

Description of a New Microsporidium of the Water Mite *Limnochares aquatica* and Establishment of the New Genus *Napamicum* (Microspora, Thelohaniidae)

J. I. RONNY LARSSON

Department of Zoology, University of Lund, S-223 62 Lund, Sweden

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The new microsporidium *Napamicum aequifilum*, a parasite of the aquatic mite *Limnochares aquatica* in Sweden, is described based on an ultrastructural investigation. The fusiform sporophorous vesicles, the pyriform spores, the unique plurilayered exospore, and the characteristic crystalline inclusions of the episporontal space are characteristics shared by *Chapmanium dispersus* (R. Larsson, 1984, *Protistologica*, 20, 547-563). The polar filament and the polaroplast differ in some ways. Some traits of ultrastructural cytology are discussed, with comments on two possible cases of reductions of an anisofilar polar filament. The taxonomic position of the species is discussed, and the new genus *Napamicum* is established for this species and for *C. dispersus*. © 1990 Academic Press, Inc.

KEY WORDS: *Napamicum* gen. nov.; *Napamicum aequifilum* sp. nov.; *Napamicum dispersus* comb. nov.; Microspora; ultrastructure; polar filament reduction; taxonomy; *Limnochares aquatica*; Acari.

INTRODUCTION

The revision of the *Thelohania*-like microsporidia by Hazard and Oldacre (1975) is a milestone in microsporidiology. For the first time ultrastructural characteristics were used more extensively for taxonomic purposes. The result was that the octosporoblastic, pansporoblastic microsporidia were split, and a series of new genera was created for them. One of the new genera was *Chapmanium*. It was established for *Chapmanium cirritus*, a parasite of the phantom midge *Corethrella brakelyi* investigated ultrastructurally, and two more species were tentatively included: *Chapmanium macrocystis* (Gurley, 1989) and *Chapmanium nepae* (Lipa, 1966). The fusiform shape of the sporophorous vesicle (SV) suggested that they might be congeneric with *C. cirritus*. Their ultrastructural cytology is still unknown.

A decade later a fourth species, *Chapmanium dispersus*, was included in *Chapmanium* (Larsson, 1984). It is a fairly common parasite of midge larvae in Sweden. The microsporidium was investigated using electron microscopy. It exhibited a uniquely layered exospore and characteristic inclu-

sions of the episporontal space. Using light microscopy characteristics, it was fairly clear that it was a *Chapmanium* species, but there were some questions about the ultrastructural cytology. Although *C. cirritus* was investigated using electron microscopy, the published micrographs are not magnified enough to reveal all details. The polaroplast was described as lamellar, but the micrographs do not reveal the construction. The exospore looks fairly thick, but the fine structure is not visible. Even if the inclusions of the episporontal space, in both *C. cirritus* and *C. dispersus*, seem to be formed by material of two different electron densities, they could not be compared in detail. Because Hazard and Oldacre (1975) had included two species tentatively in their new genus, without knowing anything about the ultrastructural cytology, there were no apparent problems including the new microsporidium of the Swedish midge larvae in *Chapmanium*. The light microscopic appearance suggested that it was a *Chapmanium* species, and even if the ultrastructural cytology could not be compared in all details, nothing spoke directly against the identification.

In 1986 a new microsporidium was isolated from the water mite *Limnochares aquatica* in Sweden. Electron microscopy studies revealed similarities with *C. dispersus*. The two microsporidia had identical exospores and identical inclusions of the episporontal space. However, they differed in the gross morphology of the polar filament: *C. dispersus*, like *C. cirritus*, has anisofilar polar filaments, while the new species has a uniformly thick filament. Undoubtedly the microsporidium of *L. aquatica* is congeneric with *C. dispersus*. Because there is no new information on *C. cirritus*, a species of *Chapmanium* which had appeared in the meantime, my original intention was to describe the parasite of the water mite as a new *Chapmanium* species. However, the reviewers doubted that the identification was correct, and one of them contacted Dr. James Becnel, Gainesville, Florida, who has access to the late Dr. Hazard's collection of microsporidia. Dr. Becnel kindly made some new micrographs, and placed them at my disposal. Even if they did not solve all of the problems, e.g., the construction of the polaroplast and the inclusions of the episporontal space are still unclear, one important difference between *C. cirritus* and the Swedish microsporidium was revealed. The uniquely layered exospore of *C. dispersus* and the microsporidium of the mite is not present in *C. cirritus*. As much speaks for the *Thelohania*-like microsporidia having exospores unique to the genus, this new information makes it clear that *C. dispersus* and the microsporidium of *L. aquatica* cannot be a *Chapmanium* species.

