FOLIA PARASITOLOGICA 52: 163–172, 2005

Microsporidia in aquatic microcrustacea: the copepod microsporidium *Marssoniella elegans* Lemmermann, 1900 revisited

Jiří Vávra[1], **Miroslav Hyliš**[2], **Miroslav Oborník**[1] and **Charles R. Vossbrinck**[3]

[1]Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic;

[2]Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic;

[3]Department of Soil and Water, Connecticut Agriculture Experiment Station, New Haven, Connecticut 06511, USA

Key words: Microsporidia, *Marssoniella*, *Cyclops vicinus*, phylogeny, ultrastructure, Trichoptera

Abstract. *Marssoniella elegans* Lemmermann, 1900, a parasite of ovarial tissues of the copepod *Cyclops vicinus* Uljanin, 1875, was studied as a representative of aquatic-clade microsporidia which form "heteroinfectious spores" (spores not infective to the original host as opposed to "homoinfectious spores" which are infective for the original host) and which thus should require an alternate host. Several structural characters of this microsporidian are similar to those of copepod morphs of microsporidia infecting mosquitoes. However, small subunit ribosomal DNA phylogeny indicates that caddis flies (Insecta, Trichoptera) might be the alternate hosts of *Marssoniella*. Ultrastructural data obtained are used to redefine the genus *Marssoniella* Lemmermann, 1900 and its type species *Marssoniella elegans.*

Aquatic microcrustacea (copepods and cladocera) are frequent hosts of microsporidia. About nine genera of microsporidia and about 50 species have been described from copepods (Bronnvall and Larsson 2001), and 9 genera with about 30 species have been described from cladocera (Larsson et al. 1996). However, the study of microsporidia goes beyond recording microsporidian diversity and establishing microsporidian classification. The challenge is to understand the biology of microcrustacean microsporidia, especially the poorly known host specificity, maintenance in habitats, and modes of transmission. Several past and recent attempts to infect aquatic microcrustacea with spores isolated from a host and fed to the same host species have failed (Vávra 1964, Green 1974, Refardt et al. 2002, Ebert unpublished). This has demonstrated that some aquatic microsporidia are different from their terrestrial counterparts, in which case perorally fed spores usually cause infection in the original host species. No explanation for the "non-infectivity" of spores in some microcrustacea was available until 1985 when it was shown that several copepod microsporidia (now known to represent the genera *Amblyospora*, *Hyalinocysta* and *Parathelohania*) are in fact mosquito parasites which are using copepods as intermediate hosts. The discovery that some microsporidia have a dixenous life cycle, that they form several kinds of spores specific for the respective hosts, was a major breakthrough in our understanding of microsporidian host specificity, life cycles and microsporidian disease epizootiology (Andreadis 1985, Sweeney et al. 1985).

Phylogeny based on the analyses of small subunit rRNA genes (SSU rRNA) has demonstrated that the mosquito microsporidia that use copepods as secondary hosts form a specific clade ("parasites of Culicidae" clade of Vossbrinck et al. 2004). A sister clade to this one, however, is another clade of microsporidia ("aquatic outgroup" of Vossbrinck et al. 2004) that infect mostly copepods and cladocera. Several members of this clade form heteroinfectious spores (see below for definition) and should have alternate hosts; however, alternate hosts for these parasites remain unknown. It is believed that the search for the eventual alternate hosts is one of the prime goals in microsporidian research as it can lead to a better understanding of the evolutionary history of microsporidia.

Marssoniella elegans Lemmermann, 1900 is a microsporidium of interest because it belongs to the "aquatic outgroup clade" mentioned above (Vossbrinck et al. 2004), forms spores non-infective for its original host, and therefore should have an alternate host. However this copepod parasite, although known for a long time, has not been completely characterized at the morphological and biological levels. This organism has been known to algologists since 1900 when Lemmermann established the genus *Marssoniella* while describing its freely floating spores as a cyanobacterium. The organism forms spore groups of mostly 4, less frequently 8,

This paper was presented at the NATO Advanced Research Workshop "Emergent Pathogens in the 21st Century: First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts", held in České Budějovice, Czech Republic, July 12–15, 2004.

Address for correspondence: J. Vávra, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic. Phone: ++420 221 951 817; Fax ++420 224 919 704; E-mail: vavrajir@natur.cuni.cz

and rarely 16 spores, arranged in a star-like configuration when released into the aquatic environment. This was the reason it was independently reported in parasitology investigations as *Gurleya* sp. (Lom and Vávra 1963, Vávra 1963) or *Gurleya elegans* (Vávra 1976, Vávra in Sprague 1977, Vávra and Barker 1980). It was the cooperation of an algologist with a parasitologist which resulted in the abolishment of the cyanobacterial genus *Marssoniella* (Komárek and Vávra 1968) and the resurrection of the original "algal" name of the organism (Sprague et al. 1992). Although the generic name *Marssoniella* is confusing —it is a senior homonym of the bryozoan genus *Marssoniella* Levinson, 1925 (see in Sprague et al. 1992)— we decided to retain the genus in order to avoid confounding the latest authoritative treatise of microsporidian genera (Canning and Vávra 2000). Although *Marssoniella* is mentioned in the publications cited above, its fine structure and phylogeny have never been fully characterized, the reason being that infected copepods occur only briefly during the season and therefore it is difficult to find enough individuals containing developmental stages of the pathogen. The objectives of this paper are thus to fill in the gaps in our knowledge about this organism, to redefine the genus and its type species, and to discuss the possible identity of its alternate host.

MATERIALS AND METHODS

Type locality. Infected copepods were collected in the carp pond Mlýnský in the vicinity of the town Mšec (county Kladno), Czech Republic. Although the infected copepods occur in many similar ponds, this one is selected as the type habitat.

Microscopy. Published methods of spore photography, smear preparation, and staining with Giemsa (Vávra and Maddox 1976, Undeen and Vávra 1997) were used. Spores were measured either from samples digitally projected at ×100 magnification on a computer screen or by the Image Splitting Eyepiece (Vávra and Maddox 1976). For electron microscopy the Hirsch and Fedorko's fixative (1968) was used as it consistently gave the best results.

Infection experiments. Freshly isolated spores (1×10^3/ml) were mixed with last-stage copepodites and adults of copepods: *Cyclops vicinus* Uljanin, 1875, *Cyclops strenuus* Fischer, 1851 and *Eucyclops serrulatus* (Fischer, 1851). Copepods were maintained for several weeks or even months in large Petri dishes containing pond water with some mud and plant debris from the original habitat.

