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Original Article

Nematofauna of red piranha *Pygocentrus nattereri* (Kner, 1958) (Characiformes: Serrasalmidae) from Amazonia, Brazil

Nematofauna de piranha-vermelha *Pygocentrus nattereri* (Kner, 1958) (Characiformes: Serrasalmidae) da Amazonia, Brasil

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Abstract

Between March and October 2008, 355 specimens of *Pygocentrus nattereri* were collected from the lowland lakes of Central Amazonia, Brazil, to study their nematode fauna. A total of 1.116 specimens of Nematoda were collected, belonging to six species. *Procamallanus* (*Spirocamallanus*) *inopinatus* was the species with the highest parasite indices. Larvae of *Anisakis* sp. have zoonotic potential and were found parasitizing the intestine and liver of *Pygocentrus nattereri*. Some of these nematode species were new records for the host *P. nattereri*. The diversity of nematodes that use *P. nattereri* as a host indicates the important role of this fish species in the maintenance of these six nematode species in the lowland lakes of Central Amazonia.

Keywords: Fish parasites, Nematoda, biodiversity, Amazonas, helminths.

Resumo

Entre março e outubro de 2008, foram coletados 355 espécimes de *Pygocentrus nattereri* para o estudo da Nematofauna, provenientes dos lagos de várzea da Amazônia Central, Brasil. Um total de 1.116 espécimes de Nematoda foram coletados, pertencentes a seis espécies. *Procamallanus* (*Spirocamallanus*) *inopinatus* foi a espécie com índices parasitários mais elevados. Larvas de *Anisakis* sp. apresentam um potencial zoonótico, e foram encontradas parasitando o intestino e o fígado de *P. nattereri*. Algumas espécies de nematoides estão sendo registradas em *P. nattereri* pela primeira vez. A diversidade de espécies de nematoides que utilizam *P. nattereri* como hospedeiro indica seu importante papel na manutenção destas cinco espécies nos lagos de várzea da Amazônia Central.

Palavras-chave: Parasitos de peixes, Nematoda, biodiversidade, Amazonas, helmintos.

Introduction

South America is undoubtedly a region where parasite biodiversity is clearly underestimated. The great ichthyological diversity of the region shows the huge dimension of the challenge for basic knowledge of helminth fauna from fish in this continent (LUQUE & POULIN, 2007). The Amazon basin is a center of diversity for most groups of Neotropical fish and it is an area of high species richness. Conservative estimates suggest there are about 3,000 fish species in this basin (ALBERT & REIS, 2011; JUNK, 2013; FROESE & PAULY, 2019; SANTOS & TAVARES-DIAS, 2017).

In recent years, parasites have been recognized as an important component of global biodiversity (POULIN & MORAND, 2004). Studies using taxonomic and systematic approaches are the key to understand how biotic and abiotic factors affect species, since the effects on a population cannot be understood without knowing the species (TAKEMOTO et al., 2009).

Nematodes constitute an important group of parasites from fish. They infect freshwater, marine and brackish water fish species and sometimes cause substantial damage to the host. Most nematodes infect fish as adults, but a large proportion of them do so as larval stages, which may affect the marketing of fish. Some nematode species can accidentally infect humans (THATCHER, 2006; ABDALLAH et al., 2012).

The red piranha (*Pygocentrus nattereri* Kner, 1858) is a carnivorous fish found in abundance in rivers in Central and South America. It is a predator with strong jaws and sharp-edged teeth, and it reaches up to 50 cm in total body length. Its high abundance, wide distribution and large muscles make it viable

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for use in human nutrition in the form of broths and filleting for sashimi (BARROS et al., 2010).

