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Parasitism by *Sphincterodiplostomum musculosum* (Digenea, Diplostomidae) metacercariae in the eyes of *Steindachnerina insculpta* (Characiformes, Curimatidae)

Parasitismo por metacercárias de *Sphincterodiplostomum musculosum* (Digenea, Diplostomidae) nos olhos de *Steindachnerina insculpta* (Characiformes, Curimatidae)

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Abstract

New occurrences and effects of parasitism by metacercariae in fish eyes have recently been discussed in many studies. The aim of the present study was to evaluate the infection levels of the eye flukes *Sphincterodiplostomum musculosum* Dubois, 1936 (metacercariae) in *Steindachnerina insculpta* (Fernádez-Yépez, 1948) from three ecosystems under the influence of the Jurumirim reservoir (Paranapanema, Taquari and Veados Rivers). A total of 174 fish specimens were sampled between April 2011 and April 2012. There were high levels of infection by *S. musculosum* metacercariae in the eyes of *S. insculpta* from all the sampled ecosystems, thus presenting broad geographical distribution. The histological analyses revealed that the metacercariae were apparently lodged behind the retina. The smaller fish from the Taquari River were less parasitized by metacercariae than the adults. This pattern can be explained by the cumulative effect of parasitism, as demonstrated by the positive correlation between the abundance of metacercariae and fish body size.

Keywords: Paranapanema River, freshwater fish, *Steindachnerina insculpta*, Brazil.

Resumo

Novas ocorrências e efeitos do parasitismo por metacercárias em olhos de peixe têm sido recentemente discutidas em muitos estudos. O objetivo deste estudo foi avaliar os níveis de infecção dos vermes de olho *Sphincterodiplostomum musculosum* Dubois, 1936 (metacercárias) em *Steindachnerina insculpta* (Fernádez-Yépez, 1948) de três ecossistemas influenciados pelo reservatório de Jurumirim (Rios Paranapanema, Taquari e Veados). Um total de 174 espécimes de peixes foi amostrado de Abril/2011 a Abril/2012. Metacercárias de *S. musculosum* mostraram altos níveis de infecção nos olhos de *S. insculpta* em todos os ecossistemas amostrados apresentando ampla distribuição geográfica. As análises histológicas revelaram que, aparentemente, estas metacercárias estavam alojadas por trás da retina. Os peixes menores do rio Taquari estavam menos parasitados por metacercárias do que os adultos. Esse padrão pode ser explicado pelo efeito cumulativo de parasitismo, evidenciado pela correlação positiva entre a abundância de metacercárias e tamanho do corpo dos peixes.

Palavras-chave: Rio Paranapanema, peixe de água doce, *Steindachnerina insculpta*, Brasil.

Introduction

The Neotropical region is one of the zoogeographical areas with the greatest diversity of freshwater fishes, comprising 4,000 species described (LÉVÊQUE et al., 2008). Approximately 2,587 species are found in Brazilian rivers (BUCKUP et al., 2007), however, parasitological studies have included only a small portion of this diversity (EIRAS et al., 2011). Fishes in their natural environment can present high diversity of parasites with pathogenic potential, although evident clinical signs are rarely shown (BACHMANN et al., 2012). This emphasizes the importance of studies on the taxonomy and occurrences of these parasites in their host organisms.

Digenetic trematodes are parasites with heteroxenous life cycles, generally using snails as intermediate hosts and involving at least two hosts. The final host may be a fish or a piscivorous mammal or bird (THATCHER, 2006; PAVANELLI et al., 2008). Species of the Diplostomidae family have been found in fishes in Asia, Europe, North America and South America (NIEWIADOMSKA, 1996). Diplostomidae metacercariae are known as eye flukes that infect

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fish eyes, and they can be found in the retina, vitreous and aqueous humor, or crystalline lens (NIEWIADOMSKA, 1996; CHAPPELL, 1995). These digeneans may be responsible for "helminthic cataracts", also known as diplostomiasis (MARTINS et al., 1999), and in extreme cases can cause severe ocular pathology associated with blindness (CHAPPELL et al., 1994). Penetration of these parasites into different structures such as the body surface, buccal cavity and gills, and migration of the larvae to the eye, can cause injuries and hemorrhages in several tissues, thereby obstructing blood vessels. These phenomena may be associated with fish death in cases of high infection rates (VALTONEN; GIBSON, 1997).

