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*Thelohania capillata* n. sp. (Microspora, Thelohaniidae) —  
An Ultrastructural Study with Remarks on the Taxonomy  
of the Genus *Thelohania* HENNEGUY, 1892

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With 35 Figures

**Key words:** *Thelohania capillata* sp. nov., Protozoa, Microspora, Ultrastructure, Taxonomy, Simuliidae

### Summary

*Thelohania capillata* sp. nov. is described from larvae of the blackfly *Odagmia ornata* (MEIGEN, 1818), collected in a small stream in the south of Sweden. Infection was restricted to the adipose tissue, which was transformed into a syncytial xenoma. The earliest stage observed was diplokaryotic merozoites, which mature directly into diplokaryotic sporonts. The first division of the sporont is a meiosis, identified by the occurrence of synaptonemal complexes, and it is usually followed by a mitotical division, resulting in a plasmodium with 8 single nuclei. The cytoplasm is further divided into 8 lobes which finally give rise to 8 rounded sporoblasts. Each sporoblast matures into a barrel-shaped spore, with a spore-wall composed of three layers, 280–350 nm thick. Most spores are octosporous microspores, dimensions 2.0–3.1 × 2.0–2.7 µm in fixed preparations, but a small number of macrospores, 3.5–4.5 × 2.8–3.0 µm, are produced in pansporoblasts with four spores. Both spore types with a single nucleus and a nearly identical fine structure. The anchoring disc is small and the straight part of the polar filament reaches the posterior pole of the spore. It is surrounded by a polaroplast composed of two structurally different parts. The anterior part is about 1/3 of the spore length, the posterior about 2/3. The polar filament is of approximately uniform thickness all over its length. In microspores the diameter is 120–140 nm, in macrospores it is slightly bigger, 125–150 nm. The polar filament of microspores is posteriorly arranged as 4–5 coils in a single layer close to the spore wall. The angle of tilt of the first filament coil is approximately 45 degrees. In macrospores the posterior part of the filament is arranged as 8 irregularly stacked coils. Sporogony occurs in a pansporoblast transversed by a fibrous material continuous from the spore wall to the pansporoblast membrane, which has a hair-like fibrous appearance. The relationship of the new species to other species of the genus *Thelohania* and to *Thelohania*-like microsporidia from blackflies is discussed.

### Introduction

Infections are commonly found in populations of backfly larvae all over the world and there are several papers dealing with this subject (e.g. STRICKLAND 1913; JÍROVEC 1943 and MAURAND 1975). In Sweden the most important pathogens are fungi of the genus *Coelomycidium* and different species of microsporidia. The microsporidium of the present report was found together with three other microsporidian species in a sample of backfly larvae collected in a small stream in the south of Sweden in the autumn of 1980. When material was processed for electron microscopy it was

clear that the microsporidium was a species of the genus *Thelohania* HENNEGUY, 1892, and not an *Amblyospora* species, the microsporidian genus which appears to be most frequently found in Simuliidae.

All microsporidian species with an octosporous sporogony inside a pansporoblast membrane have for a long time been considered to belong to the genus *Thelohania*. Today, when most microsporidiologists have realized that ultrastructural studies are necessary in dealing with these minute organisms, it has become apparent that the species previously included in *Thelohania* differ strongly at the ultrastructural level, although they might appear quite similar in the light microscope. It has also been demonstrated that there are differences in their developmental cycles. Some species have two sporogonial cycles in their development, one cycle comprising octosporous sporogony inside a pansporoblast membrane, the other cycle producing free spores in irregular numbers. By the octosporous development spores with single nuclei are produced, and the other sequence yields diplokaryotic spores. Based on a thorough knowledge of ultrastructure and development, HAZARD and OLDACRE (1975) found it necessary to split the octosporous species into 11 genera, which were included in the new family Thelohaniidae. Eight new genera were added to the previously known *Thelohania* HENNEGUY, 1892, *Parathelohania* CODREANU, 1966 and *Inodosporus* OVERSTREET and WEIDNER, 1974. For the species which had been investigated ultrastructurally the arrangement into genera was clear, but for a number of species, where knowledge of these characters was lacking, the grouping into genera had to be done more or less tentatively. However, 52 old *Thelohania* species were considered doubtful, and the generic affiliation for these had to wait until more information was available. The genus *Orthothelohania*, created by CODREANU and BALCESCU-CODREANU (1974), is a synonym for *Inodosporus* OVERSTREET and WEIDNER 1974. SPRAGUE (1977) included in the family Thelohaniidae the 11 genera recognized by HAZARD and OLDACRE (1975) as well as *Heterosporis* SCHUBERT 1969, and *Toxoglugea* LÉGER and HESSE 1924.

HAZARD and OLDACRE (1975) suspected microsporidia of the genus *Thelohania* s. str. to be parasites of Crustacea: Decapoda. It is now clear, however, that species of this genus are not restricted to crustacean hosts. There are two species from insect hosts: *T. minispora* GASSOUUMA 1972 and *T. solenopsae* KNELL, ALLEN and HAZARD 1977, which have clearly been demonstrated to belong to this genus, i.e. they have been identified using electron microscopy. The species now reported from Sweden is the third one. It has not been possible to identify this species with any octosporous microsporidium previously described from a simuliid host. The new species, which is named *T. capillata*, is described based on ultrastructural and light microscopical characteristics, and its relationship to other *Thelohania* species is discussed.

### Material and Methods

Blackfly larvae of the species *Odagmia ornata* (MEIGEN, 1818) were collected in the small stream Höje å, near the city of Lund, on October 20, 1980. The microsporidium was studied in smears, paraffin sections and by the use of electron microscopy.

For light microscopy smears and whole specimens were fixed in BOUIN-DUBOSCQ-BRASIL solution (ROMEIS 1968). Smears were fixed for about an hour, whole larvae overnight. For paraffin embedding fixed larvae were washed in 70 % ethanol, dehydrated in an ascending series of ethanols, and embedded in paraplast. Sagittal sections were cut at 5 and 10  $\mu\text{m}$ . The stainings used were GIEMSA solution, and HEIDENHAIN's iron haematoxylin (ROMEIS 1968) with a 0.5 % ethanol solution of fast green as counterstain. Measurements were made with an eye piece micrometer at 1,000 X magnification.

For electron microscopy small pieces of adipose tissue were excised and fixed in 5 % (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (*pH* 7.2) at 4 °C for 15 h. After washing in cacodylate buffer and post fixation in 2 % (w/v) osmium tetroxide in cacodylate buffer for 2 h at 4 °C the pieces were dehydrated in an ascending ethanol series. They were then stained in a solution of 0.5 % (w/v) uranyl acetate and 1 % (w/v) phosphotungstic acid in absolute ethanol for 45 min. After washing in styrene the tissue pieces were embedded in vestopal. Sections were stained with uranyl acetate and lead citrate.

## Observations

### 1. The developmental sequence of the microsporidium

The merogonial part of the development was already completed, which is not unusual in microsporidia-infected material collected from the nature. However, a small number of merozoites, the final product of merogony, could still be seen. It is not clear if there is more than one merogony in the development.

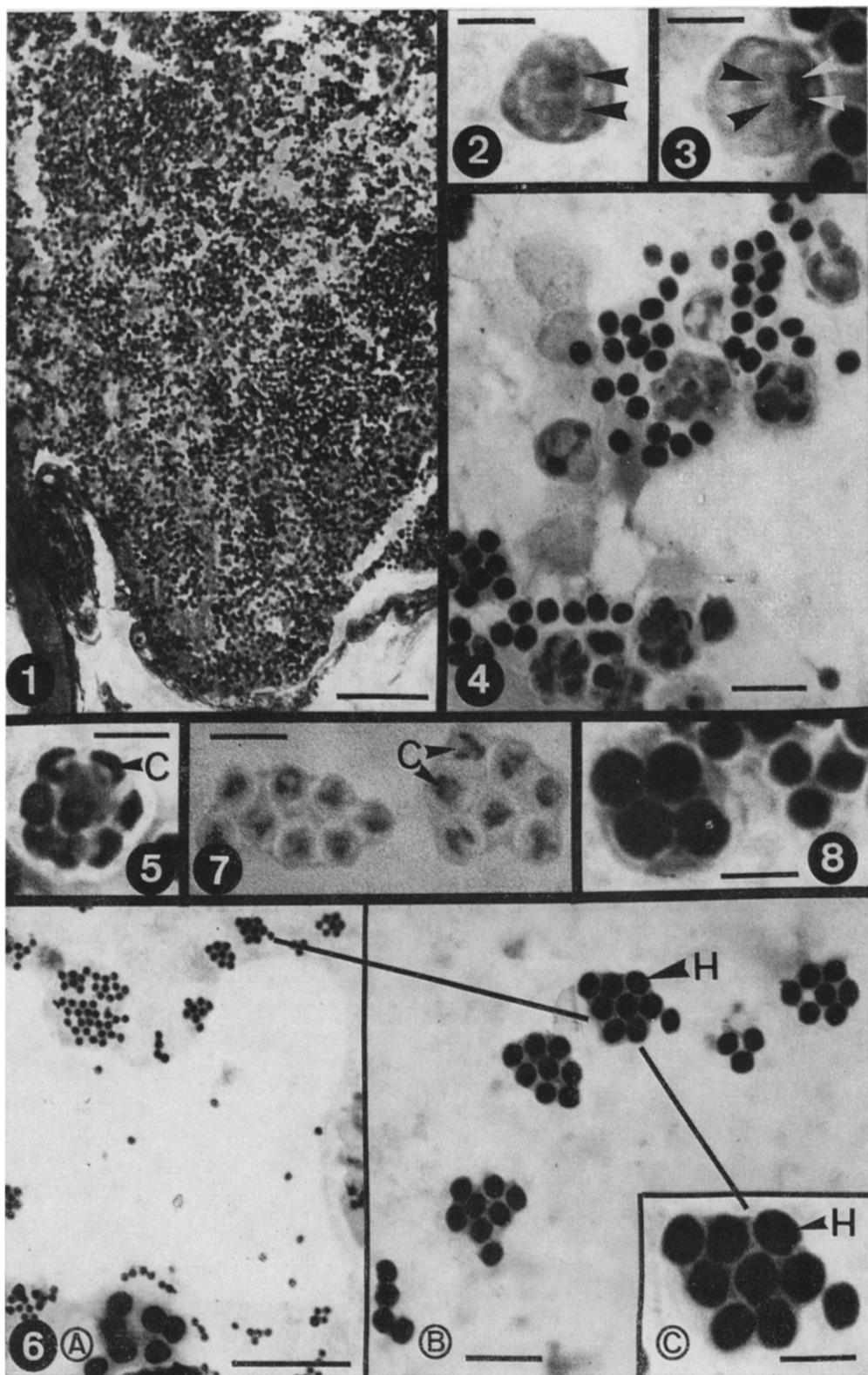
The diplokaryotic merozoites mature directly into diplokaryotic sporonts. Successive nuclear divisions result in a plasmodium with eight single nuclei. When the nuclear divisions have terminated the plasmodium splits up into eight lobes, and finally eight sporoblasts are produced. Each of these matures into a spore without further division. A pansporoblast membrane is secreted from the surface of the sporont and all sporogonial stages, including mature spores, are seen enclosed by this membrane.

