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Ultrastructure and light microscopic cytology of *Agglomerata lacrima* n. sp. (Microspora, Duboscqiidae), a microsporidian parasite of *Acanthocyclops vernalis* (Copepoda, Cyclopidae)

Annika M. Bronnvall* and J. I. Ronny Larsson

Department of Zoology, University of Lund, Helgonavägen 3, S-223 62 Lund, Sweden;
Fax: +46+46-222 45 41, E-mail: Annika.Bronnvall@zool.lu.se

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***Agglomerata lacrima* n. sp. is the first species of the genus described from a copepod host (*Acanthocyclops vernalis*.) It was studied using light- and electron-microscopy. All stages of the life-cycle had isolated nuclei. The earliest stages found were uninucleate merozoites. Sporogony produced 4–12 (mostly 8) pyriform spores by rosette-like budding within a fragile sporophorous vesicle. Live spores measured 4.4 ± 0.2 × 2.6 ± 0.2 µm, and fixed spores measured 3.7 ± 0.2 × 1.6 ± 0.2 µm. The exospore was constructed of 4 layers. The anisofilar polar filament made 5–6 coils in the posterior half of the spore. The polaroplast had an anterior part with wide lamellae (chambers), followed by a second zone of narrow lamellae. Tubule-like structures which might constitute a third polaroplast region were present immediately anterior to the first filament coil. Cytological characteristics and the generic position of the species are discussed, and it is compared to related or resembling species, and to all previously reported microsporidian species from copepods.**

Key words: *Agglomerata lacrima*; Microspora; ultrastructure; taxonomy; Copepoda

Introduction

Approximately 50 microsporidian species have been described from copepods, and as the descriptions are spread over more than hundred years, they are very heterogeneous. The earliest descriptions, which date back to the late 19th century, were restricted to light microscopic characteristics and illustrated by line drawings. The latest are based on electron microscopy, sometimes in combination with infection trials and/or molecular genetical investigations. As Copepoda, the host group, is very species-rich (about 9 000 species have been described), it can be expected that most microsporidian parasites of copepods still are undescribed.

In the spring of 1986, copepods with microsporidian infections were found in two samples collected from small pools at Sandby mosse in the south of Sweden. Closer examination revealed that the copepods of both samples were infected with the same species. The species, which is new to science, is described herein and the systematic position is discussed.

Material and methods

Copepods of the species *Acanthocyclops vernalis* Fischer, 1853 were collected in small pools at Sandby mosse (13° 25′ E, 55° 42′ N), Scania, southern Sweden

*corresponding author

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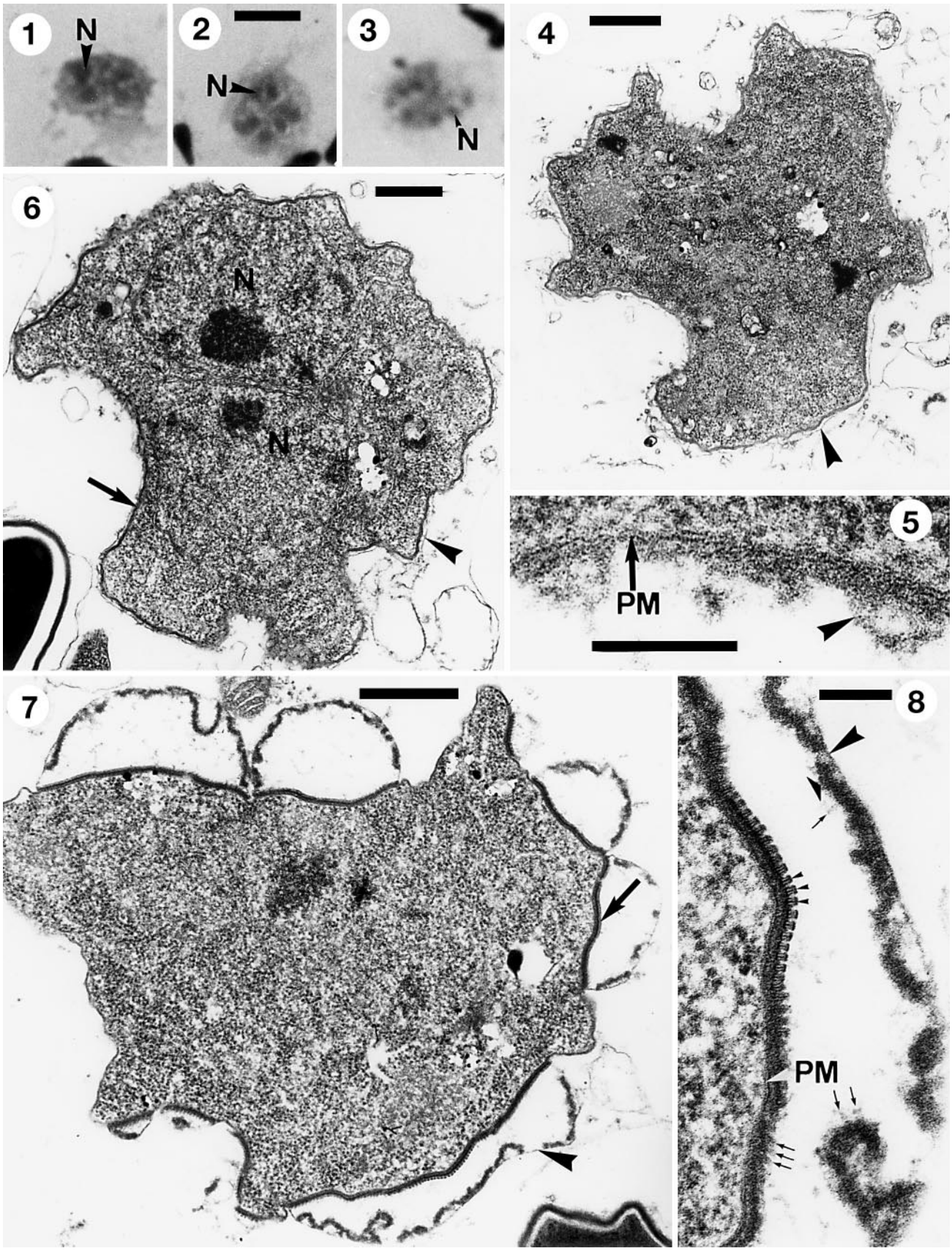
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on April 14 and May 6, 1986. These pools hold water from early autumn to late spring, but they often dry out during the summer. Specimens with anomalous coloration were selected and cut into halves.

