

Heterosporis schuberti n.sp., a New Microsporidian Parasite of Aquarium Fish

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SUMMARY

Heterosporis schuberti n.sp. is described from the myocytes of an ornamental fish, *Pseudocrenilabrus multicolor* (Cichlidae). An apparently identical species was also found in *Ancistrus cirrhosus* (Loricariidae). Early meronts – uninucleate or plurinucleate – are perhaps responsible for dissemination of the infection throughout the muscle tissue. Later development of the microsporidian takes place in a structure encased with a thick envelope, for which the name sporophorocyst is proposed. At first, it contains merogony stages. Later, sporogony stages appear, too, which eventually prevail until a voluminous sporophorocyst is packed full with sporophorous vesicles with macrospores and rather rare microspores.

Pleistophora angillarum Hoshina, 1951 reveals features similar enough to permit its reassignment to the genus *Heterosporis* Schubert, 1969.

Introduction

One of the most common parasites of freshwater aquarium fishes is *Pleistophora hyphessobryconis* Schäperlaus, 1941, a microsporidian recorded thus far from 15 host species and widely distributed in the neon tetra, *Paracheirodon inessi* [2]. Other microsporidia are very rare in ornamental fishes. *Microsporidium pseudotumefaciens* (Pflugfelder, 1952) a species of enigmatic affinities was detected in several fish species [8] but has not been found since. *Heterosporis finki* Schubert, 1969 was described from *Pterophyllum scalare* [9, 10]; this species has also been never found again. Weiser [12] considered data available on *Heterosporis* to be insufficient for a proper assignment of this genus into his classification. Issi [5]

proposed to synonymize *Heterosporis* with *Stempellia*, considering the differences too petty for an independent generic status. Kinkelin [6] found in *Brachydanio rerio* an undetermined species, a *Microsporidium* sp.

To these microsporidian species a fifth one can now be added. A massive infection with this species has been repeatedly found in the musculature of *Pseudocrenilabrus multicolor* (fam. Cichlidae). Infection with an apparently identical microsporidian was also found in material of *Ancistrus cirrhosus* (fam. Loricariidae) collected in 1982 from an aquarium fish hobbyist. The spores were reminiscent of *P. hyphessobryconis*; however, upon more careful inspection the species had to be assigned to the genus *Heterosporis*. We present its description and an emended characterisation of the genus *Heterosporis*.

Material and Methods

The infected ornamental fish, *Pseudocrenilabrus multicolor* came from the permanent breedings in the Zoology Department of the Veterinary Medical School in Hannover. Fresh spores were observed, measured and photographed. To construct a size distribution graph, a suspension of spores from the infected muscle was prepared and all spores in one fresh preparation were measured ($n = 269$). Tissue samples were fixed in 10% neutral formalin, embedded either in Technovit 7100 (Kulzer Co) or in paraplast (BDH) and stained with haematoxylin-eosin.

The size of the infected *Ancistrus cirrhosus* was 1.5 cm. The mortality was 95%. In mixed ornamental fish populations, only *A. cirrhosus* was infected. Only paraffin embedded tissue of these was available at the time of the present study. In addition to examination of stained histological sections, some samples were rehydrated, postfixed in 0.1 M cacodylate-buffered 2% osmic acid and embedded in Epon-araldite. Ultrathin sections were observed in a Philips 420 electron microscope. Although preservation of structures was poor, some information was obtained.

Results

a) Microsporidia from *Pseudocrenilabrus multicolor*

The infected fish were partly emaciated, and showed signs of distress. Large parts of trunk musculature were pervaded by the parasite stages. The earliest stages were identified as young meronts with one to three nuclei, encased in a thick envelope. Solitary early meronts without any halo of disintegrated sarcoplasm surrounding them (Fig. 1) were wedged in the myocyte. These stages were probably also responsible for dissemination of the infection throughout the musculature. There was some indication (Fig. 2) that early stages which might have several nuclei could divide along with their envelope, as described in meronts of *Pleistophora typicalis* [3]. The origin of the envelope could not be traced.

