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ECOSYSTEMS

First record of larvae of *Spiroxys* (Nematoda: Gnathostomatidae) parasitizing fishes in stream of the Caatinga domain

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Abstract: The present study is the first report of *Spiroxys* larvae parasitizing stomach and intestine of three fish species in stream of Caatinga domain, Northeast, Brazil. A total of 120 fish specimens, 40 of *Astyanax bimaculatus*, 40 of *Hoplias malabaricus*, and 40 of *Hoplosternum littorale* were examined for nematodes. A total of 633 specimens of *Spiroxys* larvae were recovered for the three fish species: 227 in *A. bimaculatus* (prevalence (%) = 48; mean abundance = 5.78 ± 1.78 (range = 0–70); and mean intensity = 11.95 ± 2.7 (range = 2–70)), 176 in *H. malabaricus* (prevalence (%) = 42.5; mean abundance = 4.40 ± 1.55 (range = 0–53); and mean intensity = 10.35 ± 2.38 (range = 2–53)) and 230 in *H. littorale* (prevalence (%) = 42.5; mean abundance = 5.75 ± 1.98 (range = 0–52); and mean intensity = 13.53 ± 3.04 (range = 1–52)). This study expands the range of the geographic distribution of nematodes of the genus *Spiroxys* and increase the list of hosts, as well as contributing to the knowledge of fish parasites biodiversity in Caatinga domain.

Key words: Freshwater fishes, nematodes, neotropical region, Salgado river basin.

INTRODUCTION

Neotropical region holds the richest freshwater ichthyofauna in the world with about 7,000 species, although this value remains uncertain or still unknown (Albert & Reis 2011, Bertaco et al. 2016). According to Buckup et al. (2007), Brazilian rivers are composed of more than 2,587 species of this world biodiversity. The fish orders Characiformes, Siluriformes and Gymnotiformes represents around 77% of the total species in Brazilian freshwater systems (Ramos 2012).

The watersheds under the Caatinga domain have specific characteristics, as intermittent and seasonal regime of its rivers, in response to scarce and irregular rainfall. These factors play an important role in the organization and functionality of aquatic ecosystems (Ab'saber 1995, Leal et al. 2003). The ichthyofauna of the watersheds of this region is the result of adaptations to biotic and abiotic specificities modeled by anthropic processes (Nascimento et al. 2014). The taxonomic collection of ichthyofauna occurring in the Caatinga domain revealed the presence of around 240 species (Rosa et al. 2005).

Proportionally to the diversity of freshwater fishes, their parasites are also significant in Brazil (Eiras et al. 2010). In this context, fishes have a number and a variety of parasites greater than any other class of vertebrates, due to their long evolutionary association (Eiras et al. 2011, Thatcher 2006). Among these parasites, nematodes are helminths that have great morphological diversity, diverse life cycles, and wide distribution in aquatic environments, being one of the most representative metazoan

groups of fish parasites in the Neotropical region (Santos et al. 2013).

Astyanax bimaculatus (Linnaeus 1758), popularly known as "yellow-tailed lambari", is a species of small size and with a short life cycle, which has a wide geographical distribution. It has an omnivorous feeding habit and serves as food for carnivorous species (Hartz et al. 1996, Garutti 2003). *Hoplosternum littorale* (Hancock 1828), popularly known as "tamoatá", is a medium-sized benthic fish. This fish species is distributed throughout the Andean East of South America, from the North, in the Amazon Basin to Buenos Aires (Hostache & Mol 1998, Sá-Oliveira & Chellapa 2002), and feeding mainly aquatic invertebrates and detritus (Winemiller 1987, Mol 1995). *Hoplias malabaricus* (Bloch 1794), popularly known as "traíra", has a wide distribution in South America. It is an agile and voracious predator, adapted to lentic environments and with a nocturnal habit (Meneguetti et al. 2013).

