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Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporidia) and extrusion of the polar tube during autoinfection

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ABSTRACT: Xenomas of the recently described new microsporidian species *Loma myrophis* parasitizing the gut tissue of the Amazonian fish *Myrophis platyrhynchus* (family Ophichthidae) were described by light- and transmission-electron microscopy. The xenoma consisted of a thin fibrillar wall that surrounded a hypertrophic host cell cytoplasm containing numerous microsporidian developmental stages and spores. Several spores showed different stages of natural extrusion of the polar tube. Numerous longitudinal and transverse sections of the extruded polar tubes were observed in developing life-cycle stages (spores excepted), the nucleus of hypertrophic host cell, the xenoma wall and surrounding fibroblasts. The extruded polar tubes were projected in all directions with no preferential orientation. These aspects suggested that autoinfection occurred within this xenoma.

KEY WORDS: Ultrastructure · Microsporidian · Xenoma · Extrusion · Polar tube · *Loma myrophis* · Parasite

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INTRODUCTION

A common reaction of hosts to invasion by a microsporidia is their laying down of membranes and cells around the parasite xenoma. This term, introduced by Weissenberg (1949), characterizes a wall of host origin surrounding the dividing parasite in the original parasitized host cell, which becomes hypertrophic with a hypertrophic nucleus. In the cytoplasm of the hypertrophic host cell (HHC), the parasite divides repeatedly, producing an enlarged xenoma containing numerous spores and other life-cycle stages (Morrison & Sprague 1981, Lom & Pekkarinen 1999). In a recent paper by Lom (2002), a complete list of microsporidians parasitic in fish was published. In this work, 14 genera and 80 named microsporidian species were catalogued. There are numerous microsporidian species, but only a few cause xenomas in fish (Dyková & Lom 1978, Lom & Pekkarinen 1999, Azevedo & Matos 2002). In addition, very few cases of autoinfection involving the extrusion of polar tubes have been observed, with extruded tubes reported only once within fish xenomas (Lom & Pekkarinen 1999). One of the first observations of sporoplasm extrusion in microsporidia was published by Lom & Vávra (1963). Later, some ultrastructural studies of the microsporidian discharges contributed to the understanding of this complex process (see Canning et al. 1992, Magaud et al. 1997, Cali & Takvorian 1999, Keohane & Weiss 1999, Shaw & Kent 1999). Some microsporidia are induced to germinate as a result of experimental environmental changes (Weidner 1972, Scarborough-Bull & Weidner 1985, Undeen & Avery 1988, Undeen & Epsky 1990). Most studies indicate that the initial activation requirements are variable, and depend mainly on the environmental changes necessary to release the triggering mechanism for extrusion of the polar tube (Undeen 1990, Undeen & Epsky 1990, Undeen & Frixioni 1990).

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In this paper, we describe both light and electron microscope observations of a microsporidian xenoma, with special reference to the ultrastructural aspects of the natural extrusion of the polar tube, suggestive of causing autoinfection.

MATERIALS AND METHODS

Specimens of the freshwater fish *Myrophis platyrhynchus* Breder, 1927 (family Ophichthidae) (Brazilian common name: Cutuca) were collected in the Amazon River (00° 35′ 38″ S, 47° 35′ 00″ W), near the city of Belém, Brazil. The internal organs were examined to detect the parasites, which appeared as a whitish xenoma in the subepithelial tissue of the midgut, containing the recently described microsporidian species *Loma myrophis* Azevedo and Matos, 2002 (family Glugeidae). For transmission electron microscopy (TEM), the xenoma and surrounding cells were fixed in 5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 10 h at 4°C, washed overnight in the same buffer at 4°C, and post-fixed in 2% OsO_4, buffered with the same buffer and at the same temperature for 8 h. After dehydration in an ethanol series (5 to 6 h in each change) and propylene oxide, the xenomas were embedded in Epon. For light microscopy (LM), semithin sections were stained with methylene blue azure II. For TEM, the ultrathin sections were contrasted with both aqueous uranyl acetate and lead citrate, and observed in a JEOL 100CXII at TEM operated at 60 kV.

RESULTS

Light microscope studies

The xenomas, small whitish nodules located in the connective tissues of the intestine, were observed after dissection. They were broadly spherical and well delimited by the xenoma wall (Fig. 1). After disruption, the spores contained within the xenomas were identified as belonging to the microsporidian species *Loma myrophis.* No overt behavioral or external morphological signs or symptoms of disease were observed in the infected fish.

