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## **BIOLOGICAL SCIENCES**

# Occurrence of metacercariae of Austrodiplostomum compactum (Lutz, 1928) (Trematoda, Diplostomidae) in Pimelodus platicirris in the Ilha Solteira Reservoir, São Paulo, Brazil

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**Abstract:** This study reports the occurrence of metacercariae of *Austrodiplostomum compactum* in *Pimelodus platicirris* from a Neotropical reservoir in the Grande River, SP, Brazil. A total of 164 fish were collected, of which 12.80% were infected with metacercariae in the eyes. The mean intensity of infection and mean abundance were 1.52±0.14 (1–3) and 0.23±0.05 (0–3), respectively. The presence of this parasite with a high intensity of infection can cause exophthalmos, retinal displacement, opacity of the lens, blindness or even death. This is the first record of ocular metacercariae for *P. platicirris*, thus increasing the number of hosts for *A. compactum*.

**Key words:** Diplostomiasis, freshwater fish, mandi, Neotropical reservoir.

# INTRODUCTION

The superfamily Diplostomoidea Poirier, 1886, comprises a group of digenetic trematodes possessing a holdfast organ found posterior to the ventral sucker, a morphological innovation unique to this trematode group. This superfamily contains over 250 described species, 88 genera, 16 subfamilies and 6 families, including the family Diplostomidae (Blasco-Costa & Locke 2017).

The biological cycle of diplostomids involves a first intermediate host (gastropod mollusks), a second intermediate host (fish) or rarely observed paratenic host (amphibians), and finally a definitive host (piscivorous birds)

(Violante González et al. 2009), in which the adult parasite is harbored in the intestine.

In general, adults of diplostomid species reproduce sexually in the intestine of infected piscivorous birds, depositing their eggs into the water through feces, with the hatching and release of miracidia (free-swimming larva) occurring within 22 days (Grobbelaar et al. 2014). Miracidia actively penetrate planorbid gastropods belonging to the genus *Biomphalaria* Preston, 1910 (in Brazil this has specifically been reported in *B. glabrata* (Say, 1818) and *B. straminea* (Dunker, 1848)) (Pinto & Melo 2013), undergo morphological alterations, such as the loss of cilia, becoming saccular with cells (sporocyst) from which several redia originate and from which the cercariae finally develop

(Thatcher 2006). The cercariae subsequently leave the snails and may remain encysted in vegetation or actively penetrate their second intermediate host (fish), commonly by the gills, reaching the eyes, brain and/or muscles through the bloodstream, where they develop into metacercariae. Finally, piscivorous birds prey the infected fish, giving continuity to the cycle (Thatcher 2006, Rassier et al. 2015).

Metacercariae of the diplostomid Austrodiplostomum compactum (Lutz, 1928) are widely distributed in the Neotropical region (Vital et al. 2016) and require at least three hosts until reaching the adult stage. Due to its low specificity, A. compactum can infect various species of freshwater fish, as described by Santos et al. (2002), Pinto et al. (2014), Costa et al. (2015), Rassier et al. (2015), Ramos et al. (2016), Vital et al. (2016), Albuquerque et al. (2017), at various sites of infection, such as the gills, swim bladder, brain, musculature and, preferably, the eyes (vitreous humor) (Ramos et al. 2013). Although the number of new records of this parasite in native fish species in Brazil is increasing (Ramos et al. 2013, Ramos et al. 2016). the potential impact of the occurrence of these metacercariae on wild fauna and transmission to animals raised in fish farms remains unknown (Pinto & Melo 2013).

Research aimed at characterizing the biology and ecology of the freshwater siluriform *Pimelodus platicirris* Borodin, 1927 is of fundamental importance for its conservation, as it is a medium-sized species with potential for artisanal fishing and human consumption. The objective of the present study was to report the occurrence of metacercariae *A. compactum* in *P. platicirris* in the Upper Paraná River basin.

