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HETEROSPORIS CICHLIDARUM N.SP (MICROSPORA), A PARASITE OF THE ORNAMENTAL CICHLID FISH *HEMICHROMIS BIMACULATUS* GILL, 1862

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Abstract

Heterosporis cichlidarum n. sp. (Microspora) was found in the striated skeletal muscles of the aquarium fish *Hemichromis bimaculatus* (Teleost, Cichlidae). Microsporidium formed voluminous sporophorocysts that contained many sporophorous vesicles (10 to 12 spores in each vesicle). Only one type of spores was present, medium size 7-8 x 4-4.5 µm (monomorphic species). During merogony, abundant secretions (granules and filaments were formed into the sporophorocyst cavity.

Introduction

Ornamental or aquarium fish may harbour numerous parasites: protozoans, helminths or crustaceans (Gratzek, 1988). Among Protozoa, Microsporida of these fish are common (Dykova, 1995), many have been described and classified in several genera: *Glugea* Thelohan, 1891, *Pleistophora* Gurley, 1893, *Heterosporis* Schubert, 1969 and the collective group *Microsporidium* Balbiani, 1884. The most common species is *Pleistophora hyphessobryconis* Schäperclaus, 1941 which caused heavy infections in many species of teleosts, such as tetras, barbs, danios, angelfish or gold fish (Canning and Lom, 1986; Lom et Corliss, 1967).

In genus *Heterosporis,* three species were now know and described (Lom et Dykova, 1992). The type species *H. finki* infected the musculature and the connective tissue of angelfish *Pterophyllum scalare* (Schubert, 1969; Michel *et al.*, 1989). Lom *et al.* (1989) gave the description of *H. schuberti* which formed a massive infection in the muscles of *Pseudocrenilabrus multicolor.* Finally, the last species was a parasite of the musculature of *Anguilla japonica*, which was named *H. anguillarum* (Hoshina, 1951; T'Sui and Wang, 1988; Lom *et al.*, 1989). We found a fourth species of the genus *Heterosporis* in the myocytes of fries of a cichlid *Hemichromis bimaculatus*. Ultrastructural and pathological studies were carried out on this microsporidium.

