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Original paper

Spirocamallanus krameri and *Contracaecum* sp. third-stage larvae (Nematoda) in *Hoplerythrinus unitaeniatus* (Erythrinidae) of eastern Amazon (Brazil)

Raul Henrique da Silva PINHEIRO[1], Vanessa Assunção TEIXEIRA[2], Marcos TAVARES-DIAS[3], Elane Guerreiro GIESE[2]

[1]Programa de Pós-graduação em Sociedade, Natureza e Desenvolvimento, Instituto de Biodiversidade e Florestas, Universidade Federal do Oeste do Pará (UFOPA), Santarém, PA, Brazil
[2]Laboratório de Histologia e Embriologia Animal, Instituto da Saúde e Produção Animal, Universidade Federal Rural da Amazônia (UFRA), Belém, PA, Brazil
[3]Empresa Brasileira de Pesquisa Agropecuária (Embrapa Amapá), Macapá, AP, Brazil

Corresponding Author: Marcos Tavares-Dias; e-mail: marcos.tavares@embrapa.br

ABSTRACT. This study aimed to investigate the presence of nematodes *Spirocamallanus krameri* and *Contracaecum* sp. in *Hoplerythrinus unitaeniatus* caught in a lake in the state of Pará, in northern Brazil, eastern Amazon region (Brazil). From 92 hosts, 83.7% were infected, and a total of 401 parasites were covered. There was a high prevalence of *S. krameri*, while the predominance was of *Contracaecum* sp. larvae. Both parasite species presented aggregated dispersion, and abundance of these parasites presented positive correlation with weight and length of the hosts. Morphological and morphometric data of third-stage larvae of *Contracaecum* sp. are described.

Keywords: aggregation, Amazon, morphology, nematode

Introduction

Biodiversity in Brazilian continental aquatic ecosystems is still imprecisely known and difficult to estimate. Among the difficulties encountered are the different non-inventoried hydrographic basins, low number of researchers, deficits in sampling and the need for taxonomic revision of several groups [1]. There is currently a consensus that the diversity of parasites in Brazil represents a large hidden fraction of the total parasite species not yet discovered on the planet [2,3].

The parasite-host-environment relationship is the result of different factors such as the evolutionary history of the hosts, the ecological interactions between them, the presence of different organisms that act as intermediate and definitive hosts, others [4]. The phylum Nematoda is a taxon of endoparasites that has a high diversity of species, but presents problems related to the quantification and taxonomic identification of the phylum, including in species of the families Anisakidae (Railliet and Henry, 1912) and Camallanidae (Railliet and Henry, 1915). However, studies of these parasites can reveal information about aspects of the diet of the hosts, their trophic level in the food web and their participation in the life cycle of parasites [5–7].

Hoplerythrinus unitaeniatus (Spix and Agassiz, 1829), is a fish of the order Characiformes and family Erythrinidae, widely distributed throughout South and Central America [6]. This fish is piscivorous as an adult, but juveniles also feed on plankton, crustaceans, insects and seeds. It is habitually active at twilight and night and does not undertake migration. In the Amazon region, it is an important fishing resource, used as food by riverside populations living along the banks of rivers, and also has important potential for commercialization in the aquarium trade [6,8].

In the Amazon region, nematode fauna in *H. unitaeniatus* consists of larvae of *Contracaecum* sp.,

Figure 1. Specimen of *Hoplerythrinus unitaeniatus* of Pará State, in eastern Amazon (Brazil)

Eustrongylides sp. and *Pseudoproleptus* sp., in addition to adults of *Spirocamallanus inopinatus* and *Guyanema seriei seriei* [5–7,9]. However, other species of nematodes are still unknown. Thus, the aim of this study was to investigate the presence of Anisakid and Camallanid nematodes in *H. unitaeniatus* captured in a lake in the municipality of Tracuateua, Pará State, northern Brazil.

