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Description of *Lanatospora tubulifera* sp. n. (Microspora, Tuzetiidae) with Emended Diagnosis and New Systematic Position for the Genus *Lanatospora*

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Summary: *Lanatospora tubulifera,* a new microsporidian parasite of the ostracod *Acanthocyclops vernalis* is described based on light microscopic and ultrastructural characteristics. The diagnosis of the genus is emended and a new family position is suggested. The microsporidium has isolated nuclei at all stages of the life cycle. Merogony gives rise to rounded plasmodia with at least four nuclei. Sporogony produces at least 8 sporoblasts by rosette-like budding. Spores are pyriform, uninucleate and measure 3.5–4 × 2 µm when fixed and stained. The exospore consists of four layers: an electron dense layer, a double membrane-like layer, a moderately dense layer and a thin, electron dense surface layer. The polar filament is isofilar and arranged in 7–9 coils close to the spore wall in the posterior half of the spore. The polaroplast is tripartite, consisting of two lamellar parts and a posterior tubular part. The spores are surrounded by individual sporophorous vesicles, connected to the exospore by tubuli.

Key Words: *Lanatospora tubulifera* n. sp.; Taxonomy; Ultrastructure; *Acanthocyclops vernalis;* Copepoda; Crustacea.

Introduction

In 1977 VORONIN described a new microsporidian parasite of the copepod *Macrocyclops albidus* which he named *Thelohania macrocyclopis.* In 1986 he created the genus *Lanatospora* for the microsporidium, and in 1989 a thorough electron microscopic description of the same species followed. In the last publication he discussed the systematic position of the new genus and placed it in the family Tetramicridae. This paper deals with a new *Lanatospora* species collected in Sweden. It is described herein and a new systematic position is suggested for the genus *Lanatospora.*

Material and Methods

Copepods of the species *Acanthocyclops vernalis* FISCHER were collected in pools at Sandby mosse, Scania, southern Sweden in May 1988. These pools hold water from early autumn to late spring, but they normally dry out during the summer. Animals with an anomalous coloration were selected and cut into halves. One half was squashed, and the preparations were air dried and fixed in Bouin-Duboscq-Brasil solution overnight. They were stained using Giemsa solution or Heidenhain's iron haematoxylin and examined for parasites (ROMEIS 1968). Permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd.).

The other half was fixed and stained for transmission electron microscopy using 2,5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 27 h. After washing with cacodylate buffer the piece was postfixed in 2% (w/v) osmium tetroxide in cacodylate buffer at 4°C for 1 h. The piece was washed with buffer, dehydrated in an ascending series of buffer-acetone solutions to absolute acetone and embedded in epon. Sections were stained using uranyl acetate and lead citrate (REYNOLDS 1963).

Results

Effect on the host and life cycle

The parasite caused an anomalous yellowish brown coloration of the host. The site of infection was the connective tissue between muscle strands and beneath the hypodermis (Fig. 12).

The microsporidium had isolated nuclei at all stages of the life cycle. The merogonial reproduction was nearly finished and only a few plasmodia representing this part of the life cycle were found. They were rounded, with at least 4, about 2.5 µm wide, nuclei. The merogonial plasmodia divided by plasmotomy producing uninucleate merozoites. It is uncertain if there is more than one cycle of merogonial divisions. The last, or possibly only, division resulted in merozoites which matured into sporonts (Figs. 2 and 4).

In squash preparations the sporonts were distinguished from the meronts by the somewhat smaller (2 µm) and more intensely stained nuclei (Fig. 6). The sporogony yielded at least 8 lightly pyriform, uninucleate spores by rosette-like budding (Figs. 7 and 8). The sporogony proceeded inside a sporophorous vesicle produced by the sporont, and the mature spores were enclosed in individual sporophorous vesicles. The only signs of the vesicles visible under the light microscope were the granular appearance of the sporogonial plasmodia, caused by the spot-wise initiation of the sporophorous vesicle (Fig. 9).

Ultrastructure of presporal stages

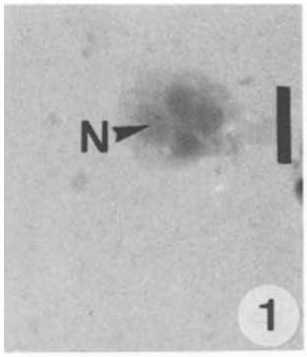
Rounded merogonial plasmodia with up to four nuclei were found in ultrathin sections (Fig. 3). The cell wall was an about 8 nm thick plasma membrane, and the approximately 20 nm thick nuclear envelopes had the normal construction (double unit membranes and a perinuclear space). The cytoplasm lacked endoplasmic reticulum but was uniformly filled with free ribosomes. During the sporogony large amounts of rough endoplasmic reticulum were formed. The reticulum was distributed irregularly throughout the cytoplasm, except around the nuclei where it formed several concentrical layers (Fig. 9). The nuclear envelopes were covered by a layer of ribosomes (Figs. 5, 9 and 10).

At the beginning of the sporogony, extremely electron dense material was secreted spot-wise outside the plasma membrane (Figs. 4 and 5), and this material was later on released in a blister-like manner (Figs. 9 and 10). In sections of the sporophorous vesicle prior to the formation of the sporoblasts, the electron dense material appeared as small granules covering the interior of vesicle (Fig. 10 and 11). These granules caused the granular appearance of the sporogonial plasmodia visible in light microscopical preparations (Figs. 6 and 7).

Inside the sporophorous vesicle the thick sporont wall was formed by secretion of another layer of electron dense material on the plasma membrane (Fig. 9). The complete sporont wall was approximately 40 nm thick and composed of five layers: an internal plasma membrane, a moderately electron dense layer, a very dense layer (about 19 nm thick), a moderately dense layer and the very thin electron dense surface layer (Fig. 11). Prior to the formation of sporoblasts, the first signs of tubuli could be seen as electron dense protrusions extending from the electron dense layer of the sporont wall (Fig. 11).

