

Light and Electron Microscopic Study of *Agglomerata volgensae* n. sp. (Microspora: Dubosqiidae), a New Microsporidian Parasite of *Daphnia magna* (Crustacea: Daphniidae)

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Summary

The new microsporidium *Agglomerata volgensae* n. sp. is described based on light microscopic and ultrastructural characteristics. The parasite invades the hypoderm and adipose tissue of *Daphnia magna* and causes hypertrophy. All life cycle stages have isolated nuclei. Sporogonial plasmodia divide by rosette-like division, producing 4–16, usually 8, sporoblasts. A sporophorous vesicle is initiated after the first nuclear division of the sporont. The fragile vesicle either collects all daughter cells of the sporont, or the vesicle divides together with the plasmodium to enclose spores in individual sporophorous vesicles. Fibril-like projections connect the exospore with the envelope of the sporophorous vesicle. Tubules, with walls of exospore material, are formed together with the sporoblasts. Mature spores are pyriform with pointed anterior pole and an obliquely positioned posterior vacuole. Unfixed spores measure 3.2–3.7×1.7–2.0 µm. The exospore is layered, approximately 38 nm thick. The polar filament is lightly anisofilar with 2–3 wide anterior coils, and 2–3 more narrow posterior coils, in a single layer of coils in the posterior half of the spore. The polaroplast has two regions: irregular wide lamellae or chambers surrounding the straight part of the filament, and more loosely arranged narrow lamellae in the coil region. The discrimination from other microsporidian species and the systematic position are briefly discussed.

Key words: *Agglomerata volgensae*; Microspora; Ultrastructure; Taxonomy; *Daphnia magna*.

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Abbreviations

A – anchoring disc; E – endospore; ER – endoplasmic reticulum; ES – episporontal space; EX – exospore; F – polar filament; G – Golgi apparatus; M – merozoite; N – nucleus; NL – nucleolus; P – plasma membrane; PA – anterior polaroplast region; PP – posterior polaroplast region; PS – polar sac; PV – polar vesicle; SE – spore; SP – sporont; SV – sporophorous vesicle; T – tubule; V – posterior vacuole

Introduction

Microsporidia of microcrustaceans (cladocerans, copepods, ostracods) have attracted an increased interest for ecologists and taxonomists during the last two decades. Profound impact on the life and reproduction of the host has been revealed and different routes of parasite transmission have been verified [3, 4]. For a number of copepod microsporidia, but not for microsporidia of cladocerans and ostracods, very complex life cycles, involving alternate hosts, have been elucidated [1]. New species have been described, and old species have been revised, provoking the establishment of a number of new genera.

More than 40 species of microsporidia have been reported from Cladocera, most of them from *Daphnia magna* and *D. pulex* [5, 8, 9, 11, 29]. Many of these reports date from the previous century. The microsporidia were described according to other principles than those in use today, which makes identification

and comparison difficult or impossible. Jírovec spent much effort on the taxonomic problems of the microsporidia of Cladocera, and he redescribed some of the old species based on newly collected material [7, 8, 9, 10]. His collection has survived and serves as valuable reference for further taxonomic studies [16, 17, 19].

In continuation of our studies of microsporidian parasites of Cladocera, we have investigated a microsporidium of *Daphnia magna* with Russian provenance. The species is clearly new to science, but the placing in a genus causes some problems. The species is briefly described herein, and the taxonomic considerations are discussed.

Material and Methods

Infected specimens of *Daphnia magna* Strauss, 1820 were collected in July and August, 1993, from a pond close to the river Volga in the town of Volgorechensk, Kostroma district, Russia.

Permanent squash preparations were lightly air-dried, fixed in methanol for 2 min, and stained using Giemsa solution [25].

For transmission electron microscopy specimens were fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C, and stored in this solution for several weeks. After washing in cacodylate buffer and post fixation in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4 °C, tissues were washed and dehydrated in an acetone series and embedded in epon. Sections were stained using uranyl acetate and lead citrate [24].

Results

Prevalence and pathogenicity

Infected cladocerans had a distinct white colour visible to the naked eye. During both months of sampling (July, August) the prevalence of infection was low with only 3% of adult specimens infected. Infection was restricted to the hypoderm and adipose tissue (Fig. 1). Infected cells retained their cellular nature but were lightly hypertrophic. Parasites exhibited some stratification in the host cells, with the most immature stages aggregated close to the periphery and with mature spores in the centre of the cells (Fig. 1).

