



Light, electron microscopy and histopathology of *Myxobolus salminus* n. sp., a parasite of *Salminus brasiliensis* from the Brazilian Pantanal[☆]

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ABSTRACT

In this report, we describe the morphology and histopathology of *Myxobolus salminus* n. sp., a parasite of the gill filaments of wild *Salminus brasiliensis* (dourado) from the Brazilian Pantanal. The small polysporic plasmodia were $\sim 100\ \mu\text{m}$ in diameter and the development was asynchronous. The mature spores were oval to pear shaped and had a smooth wall. The spore measurements were (mean $\pm$ S.D., with range in parentheses): length $10.1 \pm 0.4\ \mu\text{m}$ (9.6–10.5), width $6.1 \pm 0.4\ \mu\text{m}$ (5.8–6.6) and thickness $5.0 \pm 0.6\ \mu\text{m}$ (4.7–5.3). The polar capsules were elongated and of equal size: length $4.6 \pm 0.2\ \mu\text{m}$ (4.3–4.8) and width $1.7 \pm 0.1\ \mu\text{m}$ (1.5–1.9). The histological analysis revealed numerous plasmodia in the blood vessels of the gill filaments. The site of parasite development was the wall of the large-caliber blood vessel of the gill filament, with progressive growth towards the lumen, resulting in the obstruction of blood flow, congestion and perivascular edema. The ultrastructural study revealed that the plasmodial wall was composed of two membranes, had numerous pinocytotic canals and was in direct contact with the basement membrane of the vessel. The development of the parasite was asynchronous, with mature spores, immature spores and young developmental stages randomly distributed throughout the plasmodium. The prevalence of the parasite was 4.4%, with male and female fish being infected.

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1. Introduction

Salminus brasiliensis Cuvier, 1816 (Syn. *S. maxillosus* Valenciennes, 1849), popularly known as dourado in Portuguese or dorado in Spanish (English name = Jaw

characin), is a carnivorous fish found in the Prata basin (Argentina, Bolivia, Brazil, Paraguay and Uruguay) and in the drainage area of Lagoa dos Patos in the southern Brazilian state of Rio Grande do Sul, Brazil (Lima et al., 2003). This characid, which can reach over 1 m in length (Resende, 2003), is appreciated for its meat and is an important species in the fishing economy of the regions where it occurs (850,000 kg of dourado was commercialized in 2006) (Ibama, 2008). The dourado is also highly prized by sport fisherman and is one of the few South American fish widely recognized by the international sport fishing community (Resende, 2003). The

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dourado has been cultivated for production meat, sport fishing and as ornamental fish (Mai and Zaniboni-Filho, 2005).

More than 1500 species of Myxosporea are currently known (Casal et al., 2006) and include some of the most important fish parasites (Schmahl et al., 1989). Myxosporans infect mainly fishes, but some are also parasites of reptiles and amphibians. The genus *Myxobolus* is the most common, with >700 species described so far (Eiras et al., 2005). In South America, 26 *Myxobolus* species have been described (Eiras et al., 2005; Adriano et al., 2006, 2009; Flores and Viozz, 2007; Martins and Onaka, 2006), only one of which parasitizes *S. brasiliensis* (Molnár et al., 1998).

In this study, we used light and electron microscopy to investigate the morphology and histopathology of a new species of *Myxobolus* found to infect the gill filaments of wild, but not cultivated, *S. brasiliensis* from the Brazilian Pantanal.

2. Materials and methods

Ninety-one adult specimens of *S. brasiliensis* were collected in seven expeditions to the Pantanal Mato-Grossense in central Brazil and 90 specimens were obtained from fish farmers in São Paulo state. The wild fish were caught in the Aquidauna and Miranda rivers of the southern region ($n = 11$), in rivers and lakes of the Pantanal National Park (PARNA-Pantanal) in the central region ($n = 67$) and in the Cuiabá and Manso rivers of the northern region ($n = 13$).

