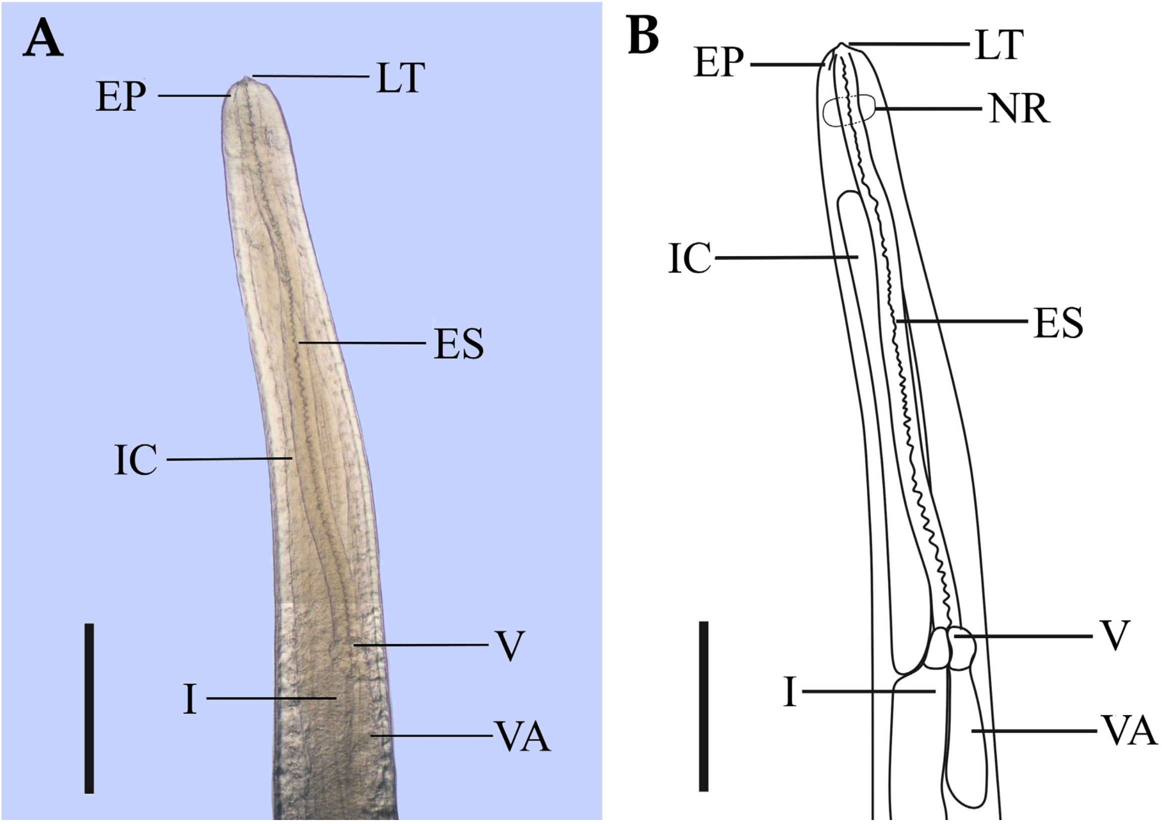

Figure 2. A- Anterior part of *Contracaecum* spp. **B** – Diagram of anterior part of *Contracaecum* spp. LT: Larval Tooth, EP: Excretory Pore, IC: Intestinal Caecum, ES: Esophagus, V: ventricle, I: Intestine, VA: Ventricular appendix. Scale Bar: 0,5 mm