In a small number of sporonts there is only one nuclear division and the plasmodium gives rise to four sporoblasts, finally resulting in a pansporoblast with only four spores.

### 2. Light microscopical appearance and histopathology

Infection was restricted to the adipose tissue and infected fat body lobes appeared to be completely filled with microsporidia (Fig. 1). These lobes were clearly visible through the semi-transparent cuticle as distinct whitish areas. Infection resulted in a decomposition of the cell-borders, and in each lobe groups of microsporidia appeared to be floating between the nuclei of the host cells. There was no sign of nuclear multiplication in the fat body induced by the microsporidia, and only a small number of nuclei could be identified in each lobe. They did not appear to be hypertrophied. The host-parasite association must be classified as a xenomena of the syncytial type (WEISER 1975). Pansporoblasts with sporoblasts or with mature spores occurred mixed with sporonts in the lobes.

In smears merozoites could not be distinguished from sporonts, and cells which could be of either type were found in a small number. These cells were rounded, with



the diameter 6—7  $\mu\text{m}$  (Fig. 2). Elongated diplokaryotic nuclei, where the longest diameter measured approximately 4  $\mu\text{m}$ , were located in the centre. In newly formed sporogonial plasmodia the four nuclei were located in the centre (Fig. 3). These nuclei were rounded and distinctly smaller than the nuclei of the sporonts, only about half the size. In sporongonial plasmodia at a later stage of development the nuclear shape was more diffuse and the nuclear material was seen as irregular spots near the periphery of the plasmodium (Fig. 4). The nuclei of older plasmodia took stain more intensely than nuclei of younger developmental stages.

Sporoblasts appeared as oval cells with a central mass of chromatin (Fig. 5). They were grouped into distinct and usually rounded pansporoblasts with the diameter 7—10  $\mu\text{m}$ . The pansporoblast membrane was not apparent, but as the material filling up the space between the sporoblasts and the pansporoblast membrane was distinctly stained, the shape of the pansporoblasts was easily observed and measurements could be made.

Mature spores were far most numerous. Dominant were microspores, with the dimensions 2.0—3.0  $\times$  2.0  $\mu\text{m}$  in fixed and stained preparations. They occurred in pansporoblasts with eight spores (Figs. 6—7). A small number of pansporoblasts

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Figs. 1—8. Light microscopical appearance of *Thelohania capillata*.

Fig. 1. A sectioned fat body lobe of a blackfly larva transformed into a syncytial xenoma by the microsporidium. Bar 50  $\mu\text{m}$ .

Fig. 2. A diplokaryotic stage, either merozoite or sporont. Bar 5  $\mu\text{m}$ .

Fig. 3. A sporongonial plasmodium with four small nuclei. Bar 5  $\mu\text{m}$ .

Fig. 4. Pansporoblasts at different stages of development, and mature spores. Bar 10  $\mu\text{m}$ .

Fig. 5. A pansporoblast with 8 sporoblasts. Bar 5  $\mu\text{m}$ .

Fig. 6. A smear from slide no. 801020-L-1 RL showing octosporous pansporoblasts. The spore selected as holotype is indicated at higher magnifications. Bars: 6A—6B 10  $\mu\text{m}$ , 6C 5  $\mu\text{m}$ .

Fig. 7. Two octosporous pansporoblasts in a hydrolyzed smear stained by the GIEMSA technique. The chromatin appears as parallel strands. Bar 5  $\mu\text{m}$ .

Fig. 8. A pansporoblast with four macrospores adjacent to some microspores. Bar 5  $\mu\text{m}$ . Stainings, except for Fig. 7, HEIDENHAINS iron haematoxylin.

Figs. 9—35. Ultrathin sections.

Fig. 9. Part of a fat body lobe with microsporidia in different stages of development. Bar 1  $\mu\text{m}$ .

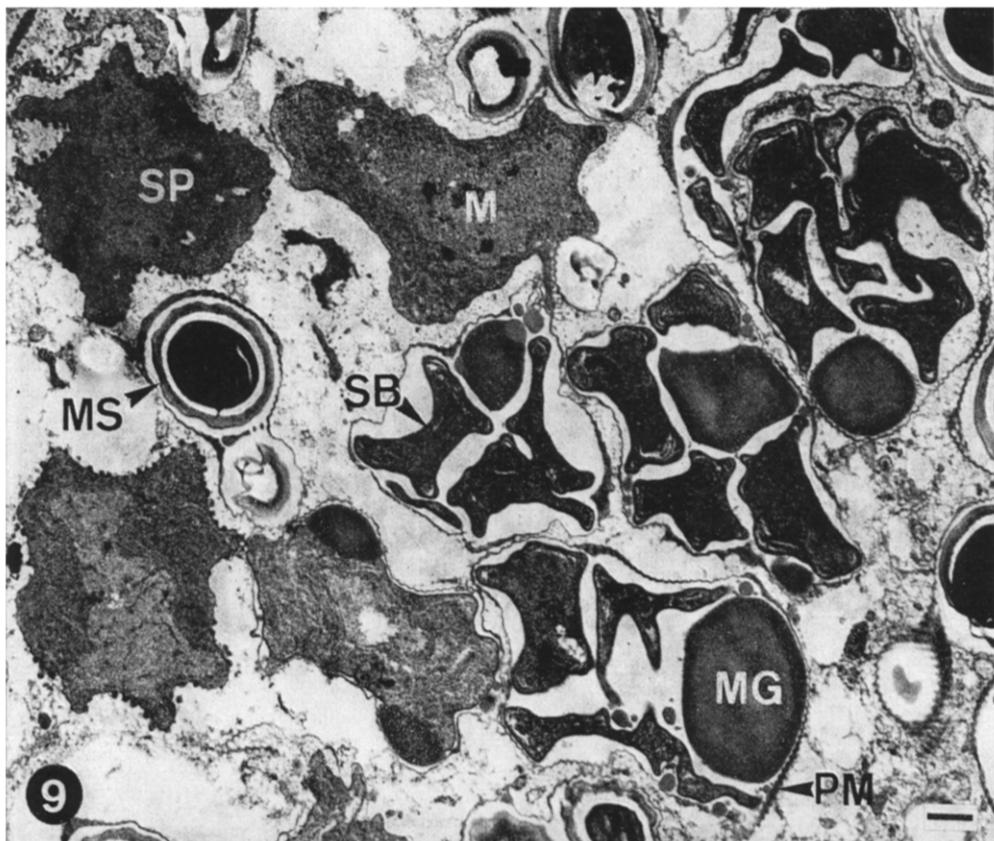
Fig. 10. A merozoite with a thin cellular membrane. Bar 0.5  $\mu\text{m}$ .

Fig. 11. A sporont with a thick cell wall and the first signs of the developing pansporoblast membrane. Bar 1  $\mu\text{m}$ .

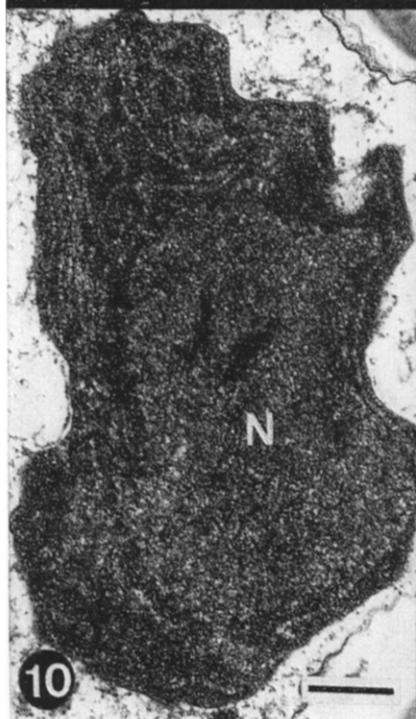
Figs. 12—15. Development of the sporont cell wall and the pansporoblast membrane.

Fig. 12. The periphery of a sporont. The pansporoblast membrane is formed from the electron-dense surface layer of the sporont. Bar 0.5  $\mu\text{m}$ .

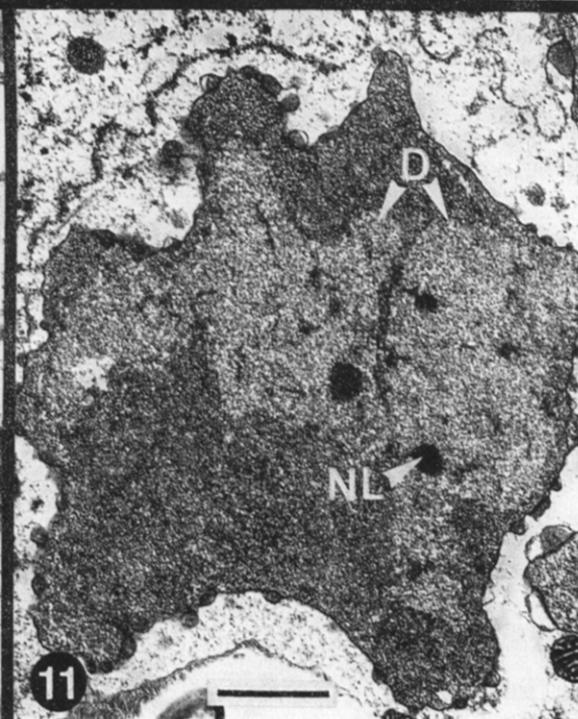
Figs. 13—15. Thin-walled vesicles close to the cellular membrane take part in the production of the sporont wall and the pansporoblast membrane. Bars 0.5  $\mu\text{m}$ .