Merogony and sporogony

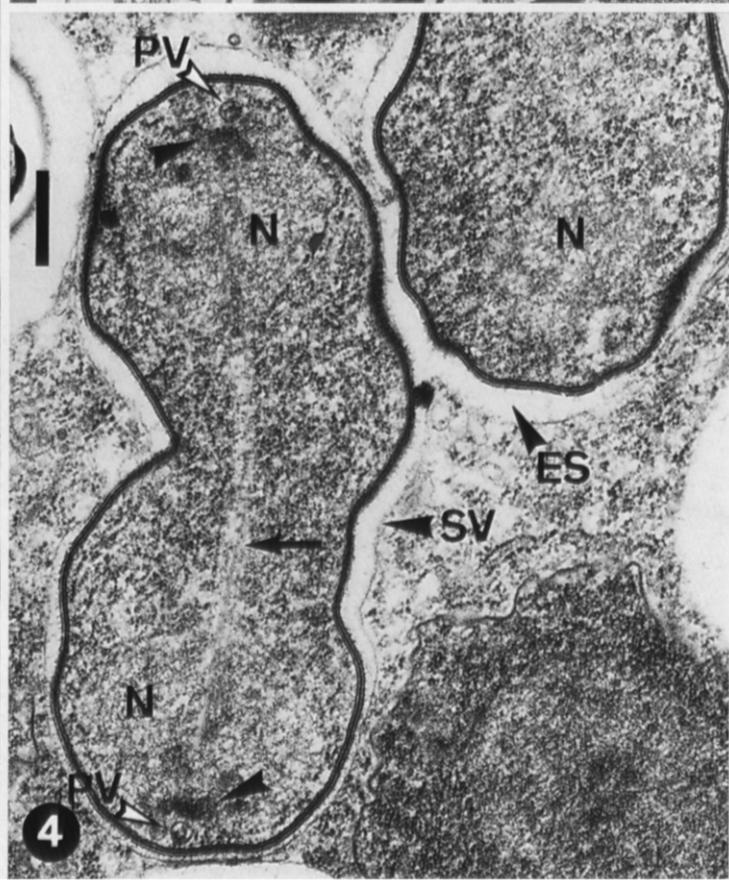
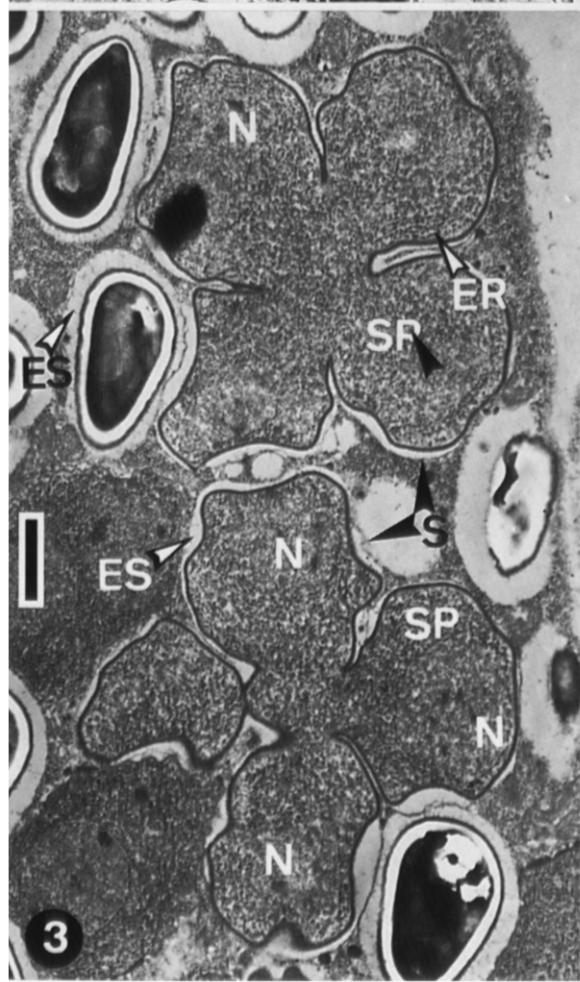
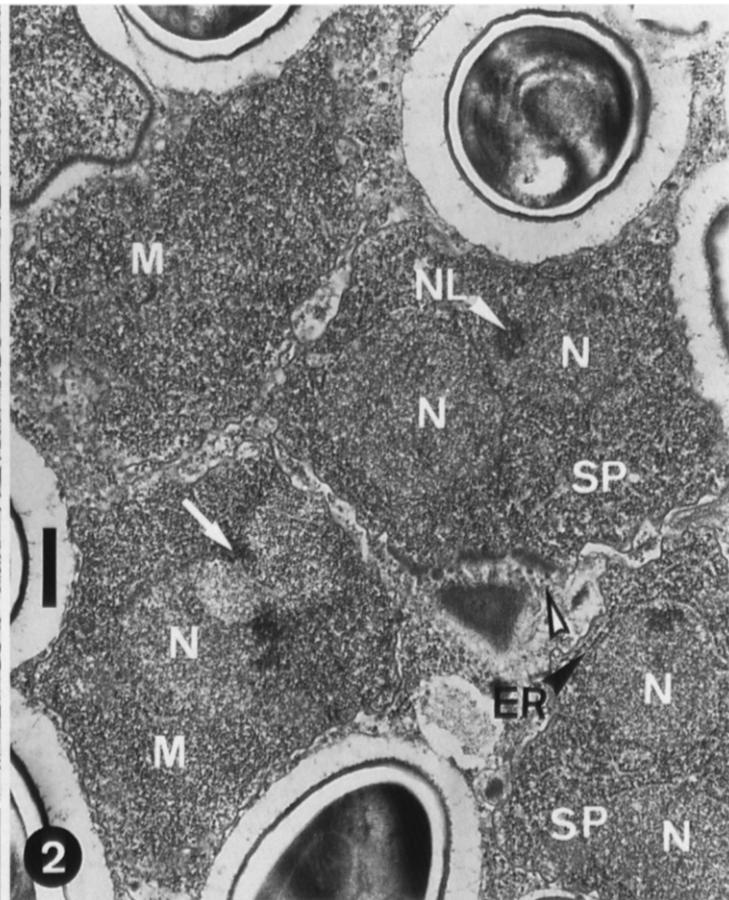
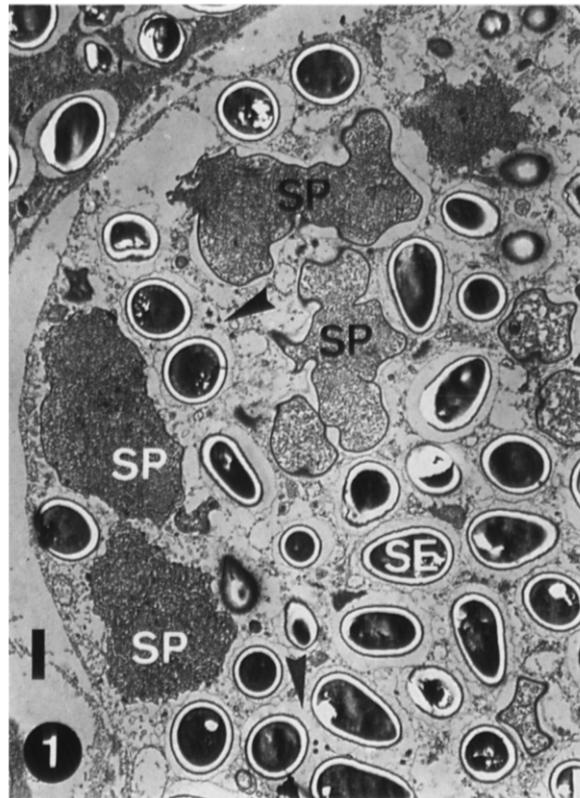
All life cycle stages, from merozoites to mature spores had isolated nuclei. Stages belonging to the merogonic reproduction were only revealed as groups of rounded to oval merozoites, measuring up to 3.3 µm in diameter (Fig. 2). Their cytoplasm was rich in free ribosomes, but there were no traces of an endoplasmic reticulum. Nuclei were uniformly granular. In some sections an electron dense nucleolus was visible (Fig. 2). The widest sectioned nucleus measured 1.7 µm in diameter. Merozoites were delimited by an approximately 8 nm thick unit membrane, without external reinforcements.