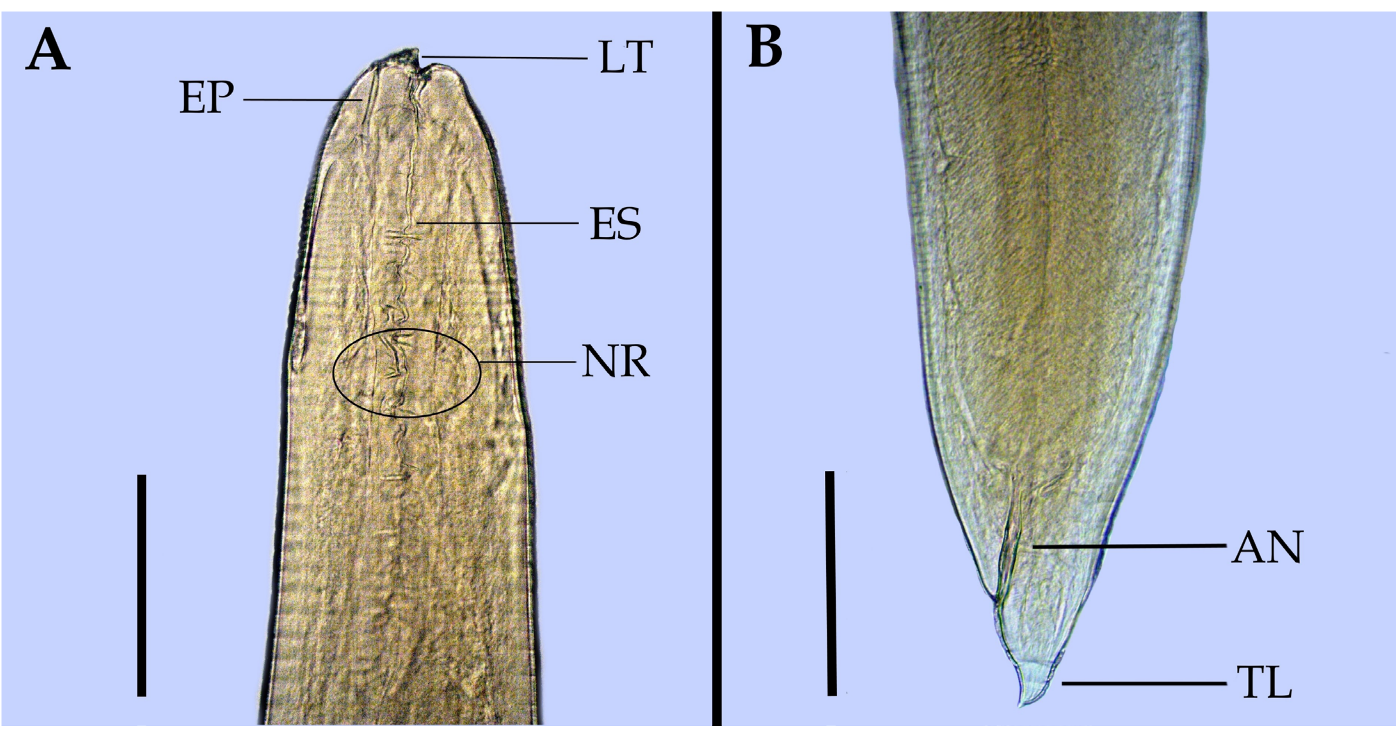

Figure 3. A - Anterior part of *Contracaecum* spp. LT: Larval Tooth, EP: Excretory Pore, ES: Esophagus, NR: Nerve Ring **B**- Posterior part of *Contracaecum* spp. AN: Anus, TL: Tail. Scale Bar: 0,2 mm

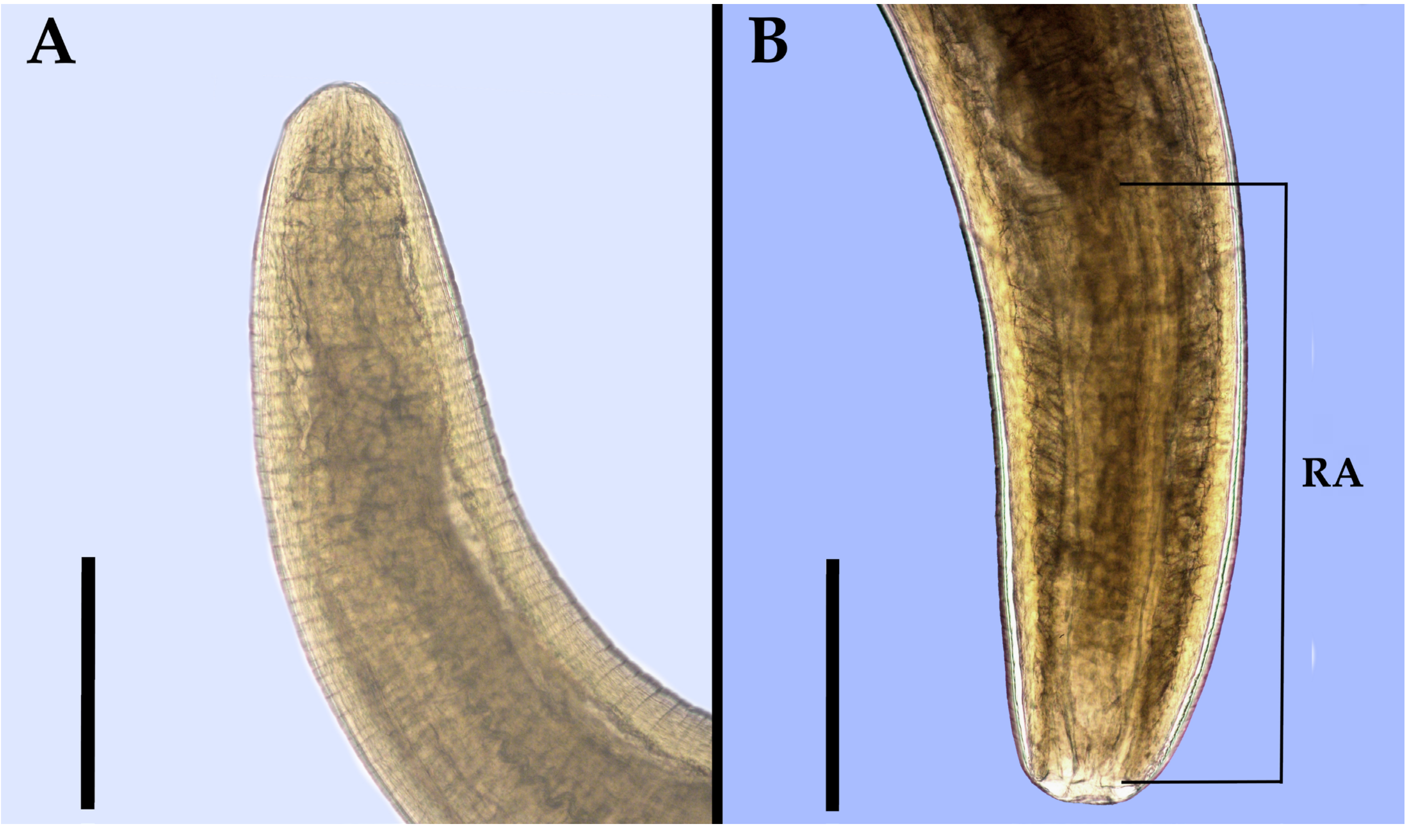

Figure 4. A – Anterior part of *Eustrongylides* spp. **B**- Posterior part of *Eustongylides* spp. RA: Rectal Ampulla

