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Microsporidian parasites in four species of carangid fishes from the Senegalese coast (West Africa)

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Hepatic microsporidiosis was observed in four species of carangid fishes from the Senegalese coast. Being unable to positively identify the parasitic species, we provisionally placed them in the collective group Microsporidium *Balbiani, 1984*. *The* Microsporidium *found in* Caranx crysos *and* Caranx senegallus *was labeled sp1, that found in* Selene dorsalis *was called sp2, and that found in* Trachurus trachurus *was called sp3*. *The Microsporidia formed cysts (xenomas) in the hepatic tissues of their hosts.*

Key words: Microsporidia, *Microsporidium,* Pisces, Carangidae, Senegal

INTRODUCTION

The occurrence of microsporidian parasites in carangid fishes from the Senegalese coast was first reported by TOGUEBAYE *et al.* (1989). In their study, they described *Microsporidium chloroscombri* in *Chlorocombrus chrysusrus*. We discovered four additional microsporidia in four distinct host species: *Caranx crysos, C. senegallus, Selene dorsalis,* and *Trachurus trachurus*. In this report, only spores of the microsporidia are described since the vegetative and sporogonic stages were not observed.

MATERIAL AND METHODS

Carangid fishes were randomly collected from the coast of Senegal (West Africa) around Dakar (Soumbedioune, Ouakam, Hann, and Yoff). Seven *C. crysos*, 18 *C. senegallus,* 38 *S. dorsalis,* and 7 *T. trachurus* were dissected and examined. The fish had no external signs of disease.

Samples of infected livers were prepared for light and electron microscopy. Fresh cysts were used to measure live spores under a light microscope. For the ultrastructure study, fresh cysts were fixed at 4°C with 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.2) for 24h and then post-fixed at 4°C with 1% osmium tetroxide in the same buffer for 1 h. After dehydration through a gradual ethanol and propylene oxide series, the cysts were embedded in SPURR resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a JEOL 100 CXII electron microscope. Semi-thin sections were stained with toluidine blue and examined under light microscopy.

For scanning electron microscopy, spores were smeared on circular cover glasses, lightly

air-dried, and fixed in 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C for 12 h. After washing in the buffer and critical point drying, the smears were covered with metallic gold and paladium and observed with a JEOL 35CF scanning electron microscope.

RESULTS

All the Microsporidia formed whitish cysts (xenomas) with a diameter of about 0.3-1 mm in the liver of their hosts.

One of the seven (14.29%) *C. crysos* was infected by Microsporidia. The cysts (xenomas) were bounded by a laminated wall and filled with mature spores (Fig. 1). Fresh spores (Fig. 2) were ovoid and measured 2.64±0.9 x 1.56±0.27 µm. Examined by transmission electron microscopy, mature spores were electron dense and uninucleate (Fig. 3). At the anterior end of the spore, the anchoring disc was central in position. The polar filament was in a single rank of 6-7 coils. The polaroplast consisted of an anterior region of closely packed membranes and a posterior region comprised of a series of more loosely packed membranes. There was a large membrane-bound vacuole containing electron-dense material at the posterior end of the spore.

Six of the 18 (33.33%) *C. senegallus* were infected. The xenomas were limited by a laminated wall and filled with mature spores (Fig. 4). The fresh spores were ovoid, measured 2.83±0.87 x 1.8±0.32 µm, and had a polar filament with 6-7 coils (Fig. 5).

Four of 38 (10.53%) *S. dorsalis* were infected. The cysts were bounded by a laminated wall and filled with mature spores (Fig. 6). The