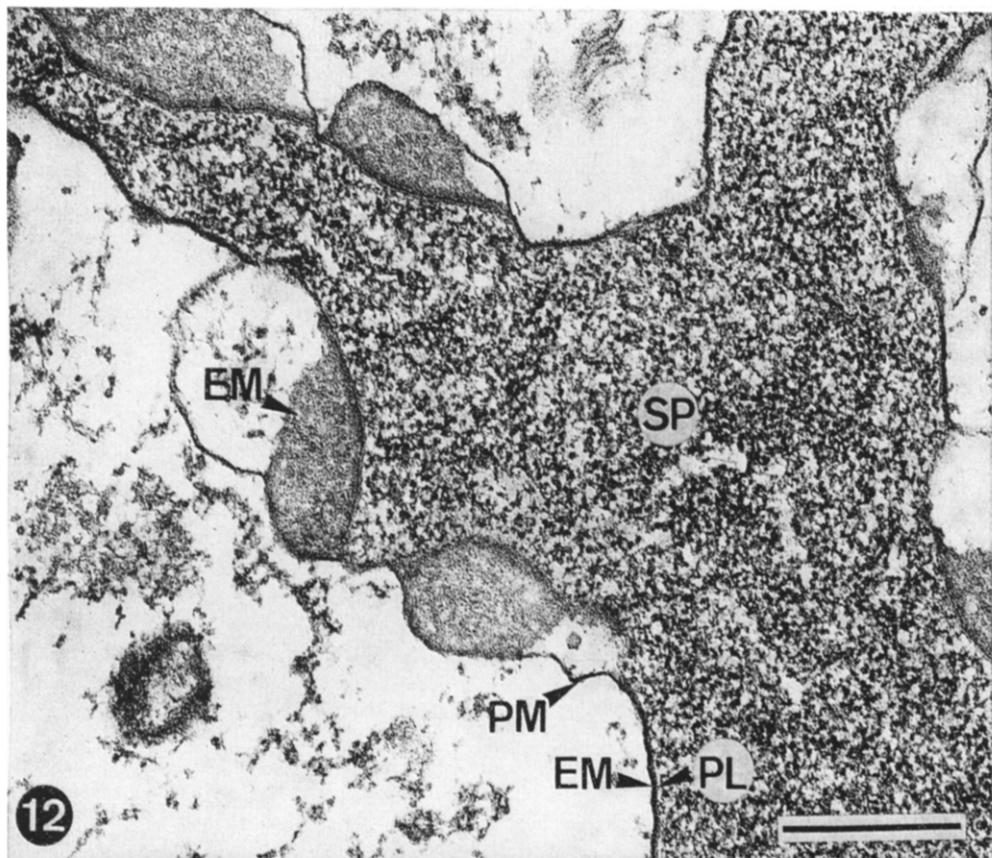
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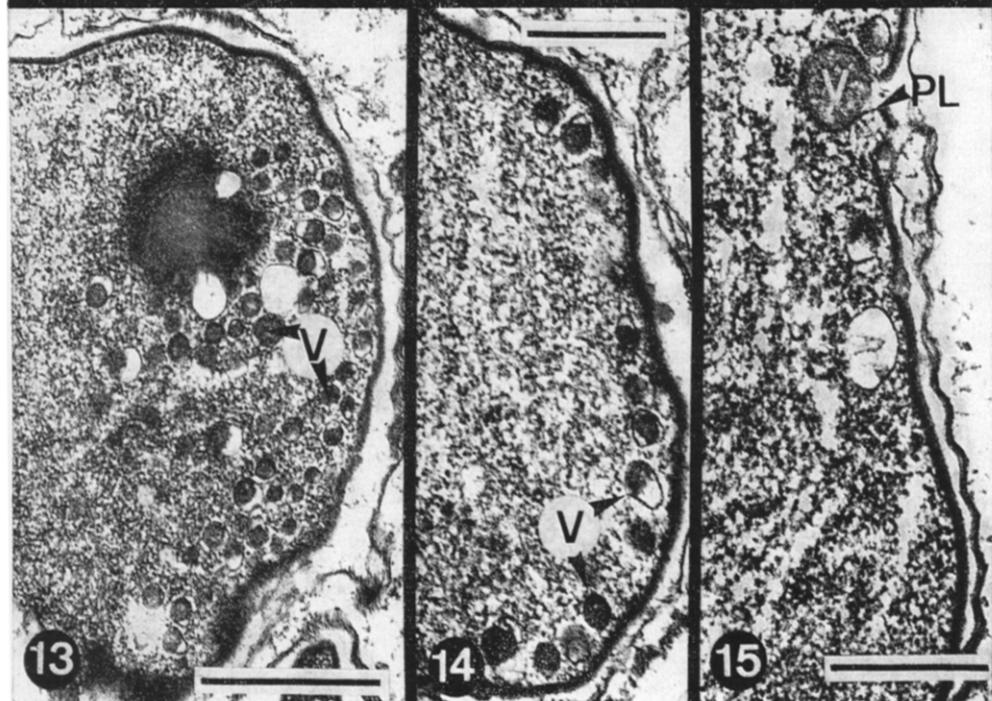
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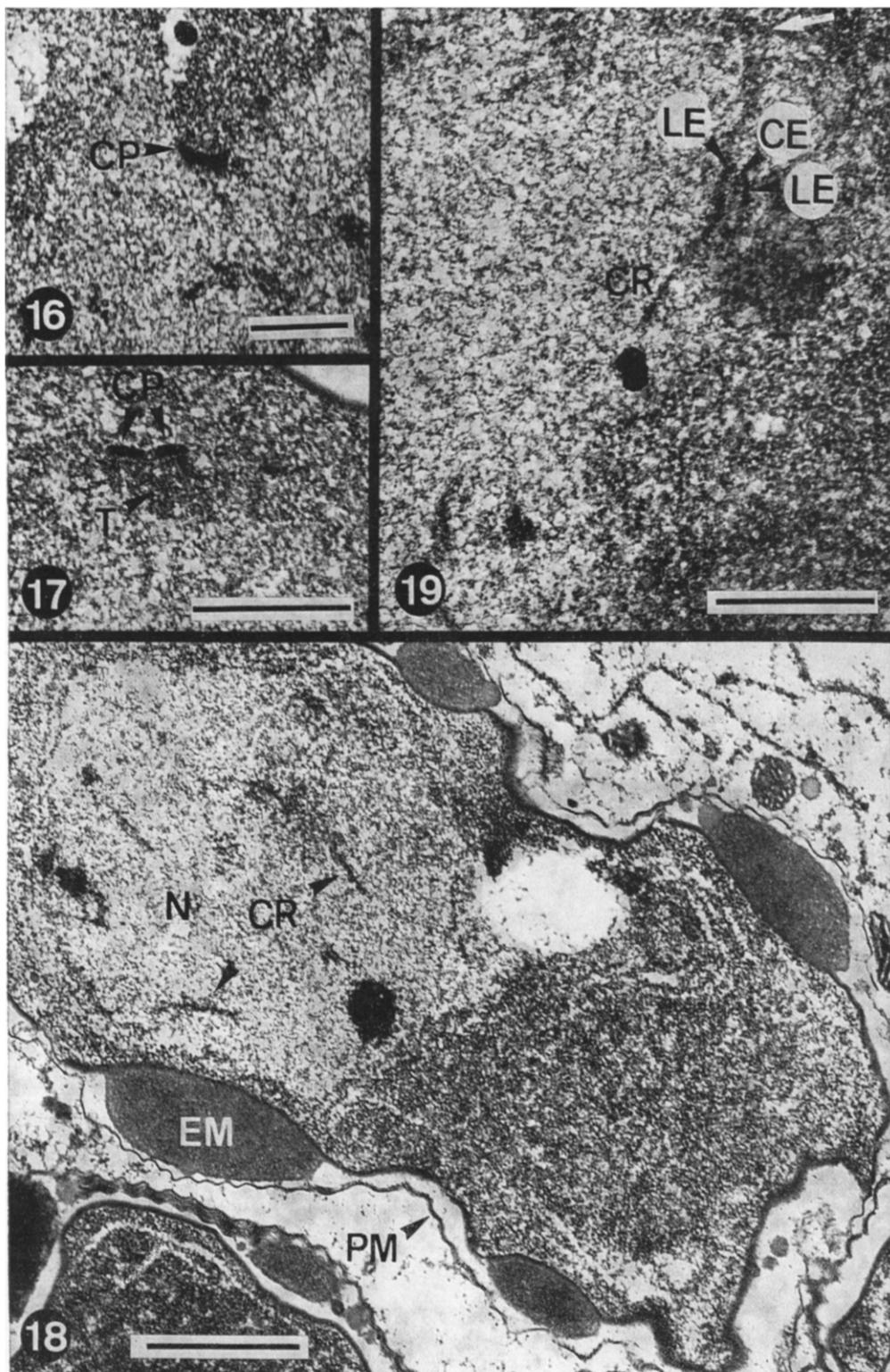
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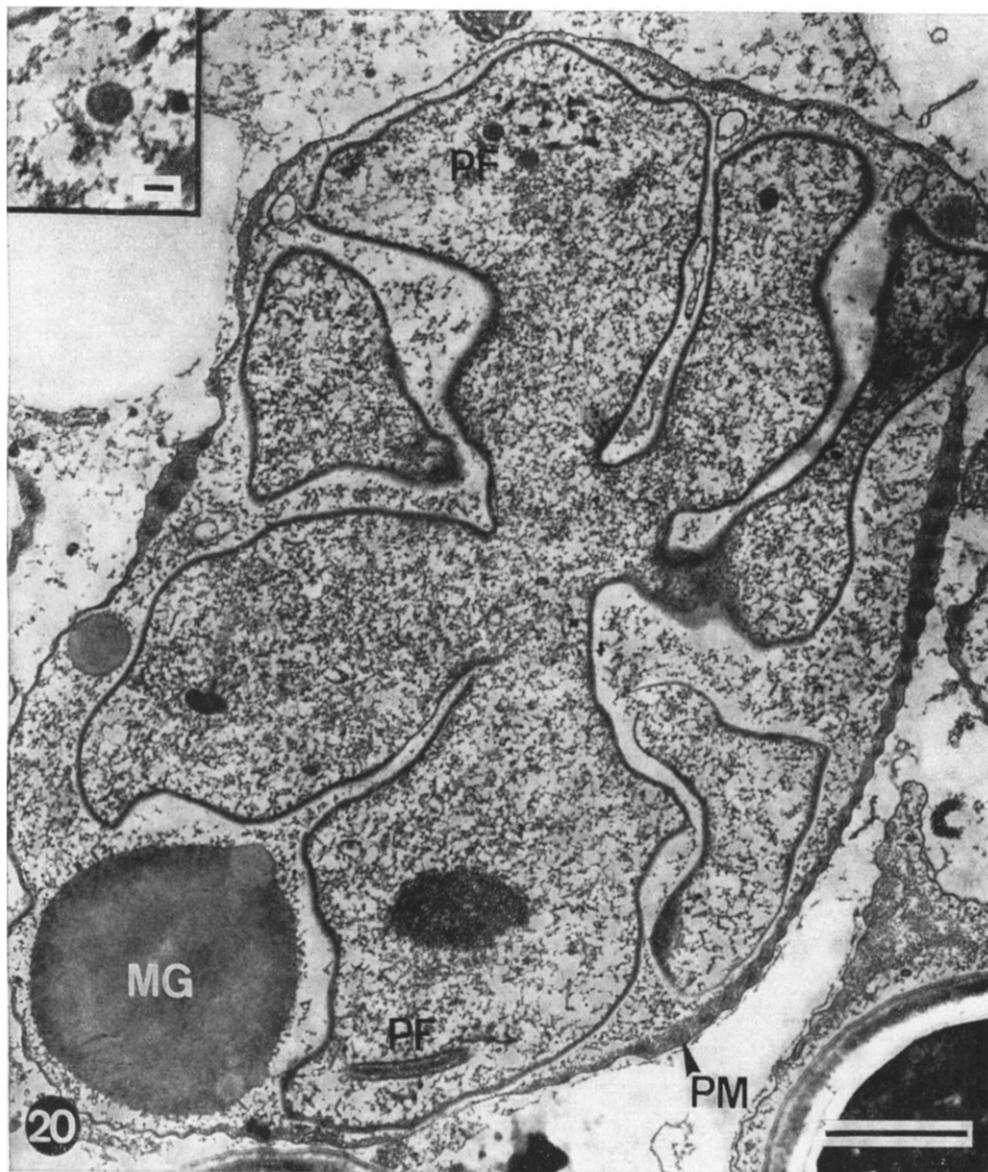


