ARTICLES
Archives of Veterinary Science
https://www.ufpr.br/

Anisakis sp. larvae in *Plagioscion squamosissimus* (Heckel, 1840) from the Curralinho municipality, Marajó Island, Pará, Brazil

Submitted: *11/02/2025*
Accepted: *25/10/2025*

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Abstract: Several reports from Brazil indicate the presence of fish parasitized by nematodes with zoonotic potential, notably those within the Anisakidae family. This research investigates the morphology, morphometry, and prevalence of *Anisakis* larvae parasitizing fish being sold in the municipality of Curralinho, in the Brazilian state of Pará. Twenty specimens of *Plagioscion squamosissimus* were directly collected from the Guajará River by fishers in the city of Curralinho. These fish were purchased, necropsied, and their organs were individually placed in Petri dishes containing saline solution for helminthological examination using a stereomicroscope. All the fish (100%) were found to be parasitized, and a total of 1,390 third-stage *Anisakis* larvae were recovered, encysted in the intestinal serosa and mesentery. The third-stage larvae were analyzed using light and scanning electron microscopy. Morphologically, the third-stage *Anisakis* larvae exhibited an anterior end featuring a dorsal lip and two poorly developed ventrolateral lips, a boring tooth, and an excretory pore situated below the boring tooth. Internally, they possessed a muscular esophagus, a long ventriculus, a terminal mucron, and a ventricular appendix, while an intestinal cecum was absent. The presence of these third-stage *Anisakis* larvae in fish sold in markets underscores their significance for public health, as these Anisakidae nematodes, especially *Anisakis* spp, are known to cause anisakiasis.
Keywords: Anisakidae, Parasite, Fish, Amazon.

1. Introduction

Anisakidae Skrjabin & Karokhin, 1945 is a large family of Ascaridoidea Railliet & Henry, 1915, with emphasis on the genera *Anisakis* Dujardin, 1845, *Contracaecum* Railliet & Henry, 1912, *Peritrachelius* (Diesing, 1851), *Pulchrascaris* Vicente & Santos, 1972, *Pseudoterranova* Mozgovoi, 1951, *Skrjabinisakis* (Mozgovoy, 1951) and *Terranova* Leiper & Atkinson, 1914, all of which act as intermediate hosts (Anderson, 2000; Klimpel & Palm, 2001; Luque et al., 2011; Borges et al., 2012; Safonova et al. 2021). Numerous records in Brazil document the presence of parasites from the family Anisakidae in marine, freshwater, and estuarine fish, and many of these records note the zoonotic potential of this group (Fontenelle et al., 2013). According to Santos (2017), parasitic diseases linked to fish consumption in Brazil often go unnoticed, mainly due to some factors, such as the mild severity of symptoms and a lack of awareness among doctors, health inspectors, and consumers.

Anisakids are parasitic bioagents that pose a significant public health risk, as they can be accidentally transmitted to humans through the ingestion of raw or undercooked fish infected by L3 larvae (Andrade-Porto et al., 2015; Eiras et al., 2016; Souza et al., 2016; Alves et al., 2019). Due to the substantial presence of parasites from the Anisakidae family in commercially important fish in Brazil, in 2010, the Ministry of Health classified the biological risk of infection by anisakids as Risk Class 2, indicating moderate risk and limited transmission potential (Brasil, 2010). According to Shih et al. (2010), the primary concern regarding these parasites is the disgust people experience when encountering them in food or during preparation, which can subsequently reduce consumption of this protein.

Pinheiro et al. (2019) highlight the scarcity of studies on Anisakidae larvae in fish in Brazil, particularly given the numerous environments that remain unstudied and the ichthyofauna present in these habitats across different states. This study aims to present the morphology and morphometry of the Nematoda Anisakidae parasite of *Plagioscion squamosissimus* (Heckel, 1840), captured in the Guajará River and sold in open-air markets in the Curralinho municipality of the Marajó Island, Pará.

