

Ultrastructural Study and Description of *Cystosporogenes deliaradicae* n. sp. (Microspora, Glugeidae), a Microsporidian Parasite of the Cabbage Root Fly *Delia radicum* (Linnaeus, 1758) (Diptera, Anthomyidae)

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SUMMARY

The microsporidium *Cystosporogenes deliaradicae* sp. nov., a parasite of the adipose tissue of adult cabbage root flies, *Delia radicum* in Denmark, is described based on light microscopic and ultrastructural characteristics. All life cycle stages have isolated nuclei. The sporogony is polysporoblastic, yielding 4–16 (most commonly 6 or 8) ovocylindrical spores, measuring 1.2–1.5 × 4.6–5.5 µm when fixed and stained. The spore wall has an approximately 43 nm thick, layered exospore with a median layer resembling a double membrane. The polaroplast is uniformly lamellar. The isofilar, 114–123 nm thick, polar filament is arranged in 11–15 (most commonly 12–13) coils in a single layer close to the spore wall. The angle of tilt is approximately 35°. The last coil touches the posterior pole of the spore. A sporophorous vesicle is produced by the sporont. The envelope is generated as a duplication of the plasma membrane. Typically the vesicle primordia grow from one pole of the sporont to the other. The episporontal space initially has granular inclusions. Later septate, persistent tubules of exospore material appear. Simultaneously with the release of sporoblasts, wide tubulus-like, non-persistent structures are formed. They are covered by regularly spaced electron-dense material. Anomalous sporogony was frequently observed, probably caused by a simultaneous parasitism by the fungus *Strongwellsea castrans*. The systematic position of the microsporidium is discussed, including the reasons for incorporating the genus into the family Glugeidae.

Abbreviations

A	= anchoring disc	N	= nucleus
D	= double layer	P	= polaroplast
E	= endospore	PM	= plasma membrane
EX	= exospore	PS	= polar sac
F	= polar filament	PV	= posterior vacuole
G	= Golgi vesicles	S	= spore
HN	= host nucleus	T	= tubular inclusion
		V	= sporophorous vesicle

Introduction

An investigation of naturally occurring pathogens of two important pest species of brassicas, the cabbage root fly (*Delia radicum*) and the turnip fly (*Delia floralis*), revealed a microsporidiosis of *D. radicum* in Denmark [7, 8]. The microsporidium produced elongated spores in sporophorous vesicles, and the first impression was that the species might belong in the genus *Octosporea* [7]. This is the genus most closely associated with flies, and up to the present time at least 17 species of various families of flies have been found to host *Octosporea* species, most commonly *Octosporea muscaedomesticae* Flu, 1911 [11, 19].

However, a closer study of the microsporidium revealed that it was an undescribed species, and it also became apparent that it was not a member of the genus *Octosporea*. The species is briefly described herein, with emphasis on the ultrastructural cytology, and it is compared to the previously known microsporidia of flies. The reasons for including the species in the genus *Cystosporogenes*, and some teratological phenomena observed, are discussed, and a family position for the genus is suggested.

Material and Methods

As part of an investigation on natural pathogens of cabbage root flies (*Delia radicum*), an extensive sampling programme on adult flies was carried out in Denmark 1991–1993. Flies were captured with a sweep-net in different cabbage cultivars on localities on the island of Sjaelland, and incubated individually in 50 ml plastic cups with 25 water agar and food supply (sugar, yeast extract and water). During a 10 day incubation period flies were checked for the presence of fungal diseases. Selected samples both including fungus-infected flies and flies without external disease symptoms were then dissected in order to document the presence of microsporidia. The following samples from two localities were checked: Sengeløse, May–June 1991: 20 specimens; May–June 1993: 33 specimens; Hegenstrup (Slangerup), August 1992: 18 specimens; June 1993: 52 specimens.

The procedure for diagnosis included processing directly for electron microscopy or freezing for a later transfer into vials with 0.2% formalin to facilitate transport. Dissection and squashing (for light microscopy) or fixation for electron microscopy took place a few days later.

Squashed pieces of infected tissues were fixed either using methanol, for later staining in Giemsa solution, or using Bouin-Duboscq-Brasil solution, for staining with Heidenhain's iron haematoxylin. All permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd., Poole, England). For details on the histological techniques used see the manual by Romeis [18]. Measurements were made with an eye-piece micrometer at $\times 1000$.

For transmission electron microscopy, small pieces of infected tissue were excised and fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 5 h. After washing in buffer and post-fixation in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 h 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 [17].

