

A *Trachipleistophora*-like microsporidium of man: its dimorphic nature and relationship to *Thelohania apodemi*

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Abstract. The structure of the human microsporidium found by Yachnis and colleagues in two AIDS patients (Am. J. Clin. Pathol. 106: 535-43, 1996) (hereafter referred to as HMY) was investigated by light and transmission electron microscopy and compared with *Thelohania apodemi* Doby, Jeannes et Raoult, 1963, a microsporidian of small rodents. The fine structure of the HMY was found to be similar to that of *Trachipleistophora hominis* Hollister, Canning, Weidner, Field, Kench et Marriott, 1996. Characteristic is the presence of a thick layer of electron dense material on the outer face of the meront plasmalemma, which is maintained during the whole life cycle and which later persists as an electron dense coat on the sporophorous vesicle (SPOV). However, HMY is distinguished from *T. hominis* during sporogony, as two types of SPOV and spores are formed in HMY. One type of SPOV contains thick-walled spores (usually 8 or more in number) with anisofilar polar filaments of 7 + 2 pattern, while the other type contains only two thin-walled spores with a smaller number (3-5) of isofilar polar filament coils. The HMY differs from *T. apodemi* which also forms SPOV with 8 spores inside, but the spores of which are larger in size and have 9 + 2 polar filament pattern.

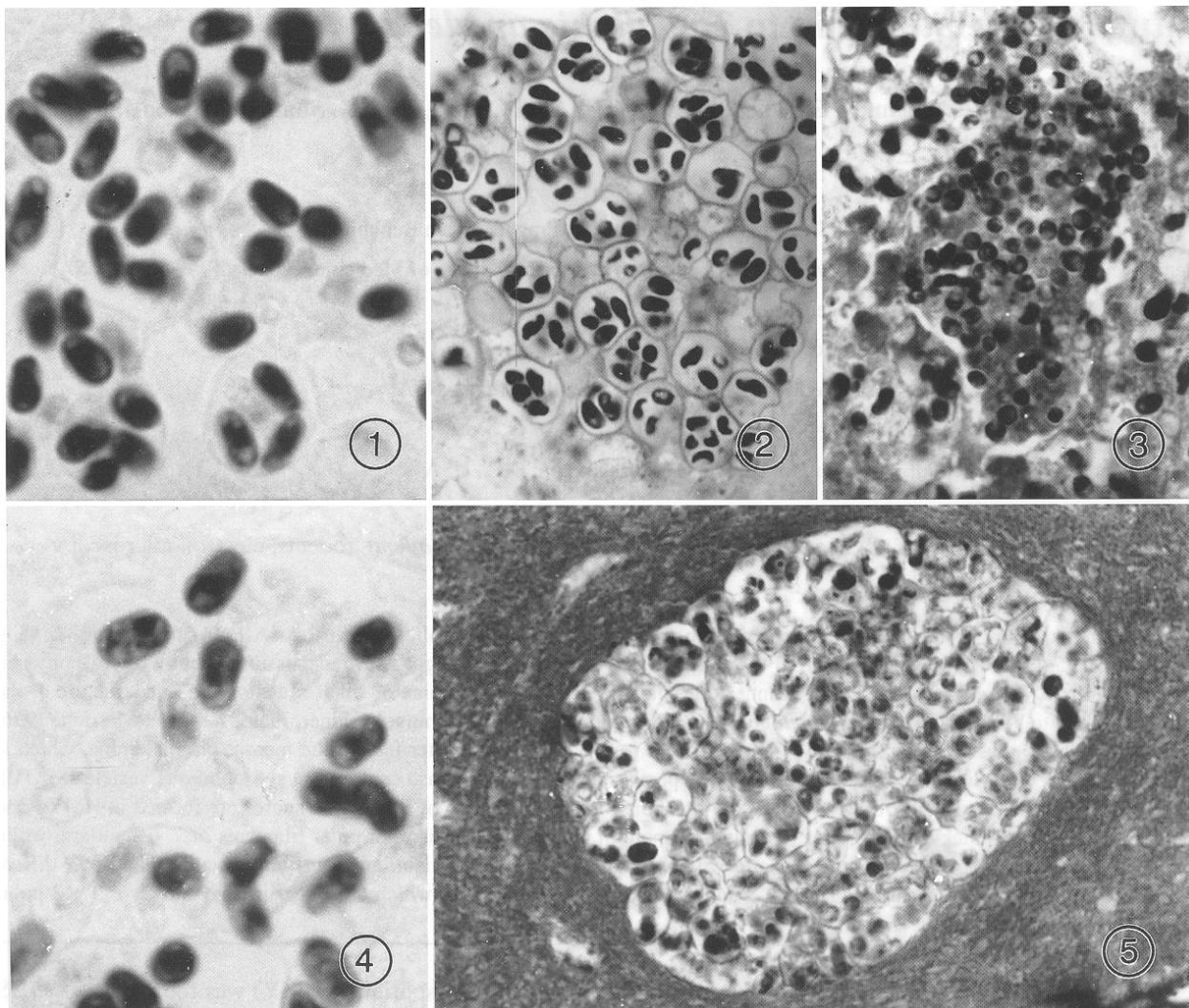
Two types of sporogonial development exist in microsporidia occurring in immunodeficient humans. Some species form spores not enclosed in sporophorous vesicles (SPOV) and, in this case, the spores are either in direct contact with the cytoplasm of the infected cell (*Enterocytozoon bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Laverne, Ravisse et Modigliani, 1985; *Nosema connori* Sprague, 1974; *Nosema* sp. of Cali et al. 1996) or are enclosed in a membrane bound parasitophorous vacuole of presumed host cell origin (*E. cuniculi* Levaditi, Nicolau et Schoen, 1923; *Encephalitozoon hellem* Didier, Didier, Friedberg, Stenson, Orenstein, Yee, Tio, Davis, Vossbrinck, Millichamp et Shadduck, 1991; *E. intestinalis* (Cali, Kotler et Orenstein, 1993) (syn. *Septata intestinalis*). In other species (*Pleistophora* spp. of Chup et al. 1993 and of Ledford et al. 1985; *Trachipleistophora hominis* Hollister, Canning, Weidner, Field et Marriott, 1996) SPOV are formed during sporogony. The SPOV, which is visible by light microscopy, encloses the spores and provides a convenient marker for classification of opportunistic microsporidia. Recently, another SPOV-