Terminology. Two general terms are introduced in order to specify the infectivity of microsporidian spores: "homoinfectious spores" are those which after isolation from a host are directly infective to the host of the same species, while "heteroinfectious spores" are those which are not infective for the same host species.

Molecular phylogeny. Small subunit ribosomal RNA gene sequence obtained by Vossbrinck et al. (2004) (GenBank Acc. No. AY090041) was compared with homologous partial sequences obtained in a detailed study of microsporidian phylogeny of tetrasporoblastic microsporidia from Trichoptera by Hyliš et al. (in preparation). The sequences of the following microsporidia were used: *Cougourdella* sp. No. 9 from *Hydropsyche fulvipes* (Curtis, 1834) (GenBank Acc. No. AY880951), *Pyrotheca* sp. No. 10 from *Plectrocnemia conspersa* (Curtis, 1834) (GenBank Acc. No. AY880955), *Episeptum* sp. No. 12 from *Potamophylax cingulatus* (Stephens, 1837) (GenBank Acc. No. AY880954), *Episeptum* sp. No. 17 from *Hydropsyche incognita* Pitsch, 1993 (GenBank Acc. No. AY880953) and *Episeptum* No. 15b from *Sericostoma personatum* (Spence, 1826) (GenBank Acc. No. AY880952). Partial sequences of SSU rDNA gene in the range 550 to 600 bp were aligned using the Clustal_X and were analysed by Maximum Parsimony (MP) using PAUP vers. 4b10 (Swofford 2000); Maximum Likelihood (ML) tree was constructed using PhyML (Guindon and Gascuel 2003).

RESULTS

Epizootiology

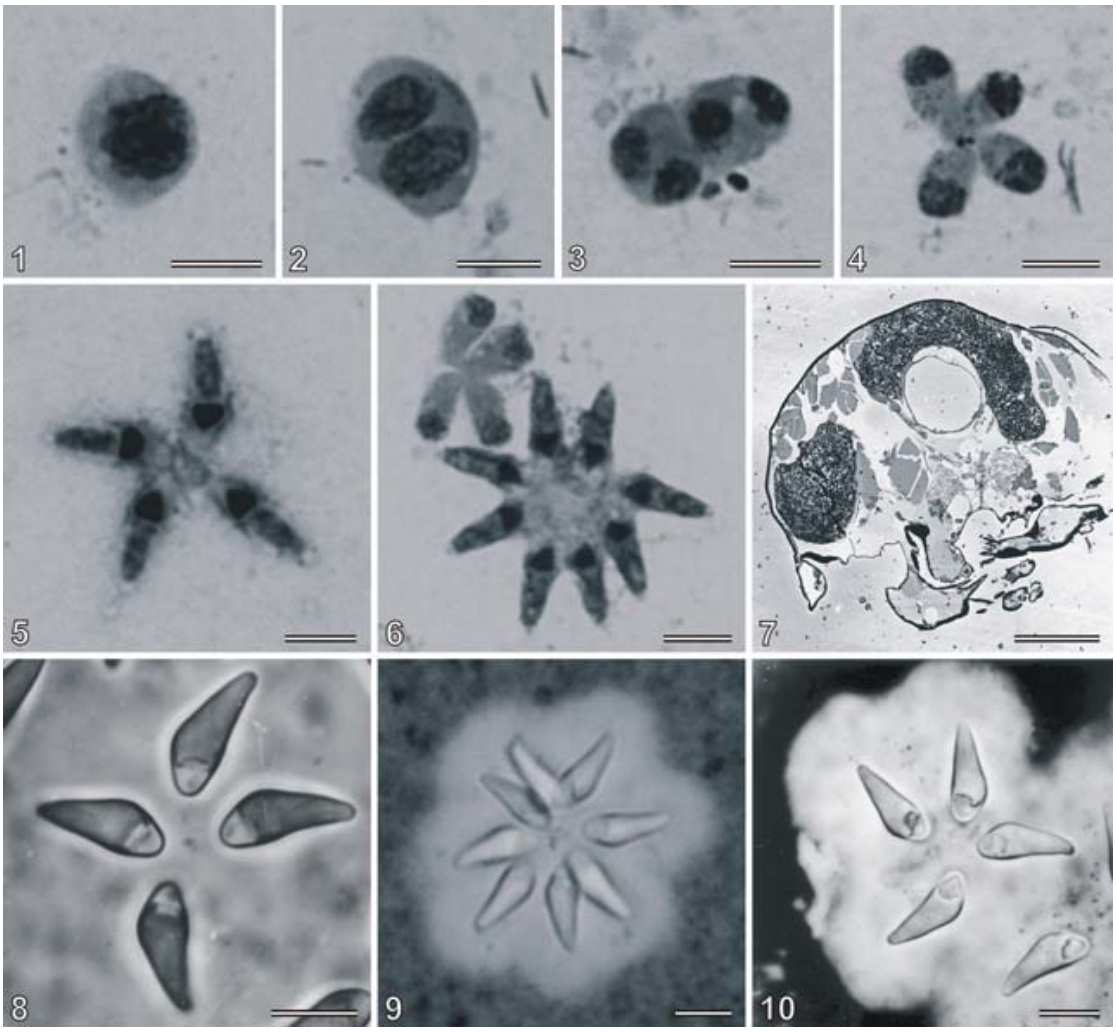
The specific host of this microsporidian is *Cyclops vicinus* Uljanin, 1875 although *C. strenuus* Fischer, 1851 has also been reported as a host (Komárek and Vávra 1968) and on one occasion, *Eucyclops serrulatus* (Fischer, 1851) was found to be infected. The specific site of infection was ovarial tissue, which is why only late copepodites or adult female copepods were found to be infected (Fig. 7). Infected tissue containing spores was visible as a white mass located in the anterior part of the copepod body. Copepods containing stages other than spores showed no visible signs of infection and thus could not be effectively selected. Infected copepods occurred in ponds from January/February until the middle of May, with the peak of infection occurring around the middle of April. Peak infection coincided with peak egg formation in uninfected copepods. Around 0.1–1% of hosts were typically infected but the prevalence of infection fluctuated widely in different years. During summer months only free floating spores or group of spores were present in the plankton of ponds (Figs. 8–10).

Infection experiments

All our attempts to infect copepods by seeding cultures with spores were unsuccessful. It thus seems that *Marssoniella* spores are of the heteroinfectious type.