Adult nematodes adults of the genus *Spiroxys* Schneider, 1866 are parasitize the gastric mucosa of freshwater turtles. Hasegawa et al. (1998) compiled 17 species in Chelonians from all geographical regions of the world. Recently, two species have been described: *Spiroxys ankarafantsika* Roca & García 2008 parasitizing *Pelusios castanoides* Hewitt 1931 and *Pelomedusa subrufa* (Lacépède 1788) in Madagascar (Roca & García 2008); and *Spiroxys sumatraensis* Purwaningsih & Mumpuni 2015 parasitizing *Amyda cartilaginea* in Indonesia (Purwaningsih & Mumpuni 2015). Table I shows all the previous records of *Spiroxys* larvae from freshwater fishes and geographical distribution.

Herein, we report new records of larvae of *Spiroxys* parasitizing three fish species in stream from Caatinga domain, Northeast, Brazil. A checklist of freshwater host species parasitized with *Spiroxys* larvae and their geographical distribution is provided, as well as a comparative morphometry table among the specimens found in the present study and a similar one available in the literature for South America.

MATERIALS AND METHODS

A total of 120 fish were captured monthly using cast and seine net from December 2018 to August 2019 in the Carás stream (7°06'37.23'' S and 39°24'21.33'' W) in the municipality of Crato, Ceará state, Brazil (Figure 1). From this total, 40 specimens of *A. bimaculatus* (4.1 to 7.9 cm); 40 of *H. malabaricus* (6.3 to 15.1 cm); and 40 of *H. littorale* (7.1 to 14.1 cm) were sampled. The fish specimens were placed in individual plastic bags and frozen. The samples were taken to the laboratory to perform parasitological analyses. The capture of fish specimens was authorized by a Permanent License for the Collection of Zoological Material (SISBIO #61328-1). All animal procedures were performed in full compliance with the Ethics Committee for Animal Experimentation (CEUA/protocols #00165/2018.1) of the Universidade Regional do Cariri (URCA). Classification and nomenclature of hosts followed Garutti & Britski (2000) and Graça & Pavanelli (2007). Vouchers specimens (*H. malabaricus* UFPB 12006; *A. bimaculatus* UFPB 12013; *H. litoralle* UFPB 12085) of fishes were deposited in the Coleção Científica de Ictiologia of Universidade Federal da Paraíba (Federal University of Paraíba - UFPB), municipality of João Pessoa, Paraíba state, Brazil.

A longitudinal incision in the ventral surface of the fish was made and all inner organs were removed and separated. The visceral cavity and all organs were surveyed for nematode parasites using a stereomicroscope. All collected nematodes were preserved in alcohol 70% and posteriorly diaphanized using lactophenol

Table I. List of host species parasitized with larvae of the genus *Spiroxys* and geographical distribution. Abbreviations: MEX = Mexico and BRA = Brazil.

Host species	Locality	Country	References
Lepisosteiformes			
Lepisosteidae			
Atractosteus tropicus Gill, 1863	Pantanos de Centla Biosphere Reserve	MEX	Bueno (2005)**
Characiformes			
Characidae			
Astyanax aeneus (Günther, 1860)	Pantanos de Centla Biosphere Reserve and, Ayuquila River, respectively	MEX	Bueno (2005)**, Salgado-Maldonado et al. (2004)
Astyanax altiparanae Garutti & Britski, 2000	Parana River	BRA	Lizama et al. (2008)**
Astyanax fasciatus (Cuvier, 1819)	São Francisco River Basin	BRA	Vieira-Menezes et al. (2017)**
Astyanax mexicanus (De Filippi, 1853)	Pánuco River Basin	MEX	Salgado-Maldonado (2008)**
Pygocentrus piraya (Cuvier, 1819)	São Francisco River Basin	BRA	Santos et al. (2009)*
Salminus hilarii Valenciennes, 1850	São Francisco River Basin	BRA	Duarte et al. (2016)**
Serrasalmus brandtii Lütken, 1875	São Francisco River Basin	BRA	Santos et al. (2009)*
Tetragonopterus chalceus Spix & Agassiz, 1829	São Francisco River Basin	BRA	Albuquerque (2009)**
Triportheus guentheri (Garman, 1890)	São Francisco River Basin	BRA	Albuquerque (2009)**
Siluriformes			
Callichthyidae			
Corydoras aeneus (Gill, 1858)	Ribeira do Feijão Basin	BRA	Tozato (2011)**
Gymnotiformes			
Gymnotidae			
Gymnotus carapo Linnaeus, 1758	Paraná River	BRA	Takemoto et al. (2009)**
Gymnotus sp. Linnaeus, 1758	Baía River	BRA	Isaac et al. (2004)**
Cyprinodontiformes			
Poeciliidae			
Poecilia butleri Jordânia, 1889	Ayuquila River	MEX	Salgado-Maldonado et al. (2004)**
Poecilia velifera (Regan, 1914)	Yucatan Peninsula	MEX	Moravec et al. (1995)**
Perciformes			
Cichlidae			
Astronotus ocellatus (Agassiz, 1831)	Lagos de várzea na Amazônia	BRA	Atroch (2016)**