Materials and Methods

Fish and collection location

Ninety-two specimens of *H. unitaeniatus* specimens (13.3 ± 2.0 cm and 42.6 ± 36.2 g) (Fig. 1) were obtained. Fish were captured by artisanal fishermen in a lake in the municipality of Tracuateua (Fig. 2), Pará State. The fish were transported in an isothermal box to the Laboratory of Universidade Federal Rural da Amazônia, Belém (PA), for necropsy.

The present study was carried out according to the recommendations of the Brazilian College of Animal Experimentation (COBEA) and with authorization of the Ethics Committee in the Use of Animals of Embrapa Amapá (Protocolo Nº 014 - CEUA/CPAFAP).

Collection, fixation and identification procedures of parasites

After collection, the fish were weighed (g) and measured for total length (cm). The fish were then necropsied for parasitological analysis. After necropsy, the gastrointestinal tract and viscera were analyzed using a stereomicroscope and a light microscope to collect endoparasites. The methodology used to fix, preserve, quantify and stain the parasites for identification as has been recommended [10]. The parasites were identified in accordance with some authors [11–16]. The ecological terms proposed in literature [17–18] were used. The correlation of the parasite abundance with length and weight of hosts was estimated using the Spearman correlation coefficient.

For morphological and morphometric analysis

Table 1. Parasite endohelminths in *Hoplerythrinus unitaeniatus* (N=92) of Pará State, in eastern Amazon (Brazil)

Parasites	P (%)	MI ± SD	MA ± SD	DF (%)	SI
Contracaecum sp. (larvae)	39.1	6.4 ± 9.2	2.5 ± 6.5	57.1	I, M, PC, L
Spirocamallanus krameri (adults)	75.0	2.5 ± 2.2.	1.9 ± 2.2	42.9	I, PC

P: Prevalence (P), MI: Mean intensity, MA: Mean abundance, SD: Standard deviation, DF: Dominance frequency, SI: Site of infection. I: Intestine, M: musculature, PC: Pyloric caecum, L: Liver

Table 2. Dispersion index (DI), statistical-*d* and discrepancy index (D) in *Hoplerythrinus unitaeniatus* (N = 92) of Pará State, in eastern Amazon (Brazil)

Parasites	DI	*d*	D	Dispersion type
Contracaecum sp. (larvae)	4.070	13.8	0.748	Aggregated
Spirocamallanus krameri (adults)	2.180	6.5	0.531	Aggregated