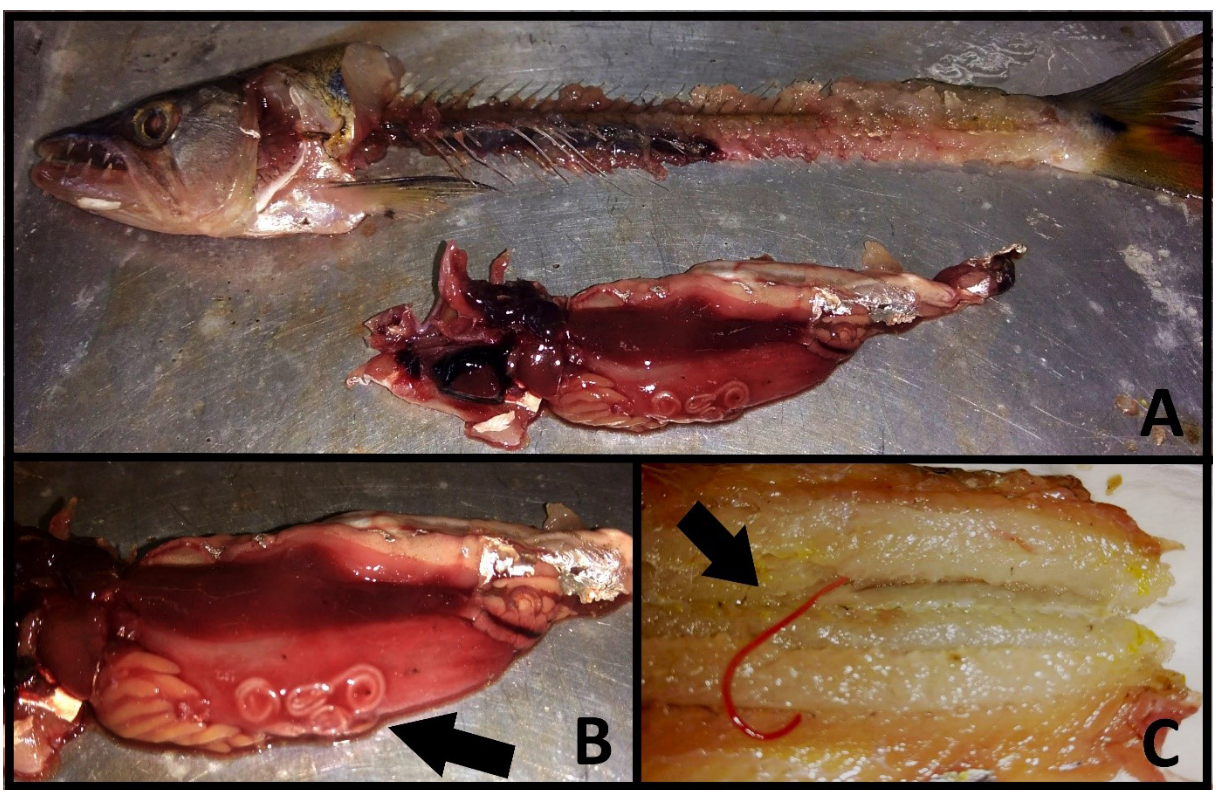

Figure 5. A- *Acestrorhynchus pantaneiro* in process of necropsy. **B-** *Contracaecum* spp. L3 stage larvae infection fixed to the stomach walls. **C-** *Eustrongylides* spp. L4 stage larvae present in the musculature of *Acestrorhynchus pantaneiro*.

DISCUSSION

Reffering to both helminth genera found in this study, it can be pointed out that this is the first record of *Contracaecum* spp. and *Eustrongylides* spp. in *A. pantaneiro* and *T. lucenai*. Eiras et al. (2010) in a wide review found fourteen host species of *Eustroungylides* spp. (larvae) including *A. lacustris*, and regarding *Contracaecum* spp. (larvae) the study found forty-two host species, again including A. lacustris, a congenus species of *A. pantaneiro*. In a more recent review by Eiras et al. (2016), *Contracaecum* spp. (larvae) was found in eighty-nine hosts, this time including a species from the *Trachelyopterus* genus, *T. striatulus*. *Eustroungylides* spp. (larvae) increased its number of hosts to thirty-six. Pantoja et al., (2016) registered *Contracaecum* spp. (larvae) in two more species of *Trachelyopterus*, *T. galeatus* and *T. coriacius*. *Eustrongylides* spp. (larvae) has not been reported in the *Trachelyopterus* genus. Concerning the reports of other helminths in general, until the present moment, this is the first parasitological study with *T. lucenai*, while *A. pantaneiro* was studied only at Argentina by Lunaschi and Drago (2004), registering one helminth species only; the trematode *Rhipidocotyle santanaensis*.

Concerning the results of parasitological descriptors, there are no studies available for comparison in the same hosts species, but as previously stated, results of phylogenetically related species can be found in the literature. In a study conducted by Carvalho et al., (2003) in the upper part of the Paraná river with *A. lacustris*, *Contracaecum* spp. (larvae) was found with a mean infection intensity of 19, higher than that of this study and a prevalence of 41.1%, lower than this study. *Eustrongylides* spp. (larvae) had a mean intensity of 2.5 which is higher than in this study and a prevalence of 3.9% which is like those found in our study. Abdallah et al., (2012) in a study performed with *A. lacustris* in state of São Paulo, from the Peixe river, reported *Contracaecum* spp. (larvae) with a mean intensity of 2.41 and prevalence of 25%, both lower than our results. Azevedo et al. (2016), in a study at the Batalha river, state of São Paulo, with *A. lacustris* found a mean intensity of 37.52 and a 96.87% prevalence, highlighting much bigger numbers than those found in this paper.

