

# Ultrastructural Study and Description of *Ordospora colligata* gen. et sp. nov. (Microspora, Ordosporidae fam. nov.), a New Microsporidian Parasite of *Daphnia magna* (Crustacea, Cladocera)

J. I. Ronny Larsson<sup>1</sup>, D. Ebert<sup>2</sup>, and J. Vávra<sup>3</sup>

<sup>1</sup>Department of Zoology, University of Lund, Sweden

<sup>2</sup>Zoologisches Institut, Universität Basel, Switzerland

<sup>3</sup>Department of Parasitology, Charles University, Prague, Czech Republic

## Summary

The new microsporidium *Ordospora colligata* gen. et sp. n. is described with emphasis on the ultrastructural characteristics. The parasite, which is superficially similar to *Glugoides intestinalis* (Chatton, 1907), invades the gut epithelium of *Daphnia magna*, where the complete development takes place in a host cell-derived parasitophorous vacuole. Merogonial and sporogonial stages occur together in the vacuole, with presporal stages at the periphery, mature spores aggregated in the centre. All life cycle stages have isolated nuclei. Merogonial plasmodia are initially rounded, later elongated, and they give rise to four merozoites. The bouts of merogony are unknown. The sporont produces two sporoblast mother cells, which often remain coupled. Each cell generates a chain of four sporoblasts. Sporophorous vesicles are not produced. Mature spores are pyriform with a pointed anterior pole and with an obliquely positioned posterior vacuole. Unfixed spores measure  $1.33-2.29 \times 2.32-3.69 \mu\text{m}$ . The exospore is uniform, about 11 nm thick. The polar filament is isofilar, 82–85 nm wide, making 5–6 coils in the posterior half of the spore. The polaroplast is uniform, composed of about 28 nm, wide lamellar components, where the lamellae are folded around each other. The discrimination from other microsporidian species and the systematic position are briefly discussed. A new family, Ordosporidae, is established and *Endoreticulatus durfortii* Martinez et al., 1993 is transferred to *Ordospora*.

**Key words:** *Ordospora colligata* gen. et sp. nov.; Ultrastructure; Taxonomy; *Daphnia magna*; Ordosporidae fam. nov.

## Abbreviations

A – anchoring disc; E – exospore; EN – endospore; F – polar filament; HN – host nucleus; N – nucleus; P – polaroplast; PM – plasma membrane; PS – polar sac;

PV – posterior vacuole; R – ribosomes; T – tubule; V – parasitophorous vacuole

## Introduction

In specimens of *Daphnia* and other cladocerans the gut wall is often infested by microsporidia. When light microscopic characters are used for the identification, the microsporidium is usually identified as the species *Pleistophora intestinalis* Chatton, 1907. This species has recently been redescribed, based upon an investigation where newly collected material was compared with the obviously only surviving slide made by Chatton [17]. The genus *Pleistophora*, with distinct cytological characteristics, the type species being a parasite of fish and all other species proven to belong to the genus being parasites of poikilothermous vertebrates, could no longer be used for classifying these parasites. The new genus *Glugoides* was established for *P. intestinalis*. It is now obvious that there is a spectrum of microsporidian species that can be erroneously identified as *Pleistophora intestinalis*.

This paper deals with a microsporidium of the *Pleistophora intestinalis*-type. The species, which is new to science, is described with emphasis on the ultrastructural characteristics, and the taxonomic position is discussed.

## Material and Methods

The host was *Daphnia magna* Strauss, 1820 collected in ponds at the following locations in United Kingdom and Germany:

1. North Oxfordshire, close to the village Ambrosden, UK (Latitude 51° 52.80' North, Longitude 1° 7.20' West). Samples had been taken on 15. 7. 1993 and in September 1994.
2. London, UK, Samples from "Children's boat pond" in Regents Park (Lat. 51° 31.58', Long. 0° 10.12'). Sampled in September 1994.
3. Lebrader Teiche near Plön, North Germany (Lat. 54° 14' North, Long. 10° 26 East). Sampled in May 1996.
4. Additional material came from laboratory lines which had been established from samples from all three localities at the stated dates.

Fresh squash preparations were made by the agar method of Hostounský and Žižka [15] and studied using phase contrast microscopy and dark field illumination. Permanent squash preparations were lightly air-dried and fixed in methanol for 15 min for Giemsa staining or in Bouin-Duboscq-Brasil (BDB) solution for at least one hour for staining with Heidenhain's iron haematoxylin. For paraffin sectioning specimens were fixed in BDB solution overnight or longer. After washing and dehydration in a graded series of ethanol specimens were cleared in butanol and embedded in Paraplast (Lancer St. Louis, MO, USA). Sections were cut more or less longitudinally at 10 µm and stained with haematoxylin. For details on the histological techniques used see the manual by Romeis [27]. All permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd., Poole, England). Measurements were made with an eye-piece micrometer at  $\times 1,000$  or using an image analysis program (Micro Macro AB, Gothenburg, Sweden).