forming microsporidian (HMY) was found in two AIDS patients by Yachnis et al. (1996). Its fine structure and structural relationship to another mammalian SPOV-forming microsporidian (*Thelohania apodemi* Doby, Jeannes et Raoult, 1963) is reported. The impetus of the study was the fact that some human opportunistic microsporidian infections have a zoonotic character (Deplazes et al. 1996).

MATERIALS AND METHODS

Buffered formalin-fixed brain tissue of two AIDS patients (Yachnis et al. 1996) was available together with paraffin-embedded tissue blocks of brain and kidneys. For transmission electron microscopy the brain tissue was further fixed in 2.5% buffered glutaraldehyde, post-fixed in 1% osmium tetroxide (OsO_4) and embedded in Spurr's epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Kidney tissue was retrieved from paraffin blocks for embedding in epoxy resin. For light microscopy, sections of paraffin embedded materials were stained with either Ehrlich's hematoxylin-eosin (HE) or Goodpasture's carbol-fuchsin (GCF) (Vávra and Maddox 1976). For *Thelohania apodemi*,

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Figs. 1-3. Light microscopic aspects of human microsporidium of Yachnis et al. (1996) (HMY) as seen in sections of a brain lesion. **Fig. 1.** High magnification view of Type I spores (Goodpasture's carbolfuchsin; $\times 2,240$). **Fig. 2.** Sporophorous vesicles containing Type I spores in semithin plastic section (methylene blue-azur II-basic fuchsin) ($\times 1,300$). **Fig. 3.** Aggregate of Type II spores (hematoxylin-eosin; $\times 1,300$). **Figs. 4-5.** Aspects of *Thelohania apodemi* as seen in sections of brain of *Apodemus sylvaticus*. **Fig. 4.** High magnification of spores (Goodpasture's carbolfuchsin; $\times 2,240$). **Fig. 5.** View of single compact brain lesion showing spores enclosed in sporophorous vesicles (hematoxylin-eosin; $\times 900$).

the original type material was available. The specimen for TEM was prepared from histological sections that had been stained with Giemsa/Colophonum. After removal of the mounting medium by soaking in xylene, sections were rehydrated to 50% ethanol and transferred via 0.1 M cacodylate buffer, into 1% OsO₄ in buffer. After dehydration in ethanol, the sections were transferred to propylene oxide, mixtures of 70 : 30 and 30 : 70 propylene oxide : Agar 100 resin (Agar Scientific) and finally Agar 100 resin. A prepolymerised block of resin was placed on top of each section and the slide was incubated overnight at 65°C for polymerisation of the new resin. The slide was then placed on a hot plate and the section, now embedded in resin, was

snapped off. Sections were cut from the exposed face of the block. For light microscopic preparations of *T. apodemi*, sections from paraffin blocks were stained with HE or GCF.