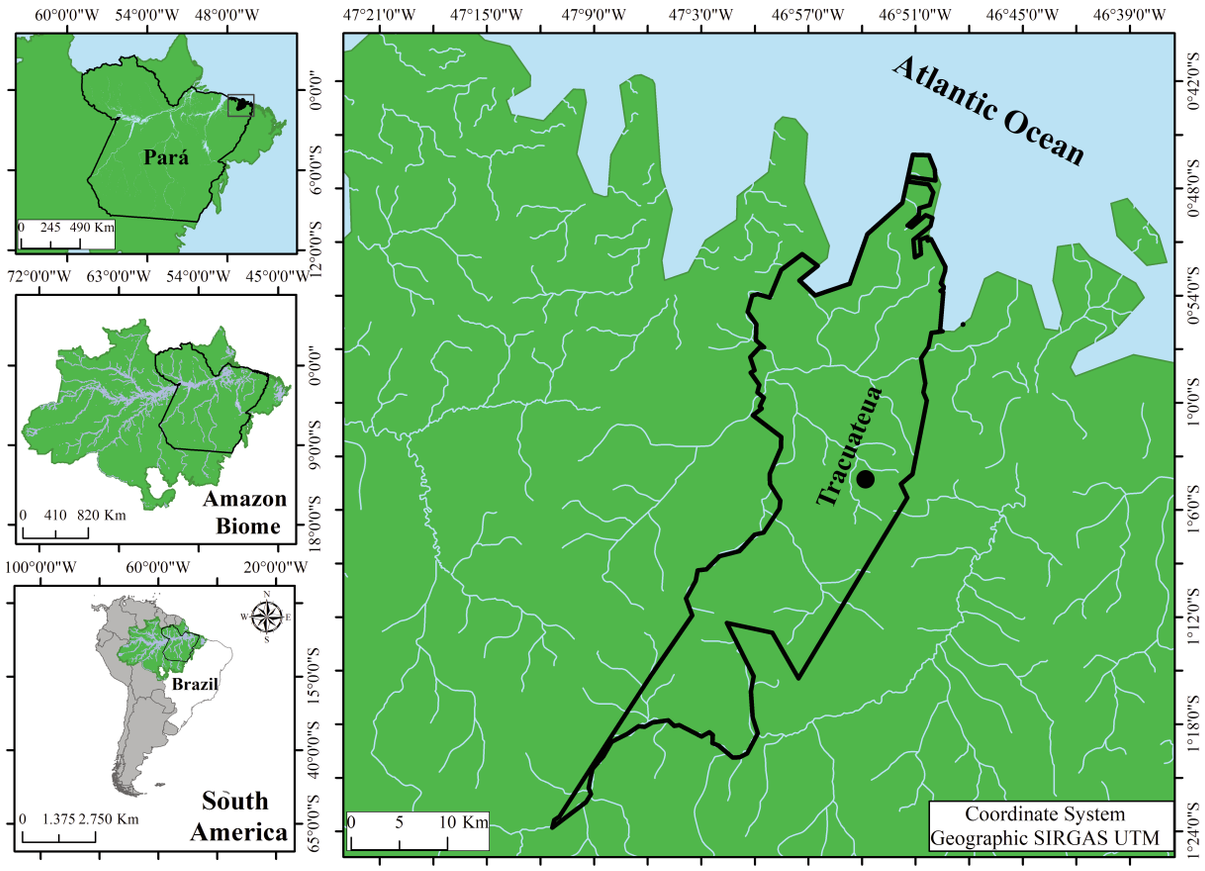

Figure 2. Geographic location of the sample collection of *Hoplerythrinus unitaeniatus* of Pará State, in eastern Amazon (Brazil)

ten larvae were dehydrated in an ethanol series, clarified with lactophenol, placed on a microscope slide under a coverslip as a temporary mount, observed using a light microscope, and photographed using a LEICA DM2500 microscope with an imaging capture system. Measurements are shown in micrometer (µm) as the mean followed by the range, or as otherwise indicated.

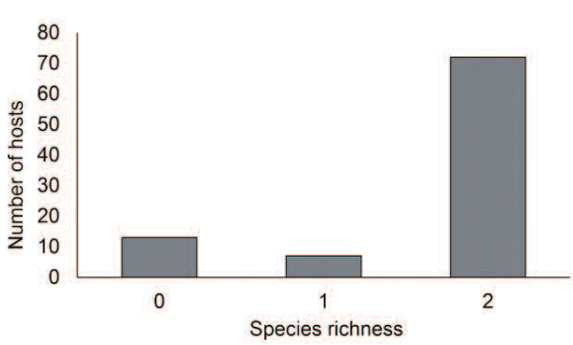

Figure 3. Species richness of parasites in *Hoplerythrinus unitaeniatus* of Pará State, in eastern Amazon (Brazil)

For scanning electron microscopy (SEM), six *Contracaecum* larvae were washed in phosphate-buffered saline (pH 7.0), post-fixed in 1% osmium tetroxide, dehydrated to the critical point of CO_2, metalized with gold-palladium, and analyzed with the VEGA 3 LMU/TESCAN scanning electron microscope at the Laboratório de Microscopia Eletrônica de Varredura, Instituto da Saúde e Produção Animal, Universidade Federal Rural da Amazônia (UFRA), state of Pará, Brazil.

Results

Parasitological data

Out of 92 hosts, 83.7% were infected and a total of 401 parasites were collected. The highest prevalence was of *S. krameri*, whereas the predominance was of larvae of *Contracaecum* sp. (Table 1). These parasite species presented an aggregated dispersion (Table 2). The abundance of parasites presented positive correlation with the weight (*rs* = 0.314, p = 0.002) and length (*rs* = 0.283, p = 0.006) of the hosts. There was a

predominance of hosts infected by two species of parasites (Fig. 3).