Light microscopy

Giemsa stained smears of copepods containing developmental stages revealed uninucleate cells around 6 μm in size with a large nucleus (3–4 μm) (Fig. 1). It is believed that these cells belonged to the beginning of the sporogonial stage of the life cycle. The nuclei divided twice giving rise first to binucleate and finally tetranucleate cells (around 9 μm in size) which divided in rosette-like fashion to produce typically 4, sometimes 8, and rarely 16 spores per cluster (Figs. 2–6). The nuclei were always monokaryotic in nature**,** although the dividing nuclei sometimes looked like a diplokaryon. In Giemsa-stained smears the nuclei in spores had a distinct triangular shape (Fig. 5).



Figs. 1–10. *Marssoniella elegans* as seen in the light microscope. **Fig. 1.** Uninucleate cell of an early sporont. **Fig. 2.** Binucleate sporont after the first nuclear division. **Fig. 3.** Tetranucleate sporont after the second karyokinesis. **Fig. 4.** Rosette-like separation of sporoblasts. **Figs. 5, 6.** The final stage of sporogony: young spores in four- and eight-cell clusters. Note the densely stained triangular nuclei and some material of the sporophorous vesicle in the centre of the star-like formation. (Figs. 1–6: Giemsa staining). **Fig. 7.** Gross-section of the copepod *Cyclops vicinus* showing two lobes of infected ovary. Methenamine-silver staining. **Figs. 8–10.** Fresh spores of *Marssoniella elegans* suspended in water. **Fig. 8.** The most common 4-cell formation. **Fig. 9.** The less frequently occurring 8-cell formation. **Figs. 9, 10.** Spores in India ink revealing the mucous material around the spores. Scale bars: Figs. 1–6, 8–10 = 5 μm; Fig. 7 = 0.125 mm.

As mentioned above, the ratio four:eight spores formed from one sporont and representing a star-like cluster when freed from the host, fluctuated in individual copepods. Typically, there were about 6–8% of clusters containing 8 spores in a copepod, occasionally, however, in some copepods this ratio varied from 3% to 40% (Komárek and Vávra 1968). No host individuals were found which contained a 100% pure population of four-cell spore clusters. Clusters containing 16 spores were extremely rare. The spore size was constant regardless of the number of spores per cluster.

The spores were lanceolate, slightly asymmetrical, uninucleate and had a large, very visible posterior vacuole (Figs. 8–10). Fresh spores measured 7.0–8.0 × 2.5–3.5 μm (Komárek and Vávra 1968); measurements with a more accurate technique (Image Splitting Eyepiece (Vávra and Maddox 1976) provided slightly higher values: 8.2 (7.7–8.8) × 3.0 (2.7–3.3) μm (n = 25). Within the copepod, the spores in the cluster were tightly compressed. Upon release from the host, the spore groups opened immediately into star-like formations (Figs. 8–10). These formations were fragile and later broke into fragments with a lesser number of spores. Mixing of spores with India ink revealed a thick layer of mucus-like material surrounding each spore and the spore groups (Figs. 9, 10). The spore groups including mucus-like material measured 25–30 μm regardless of the number of spores contained within. The nature of the mucus-like material is described below.

Electron microscopy

The youngest stages were uni- to tetranucleate cells surrounded not by a single unit membrane (as expected in microsporidial meronts) but actually by two closely adhering membranes, the outer membrane sometimes forming long, loop-like extensions which connected the two cells after cytokinesis (Figs. 11–14). We believe that these stages are early sporonts and we interpret the outer membrane and its extensions as the nascent sporophorous vesicle membrane. In sites where several sporonts adhered to each other, tetramembrane layers occurred and the host tissue was reduced to small islands among the mass of the parasite cells (Figs. 13, 14). In further development (sporogony) electron-dense material was secreted into the narrow space between the two membranes (episporontal space) and was revealed as thin dark layer on the outer surface of the sporont plasma membrane (Fig. 15). The outer membrane detached in blister-like fashion in some places and dark secretory material with periodic structure appeared within these blisters (Fig. 16). The internal fine structure of early sporonts conformed to that of other microsporidia. Nuclei in sporonts were usually single, however as many as four nuclei were found in one cell. The cytoplasm of early sporonts was rich in endoplasmic reticulum membranes bearing ribosomes and in Golgi vesicles (Fig. 15). Sporonts divided by rosette-like budding, each one of the four (or eight) buds representing future spores (Figs. 17, 18). At this stage the sporophorous vesicle membrane became more detached from the cell surface but still tightly ensheathed each cell originated by budding (Fig. 18). Secretory material in the form of small lines occurred in the episporontal space (Fig. 19). At the end of the budding process, all 4 (8) cells (be it sporoblasts or spores), resulting from the sporont division, were surrounded by a common sporophorous vesicle tightly covering the cells. Thus the sporophorous vesicle mem-brane was common to all cells resulting from the sporont, but looked like individual sporophorous vesicles at the level of a single cell (Figs. 21, 22, 24, 25). The sporoblasts showed nascent polar filament coils, individual groups of coils showing different stages of maturation into the final form found in mature spores. The first three anterior coils therefore have a slightly different substructure than the successive three coils, which differ again from the remaining four posterior coils (Fig. 20). This difference was still visible in young spores (Fig. 21).

The spore was surrounded by a 30-nm electron-dense exospore which looked like a single layer but consisted actually of two layers separated by a thin line of a denser material. The endospore was 40 nm thick (Figs. 26, 28). Numerous, very thin (about 10–15 nm), long projections extended from the exospore into the episporontal space, forming a hairy layer around the spore (Fig. 22). These hairy-like projections can be visualised by negative staining in free spores (Fig. 23) and are evidently the material appearing as mucus-like coat around the spore in the India ink preparations mentioned previously. A single, relatively large nucleus was situated in the posterior third of the spore (Fig. 21). The vesiculate polaroplast consisted of wide, flattened sacs anteriorly and large, bubble-like sacs posteriorly (Figs. 26, 27). The polar filament was anchored at the tip of the spore by a rather small anchoring disc situated within the polar sac (Fig. 26). The filament was of the isofilar type, consisting of 10–12 coils in a single row, with the posterior four coils forming an irregular double layer in mature spores (Figs. 21, 25, 28). The posterior vacuole was an irregular, electron-lucent area (Fig. 25).