RESULTS

The human microsporidium (HMY)

Light microscopy. In HE stained slides of brain two different types of spores were observed. Type I spores were darkly stained oval or slightly pyriform in shape (3.7 × 2.0 µm, fixed) (Fig. 1) enclosed in SPOV which tended to be spherical in shape (Fig. 2). Type II spores were smaller and rounder (2.4 µm, fixed) and less heavily stained. They occurred in clumps and did not

appear to be enclosed in SPOV (Fig. 3). These two spore types occurred either separately in adjacent brain cells or within the cytoplasm of the same cell. While Type I spores stained intensively red with GCF, Type II spores stained only pink and at times were almost invisible. Giemsa staining after hydrolysis with hydrochloric acid (Vávra and Maddox 1976) revealed single nuclei in both types of spores.

Electron microscopy. Developmental stages were seldom encountered in ultrathin sections because of poor preservation. They appeared as ribbon-like or oval cells containing remnants of one or more nuclei. They characteristically had a layer of electron-dense material deposited on their outer surface. The spore stages were the most common identified form. Type I spores were seen in groups of 8 or more per SPOV (Fig. 6). They had thick walls composed of a thin electron-dense, wavy exospore and a thick electron-lucent endospore. In this material, most of the spores were highly distorted and had very electron-dense contents (Fig. 6). However, occasionally the polar filament could be seen to consist of 7 thick and 2 thin distal coils, arranged in a single layer (Fig. 7). The SPOV containing spores of Type I had an approximately 60 nm thick, electron-dense, wall. Electron-dense fibres were seen extending from SPOV into the host cell cytoplasm and sometimes forming a dense fibrillar mat between neighbouring SPOV (Fig. 6).

Type II spores were seen to be surrounded by a thin-walled SPOV which had not been visible by light microscopy (Fig. 8). They were nearly round, with a very thin electron-dense exospore and a slightly thicker electron-lucent endospore. Type II spores appeared to be less electron-dense than Type I spores. Swollen membranes, a large posterior vacuole and 3-5 isofilar polar filament coils appeared to be characteristic in Type II spores (Fig. 8 and insets 8a, b). Two of the Type II spores were located in each SPOV. The SPOV walls containing Type II spores were thinner than those containing Type I spores and were not associated with any fibres (Fig. 8).

Thelohania apodemi

Light microscopy. Isolated foci of infection were seen in the brain of the European long-tailed field mouse *Apodemus sylvaticus* (Linné, 1758) in which SPOV with spores were tightly packed (Fig. 5). The number of spores in each SPOV was impossible to count with precision but was estimated to be close to 8, i.e. the number given in the original description of the organism (Doby et al. 1963). Spores arranged in groups of 8 (but also of 4 and 16) were also reported by Šebek and Weiser (1989). After GCF the spores appeared as blunt elliptical bodies, sometimes slightly constricted in middle, $4.2 \times 2.3 \mu\text{m}$ ($3.7-4.5 \times 2.1-2.6$; $n = 10$; fixed) in size (Fig. 4).

Electron microscopy. The material obtained from a stained slide was too poorly preserved for in-depth

ultrastructural evaluation. Spores appeared to have thick walls with a thin exospore (20 nm) and a rather thick endospore (120 nm). The polar filament was seen as 9 thick (120 nm) and 2 thin (88 nm) coils arranged in a

Table 1. Comparison of characters of *Trachipleistophora*-like microsporidium (HMY) and *Thelohania apodemi*