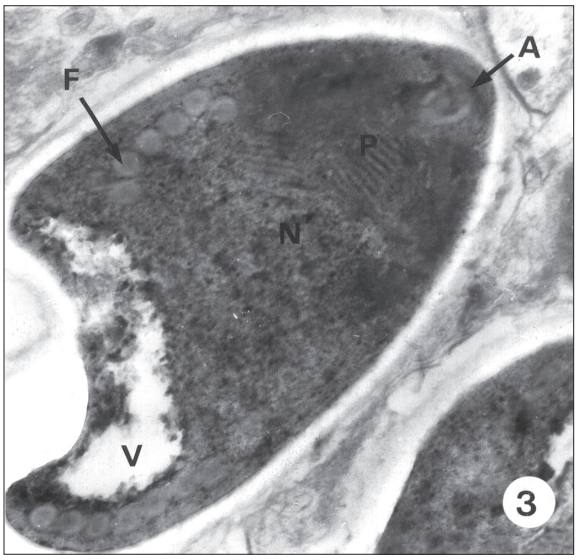
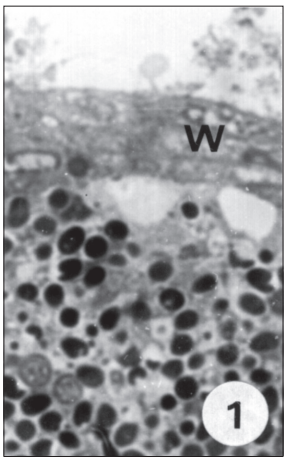


Fig. 3. Ultrastructure of a mature spore of Microsporidium *sp1 in* Caranx crysops *(x 40 000). A = anchoring disc; F = polar filament; P = polaroplast; N = nucleus; V = posterior vacuole*



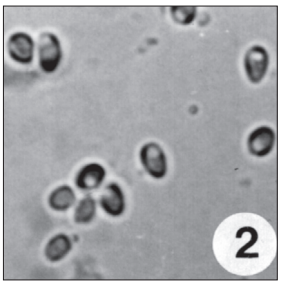


Fig. 2: Fresh spores of Microsporidium *sp1 in* Caranx crysops *(x 2000)*

*Fig. 1. Semithin section of a xenoma (*Microsporidium *sp1) containing mature spores in* Caranx crysops *(x 2000).*

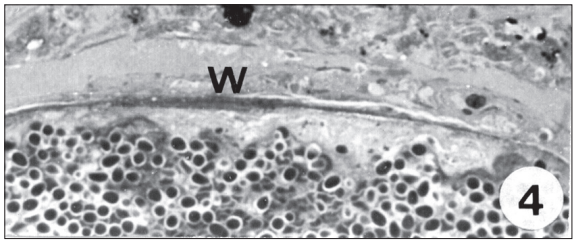


Fig. 4. Semithin section of the xenoma (Microsporidium *sp1*) *in* Caranx senegallus *(x 1000). W = wall*

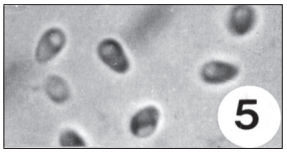


Fig. 5. Fresh spores of Microsporidium *sp1 in* Caranx senegallus *(x 2000)*

fresh spores were ovoid and measured 3.6±0.65 x 2.1±0.43 µm (Fig. 7). The surface of the spores appeared spongy under scanning electron microscopy (Fig. 8). The spores appeared uninucleate in transmission electron microscopy (Fig. 9). The anchoring disc was eccentric in position and the polar filament had 7-8 coils arranged in two layers. The polaroplast occupied approximately one-third of the spore volume and had two lamellar parts with narrow and closely packed lamellae in the anterior and wider less regularly arranged lamellae in the posterior sections.

One of the seven (14.29%) *T. trachurus* was infected. The xenomas were limited by a large laminated and filled with mature spores

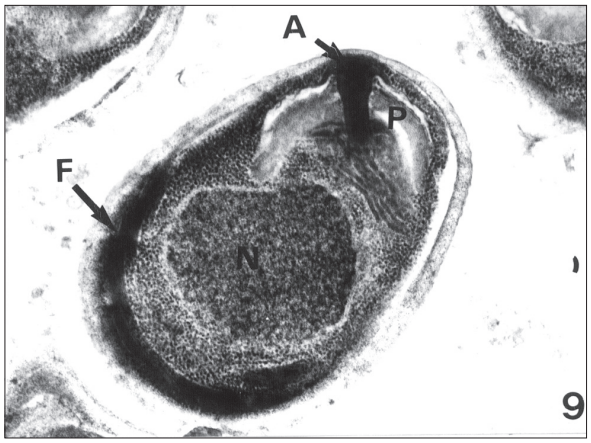


Fig. 9. Ultrastructure of a mature spore of Microsporidium *sp2 in* Selene dorsalis *(x 28 000). A = anchoring disc; F = polar filament; P = polaroplast; N = nucleus*