Phylogeny

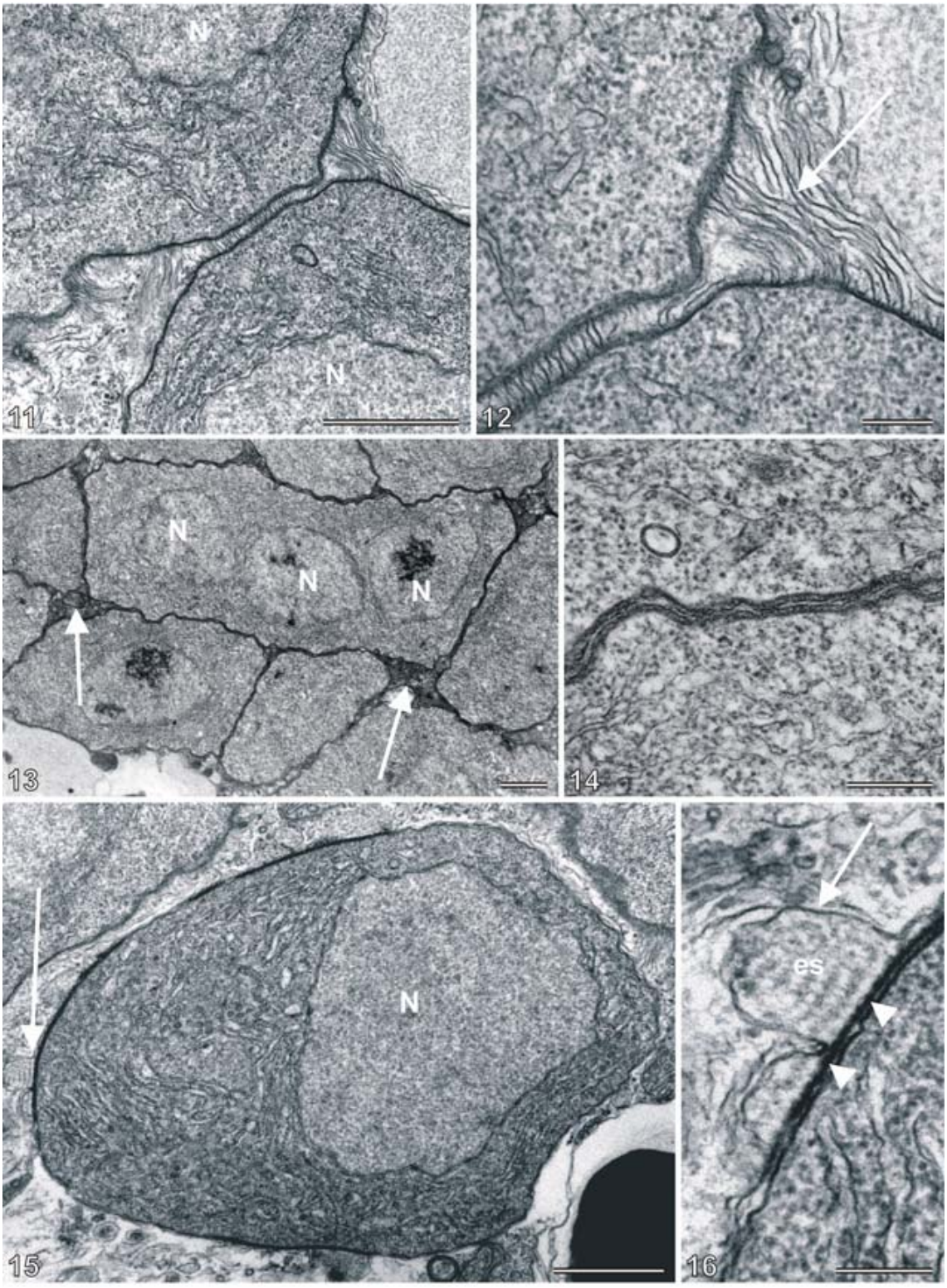
The tree based on SSU rRNA gene sequences confirms that *Marssoniella elegans* falls within microsporidian genera and species in the "aquatic microsporidia" clade of Vossbrinck et al. (2004). However, the inclusion in the tree of the caddis fly microsporidia sequences indicates that *Marssoniella* might be related to trichopteran microsporidia. *Marssoniella* and several microsporidia of caddis flies (the tetrasporoblastic genera *Cougourdella*, *Pyrotheca* and *Episeptum*) form a clade supported with high bootstrap value (MP/ML, 92/96) (Fig. 29). Although, a more detailed relationship of *Marssoniella* to the genera *Pyrotheca* and *Cougourdella* could not be resolved (*Marssoniella* × *Pyrotheca*, bootstrap 50), (*Marssoniella* × *Cougourdella* was not supported by MP bootstrap analysis), the obtained topology was, with exception of the *Trichotuzetia-Hazardia* clade, analogous for both methods used.

DISCUSSION

Taxonomy

Should the organism in question be transferred back into the genus *Gurleya*, to which it was originally assigned by Vávra (1963) and Komárek and Vávra (1967)? We believe that such action would not be justified and would only cause confusion in microsporidian taxonomy. There are two reasons for maintaining *Marssoniella* as a valid genus. First, as Larsson (1988) states, the "taxonomic handling of tetrasporoblastic microsporidia, at least from microcrustaceans, is especially

Figs. 11–16. *Marssoniella elegans*, developmental stages as seen in the electron microscope. **Fig. 11.** Portions of two early sporonts as result of cytokinesis; the outer membrane (future sporophorous vesicle membrane) is drawn into long loops connecting both cells (N – nuclei). **Fig. 12.** Detailed view of Fig. 11, clearly showing the folds of the future sporophorous vesicle (arrow). **Fig. 13.** Group of early sporonts; the cell in the centre shows three nuclei (N). Host tissue (oocyte cytoplasm) is reduced to small islands among parasite cells (arrows). **Fig. 14.** Detail of Fig. 13, showing that each early sporont is limited actually by two membranes, the outer one being the sporophorous vesicle membrane. Thus, a tetramembrane formation occurs at the contact of



two cells. **Fig. 15.** Sporont with a large nucleus (N) at the stage when the detachment of the sporophorous vesicle membrane begins. Dark material on the plasma membrane representing the future spore wall is patchily distributed on the cell surface. At arrow is the region shown in detail in Fig. 16. **Fig. 16.** Detail of Fig. 15, showing the sporophorous vesicle membrane (arrow) detached in a blister-like fashion from the cell surface. Secretory material with interrupted structure appears in the episporontal space (es). Electron-dense material covers the cell membrane (arrowheads). Scale bars: Figs. 11, 13, 15 = 1 μm; Figs. 12, 16 = 200 nm; Fig. 14 = 500 nm.