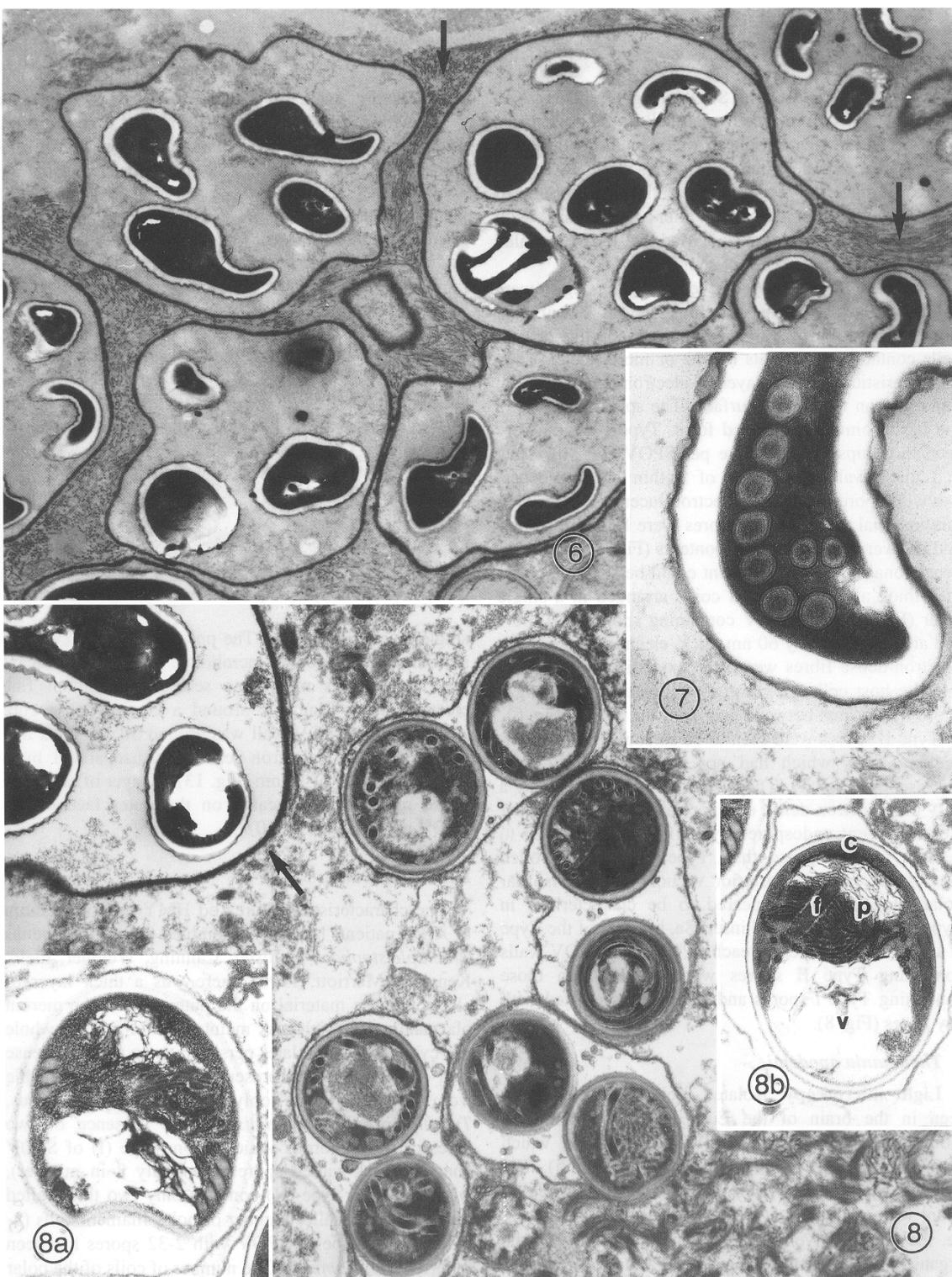
Character	HMY	<i>T. apodemi</i>
Host	<i>Homo sapiens</i>	<i>Apodemus sylvaticus</i>
Tissue	brain and many tissues	brain
Spores/SPOV	I. usually 8, or more II. 2	8 (4, 16)
Sporogony	dimorphic	monomorphic
Spore size (fixed, μm)	I. 3.7×2.0 II. 2.4	4.2×2.3
Spore shape	I. oval-pyriform II. spherical	blunt-elliptical
PF coils: thick + thin	I. 7 + 2 II. 3-5 + 0	9 + 2
Coil layers	single	single

