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Fine structure of a new species, *Loma myrophis* (Phylum Microsporidia), parasite of the Amazonian fish *Myrophis platyrhynchus* (Teleostei, Ophichthidae)

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A new species of a microsporidian, *Loma myrophis* n. sp., was found in the sub-epithelial gut tissue of the Amazonian teleost fish, *Myrophis platyrhynchus* (fam. Ophichthidae), forming small whitish xenomas. Each xenoma consisted externally of a thick wall formed by one layer of fibrous material surrounded by aggregate and concentric fibroblasts. Inside, there was a hypertrophic host cell with a hypertrophic branched nucleus surrounded by a hypertrophic cytoplasm containing intermingled life cycle stages, mainly mature spores. Among these cells several extruded polar filaments were observed. Sporonts were surrounded by numerous blisters with dense contents, which appeared to discharge their contents into the parasitophorous vacuole around the parasite. All spores were ellipsoidal and uninucleate, and measured about 3.45 × 1.71 µm (n = 50). The polar filament was isofilar and consisted of a single coil with 13–14 turns, surrounding the posterior vacuole that occupied about half of the total volume of the spore.
The xenoma, formation of the parasitophorous vacuole and the morphology of the spores were basically like those of the genus *Loma*. In this paper, we describe light and electron microscopical data of the xenoma, life cycle and the spores of a new microsporidian species, *Loma myrophis*.

Key words: Ultrastructure; Microsporidian; *Loma myrophis* n. sp.; Parasite; Fish; *Myrophis platyrhynchus*.

Introduction

The members of the phylum Microsporidia (Sprague and Becnel 1998) are strictly intracellular parasites infecting some invertebrate groups and all vertebrate classes (Canning and Lom 1986; Lom and Dyková 1992; Sprague et al. 1992; Larsson 1999). Numerous microsporidian species parasitizing fish have been assigned to the genera *Glugea*, *Pleistophora*, *Ichthyosporidium*, *Heterosporis*, *Nosemoides*, *Spraguea*, *Tetramicra*, *Loma*, *Microgemma*, *Microfilum*, *Nucleospora* (Sprague et al. 1992; Lom and Dyková 1992; Larsson 1999), *Neonosemoides* (Faye et al. 1996) and *Pseudoloma* (Matthews et al. 2001). Among the numerous microsporidian species described in fish, only some produce xenomas as a result of the host tissue reaction (Weidner 1976; Weissenberg 1976; Morrison and Sprague 1981a, 1981b; Canning et al. 1982; Takvorian and Cali 1986; Bekhti and Bouix 1985; Canning and Lom 1986; Cali and Takvorian 1999; Lom and Pekkarinen 1999; Matthews et al. 2001).

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The present light and ultrastructural studies describe some aspects of xenomas containing developmental stages and spore maturation within parasitophorous vacuoles of a new species of a microsporidian, *Loma myrophis,* of which the morphological characteristics and the taxonomic position are discussed comparatively.

Materials and Methods

Specimens of the freshwater fish *Myrophis platyrhynchus* Breder, 1927 (Teleostei, Ophichthidae) (Brazilian common name "Cutuca") were collected in the Amazon River estuary (00°35′38″ S, 47°35′00 W), near Belém (Brazil). The xenomas containing parasites were macroscopically observed as small whitish nodules in the sub-epithelial tissues of the fish gut.

For light microscopy (LM), small fragments of infected tissues and smear preparations were examined using differential interference contrast microscopy (Nomarski). For transmission electron microscopy (TEM), small fragments of the infected tissue containing the xenomas were fixed in 5% glutaraldehyde buffered with 0.2M sodium cacodylate, pH 7.2, for 10 hrs at 4 °C, washed overnight at 4 °C in the same buffer and post-fixed in buffered 2% OsO_4 for 8 hrs at the same temperature. After dehydration in a graded ethanol series, the fragments were transferred to propylene oxide and embedded in Epon. For LM, semithin sections were stained with methylene blue–azur II. For TEM, ultrathin sections were double contrasted with uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM, operated at 60Kv.

Results

Small whitish nodules (xenomas) macroscopically observed in the sub-epithelial gut tissue of the teleost fish, *Myrophis platyrhynchus*, appeared as well-defined cysts, which occurred throughout the gut.