difficult". The reason is that the genus *Gurleya* is badly defined because there are no data available on the type species *G. tetraspora* described from a *Daphnia* by Doflein (1898). In this situation, Larsson (1999) proposed to base the definition of the genus *Gurleya* on *G. daphniae*, a parasite of *Daphnia pulex* and described as a new species by Friedrich et al. (1996). If we accept this proposal, the copepod microsporidian in question is different in several structural characters, namely in having a somewhat variable number of spores per group, star-shaped spore groups as compared to an ovoid shape in *Gurleya*, and by having an isofilar polar filament. Structurally, *Marssoniella elegans* resembles several other copepod microsporidia (*Tuzetia*, *Lanatospora*, *Trichotuzetia*, copepod morphs of *Amblyospora*, *Hyalinocysta* and *Parathelohania*) by having single nuclei throughout development, sporonts and spores individually enclosed in a rather tightly fitting sporophorous vesicle ("individual sporophorous vesicle" of *Tuzetia* and *Lanatospora*), spores with a thin endospore, and a thin non-stratified exospore (the stratification of the exospore in *Marssoniella* is visible only at high magnifications and in well preserved specimens!), an isofilar polar filament, and a polaroplast with vesicular chambers. Also the fibrillar coat of the exospore resembles the much more coarse fibres found on the exospore of immature *Trichotuzetia* spores (Vávra et al. 1997). *Marssoniella* is distinct from the above-mentioned microsporidia by having a number of spores formed from a sporont (4, rarely 8 and very rarely 16) and the ability of the spore clusters to open into the star-like formation immediately upon contact with water. The mechanism that mediates this action, however, is not known. The clear halo around the spores seen in wet smears with India ink is due to the inability of carbon particles in the ink to penetrate among the fibres ornamenting the exospore. We do not know if the sporophorous vesicle membrane persists during the spore cluster inflation in water or if it bursts at that moment.

One or more *Marssoniella*?

Although the "cyanobacterial" genus *Marssoniella* as described by Lemmermann (1900) was vaguely defined, there is no doubt, that there are no cyanobacteria of this kind and that Lemmermann's *Marssoniella* is actually a microsporidium (Komárek and Vávra 1968). Although *Marssoniella elegans* was identified as the *Cyclops vicinus* and *C. strenuus* microsporidium, the question is whether this is the only organism which algologists recognize as *Marssoniella.* This question is not just academic, as *Marssoniella* is still reported by algologists as cyanobacterium occurring presently in a number of habitats as available Internet information suggests. Possibly also other aquatic microsporidia that form groups of spores that can float due to the mucus resembling exospore material could be interpreted by algologists as *Marssoniella*. As mentioned by Komárek and Vávra (1968) two other microsporidia, *Bohuslavia asterias* (Weiser, 1963) Larsson, 1985 parasitizing midge larvae and *Gurleya francottei* Léger et Duboscq, 1909 in dipteran larvae, are such candidates; possibly there are several others.

Phylogeny

According to Vossbrinck et al. (2004), *Marssoniella* belongs to a clade of "aquatic microsporidia", with its closest neighbours in the tree being microsporidia of the genus *Hazardia*, mosquito parasites that form homoinfectious spores (Canning and Vávra 2000). The clade further contains one copepod microsporidium and four microsporidian species infecting cladocera, all of them evidently forming heteroinfectious spores (Vávra 1964, Vávra and Larsson 1994, Vávra et al. 1997, Refardt et al. 2002). All these species may have a second host required for completion of their life cycles. Although the second host of *Marssoniella elegans* has not been identified, the data presented here confirm its position among water-dwelling microsporidia and suggest that Trichoptera might be another group of insects which crustacean microsporidia use as alternate hosts for dispersal and maintenance in the habitat.

Taxonomic redefinition of the genus *Marssoniella*

Marssoniella Lemmermann, 1900 (phylum Microspora Sprague, 1977, family Tuzetiidae Sprague, Tuzet et Maurand, 1977)

Diagnosis. All life-cycle stages with isolated nuclei. Merogonial reproduction probably by serial division, late meronts/early sporonts surrounded by plasma membrane to which another external membrane tightly adheres. Sporogony by rosette-like division, yielding mostly 4, sometimes 8, and rarely 16 sporoblasts. Spores lanceolate, asymmetrical, with pointed anterior pole. Posterior vacuole large, up to 1/4 of spore length, nucleus situated in posterior half of the spore. Spores produced by a single sporont are contained in a common sporophorous vesicle which adheres to individual cells, giving the impression that each spore has a sporophorous vesicle of its own. Exospore thin, covered by thick coat of very fine fibres which fill the sporophorous vesicle. Polar filament isofilar. Sporophorous vesicles released from the host swell in water and spores within form a star-like formation. Type and only species *Marssoniella elegans* Lemmermann, 1900.

Figs. 17–23. *Marssoniella elegans*, advanced sporogonial stages as seen in the electron microscope. **Fig. 17.** Sporont dividing in a rosette-like fashion into future sporoblasts. **Fig. 18.** The sporont division products have nuclei (N) typically located in the apex of the cell and their cytoplasm has a vacuolate character with abundant endoplasmic reticulum, ribosomes and Golgi-associated vesicles. Sporophorous vesicle membrane ensheaths tightly each cell (arrow). **Fig. 19.** During early sporogony the episporontal space is filled with finely granular secretory products of medium electron density forming short comma-like aggregates.