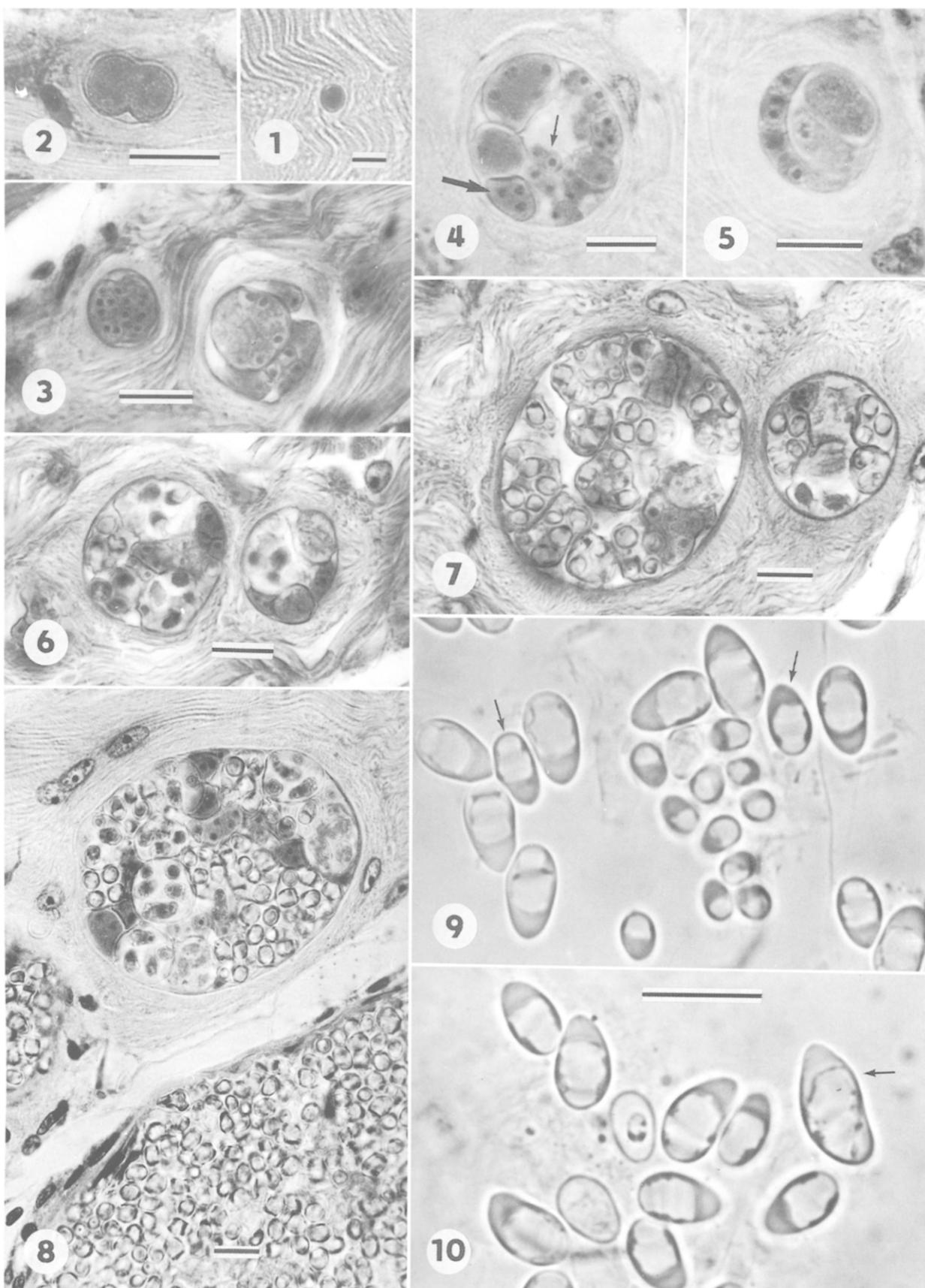
In the following period of growth, the size and number of nuclei increased and the meront became detached from its outer envelope (Fig. 3) and began to divide by plasmotomy, producing several segments (Figs. 3, 5) which in turn appeared to break down to uninucleate cells (Figs. 3, 4) and these cells in their turn may grow and divide again. Some of the cells thus produced became sporogonial plasmodia which formed their own sporophorous vesicle wall in the space within the common envelope (Figs. 4, 6).