The new microsporidium of *L. aquatica* is described here, and the new genus *Napamicum* is established for this microsporidium and for *C. dispersus*. It seems obvious that *Napamicum* and *Chapmanium* are closely related genera. Some traits on the ultrastructural cytology and the taxonomy of the species are discussed.

MATERIALS AND METHODS

The host was one specimen of the water

mite *Limnochares aquatica* (Acari, Limnocharidae), collected in a small pond at Gårdstånga, in the south of Sweden, on September 16, 1986.

Fresh smears were prepared by the modified agar method of Hostounský and Žižka (1979) and studied using phase-contrast microscopy and dark-field illumination. Permanent squash preparations were lightly air-dried and fixed in Bouin-Duboscq-Brasil solution overnight. They were stained in Giemsa solution and Heidenhain's iron hematoxylin (Romeis, 1968). Permanent squash preparations were mounted in DePeX. Measurements were made with an eye-piece micrometer at $\times 1000$.

For transmission electron microscopy, tissue fragments with microsporidia were fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (*pH* 7.2) at 4°C for 22 hr. After washing in cacodylate buffer and postfixation in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 hr at 4°C, the pieces were washed and 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.

RESULTS

Rate of Infection and Pathology

The infection was present in the last of five specimens of *L. aquatica* investigated, and there were no external signs of infection. The mite appeared to be healthy. The microsporidium was detected when the mite was routinely squashed on a microscope slide. Efforts to collect more material were unsuccessful. The spores were aggregated in a few parts of the body. Because it was impossible to make paraffin sections, details of the histopathology are unknown. Microsporidia in ultrathin sections were found in fat body cells.

Presporal Stages

Presporal stages were rare and restricted to the last phase of sporogony: SVs with

lobed multinucleate sporogonial plasmodia or with monokaryotic sporoblasts of different maturity were present. No sporonts were seen and it is unknown whether sporogony starts with a meiotic division. The morphology of the plasmodia and sporoblasts was consistent with previous observations of octosporoblastic microsporidia, especially *C. dispersus* (see Larsson, 1984).

The Mature Spore

Mature spores were lightly pyriform,

with rounded ends (Figs. 1A, 1C, 1D). Unfixed spores measured approximately $3.0 \times 5.4\text{--}5.8 \mu\text{m}$; fixed and stained spores measured $2.8\text{--}3.0 \times 4.0\text{--}5.0 \mu\text{m}$.

The spore wall measured 198–271 nm. It exhibited the normal three layers: an internal approximately 8-nm-thick plasma membrane of unit membrane construction, a thick electron-lucent endospore, and a complex, 90- to 106-nm-thick exospore (Figs. 3B, 3C). The endospore varied somewhat in thickness, but was only slightly thinner at the anterior pole (Fig. 2A). The