One half was squashed, and the preparations were air dried and fixed in Bouin-Duboscq-Brasil solution overnight. They were stained using Giemsa solution and Heidenhain's iron haematoxylin (Romeis 1968). Permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd).

The other half of the copepod was fixed for transmission electron microscopy using 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C for 42 h. After washing in cacodylate buffer the specimens were postfixed in 2% (w/v) osmium tetroxide in cacodylate buffer at 4 °C for 1 h. The pieces were washed in 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

Pathogenicity

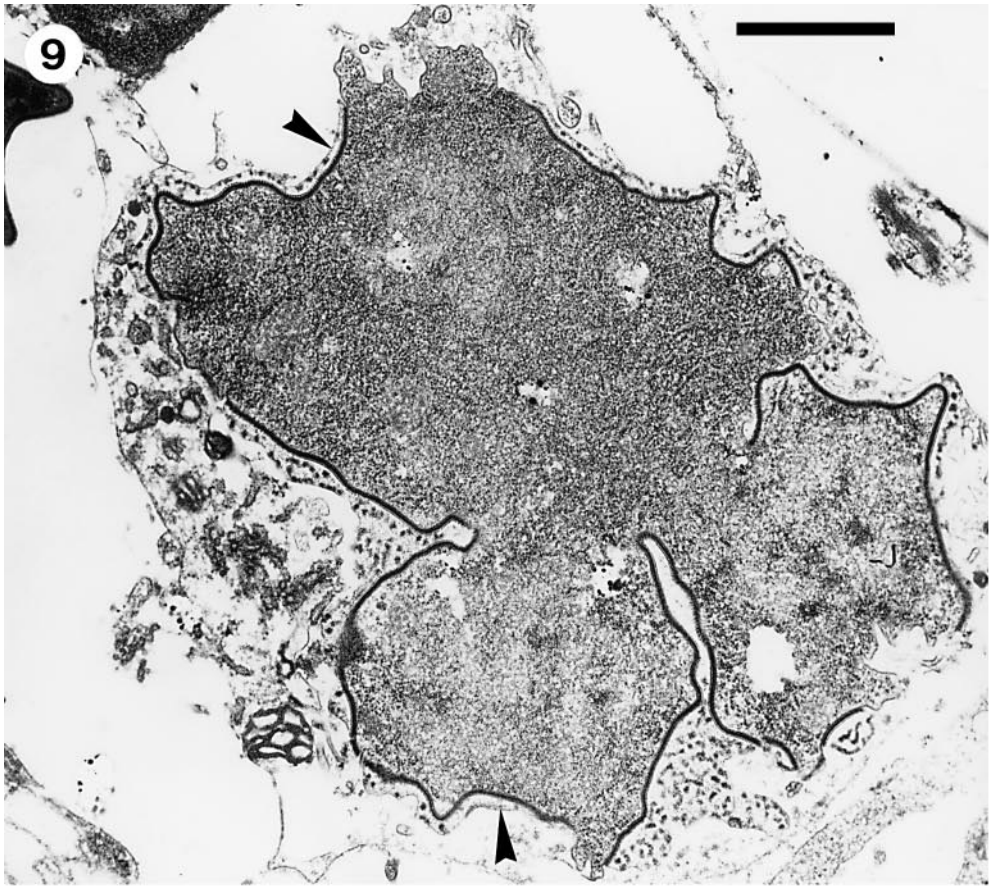
Prominent dense white spots were visible through the semitransparent cuticle of the infected copepods. Hypodermis and fat tissue were invaded by the microsporidia and free spores were found in the haemocoele. Infection was not observed in muscles or gonads. The host did not appear to suffer from the infection until at a very late stage, because even the most heavily infected specimens were still vigorous swimmers.

Presporal stages and life cycle

All life cycle stages had isolated nuclei. In all specimens studied, the merogonial reproduction was almost complete. The earliest stages found were merozoites, the final product of the merogony, which were delimited by a plasma membrane without external reinforcement. These mature into sporonts. Young sporonts could be discriminated from merozoites by their more regular rounded shape, and more compact cell wall.

Sporonts were rounded (Fig. 1–3). Numerous free ribosomes made the cytoplasm uniformly granular (Fig. 4). At the onset of sporogony, a thin (approximately 5 nm) electron-dense envelope, the primordial sporophorous vesicle, was secreted from the sporont (Fig. 5). It was released as a vesicle which followed the outline of the sporont (Figs. 4, 6). At the time of the first nuclear divisions, additional electron-dense material was secreted on the plasma membrane, inside the vesicle, to build a thick sporont wall which is the primordium of the exospore (Fig. 6). This addition of electron dense material continued throughout sporogony. As sporogony proceeded, the sporophorous vesicle started to bulge out in a blister-like fashion (Fig. 7). Inside the blisters the electron-dense sporont wall-material became organised to a layered structure, with a moderately dense 12 nm high basal layer and a more narrow (7 nm) very dense surface layer (Fig. 7–8). Sporont wall-material was formed in excess, visible as parallel strands on the surface of the sporont, as granular or fibrous projections from the surface, and as a 29–38 nm thick layer below the envelope (Fig. 7–11). The amount of released material increased until sporogony was complete (Fig. 9–11). It was obvious that the projections traversed the space from the sporont wall to the envelope (Fig. 11). After the last nuclear division, when the sporont started to bud in a rosette-like fashion (Fig. 9, 10, 12), the dense material became more evenly distributed to fill the episporontal space with a loose network (Figs. 9–11), and, at least for a short time, dense strands or fibres united the lobes of the budding