To date, 13 endohelmints species have been reported in *P. nattereri* from Brazil: the nematodes *Eustrongylides ignotus* Jäegerskiold, 1909 larvae (MORAVEC, 1998), *Eustrongylides* sp. larvae (BARROS et al., 2010; EIRAS et al., 2010; VICENTIN et al., 2013), *Contracaecum* sp. larvae (PAVANELLI et al., 2004; VICENTIN et al., 2013), *Procamallanus* (*S.*) *inopinatus* Travassos, Artigas & Pereira, 1928 (THATCHER, 2006; VICENTIN et al., 2013), *Brevimulticaecum* sp. larvae (VIEIRA et al., 2010; VICENTIN et al., 2013), *Procamallanus* sp. (BARROS et al., 2010), *Philometra nattereri* Cárdenas, Moravec, Fernandes & Morais, 2012 (CÁRDENAS et al., 2012) and Philometridae gen. sp. (VICENTIN et al., 2013); an undetermined species of Acanthocephala (EIRAS et al., 2010) and *Echinorhynchus paranensis* Machado Filho, 1959 (VICENTIN et al., 2013); the cestode *Proteocephalus serrasalmus* Rego & Pavanelli, 1990 (REGO & PAVANELLI, 1990); the metacercariae of *Clinostomum marginatum* (Braun, 1899) and *Austrodiplostomum compactum* (Lutz, 1928) (MORAIS et al., 2011).

Parasite species are a significant part of food webs, have an impact on the trophic structure and may even comprise half of the host organizational diversity (MARCOGLIESE & CONE, 1997; LAFFERTY et al., 2008; RUEHLE et al., 2017; BAIA et al., 2018). Most of the nematode parasites of fishes have a heteroxenous life cycle that includes more than one host, and fishes can act as intermediate or definitive. Considering that *P. nattereri* occupies a high position in the trophic web, this fish can harbor larvae and adults of nematodes. Parasites play fundamental roles in natural ecosystems. They can regulate host population abundance, influence the diversity and composition of communities and stabilize food webs (ALCÂNTARA & TAVARES-DIAS, 2015; OLIVEIRA et al., 2016; FERREIRA & TAVARES-DIAS, 2017). The knowledge of parasites communities of fishes, specially those with commercial interest, are of great importance since the data can be used in addition with other indicators to collaborate to the knowledge of migrations patterns, biology, and phylogeny. The aim of the present study was to describe the nematode fauna of *P. nattereri* from Central Amazonia, Brazil.

Materials and Methods

Collections in this study were authorized by the Brazilian Government's Chico Mendes Institute for Biodiversity and Conservation (ICMBIO, license number 44652-1). From March to October 2008, 355 specimens of *P. nattereri* were collected off six floodplain lakes located on the Solimões River banks Baixio (03°17'27,2" S/60°04'29,6"O); Preto (03°21'17,1" S/60°37'28,6" O); Iauara (03°36'39,2" S/61°16'33,0" O); Ananá (03°53'54,8" S/61°40'18,4" O), Campina, (03°46'15,8" S/62°20'10,3" O) and Maracá (03°50'32,8" S/62°34'32,4" O), all located between the cities of Manaus and Coari in the state of Amazonas. The nematodes collected were washed in 0.65% NaCl solution, fixed in hot AFA (2% glacial acetic acid, 3% formaldehyde and 95% ethanol 70°GL) and for light microscopical examination (LM) they were cleared in phenol, according to Eiras et al. (2000). After that, they were observed using a Zeiss Axioscope 2 or Olympus BH-2 microscope equipped with a digital camera. All measurements are in millimeters, unless otherwise stated, and the range is presented followed by the mean in parentheses. Fish names follow FishBase (FROESE & PAULY, 2019).

To describe the parasitological parameters, data related to parasite as prevalence (P), intensity (I), mean intensity (MI) and range (R) were used according to Bush et al. (1997).

Specimens studied were deposited in the Helminthological Collection of "Instituto Oswaldo Cruz", Rio de Janeiro, Brazil or in the Invertebrate Collection, Nematodes, "Instituto Nacional de Pesquisas da Amazônia" (INPA), Manaus, Brazil.

Results

A total of 355 specimens of *Pygocentrus nattereri* measuring 15.4 cm ± 3.5 in standard length and weighting 230.9 g ± 2.7 were examined, of which were collected 1,116 nematode specimens belonging to six species. Three nematode species were represented by adult specimens: *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928; *Philometra nattereri* Cárdenas, Moravec, Fernandes & Morais, 2012 and Capillariidae gen. sp. The other three nematode species were represented by larval stages: *Anisakis* sp., *Pseudoproleptus* sp. and *Brevimulticaecum* sp. (Table 1).