The genus *Sphincterodiplostomum* Dubois, 1936, includes parasites of the intestine of neotropical birds (LUNASCHI; DRAGO, 2006). *Sphincterodiplostomum* is monospecific and differs from other members of the Diplostomidae family because of the presence of a dorsal tubular invagination with a sphincter at the level of the posterior testis. The original description of *S. musculosum* was based on the morphology of immature specimens collected from the intestine of an ardeid bird *Agamia agami* (Gmelin, 1789) in Brazil (DUBOIS, 1936, 1938).

In Brazil, metacercariae of *S. musculosum* have been reported parasitizing the eyes of *Cyphocharax gilbert* (Quoy and Gaimard, 1824) in Guandu River (ABDALLAH et al., 2005), *Steindachnerina insculpta* (Fernandez-Yépez, 1948) in the Chavantes reservoir, Middle Paranapanema River (ZAGO et al., 2013), and *Hoplias malabaricus* (Bloch, 1794) in the Upper Paraná River floodplain (TAKEMOTO et al., 2009). They have also been observed in the ovaries of *Steindachnerina brevipinna* (Eigenmann and Eigenmann, 1889) in the Rosana reservoir, Low Paranapanema River (CESCHINI et al., 2010), the gills of *Prochilodus lineatus* (Valenciennes, 1836) in the Upper Paraná River floodplain (LIZAMA et al., 2006) and in the visceral cavity of *Hemisorubim platyrhynchos* (Valenciennes, 1836) in the Upper Paraná River floodplain (GUIDELLI et al., 2003).

The aim of the present study was to evaluate the infection levels of *S. musculosum* eyeflukes in *S. insculpta* from three areas under the influence of the Jurumirim reservoir, thus contributing to the knowledge of the geographical distribution and occurrence of digenetic helminths in Neotropical fishes. Our prediction was that the levels of infection of *S. musculosum* in *S. insculpta* would not differ among the ecosystems sampled.

Materials and Methods

The Jurumirim reservoir (23°12'17"S, 49°13'19"W) is the uppermost reservoir in a cascade system in the Paranapanema River and is operated as a regulator for the other ones further downstream. It is of storage type, with an approximate area of 440 km^2 (HENRY et al., 2006). The tributaries sampled were the Taquari River (23°29'21.95"S, 49°12'6.90"W), Veados River (23º15'42.4", 48°37'27.6"W) and Paranapanema River (23°15'11.9"S, 49°12'34.2"W).

Steindachnerina insculpta belongs to the Curimatidae family and is widely distributed in South America (REIS et al., 2003). The hosts were caught quarterly from April 2011 to April 2012 using gillnets of different mesh sizes with standardized effort. The individuals sampled were measured (standard length to the nearest 0.1 cm) and weighed (nearest 0.1 g).

The helminths were fixed under coverslip pressure in AFA solution (alcohol, formalin and acetic acid), preserved in 70% alcohol, stained in Mayer's carmalum, cleared in creosote and mounted in Canada balsam (EIRAS et al., 2006). The morphometric analyses were performed using a computerized image analysis system (LAS, Leica Microsystems, Wetzlar, Germany). The morphometric results (in micrometers) are presented as mean (minimum-maximum) values. Voucher specimens of the parasites were deposited in the Helminthological Collection of the Institute of Biosciences (Coleção Helmintológica do Instituto de Biociências, CHIBB), UNESP, Botucatu, state of São Paulo, Brazil.

The eyes of two specimens of *S. insculpta* sampled in the Taquari River were histologically analyzed, with the aim of determining the infection site of the metacercariae. The eyes were carefully removed, fixed with 4% formaldehyde for at least 24 hours, dehydrated in progressive ethanol solutions (70% to 95%) and impregnated with Historesin for 24 hours. The material was then sectioned at the thickness of 5 µm using a rotating microtome equipped with a glass blade, and stained with hematoxylin and eosin.

In accordance with Bush et al. (1997), the following community descriptors were calculated at the infra community level: prevalence (%), mean intensity of infection and mean abundance.