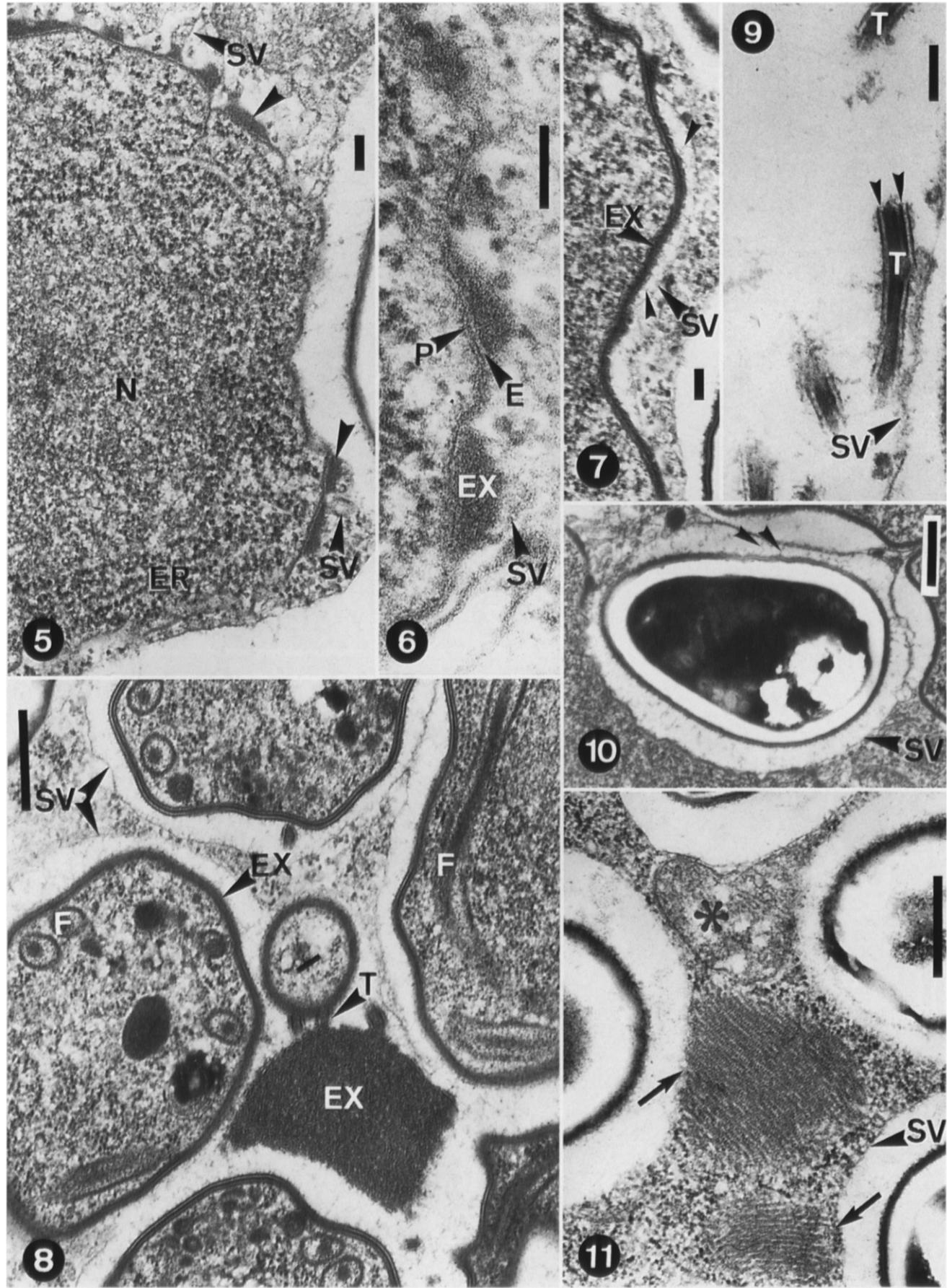
The transition from merozoite to sporont was clearly seen in ultrathin sections. At the ultrastructural level sporogonial stages could be distinguished from merozoites by two distinct features. The electron density of the cytoplasm decreased when ribosomes became associated with membranes to form a rough endoplasmic reticulum. This was arranged in concentric layers around the nuclei of sporonts (Fig. 3 & 4). The second prominent difference was the development of the complex cell wall of sporogonial stages (Fig. 2–4). Electron-dense material accumulated spot-wise externally to the plasma membrane (Fig. 2), later growing to form a continuous cover. It was originally of uniform texture, but later it split into layers (Fig. 1, 3, 4). The dense material was the exospore primordium, persisting as the surface layer also of mature spores.