Results

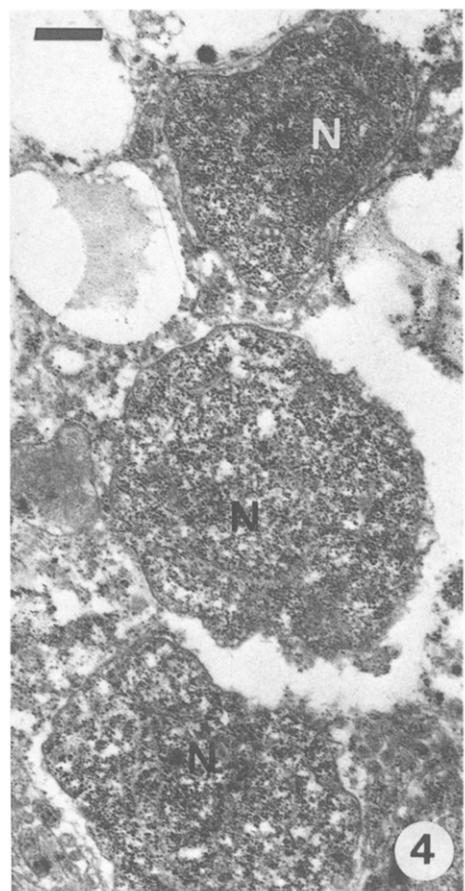
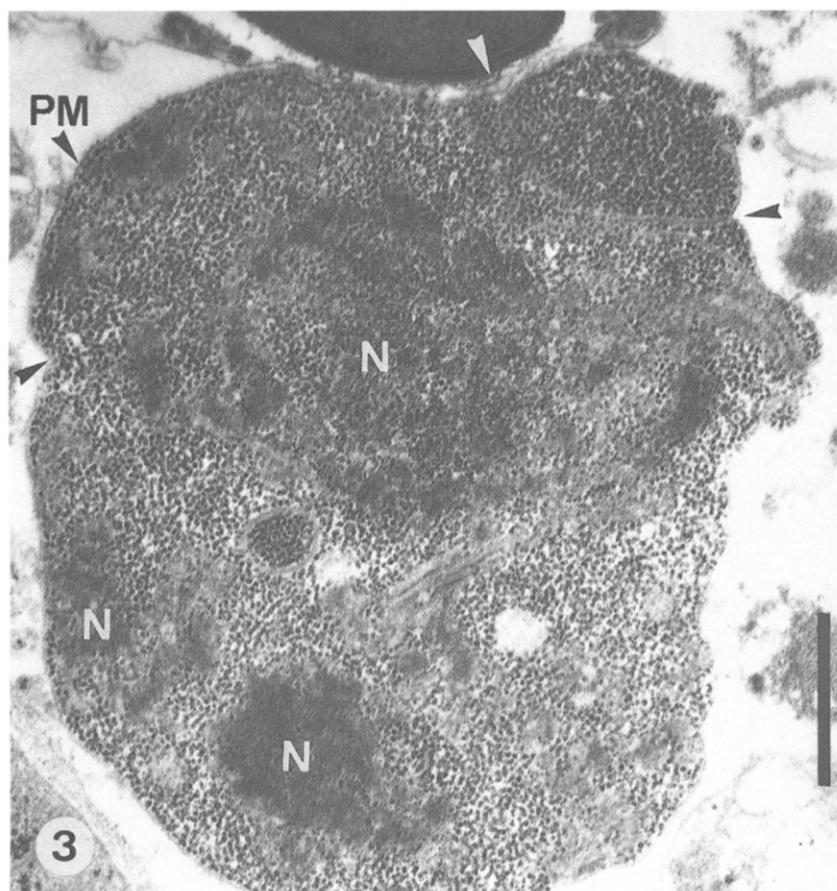
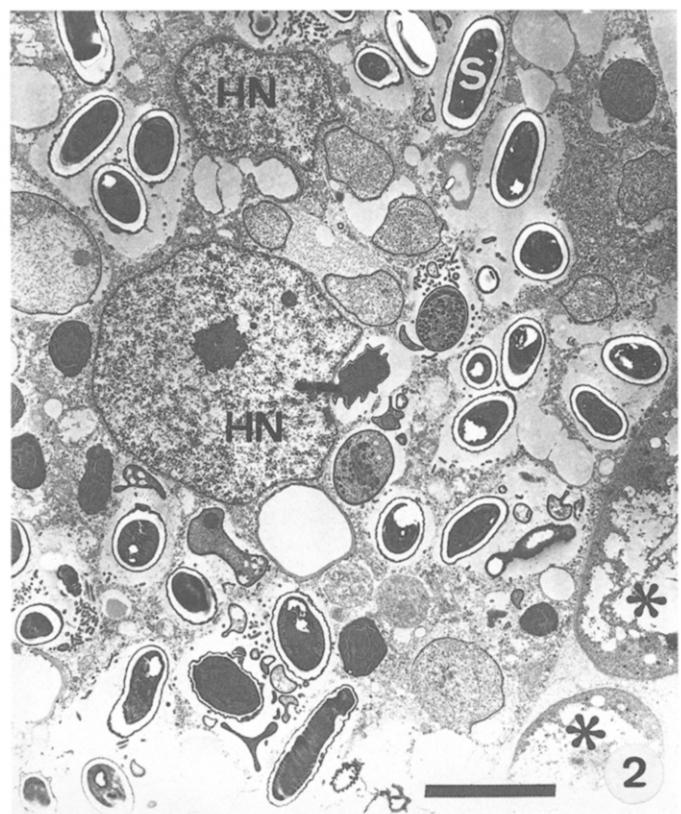
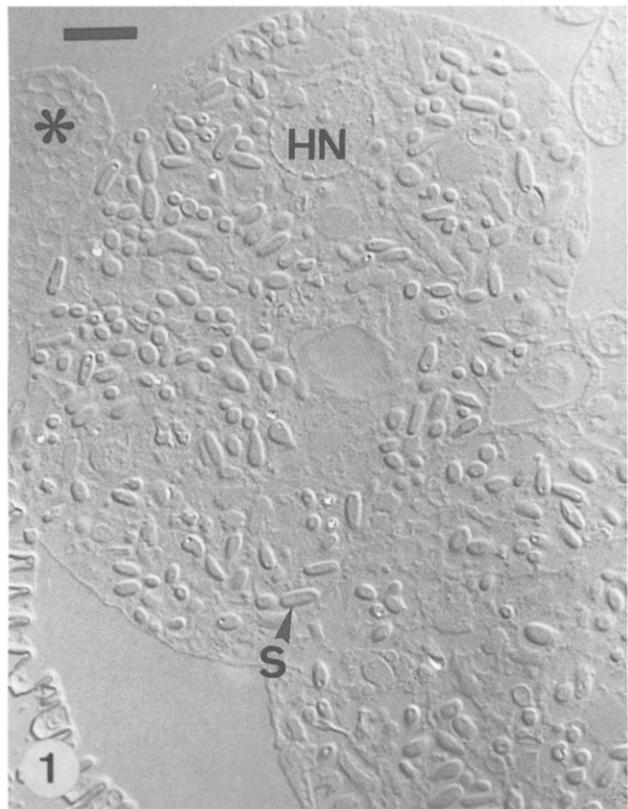
Prevalence and Pathogenicity