Table I. Continuation

Cichla kelberi Kullander & Ferreira, 2006	São Francisco River Basin	BRA	Santos et al. (2009)*
Cichlasoma fenestratum (Günther, 1860)	Papaloapan River Basin	MEX	Salgado-Maldonado et al. (2005)**
Cichlasoma grammodes Taylor & Miller, 1980	San Juan River	MEX	Salgado-Maldonado et al. (2011)**
Cichlasoma meeki Brind, 1918	Yucatan Peninsula	MEX	Moravec et al. (1995)**
Cichlasoma octofasciatum Regan, 1903	Papaloapan River Basin	MEX	Salgado-Maldonado et al. (2005)**
Cichlasoma sp.	Papaloapan River Basin	MEX	Salgado-Maldonado et al. (2005)**
Cichlasoma synspilum Hubbs, 1935	Laguna Payegua	MEX	Vidal-Martínez & Kennedy (2000)
Cichlasoma urophthalmus (Günther, 1862)	Yucatan Peninsula and Papaloapan River Basin and Lacantún River, respectively	MEX	Moravec et al. (1995)** and Salgado-Maldonado et al. (2005)** and Salgado-Maldonado et al. (2011)**
Nandopsis (Cichlasoma) istlanum	Ayuquila River	MEX	Salgado-Maldonado et al. (2004)**
Oreochromis aureus (Steindachner, 1864)	Ayuquila River	MEX	Salgado-Maldonado et al. (2004)**
Oreochromis sp.	Papaloapan River Basin	MEX	Salgado-Maldonado et al. (2005)**
Petenia splendida Günther, 1862	Pantanos de Centla Biosphere Reserve	MEX	Bueno (2005)**
Vieja argentea (Allgayer 1991).	Lacantún River	MEX	Salgado-Maldonado et al. (2011)**
Vieja intermedia (Günther, 1862)	Lacantún River	MEX	Salgado-Maldonado et al. (2011)**
Eleotridae			
Dormitator maculatus (Bloch, 1792)	Alvarado Lake	MEX	Moravec (1998)**
Mugiliformes			
Mugilidae			
Agonostomus monticola (Bancroft, 1834)	Ayuquila River	MEX	Salgado-Maldonado et al. (2004)**

*** Study that registered only *Spiroxys* sp. larvae in the host; ** Study considering *Spiroxys* sp. together with other parasites in the host.**

to visualize the inner structures using a Leica Microsystems optical microscope, after that the nematodes were stored in alcohol 70% (Amato et al. 1991). The identification of the nematodes based on morphometric and morphological characters was performed according to Moravec (1998) and Vieira-Menezes et al. (2017).