The nucleus divided at least once before the thick sporont wall was initiated (Fig. 2). Further nuclear fissions yielded a plurinucleate sporogonial plasmodium, which split in a rosette-like manner before releasing sporoblasts (Fig. 3). Rosettes observed in ultrathin sections had never more than four lobes in the section plane. The widest lobed plasmodium measured 5.7 µm. The greatest sectioned nucleus of lobed plasmodia was 1.5 µm wide. Dividing nuclei in lobes indicated that each lobe divided at least once (Fig. 4). Plasmodia with up to 16 lobes were seen in stained smears (Fig. 12). Most commonly 8 sporoblasts were formed. Sporoblasts matured to spores without further division.

Dividing nuclei exhibited up to 205 nm wide electron-dense mitotic spindle plaques, in shallow invaginations of the nuclear membrane, and bundles of about

Fig. 1–4. Pathogenicity and early development of *Agglomerata volgensae* n. sp. (electron microscopy). 1. Part of a hypertrophic fat body lobe of *Daphnia magna* with more immature developmental stages of the microsporidium close to the periphery; arrowheads indicate multisporous sporophorous vesicles. 2. Merozoites and young sporonts; arrowheads indicate initiation of the sporont wall, arrow points at a dividing nucleus. 3. Rosette-like sporogonial plasmodia and mature spores. 4. Two lobes of a sporogonial plasmodium in a common sporophorous vesicle; the thick sporont wall is complete; two nuclei are still united by mitotic spindle tubules (arrow); arrow-heads indicate spindle plaques. Scale bars: Fig. 1 and 3 = 1 µm. Fig. 2 and 4 = 0.5 µm.





18 nm wide intranuclear spindle tubules (Fig. 4). Tubules persisted in the cytoplasm uniting the nuclei some time after fission (Fig. 4). Up to 130 nm wide, in sections almost circular, polar vesicles were present in the cytoplasm close to the plaques (Fig. 4).

The sporophorous vesicle

While merogonial stages were in close contact with the cytoplasm of the host cell (Fig. 2), sporogonial stages became separated from the host cytoplasm by a sporophorous vesicle generated by the microsporidium (Fig. 3–4). In young sporonts the surface layer of the electron-dense pad-like primordia of the thick sporont wall was released in a blister-like manner (Fig. 5–6). The remaining dense material grew to a continuous cover, separated from the plasma membrane by a narrow zone of electron-lucent substance (Fig. 6–7). Both dense and lucent material were primordia of layers persisting in the spore wall. The lucent material formed the endospore layer, while the dense material built the exospore.

When the sporogonial plasmodium was distinctly lobed both the thick surface cover and the sporophorous vesicle were completely developed (Fig. 3–4). The sporophorous vesicle was delimited by an approximately 5 nm thick envelope of uniform electron dense material (Fig. 7). The episporontal space, between the envelope and the sporont wall, was a rather narrow zone (Fig. 3). The sporophorous vesicle followed closely the outline of the sporogonial plasmodium, also when lobes were present (Fig. 3). When daughter cells were released, the sporophorous vesicle normally divided together with its content to form individual sporophorous vesicles around each sporoblast and spore (Fig. 1, 10–11). However, multisporous sporophorous vesicles were also seen (Fig. 1), enclosing all sporoblasts, or a part of them, in a common sporophorous vesicle.

Fibril-like material traversed the episporontal space of the developing sporophorous vesicle, connecting the envelope with the exospore (Fig. 7–8). The fibrils persisted to some extent also in vesicles with mature spores (Fig. 10). When sporoblasts were formed a second type of inclusions appeared (Fig. 8). These were 63–85 nm

wide tubules of exospore material, extending from the exospore of the sporoblasts (Fig. 8). The number was greatest at the time sporoblasts were released, but a small number of tubules remained in vesicles with mature spores (Fig. 16). The exospore origin was also visible in the construction of the tubules. The wall of the tubules, like the exospore of the sporoblast, exhibited two electron-dense layers separated a more lucent substance (Fig. 9). The tubules were filled with an electron-dense material. Septa were not observed.

Sporophorous vesicles were externally connected to each other by dense strands of regularly arranged and possibly spiraled fibrillar material (Fig. 11).

The immature spore

The initiation and morphogenesis of the sporal organelles equalled the normal for microsporidia. The polar filament, with a cap-like polar sac on the top, grew in anterior direction from a vesicular and granular zone (normally interpreted as a Golgi apparatus) in the posterior half of the sporoblast close to the nucleus. The polar filament reached almost complete length in the immature spore (Fig. 15). Later on the anchoring apparatus developed inside the polar sac, and the polaroplast was formed in the anterior half of the spore. In the immature spore three distinct structures were visible in the Golgi zone: the reticulate material interpreted as the Golgi apparatus, an anterior area of fine granular material, and large granules of electron-dense material (Fig. 15).

The endospore layer of the spore wall was the latest structure to reach full maturity. The primordium remained unchanged from the initiation at the beginning of sporogony (Fig. 6) to the immature spore was completely polarized (Fig. 15).

The mature spore

In smears mature spores appeared isolated or occurred in small groups (Fig. 12–14). Living spores had distinct pyriform shape with pointed anterior pole (Fig. 14). Stained spores appeared more blunt (Fig. 12). Staining revealed a dark anterior spot, the polar sac, and a wide zone in the central and posterior part of the

Fig. 5–11. The sporophorous vesicle (electron microscopy). **5–6.** Initiation of the sporont wall (arrow-heads); exospore primordia are uniformly electron-dense; the first signs of the sporophorous vesicle are visible as blister-like protrusions. **7.** Detail of an older sporont exhibiting a later stage in the development of the sporophorous vesicle; arrowheads indicate fibrous material traversing the episporontal space. **8.** Sporoblasts in a common sporophorous vesicle; tubular structures connect the exospore of a sporoblast with an aggregation of electron-dense exospore material. **9.** Longitudinally sectioned tubules (arrow-heads indicate exospore component) in a sporophorous vesicle. **10.** Mature spore probably in an individual sporophorous vesicle; arrow-heads indicate fibrils traversing the episporontal space. **11.** External fibrous material (arrows) uniting sporophorous vesicles; * indicates a mitochondrion of the host cell. Scale bars: Fig. 5–7 and 9 = 100 nm. Fig. 8 and 10–11 = 0.5 µm.