Morphological and morphometric data
Camallanidae Railliet and Henry, 1915
***Spirocamallanus* Olsen, 1952**
***Spirocamallanus krameri* Petter, 1974 (Fig. 4)**

For *S. krameri*, the morphological and morphometric data, as well as additional data observed by scanning electron microscopy were as reported by [16].

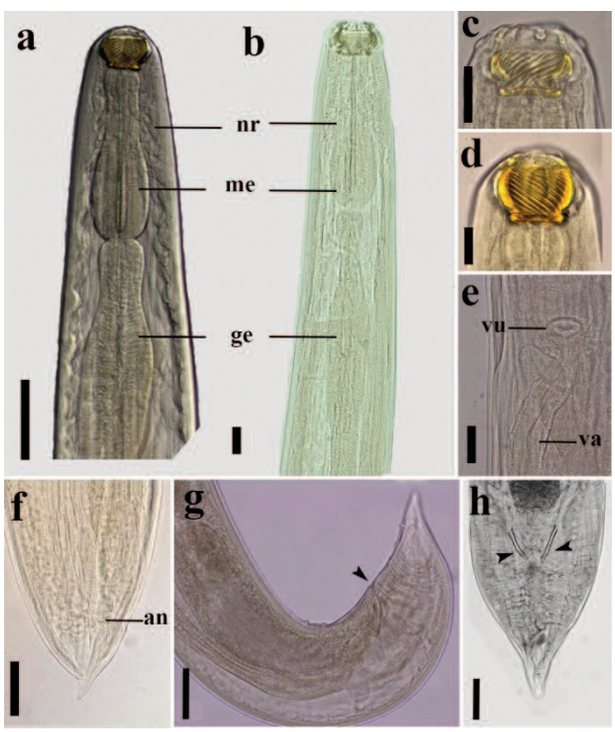

Figure 4. *Spirocamallanus krameria* parasite in *Hoplerythrinus unitaeniatus* of eastern Amazon (Brazil). **a-b.** Cephalic region of female and male respectively view: buccal capsule, nerve ring (nr), Muscular esophagus (me) and glandular esophagus (ge). **c.** Buccal capsule of males. **d.** Buccal capsule of females. **e.** Vulva (vu) and vargina (va). **f.** Tail of females view: anus (an). **g.** Tail of male, lateral views: cloacal opening (arrow head). **h.** Detail ventral of spicules (arrow head). The scale bars in **a** = 200 µm, **b** = 50 µm, **c** = 50 µm, d = 50 µm, e = 50 µm, f = 100 µm, g= 100 µm, h= 100 µm.

***Contracaecum* Railliet and Henry (1912)**
***Contracaecum* sp. (third-stage larvae) (Figs 5,6)**

Medium-sized nematodes, measuring 19 mm (15–24 mm) in length, maximum width at ventriculus of 471 (393–513), opaque-white when alive. Transversely striated cuticle present and more distinct at the extremities of the body, with anterior region interrupted by a short lateral line (Figs 5a, 6a). Cephalic extremity rounded with a small, ventral cuticular tooth, four submedian cephalic papillae surrounding the small, transverse oval oral aperture; three poorly-developed lips (Figs 5b, 6c). Excretory pore situated below the ventral cephalic tooth (Fig. 6 a-c). Muscular esophagus narrow, measuring 1.7 mm (1.5–2 mm)×95 (73–113) (Fig. 5a). Ventriculus small, rounded, size 120 (113–137)×102 (80–133); Ventricular appendix is short, measuring 365 (233–480)×103 (87–113) (Fig. 3a). Nerve ring positioned at 266 (240–317) from the anterior extremity. Deirids were observed only by SEM (Fig. 6b). Intestinal caecum, extending anteriorly to the nerve ring, measuring 1 mm (1–1.5mm)×266 (240–317) (Fig. 5a). The length ratio of the caecum and ventricular appendix is 37% (31–44%). Genital primordium indistinct. Rectum is a short hyaline tube; 3 small, unicellular rectal glands are present (Fig. 5c). Tail is conical, 158 (113–233) in length (Figs 5c, 6d).