Light microscopy

The xenomas seen in semithin sections were spherical and measured up to 160 µm (Fig. 1). At high magnification, it was observed that the xenomas were formed by a wall encircling a hypertrophic cell with a central hypertrophic nucleus surrounded by numerous spores in contact with the cytoplasm of the hypertrophic host cell (Fig. 2). After dissection and rupture of the xenomas, it was observed that they had numerous ellipsoidal spores (some hundred) identified as belonging to the phylum Microsporidia (Fig. 2, inset).

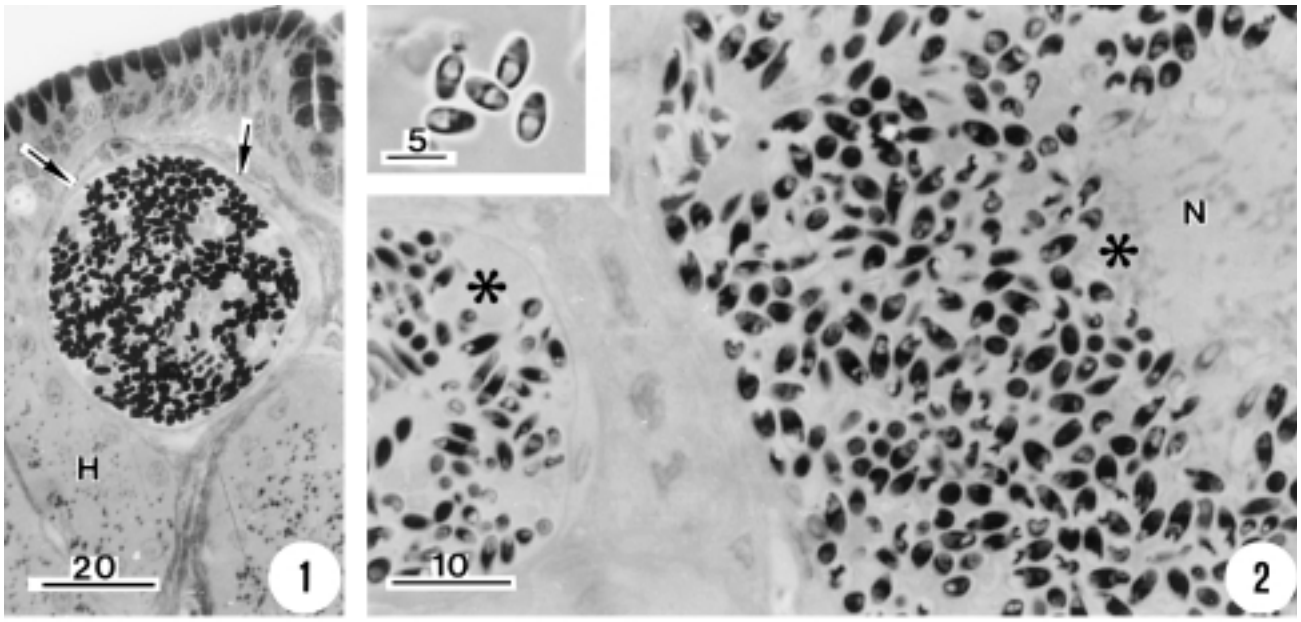
Transmission electron microscopy

The spherical xenoma was formed by a thin wall constituted by a narrow layer of a fibrous or granular substance in contact externally with some layers of compressed and concentric fibroblasts (Figs. 3, 4). In some sections it was possible to observe some collagen fibrils among the fibroblasts (Fig. 3). Different life cycle stages of the microsporidian were observed intermingled among the spores in the matrix of the xenoma (Figs. 3, 4).

Sporonts. The earliest stages proliferated by binary division (Fig. 5) into sporoblast mother cells. Early sporonts and dividing sporonts were bounded by a layer of electron dense material outside the plasmalemma and were in contact with the cytoplasm of the host cell. There was no evident associated cisterna of endoplasmic reticulum in the cytoplasm of the host cell. The sporont nuclei contained little condensed chromatin and several vacuoles were found in the granular cytoplasm (Figs. 4, 5).