The fish were caught and immediately transported alive to the field laboratory where they were measured, weighed and necropsied to parasitological examination. For taxonomic and histopathological analyses the gill filaments were fixed in 10% formalin in phosphate-buffered saline, and for ultrastructural analyses in 2.5% glutaraldehyde in cacodylate buffer. Morphological studies of the spores were based on fixed mature spores obtained from plasmodia of different dourado specimens (43 spores). The measurements were done with a computer loaded with Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope. The dimensions of the spores (in μm) were expressed as the mean $\pm$ standard deviation (S.D.). Smears containing free spores were stained with Giemsa solution and mounted in low viscosity mounting medium (CytosealTM) as permanent slides.

For histological analysis, fragments of infected gill filaments were embedded in paraffin and serial sections 4 μm thick were stained with Sirius red (Adriano et al., 2002).

For scanning electron microscopy, the plasmodia were fixed and embedded in paraffin prior to processing. Sections 10 μm thick were deposited on albumin-coated coverslips, dried in an oven for 48 h, deparaffinized with xylol, re-dehydrated, post-fixed in 1% OsO_4 in cacodylate buffer for 30 min at 4 °C, washed in the same buffer, dehydrated in ethanol, critical point dried in CO_2 , covered with metallic gold, and examined in a Joel JMS 35 microscope operated at 15 kV (Adriano et al., 2002). For ultrastructural analysis, fragments of gills containing

plasmodia were fixed in 2.5% glutaraldehyde in cacodylate buffer (12 h), post-fixed in 1% OsO_4 (2 h), dehydrated in increasing concentrations of acetone and embedded in EMbed 812 resin. Ultrathin sections double-stained with uranyl acetate and lead citrate were examined in a LEO 906 electron microscope operated at 60 kV.

3. Results

Of the 91 wild and 90 cultivated dourado specimens examined, only 4 (4.4%) of the wild specimens had plasmodia of an unknown species of *Myxobolus* in blood vessels of the gill filaments; none of the cultivated specimens contained parasites. The four infected dourado specimens (two males and two females) were caught in the central region of the Pantanal Mato-Grossense.

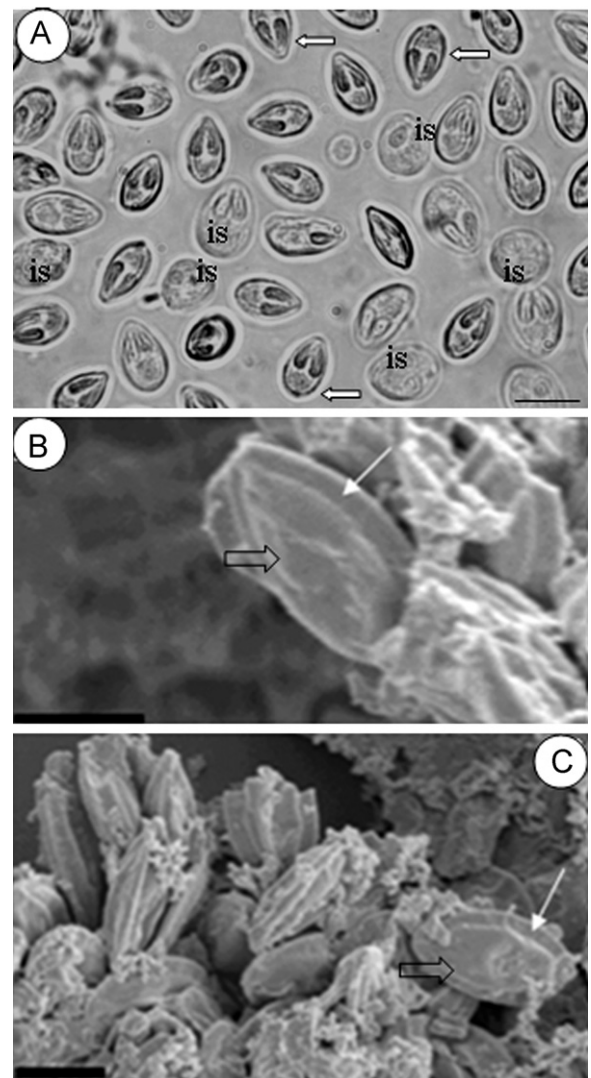


Fig. 1. Morphological aspects of *Myxobolus salminus* n. sp. spores: (A) light microscopy showing mature (arrows) and immature spores (is). Scale bar = 10 μm . (B and C) Scanning electron micrographs showing smooth valves (large arrows) and rim around the spores (thin arrows). Scale bars = 5 μm .

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