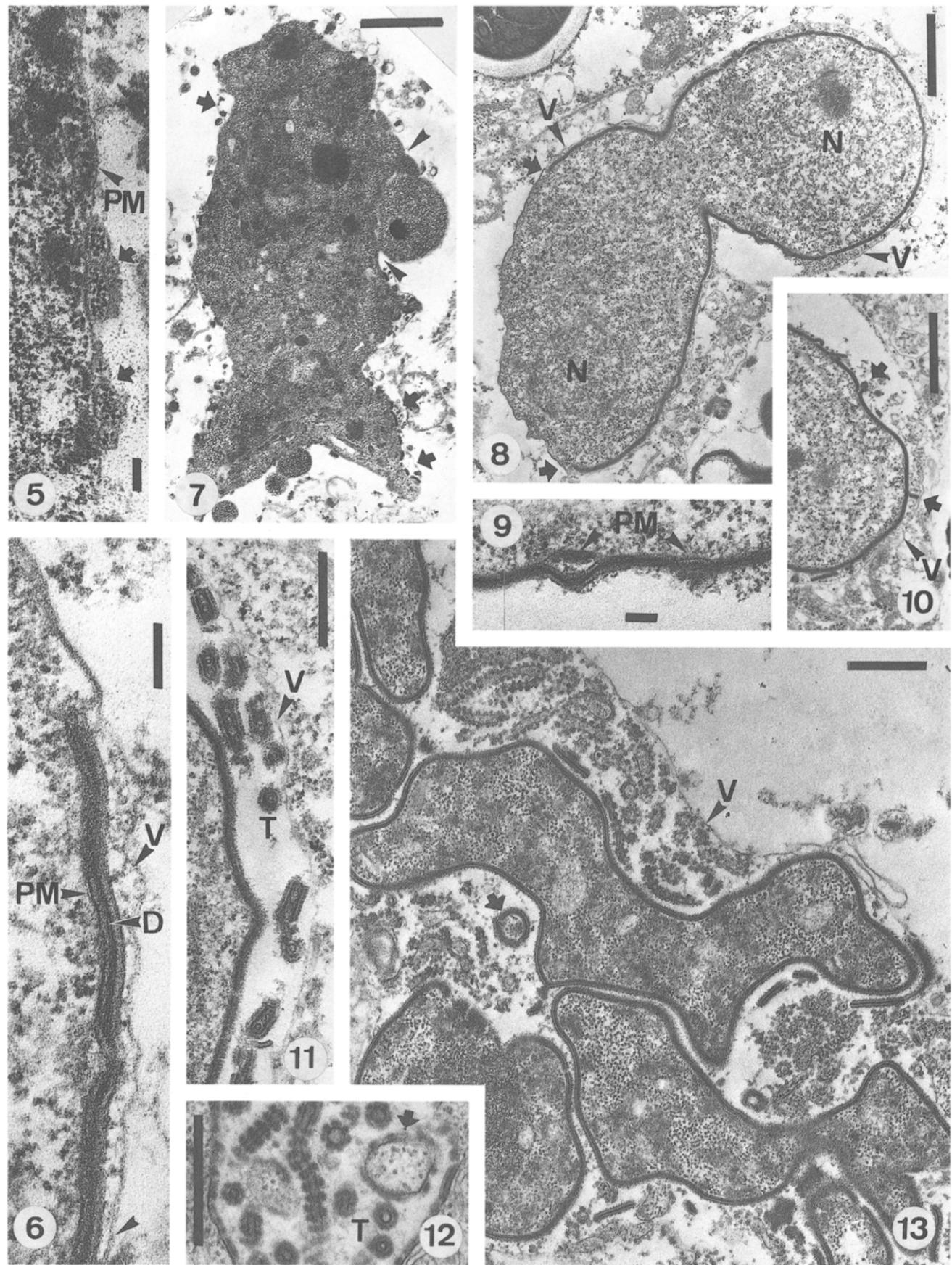
The following number of specimens per number of checked flies were found to be infected with microsporidia: Sengeløse, June 1991 (4/20), June 1993 (0/33); Hegenstrup August 1992 (1/18), June 1993 (1/52). Altogether, 6 flies out of 128 were found that were infected by microsporidia. Of those, one fly was simultaneously infected with the fungus *Strongwellsea castrans* (Zygomycetes, Entomophthorales), and one fly both with that fungus and *Bacillus thuringiensis* [8]. Both specimens with and without simultaneous infection with fungi were used for electron and light microscopy studies.

When cut open infected specimens were easily recognized by the whitish colour of the fat body lobes harbouring the microsporidium. This was the only tissue infected (Fig. 1), and the infection was especially prominent in the thorax, close to the hypoderm. Infected

Figs. 1–4. *Cystosporogenes deliaradicae* n. sp., in situ and vegetative reproduction. – Figs. 1–2. Semithin and ultrathin sections of fat body lobes filled with microsporidia. (Fig. 1 Differential interference phase contrast; Fig. 2 electron microscopy; *indicates hyphae of the fungus *Strongwellsea castrans*). – Fig. 3. Merogonial plasmodium dividing by plasmotomy (arrowheads). – Fig. 4. A line of three merozoites. Scale bars. Fig. 1 = 10 µm; Fig. 2 = 5 µm; Figs. 3–4 = 0.5 µm.