Electron microscope studies

The wall was externally surrounded by several concentric layers of compressed cells (possibly fibroblasts), some with evident signs of lysis. Among these cells, some groups of collagen fibers were observed. Internally, the xenoma matrix contained numerous microsporidian spores (Fig. 2).

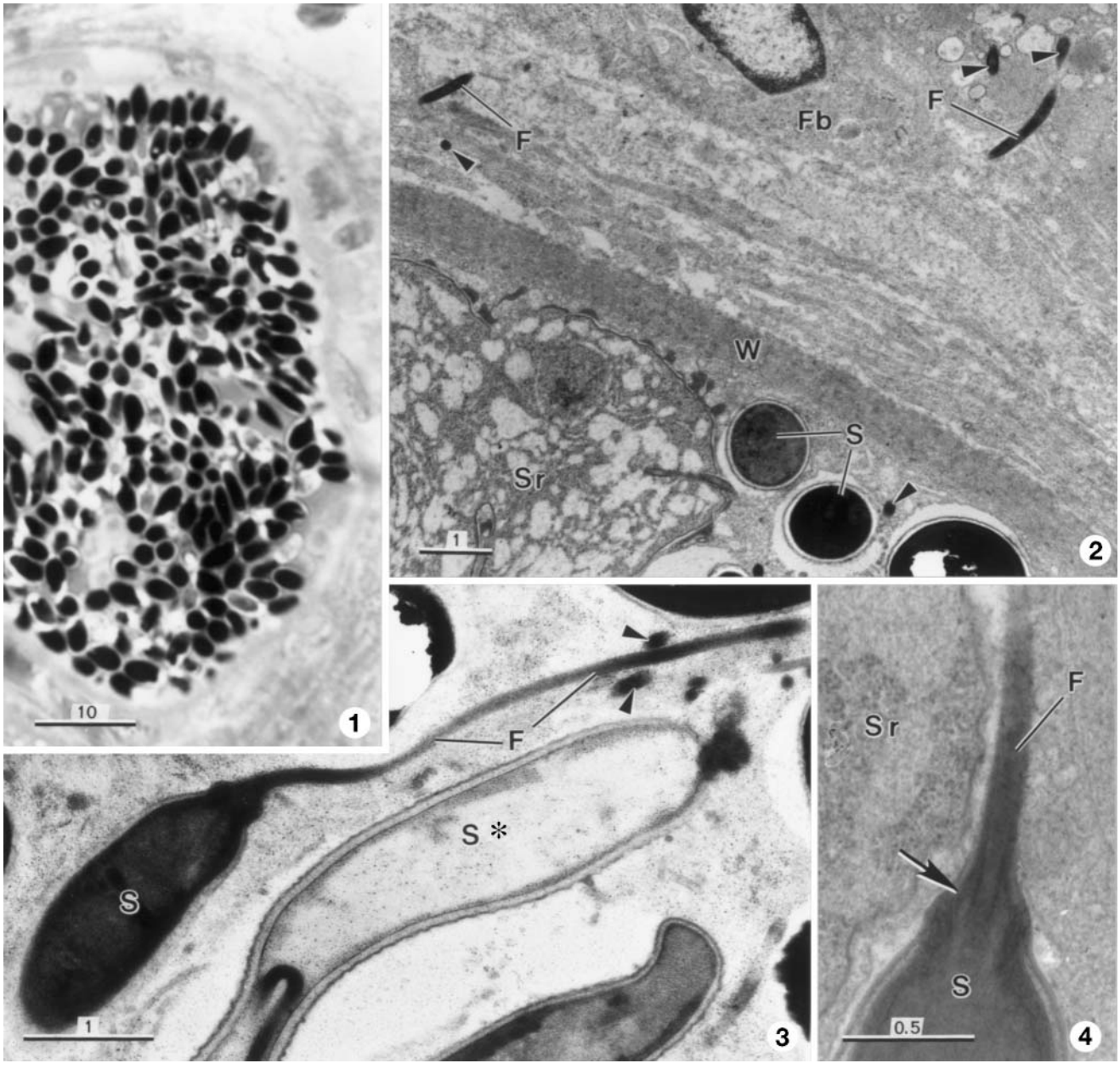
The xenoma (up to 175 µm in diameter) was bound by a thin wall of an amorphous, finely granular layer, 2 to 3 µm thick. Some extruded polar tubes (EPT) that had penetrated the xenoma wall were observed up to 10 µm away from the xenoma wall (Fig. 2). The xenoma contained different life-cycle stages and spores of the microsporidia. As the xenoma enlarged, the wall became thicker. The HHC contained a central hypertrophic branched host nucleus (HHN) located among the spores and other life-cycle stages. Among the numerous spores and other life-cycle stages, several spores with attached EPT were observed (Figs. 3 & 4). Some empty spores that had extruded their contents were also seen (Fig. 3). In addition, numerous longitudinal and transverse sections of the EPT were located within the HHN (Figs. 5 to 7). The polar tube (PT) within the spores measured 0.11 ± 0.01 µm (n = 30) in transverse sections, while the EPT measured 0.25 ± 0.08 µm (n = 25) (Figs. 8 & 9). At high magnification, the ultrastructural organization of the unextruded PT and EPT seemed similar. They were characterized by 3 circular and concentric membranous layers surrounding the lumen of PT (Fig. 9). EPT passing through the spores were never observed.