For transmission electron microscopy, infected specimens were cut in halves and treated according to one of the following procedures. Either fixation in two steps, 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2, 4 °C, 20 h), washing in cacodylate buffer, and finally 2% (w/v) osmium tetroxide in cacodylate buffer (pH 7.2, 4 °C, 1 h) (Series 930914), or direct fixation in 2% (w/v) osmium tetroxide in cacodylate buffer (pH 7.2, 4 °C, 90 min) (Series No. 950727) was performed. The fixed tissues 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 [26].

The here described gut parasite appears very similar to the previously described *Glugoides intestinalis* in light microscopy. Both parasites infect the gut epithelium of *Daphnia magna*, are horizontally transmitted through waterborne spores expelled with the faeces and are mildly pathogenic (infected hosts suffer only about 20–30% fecundity reduction). Further the geographic distribution of both parasites is overlapping and they might even be sympatric at some locations. In earlier publications the parasites had been named *Pleistophora intestinalis*, a name which has subsequently been rejected [17]. With the help of preserved material it was possible to determine which of the earlier studies on the genetics of host-parasite interactions and on the ecology of these parasites had been carried out with which species. The results described by Ebert [9, 10, 11] are obtained from work with *Glugoides intestinalis*, while the work by Mangin et al. [22] was carried out with the herein described parasite. Mangin and collaborators give a summary of the ecology and epidemiology of the here described species [22].

## Results

### Prevalence and pathogenicity

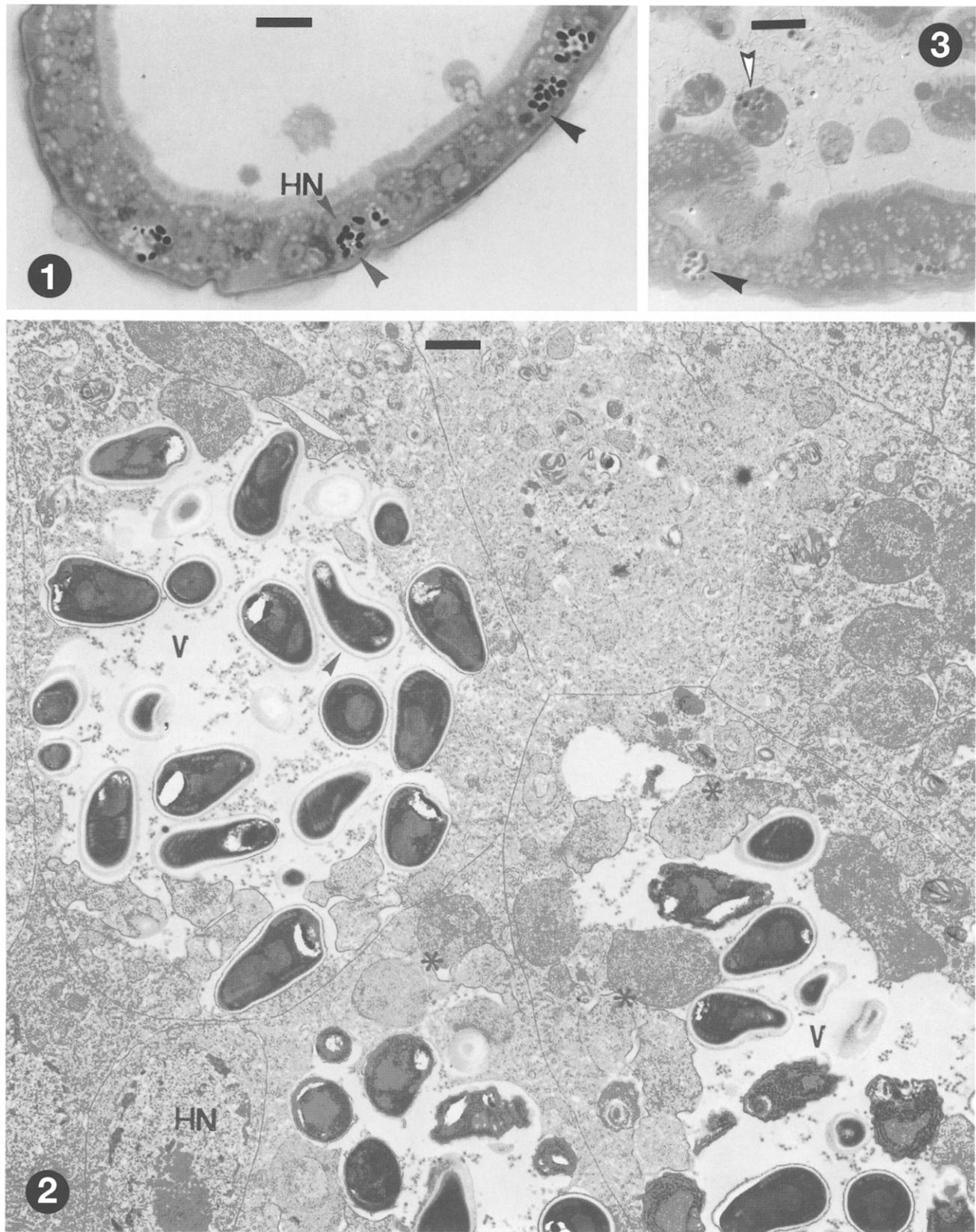
The infection appeared as isolated cells, or small groups of cells, filled with microsporidia (Fig. 1). In advanced cases of infection the centre of the epithelium cells was occupied by a large vacuole filled with microsporidia (Fig. 2). The host nucleus was dislocated to a lateral position. As the vacuole was present already around merogonial stages (Figs. 4–5) it must be interpreted as a parasitophorous vacuole produced by the host cell at the time of infection. The vacuole membrane had approximately the same thickness as the plasma membrane of the merozoites, measuring 7–8 nm, but the unit membrane nature was normally less obvious (Fig. 5). In advanced infections vacuoles contained immature stages at the periphery and mature spores in the centre (Fig. 2). Cells and nuclei were not hypertrophic. The parasites were released when microsporidia-filled epithelium cells were shed into the gut lumen (Fig. 3).