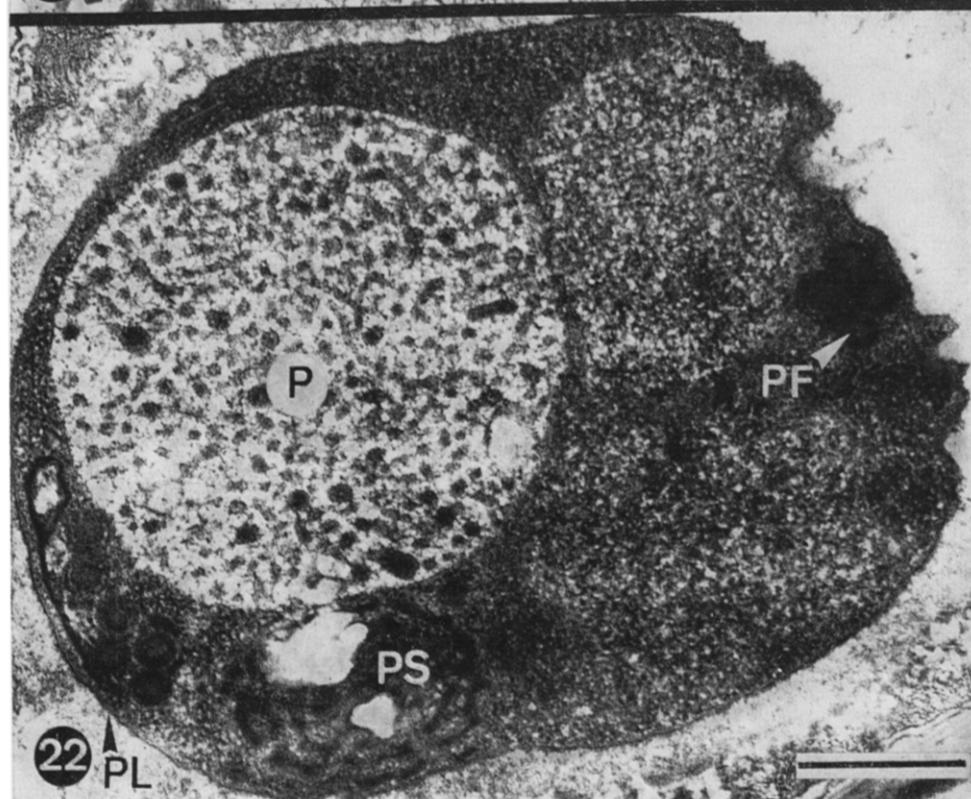
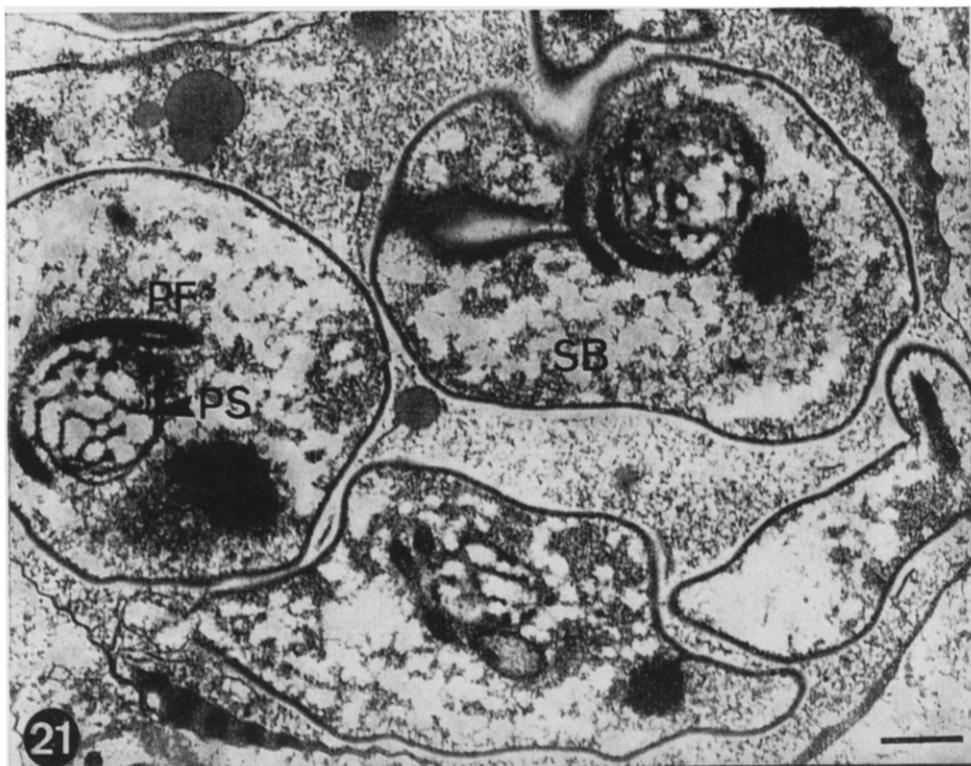
Fig. 20. A pansporoblast with a lobed sporogonial plasmodium. The first coils of the polar filament are visible, and metabolic granules are apparent in the pansporoblast cavity. Bar 1  $\mu$ m. Inset: A newly formed polar filament coil in transverse section. Bar 100 nm.

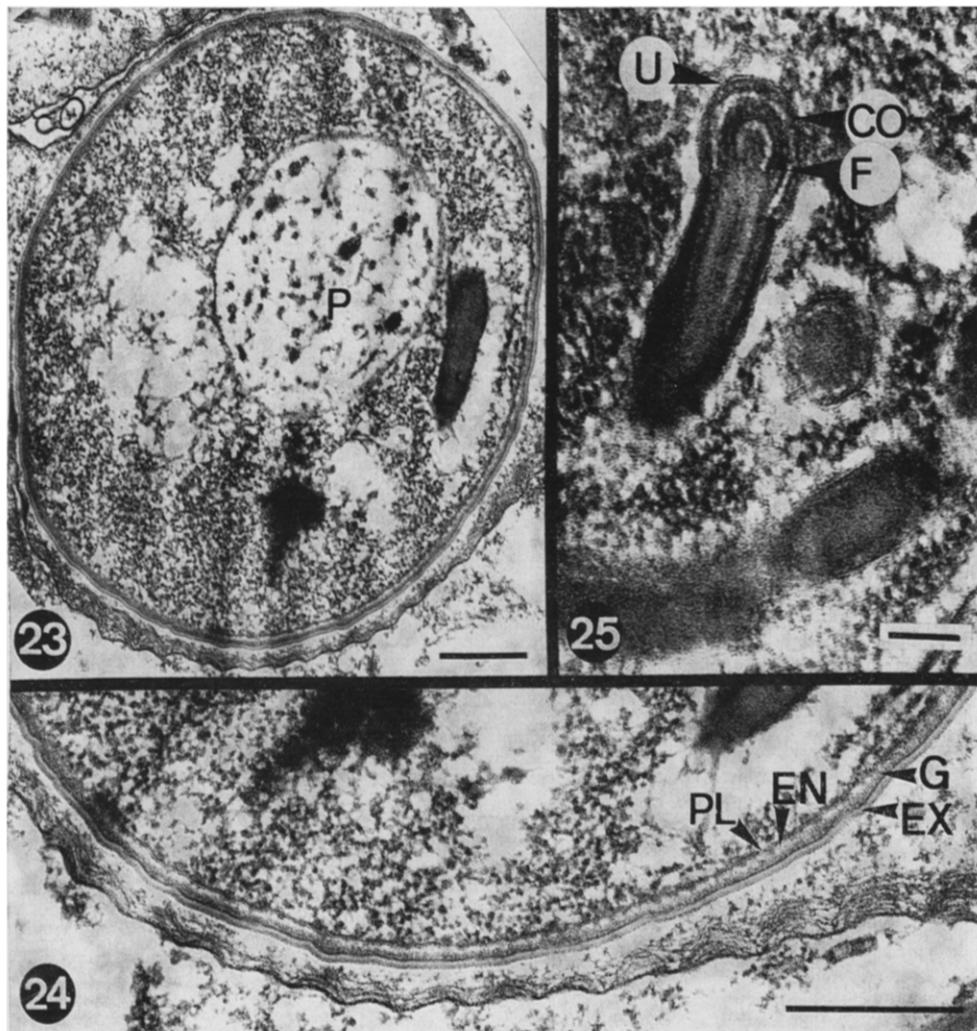
Figs. 16–19. The meiosis.

Figs. 16–17. Invaginations of the nuclear membrane with electron-dense centriolar plaques. Bars 0.5  $\mu$ m.

Fig. 18. The nuclear membrane of the dividing sporont is hardly visible, but chromosomes are apparent as dark strands. Bar 1  $\mu$ m.

Fig. 19. A synaptonemal complex with two lateral and one central element. One chromosome can be followed from the nuclear periphery (white arrow). Bar 0.5  $\mu$ m.