Late sporonts were ultrastructurally characterized by the appearance of an incomplete coat of amorphous electron-dense material, external to the plasmalemma (Fig. 6). Several extracellular blisters

Figs. 1–4. Light and electron micrographs of *Loma myrophis* n. sp. from the teleost fish *Myrophis platyrhynchus*. 1. Semithin section showing a xenoma, with numerous spores, in the sub-epithelial gut tissues (H). The xenoma is limited by a wall (arrows). 2. Semithin section of two xenomas (*) showing the hypertrophic nucleus (N) surrounded by numerous spores located inside the cytoplasm of the host cell. Inset: Some isolated living spores observed by differential interference contrast microscopy. 3. Ultrathin section of the periphery of a xenoma showing the wall (W) formed by a layer of fibrillar material surrounding numerous mature spores (S) and sporonts (*). An extruded polar filament (arrow) seems to pass through the sporont wall. Externally, in close contact with the wall, are some surrounding fibroblasts (F) and among them some collagen fibrils (C) are observed. 4. Ultrathin section of the periphery of a xenoma showing the wall (W), an early sporont (SR) and some mature spores (S), each one inside a parasitophorous vacuole (*). Externally, a fibroblast (F) and collagen fibrils are present.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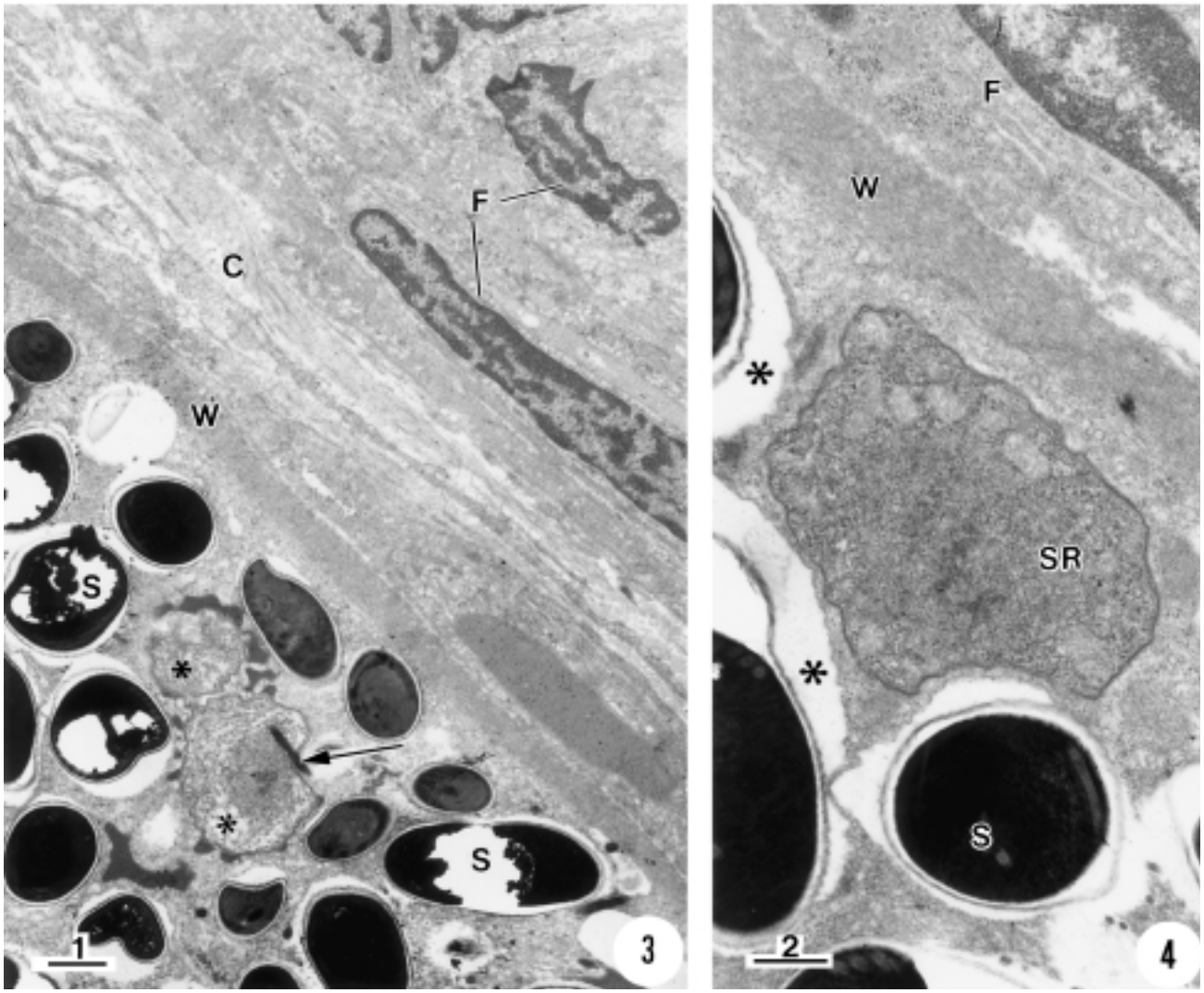
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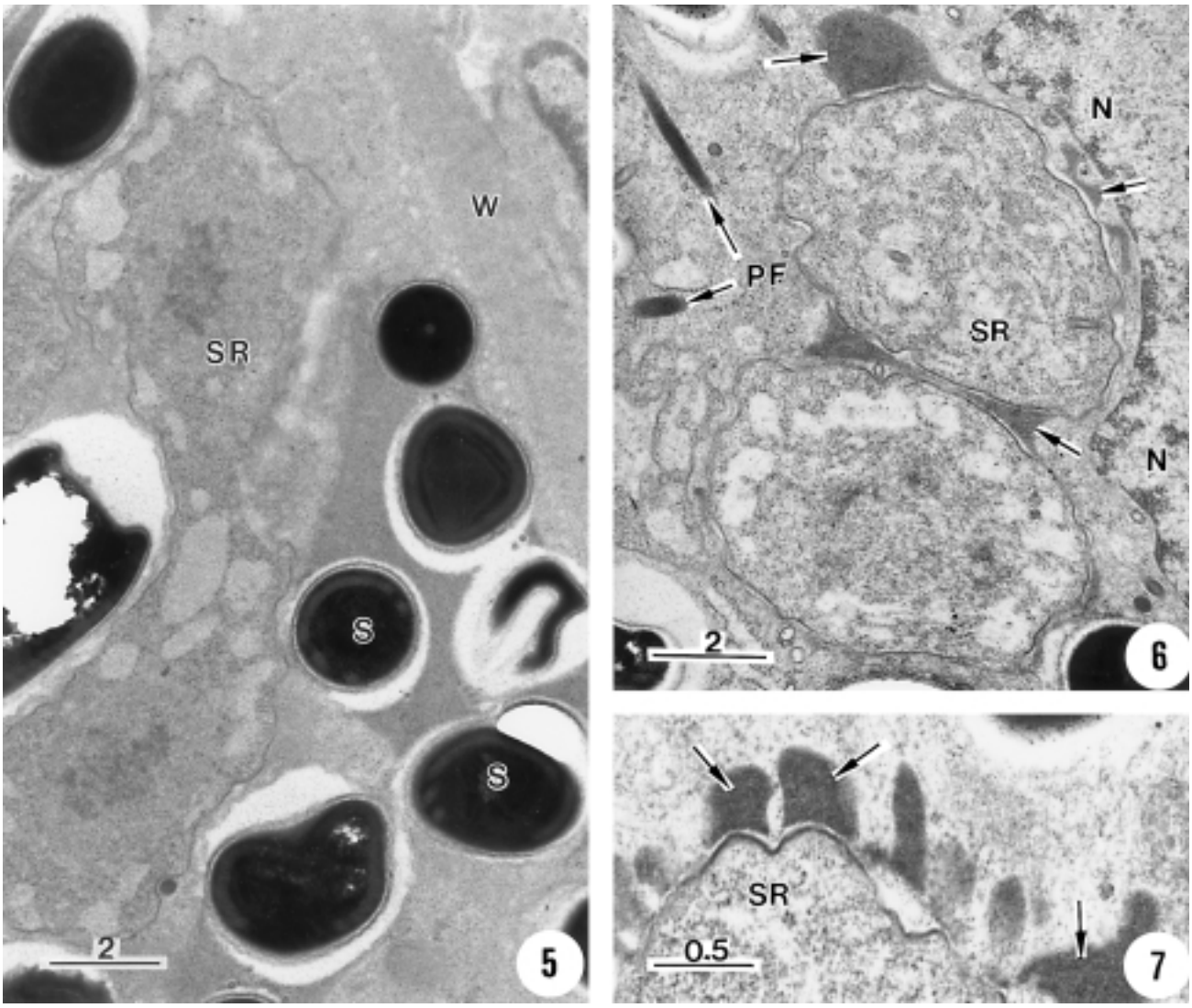
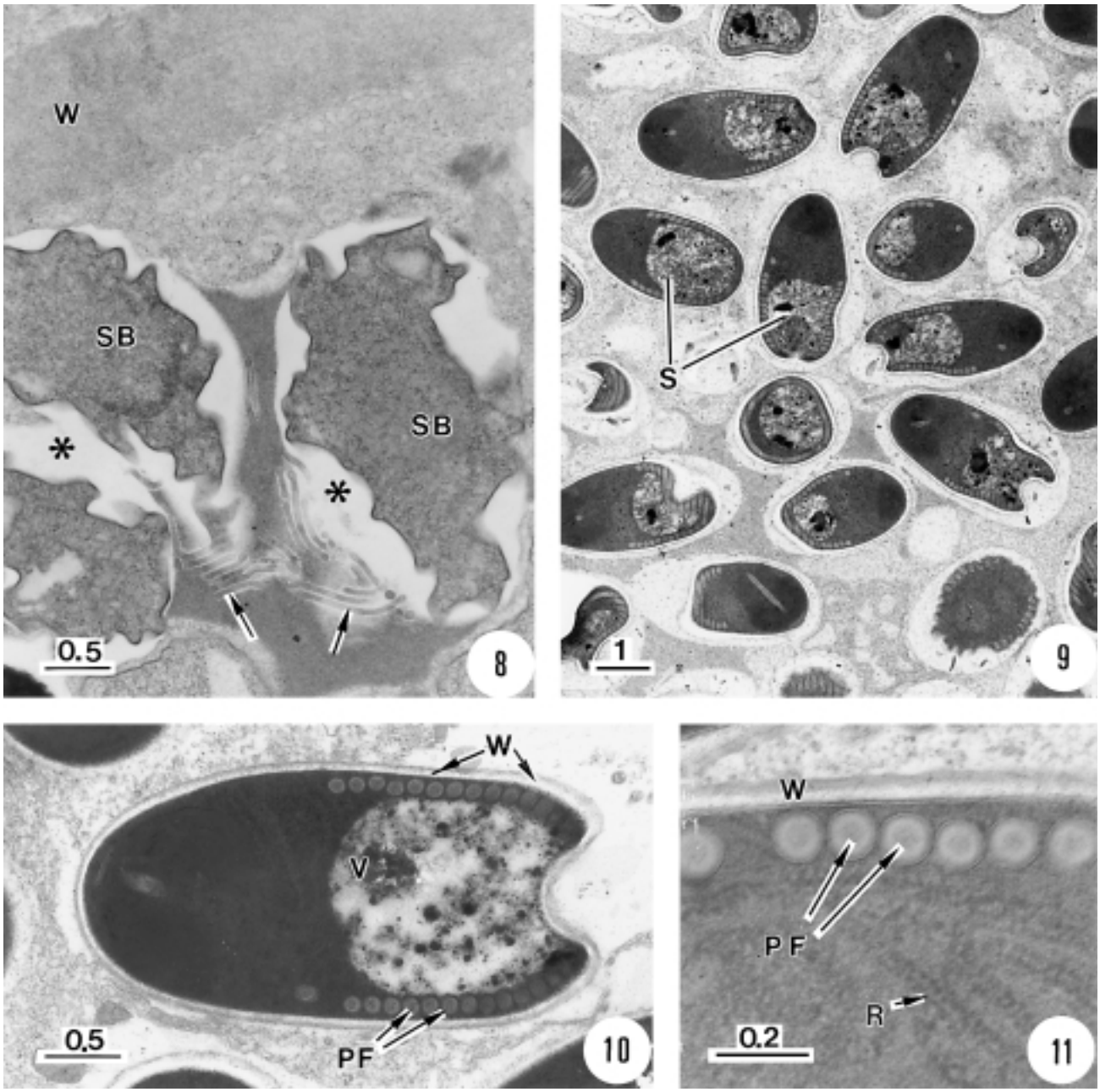