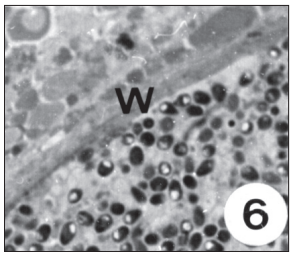


Fig. 6. Semithin section of the xenoma (Microsporidium *sp2*) *in* Selene dorsalis *(x 1000). W = wall*

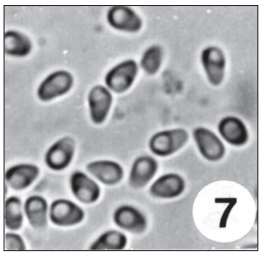


Fig. 7: Fresh spores of Microsporidium *sp2 in* Selene dorsalis *(x 2000)*

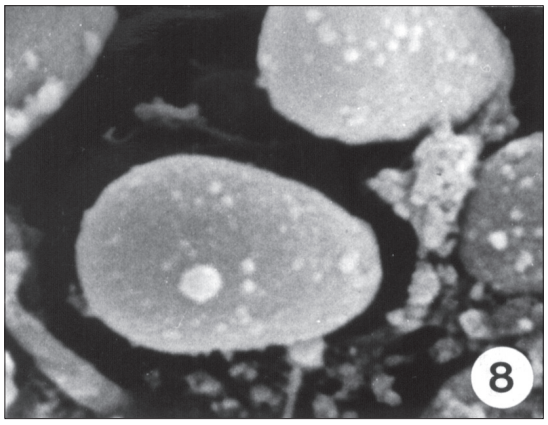


Fig. 8. Mature spore of Microsporidium *sp2 in* Selene dorsalis *seen through scanning electron microscope (x 11 000)*

(Fig. 10). The cyst wall had an external layer composed of elongated connective cells and an inner acellular layer composed of amorphous and electron dense material, seen in transmission electron microscopy (Fig. 11) Fresh mature spores (Fig. 12) were ovoid and measured 3.0±0.38 x 2.1±0.26 µm. The spore surface appeared spongy under scanning electron microscopy (Fig.13).

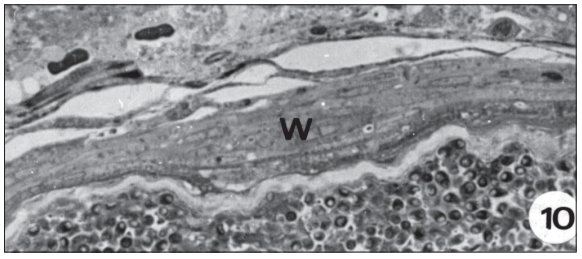


Fig. 10. Semithin section of the xenoma (Microsporidium *sp3*) *in* Trachurus trachurus *(x 1200). W = wall*

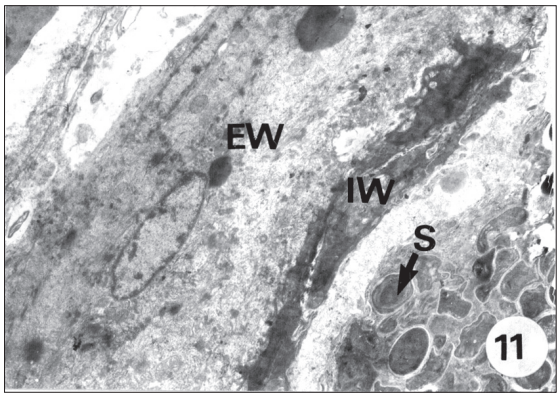


Fig. 11. Ultrastructure of a xenoma (Microsporidium *sp3*) *in* Trachurus trachurus *containing mature spores (x 4000). S = spores; EW = external layer of the wall. IW = inner layer of the wall*

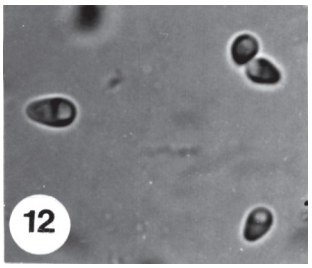


Fig. 12. Fresh spores of Microsporidium *sp3 in* Trachurus trachurus *(x 2200)*

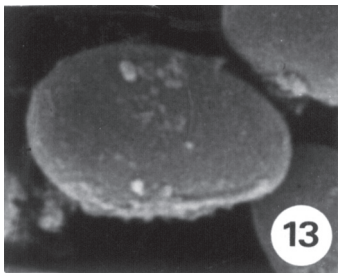


Fig. 13. Mature spore of Microsporidium *sp3 in* Trachurus trachurus*, seen in scanning electron microscope (x 12 000)*