To determine the metacercariae distribution in the host population, three size classes were separated based on the length of the fish sampled, as follows: Class I (up to 5.3 cm), Class II (from 5.4 to 6.9 cm) and Class III (greater than 7 cm). Individuals belonging to Class I and Class II were sampled only in the Taquari River. The prevalence of the three classes was compared using the Z test, and the significance level used was $p < 0.05$.

Statistical differences in parasite abundance among the three rivers studied were tested using Kruskal-Wallis variance analysis (ZAR, 1996) only for individuals belonging to class III. Spearman's rank correlation (rs) was used to investigate the length and weight relationship of the host, and the abundance of eye flukes in the fish population of the Taquari River (ZAR, 1996).

Results

A total of 174 specimens of *S. insculpta* were sampled: 95 in the Taquari River, 42 in the Paranapanema River and 37 in the Veados River. *Sphincterodiplostomum musculosum* was present in all samples belonging to Class III (prevalence = 100%), and for classes I and II in the Taquari River, the prevalences were 9.1 and 53.8 respectively (Table 1). There was a positive correlation between the abundance of metacercariae of *S. musculosum* and the length (Figure 1) and weight (Figure 2) of the host population sampled in the Taquari River.

The comparisons of prevalences among the three classes were significant for samples from the Taquari River (I *versus* II, Z = 2.489; p = 0.013) (II *versus* III, Z = 4.675; p = 0.001) (I *versus* III, Z = 8.036; p = 0.001). The abundances of *S. musculosum* in the eyes of *S. insculpta* among the three tributaries were similar (Kruskal-Wallis, H = 0.2; p = 0.9).

Table 1. Data on infection by metacercariae of *Sphincterodiplostomum musculosum* in the eyes of *Steindachnerina insculpta* sampled in the tributaries of the Jurumirim reservoir, Brazil. Sampled hosts (N), mean length ± standard error (ML ± SE), mean weight ± standard error (MW ± SE), prevalence (P%), mean abundance ± standard error (MA ± SE), mean intensity ± standard error (MI ± SE) and range (RA).

Ecological descriptors	Taquari			Paranapanema	Veados
	Class I	Class II	Class III	Class III	Class III
N	22	13	60	42	37
ML ± SE	4.0 ± 0.1	6.2 ± 0.1	9.8 ± 0.1	8.9 ± 0.7	9.7 ± 0.1
MW ± SE	1.7 ± 0.2	6.1 ± 0.2	25.8 ± 1.1	17.2 ± 0.5	20.8 ± 0.1
P (%)	9.1	53.8	100	100	100
MA ± SE	0.2 ± 0.19	1.7 ± 0.7	41.5 ± 7.1	35.3 ± 5.5	39.2 ± 9.8
MI ± SE	3 ± 1	3.6 ± 1.1	41.5 ± 7.1	35.3 ± 5.5	39.2 ± 9.8
RA	(2-6)	(1-8)	(1-286)	(2-119)	(1-337)

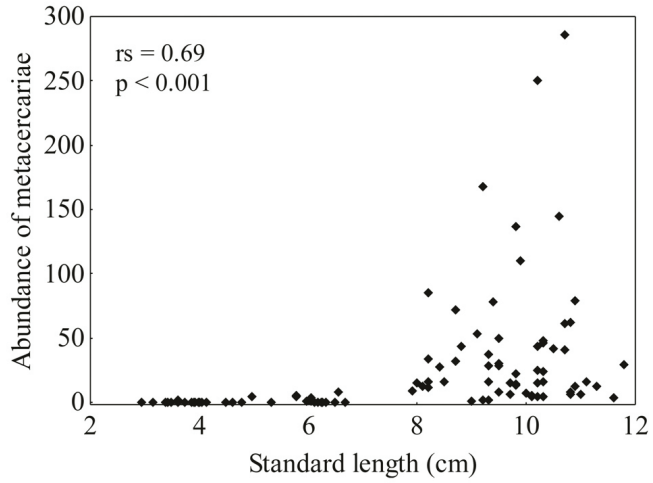

Figure 1. Correlation between the standard length of *Steindachnerina insculpta* (Fernández-Yépez, 1948) and abundance of metacercariae of *Sphincterodiplostomum musculosum* Dubois, 1936, in the Taquari River, Upper Paranapanema River, Brazil.

