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Ultrastructural and molecular characterization of a microsporidian infecting *Serranus atricauda* (Teleostei, Serranidae) in the Madeira Archipelago

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Members of the phylum Microsporidia Balbiani, 1882 are obligatory intracellular and unicellular parasites of minute dimensions, which infect many taxonomic groups. Most microsporidians infect insects and fish hosts, often causing diseases in commercially important species [1]. The blacktail comber, *Serranus atricauda* (c. n. "garoupa"), is a species of economic importance. The present study uses morphological, ultrastructural and molecular features to describe a microsporidian parasite that infects the coelomic cavity of this marine teleost fish in the Madeira Archipelago.

Spores were measured using differential interference contrast (DIC) optics. For transmission electron microscopy, fragments of the xenomas were fixed in 3% glutaraldehyde, washed overnight, and post-fixed in 2% osmium tetroxide. After dehydration in an ascending ethanol series ending with propylene oxide, the fragments were embedded in EPON. Semi-thin sections were stained with methylene blue-Azure II and ultrathin sections were contrasted and photographed using a JEOL 100CXII TEM operated at 60 kV. For molecular procedures, two fragments with 900 and 1100 bp, corresponding to the (SSU + ITS + LSU partial) region of the rRNA gene were amplified using specific primers, and then sequenced. The molecular analysis was performed with Mega 5 software, and the Neighbour-Joining (NJ) tree constructed using Kimura-2 parameters as a substitution model [2]. The microsporidian formed large whitish xenomas adhering to the hosts' visceral organs. Each xenoma consisted of a single hypertrophic cell, in the cytoplasm of which mature spores proliferated within parasitophorous vacuoles surrounded by numerous collagen fibers (Figs. 1, 2). Mature spores were ellipsoidal and uninucleate, averaging 6.5 ± 0.5 µm in length and 3.4 ± 0.6 µm in width (Fig. 2). The anchoring disk of the polar filament was subterminal, laterally shifted from the spores' anterior pole. The polaroplast surrounding the uncoiled portion of the polar filament displayed two distinct regions: a lamellar region and an electron-dense globule. The polar filament coiled in 18-19 turns, forming two rows surrounding de posterior vacuole (Fig. 3). The latter occupied about 1/3 of the spore length and contained small irregular granular masses randomly dispersed in the vacuolar matrix. The comparative analysis of the obtained rRNA gene sequence to similar microsporidian sequences determined by BLAST, identify this parasite as a member of the genus *Glugea* (Fig. 4).

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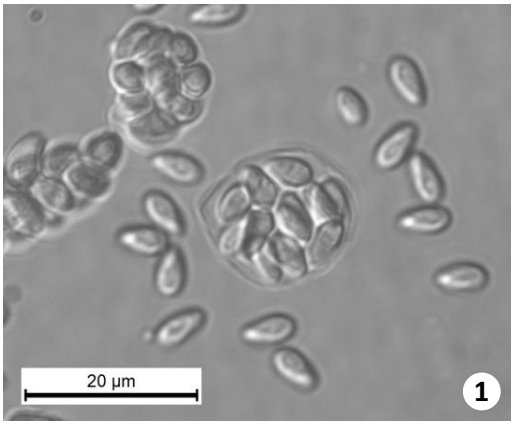

Fig. 1 - DIC optics showing free mature spores and some contained within parasitophorous vacuoles.

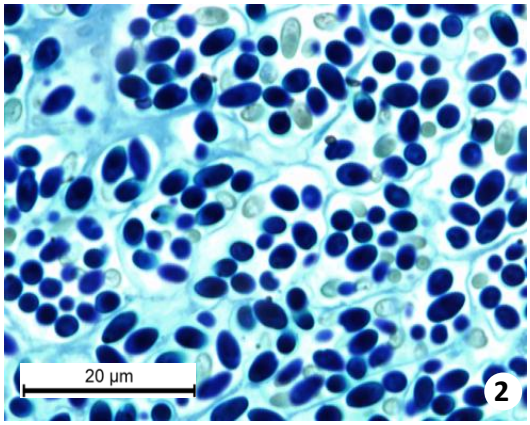

Fig. 2 - Semithin section of a xenoma showing numerous mature spores contained within parasitophorous vacuoles.

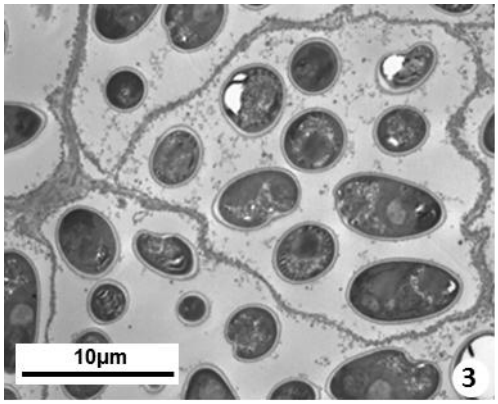

Fig. 3 - Xenoma displaying numerous ma
contained within parasitophorous vacuole

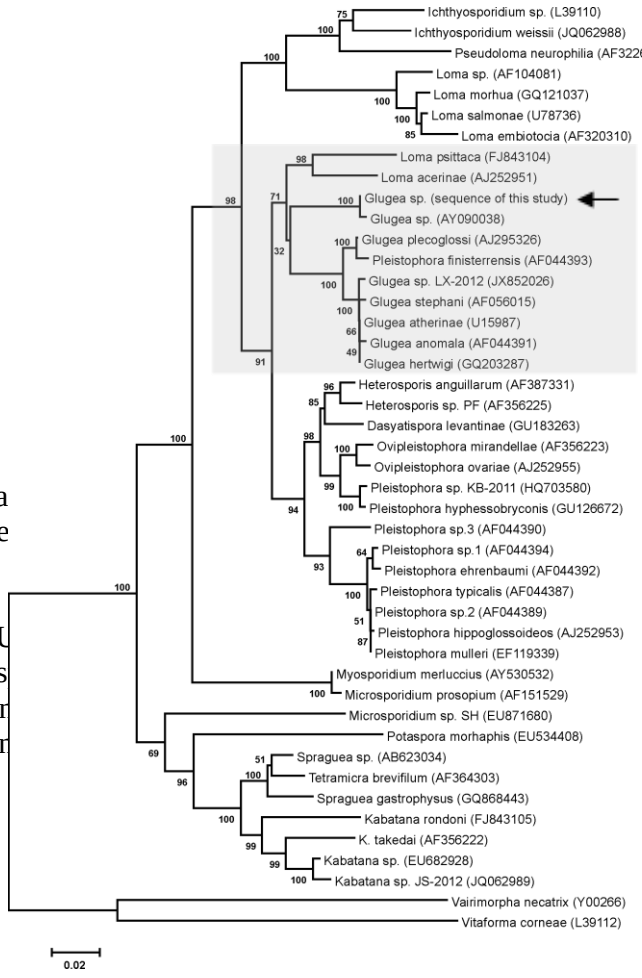

Fig. 4 - Neighbor-Joining tree for the SSU
partial LSU rRNA sequences of *Glugea* s
selected microsporidians species. GenBar
numbers in parentheses after the species r
given under the tree.

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