Procamallanus (*Spirocamallanus*) *inopinatus* was the most prevalent species (100%). Capillariidae gen. sp. was found in only one fish specimen and thus presented the lowest intensity of infection. *Anisakis* sp., *Pseudoproleptus* sp. and Capillariidae gen. sp. were reported here for the first time in *P. nattereri* (Table 1).

Considering that the nematode species found in the present study are already well described, only the main measurements with a brief description are presented here. The morphometric assessment on our material was in agreement with what had previously been reported.

Family Anisakidae Raillet & Henry, 1912

Genus *Anisakis* Dujardin, 1945

Anisakis sp. (third-stage larvae)

Table 1. Site of infection (SI), development stage (DS), prevalence (P), intensity (I), mean intensity (MI) and range (R) of nematodes collected from *Pygocentrus nattereri* in Brazil.

Parasite Species	SI	DS	P (%)	I	MI	R
Anisakis sp.*	Intestine	Larvae	8.73	159	5.13±4.28	1-40
Brevimulticaecum sp.	Intestine and Pyloric Caecum	Larvae	3.66	53	4.08±1.82	1-12
Capillariidae gen. sp.*	Intestine	Adult	0.56	2.0	1.00±0.41	1
Philometra nattereri	Oculo-orbits and Nasal Mucosa	Adult	11.83	66	1.57±1.19	1-3
Procamallanus (*S.*) *inopinatus*	Intestine and Pyloric Caecum	Adult	100	938	2.64±2.90	1-25
Pseudoproleptus sp.*	Pyloric Caecum	Larvae	1.40	57	11.40±2.70	1-50

*New host record.

Specimen deposited: Voucher 050 in the Invertebrate Collection, Nematodes, INPA, Manaus, Brazil.

Based on 10 larval specimens: Body 19-25 (23) in length, 0.54-0.63 (0.60) maximum width. Cuticle finely transversely striated. Excretory pore situated at the base of the anlagen lips. Ventral larval tooth present. Oesophagus 1.90-2.60 (2.10) in length. Ventriculus 0.50-0.60 (0.55) in length and 0.40-0.50 (0.48) wide. Nerve ring at 0.40-0.60 (0.50) from anterior region. Conical tail 0.15-0.19 (0.17) long, presenting a terminal mucron.

Remarks

Anisakiasis in human is commonly associated with seafood intake. The main genera involved are: *Anisakis* Dujardin, 1845, *Pseudoterranova* Mozgooi, 1951, *Hysterothylacium* Ward & Magath, 1917 and *Contracaecum* Raillet and Henry, 1912 (HOCHBERG & HAMER, 2010). In Brazil, *Anisakis* spp. larvae have been found in different marine host species of fish by many authors (PANTOJA et al., 2015a; DIAS et al., 2011; EIRAS et al., 2016). However, there are few records of *Anisakis* spp. larvae in fish species that inhabit freshwater and brackish water. Rodrigues et al. (2015) found *Anisakis* sp. larvae in *Plagioscion squamosissimus* (Heckel, 1840), *Brachyplatystoma filamentosum* (Lichtenstein, 1819) and *Oxydoras niger* (Valenciennes, 1821) from freshwater fish marketed in Pará, Brazil. *Anisakis* sp. third stage larvae were also found in *P. squamosissimus*, *Serrasalmus altispinis* Merckx, Jégu & Santos, 2000 and *Triportheus angulatus* (Spix & Agassiz, 1829) from Amazon region (FONTENELLE et al., 2016; MOREY & MALTA, 2016; MOREIRA et al., 2017).

Larvae of the genus *Contracaecum* Raillet and Henry, 1912 have been reported from *P. nattereri* in different localities in Brazil (BARROS et al., 2010; BENIGNO et al., 2012; VICENTIN et al., 2013). However, the present study provides the first report of larvae of *Anisakis* sp. in this fish species.

In the specimens of *P. nattereri* examined here, *Anisakis* sp. was found in the liver and intestine. However, migration of these larvae into the musculature is not impossible. Thus, there is a risk of acquiring anisakiasis through consumption of this fish in a raw, undercooked, smoked or insufficiently salted form (KLIMPEL & PALM, 2011).