2. Materials and Methods

Twenty specimens of *Plagioscion squamosissimus* [total length 14,5−22 (17,6) cm; weight 69–215 (124) g] were obtained. Fish were captured by artisanal fishers in Rio Guajará (1° 36' 39" S 50°18' 12" W), municipality of Curralinho, the island of Marajó, Pará State, Brazil. Fish were collected from November to December 2023 with the aid of a cast net and transported dead in thermal boxes filled with ice to the laboratory for necropsy. After biometric analyses, the animals were necropsied for helminths. The digestive tract of each specimen was isolated in a Petri dish containing a physiological solution and analyzed using a stereomicroscope (LEICA-ES2). The dead nematode larvae were fixed in an AFA solution (93 parts 70% ethyl alcohol, five parts formaldehyde, and two parts glacial acetic acid). For morphological and morphometric analysis, 10 larvae were dehydrated in an ethanol series, clarified with lactophenol, placed on a microscope slide under a coverslip as a temporary mount, observed with a light microscope, and photographed with a LEICA DM2500 microscope and an imaging capture system. Measurements are shown in millimeters as the mean followed by the range or as otherwise indicated. Taxonomic classification of nematodes was by Moravec (1998), Timi et al. (2001), Felizardo et al. (2009), Fontenelle et al. (2013), and Fonseca et al. (2016).

For scanning electron microscopy, six larvae were washed in phosphate-buffered saline (pH 7.0), post-fixed in 1% osmium tetroxide, dehydrated to the critical point of CO2, metalized with gold-palladium, and analyzed with the scanning electron microscope VEGA 3 LMU/TESCAN in the Laboratório de Microscopia Eletrônica de Varredura, Instituto da Saúde e Produção Animal - Universidade Federal Rural da Amazônia - UFRA, state of Pará, Brazil. The ecological indices of parasitism were used according to Bush et al. (1997) and Bautista-Hernández et al. (2015). This study was performed according to the principles of the Comissão de Ética no Uso de Animais—CEUA/UFRA (n: 7809140122).

3. Results

A total of 1390 nematodes were recovered from *Plagioscion squamosissimus*, demonstrating prevalence: 100% (20/20), mean intensity: 69.5, mean abundance: 69.5, and amplitude: 21 to 196 nematodes per fish. All specimens collected exhibited characteristics compatible with those of third-stage larvae of *Anisakis* sp. (Nematoda: Anisakidae). The parasites were encysted in the intestinal serosa and mesentery. The morphological and morphometric characteristics of the third-stage larvae of *Anisakis* are presented below.

Anisakis Dujardin, 1845

Anisakis sp. (third stage larvae) (Figures 1, 2, and 3)

(Description based on 10 larvae)

Medium-sized nematodes, measuring 11 (8–14) long, width at height of ventricle 0.17 (0.13–0.23), opaque white when alive. The cuticle is transversely striated and more distinct at the ends of the body (Figure 1A). The cephalic extremity is rounded with a small ventral boring tooth, three submedian cephalic papillae surrounding the small transverse oval-shaped oral opening, and three poorly developed lips (Figure 3A). The excretory pore is ventrally below the boring tooth (Figure 1B, 3B). Muscular esophagus, measuring 1.0 (0.8–1.0) long and 0.07 (0.05–0.09) wide (Figure 2B). Ventriculus long and cylindrical, 0.37 (0.29–0.50) long and 0.12 (0.073–0.18) wide. The entire esophagus and ventriculus length represents 13% (10–15%) of body length. The nerve ring is positioned 0.22 (0.17–0.25) from the anterior end (Figure 1B). Deirids were observed only by scanning electron microscopy (Figure 3A). Ventricular appendix and intestinal cecum absent. The rectum appears as a short hyaline tube, measuring 0.077 (0.053–0.12) (Figure 1C); three small unicellular rectal glands are present (Figure 2C). The conical tail is 0.073 (0.042–0.095) long and tapered, with marked transverse striations (Figure 3C). Mucron measuring 0.017 (0.012–0.025) long (Figure 2C).

4. Discussion

The nematodes encysted in the intestinal serosa and mesentery of *Plagioscion squamosissimus* sold at markets in the municipality of Curralinho exhibit characteristics similar to those of the genus *Anisakis*, including a boring tooth below the oral aperture between the two ventrolateral lips. Excretory pore opening beneath the boring tooth. Ventriculus length greater than width. Ventricular appendix and intestinal cecum absent. Tail conical and mucron present. According to Moravec (1998), Felizardo et al. (2009), Fontenelle et al. (2016), and Fonseca et al. (2016), these are important characteristics for diagnosing nematodes in the Anisakidae (Skrjabin & Karokhin, 1945), including Anisakis Dujardin, 1845.

With the advent of phylogenetic studies, many researchers have discussed the current classification of *Anisakis*, particularly those based solely on larval forms. The morphological similarities of *Anisakis* larvae to other genera, such as *Skrjabinisakis*, and the use of molecular data have resulted in the reclassification of some species. Safonova et al. (2021) supported by molecular data reclassified four *Anisakis* species as *Skrjabinisakis: S. physeteris* (type species) (syn. *Anisakis physeteris* and *Anisakis skrjabini* (Mozgovoy, 1949)); *S. schupakovi* (Mozgovoy, 1951); *S. brevispiculata* and *S. paggiae* (Safonova et al., 2021; Chero et al., 2023).