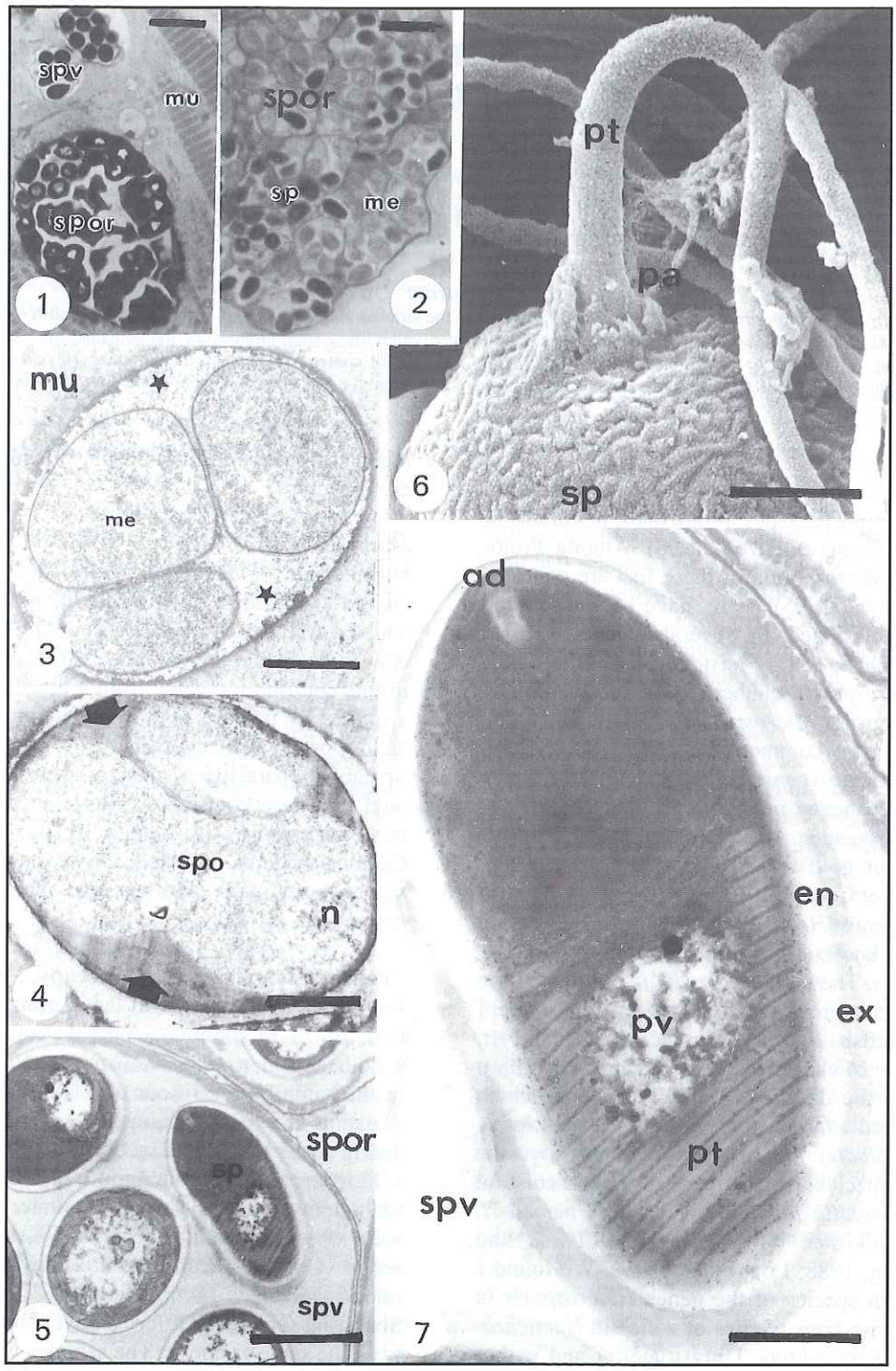
Materials and methods

Hemichromis bimaculatus Gill, 1862 is a cichlid fish widely distributed in the West and Central Africa (Teugels and Thys Van den Audenaerde, 1992). The fish examined in this contribution came from a breeding of a university in Paris (France), without indications of the African origin.

Smears of parasitised muscles were examined by phase contrast or interference contrast microscopy, or stained by the May-Grunwald-Giemsa method. Spore measurements were made with an eye piece micrometer at x 1000.

Transmission electron microscopy. Muscle fragments were fixed with 2,5% glutaraldehyde in 0,1 M sodium cacodylate buffer for 1 hour and then with 2% osmium tetroxide in the same buffer 1-hour also. After dehydratation in ethanol, fragments were embedded in Spurr resin. The sections were cut on a Reichert OM U2 microtome and stained with uranyl acetate and lead citrate. They were observed with a Jeol 200CX microscope (Central Electron Microscopy Laboratory, University Montpellier II).

Scanning electron microscopy. Fixations were the same as above. The samples were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Legend to figures (opposite)

Figure 1. Sporophorocyst (spor) and several isolated sporophorous vesicles (spv) in the host musculature (mu). (semi-thin section). bar= 12,5 µm. **Figure 2.** Sporophorocyst (spor) with merogonic (me) and sporogonic (sp) stages. (semi-thin section). bar=10 µm. **Figure 3.** Three meronts (me) in a sporophorocyst filled up with many dense granules (stars). Around the sporophorocyst, the host musculature (mu) was disorganised. bar=2 µm (TEM). **Figure 4.** One sporophorous vesicle with a sporont in division (spo) with nuclei (n) and the homogeneous matrix (black arrows) in a sporophorocyst. bar=2,3 µm (TEM). **Figure 5.** Sporophorous vesicles (spv) with mature spores (sp) inside a sporophorocyst (spor). bar=5 µm (TEM). **Figure 6.** Apical part of a spore (sp) with the extruded polar tube (pt) at polar aperture (pa). bar=0,5 µm (SEM). **Figure 7.** Spore in longitudinal section in a sporophorous vesicle (spv), with anchoring disc (ad), posterior vacuole (pv), polar tube (pt), endospore (en) and exospore (ex). bar= 1 µm (TEM).

subjected to the CO_2 critical point method, coated with gold palladium and observed with a Jeol JSM 35 (same laboratory).

Results

Implantation and general structure of the infection. No adult fish exhibited external clinical symptoms but most fries or young individuals were strongly parasitised and died. Sporophorocysts of various size were disseminated in the whole skeletal musculature of fish (Fig. 1), mainly in the caudal region. They contained different developmental stages of the microsporidium (merogonic and sporogonic stages), with numerous sporophorous vesicles (Fig. 2). The sporophorocysts measured approximately 50 to 60 µm in diameter. Several sporophorous vesicles, containing mature spores, were isolated, in direct contact with the host tissue (Fig. 1). Around the sporophorocysts, muscle fibrils were much modified and reoriented, extending perpendicularly from the surface (Fig 3). The disorganised myofibrils were connected to the sporophorocyst wall and seemed to contribute to its formation.

Developmental cycle. All stages of the cycle were located inside the sporophorocyst, with the exception of several sporophorous vesicles isolated in the musculature.