Family Acanthocheilidae Wülker, 1929

Genus *Brevimulticaecum* Mozgovoy, 1951

Brevimulticaecum sp. (third-stage larvae)

Specimens deposited: Voucher 052 in the Invertebrate Collection, Nematodes, INPA, Manaus, Brazil.

Based on 11 larval specimens: Body 4.27-5.12 (4.96) length, 175-225 µm (200 µm) width. Oesophagus 405-535 µm (504 µm) in length. Ventriculus 25-65 µm (52 µm) in length, with 4 or 5 lobes. Intestinal caecum 250-270 µm (260 µm) in length. Nerve ring and excretory pore at 125-175 µm (155 µm) and 187.5-190 µm (189 µm), respectively from anterior region. Excretory pore at 100-520 µm (129 µm) from posterior region. Anus 100-520 µm (244 µm) from posterior end. Conical tail.

Remarks

The larval stages of *Brevimulticaecum* spp. have been reported parasitizing fish, reptiles and amphibians in some geographical regions (see VIEIRA et al., 2010). In Brazil, *Brevimulticaecum* spp. larvae have been reported from the following different freshwater fishes: *Leporinus lacustris* Amaral Campos, 1945, *Leporinus friderici* (Bloch, 1794) and *Potamotrygon falkneri* Castex & Maciel, 1963 (GUIDELLI et al., 2006; LACERDA et al., 2008, 2009); *Leporinus macrocephalus* Garavello & Britski, 1988 (MARTINS et al., 2017); *Gymnotus inaequilabiatus* (Valenciennes, 1839), *Hemisorubim platyrhynchos* (Valenciennes, 1840), *Hoplias malabaricus* (Bloch, 1794), *Myleus levis* (Eigenmann & McAtee, 1907), *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829), *Serrasalmus marginatus* (Valenciennes, 1837) and *Sorubim lima* (Bloch & Schneider, 1801) (VIEIRA et al., 2010; VICENTIN et al., 2011; DEZFULI et al., 2016); *P. nattereri* (VIEIRA et al., 2010; VICENTIN et al., 2013); and *Astyanax fasciatus* (Cuvier, 1819) (VIEIRA-MENEZES et al., 2017). Vicentin et al. (2013) observed higher prevalence (19.08%) and mean intensity (117.24 ± 125.91) of *Brevimulticaecum* sp. larvae than what was observed in the present study (P = 3.66%; MI = 4.08±1.82) (Table 1).

Superfamily Trichinelloidea Ward, 1907 (1879)

Family Capilariidae Railliet, 1915

Capillariidae gen. sp.

Specimens deposited: Voucher 053 in the Invertebrate Collection, Nematodes, INPA, Manaus, Brazil.

Thin, thread-like nematodes. Cuticle bearing two longitudinal bacillary bands. Eggs barrel-shaped. Vulva near distal end of oesophagus with elevated lips.

Remarks

In Brazil, nematodes belonging to the family Capillariidae have been reported from different host fishes from different localities (LUQUE et al., 2011). In the Amazon region, only two species have been reported: *Paracapillaria* (*Paracapillaria*) *piscicola* (Travassos, Artigas & Pereira, 1928) parasitizing *Acestrorhamphus* sp. (THATCHER, 2006); and *Capillostrongyloides arapaimae* Santos, Moravec & Venturieri, 2008, parasitizing *Arapaima gigas* (Schinz, 1822) (SANTOS et al., 2008).

In the present study, only two specimens (females) in a poor condition were found, making it difficult to establish the genus and species accurately. However, this record of the family Capillariidae in *P. nattereri* for the first time contributes towards biodiversity knowledge.

Family Philometridae Baylis & Daubney, 1926

Genus *Philometra* Costa, 1845

Philometra nattereri Cárdenas, Moravec, Fernandes & Morais 2012

Specimens deposited: Paratypes (35778-35782) in the Helminthological Collection,

Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil; other paratypes (046 and 047) in the Invertebrate Collection, Nematodes, INPA, Manaus, Brazil.