Fig. 1–8. Early sporogony. 1. Sporont with 4 nuclei. 2. Sporont with dividing nuclei. 3. Sporont with 8 nuclei. 4–5. Beginning of sporogony. The granular sporophorus vesicle (arrowheads) is separating from the plasma membrane. 6. Nuclei separating after division; electron-dense material is building a thick sporont wall (arrow); the sporophorous vesicle (arrowhead) closely follows the outline of the sporont. 7. The sporophorous vesicle (arrowhead) has begun to bulge out in a blisterlike fashion; exospore primordium (arrow) is visible inside the blisters. 8. Enlarged detail of Fig. 7: Sporophorous vesicle (large arrowhead) with an internal layer of granular material (triangle). Fibrous material (arrows) projects from the sporont wall and the dense layer below the envelope. Small arrowheads indicate the regularly arranged ridges remaining after the projections have lost contact with the sporont wall. The characteristic construction is visible in sections taken perpendicular to the ridges N = nucleus, PM = plasma membrane. Scale bars: Fig. 1–3 (with common bar on 2) = 5 µm, Fig. 4, 6, 7 = 500 nm, Fig. 5, 8 = 100 nm. Fig. 1–3. Light microscopy. Heidenhain's haematoxylin. Fig. 4–8. Electron microscopy.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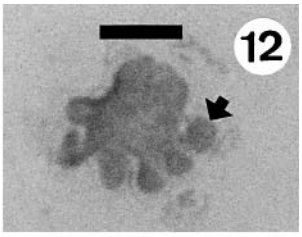
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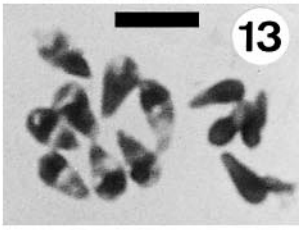
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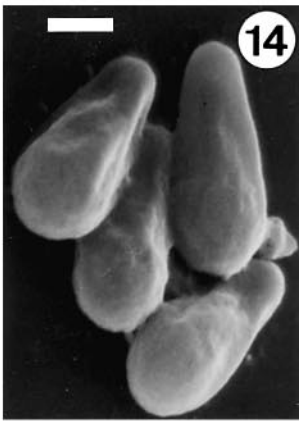
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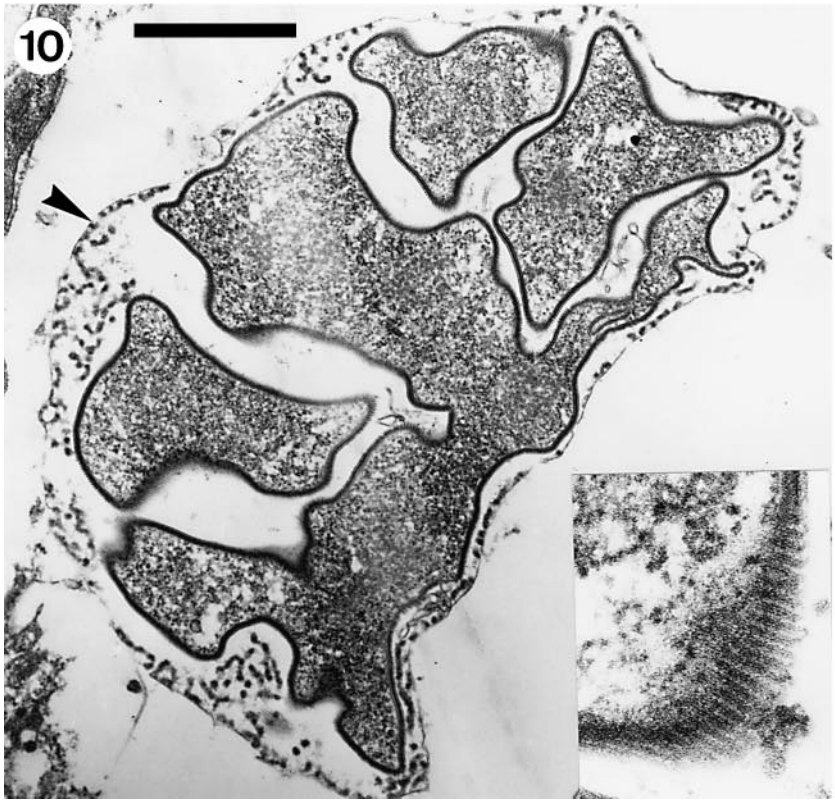
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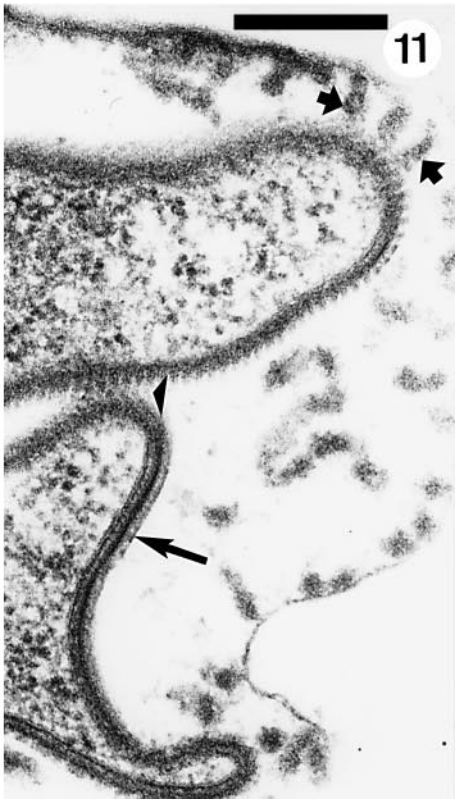
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plasmodium (Fig. 11). The parallel arrangement of strands was distinctly seen in positions where the sporont wall was sectioned tangentially (Fig. 8 arrows; 10 inset).

The projections from the sporont wall successively lost contact with the wall, leaving a system of short, parallel ridges on the surface of the sporont (Fig. 8, 11). In sections taken perpendicularly to the ridges, and to the surface of the sporont, ridges appeared as characteristic, regularly spaced, square projections. They were approximately 15 nm high and 10 nm wide, and they were separated by a gap approximately half as wide as the ridge (Fig. 8, 11). They had a characteristic construction of three narrow zones of electron-dense material separated by zones of less dense material (Fig. 8). The top of the ridge was electron-dense. The square projections later became confluent to form the surface layer of the exospore.

Sporogony produced 4–12, mostly 8, uninucleate spores (Fig. 13–14) in a common sporophorous vesicle.

