



First report of larval *Spiroxys* sp. (Nematoda, Gnathostomatidae) in three species of carnivorous fish from Três Marias Reservoir, São Francisco River, Brazil

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Abstract. The objective of this paper was to evaluate the parasitism by nematode larvae at three species of carnivorous fish in the Três Marias Reservoir, Brazil. It was verified 108 individuals of *Pygocentrus piraya* (Cuvier, 1819), 168 *Serrasalmus brandtii* Lütken, 1875, and 112 *Cichla kelberi* Kullander & Ferreira, 2006. A total of 59 individuals of nematode larvae were found in the three hosts and identified as third stage (L₃ or infective larvae of definitive host) *Spiroxys* sp.. The parasitic indexes were more elevated in the *P. piraya* and *S. brandtii*, although the prevalence and abundance of *Spiroxys* sp. were higher in smaller specimens of *S. brandtii*. This may be explained since these juveniles feed some arthropods, that serve as intermediate hosts and fish parasitized by larvae of *Spiroxys* sp. while foraging. Just two individuals of *C. kelberi* were parasitized by *Spiroxys* sp. and this rare occurrence was shown to be accidental. This record adds to the current knowledge of this nematode's life cycle and provides evidence that these carnivorous fish serve as paratenic hosts of *Spiroxys* sp. larvae in that Reservoir.

Key words: *Pygocentrus piraya*, *Serrasalmus brandtii*, *Cichla kelberi*, paratenic host fish.

Resumo: Primeiro registro de larvas de *Spiroxys* sp. (Nematoda, Gnathostomatidae) em três espécies de peixes carnívoros do Reservatório de Três Marias, Rio São Francisco, Brasil. O objetivo deste trabalho foi avaliar o parasitismo de larvas de nematóides em três espécies de peixes carnívoros no Reservatório de Três Marias, Minas Gerais. Foram examinados 108 indivíduos de *Pygocentrus piraya* (Cuvier, 1819), 168 de *Serrasalmus brandtii* Lütken, 1875 e 112 de *Cichla kelberi* Kullander & Ferreira, 2006. Um total de 59 indivíduos de nematóides larvais encontrados nas três espécies de hospedeiros foram identificadas como *Spiroxys* sp., no terceiro estágio (L₃ – larva infectante ao hospedeiro definitivo), cujos índices parasitários foram mais elevados em *P. piraya* e *S. brandtii*. Contudo, a prevalência e a abundância de *Spiroxys* sp. foram mais elevadas em espécimes menores de *S. brandtii*, pois os juvenis desta espécie se alimentam de artrópodes (com hospedeiros intermediários) e peixes parasitados pelas larvas de *Spiroxys* sp.. Apenas dois indivíduos de *C. kelberi* hospedaram larvas de *Spiroxys* sp. o que pode ter sido accidental. Os resultados encontrados neste trabalho associados ao conhecimento do ciclo de vida dos nematóides evidenciam que estes peixes carnívoros atuam como hospedeiros paratênicos das larvas de *Spiroxys* sp. no Reservatório de Três Marias.

Palavras-chave: *Pygocentrus piraya*, *Serrasalmus brandtii*, *Cichla kelberi*, hospedeiros paratênicos

Introduction

Três Marias Reservoir is located on the

Upper São Francisco River, São Francisco River Basin, in the central region of the State of Minas

Gerais, Brazil. It was inundated in 1961 and at its maximum level has a surface area of roughly 100 thousand hectares and volume of 21 billion cubic meters (Britski *et al.* 1988). It is important to regulate the level of water in the River San Francisco for navigation, control of floods, irrigation and electric power production (Sampaio & López, 2003).