Figs. 23—24. A young spore where the different layers of the spore wall are distinctly seen. Bars 0.5  $\mu$ m

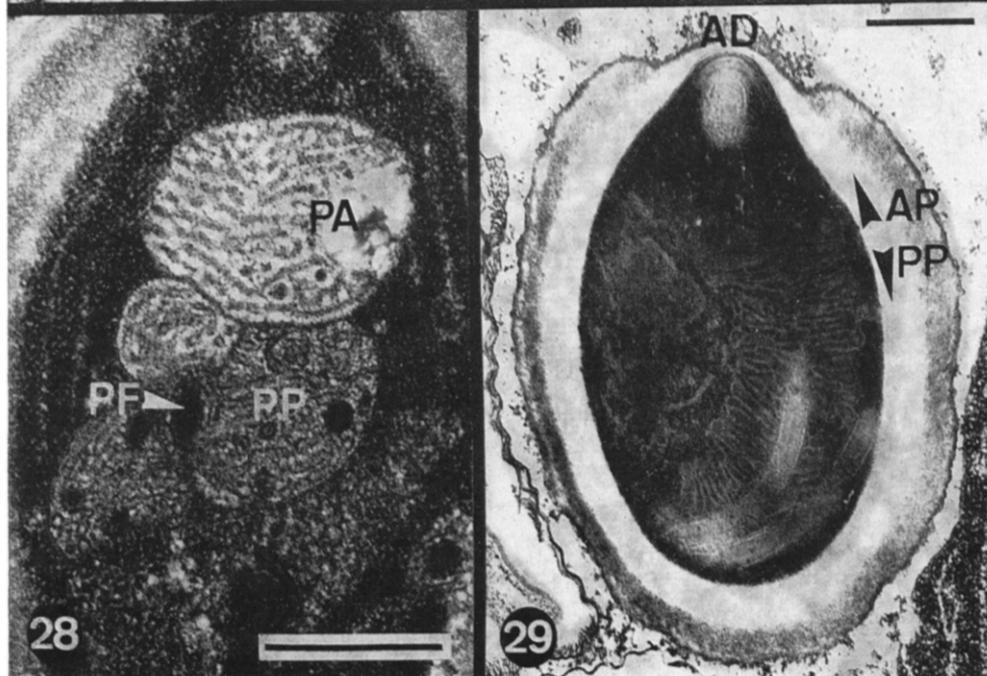
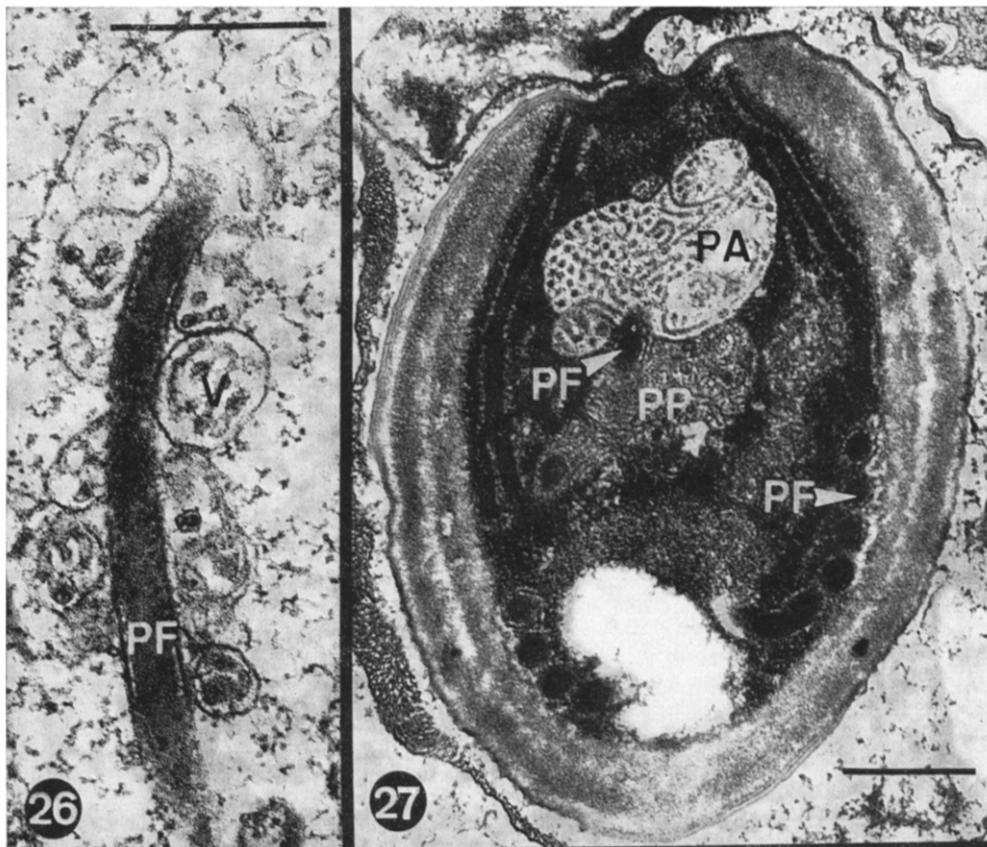
Fig. 25. Sections at different planes through polar filament coils. In the obliquely sectioned coil the surrounding unit membrane and the different internal strata are visible. Bar 100  $\mu$ m.

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Figs. 21—22. The sporoblast.

Fig. 21. A pansporoblast with sporoblasts. The polar filament coils are initiated from a spongy posterosome. Bar 0.5  $\mu$ m.

Fig. 22. A sporoblast, only surrounded by a thin plasmalemma, but with a fully developed polar filament. Bar 0.5  $\mu$ m.



contained only four macrospores (Fig. 8), measuring  $3.5-4.5 \times 3.0 \mu\text{m}$ . Both spore-types were barrel-shaped, sometimes with one end lightly rounded, and they were stained in an identical way. Using haematoxylin staining the chromatin was seen as an irregular dark mass in the centre of the spore, and at the posterior pole a weakly stained oval body usually could be identified. In some spores a small, almost clear spot was seen in the anterior end. Spores stained with the GIEMSA technique revealed the chromatin as a lobed dark area or two dark parallel strands (Fig. 7). Minute metabolic granular inclusions were seen in some of the pansporoblasts.

A number of spores at the periphery of the smears had extruded their polar filament. These were stained in a very faint tone and it was difficult to measure their length precisely. It was found to be approximately  $70 \mu\text{m}$ . In a few extruded filaments the single nucleus could be seen as a long darkly stained swelling. The nuclei of this microsporidium appeared to migrate at fast rate. The smears were only allowed to dry for one or two minutes prior to fixation. However, in this short time the filament was ejected and the nucleus passed half the way through the filament.

### 3. Ultrastructure of the sporont and the sporoblasts

In the material processed for electron microscopy merogony could be verified by the occurrence of a small number of merozoites (Figs. 9—10). These appeared as slightly irregular cells with a central diplokaryon. Both nuclei and cytoplasm were electron-dense and in the cytoplasm ribosomes occurred numerously. This stage was identified by the simple cell wall, a unit membrane about 9 nm thick.

Sporonts were seen as rounded or elongated cells (Figs. 9 and 11), with a diameter not exceeding  $9.3 \mu\text{m}$ . The cytoplasm of the newly formed sporont was densely granular, as in the merozoite stage, but during maturation it became less dense, with regularly spread ribosomes. In older sporonts a part of the ribosomes appeared to be arranged on membranes. The cell wall was 17—20 nm thick, with a layer of electron-dense material outside the plasmalemma (Fig. 12). Successively the outer layer was set free from the plasmalemma, at first appearing as blisters at irregular intervals, and finally the contact between this layer and the cell-wall was completely broken. The dense material formed a fibrous pansporoblast membrane.

A new thick sporont wall was successively produced after the formation of the pansporoblast membrane. At this stage numerous vesicles appeared in the peripheral

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Figs. 26—29. Different stages in the development of the polaroplast.

Fig. 26. The first signs of the polaroplast are thin-walled vesicles transversed by a tubular material. The vesicles are associated with the anterior region of the polar filament. Bar  $0.5 \mu\text{m}$ .

Fig. 27. The vesicles are united to the anterior polaroplast region. The posterior region, with irregularly and closely arranged tubules, is initiated below this. Bar  $0.5 \mu\text{m}$ .

Fig. 28. At a later stage the tubular material of the anterior region is arranged perpendicularly to the long axis of the spore. Bar  $0.5 \mu\text{m}$ .

Fig. 29. In a mature spore the polaroplast reaches the posterior pole. The anterior part, with regularly and closely arranged lamellae, is about one thirs of the total length. Bar  $0.5 \mu\text{m}$ .

parts of the sporont cell (Fig. 13), where they could be found in a layer close to the cellular membrane (Fig. 14). Most vesicles were small, 75—160 nm wide, and they were filled with a granular content. They appeared to communicate with the cellular membrane (Fig. 15), and they were supposed to be engaged in the production of the thick cell-wall.

The diplokaryon of the sporont was slightly less electron-dense than the cytoplasm (Fig. 11). It had usually an irregular outline and occupied more than half the volume of the sporont. The diameter did not exceed 5.1  $\mu\text{m}$ . Distinct nucleoli were regularly seen. In most nuclei signs of division were apparent. Centriolar plaques could be seen as electron-dense areas at the periphery of the nuclear membrane, often lightly invaginated (Fig. 16). The arrangement of two closely situated centriolar plaques was also observed (Fig. 17). The diameter of a plaque varied between 130—280 nm. Chromosomes were visible as dark strands (Fig. 18) and synaptonemal complexes, a feature of meiotic chromosome pairing at zygotene and pachytene, could be identified (Fig. 19). The complex was approximately 110 nm wide, with two electron-dense lateral elements, about 30 nm thick, and a less dense, thinner central element, approximately 20 nm. The occurrence of synaptonemal complexes indicated that the first division of the sporont is a meiosis.

Normally the meiosis was followed by a mitosis, resulting in a plasmodium with 8 nuclei, which was further split into 8 lobes (Fig. 20). Finally the plasmodium was broken up into 8 sporoblasts with a vesicular cytoplasm (Fig. 21), which matured into spores without further division. The morphogenesis of the sporal organelles was not completely correlated to the maturation of the spore wall, and in sporoblasts only enclosed by a thin plasmalemma a polar filament with a complete number of filament coils could be found (Fig. 22). Outside the plasmalemma a stratified spore-wall developed. In the well defined cortical part, approximately 35 nm thick, two strata of different electron density were seen (Figs. 23—24). The inner of these layers was the most electron-dense. Between the cortex and the plasmalemma a third layer with a granular structure was initiated (Fig. 24). This layer was almost clear near the plasmalemma and more granular below the cortex. The translucent part is interpreted as the first sign of the endospore layer, while the granular stratum is considered to be the innermost part of the exospore.

