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Sphincterodiplostomum musculosum (Digenea, Diplostomidae) infectando *Steindachnerina insculpta* (Characiformes, Curimatidae) no reservatório de Chavantes, Sudeste do Brasil

Aline Cristina Zago^{1*}; Lidiane Franceschini¹; Igor Paiva Ramos²; Érica de Oliveira Penha Zica¹;
 Alison Carlos Wunderlich¹; Edmir Daniel Carvalho³; Reinaldo José da Silva⁴

¹Programa de Pós-graduação em Ciências Biológicas, Instituto de Biociências, Universidade Estadual Paulista – UNESP, Botucatu, SP, Brasil

²Centro de Ciências Biológicas e da Saúde, Colegiado de Ciências Biológicas, Universidade Estadual do Oeste do Paraná – UNIOESTE, Cascavel, PR, Brasil

³Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista – UNESP, Botucatu, SP, Brasil

⁴Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista – UNESP, Botucatu, SP, Brasil

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Abstract

This study aimed to report the infection by *Sphincterodiplostomum musculosum* metacercariae in *Steindachnerina insculpta* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil. Thirty specimens of *S. insculpta* were collected and 29 were infected with *S. musculosum* metacercariae (prevalence = 96.67%) in the eyes and visceral cavity. The mean intensity of infection and abundance were 96.6 ± 29.41 (7-846) and 93.3 ± 28.6 (0-846), respectively. Positive correlation was observed between parasite abundance in the eyes and standard length ($r_s = 0.5$, $p = 0.005$), total weight ($r_s = 0.649$, $p = 0.0001$), and condition factor ($r_s = 0.439$, $p = 0.0154$). The high parasitism rates by *S. musculosum* metacercariae in *S. insculpta* can be an indicative that this fish species is highly susceptible to infection by this diplostomid, and even reflect the presence of a large abundance of the intermediate host. Moreover, *S. musculosum* metacercariae are reported for the first time in *S. insculpta*.

Keywords: Diplostomid, metacercaria, *Sphincterodiplostomum musculosum*, *Steindachnerina insculpta*, condition factor, Paraná River basin.

Resumo

O presente estudo tem como objetivo relatar a infecção por metacercárias de *Sphincterodiplostomum musculosum* em *Steindachnerina insculpta* provenientes do Reservatório de Chavantes, médio Paranapanema, município de Ipaussu, Estado de São Paulo, Brasil. Foram coletados 30 espécimes de *S. insculpta*, sendo que 29 estavam infectados por metacercárias de *S. musculosum* (prevalência = 96,67%) nos olhos e na cavidade visceral. A intensidade média de infecção e abundância apresentaram valores de $96,6 \pm 29,41$ (7-846) e $93,3 \pm 28,6$ (0-846), respectivamente. Foi observada correlação positiva entre a abundância dos parasitas presentes nos olhos com relação ao comprimento padrão ($r_s = 0,5$, $p = 0,005$), peso ($r_s = 0,649$, $p = 0,0001$) e fator de condição ($r_s = 0,439$, $p = 0,0154$). As altas taxas de parasitismo de metacercárias de *S. musculosum* em *S. insculpta* podem ser um indicativo de que esta espécie de peixe é altamente susceptível à infecção por este diplostomídeo, e até mesmo refletir a presença de grande oferta do hospedeiro intermediário. Além disso, este é o primeiro relato de parasitismo por metacercárias de *S. musculosum* em *S. insculpta*.

Palavras-chave: Diplostomídeo, metacercária, *Sphincterodiplostomum musculosum*, *Steindachnerina insculpta*, fator de condição, bacia do rio Paraná.

*Corresponding author: Aline Cristina Zago
 Instituto de Biociências, Universidade Estadual Paulista – UNESP,
 Distrito de Rubião Junior, CEP 18618-970, Botucatu, SP, Brasil
 e-mail: alinecrsthina@yahoo.com.br

Introduction

Steindachnerina insculpta Fernández-Yépez, 1948 is a native fish from Brazil and its occurrence is restricted to the Paraná River basin. This fish species belongs to the family Curimatidae and is commonly known in Brazil as *saguiru-do-rabo-amarelo* (FROESE; PAULY, 2012).

In the aquatic environment, the penetration of pathogens is facilitated and fish can be infected by numerous parasite species (KLEIN et al., 2004). Therefore, a great number of fish species can act as intermediate or definitive hosts of several parasite species (PAVANELLI et al., 2008).