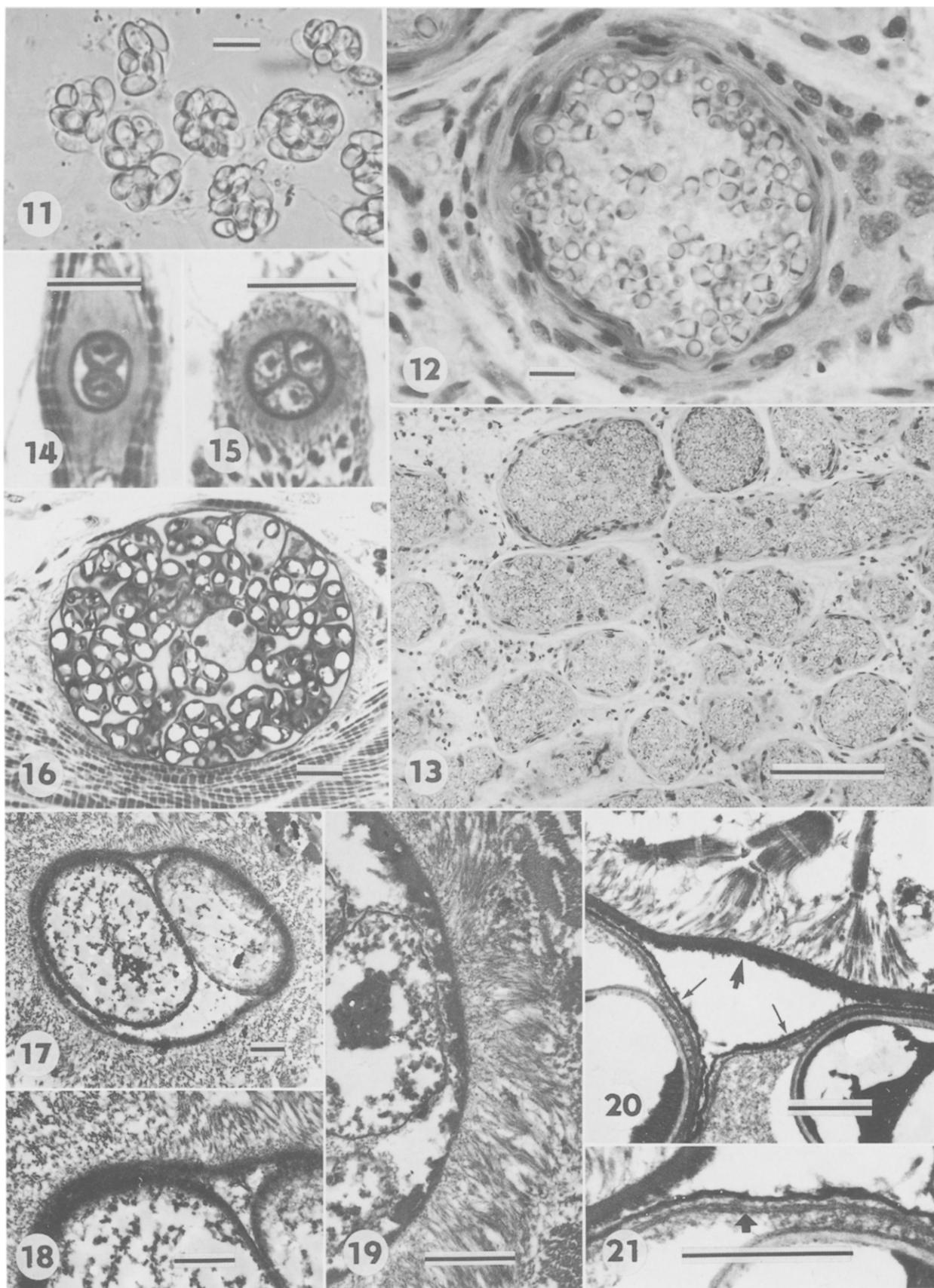
The common envelope grew thicker and enlarged continuously to accomodate the increasing volume of the stages within it (Figs. 7, 8). While some stages entered sporogony, other stayed at the meront stage which continued to divide producing new sporonts. The sporonts became sporogonial plasmodia and within their own sporophorous vesicle wall cleaved into sporoblasts which matured into spores. At this point of development the parasite appeared as a cyst-like structure with inner compartments containing stages which ranged from meronts to sporophorous vesicle with mature spores (Figs. 7, 8). We propose the term sporophorocyst for such structures. Eventually, all meronts transformed into sporogony culminating in spores and the sporophorocyst, up to 120 μm diameter, contained nothing but mature spores packaged within sporophorous vesicles of up to 15 μm diameter.