DISCUSSION

In fish, a type of host-parasite relationship involving microsporidia is often characterized by the production of a xenoma (Canning 1976, Weissenberg 1976). This complex structure is defined by the formation of a HHC containing intracellular microsporidian parasites and a HBN (Weissenberg 1949, 1968, 1976, Canning 1976, Dyková & Lom 1978). The features we observed in the xenoma of the microsporidian *Loma myrophis* (Azevedo & Matos 2002) are similar to those published in studies on other fish species (Morrison & Sprague 1981, Lom & Pekkarinen 1999).

In our observations, the presence of different sizes of xenoma, of which only the biggest with mature spores had numerous EPT, suggests that autoinfection may occur within the HHC (Morrison & Sprague 1981, Lom & Pekkaninen 1999).

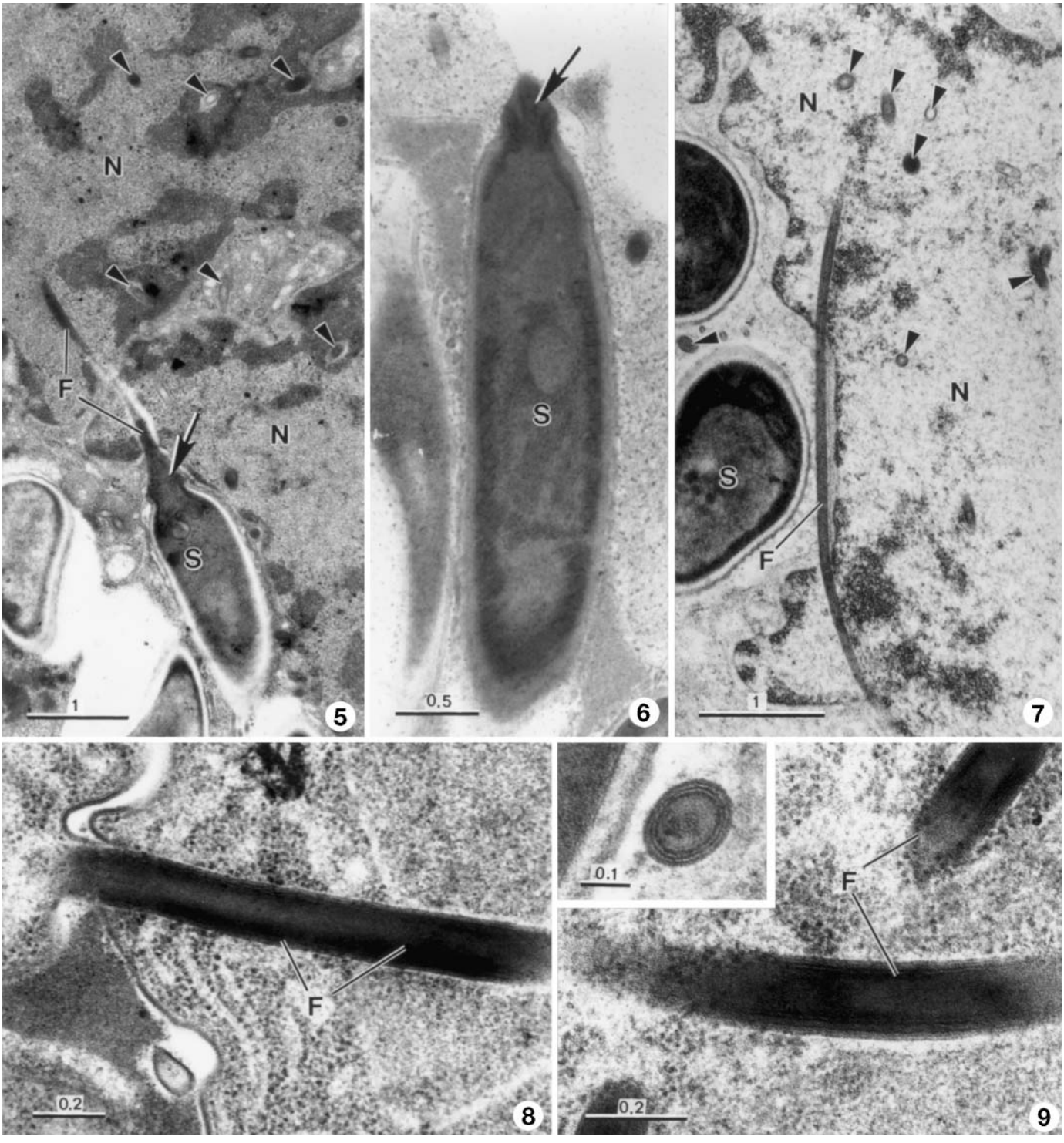
Furthermore, the presence of all developmental life-cycle stages of the microsporidia among the mature spores with no preferential position is another element that concurs with the presumption of autoinfection. Nevertheless, the presence of some EPT that have penetrated the xenoma wall and entered the surrounding host cells may signify that autoinfection is also able to occur outside the xenoma, in the surrounding host cells, possibly thereby causing the formation of other