The surface layers of the sporogonial plasmodium remained, slightly modified, as the exospore layers in the mature spore. The last layer of the spore wall, the endospore, appeared at the time the primordial polar filament was visible in the immature spore. The initia-

Figs. 1–5. The early development of *Lanatospora tubulifera* sp. n. **Fig. 1:** Rounded merogonial plasmodium with four nuclei (N) (Heidenhain's haematoxylin). **Fig. 2:** Production of uninucleate merozoites; the arrow indicates the point of division (Heidenhain's haematoxylin). **Fig. 3:** Ultrathinly sectioned rounded merogonial plasmodium with four nuclei (N). **Fig. 4:** End of mergony; the cells are still connected (arrow), but the daughter cell to the right has already matured into a sporont. Note that the sporophorous vesicle (SV) is initiated at the onset of sporogony. **Fig. 5:** A greater magnified detail of the sporont showing the primordium of the sporophorous vesicle (SV) as electron dense blisters. Note the ribosomes (R) covering the nuclear envelope. Scale bars: Figs. 1–2 = 5 µm; Figs. 3–4 = 1 µm; Fig. 5 = 500 nm.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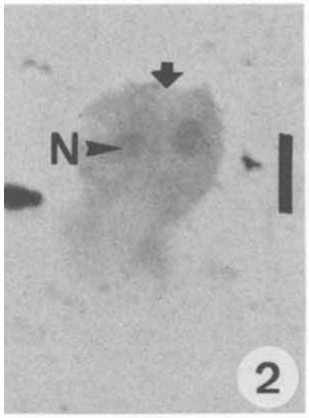