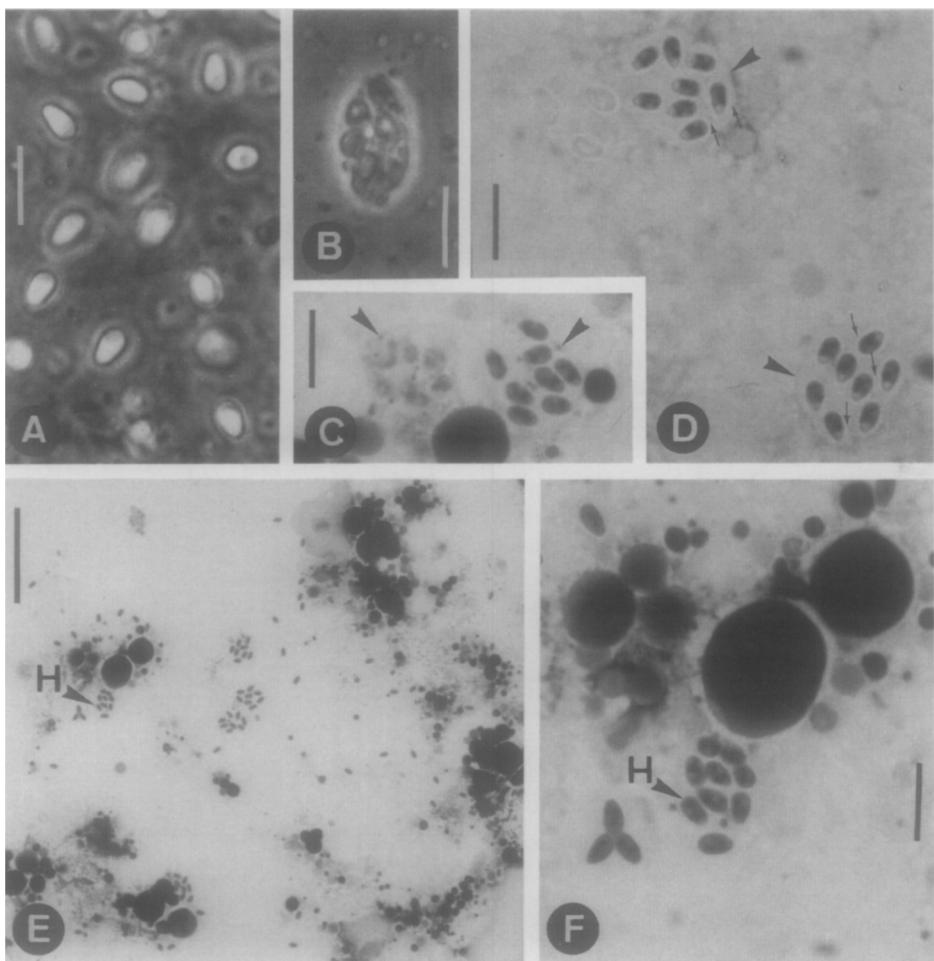


FIG. 1. Light microscopic appearance of *Napamiculum aequifilum* sp. nov. (A) Unfixed spores. (B) Unfixed sporophorous vesicle. (C) Two SVs stained with hematoxylin. Crystal-like inclusions (arrowheads) are visible. (D) Two SVs stained with Giemsa solution. Crystals are not visible. Arrowheads indicate the envelope of the SV. A clear area around the spore (arrows) suggests the presence of gelatinous material. (E, F) Localization of the holotype (H), slide No. 860916-D-2 RL (Heidenhain's hematoxylin). Bars: (A–D, F) 1 μm ; (E) 50 μm .

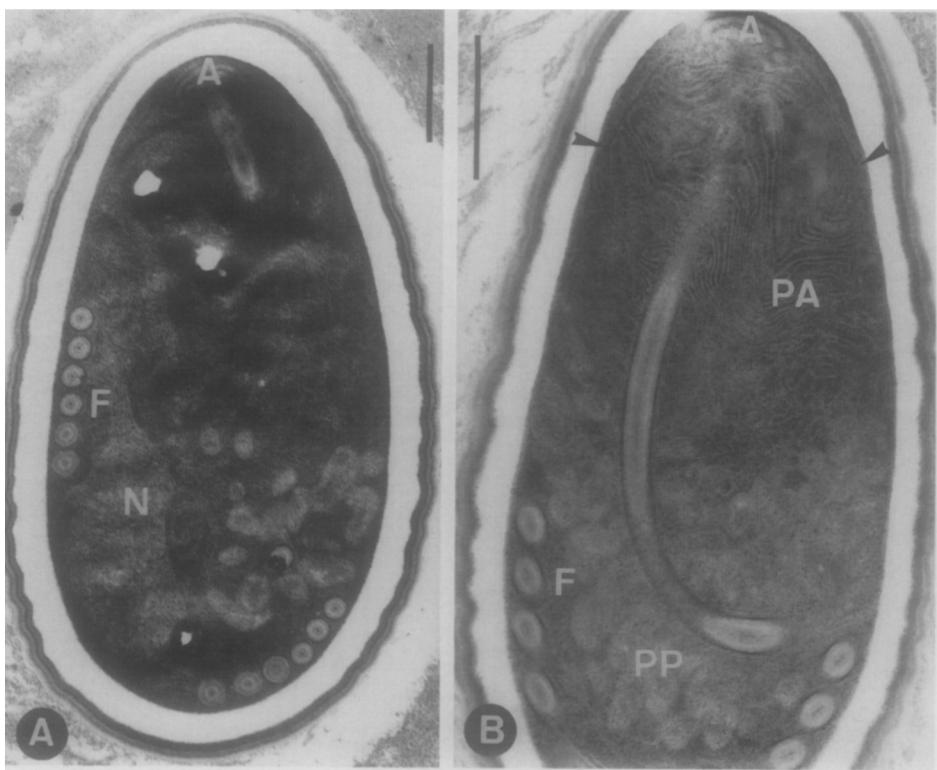


FIG. 2. Longitudinally sectioned spores of *N. aequifilum* (A, anchoring disc; F, polar filament of approximately uniform diameter; N, nucleus; PA, anterior part of polaroplast; PP, posterior part of polaroplast). Arrowheads indicate the posterior end of the polar sac. Bars: 0.5 μ m.