Figs. 1 to 4. *Loma myrophis*. Light and electron micrographs of the polar tube extrusion of the microsporidian. Fig. 1. Semithin section of part of a xenoma filled mainly with spores. Fig. 2. Ultrathin section of the periphery of a xenoma showing a sporont (Sr), spores (S), xenoma wall (W) and surrounding host tissues, with an evident fibroblast (Fb). Among these structures some transverse (arrow heads) and longitudinal sections of the extruded polar tube (EPT) (F) can be observed. Fig. 3. Ultrathin section of a late xenoma, showing a spore (S) with the EPT (F), near an empty spore (S *). In the surrounding host cytoplasm some transverse sections of the extruded tubes (arrow heads) are present. Fig. 4. A longitudinal ultrathin section of a spore (S), located near a sporont (Sr), showing details of the apical region of the spore (arrow) and the continuity of the extruded tube (F). All scale bars in µm

xenomas. In our observations, we noticed that the unextruded PT and EPT showed a great difference in diameter. We think that the EPT have a diameter larger than the unextruded PT because the extrasporal osmotic pressure is lower than the intrasporal one. This complex process was demonstrated experimentally with similar results (for review see Keohane & Weiss 1999).

Our results suggest that the EPT pierce through any of the microsporidian life-cycle stages (spores excepted), as well as into the xenoma wall, surrounding in their path the host tissues, as it occurs with other



Figs. 5 to 9. *Loma myrophis.* Ultrathin sections of the different aspects of the polar tube extrusion of the microsporidian. Fig. 5. Ultrathin section of a spore (S) with the extruded polar tube (EPT) (F) penetrating into the hypertrophic host nucleus (N). Several oblique and transverse sections (arrow heads) are observed within the nucleoplasm. Fig. 6. Ultrastructural detail of a spore (S) showing the beginning of the extrusion process (arrow). Fig. 7. Ultrathin section of a peripheral part of a hypertrophic nucleus (N) and surrounding cytoplasm contaning 2 spores (S). Some transverse and oblique sections of the EPT (arrow heads) and a longitudinal section of an EPT (F) passing sucessively through the nucleus–cytoplasm–nucleus. Figs. 8 & 9. Ultrastructural details of EPT (F), showing a longitudinal section and another oblique section passing through the sporoblasts. Inset. Detail of an EPT transversally sectioned. All scale bars in µm

different microsporidian species (Lom & Pekkarinen 1999).

The EPT observed in this autoinfection process is composed of concentric layers of different electron densities described in similar conditions (Lom 1972, Weidner 1972, Canning et al. 1992, Chioralia et al. 1998).

The occurrence of all microsporidian life-cycle stages found simultaneously in the HHC seems to be the result of autoinfection.

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