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Author(s): Carlos Azevedo and Edilson Matos

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AMAZONSPORA HASSAR N. GEN. AND N. SP. (PHYLUM MICROSPORIDIA, FAM. GLUGEIDAE), A PARASITE OF THE AMAZONIAN TELEOST *HASSAR ORESTIS* (FAM. DORADIDAE)

Carlos Azevedo and Edilson Matos*

Department of Cell Biology, Institute of Biomedical Sciences and CIIMAR, University of Oporto, Lg. A. Salazar no. 2, 4099-003 Porto, Portugal. *e-mail: azevedoc@icbas.up.pt*

ABSTRACT: We describe the microsporidian *Amazonspora hassar* n. gen., n. sp. from the gill xenomas of the teleost *Hassar orestis* (Doradidae) collected in the estuarine region of the Amazon River. The parasite appeared as a small whitish xenoma located in the gill filaments near the blood vessels. Each xenoma consisted of a single hypertrophic host cell (HHC) in the cytoplasm of which the microsporidian developed and proliferated. The xenoma wall was composed of up to approximately 22 juxtaposed crossed layers of collagen fibers. The plasmalemma of the HHC presented numerous anastomosed, microvilli-like structures projecting outward through the 1–3 first internal layers of the collagen fibrils. The parasite was in direct contact with host cell cytoplasm in all stages of the cycle (merogony and sporogony). Sporogony appears to divide by plasmotomy, giving rise to 4 uninucleate sporoblasts, which develop into uninucleate spores. The ellipsoidal spores measured 2.69 ± 0.45 × 1.78 ± 0.18 μm, and the wall measured ~75 nm. The anchoring disk of the polar filament was subterminal, being shifted laterally from the anterior pole. The polar filament was arranged into 7–8 coils in a single layer in the posterior half of the spore, surrounding the posterior vacuole. The polaroplast surrounded the uncoiled portion of the polar filament, and it was exclusively lamellar. The spores and different life-cycle stages were intermingled within the cytoplasm of the HHC, surrounding the central hypertrophic deeply branched nucleus. The ultrastructural morphology of this microsporidian parasite suggests the erection of a new genus and species.

Microsporidia Balbiani, 1882 comprises at least 144 available genera commonly found in insects, crustaceans, fish, and other vertebrates (Canning, 1976; Lom and Dyková, 1992; Sprague et al., 1992; Larsson, 1999; Sprague and Becnel, 1999). About 12 genera are reported as parasites of fish (Canning and Lom, 1986; Faye et al., 1995; Sprague et al., 1992; Larsson, 1999; Azevedo and Matos, 2001; Matthews et al., 2001). However, few microsporidian genera have been described in the gills of fish. Among them are *Glugea* Thélohan, 1891, with several species (Takvorian and Cali, 1981; Canning et al., 1982; Morrison et al., 1985; Lom and Dyková, 1992), *Loma* Morrison and Sprague, 1981, with 11 species (see Azevedo and Matos, 2001), and *Ichthyosporidium* Caullery and Mesnil, 1905, with 2 species (Lom and Dyková, 1992). These develop in xenomas that result from host tissue reactions (Weidner, 1976; Weissenberg, 1976; Morrison and Sprague, 1981a, 1981b; Bekhti and Bouix, 1985; Canning and Lom, 1986; Sprague et al., 1992; Cali and Takvorian, 1999; Larsson, 1999; Lom and Pekkarinem, 1999).

The present study of microsporidiosis in the Amazonian teleost *Hassar orestis* describes light and ultrastructural details of xenomas that contain all developmental stages, including spores, of a new genus and a new species, which induce host cell hypertrophy. The morphological characteristics and taxonomic position of the new species are discussed.