Figs. 5–13. Sporogony of *C. deliaradicae* (Figs. 5–11, 13) and *Agmasoma penaei* (Fig. 12). – Fig. 5. Initiation of the sporophorous vesicle in the young sporont (arrows). – Fig. 6. The sporophorous vesicle is formed by duplication of the plasma membrane (arrowhead indicates a position where the unit membrane nature is visible); primordia of the sporont wall (future exospore) have united to a continuous strand. – Fig. 7. The sporogony starts before all merozoites (future sporonts) have lost contact with each other (arrowheads); there are numerous primordia of the sporophorous vesicle, inside which short segments of exospore material have been initiated. – Fig. 8. A part of a sporogonial plasmodium where the polarized development of the sporont wall is visible (arrows). – Figs. 9–10. Details of the surface of the sporogonial plasmodium, exhibiting projecting exospore material (arrows). – Fig. 11. Detail of a sporophorous vesicle with septate tubular inclusions. – Figs. 12–13. Sporulating *A. penaei* (Fig. 12) and *C. deliaradicae* (Fig. 13) exhibiting identical inclusions of the sporophorous vesicle (arrows indicate wide plasmodium fragments). Scale bars: Figs. 5–6, 9 = 100 nm; Figs. 7–8, 10 = 1 µm; Figs. 11–13 = 0.5 µm.





cells were partially lysed and transformed into syncytia (Fig. 2).

Presporal Stages and Life Cycle

As the flies had been reared for at least one week before they were sampled, mature spores were dominant, and spores were the only stage observed in light microscopic preparations. The presporal development could be seen in the ultrathin sections. All life cycle stages had isolated nuclei.

The merogonial reproduction was represented by a small number of plasmodia. They were delimited by an approximately 9 nm thick unit membrane without external reinforcements (Fig. 3). The cytoplasm was uniformly granular with numerous free ribosomes, but there were only traces of endoplasmic reticulum. The largest sectioned nuclei of merogonial plasmodia measured 2.6 μm in diameter. The nuclear envelope was of traditional construction in all life cycle stages: two unit membranes, a perinuclear space, and pores. Mitosis was revealed by up to 170 nm wide, electron-dense centriolar plaques in shallow depressions of the nuclear envelope, with radiating, about 20 nm wide intranuclear microtubules. The normal division was by plasmotomy (Fig. 3). Rosette-like dividing plasmodia were not seen. Merozoites were often arranged in lines (Fig. 4). The number of merozoites per mother cell is unknown, but sections through budding plasmodia had up to 5 nuclei, and there were up to 7 merozoites in lines that could be suspected to belong to the same mother cell. The largest sectioned merozoite measured 2.5 μm in diameter, the nucleus 1.5 μm . It is unknown if there is more than one bout of merogony. The merogonial plasmodia and newly formed merozoites were not separated from the host cytoplasm by membranes, but they were often surrounded by a clear space (Fig. 4).

The last, or possibly only, generation of merozoites matured to sporonts. The plasma membrane was spot-wise duplicated, and the newly formed, approximately 9 nm thick, unit membrane was released in a blister-like manner (Figs. 5–6). The blisters, which were the primordia of the sporophorous vesicle, were initially filled with a granular substance of the same structure

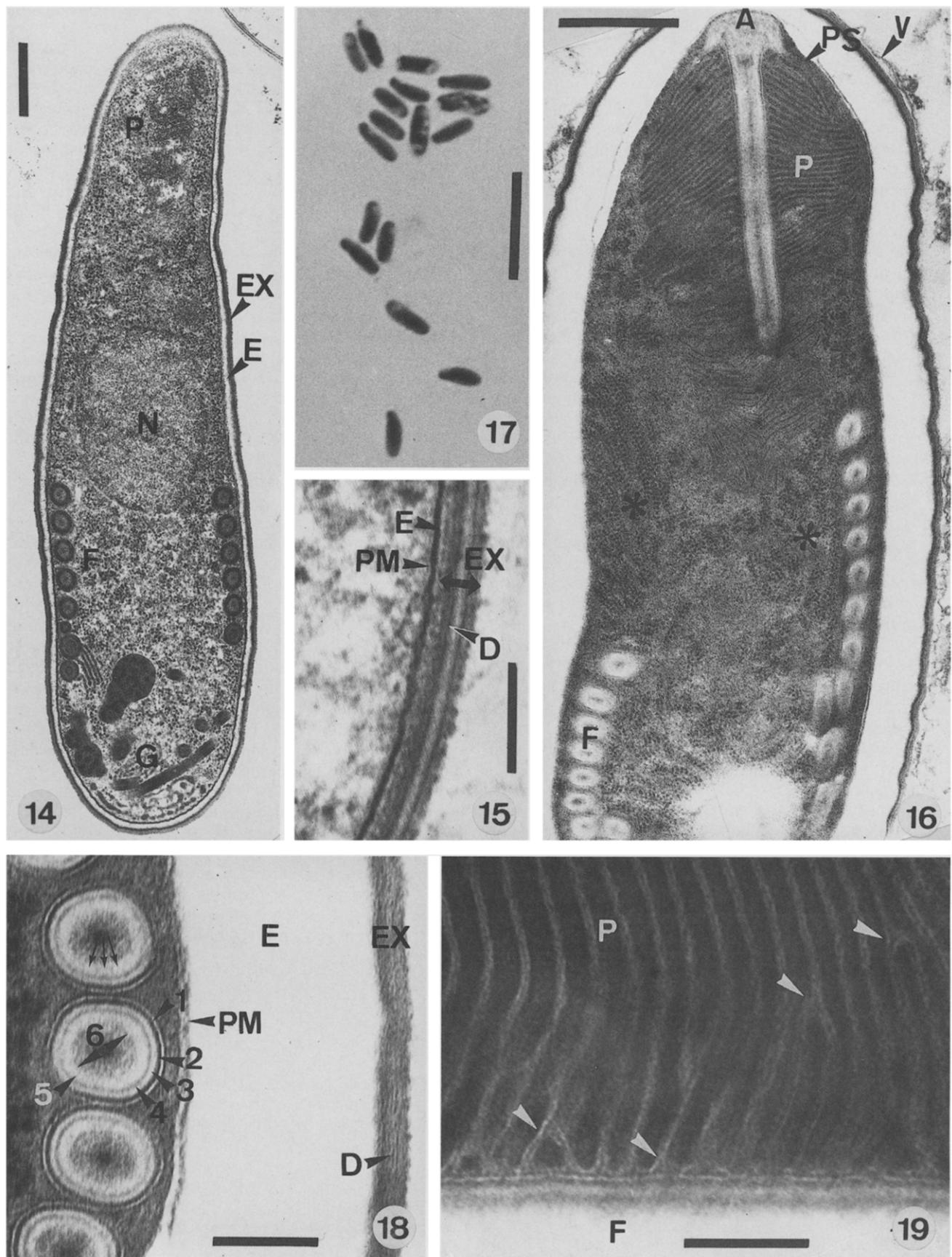
as densely packed ribosomes (Fig. 5). The primordia successively lost contact with the sporont and formed a complete vesicle, where an episporontal space separated the envelope from the plasma membrane. It was apparent that some of the merozoites matured to sporonts before all daughter cells of the merogonial plasmodium totally had lost contact with each other (Fig. 7), and the maturation was not perfectly synchronous in all daughter cells. It was also apparent that the vesicle primordia often developed in a polarized manner from one end of the sporont to the other, so that one part of the plasmodium was covered with a complete vesicle, while the opposite side still was uncovered (Fig. 8). When the vesicle primordia grew larger, the granular inclusions became less prominent (Fig. 8).