The spores varied greatly in shape and size. There were a minority of microspores, and the rest were medium-sized spores and macrospores. The microspores measured 4.2 (3.4–4.9) \times 3.1 (2.4–3.4) μm ($n = 33$) with a length : width ratio of 1.35. They were broadly ovoid with the posterior vacuole slightly exceeding the midspore length, so that the cytoplasmic contents filled less than half the spore length. This is at variance with the other spore types. The microsporous sporophorous vesicles contain about 9–27 microspores. The rest of the spores consisted of larger macrospores and medium-sized spores (Fig. 9), but these two categories overlap. If the numbers of spores in each category of length are plotted in one curve, the peak is at 7.4 μm and there is a side elevation at 5.9 μm (Fig. 22). The overall dimensions of these spores are 7 (5.4–8.8) \times 4.4 (2.9–4.9) μm ($n = 236$); the length : width ratio being 1.6. While there is gradual overlapping in size and structure, the spores at the two ends of this range appeared quite different. Macrospores about 8 μm long had a huge posterior vacuole which occupied about 2/3 of the spore length, while the cytoplasm occupied only the narrow anterior pole and encircled the vacuole as a layer with crenations which indicated the coiled polar filament (Fig. 9).

Medium-sized spores, about 6.5 μm long, had a posterior vacuole extending almost one half to 2/3 of the spore length. The ample crenations of the lateral cytoplasmic layer around the vacuole were less obvious. There were 4 to 16 medium-sized spores, and 4 to 15 macrospores per sporophorous vesicle, the average number per vesicle being 9 and 7, respectively (Fig. 11).

Figs. 1–10. *Heterosporis schuberti* n.sp. from *Pseudocrenilabrus multicolor*. — Fig. 1. Early meront in the sarcoplasm. — Fig. 2. Multinucleate early meront which seems to divide with its envelope. — Fig. 3. Left, a meront detached from the outer envelope; right, the parasite body already segmented, developing within the common envelope, now a sporophorocyst. — Figs. 4, 5. Various degree of development within the sporophorocyst; 4-uninucleate stages (fine arrow) and an incipient sporogonial plasmodium (thick arrow). — Fig. 6. Sporophorocysts containing developing sporophorous vesicles, and non-differentiated developmental stages. — Fig. 7. Sporophorocysts containing mostly mature sporophorous vesicles, in addition to a few meront stages. Note in both Figs. 6 and 7 the corona of short, hair-like myofibrils set perpendicularly to the surface of the sporophorocyst. — Fig. 8. Sporophorocyst enclosing, in addition to few sporogony stages, mature spores in sporophorous vesicles. At the bottom, a mass of spores encased by connective tissue cells in which the boundaries of separate sporophorocysts and their sporophorous vesicles have disappeared. Figs. 1 to 8, H & E stain. — Figs. 9, 10. Fresh mounts of spores. In Fig. 9, a group of microspores amidst macrospores, some of which appear as medium-sized spores (arrow). — Fig. 10. A group of macrospores, with a giant anomalous spore at right (arrow). In all Figs., bar = 10 μm .





