

***Myxobolus* sp. (Myxozoa): Ultrastructural and Phylogenetic Studies of the Eye Infection of a Brazilian Freshwater Fish (*Pimelodus maculatus*)**

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The South American continent contains one of the biggest hydrographic networks in the world, in which a great variety of ictiofauna species live. However, the number of myxosporean parasites described from Brazil is very low, especially considering that the country has one of the most diverse freshwater fish populations in the world with about 8,000 species [1]. The genus *Myxobolus* B tschli, 1882 the largest genus of the Myxobolidae family and many species have been reported, mainly parasitizing freshwater fishes throughout the world [2].

Myxosporeans infecting the eye of the freshwater teleost fish *Pimelodus maculatus* collected in the Igarac  River (Northeast Brazil) were observed using a light microscopy (LM) and small fragments of the eye were processed for transmission electron microscopy (TEM) and molecular procedures. Phylogenetic analyses were performed in MEGA 6 software, using the Neighbor-Joining method. Kimura 2-parameter as substitution model with gamma distribution (shape parameter = 1.4) was performed.

This parasite produces spherical to whitish ellipsoidal cyst-like structures (C-Ls) up to 220 nm in diameter, located in the anterior camera of eyes under the ocular cornea and in closed contact with the lateral periphery of the iris (Fig. 1a). Each C-Ls is surrounded by a complex network of anastomosed microfibrils. Some free mature spores were observed floating in the aqueous humor. Pyriform myxospores are $18.6 \pm 0.4 \mu\text{m}$ in length, $13.6 \pm 0.6 \mu\text{m}$ width and $4.9 \pm 0.6 \mu\text{m}$ thickness ((Fig. 1b). The anterior end of the spores contain two unequal-sized pyriform polar capsules measuring: (larger) $6.7 \pm 0.6 \mu\text{m}$ length, $3.6 \pm 0.4 \mu\text{m}$ width, and an isofilar polar filament with 5 to 6 turns obliquely to the longitudinal axis; (smaller) $3.8 \pm 0.4 \mu\text{m}$ in length, $2.8 \pm 0.3 \mu\text{m}$ width, and an isofilar polar filament with 3 to 4 turns, obliquely to the longitudinal axis of the polar capsules (Fig. 1c). The binucleated sporoplasm contains numerous spherical sporoplasmosomes having 0.31- 0.35 μm in diameter, constituted by a hyaline ring surrounding a central homogenous matrix. Some glycogen particles, ribosomes and a large vacuole with fine granular matrix are observed in the sporoplasm.

Based on the morphological and ultrastructural characteristics and molecular analysis (Fig. 1d) the present described parasite was compared with previously described myxozoan species it was suggested that this parasite belongs to the genus *Myxobolus* [3].

References:

- [1] E.F. Cellere, N.S. Cordeiro and E.A. Adriano, *Memorias Instituto Oswaldo Cruz* **97** (2002) p. 79.
 [2] J.C. Eiras, J. Zhang and K. Molnar, *Systematic Parasitology* **88** (2014) p. 11.
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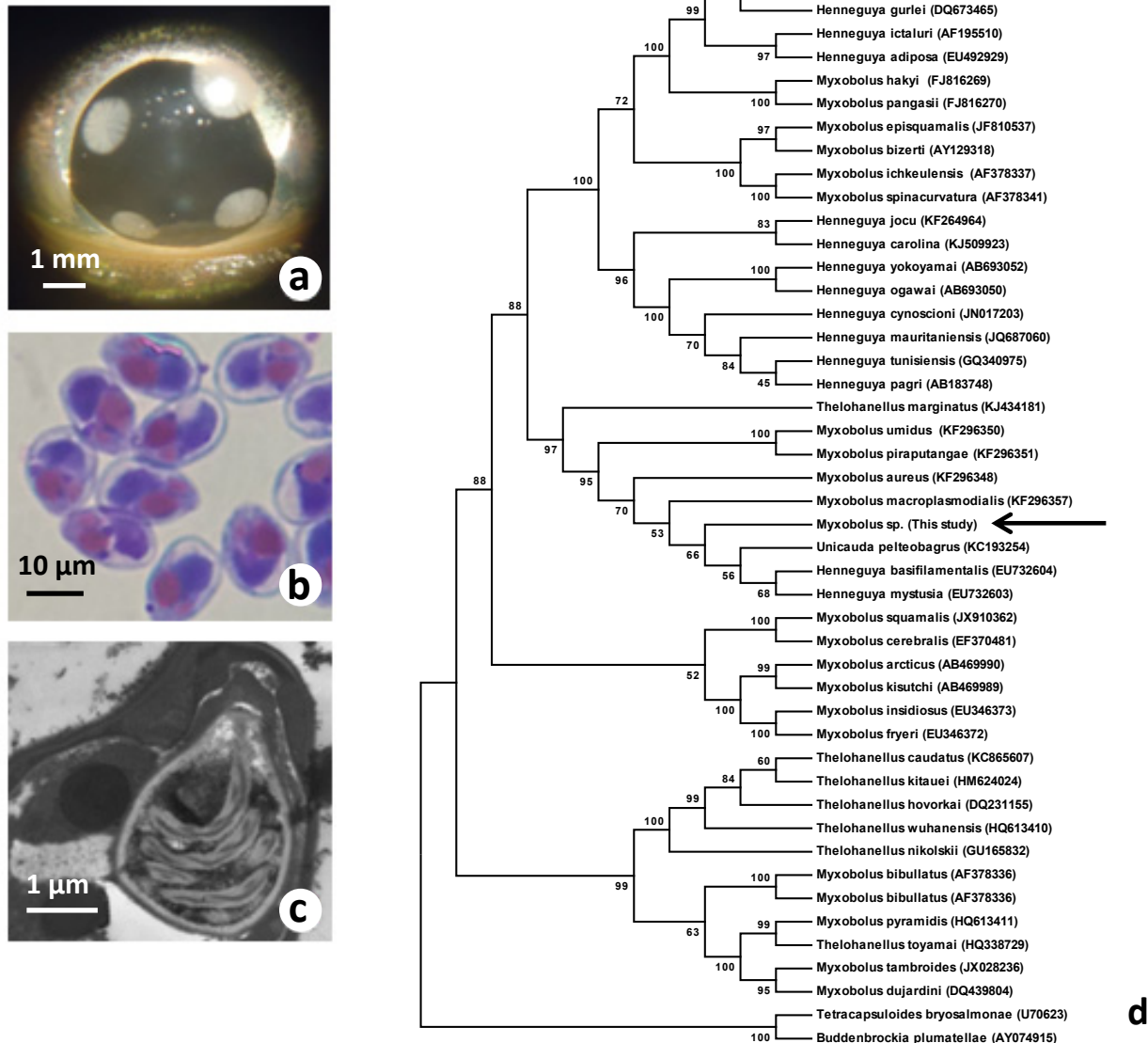


Figure 1. (a) Image of an infected eye showing four cyst-like structures. (b) Some free myxospores colored with H&E obtained from the cysts. (c) Longitudinal section of a polar capsule showing the different polar filament sections. (d) Neighbor-Joining tree for the SSU rRNA sequence of *Myxobolus* sp. and other Myxozoa species. The numbers on the branches are bootstrap confidence levels on 500 replicates.