Measurements are given in millimeters (mm) and are expressed as mean followed by minimum and maximum values in parentheses. Morphological and morphometrical analyses of nematodes were carried out using the Motic Images Plus™ software, version 2.0. Vouchers specimens of helminths (CHIBB 9197; 9198) were deposited in the Coleção Helmintológica do Instituto de Biociências (Helminthological Collection of the Institute of Biosciences of Botucatu) (CHIBB) of the Universidade Estadual Paulista (São Paulo State University - UNESP), municipality of Botucatu, São Paulo state, Brazil. The ecological descriptors of prevalence, intensity and abundance are in accordance with Bush et al. (1997).

RESULTS

A total of 633 specimens of larvae of *Spiroxys* sp. (Figures 2 and 3) encysted in the organs of the cavity, were recovered from fishes of three species studied (227 in *A. bimaculatus*, 230 in *H. littorale* and 176 in *H. malabaricus*). The parasites showed similar levels of infection in the three host species and different organs of infection (Table II). The morphometry was based on 10 specimens of nematodes in each host species of the present study (Table III).

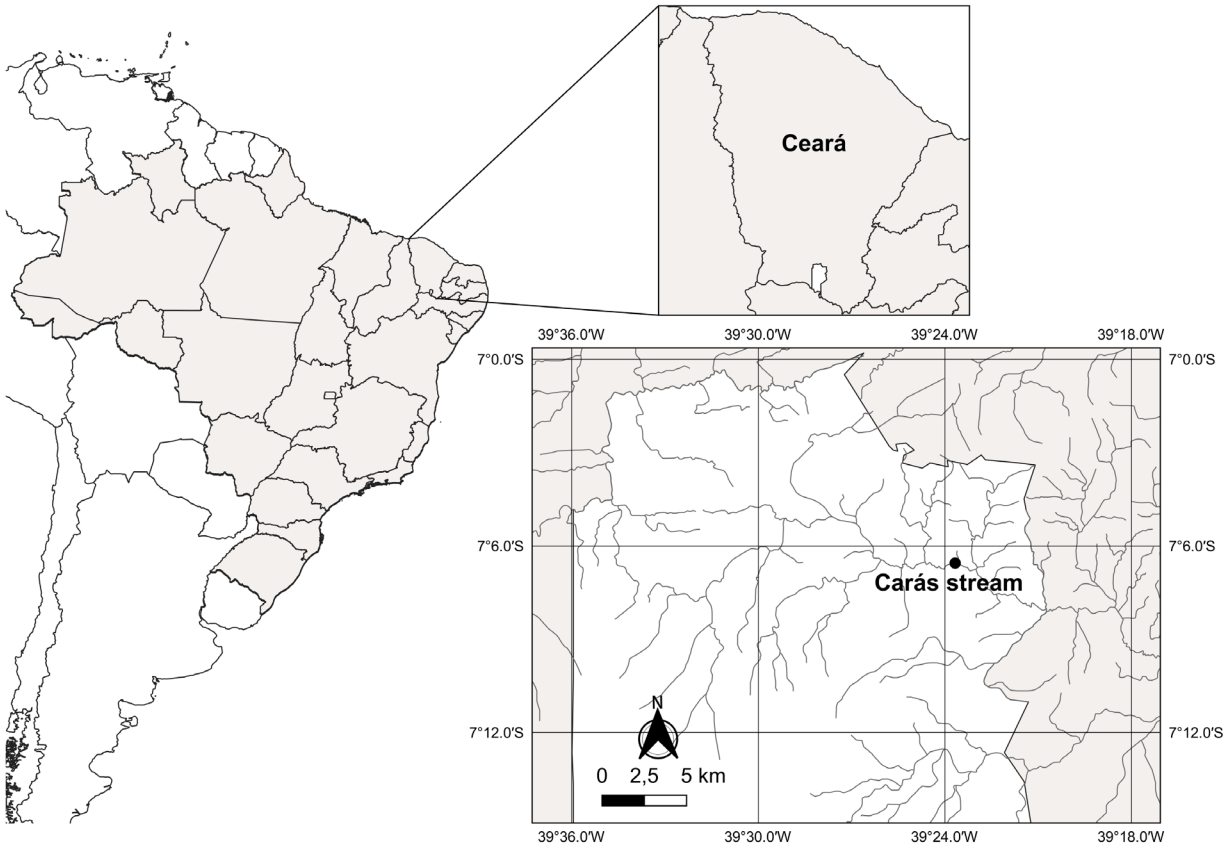

Figure 1. Sampling area, Carás stream, municipality of Crato, Ceará, Brazil.