Measurements of this species were presented in the original description (CÁRDENAS et al., 2012).

Remarks

Only three valid species of *Philometra* Costa, 1845 have so far been reported from freshwater fish in South America, all described solely from available females: *Philometra baylisi* Vaz & Pereira, 1934, from the abdominal cavity of *Pimelodus blochii* Valenciennes, 1840 in Brazil (VAZ & PEREIRA, 1934); *Philometra nattereri* Cárdenas, Moravec, Fernandes & Morais, 2012, from the ocular orbits and nasal mucosa of *P. nattereri* in the Brazilian Amazon region (CÁRDENAS et al., 2012); and *Philometra mirabilis* Moravec & Diggles, 2015 from the gonads of *Cichla mirianae* Kullander & Ferreira, 2006 (Cichliformes, Cichlidae) in the Brazilian Amazon region (MORAVEC & DIGGLES, 2015). However, the allocation of *P. baylisi* to *Philometra* is uncertain (MORAVEC, 2006). Vicentin et al. (2013) reported finding one specimen of *Philometra* sp. in the body cavity of *P. nattereri* but, according to these authors, this specimen differed in some aspects from *P. nattereri.*

Although only three valid species of *Philometra* from freshwater fishes in Brazil have been described, it can be expected that new species of this group will be discovered and described.

Family Camallanidae Railliet & Henry, 1915
Genus *Procamallanus* Baylis, 1923
Procamallanus (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928

Specimens deposited: Voucher 048 and 049 in the Invertebrate Collection, Nematodes, INPA, Manaus, Brazil.

Adults are characterized by their orange-brown, undivided, thick-walled buccal capsule in both sexes.

Males (based on 10 specimens): Body 4.30-6.00 (5.20) long by 0.22-0.36 (0.27) wide. Buccal capsule with 0.06- 0.14 (0.12) in length by 0.05-0.12 (0.09) maximum width, with 18-19 (19) ridges. Muscular and glandular regions of the esophagus are 0.27-0.37 (0.32) and 0.47-0.62 (0.52) respectively. Nerve ring at 0.02-0.13 (0.07) from the anterior extremity. Posterior end of body provided with 10 pairs of caudal papillae (4 pairs of precloacal papillae and 6 pairs of postcloacal papillae). Two equal spicules with 0.14-0.16 (0.15) long.

Females (based on 10 specimens): Body 12.00-30.25 (22.70) in length by 0.52-0.96 (0.75) in width. Buccal capsule with 0.12-0.17 (0.15) in length by 0.15-0.18 (0.16) wide, with 20-22 (19) ridges. Muscular and glandular regions of the esophagus with 0.55-0.63 (0.52) and 0.80-0.99 (0.90) respectively. Nerve ring at 0.02-0.10 (0.05) from the anterior extremity. Conical tail.

Remarks

Procamallanus (*Spirocamallanus*) *inopinatus* has been reported from different host fishes in different localities in Brazil (LUQUE et al., 2011), including the Amazon region (THATCHER, 2006; ANDRADE & MALTA, 2006; GONÇALVES et al., 2016; SANTOS & TAVARES-DIAS, 2017). This nematode species was reported parasitizing *P. nattereri* in the state of Mato Grosso do Sul, Brazil, by Pinto & Noronha (1976) and Vicentin et al. (2013). Barros et al. (2010) reported finding *Procamallanus* spp. in *P. nattereri* caught in the Cuiabá river, state of Mato Grosso, Brazil, and Benigno et al. (2012) reported finding *Procamallanus* sp. in Arari lake, Marajó island, state of Pará, Brazil.

Family Cystidicolidae Skrjabin, 1946
Genus *Pseudoproleptus* Khera, 1956
Pseudoproleptus sp. (third-stage larvae)

Specimens deposited: Voucher 051 in the Invertebrate Collection, Nematodes, INPA, Manaus, Brazil.