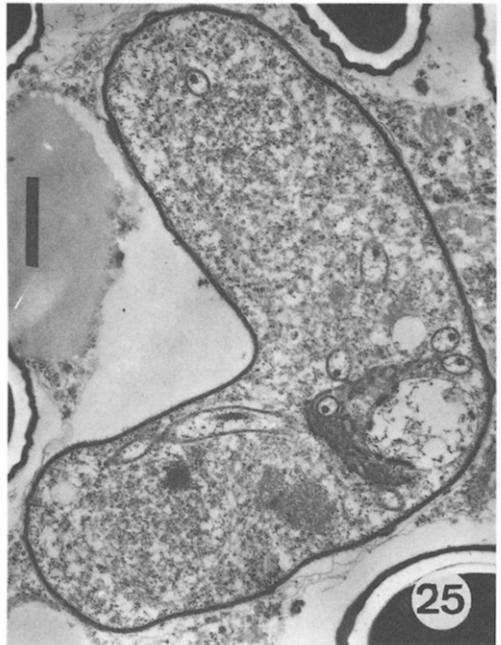
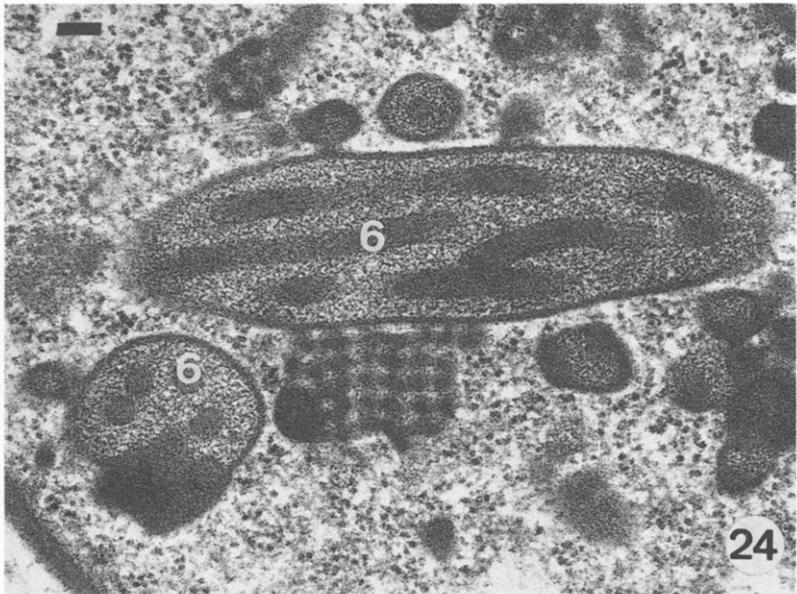
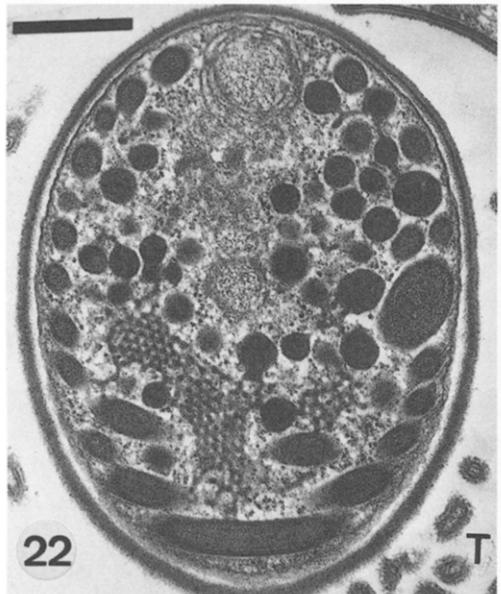
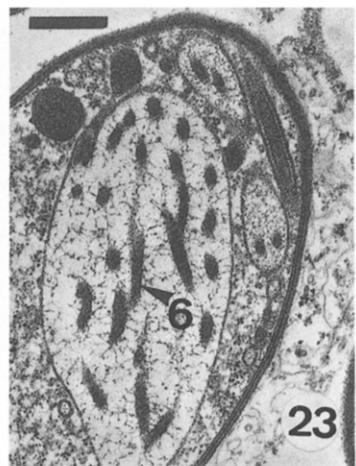
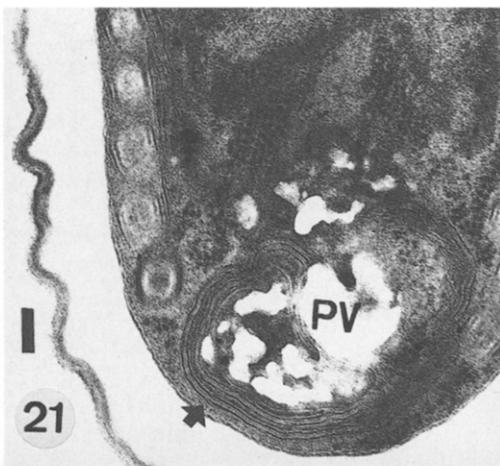
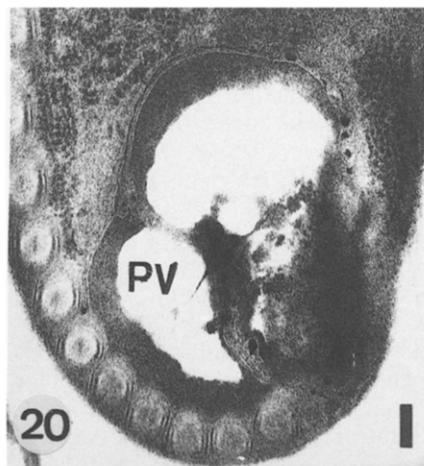
Inside the vesicle the plasma membrane was covered with electron-dense material, the future exospore layer. The primordia were initially visible as short strands (Fig. 7), but they soon became confluent to a continuous, ca. 35 nm thick, layered structure, where two equally thick moderately electron-dense layers were separated by an about 10 nm thick layer resembling a double membrane (Figs. 6, 8). The surface was covered by a thin layer of granular electron-dense material. The exospore material was produced in excess, and fragments of surplus material projected into the episporontal space (Figs. 9, 10). The surface material formed about 125 nm wide tubular inclusions of the sporophorous vesicle, with a wall identical to the exospore primordium, and with internal globular compartments (Fig. 11). These tubules were persistent and present also in vesicles containing mature spores. At the time that sporoblasts were released surplus material from the dividing plasmodium formed wider, irregular tubules (Fig. 13). They were covered by a layer of characteristic and regularly spaced electron-dense material. These tubules disappeared in a short time.