Discussion

Morphologically and morphometrically the larvae of *Contracaecum* sp. of this study show similarity with the *Contracaecum* third-stage larvae reported from different species of fish in the Paraná River and *Pygocentrus nattereri* from Rio Negro in the state of Mato Grosso do Sul (Brazil), especially in relation to body size, esophageal length, intestinal caecum, and tail [19,20]. For the northern region of Brazil has been listed the occurrence of *Contracaecum* in *Cichla* spp., *H. unitaeniatus*, *Hop - lias malabaricus*, *Pygocentrus nattereri*, *Plagio- scion squamosissimus*, *Brachyplatystoma filamen- tosum*, *Brachyplatystoma rousseauxii*, *Oxydoras niger* and *Astronotus ocellatus*, fish of states from Amazonas, Pará and Amapá, in region of the Brazilian Amazon [15]. According to previous studies, *Contracaecum* larvae have a wide geographic distribution, and besides being found in wild fish, are also found in farmed fish [21]. However, has been stated that *Contracaecum* sp. larvae can often be confused with *Hysterothylacium* sp. [22].

Spirocamallanus krameri was described by Petter [11] parasitizing *H. unitaeniatus* in French Guiana. Subsequently, Moravec et al. [23] redescribed *Spirocamallanus krameri* of *H. unita- eniatus* in Venezuela. Recently, [16] expanded the geographic distribution of this nematode to regions

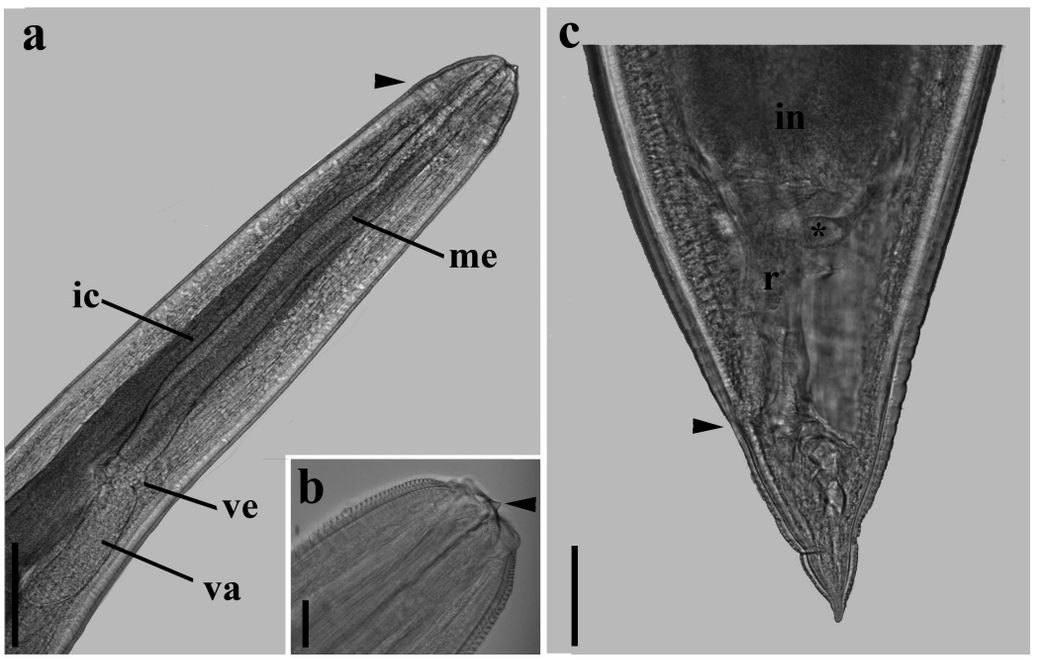

Figure 5. Third-stage larva of *Contracaecum* sp. parasite in *Hoplerythrinus unitaeniatus* of eastern Amazon (Brazil). **a.** Anterior end, showing muscular esophagus (me), cuticle with delicate transversal striations (arrow), intestinal caecum (ic), ventriculus (ve) and ventricular appendix (va). **b.** Detail of larval tooth (arrowhead). **c.** Posterior portion, showing end portion of the intestine (in), rectum (re), unicellular rectal glands (*) and anus (arrowhead), the tail without mucron. The scale bars in a = 200 μm, b = 50 μm and c = 50 μm.