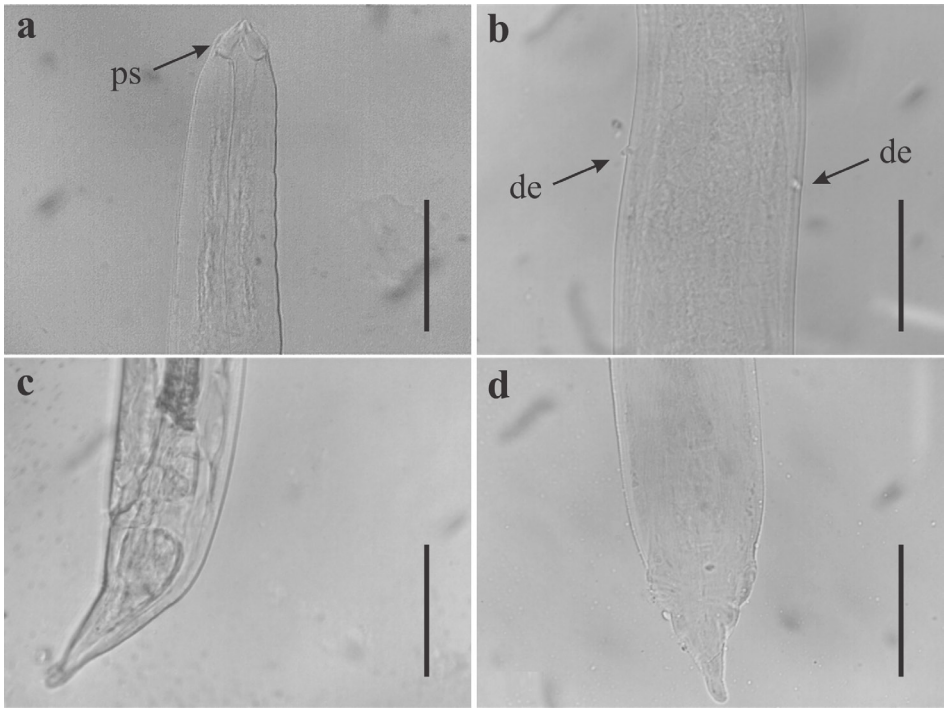

Figure 2. Photomicrographs of the *Spiroxys* sp. (larvae stage). (a) anterior end of body, lateral view; (b) anterior end of body (note deirids); (c) tail, lateral view; (d) tail, ventral view. Abbreviations: ps = pseudolabia; de = deirids. All scale bars = 50 µm.