exospore was uniformly thick, except for the granular internal border to the endospore (Fig. 3C). It was prominently layered and exhibited three wide moderately electron-dense zones of approximately equal thickness (27–29 nm) (Figs. 3B, 3C; 1, 3, 5), separated by more or less distinct more dense layers (Figs. 3B, 3E; 2, 4). The external moderately dense layer appeared to be composed of laminated material (Fig. 3C; 1, arrowheads), and it was sometimes covered with a granular substance. The narrow layer interior to this was a double layer resembling an approximately 5-nm-thick unit membrane, with a more dense internal surface (Fig. 3B; 2). The third wide layer, which bordered to the endospore, was more electron dense than the two wide external layers (Figs. 2, 3B; 5). The dense layer (Fig. 3B; 4) separating the

two wide internal zones was not always distinct (cf. Figs. 2A, 2B). Apparently a gradient in the dense material of layer 5 (Fig. 3B; 5) might result in accumulation of more dense material in the external part of the layer (Fig. 3B; 4).

The polar filament was attached to a curved anchoring disc (Fig. 3A). The widest disc observed measured approximately 410 nm. The attachment section of the polar filament was approximately 340 nm long and approximately 176 nm wide. The filament widened successively toward the anchoring disc, but the difference in width between the straight and the coiled part was small, only about 10 nm. The attachment section was not distinctly set off from the rest of the filament. The internal organization of the attachment section (Fig. 3A) corresponded closely to the construction de-