Abbreviations: SPOV – sporophorous vesicle; PF – polar filament

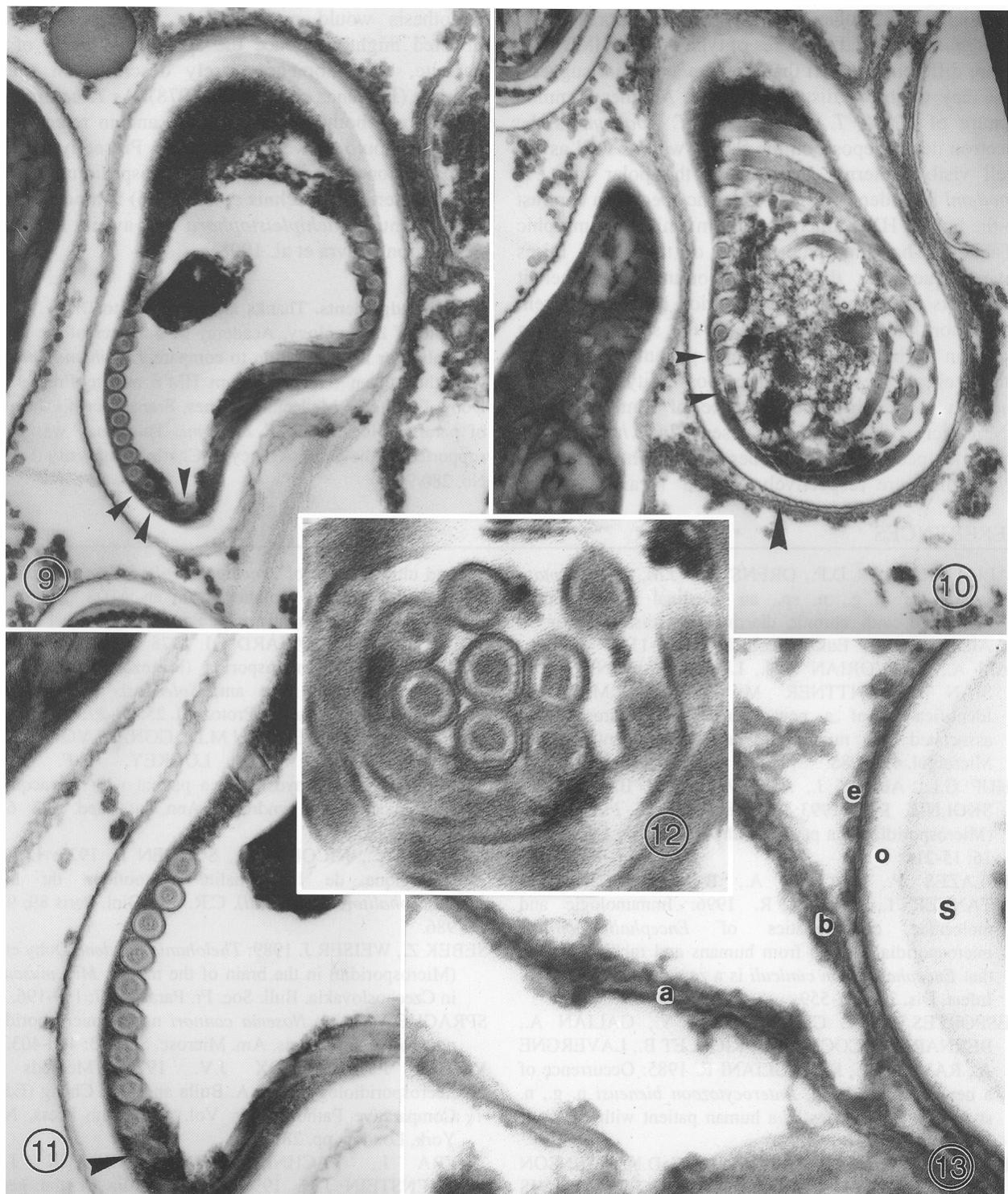
single layer (Figs. 9-11). The polar tube appeared to be surrounded by a double membrane. The electron-lucent layer below this membrane seemed to contain fine electron-lucent filaments around a central dense core (Fig. 12). The SPOV wall was around 50 nm thick and appeared as two electron-dense lines, separated by a less-dense layer of 12 nm (Fig. 13). A layer of electron-dense material was located on the outer face of the SPOV membrane (Fig. 10).