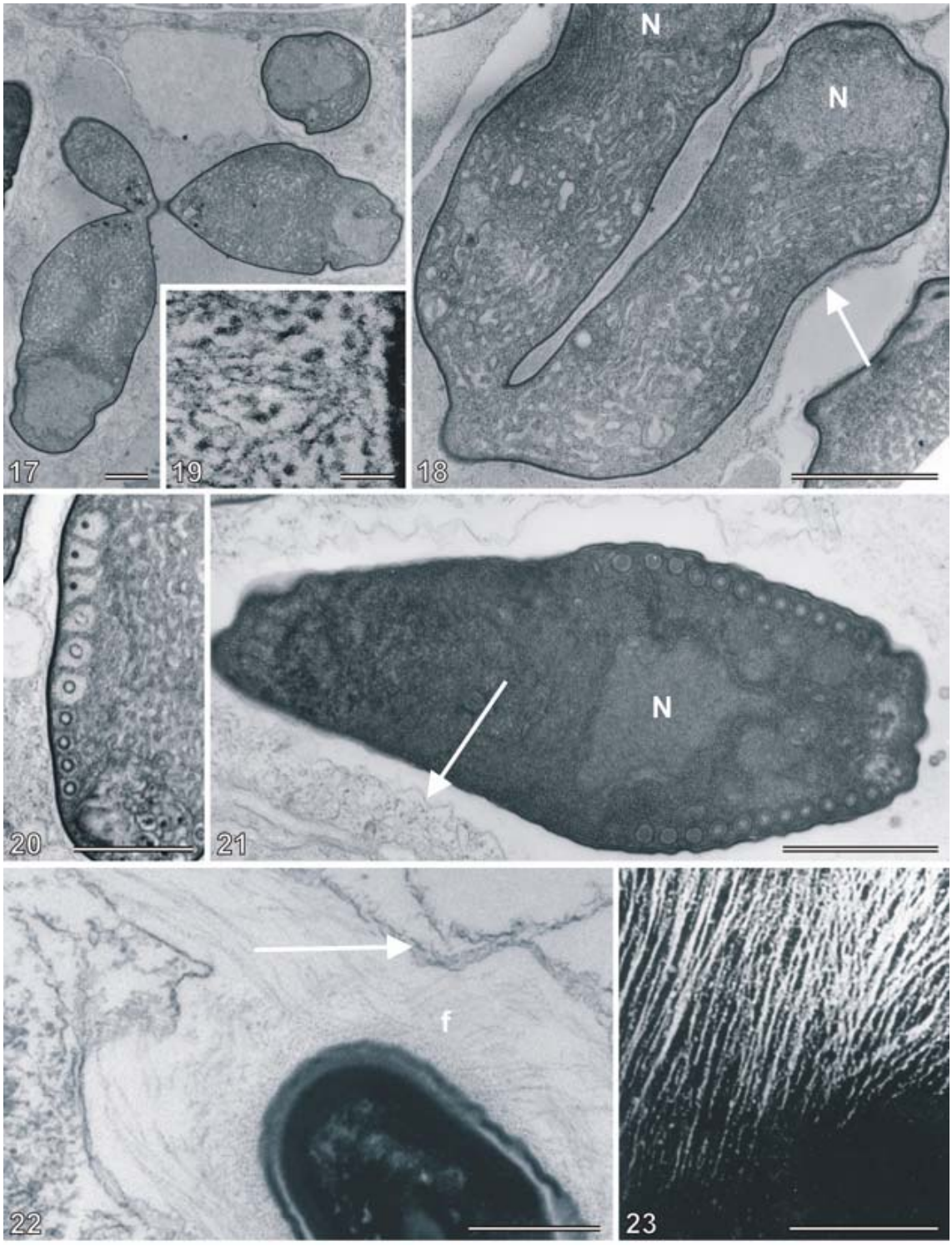
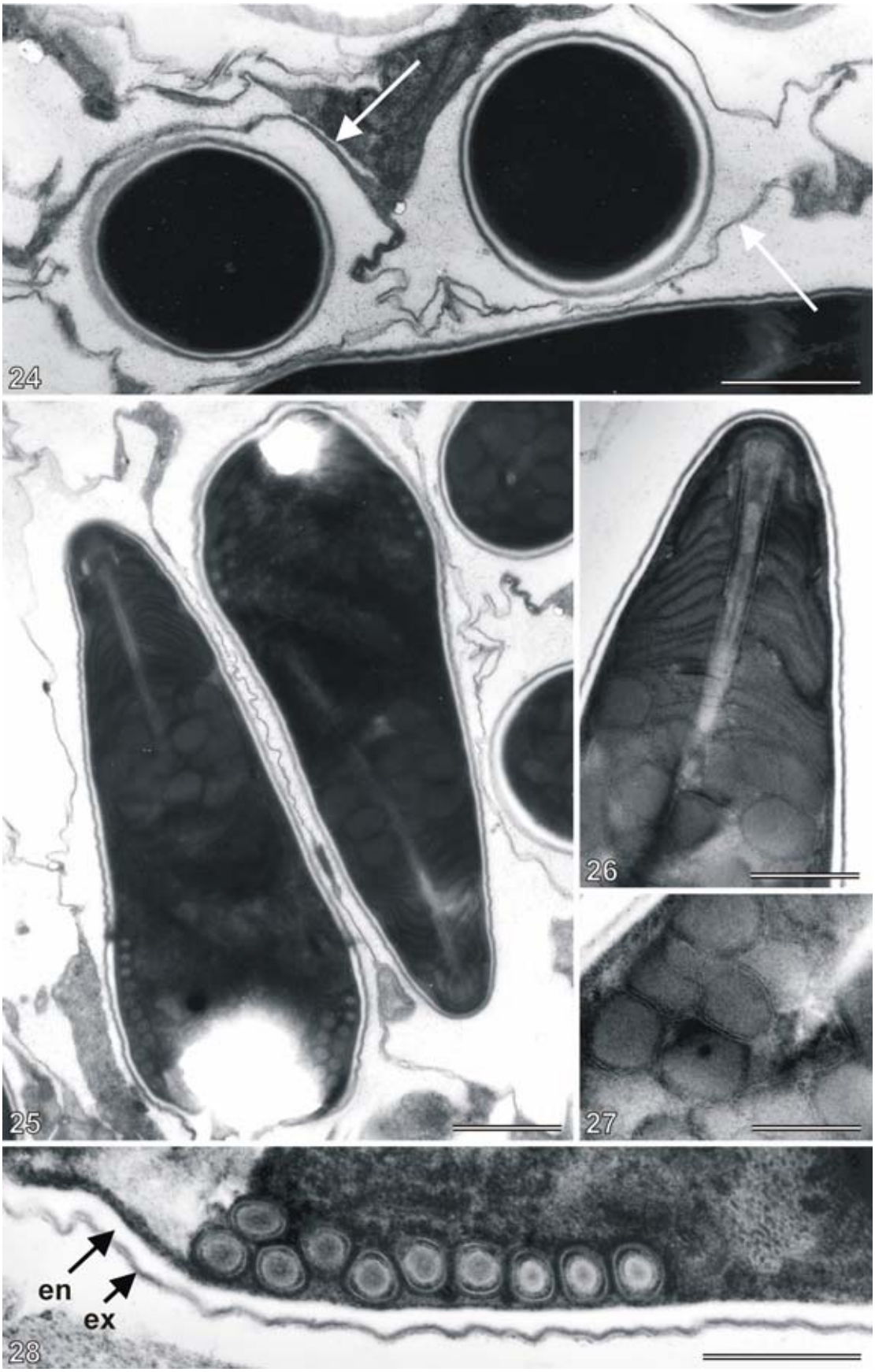


Fig. 20. Part of a sporoblast maturing into spore. The number of polar filament coils is nearly final (10 out of 11) but the substructure of coils is different for the proximal three, medium three and basal four coils, respectively. **Fig. 21.** Young spore with the final number of polar filament coils and nucleus (N) shifted towards the posterior pole of the cell. Note that individual polar filament coils have slightly different structure, reflecting the differences seen in Fig. 20. Sporophorous vesicle membrane is at arrow. **Fig. 22.** Hairy coat of very fine fibres (f) stretching from the spore surface occupies the volume of the sporophorous vesicle. This material evidently simulates the presence of the "mucus material" around floating spores seen in the light microscope. Sporophorous vesicle membrane is at arrow. **Fig. 23.** Fibres shown in Fig. 22 as revealed by negative staining. Scale bars: Figs. 17, 18, 20, 21 = 1 µm; Fig. 19 = 100 nm; Figs. 22, 23 = 0.5 µm.