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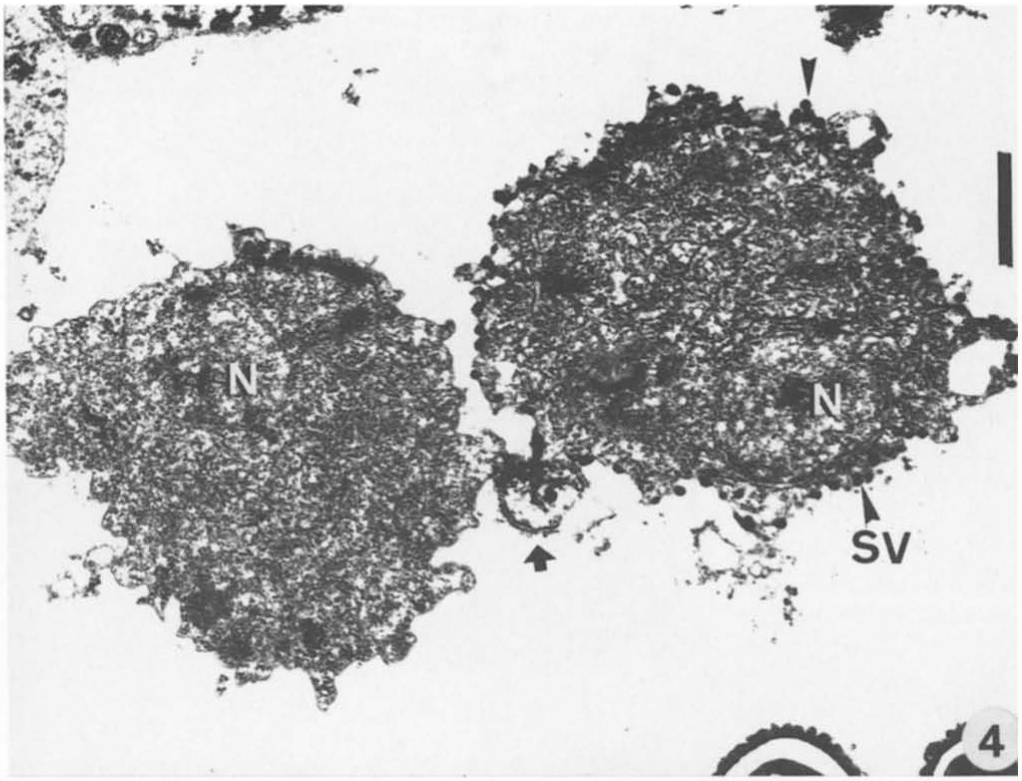
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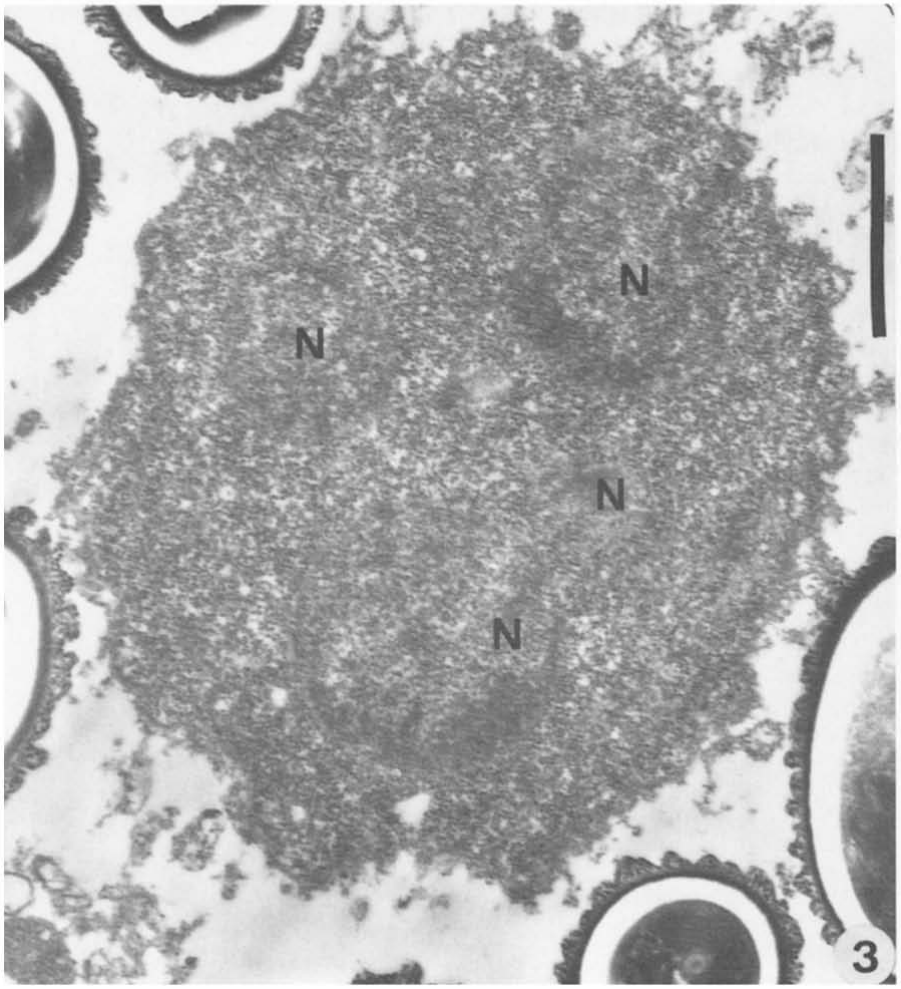
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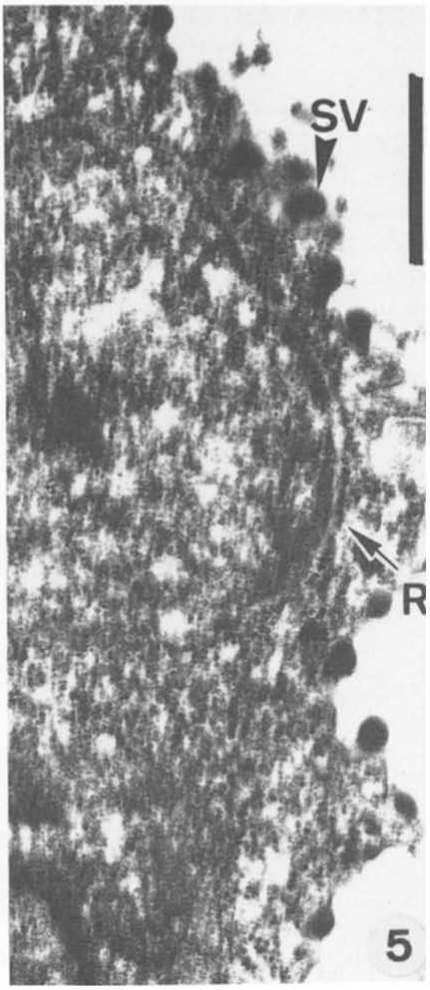
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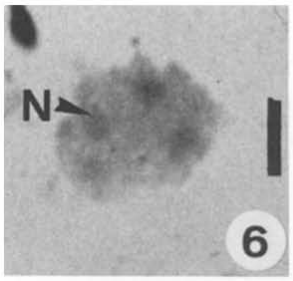
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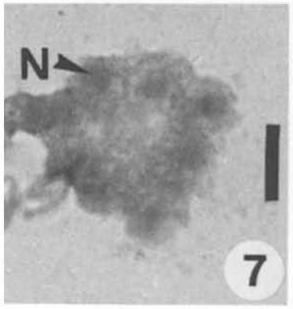
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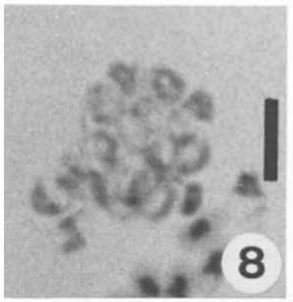
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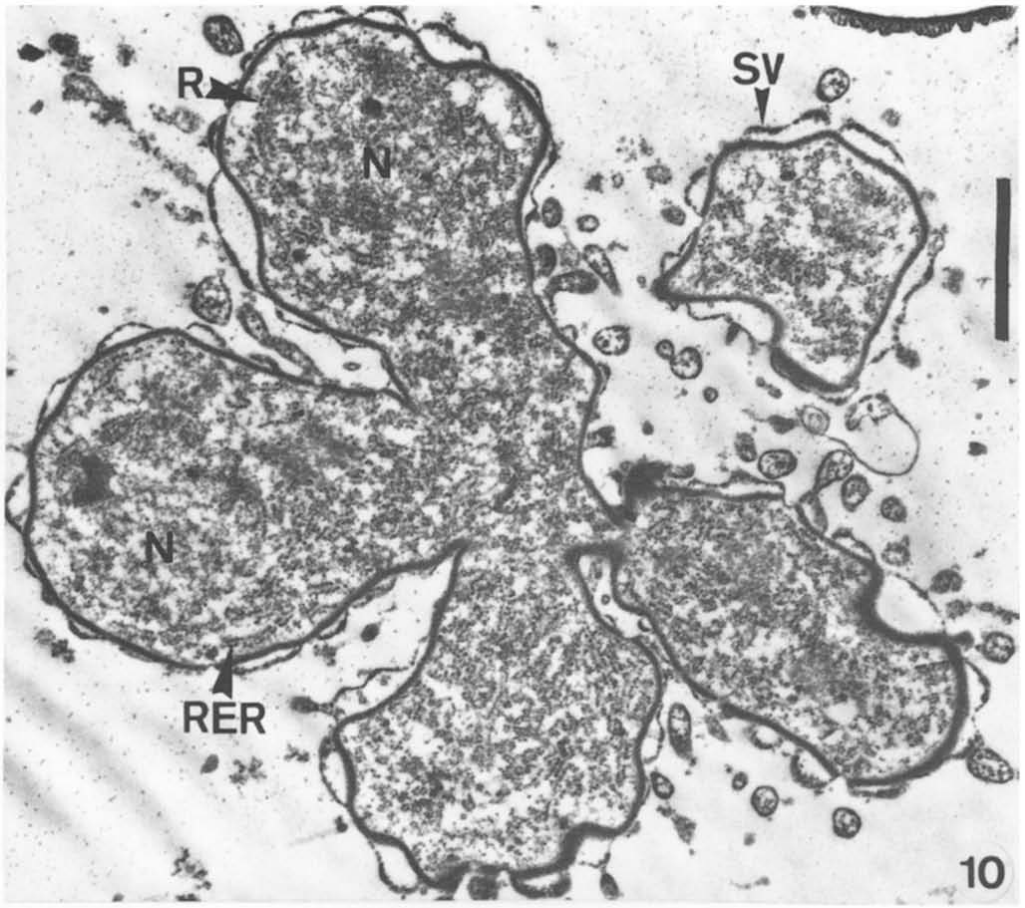
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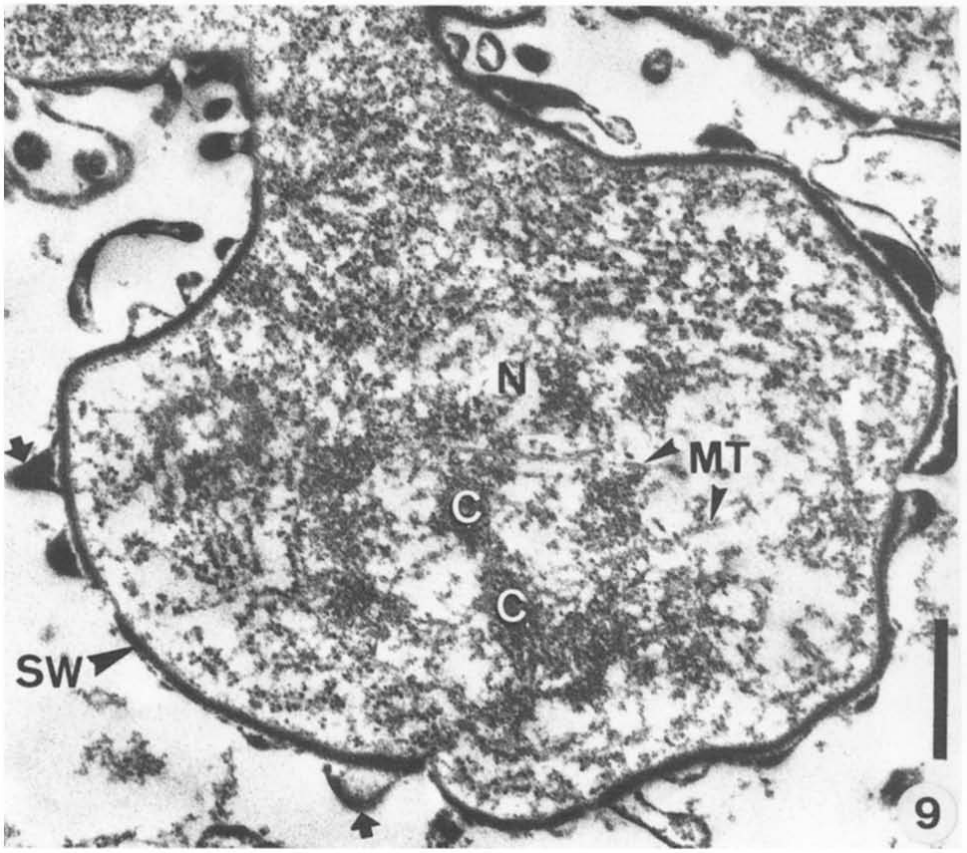
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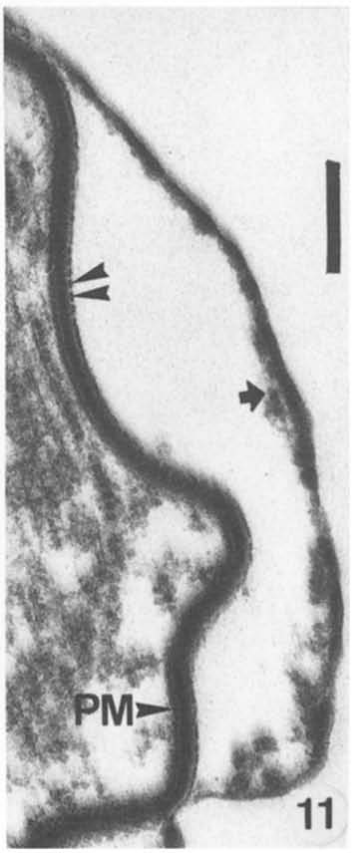
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tion and morphogenesis of the spore organelles equalled the normal for microsporidia.