Meiosis was not observed in the sporogony. The sporogonial plasmodia produced sporoblasts by plasmotomy. There were no signs of rosette-like budding cells. It was impossible to evaluate the number of sporoblasts per mother cell in ultrathin sections. However, isolated groups of spores, which could be suspected to be the offspring of the same mother cell, were present in squash preparations, and they indicated

Figs. 14–19. Spores of *C. deliaradiceae*. – Fig. 14. Immature spore. – Fig. 15. Detail of the developing spore wall at the sporoblast stage. – Fig. 16. Mature spore (* indicates polyribosomes). – Fig. 17. Light microscopical image of mature spores (Giemsa stain). – Fig. 18. Detail of a mature spore exhibiting the layers of the polar filament (1–6; arrows indicate fibril-like material) and the spore wall. – Fig. 19. Detail of the polaroplast (arrowheads indicate anastomoses). Scale bars: Figs. 14, 16 = 0.5 μm ; Figs. 15, 18–19 = 100 nm; Fig. 17 = 10 μm .

Figs. 20–27. The posterior vacuole and some examples of teratological sporogony. – Fig. 20. Posterior vacuole with unit membrane lining. – Fig. 21. Multiple membranes (arrow) surrounding a posterior vacuole. – Fig. 22. Immature spores with supernumerary coils of the polar filament. – Figs. 23–24. Immature spores with supernumerary centres (6) in the polar filament. – Fig. 25. Uncompletely separated sporoblasts. – Figs. 26–27. Mature spores with disorganized coiling. Scale bars: Figs. 20–21, 24 = 100 nm; Figs. 22–23, 26 = 0.5 μm ; Figs. 25, 27 = 1 μm .





that the sporogony yielded a variable number of daughter cells. 6 or 8 spores seem to be the most common numbers, but distinct groups with 4 to 16 spores were observed.

Newly formed sporoblasts were irregular (Fig. 13), but the shape changed successively over ovoid stages to ovocylindrical immature spores (Fig. 14). The exospore remained unchanged from the sporont stage to the mature spore, but the endospore layer developed successively during the maturation (Figs. 14–16). The morphogenesis of the sporal organelles was normal for microsporidia.

Spores

Mature spores were almost ovocylindrical (Fig. 17). They measured $1.2\text{--}1.5 \times 4.6\text{--}5.5 \mu\text{m}$ when fixed and stained. Ultrathin sections revealed that the sporophorous vesicle was persistent also when containing mature spores (Fig. 16). At this time the approximately 12 nm thick envelope had lost its unit membrane structure. A part of the distinct tubules formed at the beginning of the sporogony remained with unchanged dimensions (ca. 125 nm). Sporophorous vesicles were not seen in stained preparations.

Mature spores had a 204–248 nm thick spore wall, which at the anterior pole was reduced to less than half the thickness. The spore wall had the normal three subdivisions (Figs. 16, 18): an internal approximately 9 nm thick unit membrane, a median, wide electron-lucent endospore (this layer was reduced anteriorly), and an approximately 43 nm thick, layered exospore. The distinct exospore layers were, in outward direction: a moderately electron-dense, about 20 nm thick, layer, a double-layer resembling an approximately 7 nm thick unit membrane, and a moderately dense surface layer, covered with granular material (Fig. 18).