MATERIALS AND METHODS

Several specimens of the teleost *H. orestis* (Steindachner, 1875) (Doradidae) (Brazilian common name "Botinho") were collected in the estuarine region of the Amazon River (02°14′54″S, 49°30′12″W), near the city of Belém (Pará), Brazil. Infection was determined by the presence of xenomas located in the gill filaments. After crushing the xenomas, microsporidian spores were identified using a differential interference contrast (DIC) microscope. For transmission electron microscopy (TEM), the xenoma and surrounding tissues were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 C for 20–24 hr, rinsed overnight in the same buffer at 4 C, and postfixed in 2% OsO_4 in the same buffer at 4 C for 4 hr. After dehydration in an ascending ethanol series and propylene oxide, the xenomas were embedded in Epon (10–12 hr in each change). Semithin sections were stained with methylene blue–Azur II and observed by DIC optics. For TEM, the ultrathin sections were contrasted with uranyl acetate and lead citrate and observed under a JEOL 100CXII transmission electron microscope operated at 60 kV.

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* Laboratory of Animal Biology, Federal Rural University of the Amazonia, 66.000 Belém (Pará), Brazil.

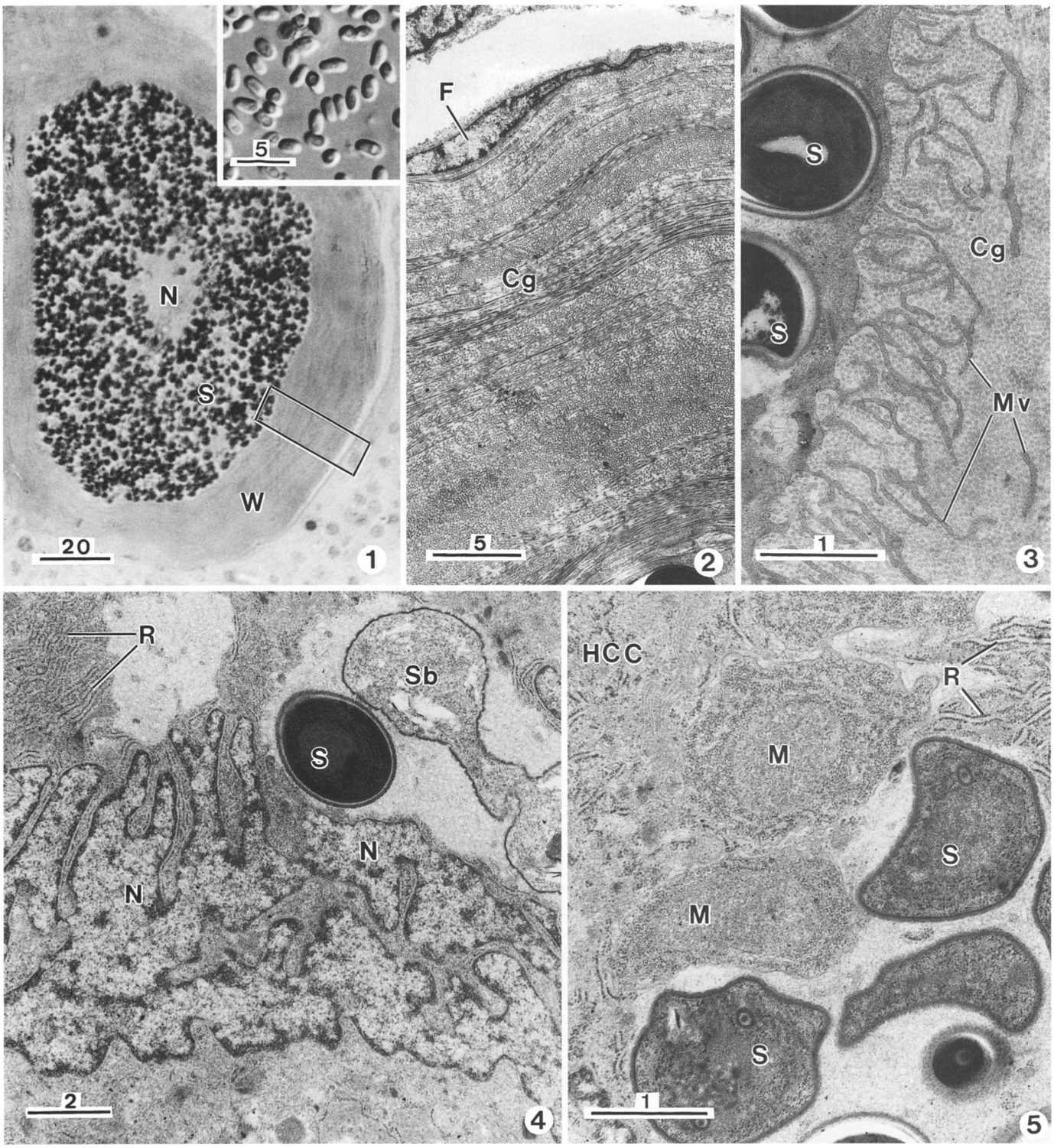
DESCRIPTION

Amazonspora hassar n. gen. and n. sp.