The mature spore

The spores were lightly pyriform (Figs. 12 and 13). Fixed and stained spores were approximately 2 µm wide at the widest part and 3.5–4 µm long. The measurement includes the tubules and the individual sporophorous vesicle which cannot be distinguished from the spore using light microscopy.

The 170–190 nm wide spore wall exhibited the usual three layers: a 30–40 nm thick exospore, a 120–140 nm thick endospore (approximately 50 nm thick at the anterior pole) and an approximately 9 nm thick plasma membrane (Figs. 15 and 16). In the center of the exospore was a double membrane-like layer (Fig. 16c), internally coated with a layer of electron dense material (15–25 nm thick) (Fig. 16d). Outside the double layer was a moderately electron dense layer (Fig. 16b) and a thin, dense surface layer (Fig. 16a). These two layers were together approximately 14 nm thick.

The polar filament was isofilar with the diameter 110–130 nm. It was arranged in a single row of 7–9 coils, close to the spore wall in the posterior half of the spore. Six layers were visible in transverse sections of the filament (Fig. 19a–f). The 40–60 nm wide centre (a) had a very electron dense core with a diffuse border in the anterior 2–3 coils. The centre was surrounded by a fibrillar layer (b), followed a very electron dense ring (c), a moderately electron dense ring (d), a thin, electron dense layer (e), and a 5 nm thick unit membrane cover (f). The angle of tilt of the most anterior coil to the long axis of the spore was about 65°. In the anchoring disc, two thin electron dense layers were separated by moderately electron dense material (Fig. 18).