24
25
26
27
en
ex
28

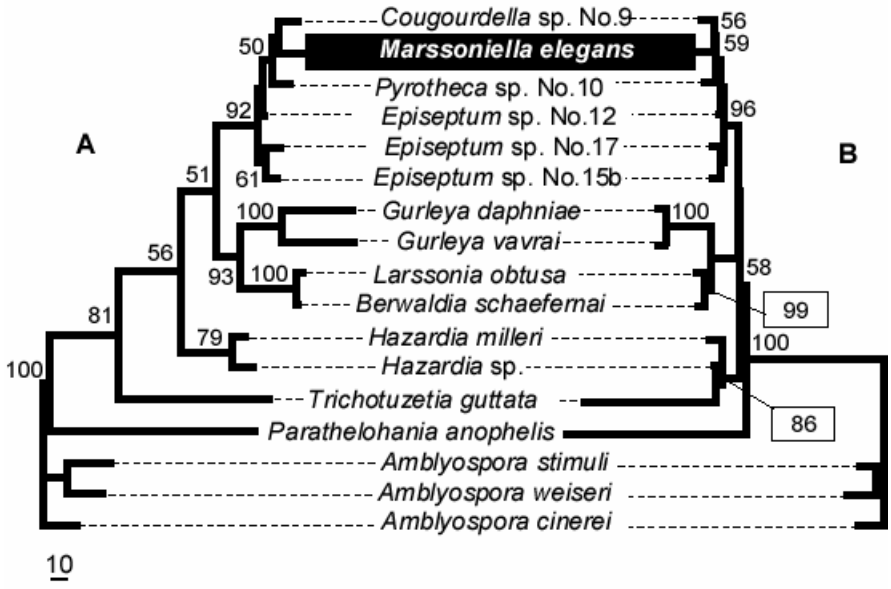


Fig. 29. Phylogenetic trees with *Marssoniella elegans*, as inferred from partial sequence of SSU rRNA. Alignment used contained 575 characters, of which 191 were parsimony informative. **A** – Maximum parsimony (MP) tree (one of two equally parsimonious). TL = 588; CI = 0.3299; RI = 0.6891; RC = 0.4617. Numbers above branches indicate MP bootstrap support (1,000 replicates). **B** – Maximum likelihood (ML) tree (loglk = –3349.08063) was constructed using GTR model for nucleotide substitutions with discrete gamma distribution in 8+1 categories. All parameters (gamma shape = 0.589; pinvar = 0.175) were estimated from dataset. Numbers above branches indicate ML bootstrap support (model HKY85, ti/tv ratio and pinvar estimated from dataset, one category of sites, 1,000 replicates).

***Marssoniella elegans* Lemmermann, 1900.** Sporonts (6 μm) originally with one relatively large nucleus (3–4 μm), their development as for the genus. Fresh spores: 8.2 (7.7–8.8) × 3.0 (2.7–3.3) μm. Fixed, Giemsa-stained spores: around 7 μm in length with large (2 μm), nucleus, triangular in shape. Spore wall thin, with endospore 40 nm thick and exospore about 30 nm thick appearing as a dense homogenous layer divided in two halves by a fine line of material of higher density. Exospore covered by a dense brush of about 10–15 nm thick filaments which fill the sporophorous vesicle. Polar filament isofilar, 130 nm in diameter with 10–12 coils in one row (young spores) or several anterior coils in one row followed by an irregular clump of posterior coils (some mature spores). Polaroplast of wide chambers anteriorly and vesicle-like sacs posteriorly. Spore groups, when released from the host, open in water into star-like formations and are surrounded by thick layer of mucus-resembling material. The mucus "capsule" with spores inside measures 25–30 μm in diameter. Spore groups non-persistent: fragments with variable number of spores floating free in water.

Type habitat: Pond Mlýnský, near Mšec, county Kladno, Czech Republic.

Hosts and site of infection: Ovarial tissues of *Cyclops vicinus* Uljanin, 1875, *Cyclops strenuus* Fischer, 1851, and exceptionally, *Eucyclops serrulatus* (Fischer, 1851).

Deposition of vouchers: International Protozoan Type Slide Collection, Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA and senior author's collection.

Acknowledgements. The authors are greatly indebted to Mrs. Eva Kirchmanová (Department of Pathology, 1st Faculty of Medicine, Charles University, Prague) for expert help in electron microscopy. The authors are also grateful for financial support received from the research project of the Institute of Parasitology, Academy of Sciences of the Czech Republic (Z60220518).

Figs. 24–28. *Marssoniella elegans*, spore fine structure. **Fig. 24.** Cross-section of two spores, members of a single spore cluster. The common sporophorous vesicle membrane (arrows) tightly ensheaths each spore giving the impression that each spore has a sporophorous vesicle of its own. **Fig. 25.** Group of two mature spores. Each spore is enclosed in a thin sporophorous vesicle. **Fig. 26.** The apex of a mature spore showing the anchoring disc and the wide anterior chambers of the polaroplast. Note the thin exo- and endospore layers of the spore wall. **Fig. 27.** Detail of the vesicular chambers of the posterior part of the polaroplast. **Fig. 28.** Mature spore has a thin, single-layer exospore 30 nm thick (arrow, ex), a relatively thin endospore (40 nm) (arrow, en) and isofilar polar filament with 10 (sometimes 11–12) coils in a single layer. The last four coils form an irregular double layer in some spores. Scale bars: Figs. 24, 25 = 1 μm; Figs. 26, 27 = 0.5 μm; Fig. 28 = 0.25 μm.

REFERENCES

ANDREADIS T.G. 1985: Experimental transmission of a microsporidian pathogen from mosquitoes to an alternate copepod host. Proc. Natl. Acad. Sci. USA 82: 5574–5577.