Among fish parasites, the larval stages of digeneans, such as diplostomid metacercariae, are important agents of diseases in these animals, because they can cause severe ocular pathology associated with cataracts and blindness or even death. Diplostomids present a complex three-host life-cycle which involves: a free-living larva (miracidium), which hatches from the egg; two asexual generations (parthenitae) in an aquatic snail; a second free-living larva (cercaria), which is released in huge numbers and is involved in the transmission to the second intermediate fish host, usually penetrating it through the gills; an unencysted larva (metacercaria), usually in the eyes (lens, humour or under the retina) of fishes; and a sexually mature, hermaphroditic adult in the intestine of piscivorous birds (VALTONEN; GIBSON, 1997).

Several species of diplostomids have been found in fish from Asia, Europe, North America, and South America (NIEWIADOMSKA, 1996). In Brazil, metacercariae of the diplostomid *Sphincterodiplostomum* sp. Dubois, 1936 have been previously reported in some fish species such as *Steindachnerina brevipinna* Eigenmann and Eigenmann, 1889 (CESCHINI et al., 2010a), *Hoplias malabaricus* Bloch, 1794, *Hemisorubim platyrhynchos* Valenciennes, 1840 (TAKEMOTO et al., 2009), *Prochilodus lineatus* Valenciennes, 1836 (LIZAMA et al., 2006) and *Cyphocharax gilbert* Quoy and Gaimard, 1824 (ABDALLAH et al., 2005). However, there are no reports on the occurrence of this metacercariae infecting *S. insculpta*. Thus, the aim of this study is to report the infection by *Sphincterodiplostomum musculosum* Dubois, 1936 metacercariae in *S. insculpta* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil.

Materials and Methods

Thirty specimens of *S. insculpta* were collected in January, 2009 in the Chavantes Reservoir (Figure 1), medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil (23° 7' 36" S and 49.59° 23' 10" W). Fish were collected using a nylon monofilament gillnet from 5:00 PM to 7:00 AM, totaling 14 hours of exposure. The fish specimens collected were transported to a laboratory