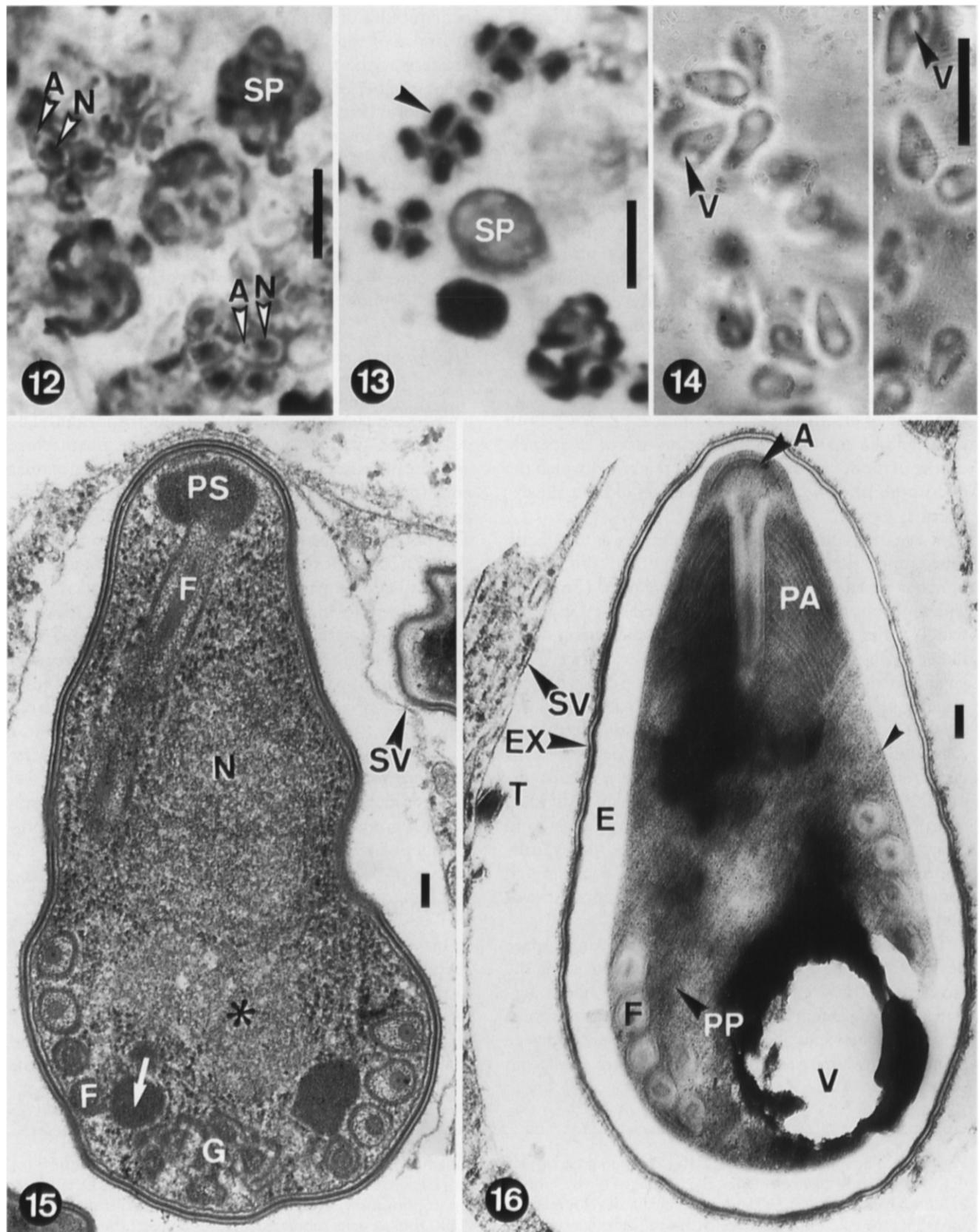


Fig. 12–16. Late sporogony and mature spores. **12.** Sporophorous vesicles with sporoblast buds and groups of mature spores; anchoring disc and nucleus are visible. **13.** Mature spores (arrow-head). **14.** Living mature spores with distinct posterior vacuole. N.B. that the shape of living spores is more slender and anteriorly more acute. **15.** Immature spore with complete exospore layer

spore, where the nucleus was visible as a more intensely stained area in the middle of the spore (Fig. 12). In living spores an approximately 1 µm wide vacuole was visible in oblique position close to the posterior pole (Fig. 14). Living spores measured 3.1–3.7×1.7–2.0 µm, fixed and stained spores 3.0–3.4×1.9–2.2 µm.