In this study, we will use the generic classification of Anisakis due to the lack of molecular data. Moravec (1998) and Moravec et al. (2016) state that the systematics of Anisakidae fish parasites have been based on adult morphology, while the systematics of larvae remain underdeveloped, making it impossible to assign more specific taxonomic levels to the larval forms.

Although this is not the first occurrence of L3 of *Anisakis* sp. found parasitizing *Plagioscion squamosissimus* in the state of Pará, this work is the first record of the occurrence of this genus in fish sold and consumed in Curralinho municipality, on Marajó Island, State of Pará. In their checklist of Anisakidae parasites in fish in Brazil, Luque et al. (2011) described the occurrence of *Anisakis typica* (Diesing, 1860) Baylis, 1920, *S. physeteris* Baylis, 1923 (syn. *A. physeteris*), *Anisakis pegreffii* Campana-Rouget & Biocca, 1955, and *Anisakis* sp., in addition to the genera *Contracaecum, Pseudoterranova, Pulchrascaris*, *Terranova*, *Goezia, and Pseudanisakis.* All were found to be parasitizing freshwater, estuarine, and marine fish in different states (RJ, CE, PR, RS) along the Brazilian coast. The larvae of *Anisakis* sp. in this study showed morphological and morphometric similarities with those described by Fontenelle et al. (2016) in *Plagioscion squamosissimus* captured in Marajó Bay and the Tapajós River, the same biogeographic region as this study.

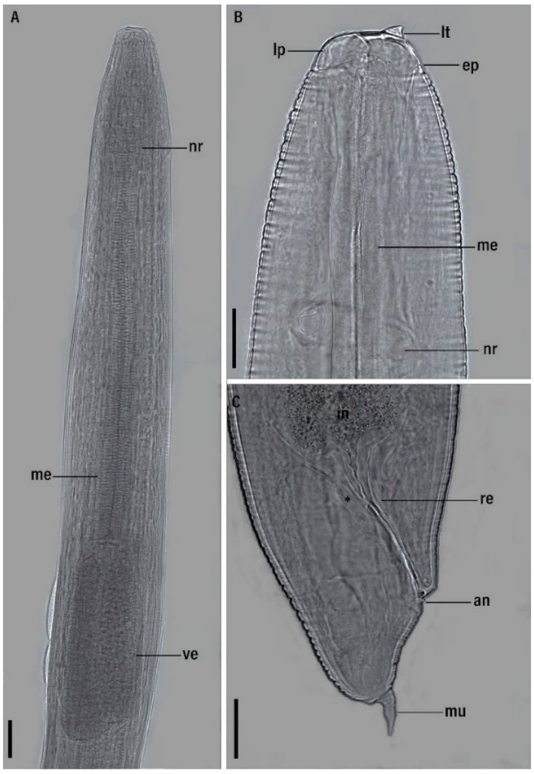

Figure 1 – Photomicrographs of L3 *Anisakis* sp. parasite in *Plagioscion squamosissimus*: A. Anterior end, showing nerve ring (nr), muscular esophagus (me) and ventriculus (ve). B. Detail of anterior end, cuticle with delicate transversal striations, lips (lp) larval tooth (lt), excretory pore (ep) and nerve ring (nr). C. Posterior portion, showing end portion of the intestine (in), rectum (re), unicellular rectal glands (*) and anus (an), the tail with mucron. The scale bars: A = 100 µm, B and C = 50 µm.

Giese (2009) recorded the parasitism and morphology of *Anisakis* larvae in *Ageneiosus ucayalensis* from the Guamá River and Guajará Bay. Reis et al. (2021), when carrying out a checklist of nematodes parasitizing fish in the Brazilian Amazon, recorded *Anisakis* larvae parasitizing the gastrointestinal tract of *Acestrorhynchus falcatus* (Bloch, 1794); *Pimelodus blochii* Valenciennes, 1840; *Plagioscion squamosissimus* (Heckel, 1840); *Pygocentrus nattereri* Kner, 1858; *Serrasalmus altispinis* Merckx, Jégu & Santos, 2000; *Triportheus angulatus* (Spix & Agassiz, 1829) in the states of Acre, Pará, and Amazonas. Despite the extensive records of *Anisakis* larvae in Brazilian ichthyofauna, few studies provide morphometric data on these larvae. This study compares the morphological and morphometric data of third-stage *Anisakis* larvae parasites found in *Plagioscion squamosissimus* from Curralinho municipality with those of larvae harvested from different hosts in Brazil, as shown in Table 1.