The *Trachelyopterus* genus has results represented by another studies. Mesquita et al., (2011) in a study with *T. strialatus* from the Gundu river, state of Rio de Janeiro, found *Contracaecum* spp. (larvae) with a mean intensity of 1.0 and a prevalence of 1.7%, lower results than the ones found here. Pantoja et al., (2016) in a study at the Amazon river basin reported *Contracaecum* spp. (larvae) with a mean intensity of 3.6 and prevalence of 13.5% for T. galeatus and a mean intensity of 2.5 and prevalence of 17.1% for *T. coriaceus*, having a higher intensity and lower prevalence than those of the current study. In the literature, it is clear that there is diversity in the given results. There are multiple explanations for these differences. According to Poulin, (2006), in the only study in which a specific attempt was made to elucidate the variation of the parasitological parameters between different communities of a same parasite, the results were inconclusive, implying that the intensity and the abundance of the parasites may be intrinsic characteristics of each parasite species, even with some variation, however, the prevalence cannot be considered as a characteristic parameter, since it can be influenced by a series of other biotic and abiotic factors. Marques and Cabral (2007) highlight that the prevalence can be influenced greatly by factors such as sampling size.

When referring to the health context, even in verified cases of infections in humans by other Anisakidae genera in other countries like Italy (Mattiucci et al., 2013), United States (Kliks, 1983; Amin et al., 2000) and Japan (Asami et al., 1965, Sonoda et al., 2015), there are no proven cases of infections in humans by larvae of the *Contracaecum* genus. However, in a study by Vidal-Martinez et al. (1994) with experimentally infected cats, the infections showed up positive and the parasite evolved until the adult phase, indicating a risk of infection in mammals.

Concerning pathogenicity, Barros et al. (2004), in a paper with experimentally infected rabbits with *C. multipapillatum* and *E. ignotus* larvae, the results showed hyperemia followed by rupture in the gastric mucosa caused by a *C. multipapillatum*, while *E. ignotus* generated hyperemia of the gastric mucosa followed by hemorrhagic gastritis, causing two rabbits to die. Instead of the *Contracaecum* genus, *Eustrongylides* genus has already been reported infecting humans. In an article published by Eberhard et al., (1989), a man from the state of New Jersey (USA) was infected by *Eustrongylides* spp. (larvae); the host was a 17-year-old young man who complained of strong pains. When receiving medical care, two worms were removed via the peritoneal cavity and intestinal perforation was verified. Although not reported in the literature for the genus *Contracaecum*, other anisakids such as *Anisakis* spp. and *Pseudoterranova* spp. besides gastric problems can also cause allergic reactions such as urticaria and angioedema (Audicana and Kennedy, 2008; Mazzucco et al., 2018).

When talking about risk of ingestion for humans, *Contracaecum* spp. (larvae) seem to be less susceptible to ingestion, since in the most cases it is found in fish viscera. Theoretically by the process of evisceration done by the fishermen before consumption the larvae would be discarded along with the viscera. However, the risk of ingestion of these larvae found in the abdominal cavity and viscera cannot be discarded. According to Fontenelle et al. (2013) these larvae have the capacity to migrate to the musculature and possess immunogenetic traits. *Eustrongylides* spp. (larvae) in the fish is at a more advanced stage, being commonly found in the musculature, which points out a greater risk of infection.

The main problem associated with parasitic infections among the local human population of the Tramandaí river basin is that, in the summer, thousands of tourists on vacation travel to the coast searching for recreation. According to Castro and Mello, (2013) the Tramandaí river basin is a complex of rivers and lagoons located very closely to the coast, having a lot of touristic potential. Given this proximity, in the period of December to March, vacationers tend to use angling fishing as a form of leisure. Most of the occasional anglers make use of the balneary and the coverage of bridges for the capture of various fish. Most are looking for native fish to consume, such as: *Rhamdia quelen*, *Hoplias malabaricus*, *Astyanax* spp., *Oligosarcus* spp. and *Geophagus brasiliensis*.

As previously mentioned, *A. pantaneiro* and *T. lucenai* are exotic species in the Tramandaí river basin. According to Latini and Petrere (2004) and Joy (2014) the presence of alien fish species is a serious threat to the native fish fauna and ecosystem, having a negative effect on the richness and abundance of the native communities. Consequently, with the lack of native fish, anglers are more likely to consume *A. pantaneiro* and *T. lucenai*. Malabarba et al. (2013) emphasizes the nutritional potential of *A. pantaneiro*, which presents a good amount of meat. However, *T. lucenai* is an abundant and unwanted fish, but in the absence of native catfish, mainly *R. quelen*, it is often consumed, since catfish are appreciated in the local cuisine in the form of soups.

Lastly, as a prophylaxis measure, Barros et al., (2008) advise that the storage of the fish for a period of 5 h at -30°C is enough to kill the larvae of *Contracaecum* spp. A similar freezing method was recommended by Deardorff and Throm (1988) to neutralize *Anisakis simplex*, another species from the Anisakidae family with high zoonotic potential. Moreover, Geller and Geller (1999) and Daschner et al. (2000) report that cooking the fish in a temperature superior to 60°C is enough to kill *A. simplex* larvae. Therefore, it is probable that by phylogenetic similarity the two methods are effective for nematode control in general. Recently the Brazilian government through a decree determines the freezing of products from fishing and aquaculture contaminated by endoparasites transmissible to man, raw consumption products to be marketed need be frozen at least -20°C for 24 hours or -35°C for 15 hours (Brasil, 2017). Thus, following the recommendations, the best way to control the zoonoses originated from fish consumption is to consume the fish well frozen or well cooked.

CONCLUSION

This study reports, in detail, the zoonotic potential of two nematode genera. It also indicates high prevalences of *Contracaecum* spp. (larvae) in both fish and points to the presence of *Eustrongylides* spp. (larvae) in the musculature of the fish; something that must be taken into consideration. Therefore, the present work is a warning so that all the possible prophylactic measures can be taken regarding the consumption of these fish, and, if possible, warn the local population of the risks of consuming the fish raw or poorly cooked. This paper also fills a knowledge gap concerning the parasitic fauna of the host species studied.