The spore

Mature spores were pyriform (Fig. 13–15). Live spores measured 4.4 ± 0.2 × 2.6 ± 0.2 µm (Fig. 15); fixed and stained spores 3.7 ± 0.2 × 1.6 ± 0.2 µm (Fig. 13).

The spore wall (Fig. 16) was of the typical construction for microsporidia, with plasma membrane, endospore, and exospore. The plasma membrane was an approximately 7 nm thick unit membrane. The endospore was electron lucent and 140–150 nm thick, except at the anterior pole above the anchoring disc, where it was considerably thinner. In the exospore four distinct layers were visible (Fig. 16 a–d): an internal, 14–18 nm thick, moderately electron dense granular layer (a) was followed by an electron dense layer (5 nm thick) (b). The dense layer appeared as a double-layer at some places. Next layer in direction outwards was 10–15 nm thick and moderately electron-dense (c). The surface layer was approximately 5 nm thick and slightly more electron dense (d). No extrusions projected from the wall of mature spores.

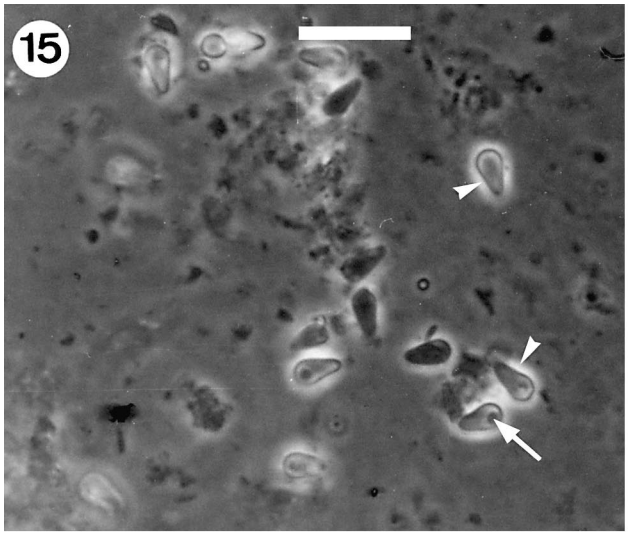
The polar filament had a straight anterior part and a posterior section with 5–6 coils, arranged in one row of coils close to the spore wall in the posterior half of the spore (Fig. 17, 19). The straight part followed the central axis to the middle of the spore, then turned towards the spore wall (Fig. 19). The most anterior coil began about one sixth from the posterior pole of the spore and coiled in anteriad direction. The angle of tilt of the first coil to the longitudinal axis of the spore was about 35° (Fig. 17), and the most anterior part of the first coil reached approximately to the middle of the spore. The zone with coils was about one sixth of the spore length. The filament was anisofilar, with the straight part and the anterior 1–2 coils slighly wider than the posterior coils. Wide coils measured 135–140 nm, narrow coils 105–110 nm.

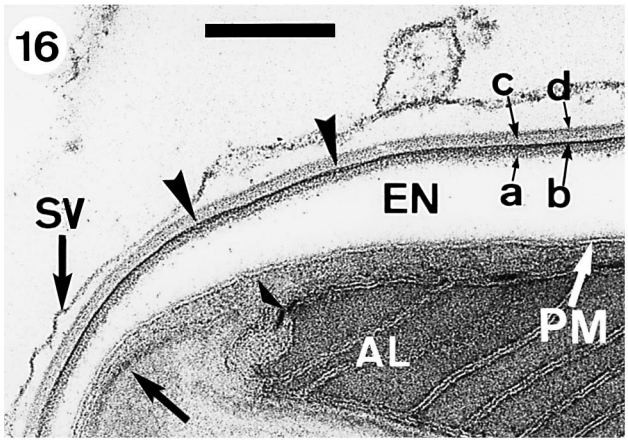
In transverse sections of the filament, 6 more or less distinct layers were visible (Fig. 18 a–f). The surface layer (a) was an approximately 5 nm thick unit membrane followed by a narrow zone (2–3 nm) of electron-dense material (b). The next layer in an inward direction was 6–8 nm thick and moderately electron-dense (c). It was followed by an electron-dense layer (about 4 nm) (d), and an electron lucent zone, up to 11 nm wide, resembling transversely sectioned fibrils (e, arrows). The centre of the filament (f) was 65–85 nm in diameter and of variable electron density. In the straight part of the filament, down to and including the point where the filament turned to make the first coil, a distinct electron-dense core was visible in the centre (Fig. 17, 19). The dense core constituted slight-

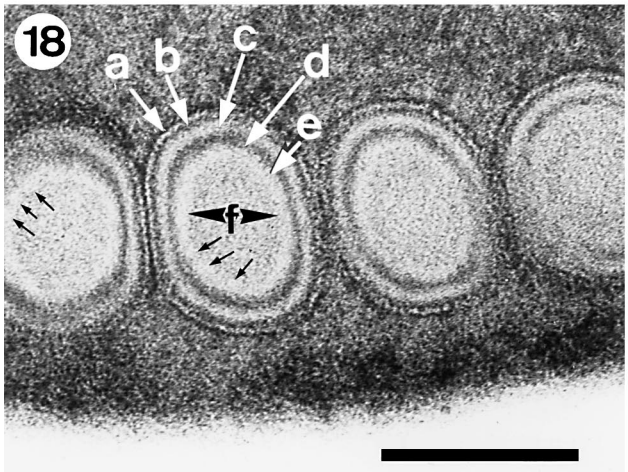
Fig. 9–14. Late sporogony and spores. 9. Lobed sporont. Strands of electron-dense material traverse the episporontal space (arrowheads indicate the sporophorous vesicle). 10. Fission of a sporont. Inset: sporont wall detail exhibiting ridges. 11. Enlarged detail of Fig. 10: Electron-dense strands (short arrows) connect the exospore primordium with the granular material inside the vesicle as well as joining the exospore primordia of adjacent lobes (triangle), all four layers of the future exospore are visible (long arrow). 12. Rosette-like budding sporont with six buds visible (arrow). 13. Group of immature spores (left) and mature spores (right). 14. Group of four spores. Scale bars: Fig. 9–10 = 1 µm. Inset on Fig. 10 and Fig. 11 (with a common scale bar on 11) = 200 nm. Fig. 12–13 = 5 µm. Fig. 14 = 1 µm. Fig. 9–11. Electron microscopy. Fig. 12–13. Light microscopy. Heidenhain's haematoxylin. Fig. 14. Scanning electron microscopy.