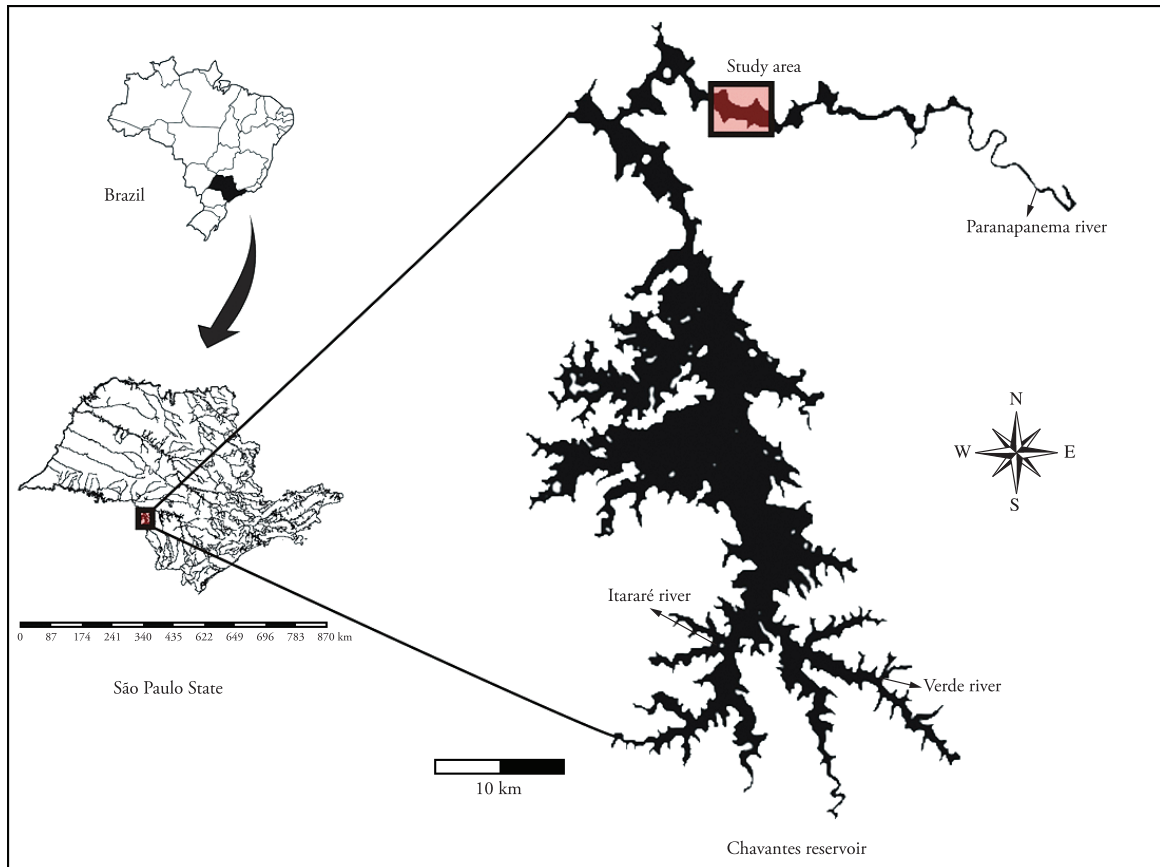


Figure 1. Map of Brazil, highlighting the State of São Paulo and the sampling area at the Chavantes Reservoir, Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil.

where they were measured (cm), weighed (g) and necropsied. Organs and body cavities of *S. insculpta* were examined and the metacercariae found were collected, fixed in alcohol-formol-acetic acid solution under cover slip pressure. After that, they were stained with carmine according to Eiras et al. (2006).

Morphometric analysis of the metacercariae was accomplished using a computerized system for image analysis (Qwin Lite 3.1, Leica Microsystems, Wetzlar, Germany). All measurements were presented in micrometers and represented by the mean \pm standard deviation (range). The ecological descriptors of parasitism (prevalence, abundance and mean intensity of infection) were calculated according to Bush et al. (1997). Spearman's rank correlation (r_s) was used to study the correlations of the abundance of parasites present in the eyes in relation to standard length, total weight and condition factor - K (LE CREN, 1951). Statistical tests were performed using SigmaStat 3.1 (Systat Software Inc., California, USA), adopting a significance level of 5%.

Voucher specimens were deposited in the Coleção Helmintológica (CHIBB: 6972-6973), of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista – UNESP, municipality of Botucatu, State of São Paulo, Brazil.

Results

The mean standard length and total weight of the fish analyzed were 12.05 (9-16.5) cm and 39.95 (13.7-74.3) g, respectively.

Twenty-nine *S. insculpta* specimens were infected by *S. musculosum* metacercariae (prevalence = 96.67%). The larvae were found parasitizing the eyes (93.33%) and visceral cavity (10%). Two thousand seven hundred ninety-nine metacercariae were collected in the studied specimens, and the mean intensity of infection and abundance were 96.6 ± 29.41 (7-846) and 93.3 ± 28.6 (0-846), respectively.

A positive correlation was observed between parasite abundance and standard length ($r_s = 0.5$, $p = 0.005$) (Figure 2), total weight ($r_s = 0.649$, $p = 0.0001$) (Figure 3) and condition factor ($r_s = 0.439$, $p = 0.0154$) (Figure 4).

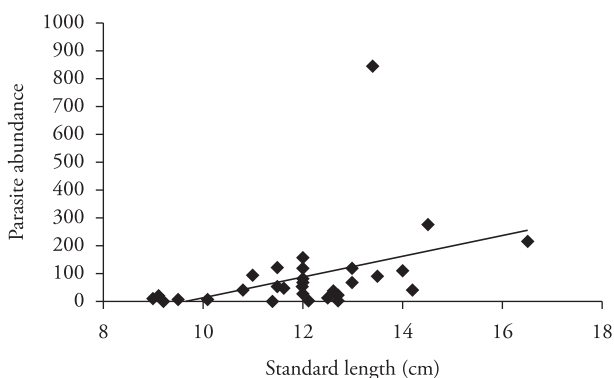


Figure 2. Correlation between standard length and abundance of *Sphincterodiplostomum musculosum* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil.

Description of *S. musculosum* metacercariae (based on 30 specimens) (Figure 5): body bipartite; forebody oval and concave ventrally; hindbody ovoid, much narrower and inserts sub-dorsally in the anterior segment. Forebody with three lobes: the median lobe is occupied by the oral sucker, and the pseudosuckers are located in the lateral lobes; circular tribocytic organ; acetabulum located below of the middle of the forebody; anterior testis asymmetrical and lateral; posterior testis larger than the anterior one, consisting of two elongated lateral masses; anterior ovary adjacent to the anterior testis; sub-terminal sphincter. The morphometric data of *S. musculosum* metacercariae is summarized in Table 1.