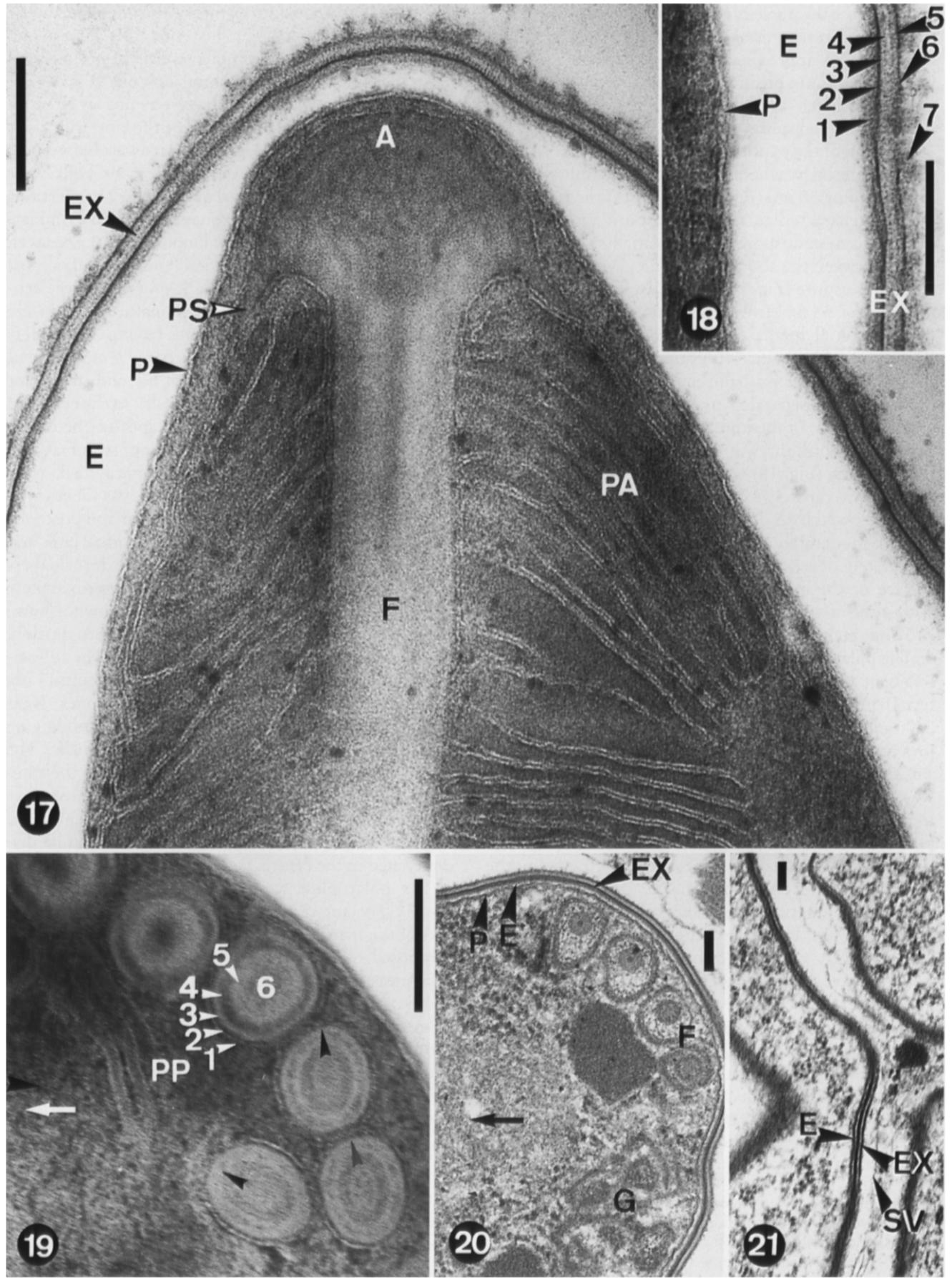
The wall of the mature spore measured up to 185 nm, except anteriorly where it was considerably thinner, not exceeding 30 nm (Fig. 16–17). It had the three subdivisions characteristic for a microsporidian spore wall with a lucent endospore layer of variable thickness inserted between the about 8 nm wide plasma membrane and the exospore (Fig. 16). The exospore measured approximately 38 nm. It was initiated as uniform electron dense material in the early sporogony (Fig. 6–7). In sporogonial plasmodia with complete thick cell wall, the exospore layer was split into two strata of electron dense material separated by a more lucent substance (Fig. 4 and 21). In the immature spore (Fig. 20) the exospore primordium was subdivided further and the final layering (Fig. 18) was reached at this stage: an internal approximately 10 nm wide zone of granular material followed by 5 distinct layers: very electron dense (3 nm), moderately dense (c. 4 nm), lucent (c. 4 nm), moderately dense (c. 4 nm), and very dense (3 nm). The surface of the exospore was an approximately 9 nm wide zone of granular dense material which formed up to 30 nm high protuberances.

The polar filament ended in the polar sac, connected to a bent anchoring disc of uniform electron dense material (Fig. 17). The widest sectioned disc measured 220 nm in diameter. The uncoiled part of the filament proceeded straight backwards in the centre of the spore for about 1/4 of the spore length, then turned sideways, and the posterior part was arranged as 5–6 coils, in a single layer of coils close to the spore wall, in the posterior half of the spore, ending close to the posterior pole (Fig. 16). The last coil was usually retracted to the interior of the row of coils (Fig. 16, 19). The angle of tilt of the most anterior coil to the long axis of the spore was about 40°. The polar filament must be characterized as slightly anisofilar (or heterofilar using the terminology by Voronin [30]). The differences in width between the wider anterior coils, and the more narrow posterior ones were not great, but differences in shape as well as in internal organization were obvious both in immature (Fig. 15, 20) and mature spores (Fig. 16, 19). The anterior coils of mature spores were almost perfectly circular in transverse sections, measuring 94–98

nm wide, the same width as that of the uncoiled anterior part of the filament. The posterior coils were ovoid, and as the long axis of sectioned coils was not directed in an identical way in neighbouring coils (Fig. 19), it was obvious that the ovoid shape was not an effect of sectioning. The widest diameter of the posterior coils varied between 90 and 94 nm, the narrowest between 72 and 74 nm. In immature spores anterior coils were wider and of more irregular shape in transverse sections (Fig. 20). At this stage three zones could be discriminated in the wide coils: an external moderately dense layer, a wide mottled median layer, and an internal, almost perfectly circular zone with a dark spot in the very centre (Fig. 20). The narrow coils of immature spores were more circular in transverse sections, having two moderately electron-dense layers separated by a mottled material (all of about the same dimensions), and an interior zone of about the same electron density as the centre of anterior coils, but lacking the dark spot in the centre (Fig. 20). The mature filament was composed of layered material and 6 subdivisions could be recognised (Fig. 19: layers 1–6). The sequence of layers was identical in all coils, and differences between anterior and posterior coils were restricted to the width of individual coils and the organization of the centre. The three external layers were identically wide in all coils: an approximately 5 nm wide unit membrane cover (1), a narrow (2–3 nm) very electron dense zone (2) and an approximately 5 nm wide fairly electron lucent layer (3). The following layer (4) was electron dense, measuring about 11 nm in anterior coils, about 5 nm in posterior ones. Next layer (5) was lucent, about 5 nm wide in all coils, giving the impression of transversely sectioned fibrils. The centre (6) of all coils was up to 40 nm wide. In the anterior coils it was fairly lucent, and in the two most anterior coils moderately dense material was present in the very centre. In posterior coils two strata could be discriminated in the centre: moderately dense and lucent.