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REFERENCES

Abdallah, V.D.; de Azevedo, K.; Carvalho, E.D.; da Silva, R.J. 2012. New hosts and distribution records for nematode parasites of freshwater fishes from São Paulo state, Brazil. Neotropical Helminthology, 6(1): 43-57.

Adams, A.M.; Murrell, K.D.; Cross, J.H. 1997. Parasites of fish and risks to public health. Revue Scientifique et technique-office international des epizooties, 16(2): 652-660.

Amato, J.F.R.; Amato, S.B. 2010. Técnicas gerais para coleta e preparação de helmintos endoparasitos de aves. In: Von Matter, S.; Straube, F. C.; de Queiroz Piacentini, V.; Accordi, I. A.; Cândido Jr, J. F Ornitologia e Conservação: Ciência Aplicada, Técnicas de Pesquisa e Levantamento. Rio de Janeiro, Technical Books, p 369-393.

Amin, O.M., Eidelman, W.S., Domke, W., Bailey, J., Pfeifer, G. 2000. An unusual case of anisakiasis in California, USA. Comparative Parasitology, 67(1): 71-75.

Arizono, N.; Miura, T.; Yamada, M.; Tegoshi, T.; Onishi, K. 2011. Human infection with *Pseudoterranova azarasi* roundworm. Emerging infectious diseases, 17(3): 555. **http://dx.doi.org/10.3201/eid1703.101350**

Artioli, L.G.S.; Maia, R. 2016. Pisces, Siluriformes, Auchenipteridae, *Trachelyopterus lucenai* Bertoletti, Pezzi da Silva & Pereira, 1995: historical occurrence and distribution extension. Check List, 6(4): 515-516. **http://dx.doi.org/10.15560/6.4.515**

Asami, K.; Watanuki, T.; Sakai, H.; Imano, H.; Okamoto, R. 1965. Two cases of stomach granuloma caused by Anisakis-like larval nematodes in Japan. The American journal of tropical medicine and hygiene, 14(1): 119-123. **https://doi.org/10.4269/ajtmh.1965.14.119**.

Audicana, M.T.; Kennedy, M.W. 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clinical Microbiology Reviews, 21(2): 360–379. **https://dx.doi.org/10.1128/CMR.00012-07**

Audicana, M.T.; Ansotegui, I.J.; de Corres, L.F.; Kennedy, M.W. 2002. *Anisakis simplex*: dangerous—dead and alive? Trends in parasitology, 18(1): 20-25. **https://dx.doi.org/10.1016/S1471-4922(01)02152-3**

Bao, M.; Pierce, G.J.; Pascual, S.; González-Muñoz, M.; Mattiucci, S.; Mladineo, I.; Strachan, N.J. 2017. Assessing the risk of an emerging zoonosis of worldwide concern: anisakiasis. Scientific reports, 7(43699): 1-17. **https://dx.doi.org/10.1038/srep43699**.

Barros, L.A.; Sigarini, C.O.; Pinto, P. R. 2008. Resistência de larvas de *Contracaecum* sp., a baixas temperaturas. Revista Brasileira de Ciência Veterinária, 15(3): 55-58. **http://dx.doi.org/10.4322/rbcv.2014.216**

Barros, L.A.; Tortelly, R.; Pinto, R.M.; Gomes; D.C. 2004. Effects of experimental infections with larvae of *Eustrongylides ignotus* Jäegerskiold, 1909 and *Contracaecum multipapillatum* (Drasche, 1882) Baylis, 1920 in rabbits. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 56(3): 325-332. **http://dx.doi.org/10.1590/S0102-09352004000300007**

Barros, L.A.; Moraes Filho, J.; De Oliveira, R.L. 2006. Nematóides com potencial zoonótico em peixes com importância econômica provenientes do rio Cuiabá. Revista Brasileira de Ciência Veterinária, 13(1): 55-57. **http://dx.doi.org/10.4322/rbcv.2014.267**

Bertoletti, J.J.; da Silva, J.P.; Pereira, E.H.L; 1992. Nota sobre o gênero *Trachelyopterus Valenciennes*, 1840, no estado do Rio Grande do Sul, Brasil (Siluriformes, Auchenipteridae). Comunicações do Museu de Ciências da PUCRS, Série Zoologia, 5(10): 169-177.

Bertoletti, J.J.; Pezzi da Silva, J.F.; Pereira, E.H.L. 1995. A new species of the catfish genus *Trachelyopterus* (Siluriformes, Auchenipteridae) from southern Brazil. Revue française d'aquariologie, 22(4): 71-74.

Brasil, 2017 Decreto nº. 9.013, de 29 de março de 2017. Dispõe sobre o regulamento da inspeção industrial e sanitária de produtos de origem animal e das outras providências. Diário Oficial da União, Brasília, 29 de março de 2017, nº. 22, Seção 3, p.47.