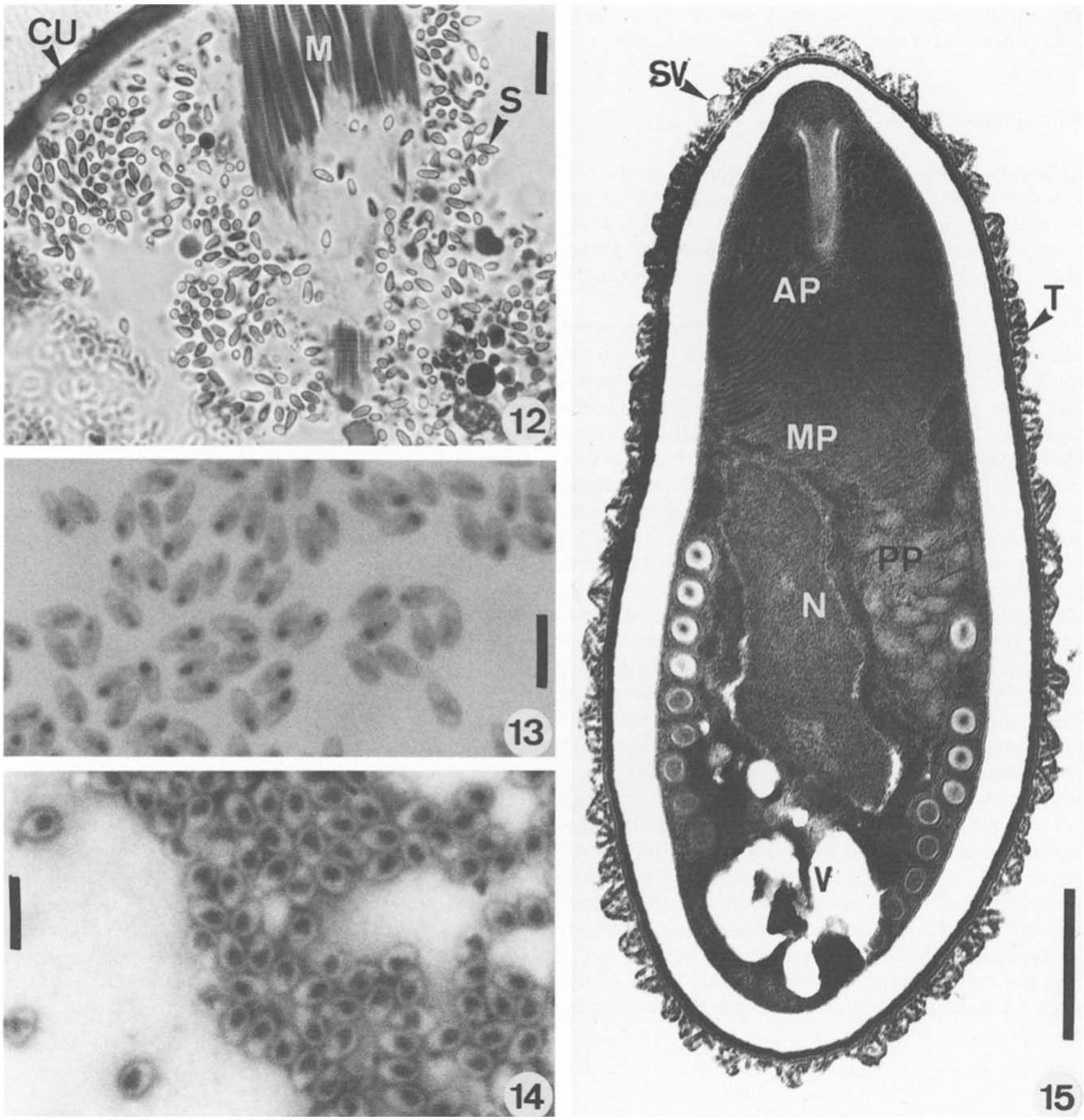
The polaroplast surrounded the uncoiled part of the polar filament and extended for about 3/5 of the spore length. The polaroplast was divided into three regions of approximately equal lengths: two lamellar and one tubular part. The compartments of the polaroplast were delimited by a unit membrane belonging to the same membrane system as the cover of the polar filament and the polar sac. The anterior lamellae were 18–21 nm thick and filled with electron dense material. The posterior lamellae were slightly more compressed (14–16 nm), and the material was slightly less electron dense. There was no distinct border between the two lamellar regions. The final region of the polaroplast consisted of 35–45 nm thick flattened tubuli, filled with still less electron dense material. These tubuli also surrounded the most anterior coil of the filament (Fig. 20). The polar sac, which enclosed the anchoring disc and covered the anterior half of the polaroplast, was filled with moderately electron dense material.

The large, irregular nucleus was situated in the middle of the spore. The electron dense cytoplasm contained strains of polyribosomes, surrounding the nucleus and the polar filament coils. The posterior vacuole, which measured 350–750 nm in diameter, was delimited by an approximately 5 nm thick unit membrane (Fig. 15). This area was never well preserved, but it contained small amounts of electron dense material.

The sphorophorous vesicle

During the production of sporoblasts, the vesicle divided together with the sporogonial plasmodium, creating individual sporophorous vesicles. The sporophorous vesicle was thin (approximately 5 nm) and of uniform electron dense material. When containing mature spores, numerous tubuli extended from the electron dense part of the exospore (Fig. 16c), connecting the spore wall with the envelope of the sporophorous vesicle (Figs. 16 and 17).

Figs. 6–11. Light microscopic, and ultrastructural aspects of the sporogony. **Fig. 6:** Rounded sporogonial plasmodium with four nuclei (N) (Heidenhain's haematoxylin). **Fig. 7:** Sporogonial plasmodium with eight nuclei (N) starting to bud (Heidenhain's haematoxylin). **Fig. 8:** Sporogonial plasmodium dividing by rosette-like budding (Giemsa stain). **Fig. 9:** Ultrastructural detail of a budding sporont. The primordium of the sporophorous vesicle grows larger but the blisters are still packed with electron dense material. Signs of mitosis are visible in the nucleus where mitotic spindel tubules (MT) connect to the chromatine (C). The production of the sporont wall (SW) is almost completed. **Fig. 10:** A slightly later sporogonial stage. The rough endoplasmic reticulum (RER) is clearly visible, and the nuclear envelope is still covered by ribosomes (R). The sporophorous vesicle (SV) is only connected to the sporont at a few positions. **Fig. 11:** Detail of the sporont wall. Note the electron dense protrusions from the electron dense layer in the sporont wall (arrowheads). The electron dense material, which filled the blisters of the sporophorous vesicle at the beginning of the sporogony, has now fragmented into granules covering the vesicle on the interior (arrow). Scale bars: Figs. 6–8 = 5 µm; Fig. 9 = 500 nm; Fig. 10 = 1 µm; Fig. 11 = 200 nm.