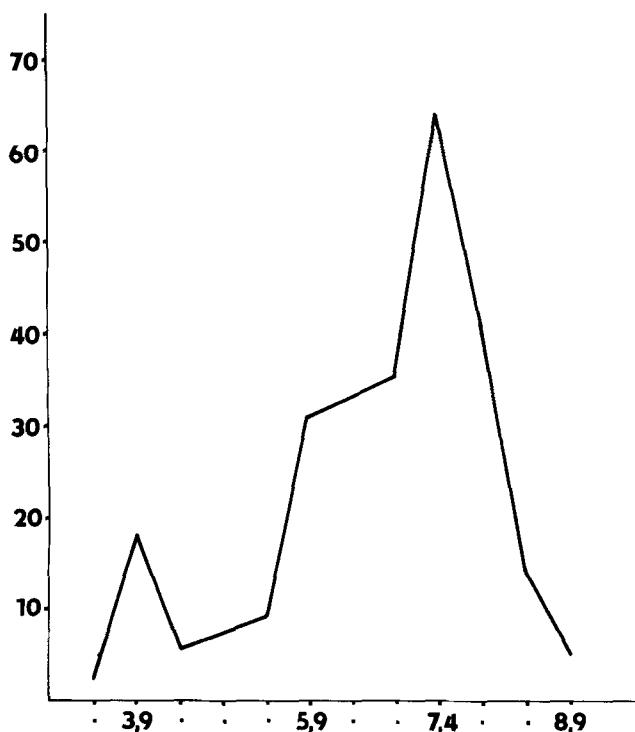


Fig. 22. Variation in spore length in a sample of 269 spores; given at intervals of 0.5 μm ; vertical = numbers of spores; horizontal = spore length.

Several anomalous spores, up to $11 \times 5.9 \mu\text{m}$, have also been observed (Fig. 10).

A single elongated spore nucleus, up to $3.5 \times 2 \mu\text{m}$ in the macrospores, is curved along the spore wall perpendicularly to the longitudinal axis. The PAS positive polar cap was small and dot-like.

Small or young sporophorocysts were surrounded by what appeared in the light microscope as modified muscle fibrils: short fibrils extended perpendicularly, like hairs, from the cyst surface. This layer of short fibrils abutted directly on the normal myofibrils of the myocyte (Figs. 6, 7). In the final stages, the walls of the sporophorous vesicle disappeared within the cyst, which was thus filled with mature spores free from any compartments. At the same time, a thick wall of connective tissue was built up around the sporophorocysts (Fig. 12). Several sporopho-

cysts were observed to have fused together to form a large cystic structure within a common connective tissue envelope.

Between the sporophorocysts there was moderate cellular infiltration (Fig. 13). As the infection progressed, many of the connective tissue capsules around the cystic structures burst and released the spores into the spaces between the myocytes. The muscle was thus converted into a disorganized tangle of damaged myocytes, cystic structures and free spores (Fig. 13).

Agglomerations of spores were also found in mesenteries and the intestine; they represented aggregates of macrophages with ingested spores.

b) Microsporidia from *Ancistrus cirrhosus*

The infection was localized in muscle bundles as in the preceding host. Aggregates of macrophages with phagocytized spores were also observed in the kidney and intestine.

Fresh spores were not observed. The material found in histological sections had the same appearance and structure as in *Pseudocrenilabrus multicolor*. The sporophorocyst was a constant feature (Fig. 16). Early meronts were seen in division within the sporophorocyst wall. Some of these early stages comprised two or three (Figs. 14, 15) uninucleate cells. Later stages corresponded fully to the development sequence in *Pseudocrenilabrus*.