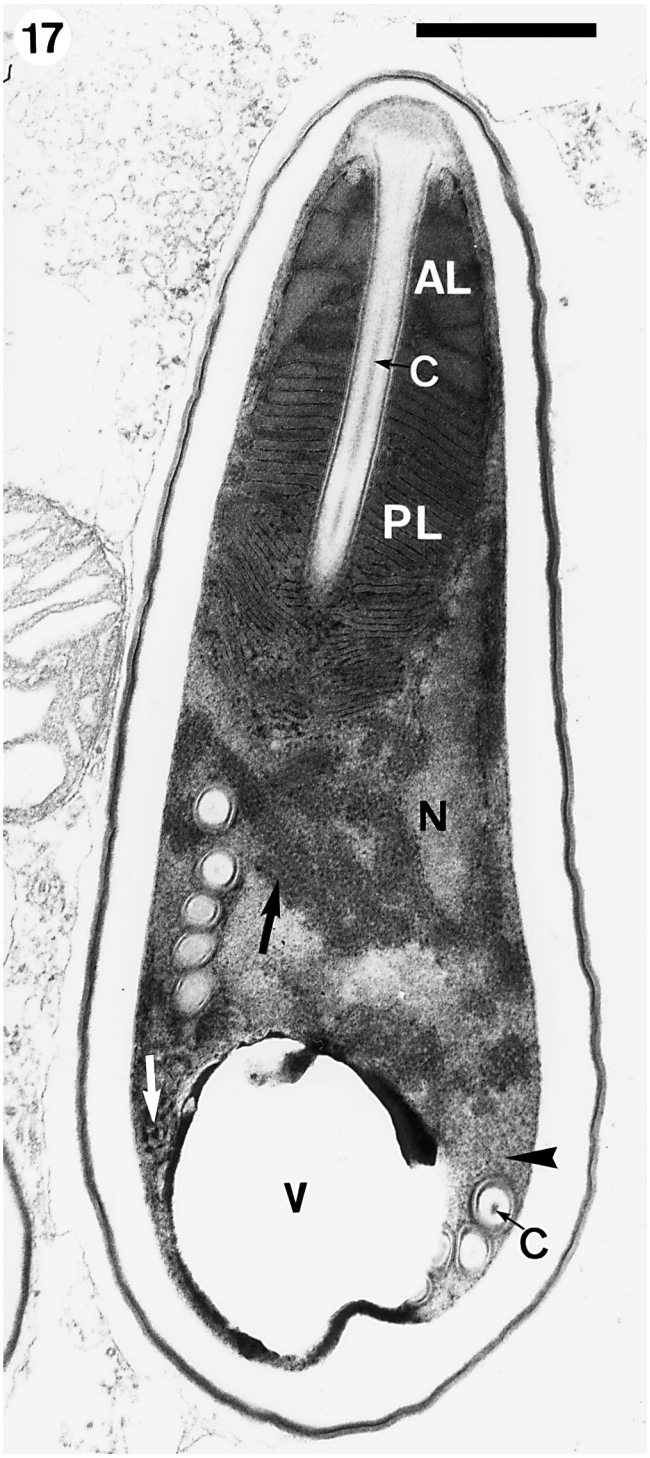
ly less than half of the diameter of the centre. The outer portion of the centre was fairly electron-lucent. No core was visible in the posterior part of the filament, instead the whole centre was uniform and moderately electron-dense (Fig. 18f).

The polaroplast surrounded the uncoiled part of the polar filament (Fig. 17, 19). It was constructed of about 5 nm thick unit membrane folds. The polaroplast was divided into two, or possibly three, parts. In the anterior part the membrane-folds









appeared as up to 170 nm wide, irregularly thick lamellae (Fig. 16). In the second part of the polaroplast the lamellae were closely packed and looked more uniform. They were about 35 nm wide in the anterior part of this polaroplast zone, but became slightly more compressed towards the posterior end of the zone. From the point where the straight filament bent to a position proximal to the start of the anterior filament coil, seemingly separated from the lamellae by a short gap, was a group of rounded, or tubular, structures which might belong to a third, tubular polaroplast part (Fig. 17, 19).

The umbrella-shaped polar sac enclosed the anchoring disc and extended back about one quarter of the spore length. It covered the anterior part, and a short section of the second part, of the polaroplast. The sac was filled with moderately electron-dense material.

The elongated nucleus was located to the midsection of the spore (Fig. 17). The longest sectioned nucleus measured about 1 µm in diameter. The cytoplasm was fairly electron dense with strands of polyribosomes surrounding the nucleus, polaroplast and polar filament. In live spores (Fig. 15) and in sections (Fig. 17), the membrane-lined posterior vacuole was visible in the posterior third of the spore. It measured up to 0.8 µm in diameter. The remainder of the organelle normally interpreted as a Golgi apparatus could be seen next to the vacuole (Fig. 17).

The mature sporophorous vesicle

The vesicle containing mature spores was thin (about 5 nm thick) and fragile (Fig. 19). There was no trace of the fibrous or granular strands characteristic for the episporontal space of the undivided or lobed sporont. In the mature vesicle different kind of tubuli, formed of excess exospore material during sporoblast formation were present (Fig. 19–20). These exhibited the same kind of stratification as the exospore (Fig. 20).

Discussion

Cytology

A few details of the cytology need comment: the organisation of the polar filament, the construction of the polaroplast, and the exospore together with the inclusions of the sporophorous vesicle.

In the straight region of the polar filament, and the first part of the anterior filament coil, an electron-dense core is present (Fig. 17). An identical core is also found in *Agglomerata sidae*, the type species (Fig. 5 A in Larsson and Yan 1988), as well as in two other species classified in this genus: *A. cladocera* (Fig. 22 in Larsson et al. 1996), and *A. volgensae* (Fig. 16 in Larsson and Voronin 2000). However, this organisation is not unique to *Agglomerata* species, and for example is also seen in *Nosema tractabile* (Fig. 17 in Larsson 1981).