Taxonomic Summary

Host: *Steindachmerina insculpta* Fernández-Yépez, 1948.

Site of infection: eyes and visceral cavity.

Locality: Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil.

Material: CHIBB (6972-6973)

Mean Intensity of infection: 96.6 ± 29.41 , up to 846 parasites found in a single host.

Prevalence: 96.67%.

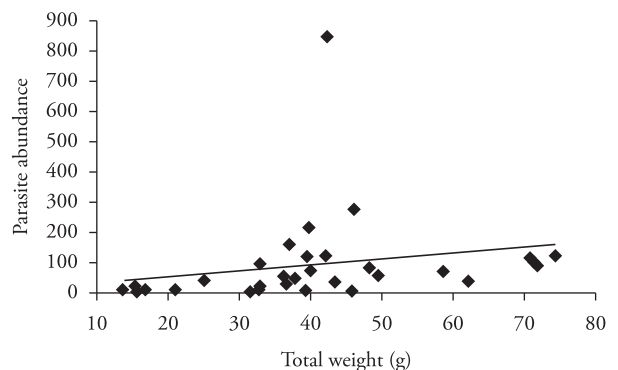


Figure 3. Correlation between total weight and abundance of *Sphincterodiplostomum musculosum* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil.

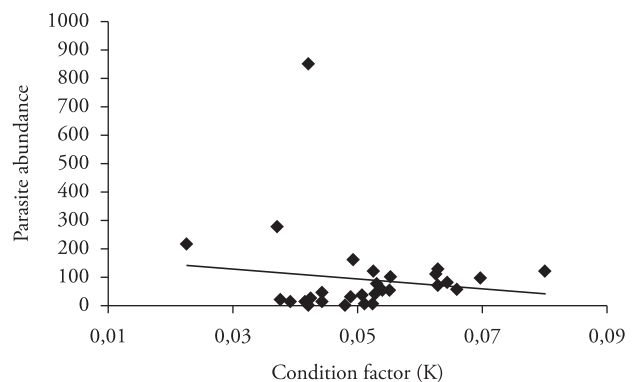


Figure 4. Correlation between condition factor (K) and abundance of *Sphincterodiplostomum musculosum* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil.

Others hosts: *Steindachnerina brevipinna* Eigenmann and Eigenmann, 1889 (CESCHINI et al., 2010a), *Hoplias malabaricus* Bloch, 1794, (TAKEMOTO et al., 2009) and *Cyphocharax gilbert* Quoy and Gaimard, 1824 (ABDALLAH et al., 2005) to *Sphincterodiplostomum musculosum* Dubois, 1936 metacercariae; and *Hemisorubim platyrhynchos* Valenciennes, 1840 (TAKEMOTO et al., 2009) and *Prochilodus lineatus* Valenciennes, 1836 (LIZAMA et al., 2006) to *Sphincterodiplostomum* sp. Dubois, 1936 metacercariae.

Discussion

The present study reports the infection by *S. musculosum* metacercariae in *S. insculpta* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil. This parasite species was reported in a few fish species (CESCHINI et al., 2010a; TAKEMOTO et al., 2009; ABDALLAH et al., 2005) in comparison with other diplostomidae,

Table 1. Comparative morphometric data between *Sphincterodiplostomum musculosum* metacercariae of the Chavantes Reservoir, Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil, and *Steindachnerina brevipinna* from the tributaries Guairacá and Corvo of the low Paranapanema River, State of Paraná, Brazil, described by Ceschini et al. (2010a).