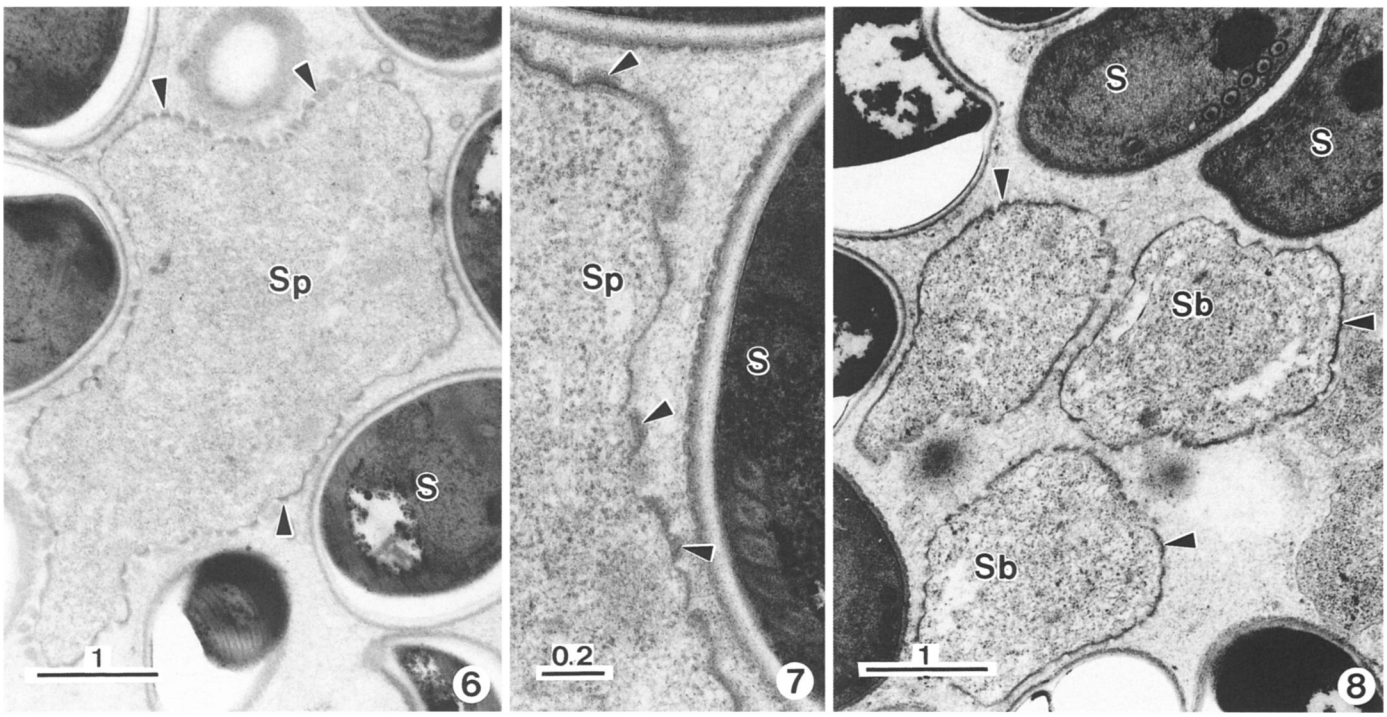
(Figs. 1–13)

Semithin sections revealed spherical to ellipsoidal whitish xenomas filled with numerous spores. Xenoma has a thick wall strengthened by several stratified collagen layers (Fig. 1); its thickness varies according to the number of layers. Characteristic microsporidian spores were clearly seen in the semithin sections by light microscopy (Fig. 1). After dissection and rupture of the xenoma, numerous isolated spores were observed by DIC optics (Fig. 1, inset). Xenoma wall (or capsule as it also has been described) completely surrounds the hypertrophic host cell (HHC) and constitutes juxtaposed layers of up to approximately 22 concentric and parallel layers of numerous collagen fibers. Several fibroblasts encase the wall externally (Fig. 2). Some compressed fibroblasts, seemingly with no function, were observed among the collagen fibers.