The polaroplast was divided into two parts (Fig. 16–17). The anterior region, surrounding the uncoiled part of the filament and ending at the anterior coil, was composed of lamellae or chambers of variable thickness, measuring 9–43 nm thick. The lamellae were composed of electron-dense material, and they were directed more or less perpendicularly from the filament. The short posterior polaroplast region, at the level of the wide filament coils (Fig. 16–17), appeared less regularly arranged. It was composed of approximately 10 nm wide lamellae.

and developing polar filament; the polar sac is filled with uniform material and there are no signs of a polaroplast; arrow points at a dense body, arrow-head at fine granular material in the proximity of the Golgi apparatus. 16. Mature spore; arrow-head points at polyribosomes. Scale bars: Fig. 12–14 = 5 µm. Fig. 15–16 = 100 nm. Fig. 12–13. Giemsa staining, Fig. 14 phase contrast, Fig. 15–16 electron microscopy.



The polar sac appeared as an almost cup-shaped structure, enclosing the anchoring apparatus and extending backwards to surround the most anterior polaroplast lamellae (Fig. 17). It was filled with material of approximately the same electron density as the polaroplast chambers. The polar sac, the polaroplast lamellae and the surface layer of the polar filament shared a continuous system of about 5 nm thick unit membranes (Fig. 17).

The nucleus was localized immediately below the equator of the spore (Fig. 12, 15). The widest sectioned nucleus measured 0.85 µm wide. The cytoplasm was dense, with strands of polyribosomes around the nucleus and close to the polaroplast and polar filament coils (Fig. 16). A vacuole was present in the coil-free area at the posterior end of the spore, distinct both in living spores (Fig. 14) and in ultrathin sections (Fig. 16). It was a real vacuole, with a membrane-lining, but this area was not always well preserved. In most spores the vacuole appeared as a mixture of empty spaces and areas of electron-dense material.

Discussion

Genus position

The cytology and development of the microsporidium described herein indicate affinities to the genus *Agglomerata* Larsson and Yan, 1988, but the correspondence is not complete.

At present time the genus *Agglomerata* comprises four species, all of them originally described in other genera [17, 20, 32]. The type species, *A. sidae* (Jírovec, 1942), was originally considered to belong in *Duboscqia* [9], a genus where the typical species are parasites of termites [22]. Of the other three species *A. cladocera* (Pfeiffer, 1895) was originally placed in *Glugea* [23], a genus now considered exclusive to microsporidia of poikilothermous vertebrates [2], *A. bosminiae* (Voronin, 1986) in *Lanatospora* and *A. simocephali* (Voronin, 1986) in *Thelohania*.

It is characteristic for *Agglomerata* that all developmental stages have isolated nuclei [20]. The sporogony proceeds by rosette-like budding, yielding a great irregular number of spores, often 16, in a persistent sporophorous vesicle. The spores are pyriform and unin-

ucleate. The exospore is multilayered, with a characteristic sequence of layers, the polar filament is lightly anisofilar, and the polaroplast is divided into three regions (wide lamellae, narrow lamellae, tubules). The sporophorous vesicles, when containing spores, have prominent tubular inclusions formed by exospore material.

The species described herein conforms with *Agglomerata* in nuclear characteristics, the sporogonial division (Fig. 3) although the number of sporoblasts usually is 8, and the shape of the spores (Fig. 12–16). Further the layered exospore (Fig. 18) is identical to the exospore of *Agglomerata*, and the tubules of the epiporal space (Fig. 9) are of the *Agglomerata*-type, exhibiting characteristic exospore material. The sporophorous vesicle is initiated in an identical way (Fig. 5–6), and the initiation is slightly delayed, like in *A. sidae* [20], in that the first vesicle primordia appear after the initial division of the sporont nucleus (Fig. 2).

However, there are two important differences. In *A. sidae*, the type species of *Agglomerata*, the persistent sporophorous vesicles are multisporous, enclosing all spores originating from one sporont [20]. The sporophorous vesicles of the microsporidium described herein are either multisporous (Fig. 1), like the typical vesicles of *Agglomerata*, or, which is the most common situation, the vesicle divides together with the sporogonial plasmodium, yielding spores in individual sporophorous vesicles (Fig. 3, 10). Sporogony in individual sporophorous vesicles is a characteristic of two microsporidian families: Tuzetiidae and Janacekidae [7, 13, 26]. However, microsporidia of these families are cytologically different from the species treated herein, most easily seen in the construction of the polaroplast and exospore.

The second difference is found in the construction of the polaroplast. The polaroplast of the species treated herein exhibits the anterior two parts of a typical *Agglomerata* polaroplast, while the last section with tubules is lacking (Fig. 16, 17, 19). The normal situation in a microsporidian genus with distinct distinguishing characters is that all species clearly belonging to the genus share a polaroplast of identical construction. It is possible that the species studied by us exhibits a reduced state, where the last third of the characteristic *Agglomerata*-polaroplast has disappeared. Obvious cases of reduction have previously been described from microsporidia [12, 14, 15].