#### 4. Morphogenesis of the polar filament and the polaroplast

The first signs of the polar filament were visible before the sporoblasts were formed from the plasmodium (Fig. 20). The filament developed in close association with a spongiuous body (Fig. 21), a structure previously described from microsporidia under the name posterosome (see e.g. VÁVRA 1976). Transverse sections through a newly formed filament coil revealed a substructure reminding of one tube inserted into another (Fig. 20). At this stage the polar filament was approximately 150 nm wide. Slightly later, in the immature spore, the filament was composed of several layers grouped into five strata, where three were electron-dense and the other two translucent (Fig. 25). The filament was surrounded by a unit membrane, which together

with two more layers formed a dense cortex. The clear space below this appeared to be composed of fibrillar or globular structures. In the middle dense region of the filament three layers could be distinguished, and separated from this region by a translucent space was a dense core with diffuse layers.

Surrounding the straight part of the polar filament of the sporoblast was a layer of thin-walled vesicles with the diameter 180—360 nm (Fig. 26). They were enclosed by a thin membrane, approximately 5 nm thick, which appeared to be of unit membrane nature, and they were transversed by a system of narrow tubules, about 35 nm in diameter. During the further development these small vesicles coalesced to a few greater vesicles (Fig. 27), which were later united to a single organelle. The arrangement of the tubules became successively more regular, almost perpendicular to the long axis of the spore (Fig. 28). This organelle is interpreted as the primordium of the anterior polaroplast region. The posterior region of the polaroplast differentiated immediately below this. The first signs were granular vesicles and at a later stage the primordia could be seen as vacuoles filled with a closely arranged tubular network (Fig. 28). In the mature spore both polaroplast regions were lamellar. The lamellae of the anterior part were thin and closely arranged, while the lamellae of the posterior region appeared more voluminous and were irregularly folded. The polaroplast was long, reaching over the entire length of the spore (Fig. 29).

##### 5. Ultrastructure of the mature spore

Mature spores were surrounded by a thick cell wall, 280—350 nm, composed of distinct layers (Fig. 30). The inner plasmalemma was a thin unit membrane, approximately 10 nm thick, and the middle layer was an electron-translucent endospore with a maximum thickness of 180 nm. Above the extrusion apparatus the endospore was thin, only about 20 nm. The exospore, the outer layer, was composed of two structurally different elements. The surface layer was electron-dense, approximately 35 nm thick, and of an identical construction to the layer surrounding the sporoblast (Fig. 24). This layer surrounded the spore completely. Between this layer and the endospore was a granular stratum, with a maximum thickness of 160 nm. This layer was missing in the anterior end of the spore. The periphery of the spore appeared slightly irregular due to the variable thickness of the granular basal layer of the exospore.

The polar filament was of uniform thickness over its entire length, 120—140 nm. Transverse sections revealed the same substructure as described in the immature spore (Figs. 25 and 30). Most prominent was a layer composed of approximately 15 electron-translucent rounded structures. In transversely sectioned filament coils it was not clear if the components of this layer were globules or fibrils. In longitudinally or obliquely sectioned coils the structure of this layer was identical to the appearance in transverse sections (Fig. 32), indicating that the subunits might be globules. VÁVRA (1975) identified in sporoblasts of *Tuzetia debaisieuxi* a layer of spiraled fibrils in the same position. The umbrella-shaped anchoring disc was relatively small, not wider than 370 nm (Fig. 29). It had a fine structure of superimposed layers

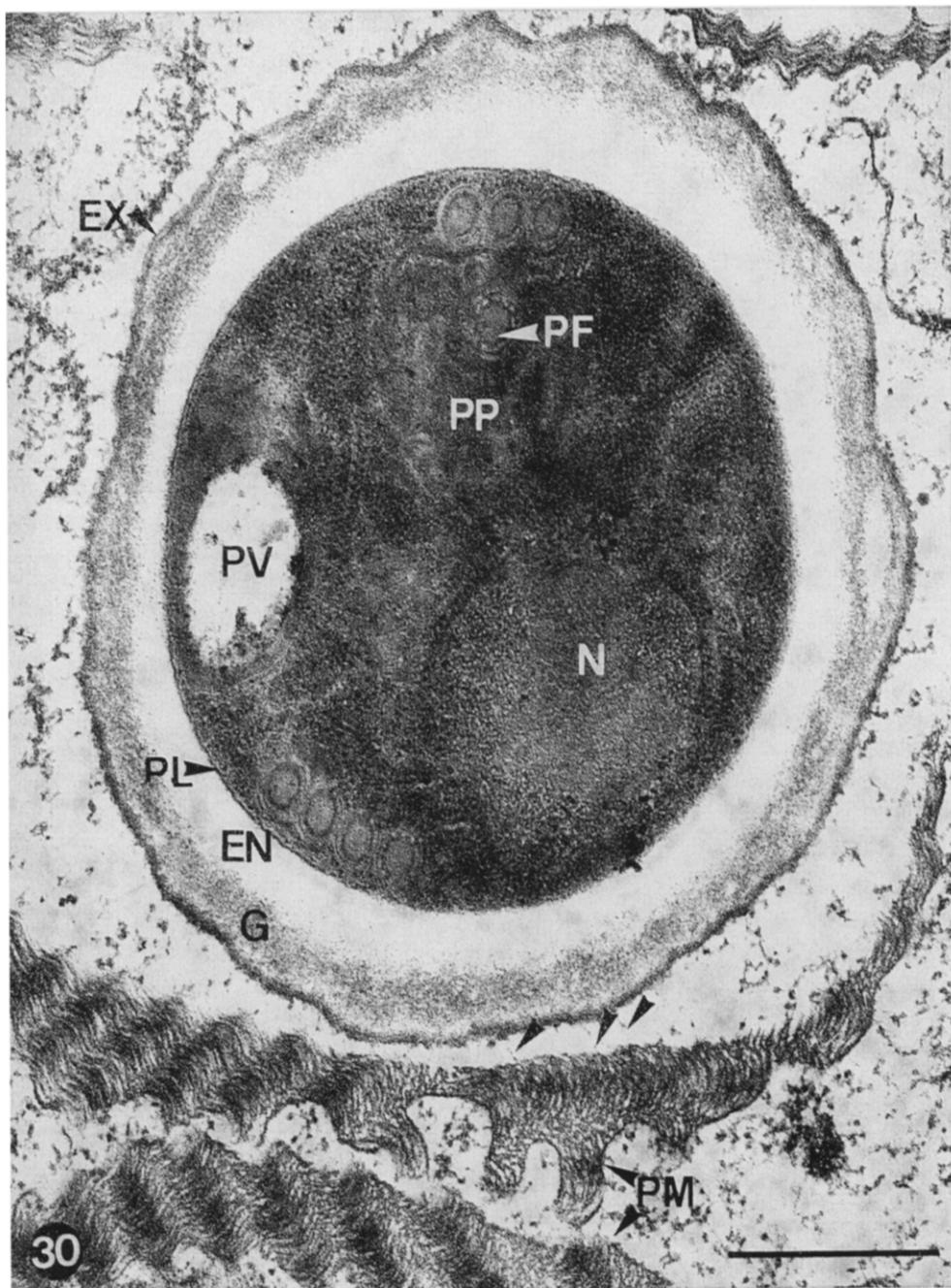
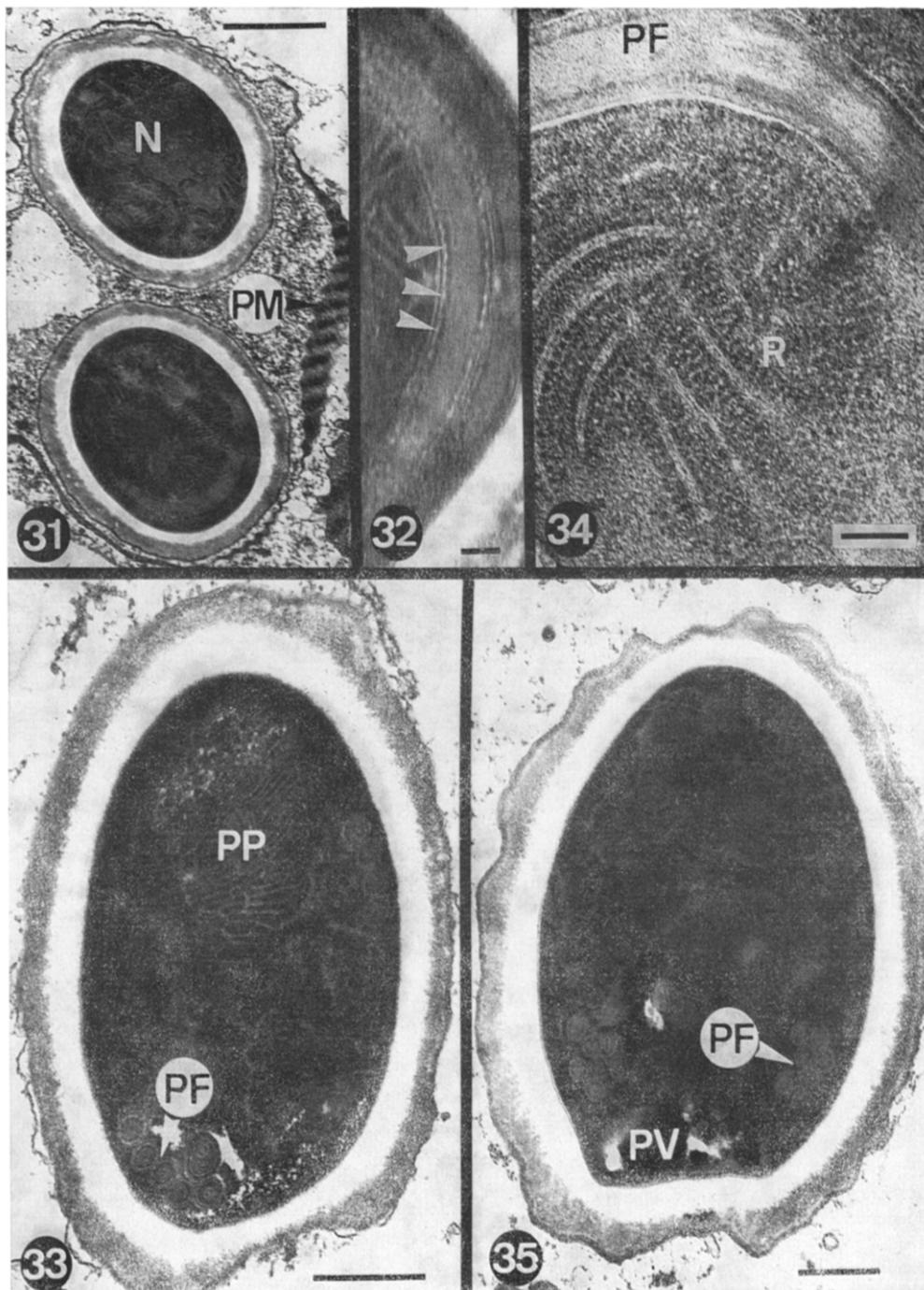


Fig. 30. A transversely sectioned mature spore with the different layers of the spore coat fully developed. The pansporoblast membrane is formed from a fibrous material which is continuous from the exospore to the pansporoblast membrane (arrows). Bar 0.5  $\mu\text{m}$ .