DISCUSSION

The characteristic features of HMY microsporidium of AIDS patients basically resembled those of the genus *Trachipleistophora* Hollister, Canning, Weidner, Field, Kench et Marriott, 1996. There was a thick layer of electron dense material on the outer face of the meront plasmalemma which was maintained during the whole life cycle and which later persisted as an electron dense coat on the SPOV. At present, HMY is distinguishable from the type and only species of the genus, *Trachipleistophora hominis*, by the presence of two types of sporophorous vesicles. One type (I) of SPOV contains thick-walled spores (generally 8 in number), while the other type (II) contains only two thin-walled spores with a smaller number of polar filament coils (3-5). Only one type of SPOV with 2-32 spores has been reported in *T. hominis*. The number of coils of the polar filaments also seems to be different: HMY has 7 thick and two thin coils, while *T. hominis* has eleven coils of the same thickness (Hollister et al. 1996). Although there are limited structural data available for *Thelohania apodemi*, it is clear that it differs from the human HMY microsporidium both at the light and TEM levels. *T. apodemi* spores are longer and broader



Figs. 6-8. Electron microscopy of human microsporidium of Yachnis et al. (1996) (HMY). **Fig. 6.** Sporophorous vesicles with Type I spores. Note dense mat of fibrils among vesicles (arrows) ($\times 9,300$). **Fig. 7.** Portion of distorted Type I spore showing characteristic anisofilar pattern with 7 thick and 2 thin, laterally displaced coils ($\times 62,000$). **Fig. 8.** Four sporophorus vesicles, each containing two Type II spores. Note absence of fibrils among vesicles and presence of sporophorous vesicle with Type I spores in the same cell (arrow). ($\times 13,000$). Inset **Fig. 8a.** detail of spore of type II showing 3-4 isofilar coils. ($\times 20,590$). Inset **Fig. 8b.** detail of spore of type II showing ascending part of polar filament (f), two regions of polaroplast (p), anchoring disc (c) and posterior vacuole (v). ($\times 18,200$).



Figs. 9-11. Electron microscopic aspects of *Thelohania apodemi*. **Fig. 9.** Nearly longitudinal section through spore showing arrangement of polar filament coils with 9 thick and 2-3 thin terminal coils (arrowheads) ($\times 25,500$). **Fig. 10.** Longitudinal section of spore showing 9 thick coils and 2 (small arrowhead) thin coils. Spore is partially surrounded by sporophorous vesicle membrane with dense material covering its outer face (large arrowhead) ($\times 21,600$). **Fig. 11.** Detail of polar filament showing its inner structure and first thin coil (arrowhead) ($\times 45,000$). **Fig. 12.** Inner structure of polar filament at higher magnification showing double outer membrane and periodical structure in electron-lucent layer beneath ($\times 120,000$). **Fig. 13.** Structure of two adjacent sporophorous vesicles (a,b) is revealed as double electron-dense line with some dense material added. S – spore inside SPOV and its exospore (e) and endospore (o) ($\times 40,000$).

(*T. apodemi*: $4.2 \times 2.3 \mu\text{m}$; HMY $3.7 \times 2.0 \mu\text{m}$). While the number of polar filament coils in spores of *T. apodemi* (9 + 2) and in spores of type I of the HMY (7 + 2) are different, the fact that they are anisofilar suggests that they might be distantly related. Another common feature of HMY, *T. hominis* and *T. apodemi* is the electron dense deposit on the SPOV wall as well as the well visible internal structure of the polar tube. *T. apodemi* is evidently a monomorphic species in contrast to the human HMY microsporidium which is dimorphic (Table 1). The dimorphic nature of the HMY raises several questions, the most important is whether it represents the simultaneous infection by two different microsporidia. The occurrence of the same life cycle stages in the brain of two unrelated patients, even in the same cell, argues against this possibility. A further question is whether the presence of dimorphism in HMY microsporidium and its absence in *T. hominis* was not influenced by the tissues in which these two organisms were respectively studied (brain tissue in

HMY and skeletal muscle cells in *T. hominis*). This hypothesis would presume that the type of tissue infected might influence the sporogonic cycle of the parasite, a phenomenon rarely described in microsporidia (Jouvenaz and Hazard 1978). As *T. hominis* has been grown both in tissue culture and in mice, future research should answer this question. Presently the most probable conclusion is that the microsporidium present in the material of Yachnis et al. (1996) is a new species of the genus *Trachipleistophora* that awaits its formal description (Vávra et al. 1998).

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