The endemic serrasalmines *Pygocentrus piraya* (Cuvier, 1819) and *Serrasalmus brandtii* Lütken, 1875 and the allochthonous cichlid *Cichla kelberi* Kullander & Ferreira, 2006, are important fishes in fisheries along the São Francisco River. *Pygocentrus piraya* is commonly known as “piranha”. It can reach a total length of 51 cm (Pinkuni 1997) and weigh over 6 kg (Ferreira *et al.* 1996). It is carnivorous, preferentially feeding on other fish (Britski *et al.* 1988, Alvim 1999), behaves opportunistically (Gomes 2002) and gregariously and generally inhabits lentic environments (Braga 1975). It is abundant in the Três Marias Reservoir (Britski *et al.* 1988). *Serrasalmus brandtii* is commonly known as “pirambeba” or “white piranha” (Jegú 2003), and can reach a total length of 31 cm and weight of 700 g (Braga 1975). According to Gomes & Verani (2003), *S. brandtii* is generally smaller than *P. piraya*. It is also carnivorous, preferentially feeding on other fish (Alvim 1999, Gomes 2002) and can tear pieces from its prey with its sharp cutting teeth (Britski *et al.* 1988). Alvim (1999) concluded that *S. brandtii* is a piscivore that feeds mainly on the fins of smaller fish. Pompeu & Godinho (2003) classify *S. brandtii* as a piscivore-insectivore species. *Cichla kelberi*, known commonly as “tucunaré” is a species originally from the Tocantins River Basin (Kullander & Ferreira 2006) that lives in lentic environments where it reproduces mainly during the rainy season (Zaret 1980). Tucunarés have been caught by professional fishermen in Três Marias Reservoir since 1982, but it is not known how the species was introduced (Magalhães *et al.* 1996).

Moreira (1994) reported *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928 in *P. piraya* from the Três Marias Reservoir. Moravec *et al.* (2008) redescribed and reported *Cystidicoloides fischeri* (Travassos, Artigas & Pereira, 1928) in *P. piraya* and *S. brandtii* from the same locality. Santos (2008) found *Spinitectus rodolphiheringi* Vaz & Pereira, 1934 in *P. piraya*., *Philometra* sp. in *S. brandtii*., *Rhabdochona* sp. in *C. kelberi*, as well as *Hysterothylacium* sp., *Goezia* sp. and *Capillostrongyloides* sp. from these carnivorous hosts.

This work constitutes the first about the

various parasitological studies of the carnivorous fish from the Três Marias Reservoir. The aim of this study was to evaluate the ecological-parasite descriptors of the nematode larvae (prevalence, intensity and mean abundance) with the biotic aspects of the hosts (sex and total length), feeding behavior and collection period (dry and wet period) of these important carnivorous fish from the Três Marias Reservoir.

Materials and Methods

The collection of hosts was as follows: 108 specimens of *P. piraya* were caught between July and August, 2004 (dry season) and December, 2004 and January, 2005 (wet season); 168 specimens of *S. brandtii* were caught between July to August, 2004 and July, 2005 (dry season) and January, 2004 and January, 2005 (wet season); and 112 specimens of *C. kelberi* were collected between August, 2004 and July to August, 2005 (dry season) and between December to January, 2004 and January, 2005 (wet season). The fishes were collected in the Três Marias Reservoir, in the area of influence of the Borrachudo River (18°12'59"S, 45°17'34"W), Upper São Francisco River, in the municipality of Três Marias, State of Minas Gerais, Brazil by fishermen from the Estação de Hidrobiologia e Piscicultura of the Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba (EPT/CODEVASF).

The specimens of *P. piraya* and *S. brandtii* were identified and classified according to Britski *et al.* (1988) and Jegú (2003), while the specimens of *C. kelberi* according to Kullander & Ferreira (2006). The nominal taxa of fish followed FishBase (Froese & Pauly 2007). The larval specimens of Nematoda were collected, fixed and processed according to Amato *et al.* (1991) and identified and classified according to Moravec (1998).

The ecological descriptors utilized in the parasitological results followed Bush *et al.* (1997). The statistical tests were only applied for the larval nematodes of fish species with prevalence of 10% or higher, following the recommendation of Bush *et al.* (1990). All the statistical analyses applied to the infrapopulations followed Zar (1996), at a significance level of $p < 0.05$.

Voucher specimens of *P. piraya*, *S. brandtii* and *C. kelberi*, were deposited in the Museu de Zoologia of the Universidade de São Paulo, São Paulo, Brazil (MZUSP: 95150, 95148 and 95149). Voucher specimens of nematode larvae from the three hosts were deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC: 36959, 35557, 36953, respectively), Rio de Janeiro, Brazil.