Figs. 12–15. Effect on the host and general spore morphology. **Fig. 12:** Semithin section showing that the spores (S) mainly are found beneath the cuticle (CU) and between the muscle strands (M). **Fig. 13:** Mature spores of *Lanatospora tubulifera* (Giemsa stain). **Fig. 14:** Mature spores of *Lanatospora macrocyclopis* (Giemsa stain). **Fig. 15:** Ultrastructure of the longitudinally sectioned spore showing the irregular nucleus (N), the large, tripartite polaroplast with anterior lamellae (AP), median lamellae (MP) and posterior tubuli (PP). At the posterior end are the remainders of the vacuole (V). The 9 coils of the isofilar polar filament are arranged in one row of coils. Tubuli (T) connect the exospore to the sporophorous vesicle (SV). Scale bars: Fig. 12 = 10 µm, Figs. 13–14 = 5 µm; Fig. 15 = 500 nm.

Discussion

Taxonomy

Somewhat 50 microsporidian species representing 20 genera have been reported from copepods. Most of these microsporidia are clearly different from the species described herein, but there are some species with a similar cytology and they are obviously closely related with this species. However, it can be concluded that the Swedish species is different from the previously described species for the following reasons:

Species of the genera *Alfvenia* (LARSSON 1983), *Mrazekia* (VÁVRA 1962; LARSSON et al 1993), and *Thelohania* (LOUBÈS 1979; VORONIN 1977, 1986; VIDTMANN 1990) differ by their partially or totally diplokaryotic life cycles, while all stages of the species treated herein have isolated nuclei.

Thelohania cyclopis (see WEISER 1945), is not known to have diplokarya and therefore it is not likely to be a *Thelohania* species. It sporulates in a sporophorous vesicle, common to all spores, and so do also *Stempellia diaptomi* (see VORONIN 1986) and species belonging to *Gurleya* (CÉPÈDE 1911; LEMMERMANN 1900; KOMÁREK & VÁVRA 1967; VORONIN 1986). *Nosemoides cyclopis* (see VIDTMANN 1990) lacks a sporophorous vesicle. The Swedish microsporidian produces individual sphorophorous vesicles.

Microsporidium leydigii (see PFEIFFER 1895; SPRAGUE 1977), *Stempellia mucilaginosus* (see VORONIN 1986), *Pleistophora cyclopis* (see LEBLANC 1930), *Ormieresia* sp. (see VIDTMANN 1990), *Duboscqia dengihilli* (see SWEENEY et al. 1993), and the species included in *Cougourdella* (HESSE 1935; VORONIN 1986, 1993), *Pyrotheca* (LEBLANC 1930; MAURAND et al. 1972; POISSON 1953), and *Amblyospora* (ANDREADIS 1988; SWEENEY et al. 1988), produce spores that are 1.5–4.5 times longer than the microsporidium described herein.

Encephalitozoon vrevi (see VORONIN 1986, 1991), produces ribbonlike sporogonial plasmodia. *Unikaryon mytilicolae* (see DUFORT et al. 1980), *Microsporidium jiroveci, Microsporidium pfeifferi* and *Microsporidium moniezi* (see VORONIN 1977), *Microsporidium* sp. (see PELL & CANNING 1993), *Nosema parva* (see MONIEZ 1887) are disporoblastic. *Holobispora thermocyclopis* (see VORONIN 1977, 1986), *Microsporidium fluviatilis* (see VORONIN 1994) and *Microsporidium cardiformis* (see VORONIN 1986) produce coupled spores. The Swedish species produces 8 or more uncoupled spores by rosette-like budding.

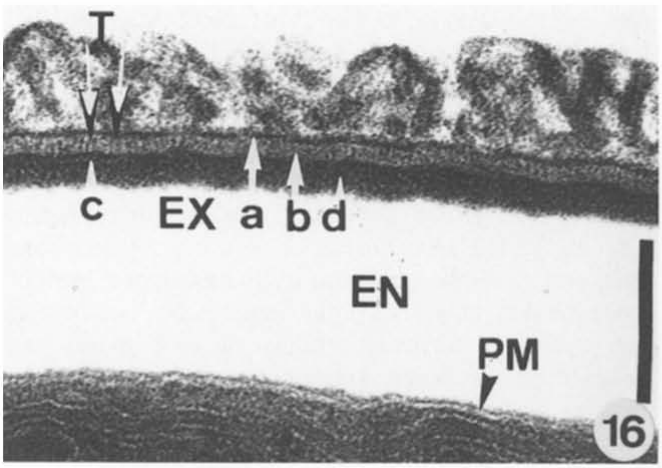
Microsporidium eucyclopis (see VORONIN 1986) and *M. schmeilii* (see PFEIFFER 1895; SPRAGUE 1977) are probably mixtures of several species, but both produce at least one spore category that is distinctly larger than the microsporidium of this paper.

Nelliemelba boeckella (see MILNER & MAYER 1982; LARSSON 1983), and the species belonging to *Tuzetia* (KUDO 1921; MAURAND 1973; MAURAND et al. 1971, 1972; SPRAGUE 1977) and *Lanatospora,* have isolated nuclei, polysporoblastic sporogony by rosette-like budding, individual sporophorous vesicles and oval-pyriform spores. However, apart from the sporophorous vesicle, *Nelliemelba boeckella* also has an additional episporal envelope produced by the exospore. Furthermore it has an anisofilar polar filament. Species belonging to *Tuzetia* normally produce up to 8 spores with unstratified exospores. There is one exception: the *Tuzetia* sp. described by MAURAND et al. (1972) which has a stratified sporont wall. The wall is said to give rise to a double membrane-like exospore and the sporophorous vesicle. However, this is obviously not a sporophorous vesicle but an extra episporal envelope, similar to those of *Nelliemelba boeckella* and *Alfvenia nuda* (see LARSSON 1983). The microsporidium treated herein differs from these species by having a stratified exospore and by lacking an additional episporal envelope.