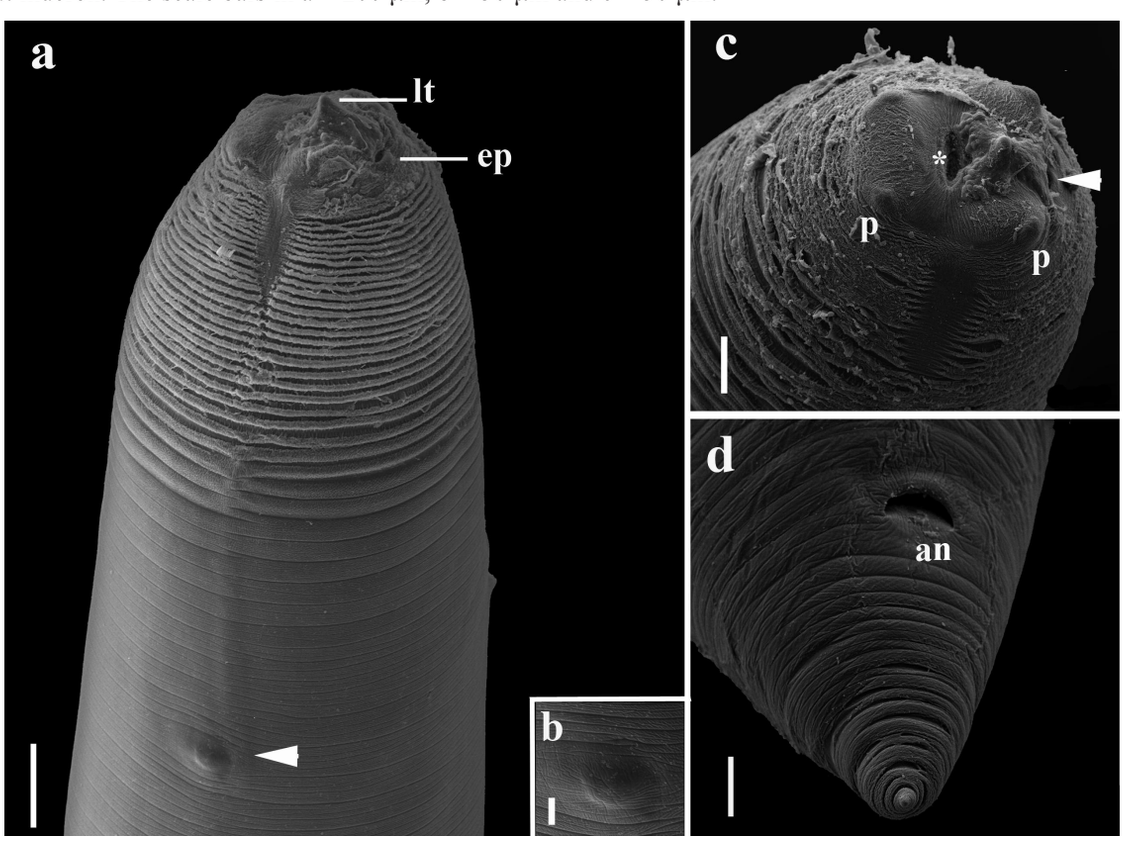

Figure 6. Scanning electron micrographs of third-stage larva of *Contracaecum* sp. parasite in *Hoplerythrinus unitaeniatus* of eastern Amazon (Brazil). **a.** Anterior end, cuticle with delicate transversal striations, larval tooth (lt), excretory pore (ep) and deirid (arrowhead). **b.** Detail of deirid. **c.** Anterior lateral view, four papillae (p), mouth opening (*), larval tooth and excretory pore (arrow). **d.** Posterior portion, anus (an), the tail without mucron. The scale bars in a = 50 μm, b = 5 μm, c = 20 μm and d = 20 μm.