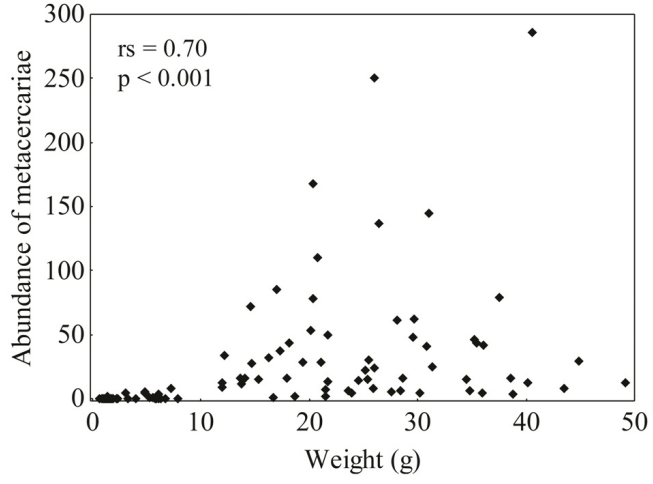

Figure 2. Correlation between the weight of *Steindachnerina insculpta* (Fernández-Yépez, 1948) and abundance of metacercariae of *Sphincterodiplostomum musculosum* Dubois, 1936, in the Taquari River, Upper Paranapanema River, Brazil.

The morphometric data on the metacercariae of *S. musculosum* and comparisons with the literature are summarized in Table 2. The histological analysis revealed that the parasites are apparently lodged behind the retina, next to the optical nerve (Figure 3).

Discussion

This study reports occurrences of metacercariae of the eye fluke *S. musculosum* in *S. insculpta* in the Paranapanema, Taquari and Veados rivers, Upper Paranapanema River. Adult forms of this helminth parasitize birds (LUNASCHI; DRAGO, 2006), and the larval forms are found in two hosts (snails and fishes). Metacercariae can infect almost all the organs or tissues of fishes (EIRAS et al., 2010).

The comparison of morphometric data of *S. musculosum* metacercariae parasitizing host populations from other localities (*S. insculpta* in the Chavantes reservoir, Middle Paranapanema River, and *S. brevipinna* in the Rosana reservoir, Lower Paranapanema River) revealed similar sizes. These results may be explained by the fact that even though these host populations are from different locations, they dwell in the same ecosystem or water body (Paranapanema River). However, several factors may act to produce significant variations in body dimensions of metacercariae, such as host species, host size and age, population size associated with intensity-dependent growth, worm condition at fixation and fixation technique (BROWN et al., 2003; FREDENSBORG; POULIN, 2005; SALDANHA et al., 2009; CHAPPELL, 1995).

Regarding comparisons of morphometric data with the literature, this study showed that the values for 'anterior testis length' were not similar to the values found by Ceschini et al. (2010) and Zago et al. (2013). The metacercariae of *S. musculosum* present two testes: the shape of the anterior testis resembles a 'walking stick' while the posterior testis is shaped like the letter 'U' upside-down; the two testes overlap and are located in the middle portion of the metacercarial hindbody (Figure 4). This disposition was represented in a line drawing by Ceschini et al. (2010), in which the shapes of the testes are very clear. However, the morphometric differences in the length of the anterior testis shown in the present study may be due to the fact that Ceschini et al. (2010) and Zago et al. (2013) used the measurements of the first portion of the anterior testis, as checked in the specimens of this study (Table 2). Furthermore,

Table 2. Comparative morphometric data (mean and range in micrometers) of *Sphincterodiplostomum musculosum* metacercariae between the present study and Ceschini et al. (2010) and Zago et al. (2013).