Plasmalemma of HHC was extended as numerous intermingled anastomosed microvillilike structures projecting from its surface toward the collagen layers without apparent order. The longest microvilli-like structures measured 3 μm and penetrated the first 1–3 internal collagen layers (Fig. 3). Spores and other life-cycle stages were located within the cytoplasm of an HHC (Figs. 4–8). This cell had a central hypertrophic, deeply branched nucleus, with which were associated several cisternae of the endoplasmic reticulum, with some cisternae penetrating between the lobes of the nucleus (Figs. 4, 10). (A schematic drawing of the xenoma wall and xenoma, illustrating all figures



FIGURES 1–5. Light and TEM aspects of *Amazonspora hassar* n. gen. and n. sp. **1.** Semithin section of a xenoma and the surrounding wall (W). The xenoma is an HHC with a central hypertrophic deeply branched nucleus (N). The cytoplasm is occupied by numerous microsporidian spores (S). The box area is enlarged in Figure 2. Some isolated living spores observed by DIC microscopy are inset. **2.** Ultrathin section of the xenoma periphery showing the xenoma wall formed by several concentric crossed layers of collagen fibers (Cg). More externally, a fibroblast (F) is present. **3.** Ultrathin section at the periphery of the HHC showing spores (S) and numerous anastomosed microvillilike structures (Mv) projecting outward among the collagen fibers (Cg). **4.** Ultrathin section of the central zone of the HHC showing the hypertrophic, deeply branched nucleus (N) and the surrounding cytoplasm containing numerous cisternae of rough endoplasmic reticulum (R), a dividing sporoblast (Sb), and a mature spore (S). **5.** Ultrathin section of the host cell cytoplasm (HCC) showing some intermingled developmental stages, as meronts (M) and immature spores (S), all in direct contact with the cytoplasm with evident ribosomes (R).



FIGURES 6–8. Ultrathin sections of some life-cycle stages of *Amazonspora hassar* n. gen. and n. sp. **6.** Ultrathin section of a sporogonial plasmodia (Sp) giving rise to sporoblasts. The periphery of the plasmalemma shows numerous blisters (arrowheads). **7.** Ultrastructural detail of the periphery of a sporogonial plasmodia (Sp) showing external blisters (arrowheads) of the plasmalemma in close contact with the cytoplasm surrounding the spores (S). **8.** Ultrathin section of some sporoblasts (Sb) showing peripheral blisters (arrowheads) formed by dense material and 2 immature spores (S).

[Figs. 1–12], formed by an HHC containing numerous spores, was made from serial UTS [Fig. 13]).

Taxonomic summary

Generic characterization: Genus *Amazonspora* with the characters of the Glugeidae. Development within an HHC (xenoma), with an external wall constituted of several concentric and parallel crossed layers of numerous collagen fibers, forming juxtaposed layers, is derived from the surrounding fibroblasts. Plasmalemma of HHC with numerous intermingled, anastomosed, microvillilike structures projecting from its surface toward the first collagen layers. Parasites develop within the hypertrophic host cytoplasm in close contact with the hyaloplasm, without any surrounding membrane (without sporophorous or parasitophorous vacuoles). Sporogony appears to divide by plasmotomy, giving rise to 4 uninucleate sporoblasts, which develop into uninucleate spores. The immature and mature spores, merogonic and sporogonic stages, are intermingled within the hypertrophic host hyaloplasm. The hypertrophic cell nucleus is central and deeply branched. The branched nucleus is associated with several cisternae of endoplasmic reticulum, with some cisternae penetrating between the lobes of the nucleus.

Specific characterization: Spores were more or less oval in shape, 2.69 ± 0.45 × 1.78 ± 0.18 μm (n = 50) (Fig. 1, and inset). A polar sac of narrow umbrellalike structure enclosed the apical anchoring disk and the anterior position of the polaroplast. Spore with a large vacuole in the posterior half of the spore. Filament that passes obliquely through the polaroplast was arranged into 7–8 coils. Polaroplast exclusively lamellar. Wall measured ~75 nm in thickness, and the exospore and the endospore measured about 37 nm each. Exospore electron dense (Figs. 11, 12). Nuclei were unpaired throughout merogony and sporogony. Sporoblasts with extracellular blisters filled with a granulofibrous material.