Buchmann, K.; Mehrdana, F. 2016. Effects of anisakid nematodes *Anisakis simplex* (sl), *Pseudoterranova decipiens* (sl) and *Contracaecum osculatum* (sl) on fish and consumer health. Food and Waterborne Parasitology, 4(2): 13-22. **https://dx.doi.org/10.1016/j.fawpar.2016.07.003**

Bush, A.O.; Lafferty, K.D.; Lotz, J.M.; Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. The Journal of parasitology (83)4: 575-583. **https://dx.doi.org/10.2307/3284227**

Castellanos, J.A.; RicardoTangua, A.; Mercado, R.; Salazar, L. 2018. First reporting of *Anisakis* sp. in the Armed Snook fish (Centropomus armatus) caught and commercialized in Buenaventura, Colombia. Infectio, 22(3): 136-140. **http://dx.doi.org/10.22354/in.v22i3.724**

Cavallero, S.; Martini, A.; Migliara, G.; De Vito, C.; Iavicoli, S.; D'Amelio, S. 2018. Anisakiasis in Italy: Analysis of hospital discharge records in the years 2005-2015. PloS one, 13(12) **https://doi.org/10.1371/journal.pone.0208772**

Cipriani, P.; Acerra, V.; Bellisario, B.; Sbaraglia, G.L.; Cheleschi, R.; Nascetti, G.; Mattiucci, S. 2016. Larval migration of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy, *Engraulis encrasicolus*: Implications to seafood safety. Food Control, 59(1): 148-157. **https://doi.org/10.1016/j.foodcont.2015.04.043**

Coyner, D.F.; Spalding; Marilyn G.; Forrester, D.J. 2002. Epizootiology of *Eustrongylides ignotus* in Florida: Distribution, density, and natural infections in intermediate hosts. Journal of Wildlife Diseases, 38(3): 483-499. **https://doi.org/10.7589/0090-3558-38.3.483**

Daschner, A.; Alonso-Gómez, A.; Cabañas, R.; Suarez-de-Parga, J.M.; López-Serrano, M.C. 2000. Gastroallergic anisakiasis: borderline between food allergy and parasitic disease—clinical and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*. Journal of Allergy and Clinical Immunology, 105(1): 176-181.

De Carvalho, S.; Guidelli, G.M.; Takemoto, R.M.; Pavanelli, G.C; 2003. Aspectos ecológicos da fauna endoparasitária de *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes, Acestrorhynchidae) da planície de inundação do alto rio Paraná, Brasil. Acta Scientiarum. Biological Sciences, 25(2): 479-483. **http://dx.doi.org/10.4025/actascibiolsci.v25i2.2043**

De La Rue, M.L.; Ceolin, L.V.; de Castro, G.; Baldisserotto, C.; Becker, B.; De Mattos, A.G.; Almeida, F.; Junior, J.P. 2010. Risco de zoonose por parasitos do trato digestório de jundiás (*Rhamdia quellen*) coletados em reservatório de água da região central do Rio Grande do Sul. Santa Maria: saúde. 36(2): 79-81. **http://dx.doi.org/10.5902/223658342316**

De Magalhães, Â.M.S.; Costa, B.S.; Tavares, G.C.; de Carvalho, S.I.G. 2016. Zoonoses parasitárias associadas ao consumo de carne de peixe cru. Pubvet, 6(2): 11-14.

De Souza, M.E.; Cardoso, E.D.O.; Leal, L.A.; De Lima, T.M.P.; Toledo, R.C.C. 2016. Anisakidose Humana: Zoonose com risco potencial para consumidores de pescado cru. Veterinária e Zootecnia, 23(1): 25-37.

Deardorff, T.L.; Throm, R. 1988. Commercial blast-freezing of third-stage *Anisakis simplex* larvae encapsulated in salmon and rockfish. The Journal of parasitology, 74(4): 600-603. **http://dx.doi.org/10.2307/3282175**

Dos Reis Sardella, C.J.; Luque, J.L. 2016. Diagnóstico morfológico e molecular de larvas de *Anisakis typica* e *Anisakis brevispiculata* em peixes do litoral do Rio de Janeiro. Brazilian Journal of Veterinary Medicine, 38(3): 124-130.

Eberhard, M.L.; Hurwitz, H.; Sun, A.M.; Coletta, D. 1989. Intestinal perforation caused by larval *Eustrongylides* (Nematoda: Dioctophymatoidae) in New Jersey. The American journal of tropical medicine and hygiene, 40(6): 648-650. **https://doi.org/10.4269/ajtmh.1989.40.648**.

Eiras, J.C.; Pavanelli, G.C.; Takemoto, R.M.; Nawa, Y. 2018. An Overview of Fish-borne Nematodiases among Returned Travelers for Recent 25 Years–Unexpected Diseases Sometimes Far Away from the Origin. The Korean journal of parasitology, 56(3): 215. **https://doi.org/10.3347/kjp.2018.56.3.215**.

Eiras, J.C; Pavanelli, G.C.; Takemoto, R.M.; Yamaguchi, M.U.; Karkling L.C; Nawa, Y. 2016. Potential risk of fish-borne nematode infections in humans in Brazil–current status based on a literature review. Food and Waterborne Parasitology, 5(1): 1-6. **https://doi.org/10.1016/j.fawpar.2016.08.002**

Eiras, J.D.C.; Takemoto, R.M.; Pavanelli, G.C.; Adriano, E.A. 2010. Diversidade dos parasitas de peixes de água doce do Brasil. Maringá: Clichetec, 333p.

Fontenelle, G.; Knoff, M.; Felizardo, N.N.; Lopes, L.M.S.; São Clemente, S.M. 2013. Nematodes of zoonotic importance in *Cynoscion guatucupa* (Pisces) in the state of Rio de Janeiro. Revista Brasileira Parasitologia Veterinária, 22(3): 281-284. **http://dx.doi.org/10.1590/S1984-29612013005000019**

Geller, M.; Geller, P. 1999. Reações alérgicas provocadas por *Anisakis simplex* após a ingestão de peixes parasitados. Revista brasileira de alergia e imunopatologia, 22(2): 60-62.

Joy, M.K. 2014. New Zealand's freshwater disaster. New Zealand Science Review, 71(4): 97–104.

Kliks, M.M. 1983. Anisakiasis in the western United States: four new case reports from California. The American journal of tropical medicine and hygiene, 32(3): 526-532. **https://doi.org/10.4269/ajtmh.1983.32.526**.