Figs. 31—35. The morphology of mature spores.

Fig. 31. A pansporoblast with two microspores. Bar 1  $\mu\text{m}$ .

Fig. 32. A longitudinal section at the periphery of a polar filament coil. The most translucent layer appears as globular structures. Bar 100 nm.

Fig. 33. A microspore with 4—5 polar filament coils. Bar 0.5  $\mu\text{m}$ .

Fig. 34. Cytoplasm of a spore with numerous membrane-associated ribosomes. Bar 100 nm.

Fig. 35. A macrospore with 8 polar filament coils. Bar 0.5  $\mu\text{m}$ .

of different electron density. The attachment of the filament was slightly swollen. The straight part of the filament reached from the anchoring disc to the posterior wall of the spore, and the terminal part was arranged as 4—5 coils in a single layer (Figs. 29 and 33). The angle of tilt of the anterior filament coil was about 45 degrees to the longitudinal axis of the spore. The straight part of the filament was completely surrounded by the polaroplast. The anterior region, with thin regularly arranged lamellae, was about one third of the polaroplast length, and in the longer posterior part the lamellae were more loosely folded (Fig. 29). The cytoplasm was densely granular, containing numerous ribosomes. They were helically arranged and in some spores it was clearly seen that they were located on membranes (Fig. 34). The single nucleus was found in the centre of the spore (Figs. 30—31), but it was not clearly visible in all spores. In the posterior part of the spore was a vacuole (Figs. 30 and 35).

Apart from microspores, which measured  $2.6-3.1 \times 2.1-2.7 \mu\text{m}$ , a small number of macrospores were found. They were  $3.5-4.0 \mu\text{m}$  long and approximately  $2.8 \mu\text{m}$  wide. They were morphologically identical to microspores, except for the polar filament. Also in macrospores the filament thickness was nearly homogeneous all over the length,  $125-150 \mu\text{m}$  in diameter, but it was arranged as 8 irregularly stacked coils in the posterior region of the spore (Fig. 35). No macrospores were sectioned in such a way to allow the angle of tilt to be calculated.

## 6. The pansporoblast

The pansporoblast membrane was formed from the cell-wall of the young sporont (Fig. 12). It appeared originally to be thin layer of dense material, not a unit membrane. Tangentially sectioned parts revealed a fibrous construction. It developed into a prominent structure of hair-like material in a wavy arrangement (Fig. 31). The content of the pansporoblast cavity appeared to be finely granular (Figs. 20 and 31), but the granules revealed to be transversely sectioned fibrils. Also in pansporoblasts with mature spores the fibrils could be seen to be continuous from the surface of the spore to the pansporoblast membrane (Fig. 30). A dense material was produced from the sporont surface inside the blister-like primordia of the pansporoblast membrane (Figs. 12 and 18). These metabolic products grew larger, originally attached to the surface, but before the sporoblasts were set free they lost contact with the surface and occurred as free crystal-like bodies in the pansporoblast cavity (Fig. 20). The granules had almost disappeared in pansporoblasts containing mature spores.

## Discussion

Although the genera of the family Thelohaniidae are based on ultrastructural characteristics usually only the fine structure of the spore is known. However, in the genera *Amblyospora* and *Thelohania* there is a number of species where more developmental stages have been investigated ultrastructurally. In the genus *Amblyospora* such studies have been published e.g. by LIU and DAVIES (1972, 1973), ANDREADIS

and HALL (1979) and LARSSON (1981). Eight *Thelohania* species have been studied using electron microscopy: *T. butleri* by VERNICK, SPRAGUE and KRAUSE (1977) and by JOHNSTON, VERNICK and SPRAGUE (1978), *T. ceccaldi* by VIVARÈS (1975), *T. contejeani* by MAURAND and VEY (1973) and by COSSINS and BOWLER (1974), *T. duorata* by KELLEY (1975), *T. maenadis* by VIVARÈS (1975, 1980), *T. minispora* by GASSOUMA and ELLIS (1973), *T. octospora* by CODREANU, CODREANU-BALCESCU and PORCHET-HENNERÉ (1974) and by VIVARÈS (1975), and *T. solenopsae* by KNELL, ALLEN and HAZARD (1977). Except for *T. solenopsae*, which is a parasite of the fire ant, *Solenopsis invicta*, and *T. minispora*, a parasite of blackfly larvae, all species are from crustacean hosts. They are clearly different from the Swedish microsporidium, not only by their occurrence in unrelated hosts, but also from ultrastructural and developmental characteristics. The hosts of the Swedish species and *T. minispora* are identical, but differences, especially in the ultrastructure of sporogonic stages and matures spores, indicate that they are different species.

The occurrence of two different sporogonial cycles in the development of the insect parasitic species *T. solenopsae* KNELL, ALLEN and HAZARD 1977, is of special interest. Octospores with single nuclei are produced in one cycle while the other yields diplokaryotic free spores. These two types of cycles have been shown to be characteristic for several insect pathogenic species of *Amblyospora*, and were e.g. described from *A. polykarya* by LORD, HALL and ELLIS (1981) and from *Amblyospora* sp. by ANDREADIS and HALL (1979). They have also been reported from *Parathelohania* by HAZARD and WEISER (1968). Usually one cycle occurs in the larval stage, the other in adults. However, there are *Amblyospora* species where both types of spores are found together in the same stage of the host, e.g. reported by HAZARD and OLDACRE (1975) from *A. callosa*. *T. capillata*, the species from Swedish blackfly larvae, produces micro- and macrospores, both with single nuclei, and both types are octospores or modified octospores. Free diplokaryotic spores are not produced in larvae. It is not known if there is a second type of sporogony in adult blackflies.

In numerous papers it has been shown that the pansporoblast cavity of microsporidia of the family Thelohaniidae is not an empty space. In several species of *Amblyospora* crystal-like metabolic granules appear in the pansporoblast, e.g. reported by HAZARD and OLDACRE (1975), ANDREADIS and HALL (1979) and LARSSON (1981). In the genus *Cryptosporina* numerous granules nearly obscure the spores (HAZARD and OLDACRE, 1975). Tubular structures were reported from the pansporoblast cavity of *Thelohania maenadis* by VIVARÈS (1980), and in *Toxoglugea variabilis* tubules of two different dimensions occur together (LARSSON 1980). Most commonly the material has been described as filamentous. Such structures have distinctly been demonstrated by the use of scanning electron microscopy (RAUSCH and GRUNEWALD 1980; VÁVRA, BARKER and VIVARÈS 1981). In both the genera *Amblyospora* and *Thelohania* the fibres appear to be continuous from the spore wall to the pansporoblast membrane. In *T. maenadis* tubules and fibrillar material are found together (VIVARÈS 1980). In *T. capillata* the material of the pansporoblast cavity is fibrillar,

no tubules were observed, and also in this species the material appears to be continuous from the exospore to the pansporoblast membrane (Fig. 30).

Synaptonemal complexes, indicative of meiosis, have been observed in some microsporidian species, but so far the occurrence of karyogamy is still an enigma. The biological consequences of meiosis in the development of microsporidia were discussed by LOUBÈS (1979), and meiosis was documented from 6 microsporidian genera belonging to different families. From *Thelohaniidae* meiosis was reported from *Thelohania fibrata* (STRICKLAND 1913), which according to micrographs in a paper by MAURAND and LOUBÈS (1978) is an *Amblyospora* species, and from an undescribed species of *Thelohania*. HAZARD, ANDREADIS, JOSLYN and ELLIS (1979) reported meiosis from one undescribed species of *Amblyospora* and from several species of *Parathelohania*. VIVARÈS (1980), in a treatise on the ultrastructure of *Thelohania maenadis* PEREZ, 1904, postulated the occurrence of meiosis in the development of this microsporidium, comparing with the condition of *Ameson pulvis* where it had been documented. In sporonts of the Swedish *Thelohania* species synaptonemal complexes have frequently been observed, indicating that the first division of the sporont is a meiosis.

The *Thelohania* species which is most closely related to the new Swedish microsporidium concerning ultrastructure and development is *T. maenadis*. VIVARÈS (1975, 1980) described for this species a sporogony which is similar to the sporogony of *T. capillata*. The nuclei of the diplokaryotic sporont divide producing a sporogonial plasmodium with 8 single nuclei, and the 8 sporoblasts are simultaneously set free. This development can be compared to the sporogony of *T. butleri* where a series of binary fissions finally result in the formation of 8 sporoblasts (JOHNSTON, VERNICK and SPRAGUE 1978). In this species there is no sporogonial plasmodium. Another similarity between the Swedish species and *T. maenadis* is the early development of the polar filament. In both species the first filament coils are observed already in the lobed sporogonial plasmodium. In many microsporidia the polar filament is not beginning to develop until the sporoblasts have been separated from the plasmodium. Further similarities between the species are e.g. the occurrence of both micro- and macrospores and the fine structure of the spore wall. However, there are also morphological differences between these two species, e.g. spore dimensions and the number of polar filament coils.

There are several microsporidia more or less clearly related to the family *Thelohaniidae* described from Simuliidae. Originally described in the genus *Thelohania* are: *T. avacuolata* GASSOUMA, 1972, *T. bertrami* GASSOUMA, 1972, *T. canningae* GASSOUMA, 1972, *T. minuta* GASSOUMA, 1972, *T. simulii* GASSOUMA, 1972, and *T. columbaczense* WEISER, 1960. *T. minuta* GASSOUMA, 1972, is homonymous with *T. minuta* KUDO, 1924, a different microsporidian species, and the species by GASSOUMA was renamed by SPRAGUE (1977) to *T. minispora*. Three species originally described in the genus *Glugea* have been transferred to *Thelohania*: *T. bracteata* (STRICKLAND 1913), *T. fibrata* (STRICKLAND 1913) and *T. varians* (LÉGER 1897). HAZARD and OLDACRE (1975) revised the microsporidia close to *Thelohania*, and both *T. fibrata* and *T. varians*

were considered not to belong to the family Thelohaniidae. The species described by GASSOUMA were not treated in the revision. These species were described solely based on sporal dimensions. The spores were of identical shape, except for *T. avacuolata*, which had truncate oval spores instead of elliptical. The astonishing fact that GASSOUMA did not recognize any previously known microsporidian species in the samples studied was pointed out by MUU (1977). In this paper it was clearly demonstrated that the species described by GASSOUMA, using sporal dimensions as distinguishing characters, readily fit into the variation of the previously known species, and the species were synonymized in the following way: *T. minuta* and *T. avacuolata* are synonyms for *T. bracteata* (STRICKLAND 1913), *T. bertrami* and *T. canningae* are synonyms for *T. varians* (LÉGER 1897), and *T. simulii* is a synonym for *T. fibrata* (STRICKLAND 1913). However, it was overlooked that GASSOUMA and ELLIS in 1973 had published micrographs from two of the *Thelohania* species described by GASSOUMA (1972). With this information available the species cannot be synonymized in the way it was done by MUU (1977). The micrographs clearly reveal that *T. canningae* is an *Amblyospora* species, and *T. minispora* is a species of the genus *Thelohania*. For that reason *T. minispora* must be acknowledged as a valid species and not a synonym for *T. bracteata* (STRICKLAND 1913).