Type host: Hassar orestis (Steindachner, 1875) (Teleostei, Doradidae).

Site of infection: Gills.

Type locality: Estuarine region of the Amazon River near the city of Belém (Pará), Brazil (02°14′54″S, 49°30′12″W).

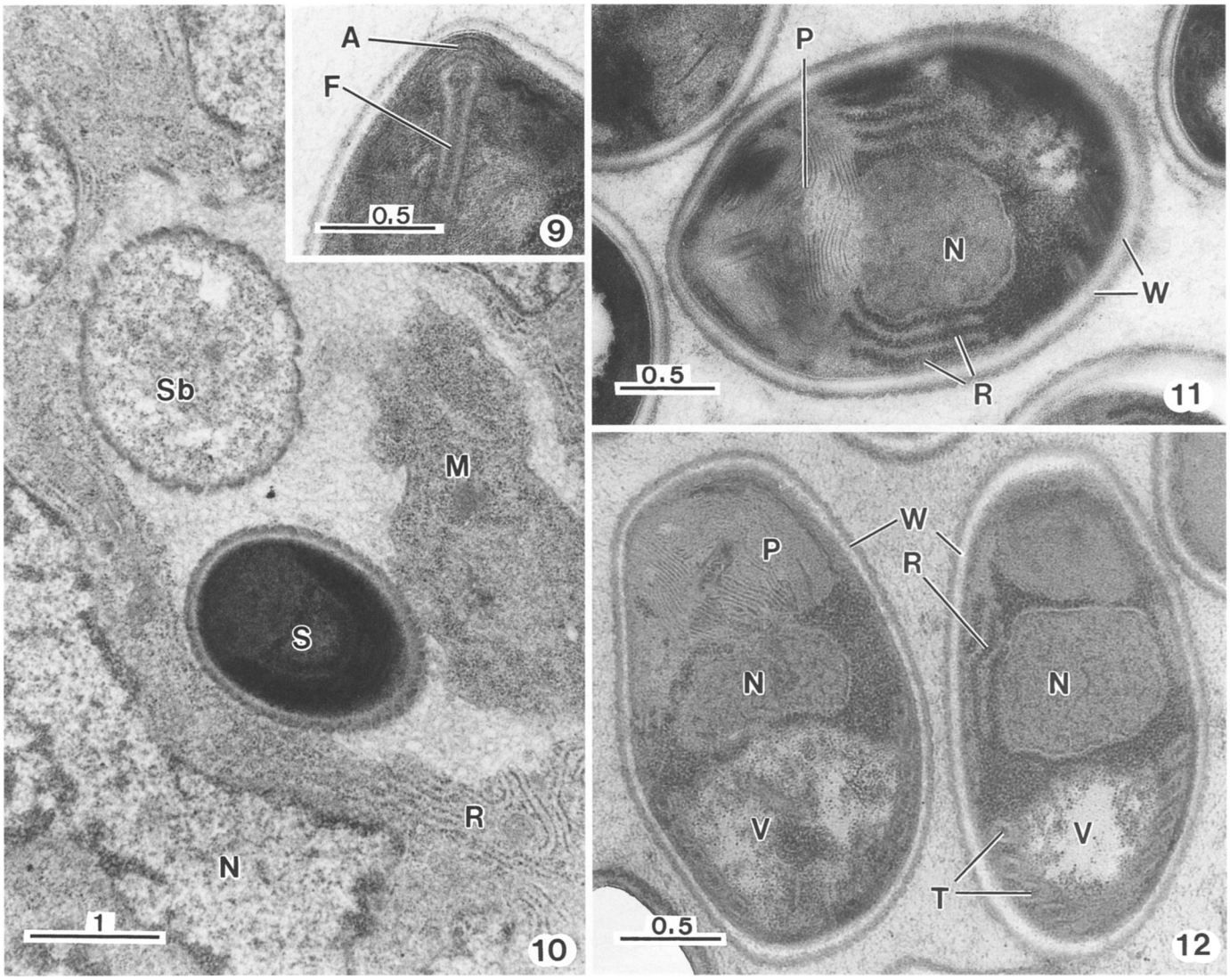
Type specimens: A slide and 2 glass slides of semithin sections of the xenoma were deposited in the International Type Slide Collection at Smithsonian Institution, Washington, D.C. 20560 (USNM no. 1009069). Specimens embedded in plastic Epon are in the collection of the first author.