Data and measures (µm) of <i>Sphincterodiplostomum musculosum</i> metacercariae	Host	
	<i>Steindachnerina insculpta</i> ^a	<i>Steindachnerina brevipinna</i> ^b
	Mean ± SD (range)	Mean (range)
Locality	Chavantes Reservoir, medium Paranapanema River, State of São Paulo, Brazil	Tributaries Guairacá and Corvo of the low Paranapanema River, State of Paraná, Brazil
Number of metacercariae	30	15
Body		
Length	2734 ± 508.8 (2151-3982)	2553 (1775-3075)
Width	1272.4 ± 231 (914.4-1844.3)	1396 (1075-1675)
Anterior segment		
Length	1642.6 ± 330.9 (1133.9-2346.9)	1530 (1150-1900)
Width	1272.4 ± 230.9 (914.4-1844.3)	1508 (1075-1675)
Posterior segment		
Length	1087.4 ± 259.9 (810.9-1811.1)	1033 (600-1375)
Width	572.8 ± 79.4 (452.8-745.4)	665 (500-800)
Pharynx		
Length	114.8 ± 27.5 (89.6-218.5)	104 (84-117)
Width	73.1 ± 16.1 (47.9-113.4)	54 (45-84)
Oral sucker		
Length	142.4 ± 34.5 (96.3-292.6)	145 (100-200)
Width	180.5 ± 31.5 (134.8-313.6)	213 (130-200)
Lateral pseudosucker (1)		
Length	216.4 ± 57.7 (161.5-475.9)	-
Width	261.1 ± 46.2 (186.8-438.4)	-
Lateral pseudosucker (2)		
Length	217.9 ± 56.6 (1149.8-461.4)	-
Width	252.8 ± 50.4 (168.3-454.5)	-
Ventral sucker		
Length	142.4 ± 34.5 (96.3-292.6)	162 (130-210)
Width	180.5 ± 31.5 (134.8-313.6)	213 (180-250)
Tribocytic organ		
Length	391.5 ± 85.1 (246-644.6)	384 (290-440)
Width	494.9 ± 107.2 (321.2-732.4)	490 (350-650)
Anterior testis		
Length	285.4 ± 62.2 (199.8-452.7)	217 (130-390)
Width	293.2 ± 49.8 (209.4-414.3)	307 (250-350)
Posterior testis		
Length	310.1 ± 58.9 (210-446.6)	290 (210-400)
Width	385.1 ± 79.6 (275.2-557.9)	410 (200-510)

^aPresent study (CHIBB: 6972-6973); ^bCeschini et al. (2010a); Range = min-max; SD = standard deviation (mean).