Results and Discussion

Of the 108 specimens of *P. piraya* collected, 56 were males with average total length of 18.1 ± 4.4 cm (11.1 to 30.5 cm) and average weight of 169.8 ± 180.1 g (21.0 to 835.0 g), and 52 were females with average total length of 21.4 ± 6.0 cm (13.0 to 34.0 cm) and average weight of 323.4 ± 308.4 g (40.0 to 1225.0 g). Of the 168 specimens of *S. brandtii* collected, 55 were males with average total length of 16.2 ± 3.5 cm (9.5 to 27.0 cm) and average weight of 96.9 ± 96.4 g (11.0 to 500.0 g), and 113 were females with average total length of 16.9 ± 4.6 cm (8.5 to 29.5 cm) and average weight of 125.5 ± 142.9 g (7.0 to 657.0 g). Of the 112

specimens of *C. kelberi* collected, 59 were males with average total length of 29.6 ± 6.2 cm (18.0 to 48.0 cm) and average weight of 419.2 ± 285.3 g (85.0 to 1540.0 g), and 53 were females with average total length of 28.4 ± 4.4 cm (20.0 to 35.5 cm) and average weight of 366.9 ± 179.7 g (85.0 to 684.0 g).

A total of 59 specimens (27 in *P. piraya*, 30 in *S. brandtii* and two in *C. kelberi*) of nematode larvae found in the three hosts were identified as third stage (L₃) *Spiroxys* sp.; prevalence was higher in *S. brandtii* and the mean intensity and mean abundance were higher in *P. piraya* (Table I).

Table I. Prevalence (P), intensity range (IR), mean intensity (MI) and mean abundance (MA), with the respective standard deviation (SD), and site of infection (C = coelome, IC = intestinal cecum, E = stomach, AG = anterior gut, MG = middle gut, GB = gall bladder) of *Spiroxys* sp. from three hosts, piscivorous fish from Três Marias Reservoir, Upper São Francisco River, Brazil.

| Hosts | Parasitic indexes of <i>Spiroxys</i> sp. | | | | |
|-----------------------------|--|-----|-----------|------------|-------------------|
| | P (%) | IR | MI±SD | MA±SD | Site of infection |
| <i>Pygocentrus piraya</i> | 12.9 | 1-6 | 1.93±1.49 | 0.25±0.83 | C, IC, AG, GB |
| <i>Serrasalmus brandtii</i> | 13.1 | 1-4 | 1.36±0.85 | 0.18±0.55 | C, E, AG, MG |
| <i>Cichla kelberi</i> | 1.8 | 1 | 1.0 | 0.018±0.13 | C, MG |

The prevalence and mean abundance of *Spiroxys* sp. were not influenced by sex, total length and collection period of *P. piraya*. The prevalence and mean abundance of these larvae were also not influenced by either sex or collection period of *S.*

brandtii. On the other hand, the prevalence and mean abundance of *Spiroxys* sp. were higher in smaller fish (Table II). As only two specimens of *C. kelberi* were parasitized by *Spiroxys* sp., it was not possible to conduct the statistical analyses for these specimens.

Table II. Analysis of the parasitic indexes (P= prevalence, A= abundance) of *Spiroxys* sp. under the possible influence of sex, total length and collection period of two serrasalmine fish from Três Marias Reservoir, Upper São Francisco River, Minas Gerais, Brazil.

| | <i>Pygocentrus piraya</i> | | <i>Serrasalmus brandtii</i> | |
|-----------------------|-------------------------------|-----------------------------|-------------------------------|--------------------------------|
| | P | A | P | A |
| Sex | $\chi^2 = 0.02$ $p = 0.89$ | $U = 1425.00$ $p = 0.84$ | $\chi^2 = 0.68$ $p = 0.40$ | $U = 2922.00$ $p = 0.52$ |
| Total length | $r = -0.14$ $p = 0.74$ | $r_s = 0.001$ $p = 0.99$ | $r = -0.94$ $p = 0.0003^*$ | $r_s = -0.25$ $p = 0.001^*$ |
| Collect period | $\chi^2 = 0.62$ $p = 0.43$ | $U = 1318.00$ $p = 0.37$ | $\chi^2 = 0.75$ $p = 0.38$ | $U = 3304.50$ $p = 0.47$ |

*Significant values: $p < 0.05$; χ^2 : Chi-square with Yates correction; U : Mann Whitney test; r : Pearson's correlation; r_s : Spearman's correlation rank.