The polar filament was attached to a layered anchoring disc at the anterior pole of the spore (Fig. 16). The greatest sectioned disc measured 319 nm in diameter. The filament proceeded backwards, in the centre of the spore, for about one third of the spore length and then turned slightly sideways to a position between the middle and the posterior third of the spore, where it began coiling up with the coils directed anteriorly. Most spores had 12 or 13 coils, but exceptionally 11, 14 or 15 coils were present (Fig. 16). The coils were arranged in a single layer close to the spore wall. In spores where the row of coils was sectioned perpendicularly to the coils, it measured about one third of the spore length. The angle of tilt of the anterior filament coil to the long axis of the spore was approximately 35°. The last coil touched the posterior pole of the spore. The polar filament was isofilar, 114–123 nm in diameter. Transverse sections revealed a distinct sequence of layers of different electron density and thickness (Fig. 18, numbers 1–6): a c. 5 nm thick unit membrane cover (1), a strand of electron-dense material of the same thickness (2), a moderately dense, approximately 14 nm wide layer (3), an approximately

equally thick but more dense layer (4), an equally thick, lucent layer consisting of structures that resembled fibrils (5), and the centre (6), measuring about half the diameter of the filament. In this zone moderately dense material was stratified in an indistinct way, but there was usually more distinct dark material in the very centre.

The polaroplast surrounded the straight part of the filament and ended at the level of the anterior coil (Fig. 16). It was of uniform construction, composed of 19–25 nm wide, regularly arranged electron-dense lamellae. The lamellae were delimited by a unit membrane identical to the unit membrane cover of the polar filament. There were anastomoses between the membranes of adjacent lamellae as well as between lamellae and the cover of the filament (Fig. 19). The anchoring disc was enclosed in an umbrella-shaped polar sac, where the posteriorly directed narrow folds enclosed a few of the anterior polaroplast lamellae (Fig. 16). The nucleus, which was rounded and localized to the centre of the immature spore (Fig. 14), was pushed to the posterior half of the spore and compressed laterally upon maturation. Sectioned nuclei measured up to 2 μm long. At the posterior pole was a membrane-lined vacuole filled with dense material (Figs. 20–21). The widest sectioned vacuole measured 0.8 μm . Occasionally concentrical layers of membranes enclosed the vacuole (Fig. 21). The cytoplasm of immature spores had numerous ribosomes (Fig. 14), while the cytoplasm of mature spores was packed with strands of polyribosomes (Fig. 16).

Teratological Sporogony

Teratological spores were numerous in hosts with mixed infection of microsporidia and the fungus *Strongwellsea castrans*. Teratologies included supernumerary and undifferentiated filament coils (Fig. 22), anomalous coils with increased number of centres (Figs. 23–24), incompletely separated sporoblasts (Fig. 25), and mature spores with disorganized coiling (Figs. 26–27) and shape (Fig. 27). Teratological spores were especially common close to hyphae of *S. castrans*.

Discussion

Cytology

Few traits of the cytology of this microsporidium need comments. Compartmentalized tubular material of the episporontal space (Fig. 11) is not unique to this microsporidium. Similar structures are for example formed by *Glugea stephani* [21]. However, the more irregular tubular material, appearing when the sporoblasts are released, is of an unusual type (Fig. 13). We have seen identical inclusions only once: produced by *Agmasoma penaei* (Fig. 12), an enigmatic microsporidium that has been placed in the family Thelohaniidae even if it has not been proven that the presporogonial development is diplokaryotic [10].

It is not unusual among the disporoblastic *Bacillidium*-like microsporidia that a small proportion of the sporonts fail to release the sporoblasts, yielding anomalous spores of triskelion-like shape. This has been reported e.g. for *Bacillidium strictum* [15] and *Rectispora reticulata* [14]. However, the proportion of anomalous spores seen in the microsporidium described herein is unusually high, and the anomalies are not restricted to incomplete fission. Anomalies were only seen in connection with simultaneous fungal infection. It cannot be excluded that the anomalies were induced by substances produced by the fungus, for deformed spores were frequent in the proximity of the hyphae. Toxic effects of a similar type were revealed in a study of *Nosema apis* by Liu and Myrick, where treatment of the host (honey bees) with the chemotherapeutic itraconazole disturbed the development of the polar filament [16]. It is interesting to notice the supernumerary central regions of the polar filament (Figs. 23–24). This reveals that the central part of the filament is a distinct structure, not just a cavity filled with more or less electron-dense material. Anomalous supernumerary filament centres have previously been observed in microsporidian spores, for example in *Edhazardia aedis* (Fig. 50 in [1]).

Taxonomy

Ten species of microsporidia are primarily parasites of flies, and in addition a few microsporidia of other hosts, like *Nosema apis* and *Nosema bombycis*, have been reported from flies. The genus *Octosporea* is especially closely associated with this group of insects. The type species, *O. muscaedomesticae* Flu, 1911, is a parasite of flies [6], and so are three more named species: *O. monospora* Chatton & Krempf, 1911 [4], *O. antiquae* Issi, Radischcheva & Dolzhenko, 1983, and *O. deliae* Issi, Radischcheva & Dolzhenko, 1983 [11]. The other microsporidian parasites of flies are: *Campanulospora deliae* Issi, Radischcheva & Dolzhenko, 1983 [11], *Microsporidium thomsoni* (Kramer, 1961) Sprague, 1977 [12], *Nosema kingi* Kramer, 1964 [13], and three doubtful microsporidia reported by Fantham and Porter [9] and described as new species by Weiser, 1961 [22]: *Telomyxa muscarum* Weiser, 1961, *Toxoglugea calliphorae*, Weiser, 1961, and *Toxoglugea porterae* (Weiser, 1961) Sprague, 1977. Three of the species use flies of the genus *Delia* as hosts: *C. deliae*, *O. deliae* and *O. antiquae*. All these species are reported to produce diplokaryotic spores. The diplokaryon might be difficult to recognize using light microscopy alone, but when clearly proven, it tells the difference to the species described herein clearly. The micrograph of *O. deliae* shows the diplokaryon distinctly (Fig. 1 : 3 in [11]). *Campanulospora deliae* produces characteristic bell-shaped spores (Fig. 1 : 1 in [11]). The sporogony of the third species, *O. antiquae*, yields a variable number of ovocylindrical spores (2, 4 or 8), like in the species treated herein, but they are distinctly smaller, measuring 0.6–0.8 × 3.5–