BRONNVALL A.M., LARSSON J.I.R. 2001: Ultrastructure and light microscopic cytology of *Agglomerata lacrima* n. sp. (Microspora, Duboscqiidae), a microsporidian parasite of *Acanthocyclops vernalis* (Copepoda, Cyclopidae). Eur. J. Protistol. 37: 89–101.

CANNING E.U., VÁVRA J. 2000: Phylum Microsporidia Balbiani, 1882. In: J.J. Lee, G.F. Leedale and P. Bradbury (Eds.), The Illustrated Guide to the Protozoa (2nd Edition), Vol. 1. Society of Protozoologists, Lawrence, Kansas, pp. 39–126.

DOFLEIN F. 1898: Studien zur Naturgeschichte der Protozoen. III. Ueber Myxosporidien. Zool. Jahrb., Abt. Anat. Ontog. Thiere 11: 281–350.

FRIEDRICH C., WINDER O., SCHAFFLER K., REINTHALER F.F. 1996: Light and electron microscopy study on *Gurleya daphniae* sp. nov. (Microspora, Gurleyidae), a parasite of *Daphnia pulex* (Crustacea, Phyllopoda). Eur. J. Protistol. 32: 116–122.

GREEN J. 1974: Parasites and epibionts of Cladocera. Trans. Zool. Soc. Lond. 32: 417–515.

GUINDON S., GASCUEL O. 2003: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52: 696–704.

HIRSCH J.G., FEDORKO M.E. 1968: Ultrastructure of human leucocytes after simultaneous fixation with glutaraldehyde and osmium tetroxide and "postfixation" in uranyl acetate. J. Cell Biol. 88: 615–627.

KOMÁREK J., VÁVRA J. 1967: Identity of a blue-green alga *Marssoniella elegans* with a microsporidium *Gurleya* sp. J. Protozool. 14 (Suppl.): 36.

KOMÁREK J., VÁVRA J. 1968: In memoriam of *Marssoniella* Lemm. 1900. Arch. Protistenkd. 111: 12–17.

LARSSON J.I.R. 1988: Identification of microsporidian genera (Protozoa, Microspora) – a guide with comments on the taxonomy. Arch. Protistenkd. 136: 1–37.

LARSSON J.I.R. 1999: Identification of microsporidia. Acta Protozool. 38: 161–197.

LARSSON J.I.R., EBERT D., VÁVRA J., VORONIN V.N. 1996: Redescription of *Pleistophora intestinalis* Chatton, 1907, a microsporidian parasite of *Daphnia magna* and *Daphnia pulex*, with establishment of the new genus *Glugoides* (Microspora, Glugeidae). Eur. J. Protistol. 32: 251–261.

LEMMERMANN E. 1900: Beitraege zur Kenntnis der Planktonalgen. IX. *Lagerheimia Marssonii* nov. spec., *Centratractus belonophora* (Schmidle) nov. gen. et spec., *Synedra limnetica* nov. spec., *Marssoniella elegans* nov. gen. et spec. Ber. Dtsch. Bot. Ges. 18: 272–275.

LOM J., VÁVRA J. 1963: Mucous envelopes of spores of the subphyllum Cnidospora (Doflein, 1901). Acta Soc. Zool. Bohemoslov. 27: 4–6.

REFARDT D., CANNING E.U., MATHIS A., CHENEY S.A., LAFRANCHI-TRISTEM N.J., EBERT D. 2002: Small subunit ribosomal DNA phylogeny of microsporidia that infect *Daphnia* (Crustacea: Cladocera). Parasitology 124: 381–389.

SPRAGUE V. 1977: Systematics of the microsporidia. In: I.A. Bulla and T.C. Cheng (Eds.), Comparative Pathobiology. Vol. 2. Plenum Press, New York, London, pp. 1–510.

SPRAGUE V., BECNEL J.J., HAZARD E.I. 1992: Taxonomy of phylum Microspora. Crit. Rev. Microbiol. 18: 285–395.

SWEENEY A.W., HAZARD E.I., GRAHAM M.F. 1985: Intermediate host for an *Amblyospora* sp. (Microspora) infecting the mosquito *Culex annulirostris*. J. Invertebr. Pathol. 46: 98–102.

SWOFFORD D.L. 2000: PAUP* phylogenetic analysis using parsimony (*and other methods). Software Programme distributed by Sinauer Associates Sunderland, MA, USA.

VÁVRA J. 1963: Spore projections in Microsporidia. Acta Protozool. 1: 153–155.

VÁVRA J. 1964: A failure to produce an artificial infection in cladoceran Microsporidia. J. Protozool. 11 (Suppl.): 35–36.

VÁVRA J. 1976: Structure of the microsporidia. In: I.A. Bulla and T.C. Cheng (Eds.), Comparative Pathobiology. Vol. 1. Plenum Press, New York, London, pp. 1–85.

VÁVRA J., BARKER R.J. 1980: The microsporidian mucocalyx as seen in the scanning electron microscope. Folia Parasitol. 27: 19–21.

VÁVRA J., LARSSON J.I.R. 1994: *Berwaldia schaefernai* (Jirovec, 1937) comb. n. (Protozoa, Microsporida), fine structure, life cycle and relationship to *Berwaldia singularis* Larsson, 1981. Eur. J. Protistol. 30: 45–54.

VÁVRA J., LARSSON J.I.R., BAKER M.D. 1997: Light and electron microscopic cytology of *Trichotuzetia guttata* gen. et sp. n. (Microspora, Tuzetiidae) a microsporidian parasite of *Cyclops vicinus* Uljanin, 1875 (Crustacea, Copepoda). Arch. Protistenkd. 147: 293–306.

VÁVRA J., MADDOX J.V. 1976: Methods in microsporidiology. In: I.A. Bulla and T.C. Cheng (Eds.), Comparative Pathobiology. Vol. 1. Plenum Press, New York, London, pp. 281–319.

UNDEEN A., VÁVRA J. 1997: Research methods for entomopathogenic protozoa. In: L.A. Lacey (Ed.), Biological Techniques. Manual of Techniques in Insect Pathology. Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, pp. 117–151.

VOSSBRINCK C.R., ANDREADIS T.G., VAVRA J., BECNEL J.J. 2004: Molecular phylogeny and evolution of mosquito parasitic Microsporidia (Microsporidia: Amblyosporidae). J. Eukaryot. Microbiol. 51: 85–95.

Received 29 September 2004 Accepted 31 January 2005