Klimpel, S.; Palm, H.W. 2011. Anisakid nematode (Ascaridoidea) life cycles and distribution: increasing zoonotic potential in the time of climate change? In: Melhorn, H. Progress in parasitology. Berlin, Springer. p 201-222 **https://doi.org/10.1007/978-3-642-21396-0_11**

Latini, A.O.; Petrere Jr, M. 2004. Reduction of a native fish fauna by alien species: an example from Brazilian freshwater tropical lakes. Fisheries management and Ecology, 11(2): 71-79. **https://doi.org/10.1046/j.1365-2400.2003.00372.x**

Lunaschi, L.I.; Drago, F.B. 2004. Descripción de una especie nueva de *Tylodelphys* (Digenea: Diplostomidae) parásita de *Podiceps major* (Aves: Podicepedidae) de Argentina. Anales del Instituto de Biología Serie Zoología, 75(2): 245-252.

Maia, R.; Artioli, L.G.; Hartz, S.M. 2013. Diet and reproductive dynamics of *Trachelyopterus lucenai* (Siluriformes: Auchenipteridae) in subtropical coastal lagoons in southern Brazil. Zoologia (Curitiba), 30(3): 255-265. **http://dx.doi.org/10.1590/S1984-46702013000300002**

Malabarba, L.R.; Carvalho Neto, P.; Bertaco, V.D.A.; Carvalho, T.P.; Santos, J.D.; Artioli, L.G. S. 2013. Guia de identificação dos peixes da bacia do rio Tramandaí. Porto Alegre: Via Sapiens, 140p.

Marques, J.F.; Cabral, H.N. 2007. Effects of sample size on fish parasite prevalence, mean abundance and mean intensity estimates. Journal of applied Ichthyology, 23(2): 158-162. **https://doi.org/10.1111/j.1439-0426.2006.00823.x**.

Masson, M.L.; Pinto, R.D.A 1998. Perigos potenciais associados ao consumo de alimentos derivados de peixe cru. Boletim CEPPA (Curitiba), 16(1): 71-84.

Mattiucci, S.; Nascetti, G. 2007. Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. Veterinary Parasitology, 148(1): 43-57. **https://doi.org/10.1016/j.vetpar.2007.05.009**

Mattiucci, S.; Fazii, P.; De Rosa, A.; Paoletti, M.; Megna, A.S.; Glielmo, A.; Sorrentini, I. 2013. Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy. Emerging Infectious Diseases, 19(3): 496. **https://doi.org/10.3201/eid1903.121017**

Mazzucco, W.; Raia, D.D.; Marotta, C.; Costa, A.; Ferrantelli, V.; Vitale, F.; Casuccio, A. 2018. Anisakis sensitization in different population groups and public health impact: A systematic review. PloS one, 13(9): 1-23. **https://doi.org/10.1371/journal.pone.0203671**

Mcclelland, G. 2002. The trouble with sealworms *Pseudoterranova decipiens* species complex, (Nematoda): a review. Parasitology, 124(7): 183-203. **https://doi.org/10.1017/S0031182002001658**.

Mello, R.; Castro, D. 2013. Atlas Ambiental da Bacia Hidrográfica do Rio Tramandaí. Porto Alegre: Via Sapiens. 180p.

Melo, F.T.D.V.; Melo, C.D.S.B.; Nascimento, L.D.C.S.D.; Giese, E.G.; Furtado, A.P.; Santos, J.N.D. 2016. Morphological characterization of *Eustrongylides* sp. larvae (Nematoda, Dioctophymatoidea) parasite of *Rhinella marina* (Amphibia: Bufonidae) from Eastern Amazonia. Revista Brasileira de Parasitologia Veterinária, 25(2): 235-239. **http://dx.doi.org/10.1590/S1984-29612016024**

Meneguetti, D.U.D.O.; Laray, M.P.D.O.; Camargo, L.M.A. 2013. Primeiro relato de larvas de *Eustrongylides* sp. (Nematoda: Dioctophymatidae) em *Hoplias malabaricus* (Characiformes: Erythrinidae) no Estado de Rondônia, Amazônia Ocidental, Brasil. Revista Pan-Amazônica de Saúde, 4(3): 55-58. **http://dx.doi.org/10.5123/S2176-62232013000300008**

Mesquita, R.L.B.; Azevedo, R.K.; Abdallah, V.D.; Luque, J.L. 2011. Ectoparasites as numerical dominant species in parasite community of *Trachelyopterus striatulus* (Siluriformes: Auchenipteridae) from Guandu River, Southeastern Brazil. Brazilian Journal of Biology, 71(3): 623-627. **http://dx.doi.org/10.1590/S1519-69842011000400006**

Meurer, S.; Zaniboni-Filho, E. 2012. Reproductive and feeding biology of *Acestrorhynchus pantaneiro* Menezes, 1992 (Osteichthyes: Acestrorhynchidae) in areas under the influence of dams in the upper Uruguay River, Brazil. Neotropical Ichthyology, 10(1): 159-166. **http://dx.doi.org/10.1590/S1679-62252012000100015**

Moravec, F. 1998. Nematodes of freshwater fishes of the Neotropical region. Praga: Academia. 464 p.

Nadler, S.A.; D'Amelio, S.; Dailey, M.D.; Paggi, L.; Siu, S.; Sakanari, J.A. 2005. Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern Pacific marine mammals. Journal of Parasitology, 91(6): 1413-1430. **http://dx.doi.org/10.1645/GE-522R.1**

Narr, L.L.; O'Donnell, J.G.; Libster, B.; Alessi, P.; Abraham, D. 1996. *Eustrongylidiasis* - a parasitic infection acquired by eating live minnows. The Journal of the American Osteopathic Association, 96(7): 400-402. **https://doi.org/10.7556/jaoa.1996.96.7.400**.