The electron microscopic observation of re-embedded material showed the electron dense wall of the sporophorocyst (Figs. 17 to 19) to be about 130–250 nm thick. It had an amorphous structure, with a smooth outer surface and an uneven inner surface (Fig. 19). The cell membrane of the early developmental stages had a similar opaque coat, and it is likely that the sporophorocyst was derived from it (Fig. 18). The wall of spore-containing mature sporophorous vesicles had an outer, electron opaque amorphous layer about 40–50 nm across, subtended by a bilaminar envelope of the same thickness (Figs. 20, 21). The sporophorocyst envelope had a corona of perpendicularly oriented fibrils which were in fact frayed myofibrils attached to the sporophorocyst surface (Figs. 19, 20). In mature spores, the anchoring disc was situated exactly in the middle of the apex of the spore. The polar tube made from 40 to 42 turns around the walls of the posterior part of the spore.

- ◀ Figs. 11–13. *H. schuberti* from *Pseudocrenilabrus multicolor*. – Fig. 11. Fresh mount of sporophorous vesicles with macrospores. – Fig. 12. Sporophorocyst encased by layers of connective tissue; sporophorous vesicle walls have disappeared. H & E. – Fig. 13. Muscle tissue replaced by cystic structures full of mature spores. H & E.
- Figs. 14–21. *H. schuberti* from *Ancistrus cirrhosus*. – Figs. 14–16. Semithin sections stained with toluidine blue. – Figs. 17–21. Electron micrographs of re-embedded material. – Figs. 14, 15. Early meronts within sporophorocysts. – Fig. 16. Sporophorocyst with mature spores. – Fig. 17. Early meront dividing within the sporophorocyst wall. – Fig. 18. Detail of the preceding. – Fig. 19. Corona of short fibres on the surface of the sporophorocyst. – Fig. 20. Part of the periphery of the sporophorocyst to the surface of which are attached frayed fibers of the myocyte. Large arrow points to the sporophorocyst wall, small arrows point to parts of two sporophorous vesicles. – Fig. 21. Detail of Fig. 20; arrow points to the outer dense layer of the sporophorous vesicle, subtended by a bilaminar membrane. – In Figs. 11–16, bar = 10 μm ; in Fig. 13, bar = 100 μm , and in Figs. 17–21, bar = 1 μm .

Discussion

A comparison of the developmental stages of the parasites from *Pseudocrenilabrus* and *Ancistrus* shows that these populations are probably conspecific. We say this cautiously, since we did not observe fresh spores from the latter host, but all features indicate identity.

This species clearly differs from *Pleistophora hyphessobryconis* by the presence of micro- and macrospores and by the absence of a clear halo of disintegrated sarcoplasm around the parasites [2]. It differs from all other species of *Pleistophora* by the sporophorous vesicles being contained within a common envelope inside the sarcoplasm. The origin of this envelope could not be safely elucidated: ultrastructural observation of the unsatisfactorily preserved material from *Ancistrus cirrhosus* indicated that it was produced at the early meront stage. We propose to call it a sporophorocyst to differentiate it from other microsporidian cyst-like structures; it certainly differs from the "sporogony vacuole" as defined by Vávra and Sprague [11] since it contains both meronts and sporonts. The difference between meronts and early sporonts in the genus *Pleistophora* is small, amounting merely to the addition of another layer to the dense coat enveloping the meront and to cytoplasmic changes [3]. A similar continuous transition between meronts and sporonts is also known in *Vavraia* where the sporophorous vesicle wall forms in merogony as a thin amorphous layer which turns later, during sporogony, into a two-layered structure [7]. The precise distinction between meronts and sporonts in the sporophorocyst in *Heterosporis schuberti* is impossible to define at the moment, and stages which have entered sporogony can only be recognized when the sporophorous vesicle wall is laid down.

