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Ultrastructural and molecular characterization of Glugea serranus, a microsporidian parasite infecting the blacktail comber

Microsporidians (phylum Microsporidia Balbiani, 1882) are unicellular organisms that develop as obligatory intracellular parasites in different animal hosts, particularly in arthropods and fish. To date, about 187 genera have been described, 21 of which parasitize freshwater and marine fish worldwide, frequently causing diverse pathologies in commercially important species. A microsporidian parasite infecting the peritoneum of the blacktail comber *Serranus atricauda*, a commercially important fish collected from the Madeira Archipelago (Portugal), was recently described on the basis of morphological, ultrastructural, and molecular features. For transmission electron microscopy (TEM), small fragments of infected tissue were fixed in 5% glutaraldeyde buffered with 0.2 M sodium cacodylate, post-fixed in 2% OsO4 in the same buffer, dehydrated in an ascending series of ethanol, and embedded in Epon. Ultrathin sections were observed using a JEOL 100CXII TEM operated at 60 kV. For molecular analysis, isolated spores were preserved in 80% ethanol, and its genomic DNA extracted. The rRNA genes were sequenced using microsporidian-specific primers. Phylogenetic analyses were performed in MEGA 5.05, using maximum likelihood and neighbor-joining methodologies.

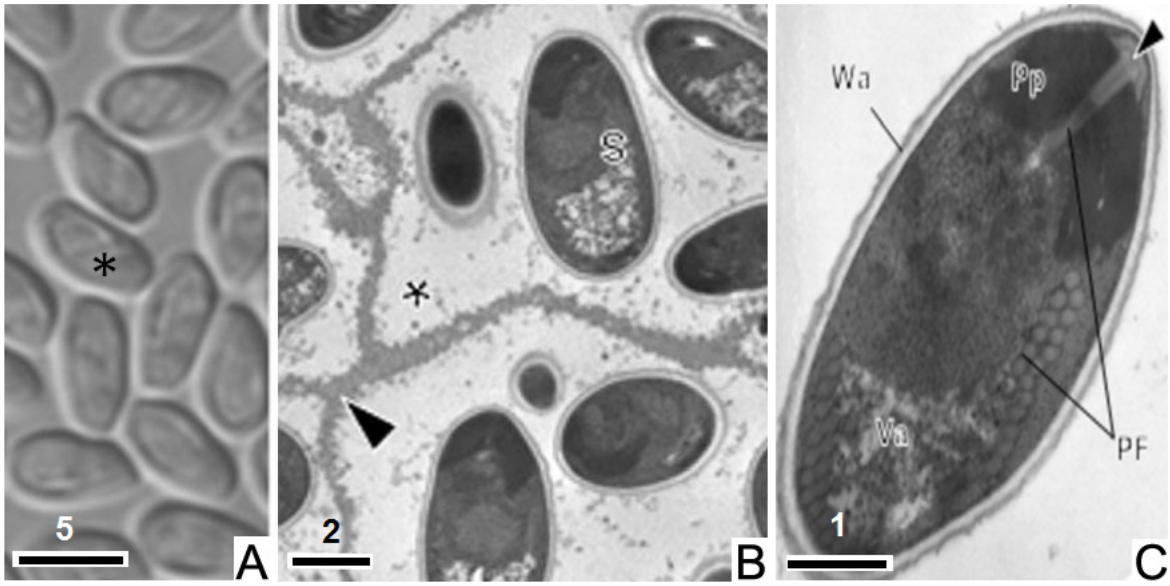

Fig. 1. A.Differential interference contrast micrograph showing several fresh spores (*). B. TEM micrograph depicting the spores (S) developing within parasitophorous vacuoles (*). Notice the wall (arrowhead) formed by numerous collagen fibers. C. Longitudinal section of a spore revealing its ultrastructural organization: Wa – Spore Wall; Pp – Polaroplast; arrowhead – Anchoring Disc; PF – Polar Filament; Vacuole – Va.

The parasite formed large whitish xenomas that adhered to the peritoneum of the host. Each xenoma consisted of a single hypertrophic cell, in the cytoplasm of which mature spores proliferated within parasitophorous vacuoles surrounded by numerous collagen fibers. Mature spores formed by an external wall surrounding an extrusion apparatus and a single nucleus. Spores ellipsoidal, measuring 6.5 ± 0.5 µm in length and 3.4 ± 0.6 µm in width (Fig. 1A). The anchoring disk of the polar filament was subterminal,

laterally shifted from the anterior pole of the spore. The isofilar polar filament coiled in 18–19 turns, forming two rows that surrounded the posterior vacuole. The latter occupied about one third of the spore length. The polaroplast surrounding the apical and uncoiled portion of the polar filament displayed two distinct regions: a lamellar region and an electron-dense globule (Fig. 1B,C). The molecular analysis revealed the parasite clustering alongside several species of the genus *Glugea*, with significant similarity to *G. nagelia*, *G. jazanensis*, *G. arabica*, and *G. epinephelusis*.

Both the morphologic and molecular data acquired identified the parasite as a microsporidian of the genus *Glugea*. Further comparisons of host specificity, habitat, and specific morphological and ultrastructural traits, allowed the erection of a new species, *Glugea serranus*.

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