## MATERIALS AND METHODS

The study was carried out in the Ilha Solteira Reservoir, in the municipal region of Santa Clara D'Oeste, (50°55'57'60''W and 20°02'33.62"S) on the Can-Can branch of the Grande River, São Paulo state, Brazil (Figure 1). The specimens of P. platicirris were collected bimonthly from September 2014 to October 2016 with the aid of gill nets of different meshes (3 to 16 cm between adjacent nodes) in an area adjacent to a fish farm. The fish were euthanized, frozen and sent to the laboratory of the Department of Biology and Animal Science, School of Engineering, Ilha Solteira (FEIS) (SISBIO n° 42229-1, CEUA/ FEIS nº 001/2014, SisGen n° A278D23). The metacercariae were recovered from the eyes. fixed under pressure between a lamina and coverslip, and preserved in 70% ethanol (Eiras et al. 2006). Then, the metacercariae were stained with hydrochloric carmine and clarified in eugenol for identification using an optical microscope with a coupled camera connected to a computerized image analysis system (Optical Microscope with AXIOCAM MRc5 ZEISS camera). The morphological identification followed the recommendations of Ostrowisky de Núñez (2017). The following parasitological parameters were calculated: prevalence (P), mean abundance (MA) and mean intensity of infection (MII) (Bush et al. 1997), being are expressed as mean, followed by standard error and the range (minimum maximum) in parentheses.

Voucher specimens of *P. platicirris* were deposited in the DZSJRP Fish Collection of UNESP, municipality of São José do Rio Preto (DZSJRP-Pisces 21316) and *A. compactum* were recorded in the Helminthological Collection of the Institute of Biosciences of Botucatu (CHIBB – 8458).

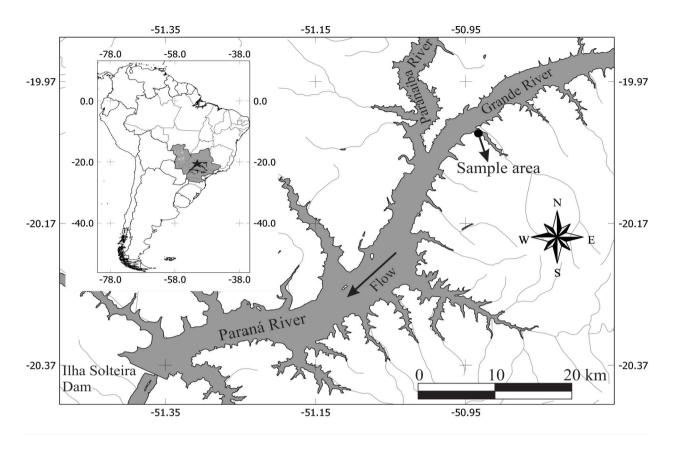


Figure 1. Study area on the Can-Can branch of the Ilha Solteira Reservoir, Grande River, São Paulo state, Brazil.

# **RESULTS**

The morphology of the recovered metacercariae followed the redescription proposed by Ostrowski de Núñez (2017): body bipartite; forebody spatulate, slightly concave ventrally; hindbody very short, with a small conical segment. Oral sucker subterminal, two lateral pseudosuckers well developed, on each side of oral sucker; ventral sucker absent. Small pharynx, esophagus short, ceca simple, reaching until level of genital primordia. Holdfast organ (= tribocytic organ) elliptical and bilobed. Genital primordia poorly developed, differentiated in two small testes, ovary not distinct.

A total of 164 specimens of *P. platicirris* were captured, with a mean standard length of  $26.98 \pm 0.28$  (15–47 cm) and a mean total mass

of 378.22  $\pm$  12.76 (78.99–912.36 g). As for the parasitological parameters, 21 were infected with metacercariae of *A. compactum* (prevalence 12.80%), with a mean intensity of infection and mean abundance of 1.52  $\pm$  0.14 (1–3) and 0.23  $\pm$  0.05 (0–3), respectively.