### Presporal stages and life cycle

All life cycle stages had isolated nuclei of normal eukaryotic type, delimited by double unit membranes separated from each other by a perinuclear space. The nucleoplasm was granular and almost similar to the cytoplasm (Figs. 4, 9). Merogonial stages had an electron-dense cytoplasm with densely packed ribosomes and with traces of a rough endoplasmic reticulum (Figs. 4–5). Uninucleate merozoites were rounded or ovoid (Fig. 4). Sectioned merozoites measured up to 2.8 µm in diameter, the central nucleus up to 1.6 µm (sectioned). The merogony produced small plasmodia with four nuclei. They were initially rounded (Fig. 6), but the shape changed to elongated before the four merozoites were released (Fig. 7). Sectioned elongated plasmodia measured up to 1.6 µm wide, sectioned circular nuclei up to 1.2 µm in diameter. The bouts of merogony are unknown, but the occurrence of merogonial and sporogonial stages together in the same parasitophorous vacuole indicated that there must be repeated merogonial divisions. The last generation of merozoites matured to sporonts. Meiosis was not observed.

In ultrathin sections sporogonial stages were discriminated by their less dense cytoplasm (Figs. 8–9). The rough endoplasmic reticulum was still weakly developed (Fig. 9). The cell surface became coated with electron-dense secretions in a layer slightly thicker than the plasma membrane (Figs. 9–10). The dense material was secreted simultaneously over a wide area of the surface of the sporont (Fig. 9), not spot-wise or as ridges.

Already in the initial phase of the sporogony tubular material, measuring 63–65 nm in diameter, was pro-



duced by the sporont or sporoblast mother cell (Fig. 9). It was formed from surplus wall material and exhibited the layers of the sporont wall. When sporoblasts were formed the tubules connected the division products (Fig. 12). The tubules were persistent and visible also in the proximity of mature spores (Fig. 2). The initiation and morphogenesis of the organelles of the spore followed the normal pathway for microsporidia.

The earliest sporogonial stages visible in light microscopic preparations were binucleate, but not diplokaryotic, elongated oval cells (Fig. 13a). These cells were often associated pairwise. They are interpreted as two sporoblast mother cells, produced by the initial division of the sporont. The nuclei of each cell divided once more, and finally each sporoblast mother cell fragmented into a chain of four sporoblasts (Fig. 14b–c). The failing separation of the two sporoblast mother cells made the two chains remain connected, and in carefully made squash preparations also mature spores were seen as chains of 8 cells (Fig. 13d). Chains of sporoblasts and spores were usually curved in light microscopic preparations (Figs. 13b–d, 16), and a curved arrangement was also visible *in situ* in sectioned cells (Fig. 11). The curved outline of the chain was often interrupted at the point where the two sporoblast mother cells were connected (Figs. 13b, 16). It is remarkable that even completely mature spores formed a chain, where the spores not only were adhering externally, but were communicating by cytoplasmic bridges (Fig. 14).

## Spores

Mature spores were pyriform with pointed anterior pole (Figs. 15–16), sometimes slightly curved (Figs. 2, 15, 16, 18). A posterior vacuole was visible in an

oblique position close to the posterior pole of living spores (Fig. 15). In stained spores the nucleus could be visualized as a dark spot in, or slightly below, the equatorial area (Fig. 16). Unfixed spores measured  $1.33\text{--}2.29 \times 2.32\text{--}3.69 \mu\text{m}$  ( $n = 50$ ), fixed and stained  $1.12\text{--}1.84 \times 2.08\text{--}3.19 \mu\text{m}$  ( $n = 50$ ).