3.7 µm (fixed and stained) [11]. It must be concluded that the microsporidium of the cabbage root fly in Denmark is an undescribed species.

Isolated nuclei in all developmental stages, combined with a sporogony that yields a variable number of uninucleate ovocylindrical spores in sporophorous vesicles, suggest the genus *Cystosporogenes* Canning, Barker, Nicholas & Page, 1985 [2]. Both the type species, *C. operophterae* (Canning, 1960) Canning et al., 1985 [2], and a second species assigned to the genus, *Cystosporogenes pristiphorae* (Smirnoff, 1966) Darwishi, Brooks & Moore, 1992 [5], are parasites of terrestrial hosts (winter moth, *Operophtera brumata*, and larch sawfly *Pristiphora erichsoni*, respectively), like the species treated herein. Both species have been investigated ultrastructurally, but we lack detailed descriptions of the mature spores for both species. It is seen, however, that the type species and the species treated herein have spores of approximately the same shape and isofilar polar filaments with one row of coils. Also the exospore layers seem to be identical (c.f. Fig. 14 in [2]). Furthermore, in the episporontal space, visible in the same micrograph, is one tubular exospore-derived inclusion of identical type to the tubules produced by the microsporidium described herein. There might be one difference of taxonomic importance between the type species and the Danish microsporidium. According to the description, all developmental stages of the type species are separated from the cytoplasm of the host cell by a membranous structure of unknown origin [2]. This membrane-bound cavity is called "sporophorous vesicle" in the description, even if it might be a structure different from the normal sporophorous vesicle of microsporidia. The micrographs of the description are not distinct enough to allow detailed studies of envelopes and membranes. It is apparent, however, that the plasmodial cell of Fig. 4 in [2] which is called a "meront" exhibits different electron density of the cell wall on the right and left sides of the plasmodium, which suggests that the stage might be an early sporont and that the membrane projections visible might belong to a sporophorous vesicle of traditional type. The illustration resembles the sporont of Fig. 8 in this paper, which exhibits the characteristic development of the sporont wall: progressively from one side to the other. Since the only difference that might exclude the microsporidium treated herein from the genus *Cystosporogenes* is a membranous envelope of unknown history, we believe that the best solution will be to assign our microsporidium to the genus *Cystosporogenes* until new information about the type species might provide more substantial distinguishing characters.

In the recent new classification of the microsporidia by Sprague and colleagues, the genus *Cystosporogenes* was placed in a family of incertae sedis position [20]. However, we believe that the family Glugeidae Thélohan, 1892 could accommodate the genus. The type genus of the family is *Glugea* Thélohan, 1891. The type species, *Glugea anomala*, has been investigated using modern techniques by Canning and colleagues [3].

All life cycle stages have isolated nuclei, the spores are uninucleate, the sporogony is polysporoblastic, and the spores are enveloped in a sporophorous vesicle produced by the sporont. The envelope is formed by duplication of the plasma membrane, and the episporontal space has granular and tubular inclusions. Further the illustrations show an exospore of similar, or possibly identical, construction to the exospore seen here. *Cystosporogenes* is not a synonym of *Glugea*, but a distinct genus which could be placed in the family Glugeidae.

Description

Cystosporogenes deliaradicae sp. nov.

Merogony. Multinucleate plasmodia with isolated nuclei produce a small number of uninucleate merozoites by plasmotomy. Merozoites are often lined in the tissue.

Sporogony. Sporogonial plasmodia with isolated nuclei produce 4–16 (usually 6 or 8) uninucleate sporoblasts by plasmotomy.

Spore. Ovocylindrical, measuring 1.2–1.5 × 4.6–5.5 µm (fixed and stained). The spore wall is thick with a layered, approximately 43 nm thick exospore, where one of the median layers resembles a double-membrane. The polar filament is isofilar, 114–123 nm wide, with 11–15 (usually 12–13) coils in a single layer close to the spore wall. The coils occupy about one third of the spore length. The last coil touches the posterior pole of the spore. The angle of tilt is approximately 35°. The polaroplast is uniformly lamellar, surrounding the uncoiled part of the polar filament. The elongated nucleus is localized to the region with filament coils.

Sporophorous vesicle. Initiated at the beginning of the sporogony as a duplication of the plasma membrane. The development is characteristic, normally proceeding from one side of the sporont to the other. The approximately 12 nm thick envelope of vesicles with mature spores has lost its unit membrane structure. Septate, about 125 nm wide tubules with wall of exospore material persist also in vesicles with mature spores.

Host tissues involved. Fat body.

Type host. *Delia radicum* (Linnaeus, 1758) (Diptera, Anthomyidae), adults.

Type locality. Hegnstrup, Slangerup, Sjælland, Denmark.

Type series. Syntypes on slides No. 930812-A8-(1–4).

Deposition of types. In the International Protozoan Type Slide Collection at Smithsonian Institution, Washington, DC, in the collection of Dr. Jaroslav Weiser, Prague, and in the collection of R. Larsson.

Etymology. *deliaradicae* alluding to the type host.

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