***S. musculosum* measurements (CHIBB 7179)**	**Present study**	**Ceschini et al. (2010)**	**Zago et al. (2013)**
	Steindachnerina insculpta	***Steindachnerina brevipinna***	***Steindachnerina insculpta***
	N = 30	**N = 15**	**N = 30**
Locality	Jurumirim reservoir, Upper Paranapanema River	Rosana reservoir, Low Paranapanema River	Chavantes reservoir, Middle Paranapanema River
Body			
Length	2818.2 (1973.9-3630.5)	2553 (1775-3075)	2734 (2151-3982)
Width	1435.6 (999-1775.6)	1396 (1075-1675)	1272.4 (914.4-1844.3)
Forebody			
Length	1682.9 (1241.5-2249.8)	1530 (1150-1900)	1642.6 (1133.9-2346.9)
Width	1435.6 (999-1775.6)	1508 (1075-1675)	1272.4 (914.4-1844.3)
Hindbody			
Length	1114 (734.5-1462.8)	1033 (600-1375)	1087.4 (810.9-1811.1)
Width	645.6 (502.9-750.4)	665 (500-800)	572.8 (452.8-745.4)
Pharynx			
Length	107 (72-104)	104 (84-117)	114.8 (89.6-218.5)
Width	70.6 (42.9-178.8)	54 (45-84)	73.1 (47.9-113.4)
Oral sucker			
Length	137.2 (80.7-167.4)	145 (100-200)	142 .4 (96.3-292.6)
Width	169.7 (115.1-173.5)	213 (130-200)	180.5 (134.8-313.6)
Pseudosuckers			
Length	223.8 (159.5-342.3)	-	216.4 (161.5-475.9)
Width	251.1 (212.2-342.5)	-	261.1 (186.8-438.4)
Ventral sucker			
Length	181.1 (120.9-217.3)	162 (130-210)	142.4 (96.3-292.6)
Width	224.4 (192.3-256)	213 (180-250)	180.5 (134.8-313.6)
Tribocytic organ			
Length	404.8 (210.2-604.9)	384 (290-440)	391.5 (246-644.6)
Width	444.5 (287.9-573.6)	490 (350-650)	494.9 (321.2-732.4)
Anterior testis			
First portion length	275 (144.7-414)	-	-
Length	485.7 (262.7-717.4)	217 (130-390)	285.4 (199.8-452.7)
Width	332.4 (267-433.2)	307 (250-350)	293.2 (209.4-414.3)
Posterior testis			
Length	284.8 (156.9-469.6)	290 (210-400)	310.1 (210-446.6)
Width	448.9 (315.4-525.9)	410 (200-510)	385.1 (275.2-557.9)
Ovary			
Length	75.5 (59.3-99.7)	-	-
Width	95.9 (66.2-135.8)	-	-

this study provides complementary data on the measurements of the ovary, which had not been shown before.

The occurrence of high numbers of parasites in the eye of *S. insculpta* (per host), indicates that metacercariae of *S. musculosum* are well adapted to this host. Zago et al. (2013) also suggested that *S. insculpta* is highly susceptible to infection by this parasite. Moreover, we raise the hypothesis that higher average temperature is an important factor for high levels of infection in the population of *S. insculpta* in the Jurumirim reservoir, Paranapanema River. The Jurumirim reservoir presents a mean temperature of 22.8 °C (range 20.9 to 24.9 °C) (GRANADO; HENRY, 2012). According to Valtonen and Gibson (1997), transmission and production of cercariae is primarily regulated by temperature. Cercariae of species of the genus *Diplostumum* emerge into the water only at temperatures above 10 °C (BERRIE, 1960; WOOTTEN, 1974; BRASSARD et al., 1982; STABLES; CHAPPELL, 1986). Moreover, the optimum temperature for the establishment of metacercariae is 17.5 °C (STABLES; CHAPPELL, 1986).

During ontogeny, changes to the behavior and biology of fish occur (TAKEMOTO et al., 1996). Changes to the diet can include a large number of items used in the life cycle of parasites, such as intermediate hosts or even aquatic plants containing parasite structures (larvae and eggs), resulting in higher parasite abundance in larger fishes. It is expected that parasites cause low damage to the host, and thus the fish can increase in size and weight regardless of parasitism, and harbor a larger quantity of parasites (POULIN, 1998). In this study, the levels of infection by metacercariae of *S. musculosum* in *S. insculpta* (size class III)