from Brazil. The genus *Spirocamallanus* is characterized by the presence of spiral ridges in the buccal capsule, both in males and females [24,25]. In Brazil, until recently, only *Spirocamallanus inopinatus* Travassos, Artigas & Pereira, 1928; *Spiroczma Uanus Spirocamallanus iheringi* Travassos, Artigas and Pereira, 1928; *Spirocamallanus rarus* Travassos, Artigas and Pereira, 1928; *Spirocamallanus amaralis* Vaz and Pereira, 1934; *Spirocamallanus hilarii* Vas and Pereira, 1934; *Spirocamallanus barroslimai* Pereira, 1935; *Spirocamallanus*. *pereirai* (Annereaux, 1946); *Spirocamallanus macaenses* Vicente and Santos, 1972; *Spirocamallanus intermedius* (Pinto, Fábio, Noronha and Rolas, 1974); *Spirocamallanus solani* Pinto, Fábio, Noronha and Rolas, 1975; *Spirocamallanus cruzi* Guimarães, Cristófaro and Rodrigues, 1976; *Spirocamallanus paraensei* Pinto and Noronha, 1976; *Spirocamallanus pexatus* Pinto, Fábio, Noronha and Rolas, 1976; *Spirocamallanus caballeroi* Bashirullah, 1977 (Pavanelli et al. 2004); *Spirocamallanus halithophus* (Fusco and Overstreet, 1978); *Spirocamallanus pintoi* Kohn and Fernandes, 1988; *Spirocamallanus freitasis* Moreira Oliveira and Costa, 1991 and *Spirocamallanus belenensis* Giese, Santos and Lanfredi, 2009 had been found parasitizing different orders of fish in marine, fresh and brackish water environments [26].

Fish parasites represent a large portion of aquatic biodiversity and can be directly affected by the environment or indirectly affected through their hosts. They are thus a significant part of food webs and have an impact on the trophic structure of their hosts [7,8,27]. Of the specimens of *H. unitaeniatus*, 83.7% were parasitized with *S. krameri* and *Contracaecum* sp. larvae, but the prevalence of *S. krameri* was high. High prevalence of *Contracaecum* sp., *Pseudoproleptus* sp., *S. inopinatus* and *G. seriei seriei* has also been reported for *H. unitaeniatus* from Vila Nova River in the state of Amapá, in eastern Amazon [7]. Larvae of *Contracaecum* are found in diverse species of fish across from Brazil, due to its lack of parasitic specificity [15]. In addition, *S. krameri* and *Contracaecum* sp. had an aggregated dispersion, a pattern also for this host of the eastern Amazon [6,7]. Overdispersion suggests that the parasite-host relationship is stable and is influenced by environmental factors relating mainly to spatial-temporal changes in physicochemical parameters and to differentiated host susceptibility to parasites due to differences in immunological, behavioral and genetic characteristics [6.7,28].

In host-parasite interactions, the host body provides a microhabitat for parasite species. Host body size constitutes an important variable influencing their parasitic ecology [7–27] and may be considered a determinant factor in parasite abundance, although it generally explains only a portion of the variance in the abundance of parasites infecting different host species. In *H. unitaeniatus*, the abundance of parasite nematodes was positively correlated with the size of hosts. [7] also reported a similar correlation for *Contracaecum* sp. in *H. unitaeniatus*.

The occurrence of *Contracaecum* sp. and *S. krameri* in fish used as food by riverine human populations in the state of Pará, serves as an alert for better inspection of fish at fairs and markets [29] stated that Brazilian legislation related to infection prevention is not very clear. Therefore, given that several species of fish are infected by this anisakid with zoonotic potential, these infections could be a public health problem.

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