DISCUSSION

Currently, microsporidia parasites of fish include the collective group *Microsporidium* Balbiani, 1884, and 17 genera: *Amazonspora* Azevedo and Matos, 2003; *Glugea* Thélohan, 1891; *Heterosporis* Schubert, 1969; *Ichthyosporidium* Caullery and Mesnil, 1905; *Kabatana* Lom, Dyková and Tonguthai, 2000; *Loma* Morrison and Sprague, 1981; *Microfilum* Faye, Toguebaye and Bouix, 1991; *Microgemma* Ralphs and Matthews, 1986; *Neonosemoides* Faye, Toguebaye and Bouix, 1996; *Nosemoides* Vinckier, 1975; *Nucleospora* Hedrick, Groff and Baxa, 1991; *Ovipleistophora* Pekkarinen, Lom and Nilsen, 2002; *Pleistophiora* Gurley, 1893; *Pseudoloma* Matthews, Brown, Larison, Bishop-Stewart, Rogers and Kent, 2001; *Spraguea* Sprague and Vávra, 1976; *Tetramicra* Matthews and Matthews, 1980 (CANNING & LOM, 1986; RALPHS & MATTHEWS, 1986; FAYE *et al*., 1991, 1996; HEDRICK *et al*., 1991; CANNING & VAVRA, 2000; LOM *et al*., 2000, MATTHEWS *et al*., 2001; PEKKARINEN *et al*., 2002; AZEVEDO & MATOS, 2003; LOM & NILSEN, 2003). CANNING & LOM (1986) listed two additional genera: *Thelohania* Henneguy, 1892 and *Mrazekia* Léger and Hesse, 1916. However, according to LOM & NILSEN (2003), the single finding of *Mrazekia* in fish was probably accidental and the report of a *Thelohania* species in fish must be reinvestigated since this genus is typical of arthropods.

The previously described microsporidium from carangid fishes was *Microsporidium chlotroscombri* Toguebaye, Marchand and Faye, 1989, found in the liver of *Chloroscombrus chrysurus*. This species differs from the species described in our report because its spores are pyriform, measure 3.4 x 1.6 µm, have a lamellar and vesicular polaroplast, and are contained within vacuoles and cysts (TOGUEBAYE *et al.* 1989).

In the present study, no developmental stages were observed and, for technical reasons, we were unable to analyze SSU rDNA to compare the sequence of our microsporidia with already sequenced species. Therefore, we were unable to identify the microsporidia species and assigned them to the collective group, *Microsporidium*. Similar morphology and ultrastructure of the mature spores indicate that the same species infected *C. crysos* and *C. senegallus*; their spores had the same shape and size and their hosts belong to the same genus. The species found in *S. dorsalis* and *Tc .trachurus* were distinct and differed from the species found in *C. crysos* and *C. senegallus* in spore size and ultrastructure.

Because of differences between the microsporidia and the unknown pre-spore stages, we propose provisionally designating these species *Microsporidium* sp1 for the species found in *C. crysos* and *C. senegallus*, *Microsporidium* sp2 for the species found in *Selene dorsalis,* and *Microsporidium* sp3 for the species found in *T. trachurus*.

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Pojava parazitskih mirkrosporidija (Microsporidia) na četiri vrste riba iz porodice Carangidae u obalnom području Senegala

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SAŽETAK

U obalnim vodama Senegala zabilježena je na 4 vrste riba mikrosporidioza jetre. Privremeno su parazitske vrste svrstane u zajedničku skupinu, *Microsporidium* Balbiani, 1984. *Microsporidium* u ribama *Caranx crysos* i *C. senegallus* nazvan je sp1, u ribi *Selene dorsalis*, sp2, a *Microsporidium* u ribi *Trachurus trachurus* predstavlja sp3. Microsporidia stvaraju ciste u jetrenim tkivima svojih domaćina.

Ključne riječi: Microsporidia, *Microsporidium*, Pisces, Carangidae, Senegal