Prevalence: Fifteen to 40 adult fishes (37.5%) were parasitized.

Etymology: This genus is named with reference to the Amazon River region where the host fish were obtained. ''Hassar'' derives from the generic name of the host species.

Remarks

Our observations demonstrate that the ultrastructure of the different life-cycle stages and the morphology of the spores correspond to Microsporidia (Larsson, 1999; Sprague and Becnel, 1999; Vávra and Larsson, 1999), Microsporidea, and Microsporida (Sprague et al., 1992). The ultrastructural organization observed in the xenoma, mainly the xenoma wall, the HHC, and the developmental stages of the microsporidian spe-



FIGURES 9–12. Ultrathin sections of some life-cycle stages of *Amazonspora hassar* n. gen. and n. sp. **9.** Ultrastructural detail of the apical zone of a mature spore showing the anchoring disk (A) and anterior portion of the polar filament (F). **10.** Ultrathin section of a peripheral region of the hypertrophic, deeply branched nucleus (N) surrounded by several cisternae of the endoplasmic reticulum (R), a meront (M), a sporoblast (Sb), and a mature spore (S). **11, 12.** Ultrathin section of mature spores showing different microsporidian structures, such as the wall (W), polaroplast (P), polyribosomes (R), nucleus (N), posterior vacuole (V), and some sections of the polar tube (T).

cies described herein, correspond to species of Glugeidae Thélohan, 1892 (Lom and Dyková, 1992; Sprague et al., 1992).

Some ultrastructural aspects of our results seem to be similar to those of *Glugea* Thélohan, 1891 and *Loma* Morrison and Sprague, 1981 because they show some ultrastructural resemblances (Shaw and Kent, 1999). Molecular data clearly indicate that these 2 genera are closely related (Cheney et al., 2001). The recently created *Pseudoloma neurophila* shows some similarities with *Glugea* and *Loma*. However, it differs from these genera because the spores are segregated into clusters of up to 16 and appear to develop within a true sporophorous vesicle (Matthews et al., 2001).

Principal differentiating characters of *Glugea* and *Loma* are found with xenoma (host cell), where parasites develop. The xenoma is a complex and important structure that possesses the distinguishing morphological features of the genus (Sprague et al., 1992). In *Glugea*, the xenoma wall consists of stratified layers of the surface coat, the nucleus is branched and peripheral, whereas the merogonic stages are peripheral and the sporogonic stages, central. In *Loma*, the xenoma wall is uniformly granular, the host cell nucleus is central and deeply lobed, and the merogonic and sporogonic stages are intermingled. The envelope surrounding the group of spores is host derived and is not a sporophorous vesicle (Lom and Pekkarinem, 1999; Cheney et al., 2001).

The present observations compare well with the description of *Glugea*, *Loma*, and species of these genera. Our descriptions reveal some ultrastructural characters not evident in previously described genera. However, these 2 genera have characters in common; the most important differences observed in the present study are related to the differentiation of HH plasmalemma into numerous anastomosed microvillilike structures. These structures resemble the organization observed in *Ichthyosporidium* (Sprague and Vernick, 1974), *Tetramicra* (Matthews and