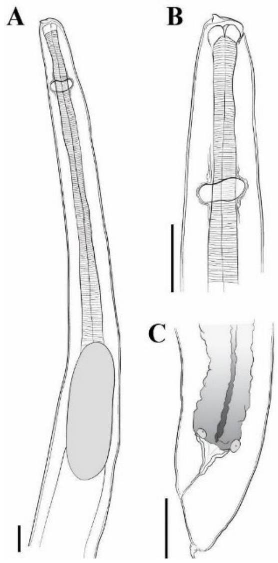

Figure 2 – Drawings of L3 of *Anisakis* sp. Parasite in *Plagioscion squamosissimus*. The scale bars: A, B and C = 100 µm.

https://doi.org/10.5380/avs.v30i4.98412

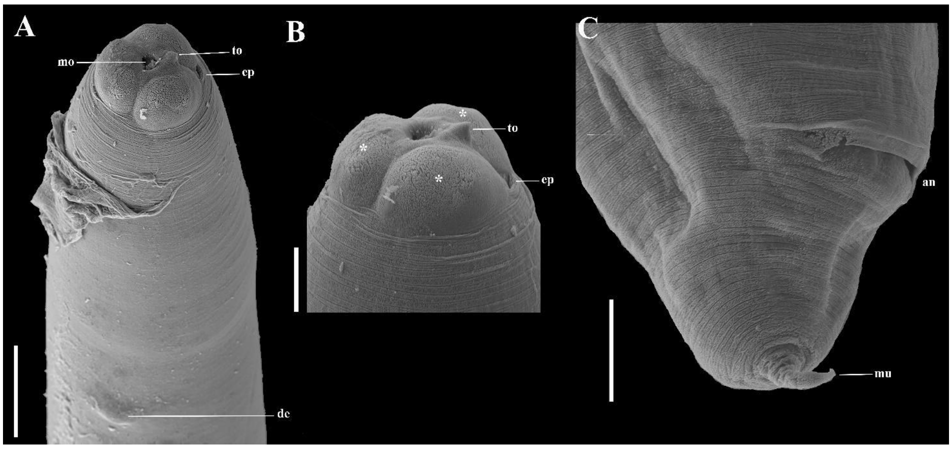

Figure 3 – Scanning electron micrographs of L3 *Anisakis* sp. parasite in *Plagioscion squamosissimus*: (A) Cephalic region showing evidence cuticle with transversal striations, three lips, mouth opening (mo), provided with boring tooth (to), excretory pore (ep) and deirid (de); (B) Side view of cephalic region with papillae (*), larval tooth (to) and excretory pore (ep); (C) Posterior portion, anus (an), the tail with mucron. The scale bars: A = 50 µm, B and C = 20 µm.

Character	Third stage larvae the *Anisakis* spp.					
Hosts larvae	*Plagioscion squamosissimus*	*Paralichthys isosceles*	*Ageneiosus ucayalensis*	*Cichla monoculus*	*Pygocentrus nattereri*	*Hypophthalmus marginatus*
Length[a]	8.0–14.0	15.3–16.0	11.8–15.9	9.80–17.05	19–25	9.32–13.87
Width	0.13–0.23	0.35–0.37	0.17–0.23	0.19–0.35	0.54–0.63	0.27–0.32
Nervous ring	0.17–0.25	0.26–0.28	0.23–0.24	0.19–0.21	0.4–0.6	0.10–0.25
Esophagus [L, ab]	0.8–1.0	1.53–1.62	–	0.81–1.18	1.9–2.6	0.74–1.10
Ventriculus [L,b]	0.29–0.50	–	0.36–0.46	0.28–0.47	0.5–0.6	0.33–0.42
Ventriculus [W,b]	0.073–0.18	0.22–0.25	0.11–0.15	0.09–0.17	0.4–0.5	0.13–0.16
Tail	0.042–0.095	0.07–0.08	0.17	0.06–0.24	0.15–0.19	–
Mucron	0.012–0.025	0.02–0.03	–	–	–	–
N	10	3	15	–	10	3
Reference	From this study	Felizardo et al. (2009)	Giese (2010)	Santana et al. (2017)	Morais; Cárdenas; Malta (2019)	Cárdenas et al. (2021)

Table 1 – Morphological and morphometric comparison of third-stage larvae of *Anisakis* collected from fish in Brazil.
[a]Measurements in millimeters unless indicated; The parameter number of buds is given in amplitude. [b]Abbreviations: L: length, W: width.

