

## Ultrastructure and phylogeny of *Thelohanellus* sp. (Myxozoa: Myxosporea) infecting the gills of *Hypophthalmus marginatus* (Actinopterygii: Pimelodidae), a fish from the Amazon River

Rocha, S.<sup>\*</sup>, Matos, E.<sup>\*\*</sup>, Velasco, M.<sup>\*\*</sup>, Casal, G.<sup>\*\*\*</sup>, Alves, A.<sup>\*\*\*\*</sup> and Azevedo, C.<sup>\*\*\*\*\*</sup>

<sup>\*</sup> Laboratory of Animal Pathology, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/UP), University of Porto, PORTUGAL.

<sup>\*\*</sup> Carlos Azevedo Research Laboratory, Federal Rural University of Amazonia (UFRA), Belém, BRAZIL.

<sup>\*\*\*</sup> Department of Sciences, High Institute of Health Sciences - North (CESPU), Gandra, PORTUGAL.

<sup>\*\*\*\*</sup> Department of Microscopy, Institute of Biomedical Sciences Abel Salazar (ICBAS/UP), University of Porto, PORTUGAL.

Email : sonia.oliveira.rocha@gmail.com

Myxosporidians (Myxozoa Grassé, 1970) are important microparasites of fishes worldwide. Amongst the family Myxobolidae Thélohan, 1892, the genus *Thelohanellus* Kudo, 1933 represents a small group of generally histozoic and highly host-specific pathogens, which have mainly been described on the basis of light microscopy. Nowadays, the combination of morphological and molecular features constitutes a prerequisite for the description of myxosporidians; a practice that has been allowing the unraveling of taxonomic and phylogenetic trends [1-3]. The study here presented relies on ultrastructural and molecular data to characterize a *Thelohanellus* sp. infecting the gills of a teleost fish from the Amazon River.

Thirty-nine specimens of *Hypophthalmus marginatus* Spix & Agassiz, 1829 were microscopically analyzed. Infected samples were observed using the differential interference contrast (DIC) optics for measurements of myxospores, and prepared for transmission electron microscopy. The SSU rRNA gene was sequenced using both eukaryotic and myxozoan-specific primers. For inferring phylogenetic relationships, the Neighbour-Joining (NJ) method was performed, using Kimura-2 parameters as a substitution model.

Prevalence of infection was estimated at 49%. Whitish cysts formed ellipsoidal masses up to 250 µm in diameter (Fig. 1). Myxospores were pyriform, with rounded anterior and posterior ends, measuring  $17.1 \pm 0.6$  µm in length,  $6.9 \pm 0.4$  µm in width and  $5.1 \pm 0.5$  µm in thickness (Fig. 2). A single ellipsoidal polar capsule,  $9.0 \pm 0.3$  µm long and  $6.1 \pm 0.4$  µm wide, was located at the anterior pole, eccentric to the myxospores' longitudinal axis, and containing a polar filament coiled in 4-5 irregular coils and surrounded by an electron-dense material (Fig. 3). The sporoplasm was located at the myxospores' posterior pole and contained two nuclei surrounded by an irregular dense matrix displaying numerous sporoplasmosomes and several vacuoles. The NJ phylogenetic tree revealed *Thelohanellus* sp. clustering among other myxobolids, namely *Henneguya* and *Myxobolus* (Fig. 4).

The ultrastructural aspects and molecular analysis of the SSU rRNA gene indicate this parasite as a possible new species of *Thelohanellus*, the first of its genus to be described from South America. It is further noticed that the genus *Thelohanellus* is paraphyletic, clustering within the subclades of the speciose *Henneguya-Myxobolus*. However, the scarcity of molecular data concerning *Thelohanellus* species, as well as the paucity of reported species in some clades continues to hamper the implementation of a new taxonomic systematic for these three genera.

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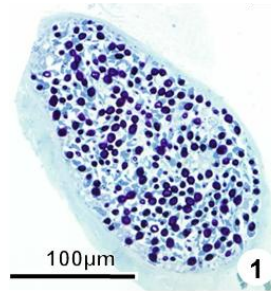
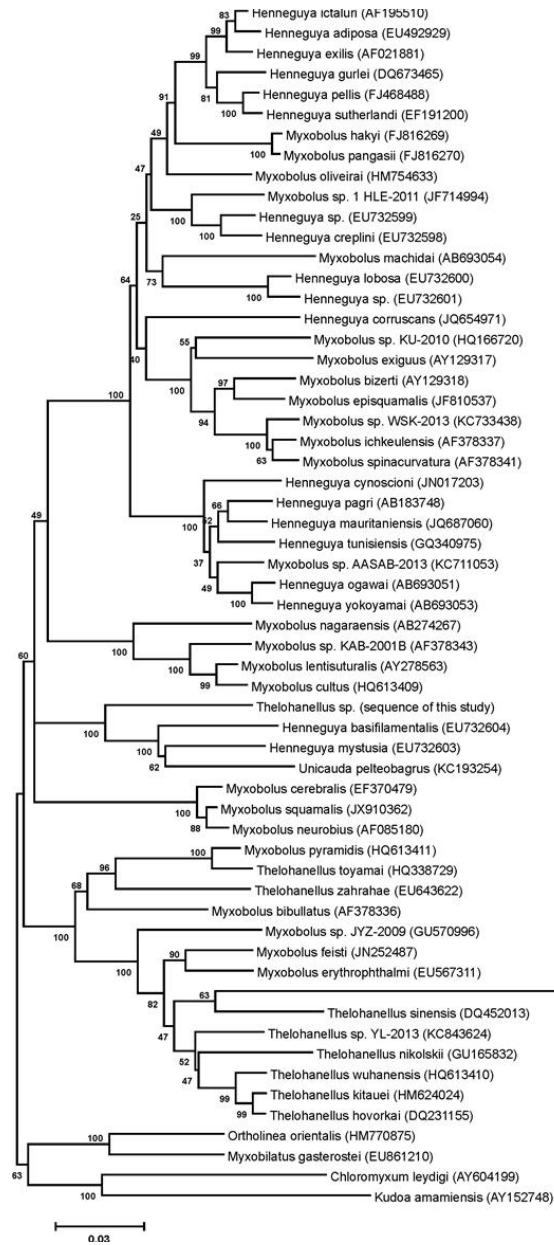


Figure 1. Semithin section of a cyst containing numerous myxospores.

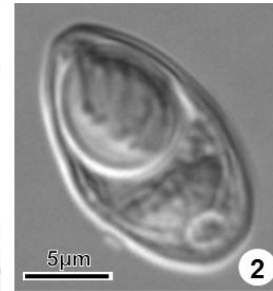


Figure 2. Free fresh mature myxospore observed under DIC optics.

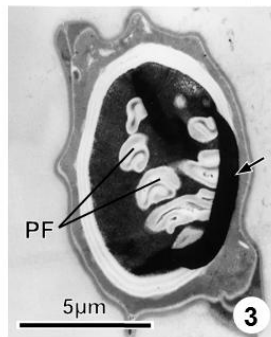


Figure 3. Transmission electron micrograph of a myxospore in transverse section. Notice that the polar capsule contains an electron-dense material (arrow) that surrounds the polar filament (PF).

Figure 4. Neighbor-Joining tree for the SSU rRNA sequence of *Thelohanellus* sp. and other selected myxosporidians. GenBank accession numbers in parentheses after the species name; scale given under the tree.