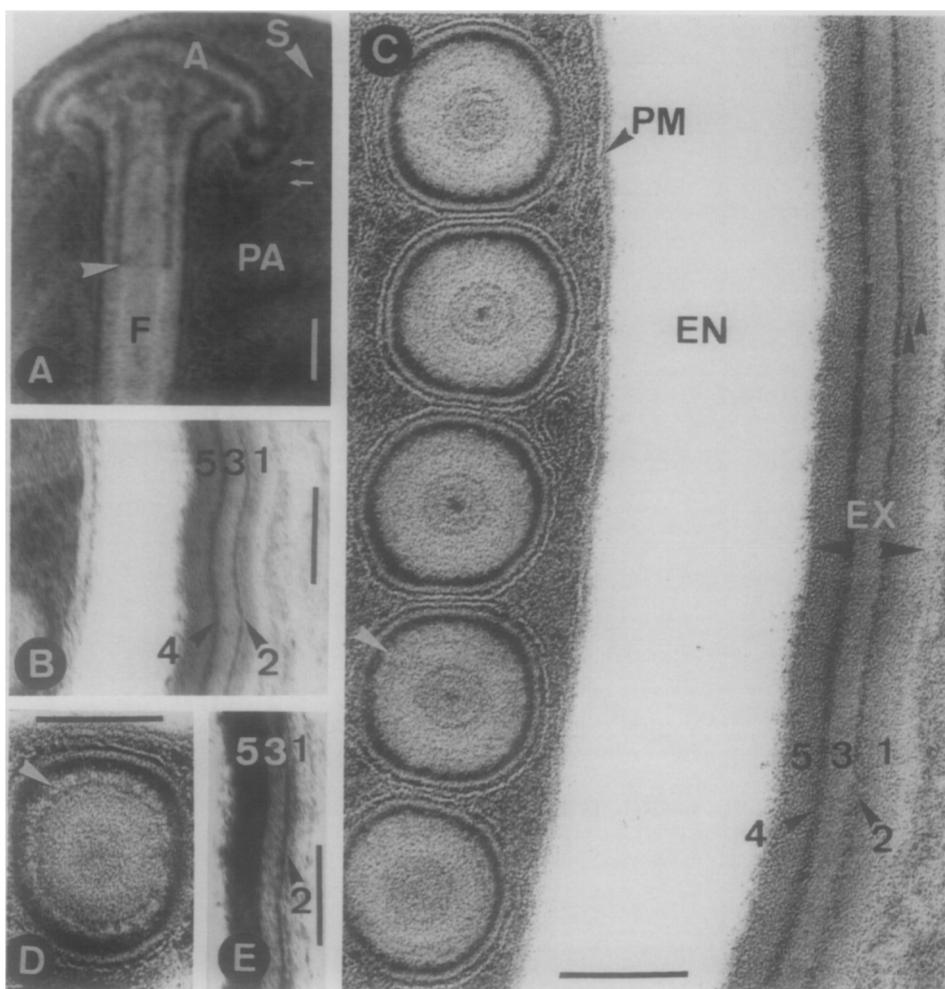


FIG. 3. Ultrastructural details of mature spores. (A-C) *N. aequifilum*: (A) Longitudinal section through the anterior part of the extrusion apparatus. The arrowhead indicates the posterior end of the attachment section of the polar filament (F). The most anterior lamellae of the polaroplast (PA) are closely packed (arrows) (A, anchoring disc; S, polar sac). (B) Longitudinal section through the spore wall. The layers of the exospore are numbered 1-5; layer 2 is the double layer. (C) Longitudinal section through the coiled part of the polar filament and the spore wall. The white arrowhead on the left indicates the fibril-like layer of the filament. The layers of the exospore are numbered 1-5; stratification of the surface layer is indicated by black arrowheads (EN, endospore; PM, plasma membrane). (D, E) *N. dispersus*: (D) Transversely sectioned wide coil of the polar filament; the arrowhead indicates the fibril-like layer. (E) Longitudinal section through the exospore, showing the five layers; layer 2 is the double layer. Bars: 100 nm.

scribed for *C. dispersus* (see Larsson, 1984, Figs. 21-25). The enclosing polar sac was fairly narrow, and it extended backward for about one-quarter of the length of the spore (Fig. 2B). The coiled part of the polar filament began approximately one-third from the anterior pole. The six or seven 149- to

165-nm-wide coils were packed in a single layer close to the spore wall (Fig. 2A). The filament was not constricted. However, a part of the spores had one intermediate coil that was slightly wider than the neighboring coils (cf. Figs. 2A and 3C). The angle of tilt of the anterior coil to the long axis of the

spore was about 40°. The transversely sectioned filament revealed concentric layers of varying electron density, in the outward direction: a central, moderately dense zone with concentric bands, a moderately dense zone suggesting longitudinal fibrils, a prominent, dense zone interrupted by a less dense area, and a surface layer resembling an approximately 5-nm-thick unit membrane (Fig. 3C).

The polaroplast surrounded the uncoiled part of the polar filament. It was composed of two different parts (Fig. 2B): an anterior polaroplast of irregular shape, up to 150 nm wide, lamellae, extending backward approximately to the middle of the spore, and a posterior section of loosely arranged tubules or narrow sacs, not wider than 140 nm. The most anterior lamellae were so closely packed that there seemed to be no lumen at all (Fig. 3A). The sac-like polaroplast extended backward approximately to one-quarter from the posterior pole of the spore. The polar sac, the compartments of the polaroplast, and the surface layer of the polar filament had equally constructed unit membrane linings about 5 nm thick.

The elongated and sometimes constricted nucleus was oriented obliquely in the widest part of the spore. The cytoplasm was granular with the normal strands of membrane-associated ribosomes surrounding the nucleus, the polaroplast, and the polar filament. A well-defined posterior vacuole was not observed.

The Sporophorous Vesicle

Uncompressed SVs were fusiform (Fig. 1B). The largest unfixed vesicle measured $7.2 \times 17.9 \mu\text{m}$. Fixed and stained vesicles, compressed with all spores in the same plane, were more or less oval (Fig. 1D). The largest squashed and fixed vesicle measured 7.0–12.0 μm . The spores were not arranged in the vesicles in a particular way.

The envelope was about 20 nm thick. In vesicles with sporogonial plasmodia, three types of inclusions were present in the episporontal space: a finely granular or fibrous

material, 35- to 44-nm-wide tubules exhibiting the layers of the sporont wall, and more or less rounded crystal-like aggregates. In older SVs the tubules had disappeared, and the granular or fibrous material was organized as thin fibers, continuous from the spore wall to the envelope of the vesicle (Fig. 4A). The crystal-like aggregations were reduced in numbers, but were present also in vesicles with mature spores (Figs. 1C, 4A). They looked identical in vesicles with mature spores from initiation to the final stage and were composed of material of two electron densities (Fig. 4B). The crystals were clearly visible with hematoxylin stain (Fig. 1C), but were almost invisible in Giemsa-stained preparations (Fig. 1D).