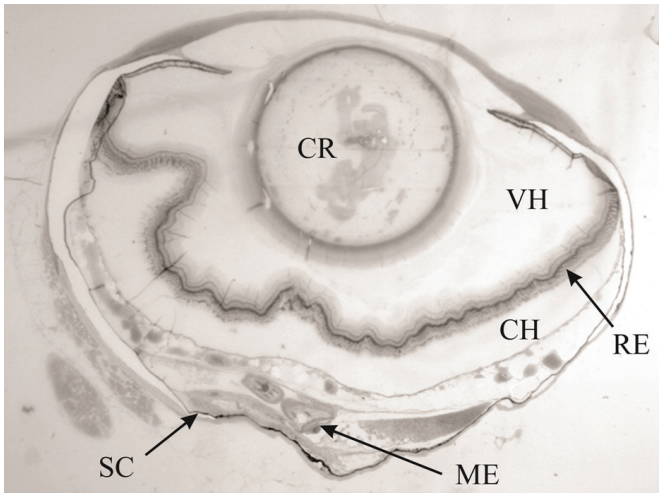

Figure 3. Histological section of the eye of *Steindachnerina insculpta* (Fernández-Yépez, 1948) and site of infection of *Sphincterodiplostomum musculosum* Dubois, 1936, metacercaria. CR = crystalline lens; VH = vitreous humor; RE = retina; CH = choroid; ME = metacercariae; SC = sclera.

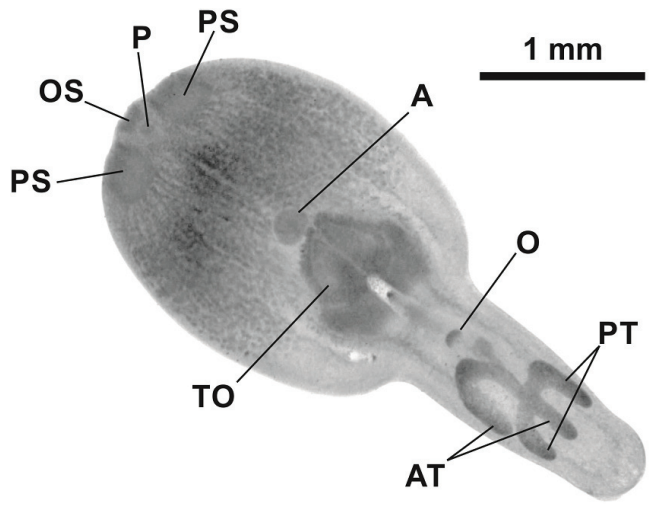

Figure 4. Specimen of *Sphincterodiplostomum musculosum* Dubois, 1936, metacercaria in the eye of *Steindachnerina insculpta* (Fernández-Yépez, 1948). Note the morphology and disposition of anterior (AT) and posterior (PT) testes. PS = pseudo suckers; P = pharynx; OS = oral sucker; A = acetabulum (ventral sucker); TO = tribocytic organ; O = ovary. Scale bar = 1 mm.

from the three localities did not show differences. Smaller hosts collected in the Taquari River showed lower levels of parasitism, compared with larger hosts. In this case, a significant positive correlation between the standard length and weight of the host and the abundance may occur as a result from the temporal accumulation process (YAMADA et al., 2012), i.e. the length of exposure of *S. insculpta* to metacercariae of *S. musculosum*.

Digenean trematodes have a complex life cycle in which at least three hosts are involved. The second stage (cercaria) infects the second intermediate fish host, in elevated numbers, usually through the gills, and develops an unencysted larva (metacercaria). Metacercariae of Diplostomidae infecting fish eyes are usually found in the retina, vitreous humor, aqueous humor and/or crystalline lens (YAMADA et al., 2008; ZICA et al., 2009) and they can be easily observed with the aid of a stereomicroscope or even with the naked eye. In one histological section of the present study, metacercariae of *S. musculosum* were observed lodged behind the retina. Nonetheless, further studies are necessary in order to be sure that the site of infection of *S. musculosum* differs from that of other eye flukes.

There are gaps in the knowledge of the life cycle of these parasites that might be fully understood based on studies on the helminth fauna of piscivorous birds. Thus, it is possible that metacercariae of *S. musculosum* use *S. insculpta* and *S. brevipinna*, which are both abundant in the Paranapanema River, as intermediate hosts in order to reach the maximum biotic potential, and complete their life cycle in ardeid birds, which are common in South America. Therefore, future studies on the helminth fauna of birds are suggested in order to better understand the life cycle of this parasite.

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