There are two other genera with an envelope around the developing sporophorous vesicles. In *Helmichia*, sporophorous vesicles are confined within a membrane-bound vesicle [7] but all stages grow within a vesicle bounded by a membrane-like envelope [1]. In both *Helmichia* and *Cystosporogenes*, there are other important differences separating it from the parasite of *Pseudocrenilabrus*.

For an adequate comparison with *Heterosporis finki*, we examined the slides kindly supplied a long time ago by the author of the description, the late Dr. G. Schubert. We could thus examine some features not made clear in his paper (1969) [9]. In *H. finki*, there is also a sporophorocyst containing, in the late stage of development, a mass of sporophorous vesicles enclosing spores some even with persisting meront stages. Schubert [10] described neither the ultrastructure of the sporophorocyst wall nor that of the immature sporophorous vesicle wall, so that we cannot compare it with our findings in the material from *Ancistrus cirrhosus*.

The sporophorocyst represents the pivotal feature indicating that the two species are congeneric. Other features are listed in Table 1. They clearly prove that both species belong to the same genus yet show important species differences, which enable us to establish the *Pseudocrenilabrus* parasite as a new species, *Heterosporis schuberti* sp.n. in honour of the late Dr. G. Schubert from Stuttgart, a distinguished fish parasitologist.

In 1951, Hoshina [4] described the species *Pleistophora anguillarum* as a serious pathogen of the trunk musculature of Japanese eels, *Anguilla japonica*. His account of the life cycle of this parasite contains some enigmatic points (meronts in intestinal epithelial cells, large amoeboid meronts, chain-like meronts in the connective tissue) which do not fit into the developmental cycle of the

Table 1. A comparison of the two *Heterosporis* species

species	<i>Heterosporis, finki</i> Schubert, 1969	<i>Heterosporis schuberti</i> n.sp.	
host	<i>Pterophyllum scalare</i> (Cichlidae)	<i>Pseudocrenilabrus multicolor</i> (Cichlidae)	<i>Ancistrus cirrhosus</i> (Loricariidae)
site of infection	connective tissue cell, undergoes hypertrophy	myocyte, no hypertrophy	myocyte, no hypertrophy
earliest meronts observed	several uninucleate cells within a common envelope	single cells completely filling one envelope each	single cells completely filling one envelope each
sporogony	stages mixed with meronts in a sporophorocyst	stages mixed with meronts in a sporophorocyst	stages mixed with meronts in a sporophorocyst
spore shape	ovoid, posterior flat	ovoid	ovoid
sporophorocyst wall	50 to 200 nm thick	up to 250 nm thick	no data
microspores	3 × 1.5 µm 16 and more per vesicle	4.2 × 3.1 µm 9 to 26 per vesicle	fresh spores not available
macrospores	7–9 × 2–3 µm 8 spores per vesicle	4.9–8.8 × 2.9–4.9 µm 4–16 per vesicle	
number of polar tube coils in macrospores	31–36	not detected	40–42

microsporidian. It is clear, however, that this parasite has many features in common with the genus *Heterosporis*: there is a sporophorocyst wall enveloping the early meront (Hoshina's Fig. 11 corresponds to our Fig. 2). This wall later contains meronts and developing sporophorous vesicles (Fig. 24 of Hoshina exactly corresponds to our Fig. 5). The parasite divides in the sporophorocyst in the same way it does in *H. schuberti*. There are macrospores, mostly 4 to 8 per sporophorous vesicle, sometimes up to 27, and microspores, 16 and more per one sporophorous vesicle. Because of the presence of the sporophorocyst and the way the parasite divides within its wall, we propose to transfer *P. anguillarum* into the genus *Heterosporis* Schubert, 1969 as *H. anguillarum* (Schubert, 1969) comb. nov. The genus *Heterosporis* thus comprises three species.

The emended diagnosis of the genus *Heterosporis* is as follows: Nuclei isolated throughout the cycle. Meronts are encased in a distinct wall of parasite origin, which grows as the parasite develops. This wall later contains merogony and sporogony stages until all meronts transform into sporonts and spores. Micro- and macrospores present. Parasites of fish.

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