*Thelohania bracteata* (STRICKLAND 1913) was considered by HAZARD and OLDACRE (1975) to be an *Amblyospora* species, based on the illustrations of the original description. They also discussed the difficult matter of clearly recognizing this species. They reported that they had several new microsporidia of the genus *Amblyospora* from blackflies which could be clearly distinguished using ultrastructural characteristics. However, the description of these had to wait until a neotype of *A. bracteata* was available and had been ultrastructurally investigated. They were also of the opinion that the species from Europe identified as *A. bracteata* (STRICKLAND 1913) represented new and undescribed *Amblyospora* species. Although the spores of *A. bracteata* are of a comparable size to the spores of the Swedish microsporidium, this species can be excluded from the discussion as belonging to a different genus.

The spore length of *T. fibrata* (STRICKLAND 1913) reported in the original description was 5.8—6.6  $\mu\text{m}$  for the microspores, 7.8—9  $\mu\text{m}$  for the macrospores, considerably bigger than the spores of *T. capillata*. Further the micrographs from a species considered by MAURAND and LOUBÈS (1978) to be identical with *T. fibrata* reveal that this is a species of the genus *Amblyospora*.

*Thelohania columbaczense* WEISER, 1960, has spores of about the same size as the Swedish species. There are, however, several distinguishing characters between the two species. The sporont of *T. columbaczense* is originally uninuclear, the spores are pyriform and have double nuclei. The pansporoblast membrane is dissolved, and the number of spores produced in each pansporoblast is usually 8, but 12 or 16 can also be found. All these characters are different in *T. capillata*.

*Thelohania varians* was described by LÉGER (1897) in a very brief way and there are no illustrations included in the description. The host was larvae of *Simulium ornatum* MEIGEN collected in France, and infected larvae were recognized on the

white and swollen abdomen. The fat body was the only tissue parasitized. The spores were oval, refractive, and of two different sizes. Microspores, 4—5  $\mu\text{m}$  long, were produced in groups of 8, macropores, about 8  $\mu\text{m}$  long, were produced in an indefinite number. Both types of spores were enclosed by thin membranes. Stages of sporogony showing one, two, four or eight nuclei were also recognized. These characters are very general for a microsporidium of the family Thelohaniidae, and they are clearly not sufficient to distinguish this species from related species of microsporidia. The microspores are slightly bigger than the microspores of the Swedish species, and the macropores are considerably bigger and they are produced in a different number. There is no reason to believe that these species are identical.

The micrographs of *T. minispora* published by GASSOUMA and ELLIS (1973) are of a small size, the quality is not very high, and the verbal information of the paper is brief. However, it is apparent that there are both similarities and differences between *T. minispora* and *T. capillata*. The sporoblasts are formed in an identical way in the two species, and the number of polar filament coils are identical in microspores of *T. capillata* and the spores of *T. minispora*. Mature spores of *T. minispora*, which measured  $3.2 \pm 0.8 \times 2.9 \pm 0.7 \mu\text{m}$ , are slightly bigger than the microspores of *T. capillata*, but they are smaller than the macropores. In *T. minispora* all spores are of the same type, and they appear to be slightly more elongated than the spores of *T. capillata*. There are also differences in the histopathology of the two species. Adipose tissue infected with *T. minispora* was not milky white and the fat cell nuclei were markedly hypertrophied. Larvae infected with the Swedish microsporidium were distinctly white and the nuclei appeared not to be hypertrophied.

Dimensions of the polar filament and the spore wall are slightly different, but these differences might be explained by the different fixatives used. The polar filament of *T. capillata* has a long straight part, reaching the posterior pole of the spore. In the other species the straight part is apparently shorter, judged both from the information of the micrographs and the text. The angle of tilt of the anterior coil is approximately 45 degrees in the Swedish species, but in micrographs of *T. minispora* the anterior coil appears to be perpendicular to the long axis of the spore. The polaroplast of *T. capillata* is long, completely surrounding the straight part of the polar filament, in the other species it occupies only the anterior 1/3 of the spore length. The spores of *T. minispora* were fixed with permanganate, which is a good fixative for membranes, so it is not plausible that a part of the polaroplast had been destroyed during the fixation. The two microsporidia differ also in the structure of the pansporoblast membrane. This is very prominent in *T. capillata*, and it is distinctly seen in all stages of sporogony. In the micrographs of *T. minispora* the pansporoblast membrane is not visible, neither is it mentioned in the text. The different stages of sporogony can be identified, and it must be concluded that the pansporoblast membrane of this species is a more delicate structure than the wavy, fibrous pansporoblast membrane of *T. capillata*. All these accumulated differences can hardly be interpreted in another way than the two species are different.

In a paper on microsporidia from Newfoundland blackflies VÁVRA and UNDEEN (1981) reported 8 species, 6 previously known and two unidentified. They also discussed the synonymy of the microsporidia from blackflies, and stressed the importance of not describing new species solely based on minute morphological differences. Of course, it is important not to describe a new species without sufficient information available, but it is doubtful if it is much better to report the presence of a species which, like *T. varians* (LÉGER 1897), hardly can be clearly identified. Several examples can be found in the literature on microsporidia, where different authors state that they have been working with the same species, but where the micrographs clearly reveal that the species are different. In a thesis by MAURAND (1973) there is a micrograph illustrating the spore of a species identified as *Thelohania opacita* KUDO, 1922. This spore, which is of the *Amblyospora* type, shows 10.5 polar filament coils, the anterior four coils are wider than the posterior ones. HAZARD and OLDACRE (1975) state that the spore of *A. opacita* (KUDO 1922) has 8 filament coils, where the anterior 3.5 are wider. Are these two species identical? It is also quite clear that the species which CODREANU, CODREANU-BALCESCU and PORCHET-HENNÉRÉ (1974) identified as *Thelohania octospora* HENNEGUY, 1892, and for which they proposed the new genus *Orthothelohania*, is quite different from the microsporidium which VIVARÈS (1975) identified as *T. octospora* HENNEGUY, 1892. The micrographs reveal strikingly the difference.

Microsporidian species which are insufficiently described, and where no type material is available, must be treated very carefully to avoid confusion. The establishment of a neotype and a redescription of the species, with sufficient details to allow identification, are necessary steps that must be taken before the species can be more widely recognized. In the family Thelohaniidae, like the case of most microsporidia, knowledge of ultrastructural details are necessary for the identification of the species. In this family knowledge of the fine structure is also necessary for a correct attribution to the genus. *Thelohania capillata*, the species described in this paper, is different from the microsporidian parasites of blackflies which can be clearly identified, as well as it is different from *T. varians* judged from the brief and vague characters of the original description. The species is also undoubtedly different from previously described *Thelohania* species from other hosts than Simuliidae.

### Description

*Thelohania capillata* n. sp.

Host species: *Odagmia ornata* (MEIGEN, 1818) (Diptera: Simuliidae)

Host tissues involved: fat body, which develops into a syncytial xenoma.

Merogonial stages: not recognized except for diplokaryotic merozoites, the final stage of merogony.

Sporogony: only pansporoblastic sporogony was observed in infected larvae. The occurrence of a second sporogonial sequence with free spores not verified. A diplokaryotic sporont undergoes a meiotic and usually a subsequent mitotic division producing a sporogonial plasmodium with 8 single nuclei. From the plasmodium 8 sporoblasts are formed, each of which develops into a

spore without further division. In a small number of sporonts four macrosporoblasts are formed directly after the meiosis.

**Spores:** in fixed and stained smears barrel-shaped with one end lightly rounded. Dimensions: microspores  $2.0-3.1 \times 2.0-2.7 \mu\text{m}$ , macrospores  $3.5-4.5 \times 2.8-3.0 \mu\text{m}$ . Microspores are produced in a pansporoblast with 8 spores, macrospores in a pansporoblast with four spores. Spore-wall 280–350 nm thick, thinner above the anchoring disc. The straight part of the polar filament long, reaching the posterior pole of the spore. Diameter of the filament in microspores 120–140 nm, in macrospores 125–150 nm. In microspores the posterior part of the filament is arranged as 4–5 coils in a single layer close to the cell wall. The angle of tilt of the anterior coil is approximately 45 degrees. In macrospores the posterior part of the filament is arranged as 8 irregularly stacked coils in the posterior part of the spore. The polaroplast is structurally differentiated into two parts, the anterior about 1/3 of the spore length, the posterior 2/3, reaching the posterior pole of the spore. Micro- and macrospores with a single nucleus.

**Pansporoblast:** pansporoblast membrane prominent, composed of a fibrous hair-like material. The pansporoblast cavity filled with a fibrous material, continuous from the spore-walls to the pansporoblast membrane. Crystal-like metabolic granules are seen only in young pansporoblasts.

Type locality: Höje å, a small stream in the south of Sweden, near the city of Lund.

Types: holotype (Fig. 6C) on slide no. 801020-L-1 RL, paratypes on slides no. 801020-L-(1-15) RL.

Deposition of types: The slide with the holotype in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, U.S.A. Paratypes in the collections of Dr. E. HAZARD, Gainesville, Florida, Dr. V. SPRAGUE, Solomons, Maryland, Dr. J. WEISER, Prague, and in the collection of the author.

Etymology: *capillata* alluding to the hair-like appearance of the pansporoblast membrane.

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### Abbreviations

AD	anchoring disc	N	nucleus
C	chromatin	NL	nucleole
CE	central element	P	polaroplast
CO	cortical layer	PA	anterior region of the polaroplast
CP	centriolar plaque	PF	polar filament
CR	chromosome	PL	plasmalemma
D	diplokaryon	PM	pansporoblast membrane
EM	electron-dense material	PP	posterior region of the polaroplast
EN	endospore	PS	posterosome
EX	exospore	PV	posterior vacuole
F	fibrillar layer	R	ribosomes
G	granular layer	SB	sporoblast
H	holotype	SP	sporont
LE	lateral element	T	tubule
M	merozoite	U	unit membrane
MG	metabolic granule	V	vesicle
MS	mature spore		

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