Figs. 5–7. Transmission electron micrographs of spores and different life cycle stages of *Loma myrophis* n. sp. in the gut tissue of *Myrophis platyrhynchus*. 5. Dividing sporonts (SR) located near the xenoma wall (W) among some mature spores (S). 6. Maturing sporonts (SR) with extracellular blisters (arrows) filled with a granulo-fibrous material. Extruded polar filaments (PF) are present near the sporonts. 7. Ultrastructural details of the extracellular blisters (arrows) surrounding the sporont (SR).

of dense material were present surrounding the sporonts (Figs. 6, 7). This dense material accumulated in the parasitophorous vacuole around the sporonts (Fig. 8).

Sporoblasts. During sporulation a well-developed parasitophorous vacuole was evident. Each sporoblast mother cell divided by binary division into 2 uninucleate sporoblasts (Fig. 8). During this phase tubular structures appeared within the dense matrix between the developing sporoblasts (Fig. 8); these tubules were organized in parallel groups and measured 50–55 nm in diameter (Fig. 8). The sporoblasts were characterized by the formation of the primordia of the polar filament. At the last phase of the maturing process, the sporoblasts broke the connection to the host cell cytoplasm and became free in the parasitophorous vacuole (Fig. 8). The xenoma wall was now thicker and denser (Fig. 8). The nucleus occupied a central position and the polar tube was coiled posteriorly, while the vacuole differentiated (Fig. 8).



Figs. 8–11. Ultrathin sections of different life cycle stages of *Loma myrophis* n. sp., parasite in the gut tissue of *Myrophis platyrhynchus*. 8. A group of maturing sporoblasts (SB) within a parasitophorous vacuole (*), where several tubular structures (arrows) are present amongst the dense matrix. The sporoblasts are sometimes located near the wall (W). 9. Several spores (S) sectioned at different levels, showing some microsporidian specific structures. 10. Longitudinal section of a spore, showing the wall (W), the polar filament (PF) with 13–14 turns and the posterior vacuole (V). 11. Detail of the transverse section of the polar filament (PF). In the cytoplasm, the polyribosomes (R) are arranged as a tape-like structure.

Spores. The unfixed spore had an ellipsoidal shape and was 3.45 (3.22–3.70) × 1.71 (1.59–1.81) µm (n = 50) (Fig. 2, inset). The spore wall consisted of a ~25 nm thick electron-dense exospore and an electron-lucent endospore with the same thickness (Figs. 9, 10, 11). These spores were uninucleate and the nucleus occupied a position between the apical polaroplast and the basal vacuole (Figs. 9, 10).

Around the nucleus, some layers of helically arranged aggregates of polyribosomes were observed (Fig. 11). The polar filament, measuring 100–110 nm in diameter (Fig. 11), was isofilar and consisted of a single coil with 13–14 turns surrounding the posterior vacuole that occupied about half of the total volume of the spore (Figs. 9, 10).

Ultrathin sections revealed that some spores had lost their contents in the xenoma. Several extruded polar filaments were found among the different life cycle stages and some others were passing through the developing sporoblast cells (not the spores) (Figs. 3, 6), including the hypertrophic nucleus of the host cell.

Discussion

Among the fish microsporidian genera, *Glugea*, *Loma* and *Pseudoloma* show some ultrastructural features similar to those illustrated in our results. The recently erected new genus and species *Pseudoloma neurophila* found in central nervous systems differs from *Glugea* and *Loma* because the spores were clearly segregated into clusters of up to 16 spores and appeared to develop within a true sporophorous vesicle (Matthews et al. 2001). Although the distinction between the genera *Glugea* and *Loma* is not very clear (Larsson et al. 1996; Cali and Takvorian 1999; Lom and Pekkarinen 1999), the developmental stages, mature spores and xenoma wall observed by Nomarski optics and by serial ultrathin sections revealed a closer resemblance to features of the genus *Loma* (Lom and Pekkarinen 1999). The principal differentiating characters of the genera *Glugea* and *Loma* relate to the xenoma (host cell) within which the parasites develop. In *Glugea* the xenoma wall consists of stratified layers of surface coat, the host cell nucleus is branched and peripheral, while merogonic

Table 1. Comparative characters of the spore of *Loma* species parasites of fish.