According to Moravec (1998), *Spiroxys* Schneider, 1866 is represented by seven species that infect freshwater chelid turtles of Central and North America. Of these, four have been reported in Mexico: *Spiroxys contortus* (Rudolphi, 1819), *S. corti* Caballero, 1935, *S. susanae* Caballero, 1941 and *S. triretrodens* Caballero & Zerecero, 1943. Because the morphological characteristics at the

species level only become evident in adult specimens, it was only possible to identify the larvae to the genus level. Freshwater fishes are paratenic hosts of these larvae. Moravec (1998) suggested the possibility that the larvae of *Spiroxys* found in fish are *S. contortus*, a well-distributed and common species.

Moravec *et al.* (1995) inventoried the

Spiroxys present in the coelomic cavity, mesentery and intestines of *Cichlasoma meeki* (Brind, 1918), *C. urophthalmus* (Günther, 1862), *Poecilia velifera* (Regan, 1914), *Poecilia* sp. and *Astyanax fasciatus* (Cuvier, 1819) in the state of Yucatan, Mexico. Mendoza *et al.* (2004) reported these species in *Dormitator maculatus* (Bloch, 1792) (paratenic host) from Alvarado Lake, Mexico. In Brazil, Isaac *et al.* (2004) found larvae of *Spiroxys* in *Gymnotus* spp. caught in the Baía River in the State of Mato Grosso do Sul. In the present work, these larvae were found in the stomach, intestinal cecum, anterior and middle gut and gall bladder of the three host carnivores examined, two of them endemic serrasalmine of the São Francisco River Basin and one an *allochthonous* cichlid native to the Tocantins River Basin. Therefore, besides the possibility that smaller “piranha” ingest greater quantities of arthropods, our findings suggest that carnivorous fish also prey on foraging fish parasitized by larvae of *Spiroxys* sp. (one of the authors of this study previously found *Spiroxys* larvae in foraging fish in the Três Marias Reservoir) and are thus acting as paratenic hosts of these nematodes in Três Marias Reservoir.

Despite the possibility of behavioral differences between male and female fish, mainly during the spawning season when females generally become stressed and vulnerable to infections, there was no difference in the parasitic indexes of the larvae of *Spiroxys* sp. between the male and female carnivorous fishes studied here.

Spiroxys sp. was significantly more prevalent and abundant in smaller specimens of *S. brandtii*. This can be explained by the piscivorous-insectivorous feeding habit of juvenile “pirambeas”, as reported by Pompeu & Godinho (2003), including the intermediate host species of this nematode among the various arthropods eaten. According to Moravec (1998), fish act as paratenic hosts of these larvae (L₃ or infective larvae of the definitive host), when feeding on infected aquatic insects (intermediate hosts), and the definitive hosts are freshwater chelid turtles. This study, besides the finding that smaller “pirambeas” can ingest greater quantities of arthropods, suggests that they also feed on foraging fish parasitized by larvae of *Spiroxys* sp., meaning these fish act as paratenic hosts of these nematodes in Três Marias Reservoir.

According to Paperna (1996), if nematode larvae are not eliminated by the host's immune system or by some regulatory system of the parasite intensity, they can remain viable for a long time until reaching their definitive hosts. The low intensity of *Spiroxys* sp. found in the three hosts analyzed, particularly in *C. kelberi* (where only two

specimens were parasitized), can be explained by the elimination of these larvae by the immune system of these hosts, or also by the sporadic feeding on these larvae by the fish species studied.

The three carnivorous species were all infected by *Spiroxys* sp. larvae, but the parasitism pattern between the two serrasalmine endemic to the São Francisco River Basin was similar, that is, there was no quantitative difference between them. However, in *C. kelberi* these larvae were rare and the parasitism was probably accidental in this cichlid.

Despite the results found in this study, it is probable that the diets of the serrasalmine fish (based on arthropods infected with *Spiroxys* larvae) were similar throughout the year in the Três Marias Reservoir since the prevalence and abundance of these larvae were not influenced by the fish collection period.

Besides being considered definitive hosts of some parasite species, these findings indicate that the carnivorous fish from the Três Marias Reservoir analyzed in this study can be classified as paratenic hosts of *Spiroxys* larvae, indicating the complexity of the host-parasite relationship in the aquatic system in question.

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