The remaining species, *Lanatospora macrocyclopis* (see VORONIN 1977, 1986, 1989), which is the type species of the genus, is ultrastructurally very similar to the species described herein. Clear differences are, however, the eggshaped spores which are 3.0–3.3 µm long, while the spores of the species described herein are more pyriform and 3.5–4.0 µm long (Figs. 13 and 14). Furthermore, the endospore of *L. macrocyclopis* is 20–40 nm thinner, and the remainder of the internal moderately electron dense layer of the exospore is more obvious. On the average, *L. macrocyclopis* has one polar filament coil less than the species described herein.

Another species belonging to this genus has been found in a cladoceran: *L. bosminae* (see VORONIN 1986). It was described briefly and illustrated only by one light micrograph. It has pyriform spores, measuring 2.9–3.6 ×1.7–2 µm. Since the genus *Lanatospora* cannot be identified without EM, it is unknown if *L. bosminae* really belongs to the genus. An ultrastructurally very similar microsporidian, identified as *Microsporidia obtusa* MONIEZ, 1887, is also a parasite of cladocerans (VIDTMANN & SOLOKOVA 1994; own observations). It also has an exosporal coat, which, however, is not of the same construction as for *Lanatospora.*

When comparing the life cycles and ultrastructure, two features of the microsporidium described herein appear inconsistent with the diagnosis of the genus *Lanatospora.* In the description of the genus *Lanatospora* it was stated that no sporophorous vesicle is produced. However, this is an obvious misinterpretation, because the electron micrographs accompanying the ultrastructural description clearly show that a sporophorous vesicle is initiated and that it develops exactly in the same way as in the genus *Tuzetia,* and as it has been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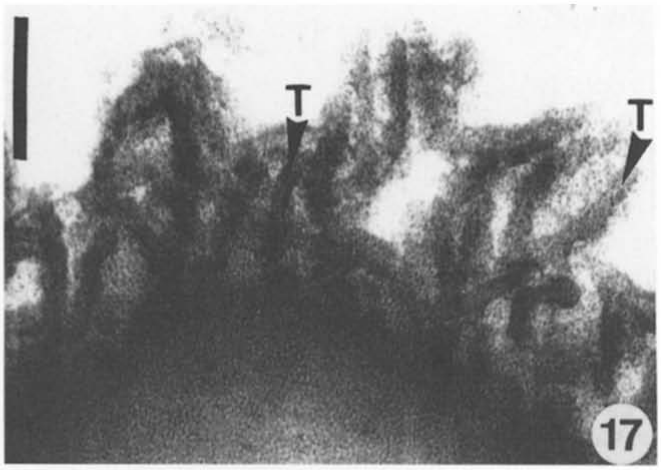
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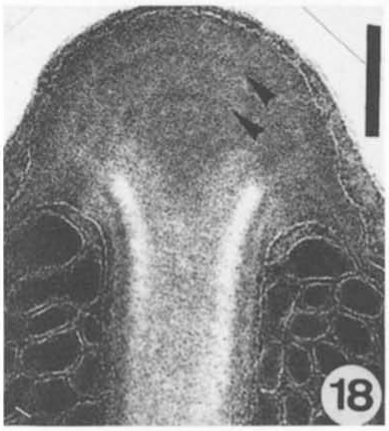
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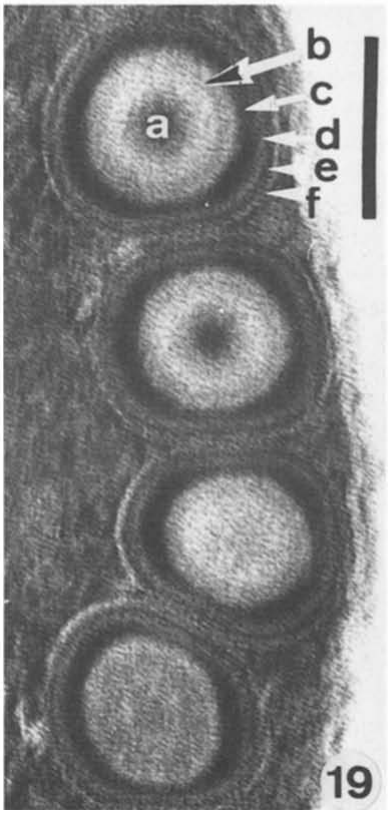
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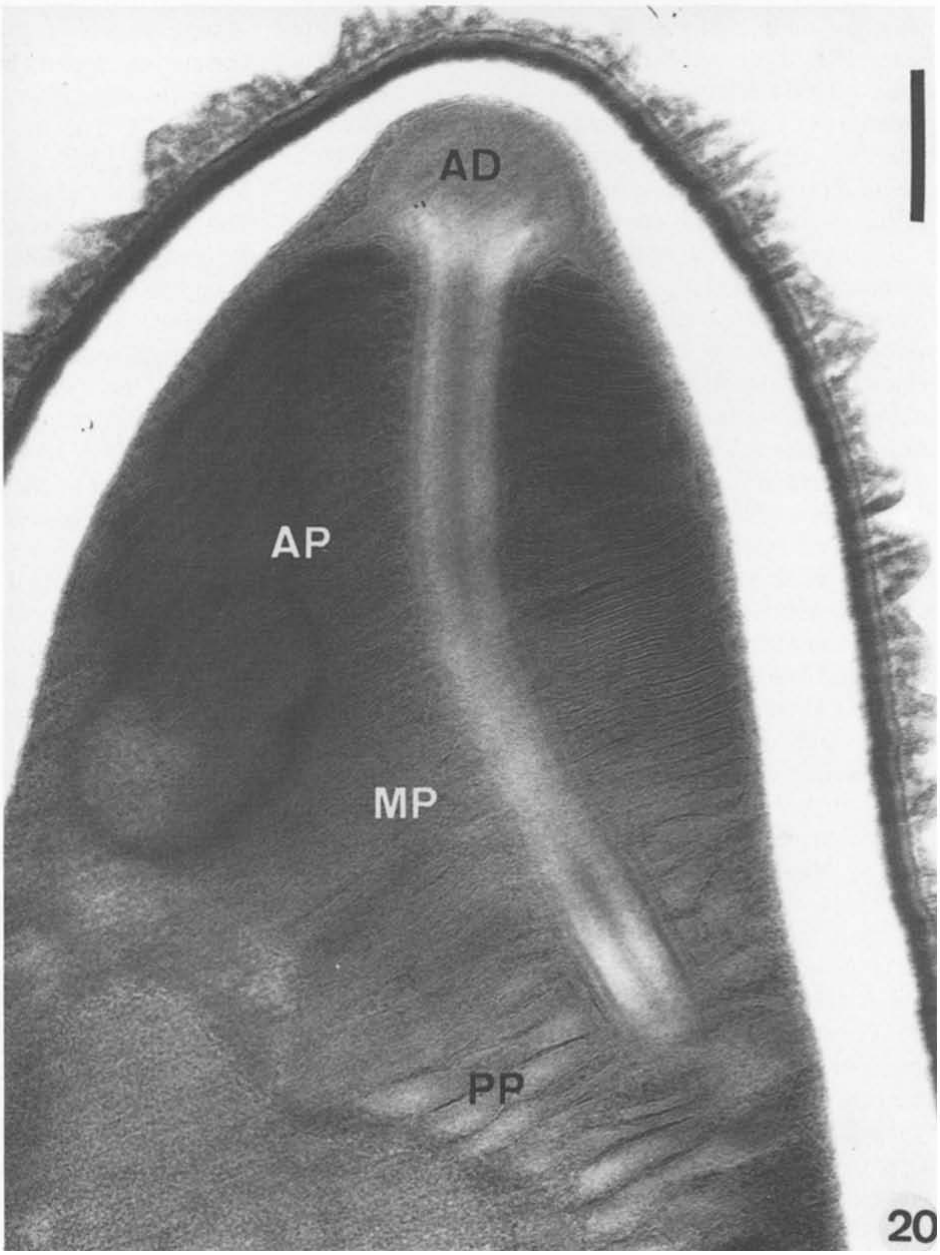
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observed in the species treated herein. The final condition, with tubuli connecting the envelope to the exospore, is also identical between *L. macrocyclopis* and the Swedish species.