The polaroplast of *A. sidae* , with three regions, was at the time of description of unique construction (Fig. 6 in Larsson and Yan 1988). An anterior part with wide lamellae was followed by a section with considerably narrower lamellae. These two regions join each other. Separated from the lamellae by a distinct gap was a posterior third region composed of tubular structures. An identical polaroplast was later observed in *A. cladocera* (Fig. 22, 24–26 in Larsson et al. 1996). However, in the latest described *Agglomerata* species, *A. volgensae*, the tubular region is lacking which was interpreted as a reduction (Larsson and Voronin 2000).

Fig. 15–18. The mature spore. 15. Living spores. The spores are surrounded by a gelatinous cover (arrowheads), and the posterior vacuole is visible (arrow). 16. Section of the anterior end of a spore showing the layers of the spore wall: plasma membrane (PM), endospore (EN), exospore with four layers (a – d), and the anterior region of the polaroplast (AL). The arrowheads mark places where the second layer of the exospore appears as a double-layer. The anchoring disc (arrow), and the most anterior part of the polar sac (triangle) are visible. SV = sporophorous vesicle. 17. Longitudinal section of a spore. The polaroplast has wide anterior lamellae (AL), narrow posterior lamellae (PL); tubular material (arrowhead) close to the anterior filament coil might be a third polaroplast part. The reticulate structure (white arrow) close to the posterior vacuole (V), is probably the remainder of the Golgi apparatus. Polyribosomes (black arrow) surround the nucleus (N), polar filament and polaroplast. An electron-dense core (C) is visible in the straight part of the polar filament, terminating in the anterior coil. 18. Transverse section of the polar filament coils; a–f indicate the layers visible, arrows point at fibrous components. Scale bars: Fig. 15 = 10 µm, Fig. 16 = 200 nm, Fig. 17 = 500 nm, Fig. 18 = 100 nm. Fig 15. Phase contrast. Fig 16–18. Electron microscopy.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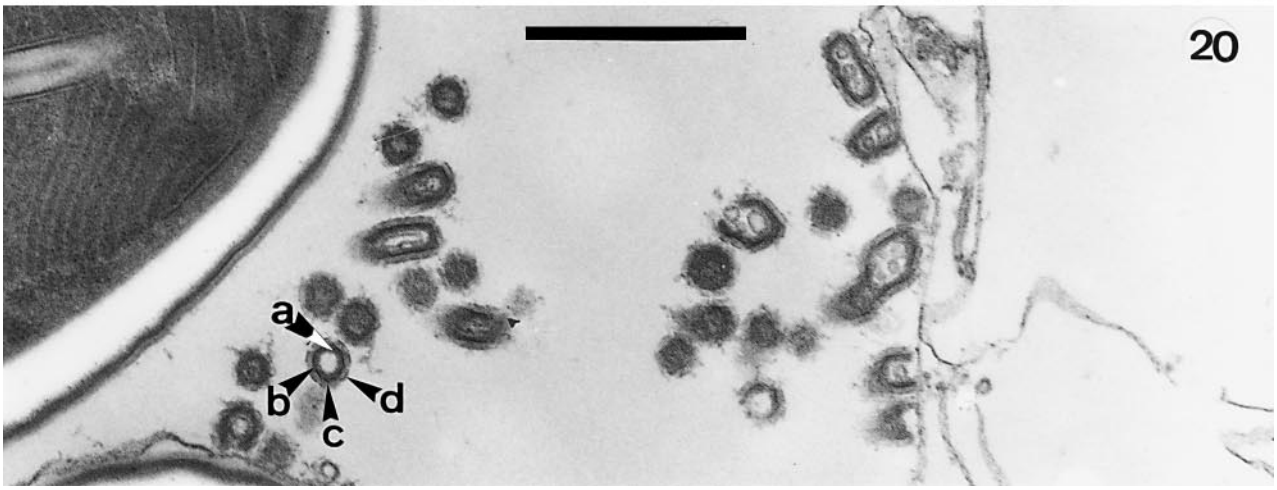
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The species described herein has a polaroplast with two distinct parts, equal to the anterior regions of a typical *Agglomerata* polaroplast. In addition rounded, or tubular, structures were seen immediately anterior to the first filament coil which is the correct position for the third tubular part of an *Agglomerata* polaroplast (Fig. 17, 19). Tripartite polaroplasts are known from other microsporidia. In *Binucleospora elongata* a bipartite lamellar polaroplast (narrow and wide lamellae – opposite to the arrangement of *Agglomerata)* is directly followed by a tubular region (Bronnvall and Larsson 1995a). In *Lanatospora tubulifera* a bipartite lamellar polaroplast of the *Agglomerata*-type is followed by a section with tubules (Bronnvall and Larsson 1995b). However, in this case the distinct *Agglomerata*-gap is absent.

The exospore and the inclusions of the sporophorous vesicle are closely connected as they are formed by the same secretory products during sporogony. A great variety of inclusions have been described from various vesicle-forming microsporidia. The exospore of *Agglomerata sidae* is a layered structure, and regularly spaced electron-dense material projects from the exospore to the envelope of the sporophorous vesicle (Fig. 2 and 4 in Larsson and Yan 1988). In the early phase of the sporogony the strands appear granular or fibrous. Later, when sporoblasts are individualised, inclusions are distinctly tubular, exhibiting the same layers as the exospore. Identical tubules are also formed by *A. cladocera* (Fig. 10 and 13 in Larsson et al. 1996) and the species described herein (Fig. 19, 20). There is also a striking similarity between *A. sidae* (Fig. 2 D and 4 B in Larsson and Yan 1988) and the species described in the present paper in the way the parallel ridges and strands of exospore material are formed in the early sporogony (Fig. 10 inset).

The exospore can be initiated in various ways. In the genera *Nosema* (Fig. 10–11 in Larsson 1981), *Encephalitozoon* and *Unikaryon* (which lack sporophorous vesicles) the exospore is initiated as parallel strands of electron-dense material which later become confluent to a uniform electron-dense exospore. In *A. sidae* the primordia of the exospore appear as wide fields of uniform electron-dense material, which later become stratified, a common way of exospore initiation (Larsson and Yan 1988). In sections of sporonts the surface of the primordial exospore appears somewhat undulated, caused by more or less parallel strands of projecting granular material (Fig. 2 C and D in Larsson and Yan 1988). The exospore of the species described herein is initiated in the same way as in *A. sidae*: originally as wide fields of uniform electron-dense material (Fig. 5–6) which at a later stage become stratified (Fig. 7–8). After the projections have lost contact with the sporont wall, the remainder are visible as regular ridges which later become confluent. However, these regularly spaced ridges have a particular construction with alternating strands of electron-dense and more lucent material – an arrangement unique to this species.