Based on 10 larval specimens: Body filiform, 12.25 - 24.70 (20.76) long and 149-150 (150) wide. Anterior end of body with helmet-like cuticular structure with 32-35 μm (33 μm) in length from anterior end. Vestibule including prostom 177-187 μm (181 μm) in length. Length of muscular esophagus 0.690-0.695 (0.692); length of glandular esophagus 2.66-4.18 (3.33). Nerve ring and excretory pore 250-275 μm (262 μm) and 507-540 μm (522 μm), respectively, from anterior extremity. Anus 100 to 145 μm (122 μm) from posterior extremity.

Remarks

In Brazil, third-stage larvae of *Pseudoproleptus* sp. were reported for the first time in the prawn *Macrobrachium amazonicum* (Heller, 1862) from Mexiana island (Amazon river delta), state of Pará (MORAVEC & SANTOS, 2009). Subsequently, the third-stage larvae of this nematode were reported in the *Satanoperca jurupari* Heckel, 1840 and *Ageneiosus ucayalensis* Castelnau, 1855 (MELO et al., 2011), *Aequidens tetramerus* (Heckel, 1840) (TAVARES-DIAS et al., 2014), *Mesonauta acora* Castelnau, 1855 (PANTOJA et al., 2015b), for second time from a fish in the Neotropical region. We agree with Melo et al. (2011) that this fish should be considered a paratenic host for this nematode species.

Discussion

The parasite community structure of fish is mostly influenced by host physiology, ecology and phylogeny (HOSHINO et al., 2016). Differences in species richness and diversity of parasites may also be a result of the host's individual responses to parasitism and transmission rates, among other factors (TAKEMOTO et al., 2009; TAVARES-DIAS et al., 2013; SHAH et al., 2014). Therefore, biotic factors such as the host immunity may have a differential effect on a fish-parasite environment system (NEVES et al., 2016).

The trophical level of *P. nattereri*, together with the large population of this species in lowland environments, shows that it is a species that can act as an intermediate, paratenic or definitive host for several species of nematode, thereby facilitating completion of their life cycles. This assertion is corroborated by the presence of parasite species, both in adult form (such as *Procamallanus* (*Spirocamallanus*) *inopinatus*) and in larval form (such as *Pseudoproleptus* sp., *Brevimulticaecum* sp. and *Anisakis* sp.) in the present study.

Pygocentrus nattereri is a very abundant species in the Amazon region and is widely used in feeding the regional population. Thus, it is considered to be a commercial species (SANTOS et al., 2006). With increasing consumption of this species and a lack of sanitary inspection to detect pathogens, there is a risk of increasing

incidence of zoonoses, especially because of the popularization of its use in prepared dishes based on raw fish (BARROS et al., 2007; MORAIS et al., 2011). The presence of *Anisakis* sp. in *P. nattereri* in the present study demonstrates that the use of raw fish or undercooked fish in food presents an eminent risk of zoonoses.

The diversity of fish species in Brazil, including both freshwater and marine species, is one of the richest in the world, especially concerning freshwater fish. Fish diversity is not in itself a risk factor for fish-borne nematode infections, and probably not all the species serve as hosts. However, the great number of fish species infected, especially in the case of economically important species, increases the risk that humans will ingest fish infected with nematodes (EIRAS et al., 2016).

Previous studies showed that infrapopulations of metazoan endoparasites of *P. nattereri* presented low parasite indexes, such as prevalence and abundance, except in the case of *P.* (*S.*) *inopinatus* (CARVALHO et al., 2004; BARROS et al., 2010; VICENTIN et al., 2013). In the present study, similar parasite indexes were found in *P. nattereri* from the Solimões river. The present findings can be explained by similarities in the environment, in which all hosts were collected from natural rivers. The same nematode species, *P.* (*S.*) *inopinatus*, showed the highest values, demonstrating that specimens of *P. nattereri* from different rivers had the same opportunities to find the intermediate hosts with infective stages.

Fish of wild populations have a more diversified diet than those in manmade lakes and reservoirs, which can positively affect the composition of endoparasites because the diet can include numerous animals that act as intermediate and/or paratenic hosts (PEREIRA et al., 2018). In the present study, Capillariidae gen. sp., the third-stage larvae of *Anisakis* sp., *Pseudoproleptus* sp. and *Brevimulticaecum* sp. are reported for the first time in *P. nattereri*, thus contributing to biodiversity knowledge.

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