Plagioscion squamosissimus from the Guajará River (freshwater environment) presented 100% prevalence of *Anisakis* larvae infection and a total infection intensity of 1390 larvae encysted in the intestinal serosa and mesentery. Souza et al. (2020) evaluated the presence of *Plagioscion squamosissimus* parasites caught in the Tapajós and Amazonas rivers. They found a prevalence of over 75% in both locations in Pará. The high prevalence rate found in this study does not reflect other records found in the literature for *Plagioscion squamosissimus*; Rodrigues et al. (2015) recorded 50% parasitism in specimens captured in Colares municipality and 49% of parasitism in specimens in Vigia municipality, and Fontenelle et al. (2016) recorded 28.57% of larvae in specimens captured in the Tapajós River and 23.33% of parasitism in fish captured in Marajó Bay, all in the same biogeographic region as this study.

Other Amazonian fish (Freshwater) act as intermediate or paratenic hosts for *Anisakis* larvae: Giese (2010) recorded a prevalence of 2.9% for *Ageneiosus ucayalensis*; Rodrigues et al. (2015), 9.09% for *Brachyplatystoma filamentosum* and 5% *Oxydoras niger*; Moreira et al. (2017) 3.49% for *Triportheus angulatus*; Santana et al. (2017) 13.15% for *Cichla monoculus*; Morais, Cárdenas & Malta (2019) recorded 8.73% of parasitisms in *Pygocentrus nattereri* and Cárdenas et al. (2021) 54.54% for *Hypophthalmus marginatus*. Takemoto & Lizama (2010) already warned about the low host specificity of Anisakidae nematodes, especially in the larval stage. Although there is no confirmation at the generic level, Melo et al. (2014) and Rabelo et al. (2017) found a 100% prevalence of anisakid larvae in *Plagioscion squamosissimus* collected in the Xingu River, municipality of Altamira, and in the Guajará Bay, in Belém, respectively.

Plagioscion squamosissimus is a carnivorous fish whose biological cycle can occur in fresh and estuarine water. It is a species restricted to South America, originating from the Amazon basin, and has been introduced in other states of Brazil (Boujard et al., 1997; Rocha et al., 2016). The diet of this species changes throughout its growth; juveniles feed on crustacean larvae (especially *Macrobrachium*), aquatic insects, and copepods, while adults are active fish hunters (Froese & Pauly, 2024). In this context, *Plagioscion squamosissimus* can be infected by consuming different items in the parasite's life cycle. Species of *Anisakis* have a complex life cycle. In the adult stage of the parasite, it lives in marine mammals, mainly cetaceans (ziphiids, delphinids, sperm whales, or a wide array of delphinoid odontocetes and mysticetes), while planktonic or semi-planktonic crustaceans act as first intermediate hosts of the parasite, and fish and squid represent intermediate/paratenic hosts (Mattiucci & Nascetti, 2008; Kuhn et al., 2016; Cipriani et al., 2022). According to Rabelo et al. (2017), the cetaceans *Sotalia fluviatilis* and *Inia geoffrensis* can act as definitive hosts for *Anisakis* species in the Amazon basin.

This work is vital because it involves a species widely consumed in the northern region and is essential to the ecological flow of Amazonian aquatic environments. Pavanelli et al. (2013) warn about the parasitic diversity of fish, reporting that less than 25% of the Brazilian ichthyofauna has been inventoried to understand its parasitic fauna, with the Amazon and Paraná regions among the most important for research on parasites of aquatic organisms. However, other areas in Brazil remain a vast field to be explored.

5. Conclusion

This study records the occurrence of *Anisakis* sp. larvae parasitizing *Plagioscion squamosissimus* in Pará. Sanitary inspection measures and the consumption of properly cooked fish are important to prevent harm to human health from the ingestion of this nematode's larvae.

Acknowledgments: The authors are grateful to the Laboratório de Recursos Naturais do Instituto Federal do Pará, Campus Breves; to the Laboratório de Histologia e Embriologia Animal and Laboratório de Microscopia Eletrônica de Varredura – Instituto da Saúde e Produção Animal – Universidade Federal Rural da Amazônia – UFRA, campus Belém, State of Pará, Brazil, for the use of the scanning electron microscope. Dra. Elane Giese was supported by a research fellowship from the Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq-Brasil) (#304593-2023-0). Dr. Raul Henrique da Silva Pinheiro was supported by a Post-doctoral researcher fellowship of the Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq, Brazil) (#171021/2023-1), developed at the Laboratório de Histologia e Embriologia Animal of Universidade Federal Rural da Amazônia (Pará, Brazil).

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