Generic position

Many microsporidian genera contain microsporidia of copepods, but our knowledge of these species is rarely great enough to verify their placing in a correct genus. The most well-known microsporidia of copepods belong to the two polymorphic genera *Amblyospora* and *Parathelohania*. These microsporidia use copepods and mosquitoes as alternate hosts. *Amblyospora* is most extensively studied (Becnel and Andreadis 1999). The spore morph of *Amblyospora* produced in copepods is large and lanceolate with a unique vesicular polaroplast. *Parathelohania* spores from copepods are similar at the light microscopic level (Larsson 1999). Their ultrastructure is unknown. The microsporidium treated herein has a spore morphology distinctly different from the copepod morphs of these polymorphic genera.

Fig. 19–20. Ultrastructure of the mature sporophorous vesicle. 19. Sporophorous vesicle (black arrowhead) with three spores visible. The polar filament turns to the side at the middle of the spore (large white arrowhead), and continues to about 1/6 from the posterior end of the spore before making the first coil (in anteriad direction). The electron dense core of the filament is visible in the straight part and in the first coil (white arrows). Small white arrowheads point at tubuli which might belong to a third polaroplast region. Tubular projections formed from excess exospore material are visible in the episporontal space (black arrows). 20. Enlarged detail of Fig. 19 exhibiting the exospore stratification (a–d) of the tubuli. Scale bars: Fig. 19 = 1 µm, Fig. 20 = 500 nm.

There are 9 microsporidian genera from invertebrate hosts that produce sporogonial plasmodia with isolated nuclei, which divide by rosette-like budding to yield uninucleate, pyriform spores within a sporophorous vesicle: *Tuzetia* Maurand et al., 1971, *Nelliemelba* Larsson, 1984, *Gurleya* Doflein, 1898, *Flabelliforma* Canning et al., 1991, *Lanatospora* Voronin, 1986, *Episeptum* Larsson, 1986, *Larssonia* Vidtmann et Sokolova, 1994, *Trichotuzetia* Vávra et al., 1997 and *Agglomerata* Larsson et Yan, 1988. By distinct differences 7 of these are excluded for the microsporidium treated herein.

The exospore of *Tuzetia* is uniformly electron-dense, and the spores are enclosed in individual sporophorous vesicles (Larsson 1984). The sporophorous vesicles of *Nelliemelba boeckella* (Milner et Mayer, 1982), the only species of the genus, are also individual and in addition an extrasporous coat is inserted between the spore and the sporophorous vesicle (Larsson 1984). The genus *Gurleya* is tetrasporoblastic. There is no modern investigation of the type species, *G. tetraspora* Doflein, 1898, but in the description of *Gurleya daphniae* Friedrich et al., 1996, a parasite of microcrustaceans as is the type species, it was shown that the sporogony proceeds in two steps (Friedrich et al. 1996). After the first nuclear division, the plasmodium becomes dumbbell-shaped with one nucleus at each pole. The second division yields two nuclei at each pole. Finally cytoplasmic fission occurs at each end. The species described herein yields multinucleate sporogonial plasmodia which divide by typical rosette-like budding: i.e. after completion of the nuclear divisions daughter nuclei migrate to the periphery of the plasmodium where numerous buds, each with one nucleus, are formed.

According to the description the pattern of division in *Flabelliforma* is fan-like, i.e. all the lobes of the budding sporont are directed to one side (Canning et al. 1991). Little is known about the mature spore. In Fig. 33 of the description a series of closely packed polaroplast lamellae are visible at the anterior pole of the spore, and cytoplasmic structures resembling wider lamellae or tubules seem to be present anteriorly to the most anterior polar filament coil (Canning et al. 1991). Further the sporophorous vesicle is formed without production of blister-like protrusions. Mature spores of *Lanatospora* have, as the name suggests, a persistent, thick, tubular and "woolly" exosporal coat which connects to the individual sporophorous vesicles (Voronin 1989; Bronnvall and Larsson 1995b). Species of *Episeptum*, so far only found in caddis flies (Trichoptera) are tetrasporoblastic (Larsson 1999). The type species has a distinct, compartmentalized, external layer of the exospore, somewhat resembling the spore coat of *Lanatospora* (Larsson 1986). *Trichotuzetia guttata* Vávra et al., 1997 is the only described species of the genus. Prior to formation of the complete sequence of layers of the future exospore, the sporont of *T. guttata* produces a multitude of long fibrils of unique construction traversing the episporontal space. At high magnification the fibrils show a characteristic electron-dense transverse striation (Vávra et al. 1997).

Two genera remain: *Larssonia* and *Agglomerata*. The distinction between these two genera is not clear, and some species have been more or less temporarily assigned to *Agglomerata*. *Larssonia obtusa* (Moniez, 1887), so far the only species of the genus, produces spores with exosporal coats of thin tubules or fibrils, the spores are enclosed in individual sporophorous vesicles, and, according to the description of the genus, a brief diplokaryotic stage occurs between merogony and sporogony (Vidtmann and Sokolova 1994). These features are not shared by the Swedish microsporidium.

In the early part of the discussion it was concluded that the cytology of the species treated herein corresponds fairly well with the characteristics of *Agglomerata*, and the only prominent difference concerns the persistence of the sporophorous vesicle. The sporont of *A. sidae* produces a persistent, rounded sporophorous vesicle where the complete spore yield of the sporont is connected by prominent tubular projections to each other and to the envelope, while the species treated herein sporulates in fragile sporophorous vesicles. That difference alone does not justify the establishment of a new genus, and therefore we decide to assign the microsporidium to the genus *Agglomerata*.