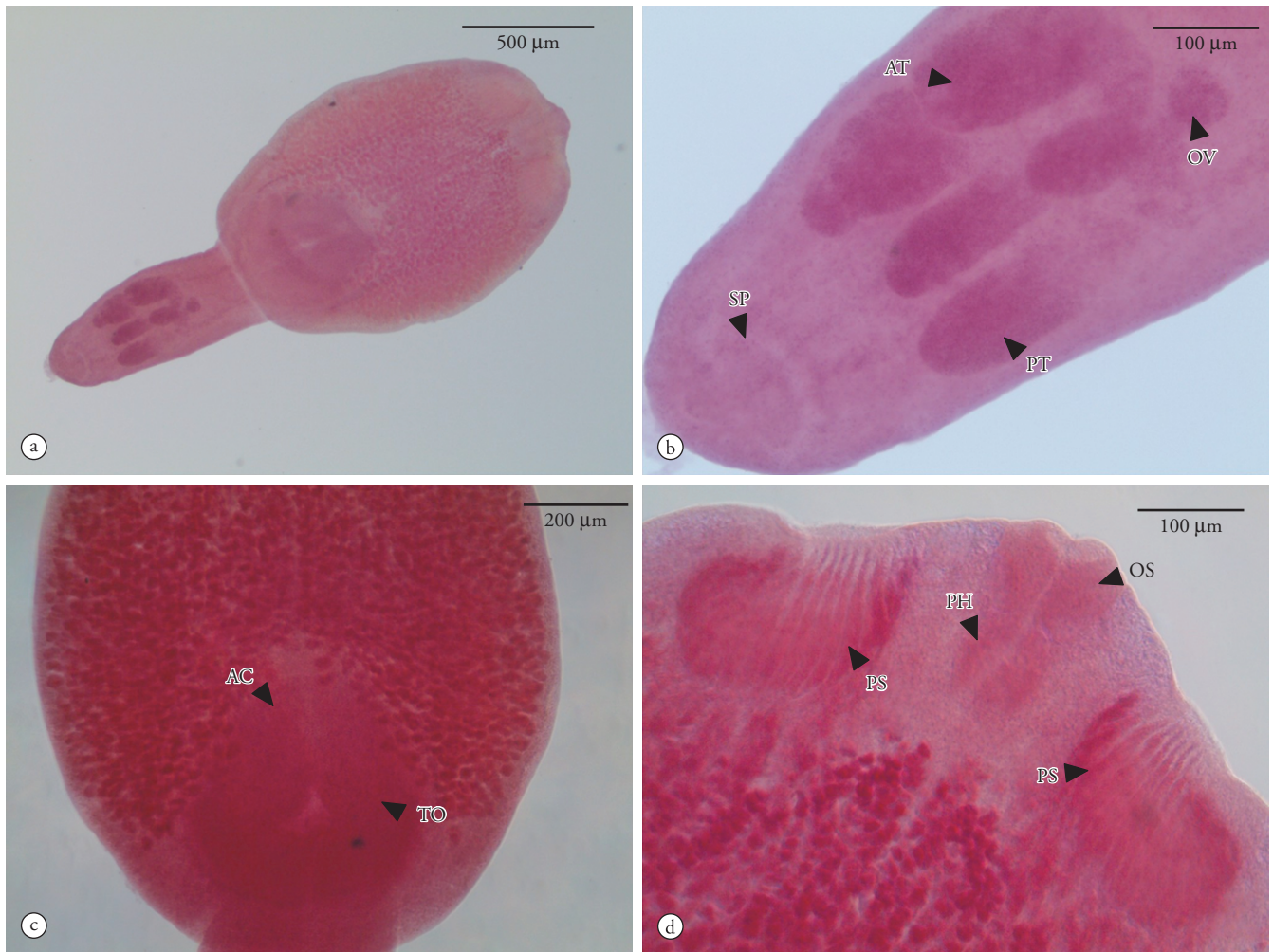


Figure 5. *Sphincterodiplostomum musculosum* metacercariae found in *Steindachnerina inculpta* from the Chavantes Reservoir, medium Paranapanema River, municipality of Ipaussu, State of São Paulo, Brazil. a) total body; b) anterior testis (AT), posterior testis (PT), ovary (OV), sphincter (SP); c) acetabulum (AC), tribocytic organ (TO); d) oral sucker (OS), pseudosucker (PS), pharynx (PH).

such as *Austrodiplostomum compactum*, which was previously reported in approximately 25 fish species (MACHADO et al., 2005; EIRAS et al., 2010; PAES et al., 2010). Furthermore, the studied *S. inculpta* specimens presented high parasitism rates of *S. musculosum*, indicating that this fish species is high susceptible to infection by this parasite.

The morphological and morphometric data observed in this study to *S. musculosum* metacercariae from *S. inculpta* corroborated the data presented by Ceschini et al. (2010a), who found a high quantity of this diplostomid in the ovaries of *S. brevipinna*.

The abundance of *S. musculosum* in the eyes of *S. inculpta* was positively correlated to the standard length and total weight. Similar results were found by Ceschini et al. (2010b), who observed positive correlation between the abundance of *S. musculosum* and the standard length of *S. brevipinna* from the tributaries Guairacá and Corvo, in the low Paranapanema River, State of Paraná, Brazil. According to Ceschini et al. (2010b), these results support the idea of cumulative effect of parasites by increasing the area of infection with the growth of the host, as mentioned by Luque et al. (1996), and with the increase of the standard length

with the ontogenetic development of the host, the area of the body surface also increases, as well as the possibility of occurring the process of active penetration of cercariae. This pattern was also observed by Paes et al. (2010) and Machado et al. (2005) in relation to other diplostomidae, such as *Austrodiplostomum compactum* metacercariae.

Positive correlation was also observed between the condition factor (K) and the abundance of *S. musculosum* in the eyes of *S. inculpta*. The condition factor is given by the relationship between the individual's weight and length and it is an important indicator of the fish health, reflecting recent nutritional conditions and the interactions between the fish and the biotic and abiotic factors (LE CREN, 1951; SATAKE et al., 2009). Thus, the results observed in this study suggest that the parasitism by *S. musculosum* could not affect the physiological status, health or welfare of the studied *S. inculpta* specimens.

Sphincterodiplostomum musculosum found in this study were in the larval stage, suggesting that this fish species occupies an intermediate position in the food chain and may be part of the diet of piscivorous birds (definitive hosts of this parasite species).

In this study, a great number of *S. musculosum* metacercariae was found parasitizing the eyes of *S. insculpta*. According to Evans et al. (1976), around 40 metacercariae of diplostomids per eye may be responsible for cataract or blindness in fish (depending on the size of the host). In our study, no histopathological study was performed to analyze whether the *S. insculpta* specimens were affected by the parasites. However, the fact that the metacercariae were found parasitizing the eyes at high infection rates makes the fish an easier prey for capture by the definitive host (commonly piscivorous birds), so the parasite can complete its life cycle faster (ABDALLAH et al., 2005).

In summary, the high parasitism rates by *S. musculosum* metacercariae in *S. insculpta* can be an indicative that this fish species is highly susceptible to infection by this diplostomid, and even reflect the presence of a large abundance of the intermediate host. Moreover, *S. musculosum* metacercariae are reported for the first time in *S. insculpta*.

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