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Ultrastructural and phylogenetic study of Microsporidia parasites infecting the trunk muscle of two marine fishes from the East Atlantic Ocean water

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Abstract

Background: Microsporidia are small, obligate intracellular parasites with unique cellular and molecular features infecting both invertebrates and vertebrates worldwide. Common in fish and crustaceans, they make sanitary control crucial for public health and the aquaculture industry. Nevertheless, studies focusing on aquatic organisms from Portuguese fauna remain limited [1,2]. **Objective:** This study aimed to expand current knowledge on the diversity of microsporidians infecting commercially important teleost fish species captured along the northern Atlantic coast of Portugal. **Methods:** Specimens of the three-bearded rockling *Gaidropsarus vulgaris* and pout *Tripsopterus luscus* were necropsied and infected tissue was photographed using differential interference contrast microscopy for morphological characterization. Samples were processed for histology, transmission electron microscopy and molecular analyses targeting the 18S and 28S ribosomal ribonucleic acid (rRNA) genes, including the internal transcribed spacer region. Positive polymerase chain reaction products were cloned, sequenced, and analyzed using BLAST in MEGA11 software. Phylogenetic relationships were inferred using Maximum Likelihood and Bayesian Inference methods [3]. **Results:** In both infections, no xenoma formation was observed. Instead, a generalized degradation of trunk muscle myofibrils was evident. The infections were predominantly characterized by late sporogonic stages developing in direct contact with the host cell cytoplasm. The two *Microsporidium* species identified could be distinguished based on spore size and shape, and ultrastructural characteristics, particularly the number of polar tube coils and the patterning of the spore surface. Molecular and phylogenetic analyses revealed that the obtained ribosomal deoxyribonucleic acid (rDNA) sequences showed strong affinity with members of the genera *Microgemma*, *Spraguea*, and *Tetramicra*. **Conclusions:** The obtained rDNA sequences cluster within the Marinosporidia clade, grouping with *Tetramicra brevifilum* (AF364303) and *Microgemma caulleryi* (AY033054). The ultrastructural characteristics observed during the late sporogonic stages, together with tissue tropism, genetic distances among related parasites, and phylogenetic data, indicate that the two *Microsporidium* spp. described herein are closely related. These organisms exhibit a high sequence identity, with 99.7% similarity between them and 99.4% similarity to *Tetramicra brevifilum*.

Keywords: marine fishes; parasites; microsporidia

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