Netto, M.T. 2009. Curso de boas práticas de fabricação de alimentos. Florianópolis: Secretaria do Estado da Saúde de Florianópolis, 322p.

Okumura, M.P.M.; de Pérez, A.C.A.; Espíndola Filho, A. 1999. Principais zoonoses parasitárias transmitidas por pescado-revisão. Revista de Educação Continuada em Medicina Veterinária e Zootecnia do CRMV-SP, 2(2): 66-80.

Pantoja, W.M.D.F.; Silva, L.V.F.; Tavares-Dias; M. 2016. Are similar the parasite communities structure of *Trachelyopterus coriaceus* and *Trachelyopterus galeatus* (Siluriformes: Auchenipteridae) in the Amazon basin?. Revista Brasileira de Parasitologia Veterinária, 25(1): 46-53. **http://dx.doi.org/10.1590/S1984-29612016016**

Porto, S.M.; Cárdenas, M.Q.; Martins, M.L.; Oliveira, J.K.Q.; Pereira, J.N.; Araújo, C.S.O.; Malta, J.C.O. 2015. First record of larvae of *Hysterothylacium* (Nematoda: Anisakidae) with zoonotic potential in the pirarucu Arapaima gigas (Osteichthyes: Arapaimidae) from South America. Brazilian Journal of Biology, 75(4): 790-795. **http://dx.doi.org/10.1590/1519-6984.22213**

Poulin, R. 2006. Variation in infection parameters among populations within parasite species: intrinsic properties versus local factors. International Journal for Parasitology, 36(8): 877-885.

Prado, S.P.T.; Capuano, D.M. 2006. Relato de nematóides da família Anisakidae em bacalhau comercializado em Ribeirão Preto, SP. Revista da Sociedade Brasileira de Medicina Tropical, 39(6): 580-581. **http://dx.doi.org/10.1590/S0037-86822006000600016**

Santana, H.P.; Dos Anjos, C.S.; Morais, A.M.; De Oliveira Malta; J.C. 2017. Nematode larvae with zoonotic importance found in peacock bass *Cichla monoculus* (Spix & Agassiz, 1831) from floodplain lakes in Central Amazon. Revista Brasileira de Zoociências, 18(2): 71-75.

Schifino, L.C.; Fialho, C.B.; Verani, J.R. 2004. Fish community composition, seasonality and abundance in Fortaleza lagoon, Cidreira. Brazilian Archives of Biology and Technology, 47(5): 755-763. **http://dx.doi.org/10.1590/S1516-89132004000500011**

Sonoda, H.; Yamamoto, K.; Ozeki, K.; Inoye, H.; Toda, S.; Maehara, Y. 2015. An Anisakis larva attached to early gastric cancer: report of a case. Surgery today, 45(10): 1321-1325. **http://www.doi.org/10.1007/s00595-014-1012-3**.

Spalding, M.G.; Forrester, D.J. 1993 Pathogenesis of *Eustrongylides ignotus* (Nematoda: Dioctophymatoidea) in ciconiiformes. Journal of Wildlife Diseases, 29(2): 250-260. **https://doi.org/10.7589/0090-3558-29.2.250**.

Tavares, L.E.R.; Campião, K.M.; Costa-Pereira, R.; Paiva, F. 2017. Helmintos endoparasitos de vertebrados silvestres em Mato Grosso do Sul, Brasil. Iheringia Série Zoologia, 107(1): 1-14.

Valles-Vega, I.; Molina-Fernández, D.; Benítez, R.; Hernández-Trujillo, S.; Adroher, F.J. 2017. Early development and life cycle of *Contracaecum multipapillatum* from a brown pelican *Pelecanus occidentalis* in the Gulf of California, Mexico. Diseases of aquatic organisms, 125(3): 167-178. **https://doi.org/10.3354/dao03147**.

Vidal-Martinez, V.M.; Osorio-Sarabia, D.; Overstreet, R.M. 1994. Experimental infection of **Contracaecum multipapillatum** (Nematoda: Anisakinae) from Mexico in the domestic cat. The Journal of parasitology, 1(8): 576-579.

Walderhaug, M. 2007. Bad Bug Book: Foodborne pathogenic microorganisms and natural toxins handbook. U.S.A: F.D.A, food and drug administration (departament), p 2009.

Weitzel, T.; Sugiyama, H.; Yamasaki, H.; Ramirez, C.; Rosas, R.; Mercado, R. 2015. Human infections with *Pseudoterranova cattani* nematodes, Chile. Emerging infectious diseases, 21(10): 1874. **https://doi.org/10.3201/eid2110.141848**

Xiong, F.; Wang, G.T.; Wu, S.G.; Nie, P. 2009. Development of *Eustrongylides ignotus* (Nematoda: Dioctophmida) in domestic ducks (*Anas platyrhynchos domestica* (L.)). Journal Parasitology, 95(5): 1035-1039. **https://doi.org/10.1645/GE-2057.1**

Yanong, R.P. 2002. Nematode (roundworm) infections in fish. Gainesville: University of Florida, Cooperative Extension Services. 10.

Younis, A.E.; Saad, A.I.; Rabei, J.M. 2017. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in four teleostean species from Lake Nasser, Egypt: morphological and molecular studies. The Journal of Basic and Applied Zoology, 78(1): 9. **https://doi.org/10.1186/s41936-017-0012-4**

Zullo, A.; Hassan, C.; Scaccianoce, G.; Lorenzetti, R.; Campo, S.M.; Morini, S. 2010. Gastric anisakiasis: do not forget the clinical history. Journal of Gastrointestinal Liver Diseases, 19(4): 359.