The other inconsistency is in the merogony. *Lanatospora* was described as to produce elongated merogonial plasmodia. However, rounded plasmodia with four nuclei, cytologically identical to the ones we have found, were also observed by VORONIN (1989). He concluded that these were either stages preceeding the ribbonlike meronts or abortive merogonial forms. The only stages observed by us were rounded merogonial plasmodia and sporogonial stages. We have no reason to believe that the rounded meronts are abortive stages. Most likely they are stages following the ribbonlike meronts.

The other features of the species described herein are consistent with the definition of the genus: isolated nuclei, sporogony by rosette like budding producing 6–16 spores, isofilar polar filament and a "woolskin-like exosporal coat".

This allows us to conclude that the microsporidian described herein is a new species, belonging to the genus *Lanatospora.* It is necessary to amend the diagnosis of the genus *Lanatospora,* stating that the spores are enclosed in individual sporophorous vesicles. As the sporogony of *Lanatospora* is of the type characteristic for the family Tuzetiidae, we agiee with SPRAGUE and colleagues (1992) that this would be the proper systematic position for the genus.

Description

Lanatospora VORONIN, 1986, emended diagnosis

All developmental stages with isolated nuclei. Two merogonies. The first produces ribbon-like merogonial plasmodia with several nuclei in linear arrangement. The second yields rounded merogonial plasmodia with four nuclei. Polysporous sporogony by rosette-like budding, producing a variable number of up to 16 spores per sporont. Exospore with a woolskin-like cover. Polar filament is isofilar. Sporophorous vesicle divides with the sporogonial plasmodium enclosing the spores in individual vesicles.

Lanatospora tubulifera sp. n.

Merogony: The initial merogonial cycle not observed. In the second merogonial division rounded plasmodia with at least four nuclei divide by plasmotomy.

Sporogony: As for genus. At least 8 spores per sporont.

Spore: Pyriform, measuring 3.5–4 × 2 µm when fixed and stained. Exospore constructed of 4 layers: A very electron dense layer, a double membrane like layer, a moderately electron dense layer and a very thin, electron dense surface layer. Polar filament isofilar, 110–130 nm in diameter, arranged in a single row of 7–9 coils in the posterior half of the spore. Polaroplast tripartite: anterior part composed of thicker lamellae, median part of slightly more compressed lamellae, and posterior part tubular.

Sporophorous vesicle: Thin, electron dense, connected to the exospore by numerous tubuli which cause the "woolskin-like" look of the exospore.

Site of infection: Connective tissue between muscle strands and beneath the hypodermis.

Type host: *Acanthocyclops vernalis* FISHER (Crustacea, Copepoda, Cyclopidae).

Type locality: Temporary pools at Sandby mosse, Scania, southern Sweden.

Types: Syntypes on slides # 880509–C–(1–2).

Deposition of types: In the International Protozoan Type Slide Collection at Smithsonian Institution, Washington D.C., USA (USNM) and in the collection of the senior author.

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Figs. 16–20. Ultrastructure of the mature spore. **Fig. 16:** Longitudinally sectioned spore wall showing the plasma membrane (PM), and the endospore (EN) and exospore layers (EX). The exospore is composed of a very thin electron dense layer (a), a moderately electron dense layer (b), a double membrane like layer (c), and a wide electron dense layer (d). The tubuli (T) of the exosporal coat extend from the wide electron dense layer. **Fig. 17:** Obliquely sectioned detail of the spore wall showing the tubuli (T). **Fig. 18:** The anchoring disc is constructed of two thin, electron dense layers (arrowheads) separated by moderately electron dense material. **Fig. 19:** Transverse section of the polar filament revealing the internal organization: (a) the center, with an electron dense core in the first two coils; (b) the fibrillar layer; (c) the electron dense ring; (d) the moderately electron dense ring; (e): the narrow electron dense layer; (f) the unit membrane cover. **Fig. 20:** Longitudinal section of the polaroplast showing the two lamellar parts (AP, MP) and the tubular part (PP). Anteriorly is the anchoring disc (AD) visible. Scale bars: Fig. 16–19 = 100 nm; Fig. 20 = 200 nm.

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