DISCUSSION

The Ultrastructural Cytology

A comparison between the microsporidium described herein and *C. dispersus* (see Larsson, 1984) reveals a set of similarities and two prominent differences.

The two microsporidia share the complex, layered exospore, and the dimensions of the different layers are nearly the same (Figs. 3B, 3C, 3E). The concentric banding of the transversely sectioned polar filament is identical (Figs. 3C, 3D). Further the fine structures of the anterior part of the extrusion apparatus (the attachment section of the polar filament and the connection to the anchoring disc) are similar (Fig. 3A; Larsson, 1984, Figs. 21–25).

The differences lie in the gross morphology of the polar filament and the fine structure of the polaroplast.

The polar filament of *C. dispersus* is anisofilar, with four of five wide anterior cells and eight to ten narrow posterior coils. The microsporidium of this paper has an almost uniform filament, without a constriction. As the two species share unique cell constructions (plurilayered exospore, internal organization of the polar filament), it seems improbable that one species has an aniso-

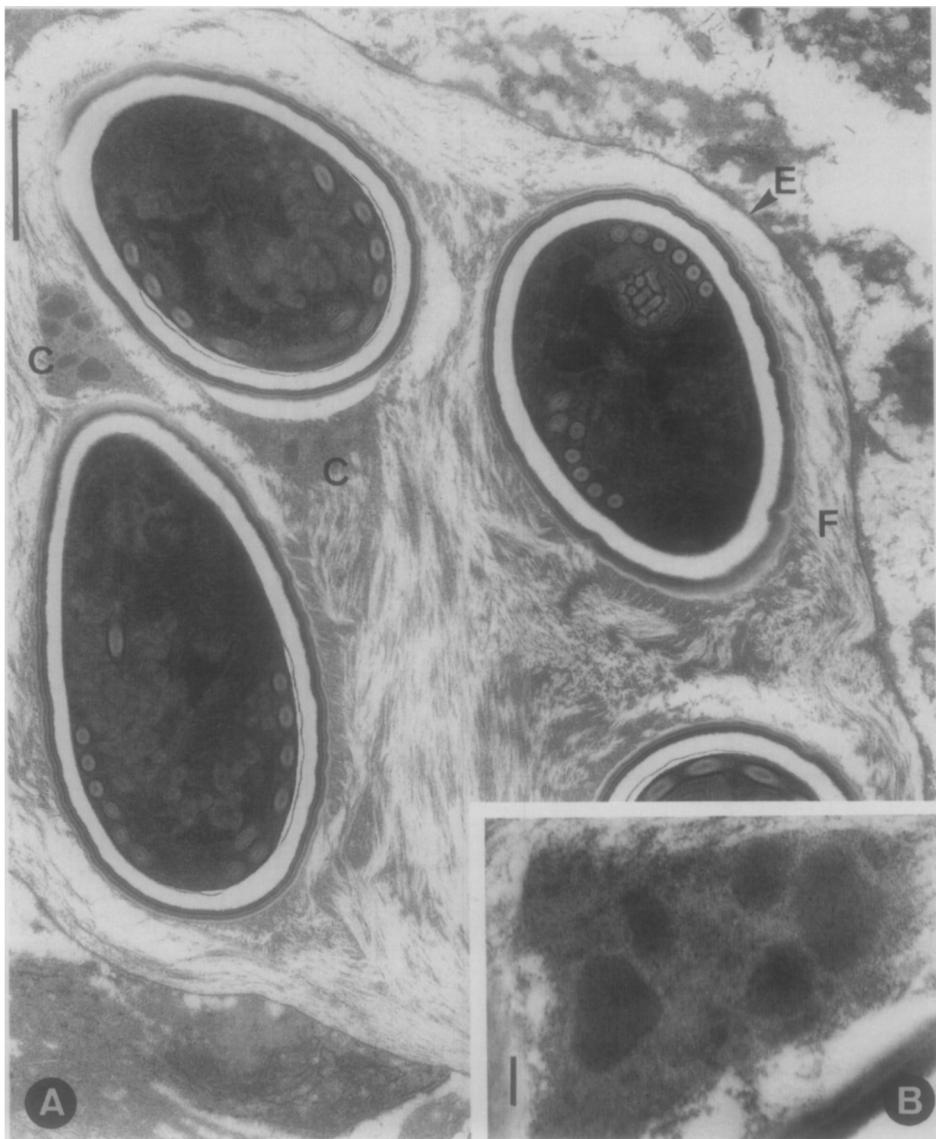


FIG. 4. (A) The sporophorous vesicle of *N. aequifilum* (C, crystal-like inclusions; E, envelope of the vesicle; F, fibrils). Bar: 1 μ m. (B) A crystal-like inclusion, composed of material of two electron densities. Bar: 100 nm.