Fig. 17–21. Ultrastructure of the spore. 17. Anterior end of a mature spore exhibiting the anchoring apparatus in the polar sac and the anterior region of the polaroplast. 18. Detail of the spore wall; the seven distinct layers of the exospore are visible. 19 (arrows of Fig. 19–20 point at the anterior pole of the spore). Posterior region of the polaroplast and transversely sectioned polar filament coils, layers 1–6 are visible; arrow-heads indicate the anterior surface of posterior filament coils, the ovoid shape is not an effect of sectioning. 20. Spore wall and polar filament of the immature spore; arrow indicates the granular material anterior to the Golgi apparatus. 21. The layered exospore of a late sporogonial plasmodium with complete sporophorous vesicle. Scale bars: Fig. 17–21 = 100 nm.

Agglomerata is the only genus to which the species described herein shows clear affinities. If it is stressed that the variable development of the sporophorous vesicle and the polaroplast with two regions are important deviations from the typical *Agglomerata* cytology, the only solution would be to establish a new genus. However, we believe that a conservative approach would be the best solution and the species is therefore included, at least temporarily, in the genus *Agglomerata*.

Species status

More than 40 species of microsporidia are known to be more or less host specific parasites of cladoceran hosts [8, 9, 11, 29, 31]. The oldest descriptions [21, 23], made more than 100 years ago, belong to the early history of microsporidiology. They are very superficial and no type material has survived. These species cannot be recognised with certainty. However, in some cases, like *Glugea cladocera II* Pfeiffer, 1895, evaluation of newly collected material has connected the names to precise redescriptions [7, 9, 10, 19]. At least 25% of the microsporidia of cladocerans are difficult or impossible to identify.

Daphnia magna has been reported to be host of 9 species of microsporidia. Seven of these species are distinctly different from the species treated herein, by cytological differences and in some cases also by affinity to other host tissues: two *Agglomerata* species (*A. sidae* [20], *A. cladocera* [17]); two parasites of the gut epithelium (*Glugoides intestinalis* [19] and *Ordospora colligata* [18]); *Octosporea bayeri* [7] with binucleate spores; and two species in other genera defined by ultrastructural characters (*Flabelliforma magnivora* [16] and *Larssonia obtusa* [8, 28]). The remaining two species, *Microsporidium elongatum* [7, 21] and *Pleistophora ellipsoidea* [27], need closer comparison.

Microsporidium elongatum produces uninucleate elliptical spores measuring $5 \times 2 \mu\text{m}$ [21]. Even if this is all we know about the species, the larger spore size tells the difference to the species described herein.

The sporogonial plasmodium of *Pleistophora ellipsoidea* is multinucleate, elongate-ovoid, and it divides by rosette-like budding inside a fragile sporophorous vesicle [27]. The living spores are ellipsoid, measuring $4.6 \times 2.8 \mu\text{m}$. Even if this species obviously does not belong to *Pleistophora*, the fragile sporophorous vesicles and the invertebrate host speak for that, the larger spores of a different shape indicate the difference to the species treated by us.

The microsporidium treated herein is also clearly different from the two *Agglomerata* species which are parasites of other cladocerans than *D. magna*. *A. simocephali*, a parasite of *Simocephalus vetulus*, has greater spores with a greater number of filament coils

(Voronin, unpublished). *A. bosminiae*, a parasite of *Bosmina* species, produces spores of approximately the same size and with a similar number of filament coils (Voronin, unpublished). However, this species differs by producing a thick fibrous material in the episporal space.

The microsporidium of *Daphnia magna* described in this paper is clearly new to science, and we name it *Agglomerata volgensae*.

Description

Agglomerata volgensae n. sp.

Merogony: Merogonial reproduction only observed as groups of rounded-oval uninucleate merozoites.

Sporogony: Sporont uninucleate. Sporogonial plasmodia with isolated nuclei divide by rosette-like budding producing 4–16, usually 8, sporoblasts.

Spores: Pyriform with pointed anterior pole. Living spores measure $3.1\text{--}3.7 \times 1.7\text{--}2.0 \mu\text{m}$, fixed and stained $3.0\text{--}3.4 \times 1.9\text{--}2.2 \mu\text{m}$. Spore wall up to 185 nm thick, with an about 38 nm wide stratified exospore layer coated by thin fibrous material. Polar filament with 5–6 lightly anisofilar coils in one row of coils in the posterior half of the spore. Anterior 2–3 coils are 94–98 nm wide, in transverse section of circular shape; posterior 2–3 coils are ovoid in transverse section, measuring 90–94×72–74 nm. The angle of tilt of the most anterior coil to the long axis of the spore is approximately 40° . The polaroplast has two subdivisions: irregularly wide lamellae or chambers in the anterior part, ending at the level of the anterior filament coil, and loosely arranged more narrow lamellae in the short posterior part at the level of the wide filament coils. A single nucleus at the level of the anterior coils. Distinct posterior vacuole visible in living spores.

Sporophorous vesicle: Multisporous, enclosing all or a part of the daughter cells of the sporogonial plasmodium, or individual, enclosing each spore in a vesicle of its own. Episporal space traversed by narrow fibrils and by a small number of wide tubules with walls of exospore material.

Host tissues involved: Hypodermic and adipose tissue.

Type host: *Daphnia magna* Strauss, 1820 (Crustacea, Cladocera, Daphniidae).

Type locality: A pond, close to the river Volga, in the town Volgorechensk, Kostroma district, Russia.

Types: Syntypes on slides No. 930714 (1–5).

Deposition of types: In the International Protozoan Type Slide Collection at Smithsonian Institution, Washington D. C., and in the collections of V. N. Voronin, State Research Institute for Lake & River

Fisheries, Saint-Petersburg, and J. I. R. Larsson, Department of Zoology, Lund.

Etymology: Derived from the river Volga where the microsporidium was found.

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