Loma	Host Tissue	Spore		PF		Spore Form
		L	W	coils	row	
L. branchialis (= *L. morhua*)	gill	4.2	2.0	16/17	*i*	ell/ov
(Morrison and Sprague 1981a)	gill	~6	4	16/19		------
L. salmonae	gill	3.7	2.2	12–14		py/ell
(Putz et al. 1965)	gill	4.4	2.3	14–17		ell/el
L. fontinalis (Morrison and Sprague 1983)	gill	3.7	2.2	12–14		------
L. dimorpha (Loubès et al. 1984)	digestive	4.5	1.8–2.0	13–15	*i*	ov/ell
L. diplodae (Bekhti and Bouix 1985)	gill	4.17	2.22	17–18		ov
L. trichiuri (Sandeep and Kalavati 1985)	gill	3.0	2.0	------		py
L. camerounensis (Fomena et al. 1992)	digestive	3.96	2.16	11–12		ov
L. boopsi (Faye et al. 1995)	liver-intestine	3.70	2.40	12–14 or 16–18	*i*	ov
L. embiotocia (Shaw et al. 1997)	gill	4.8	2.6	14–18		ov
L. acerinae (Lom and Pekkarinen 1999)	intestine	4.64	2.19	11–23	*i*	ell/el
L. myrophis n. sp.	gut	4.06	1.61	13–14	*i*	ell/el

i = isofilar; el = elongated; ell = ellipsoidal; ov = ovoid; py = pyriform.

stages are peripheral and sporogonic stages are located centrally. In *Loma*, by contrast, the xenoma wall is uniformly granular, the host cell nucleus is central and deeply lobed, and parasite merogonic and sporogonic stages are intermingled (Lom and Pekkarinen 1999; Cheney et al. 2001).

The results described here, and especially those regarding the developmental stages, are consistent with the morphology and ultrastructural data described for some species of the genus *Loma* listed in Table 1. When comparing the previously described species of the genus *Loma* with our results, there are some variations in the morphology of the spores and developmental stages. The internal organization of the spores and the ultrastructure of the xenoma wall showed important differences in comparison with the various species of the genus *Loma*. Details of the original descriptions of the spores of different species are given in Table 1. Thus, we conclude that the microsporidian parasite of the fish *Myrophis platyrhynchus* is a new species of the genus *Loma*, and we propose to name it *Loma myrophis*.

The phenomenon of natural and stimulated extrusion of the microsporidian polar filament has been described previously (Dyková and Lom 1978; Lom and Pekkarinen 1999). The presence of extruded polar filaments among the different developmental stages inside the xenomas of this new microsporidian species, seems a strong argument to reinforce the view that autoinfection occurs in this new species.

Description

Loma Morrison and Sprague, 1981
Loma myrophis n. sp. (Figs. 1–11)

Type host: *Myrophis platyrhynchus* (Teleostei, Ophichthidae)

Transmission: No data.

Site of infection: Sub-epithelial gut tissue forming a xenoma.

Type locality and prevalence: Amazon River estuary (00°35′38″S, 47°35′00″W), near Belém, Brazil. Natural prevalence of 34% (17 of 50 fishes).

Sporogony: Uninucleate sporoblasts. Parasitophorous vacuole evident at the beginning of this stage. Multiporous sporogony within the parasitophorous vacuole, producing a variable number of spores.

Spores: The spores and other developmental stages occur simultaneously in a hypertrophic whitish xenoma measuring up to 160 µm. The spores are ellipsoidal and uninucleate, measuring about 3.45×1.71 µm with an isofilar polar filament of a single coil with 13–14 turns. The posterior vacuole of the spore occupies about half of the total volume of the spore. The spore wall consists of an electron-dense exospore and an electron-lucent endospore, each about 25 nm thick.

Pathology: Host cells are enlarged in the form of macroscopic spherical cysts (xenomas) located within the sub-epithelial gut tissue. The proliferative forms of the parasite develop in contact with the host cytoplasm.

Deposition of Type Material: A slide and two glass slides of semithin sections of the xenoma were deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution, Washington D.C. 20560 (No. USNM-51.556). Glass slides with semithin sections and specimens embedded in plastic resin (Epon) are in the collection of the first author.

Etymology: The specific epithet derives from the generic name of the host species.

Remarks: Macroscopical and histological examination of the parasitized fish revealed that this newly identified microsporidian occurs within multiple xenomas located in the sub-epithelial gut tissue. The proliferative forms of the parasite develop in contact with the host cytoplasm.

Developmental stages were observed dispersed throughout the spore-filled xenoma. Among the developmental stages several extruded polar filaments were found passing through these cells (except the spores), including the hypertrophic nucleus of the host cell. This study provides the first record of microsporidians in this host species of the Amazon River.

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