# **DISCUSSION**

These results may be associated with the availability of organic matter from fish farming deposited in the sediment (Beveridge 2004), increasing the abundance of gastropod mollusks in these areas (Edgar et al. 2005). This increase has also been reported by authors such as Oliveira & Krau (1970), Barbosa (1983) and Callisto et al. (2005) in environments not used by fish farming but with a high content

of organic matter. Moreover, according to Karvonen et al. (2006), the dynamics of infection of *Diplostomum spathaceum* (Rudolphi, 1819) (Diplostomidae) in fish are mainly related to population variations in the first intermediate host (Gastropoda). Thus, the presence of fish farming may directly affect the life cycle of *A. compactum*, as proposed by Ramos et al. (2014).

These data corroborate the observations of Ramos et al. (2013), Ramos et al. (2016) and Barrett et al. (2019) which reported that fish farm contributed with resources, in the form of organic matter, to the aquatic environment, attracting the local biota and altering the abundance of intermediate hosts (gastropods and fish). These, in turn, may attract the definitive hosts (piscivorous birds), resulting in an increase in the dissemination/availability of infective forms of *A. compactum* in areas near to fish farms. In this way, wild fish attracted to these areas most likely are more susceptible to contact with infecting forms and can thus be infected, as may have occurred with *P. platicirris*.

The occurrence of metacercariae of A. compactum in P. platicirris was similar to the infection values reported for Pimelodus maculatus Lacepède, 1803, from the Paranapanema River, Chavantes Reservoir, São Paulo (Ramos et al. 2013), in the Itajaí-Açu River in Blumenau, Santa Catarina (Bachmann et al. 2007) and in the Guandu River, Rio de Janeiro (Santos et al. 2007). We can therefore infer that P. platicirris and P. maculatus have similar susceptibility to the metacercariae of A. compactum, due to the fact that they are congeneric, have similar alimentary habits and occupy the same habitat (Silva 2015).

The occurrence of metacercariae of *A. compactum* has been recorded in at least 43 freshwater species fish in Brazil, demonstrating their low parasitic specificity (Ramos et al. 2013, Sabas & Brasil-Sato 2014, Costa et al. 2015,

Kubitza & Campos 2014, Ramos et al. 2016 and Vital et al. 2016). They are often found in the eves of fish (the second intermediate host). and may in extreme cases cause exophthalmos. retinal displacement, lens opacity and blindness (Seppälä et al. 2004). However, penetration of the cercariae into different structures such as the surface of the body, the fins, the buccal cavity and gills, together with migration to the eyes, can cause lesions and hemorrhages in various tissues, as well as obstruction of the blood vessels, phenomena that may be associated with fish death in cases of high infection rates (Silva-Souza 1998). The reduction of visual acuity has also been reported, with a consequent reduction in feeding activity (Seppälä et al. 2004). In addition, blindness or poor vision makes fish more susceptible to predation, facilitating the transmission of the parasite to the definitive host (birds).

This is the first record of the occurrence of metacercariae of *A. compactum* in *P. platicirris*, contributing to the knowledge of the distribution of this parasite and providing the first information on the parasitological aspects of this Neotropical fish. The occurrence of this parasite in wild fish near fish farms, together with its low specificity, raises concerns about the potential impacts of infection and dissemination to other species of wild fish.

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# **Author contributions**

Main author, general structure of the manuscript and discussion of the results: Denis William Johansem de Campos; contributed to the laboratorial analysis: Letícia de Oliveira Manoel and Denis William Johansem de Campos; participated in the sampling of fish: Rosicleire Veríssimo-Silveira, Letícia de Oliveira Manoel and Cristiéle da Silva Ribeiro; took part in the structuration: Letícia de Oliveira Manoel and Lidiane Franceschini also contributed to parasite identification and laboratorial analysis; revision of the manuscript: Rosicleire Veríssimo-Silveira and Cristiéle da Silva Ribeiro; Igor Paiva Ramos acted as supervisor of Mr. Campos during the structuration, scientific discussions and interpretation contained in this manuscript.