Agglomerata species

In addition to the type species discussed above, four more species have been assigned to the genus *Agglomerata*. All five are parasites of Cladocera. Three of these are easily distinguished from the present species. Apart from the distinctly tripartite polaroplast, mature spores of *A. cladocera* (Pfeiffer, 1895) have an exosporal coat similar to the one

found in *Larssonia obtusa* (Larsson et al. 1996), *A. bosminae* (Voronin, 1986), originally placed in the genus *Lanatospora*, has a thick woolly exospoal coat (Voronin 1989a; Voronin 1999), and *A. simocephali* (Voronin, 1986) has thin filamentous protrusions from the exospore and a greater number of filament coils (Voronin 1989b).

The polaroplast of *A. volgensae* Larsson et Voronin, 2000 is clearly bipartite and the sporophorous vesicle follows the outline of the spores. The vesicle either divides together with the sporont to form individual sporophorous vesicles, or, occasionally, remains as a common vesicle. Living spores are approximately 1 µm shorter than spores of the Swedish species, and transverse sections of the last three filament coils show an internal construction which is different from the anterior coils (Larsson and Voronin 2000).

It is obvious that the present microsporidium is different from other species assigned to the genus *Agglomerata*.

Microsporidia of copepods

Approximatly 50 species of microsporidia have been described from copepods. Some of them have already been discussed and the differences from the species treated herein have been stated. If we further disregard species placed in genera with distinct characteristics, which clearly tell that they are different from *Agglomerata*, and those species where the spores distinctly differ from the Swedish species by their shape and/or size, 10 species remain for comparison: *Gurleya macrocyclopis* Voronin, 1986, *Thelohania cyclopis* Weiser, 1945, *Tuzetia cyclopis* (Kudo, 1921), *Gurleya lopukhinae* Voronin, 1986, *Microsporidium schmeilii* (Pfeiffer, 1895), *Microsporidium jiroveci* (Voronin,1977), *Gurleya richardi* Cépède, 1911, *Flabelliforma diaptomi* (Voronin, 1977), *Nosemoides cyclopis* Vidtmann, 1990 and *Thelohania kaunensis* Vidtmann, 1990. These species, at least those that can be identified clearly, also differ from the species treated here.

Gurleya macrocyclopis produces broadly oval spores by tetrasporoblastic sporogony in a persistent sporophorous vesicle and the anisofilar polar filament is arranged in 12 coils (Voronin 1986; Voronin 1996a). *Thelohania cyclopis*, which is mainly octosporoblastic, produces 5 µm long, oval to pyriform spores within a persistent, elongated sporophorous vesicle (Weiser 1945). *Tuzetia cyclopis* produces pyriform spores, 4.2–4.7 µm long, with a distinctly longer polar filament arranged in 13 coils (Kudo 1921; Loubès 1979). *G. lopukhinae* produces pyriform spores that measure 2.3–3.6 × 1.7–2.2 µm (Voronin 1986), considerably smaller than spores of the present species, and the polar filament has 7 coils (Voronin 1989b). *M. schmeilii* is probably a conglomerate of several species (Sprague 1977) and can not be recognized. It was originally described from three different hosts (2 cladocerans and one not specified copepod), and no type host was designated (Pfeiffer 1895). The spore length was described to vary from 4 to 8 µm. However, all spores were of the same size in each host species. Which spore length was found in which host was not specified. *M. jiroveci* has elongated pyriform spores measuring 5.5–6.7 × 2.4–2.7 µm (Voronin 1977). *G. richardi* might be a mixture of two species or a dimorphic species, as both round and oval spores are found. Both sporetypes are enclosed in tetrasporoblastic persistent sporophorous vesicles (Cépède 1911). *F. diaptomi* (Voronin 1977, Voronin 1996b) has a lamellar polaroplast with wider lamellae anteriorly. It differs from the species treated herein by the smaller size of the living spores, 3.7 ± 0.3 × 1.9 ± 0.2 µm, and a polar filament with 7–8 coils. Finally, spores of both *T. kaunensis* and *N. cyclopis* are described as oval (Vidtmann 1990). A line drawing of *T. kaunensis* shows a spore shape clearly different from the species of this article. The anterior pole of these spores is distinctly blunt in comparison with the elongated narrow anterior end of the spores of the Swedish microsporidium. Also the line drawings of *N. cyclopis* show spores with more blunt anterior poles.

The conclusion must be that the species described herein is new to science.

Description

■ *Agglomerata lacrima* sp. nov.

Merogony: Uninucleate merozoites were the only merogonial stages observed.

Sporogony: Sporonts with isolated nuclei produce 4–12, mostly 8, sporoblasts by rosette-like budding. Granular or fibrous extrusions from the sporont connect the sporont wall with the sporophorous vesicle. Projections lose contact with the sporont wall. Remaining material appears as regularly arranged, 15 nm high, parallel ridges.

Spore: Uninucleate and pyriform. Fresh spores measure 4.2–4.6 × 2.4–2.8 µm; fixed and stained spores 3.7 ± 0.2 × 1.6 ± 0.2 µm. The posterior vacuole is approximately 0.8 µm in diameter in fresh spores. The exospore is 35–40 nm thick with four visible layers. The polar filament is arranged in 6 coils and the angle of tilt is about 35°. The filament is anisofilar. The straight part and anterior 1–2 coils measure 135–140 nm in diameter, the posterior coils 105–110 nm. The polaroplast is bipartite or possibly tripartite. The anterior part consists of wide lamellae, or chambers, up to 170 nm thick, the posterior part of narrow, up to 35 nm thick, lamellae. Tubule-like structures might represent a third polaroplast region.

Sporophorous vesicle: Encloses the complete spore yield of the sporont. Envelope fragile, about 5 nm thick. Episporontal space of mature vesicles with tubuli formed of excess exospore material.

Site of infection: Hypodermis and fat tissue; free spores in the haemocoele.

Type host: *Acanthocyclops vernalis* Fischer, 1853 (Crustacea, Copepoda, Cyclopidae).

Type locality: Pools at Sandby mosse (13° 25′ E, 55° 42′ N), Scania, Sweden.

Types: Syntypes on slides 860419-B-(1–4).

Deposition of types: In the International Protozoan Type Slide Collection at Smithsonian Institution, Washington D. C., U.S.A., and in the collection of the senior author.

Etymology: The latin noun lacrima (tear) alludes to the shape of the spore.

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