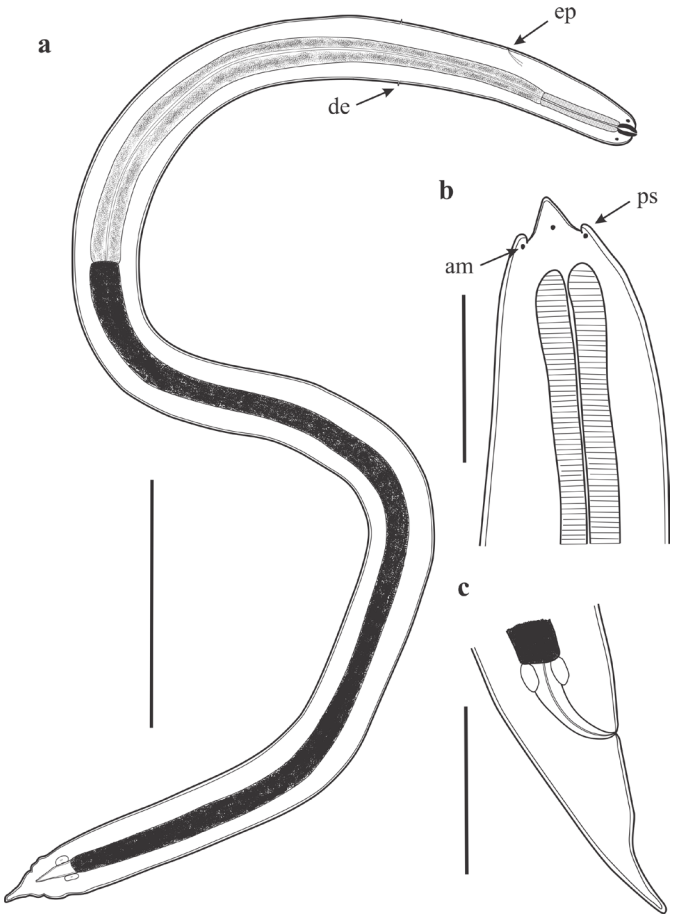

Figure 3. Line drawing of the *Spiroxys* sp. (larvae stage). (a) whole mount, ventral view; (b) cephalic end, lateral view; (c) tail, lateral view. Abbreviations: ep = excretory pore; de = deirids; am = amphid; ps = pseudolabia. Scale bars: a = 0.5 mm; b and c = 0.1 mm.

Table II. Prevalence (P(%)), mean intensity of infection (MI) and mean abundance of infection (MA) with the respective site of infection (IC = intestinal cecum, I= intestine, E = stomach) of larvae of *Spiroxys* sp. parasitizing three hosts species in Carás stream municipality of Crato, Ceará state, Brazil.

Host species	P (%)	MI ± SE	Range	MA ± SE	Range	Site of infection
Astyanax bimaculatus	48	11.95 ± 2.7	2–70	5.78 ± 1.78	0–70	IC, E, I
Hoplosternum littorale	42.5	13.53 ± 3.04	1–52	5.75 ± 1.98	0–52	E, I
Hoplias malabaricus	42.5	10.35 ± 2.38	2–53	4.40 ± 1.55	0–53	IC, I

Table III. Comparative morphometry (mm) based on 10 specimens of *Spiroxys* sp. parasites of *Astyanax bimaculatus*, *Hoplosternum littorale* and *Hoplias malabaricus* (present study) and *Astyanax fasciatus* (Cuvier, 1819) from the upper São Francisco River, State of Minas Gerais, Brazil.

Characters	*A. bimaculatus* present study	*H. littorale* present study	*H. malabaricus* present study	*A. fasciatus* Vieira-Menezes et al. (2017)
Body [L]	2,308 (1,310–3,230)	2,136 (1,590–2,440)	1,980 (1,750–2,110)	2,145 (1,725–2,871)
Body [W]	0.082 (0.060–0.110)	0.070 (0.070–0.090)	0.068 (0.050–0.080)	0.056 (0.043–0.069)
Muscular esophagus [L]	0.110 (0.070–0.170)	0.132 (0.080–0.170)	0.130 (0.110–0.160)	0.115 (0.080–0.150)
Glandular esophagus [L]	0.500 (0.380–0.710)	0.576 (0.480–0.750)	0.584 (0.450–0.660)	0.599 (0.212–0.818)
Pseudolabia	0.023 (0.020–0.030)	0.190 (0.010–0.020)	0.021 (0.020–0.030)	0.019 (0.018–0.021)
Nerve ring [A]	0.095 (0.070–0.120)	0.111 (0.080–0.130)	0.104 (0.090–0.120)	0.175
Excretory pore [A]	0.180	0.156 (0.130–0.170)	0.170 (0.140–0.220)	0.200 (0.175–0.228)
Tail	0.050 (0.040–0.080)	0.050 (0.040–0.070)	0.050 (0.040–0.060)	0.084 (0.063–0.118)
Deirids	0.260 (0.240–0.280)	*	0.270	0.325 (0.288–0.365)

[L] = length; [W] = width; [A] = distance from the anterior end of body; * = structure not observed.