filar polar filament, while the other has an isofilar filament. The uniformly thick filament of the species treated herein is probably an anisofilar filament where the distal narrow part has disappeared. A similar condition was found in *Amblyospora capillata*, which originally was described as a *Thelohania* species, mostly based on the uni-

formly thick polar filament (Larsson, 1983, 1988). The genus *Amblyospora* has uniquely shaped spores and an exospore of unique construction (like the present microsporidia have), characters present in *A. capillata*. Because the polar filament of the genus *Amblyospora*, where numerous species have been investigated using electron

microscopy, is anisofilar, the uniform filament of *A. capillata* must be interpreted as a reduced anisofilar polar filament.

The polaroplast of *C. dispersus* is a uniformly constructed organelle, with wide, closely packed, more or less sac-like compartments (Larsson, 1984, Figs. 14–15). The species described herein has a polaroplast with two regions: an anterior part with tight, wide lamellae or sacs, and a posterior region with more irregularly spaced tubule-like sacs (Fig. 2B). The entire polaroplast of *C. dispersus* probably corresponds to the anterior part of the polaroplast of the present species.

Crystal-like material is present in SVs of a number of *Thelohania*-like microsporidia, at least in the early phase of the sporogony. Crystals are found in the genera with the most complex exospores, and they are probably formed from the same material that builds the layers internal to the double-layer of the exospore. *Amblyospora*, *Parathelohania*, and *Hyalinocysta* have one electron-dense layer internal to the double layer, and their crystals are uniformly electron dense. *C. dispersus* and the species described here have two layers of different electron density (Figs. 3B, 3C, 3E) internal to the double layer and crystals composed of two different electron densities (Fig. 4; Larsson, 1984, Figs. 26, 28). Also *Cryptosporina brachyfila* has prominent crystal-like inclusions, but fine structure of the inclusions and the fine structure of the exospore are not revealed in the description (Hazard and Oldacre, 1975).

It is obvious that the polaroplast and the polar filament are variable organelles, which should be used with care in taxonomy. Characteristics from the exospore and the SV appear to be more constant.

Taxonomy

The genus *Chapmanium* was characterized by the fusiform SVs with persistent envelope, the pyriform octospores, the lamellated polaroplast, and the anisofilar polar filament (Hazard and Oldacre, 1975). Obvi-

ously the shape of the SV was considered most important, for two more species were tentatively assigned to the genus by this characteristic alone (Hazard and Oldacre, 1975). At the light microscopic level *C. dispersus* and the species described herein are similar to *C. cirritus*, the type species of *Chapmanium*, but the prominent differences in the exospores clearly indicate that we are dealing with two different genera. There is no other genus of microsporidia with a layered exospore of this type. It is therefore necessary to create a new genus for the Swedish microsporidia. This situation, with two genera with similar light microscopic cytology but with clear ultracytological differences creates a problem concerning the generic position of *C. macrocystis* (Gurley, 1893) and *C. nepae* (Lipa, 1966). Do they belong in *Chapmanium* or *Napamicum* or in one or two undescribed genera?

The spores of the species described herein are considerably larger than spores of *C. nepae*, but of comparable size to spores of *C. dispersus* (Lipa, 1966; Larsson, 1984). However, the differences at the ultrastructural level clearly show that the species is different from *C. dispersus*. Gurley (1893) did not mention the size of the spores of *C. macrocystis*, a parasite of a freshwater shrimp, and the species can probably not be identified. Kudo (1924) considered the species to be doubtful. Undoubtedly the species described herein is different from the other species with fusiform SVs that can be clearly identified.