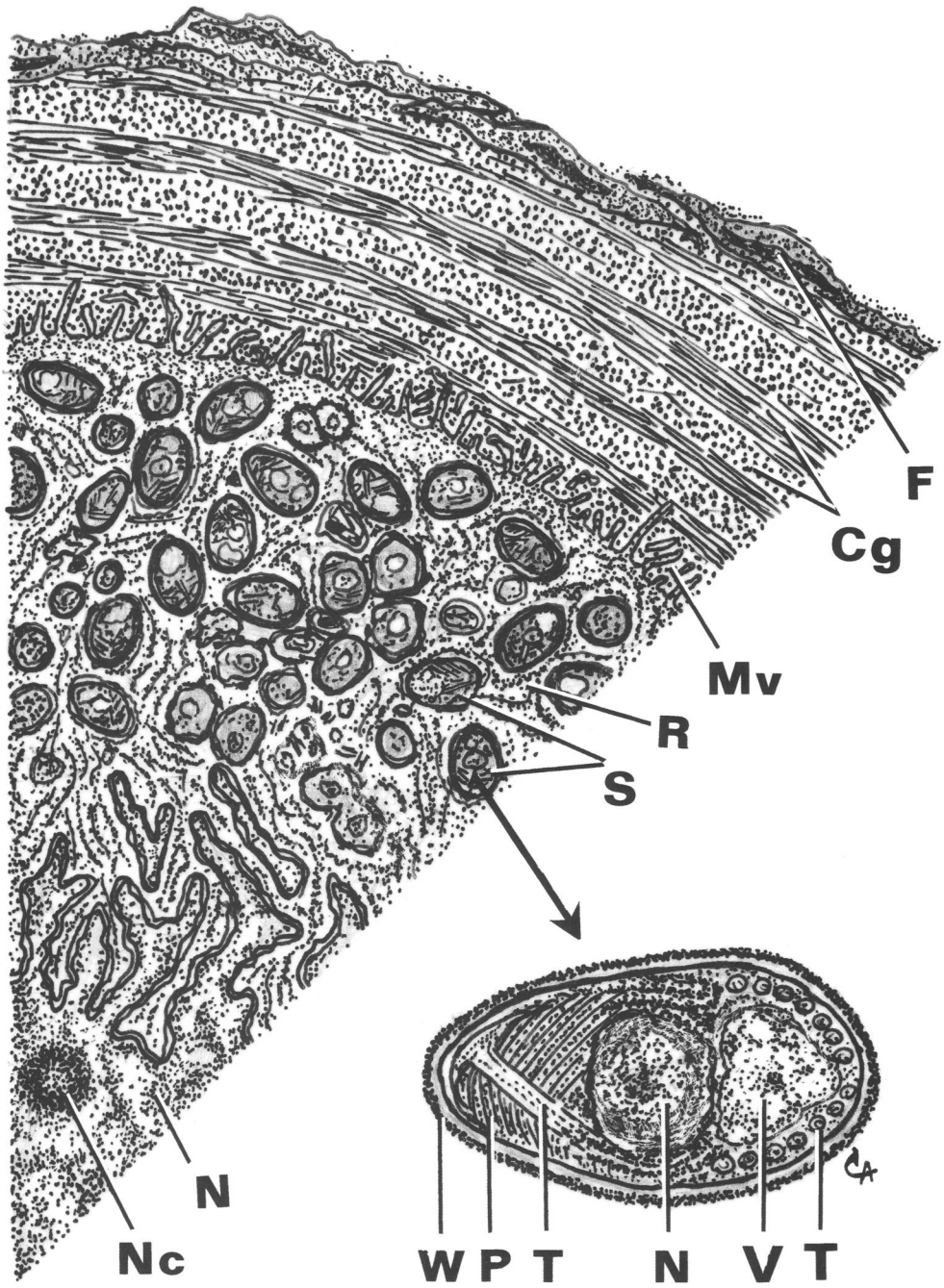


FIGURE 13. Semischematic drawing of a section of the xenoma showing several layers of collagen fibers (Cg) external to the xenoma, surrounding the HHC, with evidence of peripheral microvillilike structures (Mv), and different life cycles, including spores (S), intermingled inside the cytoplasm, where several polyribosomes (R) are present. The hypertrophic deeply branched nucleus (N) contains a nucleolus (Nc).

Matthews, 1980), and *Microfilum* (Faye et al., 1991). However, the microvilli of *A. hassar* xenoma, forming a layer intermingled with collagen fibers at the periphery of the HHC, is an ultrastructural organization that has never been reported in any microsporidian xenoma. In addition to some similar ultrastructural data, our results suggest that this microsporidium does not fit into any of the known genera. For this reason, we propose a new genus and a new species, *A. hassar*.

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