Meronts and merogony. Early meronts observed during the development were plasmodia with different sizes. The cytoplasm appeared granular due to numerous ribosomes (Fig. 3). These plasmodia divide through plasmotomy. In the same sporophorocyst, there was no synchronisation between the different stages. The course of merogony was marked by the structural reorganisation of the membrane surrounding the meront. This membrane was thin at the beginning of merogony but, later, it split to form a typical thick wall with small clear spheres. At the same time, many secretions were synthesised in the sporophorocyst, outside the meronts: many dense granules filled up the sporophorocyst cavity (Fig. 3) and, then parallel filaments focused around the meronts.

Sporonts and sporogony. The beginning of sporogony was marked by the retraction of the parasite plasma membrane inside the sporophorous vesicle wall (Fig. 4). Formations produced by the sporogonial plasmodium accumulated in the vesicle: they constituted an amorphous and dense material which filled up the sporophorous vesicle cavity (Fig. 4). Sporogonial plasmodia divided through plasmotomy (Fig. 4) and, at last, sporophorous vesicles contained ten to twelve uninucleate sporoblasts or spores (Fig. 5).

Sporogenesis and spores. At the end of sporogony, uninucleate sporoblasts were formed by successive fragmentations of plasmodia. When matured, uninucleate spores (Fig. 6 and 7) measured 7-8x4-4.5 µm and displayed a very conspicuous posterior

Table 1 Principal characteristics of *Heterosporis* species

Species	*Heterosporis finki*	*Heterosporis schuberti*	*Heterosporis anguillarum*	*Heterosporis cichlidarum*
	Schubert, 1969	Lom, Dykova, Körting & Klinger, 1989	(Hoshina, 1951)	Coste et Bouix, present work
Host	*Pterophyllum scalare*	*Pseudocrenilabrus multicolor Ancistrus cirrhosus*	*Anguilla japonica*	*Hemichromis bimaculatus*
Infection	Connective tissue cells, myocytes	Myocytes	Myocytes	Myocytes
Development	Meronts and sporonts (inside SPV) mixed in a sporophorocyst	Meronts and sporonts (inside SPV) mixed in a sporophorocyst	Meronts and sporonts (inside SPV) mixed in a sporophorocyst	Meronts and sporonts (inside SPV) mixed in a sporophorocyst
Spore (m=microspores, M= macrospores)	Ovoid, elongate, posterior flat m=3x1.5 µm, 16 and more per SPV. M=7-9x2-3 µm 8 per SPV	Ovoid m=4.2x3.1 µm 9 to 26 per SPV. M=4.9-8x2.9-4.9 µm, 4 to 16 per SPV	Ovoid, elongate m=3.5x2.4 µm M=7.8x4.5 µm	Ovoid, slightly pyriform. Only one type of spore 7-8x4-4,5 µm, 10 to 12 per SPV
Number of polar tube coils in macrospores (or spores)				
	31 to 36	40 to 42	-	30 to 39

vacuole, as seen by transmission electron microscopy (Fig. 7). Under the anchoring disc, shaped like a mushroom cap, the polar tube was isofilar (50-70 nm in diameter) and described 30 to 39 coils (Fig. 7). The polaroplast was distinct and represented by the lamellar part. Polyribosomes were clearly defined between the varied organelles.

Discussion

Among microporidia, three genera infect muscle cells of fish: *Pleistophora* Gurley, 1893, *Heterosporis* Schubert, 1969 and *Microsporidium* Balbiani, 1884 (Sprague, 1977). The species described in *Hemichromis bimaculatus* is undoubtedly a species of the genus *Heterosporis,* as defined by Schubert (1969) and confirmed in its diagnosis by Lom *et al.,* (1989). The following characteristics support this generic identification: nuclei are isolated at all stages of development; meronts are enclosed within a sporophorocyst of parasite origin; merogony and sporogony stages are situated inside the sporophorocyst; sporonts and spores are encased within numerous separate sporophorous vesicles.

Three species of the genus *Heterosporis* are know, the characteristics of each are given on table 1.These species are very close by their development cycle and their diverse mensurations. The species described in the cichlid *Hemichromis bimaculatus* belongs to the genus *Heterosporis:* single nuclei, sporophorocysts, sporophorous vesicles, asynchronism of the development cycle are the characteristics that support this assessment. However, the double sporogonic sequence leading to two types of spores (macrospores and microspores) is absent in the species of *H. bimaculatus* although it has been described in *H. finki, H. schuberti or H. anguillarum.* Only one type of spores occurred, 7-8 x 4-4,5 µm in size. So, it is difficult to

compare this microsporidium with other forms of *Heterosporis.* For these reasons, we think that it is a new species and we propose that it should be named *Heterosporis cichlidarum,* after the family of fish host, i.e. Cichlidae.

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