DISCUSSION

In the genus *Spiroxys*, 19 species are allocated that parasitize the intestinal mucosa of freshwater turtles (Hasegawa et al. 1998, Roca & García 2008, Purwaningsih & Mumpuni 2015). In the life cycle of *Spiroxys*, a turtle is considered the definitive host, the copepods or aquatic insects are the intermediate host (Moravec 1998), while freshwater fish act as paratenic hosts (Santos et al. 2009). According to Moravec et al. (1995), *Spiroxys* specific characteristics are very noticeable in adults specimens. In contrast, larvae recovered from paratenic or intermediate hosts cannot be identified at species level based solely on morphological characteristics. There is a possibility that *Spiroxys* larvae recorded in fish, including those of the present study, are *Spiroxys contortus* (Rudolphi 1819), a well distributed and common species (Moravec 1998). However, the lack of records in the literature for larval stages

makes species recognition difficult. Furthermore, the identification of species level is possible only in adult specimens. This is evidenced by several studies that present identification only at genus level.

In the present work, *Spiroxys* larvae were found encysted in the intestinal caecum, stomach and intestine of *A. bimaculatus*, in the stomach and intestine of *H. littorale* and in the intestinal caecum and intestine of *H. malabaricus*, showing similarity in the infection sites (see Table II). This parasite recovered in the three host species examined of the present study are morphologically similar and have similar measurements to the larvae described by Vieira-Menezes et al. (2017). Table III shows the comparative measurements among the species found in this study and that of Vieira-Menezes et al. (2017) for parasites from *Astyanax fasciatus* in the São Francisco River, Brazil.

The feeding habit of *A. bimaculatus* and *H. littorale* composed mainly of aquatic invertebrates, considered intermediate hosts (Caldeira et al. 2007, Suzuki & Orsin 2008) and carnivore of *H. malabaricus* can play an important role in the transmission (Montenegro et al. 2012). Probably, these feeding behaviors may be associated with forms of infection by *Spiroxys* sp. in the Carás stream, as well as being associated with infections by other nematode larvae, according to the records of Takemoto et al. (2009), Abdallah et al. (2012), Acosta et al. (2015), Camargo et al. (2016) and Vieira-Menezes et al. (2017).

The nematode larvae found in this study were identified as *Spiroxys* sp. due to the presence of a cephalic extremity provided with two pseudolips; a small muscular esophagus however larger than glandular esophagus; a nervous ring surrounding the muscular esophagus at its anterior end; an excretory pore located just below the anterior end of the glandular esophagus; a pair of deirids located in the esophagus glandular; and a conical tail with the rounded tip. Such features fall into the morphological characteristics of nematodes of *Spiroxys* (Moravec 1995). Figures 2 and 3 show the *Spiroxys* larvae found in this study, demonstrating the main diagnose characteristics of the genus.

Spiroxys larvae have been found parasitizing fish species of the families Cichlidae, Lepisosteidae, Callichthyidae, Eleotridae, Gymnotidae, Mugilidae, Poeciliidae and Characidae. These results have reported this parasite only in freshwater fishes from Mexico and Brazil (see Table I for details). Therefore, it is interesting to state the necessity to investigate fishes from others localities, increasing the knowledge of biogegraphic limits of this genus. This study is the first record of *Spiroxys* larvae parasitizing *A. bimaculatus*, *H. littorale* and *H. malabaricus*, in addition in a new locality (a stream from Caatinga domain).

Corroborating with Moravec (1998) and Santos et al. (2009), the three hosts species of the present study act as paratenic hosts for nematode larvae of this genus. Furthermore, this study expands the range of the geographic distribution of nematodes of the genus *Spiroxys* and increase the list of hosts, as well as contributing to the knowledge of fish parasites biodiversity in Caatinga domain.

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M.N.M.C., W.B.B.S. and B.A.F.S. identified the species, mounted the material and wrote the manuscript; and F.H.Y. identified the species, wrote the manuscript and reviewed the final manuscript.