Fifteen species of microsporidia are parasites of mites, and in addition Dissanaike (1958) claimed to have experimentally infected mites with the tapeworm parasite *Nosema helminthorum*. Three species (*Nosema sperchoni*, *Cryptosporina brachyfila*, and *Gurleya sokolovi*) are parasites of water mites. Eight *Nosema* species, described by Weiser (1956), Lipa (1962, 1982), Purriini and Bäumler (1976, 1977), and Purriini and Weiser (1981), lack SVs, and most of them have diplokaryotic spores. The

four *Pleistophora* species described by Purrini and Weiser (1981) are probably not *Pleistophora* species, but none is reported to have octospores. *Thelohania microtritiae* has spherical-oval octospores and spherical SVs found in pseudocysts (Purrini and Weiser, 1981). *Cryptosporina brachyfila* is octosporous, having oval SVs with abundant crystal-like inclusions, nearly of the size of the spores, and present also in vesicles with mature spores (Hazard and Oldacre, 1975). All these microsporidia are clearly different from the species from Sweden.

The interesting microsporidium *Gurleya sokolovi* shares the host, *Limnochares aquatica*, with the present species (Issi and Lipa, 1968). The characteristic pyriform spores are produced by octonucleate sporogonial plasmodia, but the species is actually tetrasporous, in that half of the sporoblasts degenerate. The SVs are elongated. Undoubtedly these microsporidia are different species. Whether or not they belong in the same genus cannot be solved without an ultrastructural investigation of *G. sokolovi*.

TAXONOMIC SUMMARY AND DESCRIPTION

Napamichum gen. nov.

Diagnosis. Merogony diplokaryotic. Merogonial plasmodium divides into numerous merozoites. Diplokaryotic sporont divides meiotically. Eight monokaryotic sporoblasts usually bud off simultaneously. Spores are lightly pyriform. The polaroplast has uniform sac-like compartments or an anterior lamellar region and a posterior section with sac-like compartments. The exospore is plurilayered with three wide layers of approximately identical thickness, separated by two narrow layers. The internal wide layer is the most electron dense. The two wide external layers are separated by an approximately 5-nm-thick double layer with a unit membrane appearance. The polar filament is anisofilar or of ap-

proximately uniform diameter. The sporophorous vesicle is fusiform, squashed with all spores in the same plane more or less ovoid. The envelope is uniform and persistent. The episporontal space of newly formed SVs contains fibrous, crystalline, and wide tubular inclusions. The wide tubules display the layers of the exospore. SVs with mature spores have fibrils and a small number of crystalline inclusions. Crystals are composed of material of two electron densities. Only one sporogonial sequence is observed.

Etymology. Anagram of *Chapmanium*.

Remark. The diagnosis of the new genus is principally based on the most thoroughly investigated species, *N. dispersus*.

Species

1. *N. dispersus* (Larsson, 1984) comb.
nov., type species

Synonym. *Chapmanium dispersus* (Larsson, 1984)

2. *N. aequifilum* sp. nov.

Merogony. Not observed.

Sporogony. Sporogonial plasmodia with eight nuclei produce eight monokaryotic sporoblasts by rosette-like budding.

Spores. Spores are pyriform with rounded ends. Unfixed spores are up to 3.0 μm wide and 5.4–5.8 μm long; fixed and stained spores are 2.8–3.0 \times 4.0–5.0 μm . Spore walls are 198–271 nm thick, with a 90- to 106-nm-thick plurilayered exospore. The polar filament is 149–165 nm wide, without constriction, and is arranged in six or seven coils in a single layer close to the spore wall. The coiled part begins about one-third from the anterior pole of the spore. The angle of tilt is about 40°. The polaroplast has two parts: anterior tight, irregular wide lamellae, and posterior more loosely arranged tubule-like sacs. A single nucleus occurs in the widest part of the spore.

Sporophorous vesicles. Uncompressed vesicles are fusiform; squashed vesicles

with all spores in the same plane are ovoid. Unfixed vesicles have dimensions up to 7.2 × 17.9 µm; fixed and stained vesicles have dimensions up to 7.0 × 12.0 µm. Spores are not arranged in any particular way. Crystals of the episporontal space are prominent in hematoxylin stainings and hardly visible in Giemsa stainings.

Host tissues involved. Except for fat cells, unknown.

Type host. *Limnochares aquatica* (Acari, Limnocharidae).

Type locality. A pond at Gårdstånga, Scania, in the south of Sweden.

Types. Holotype (Figs. 1E, 1F) on slide No. 860916-D-2 RL, paratypes on slides No. 860916-D-(1-8) RL.

Deposition of types. The slide with the holotype is in the International Protozoan Type Slide Collection at Smithsonian Institution (Washington, DC). Paratypes are in the collections of Dr. Jaroslav Weiser, Prague, Czechoslovakia, and in the collection of the author.

Etymology. The species name alludes to the unconstricted polar filament.

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