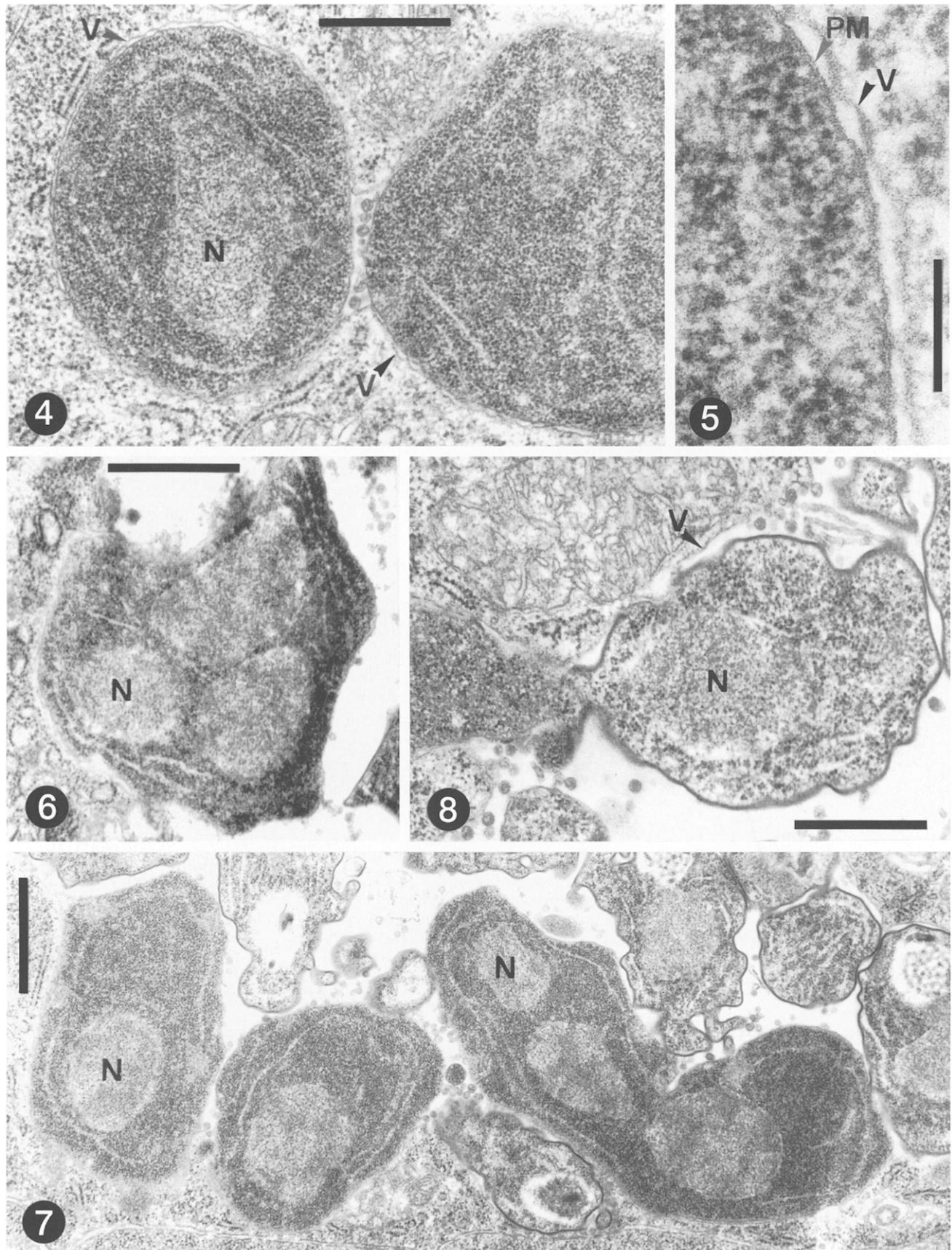
Mature spores had a  $106\text{--}132 \text{ nm}$  thick spore wall, which was considerably thinner at the anterior pole, down to  $49 \text{ nm}$  (Fig. 18). The spore wall had the normal three subdivisions (Fig. 19). The internal layer was an about  $8 \text{ nm}$  thick plasma membrane. This layer must be considered to belong to the spore wall since the membrane remains as the internal component of the spore wall of the empty spore after the sporoplasm has been ejected. The median endospore layer was translucent, and weaker development of this layer caused the reduced thickness of the spore wall anteriorly. The surface layer, the exospore, was about  $11 \text{ nm}$  thick and uniformly electron-dense.

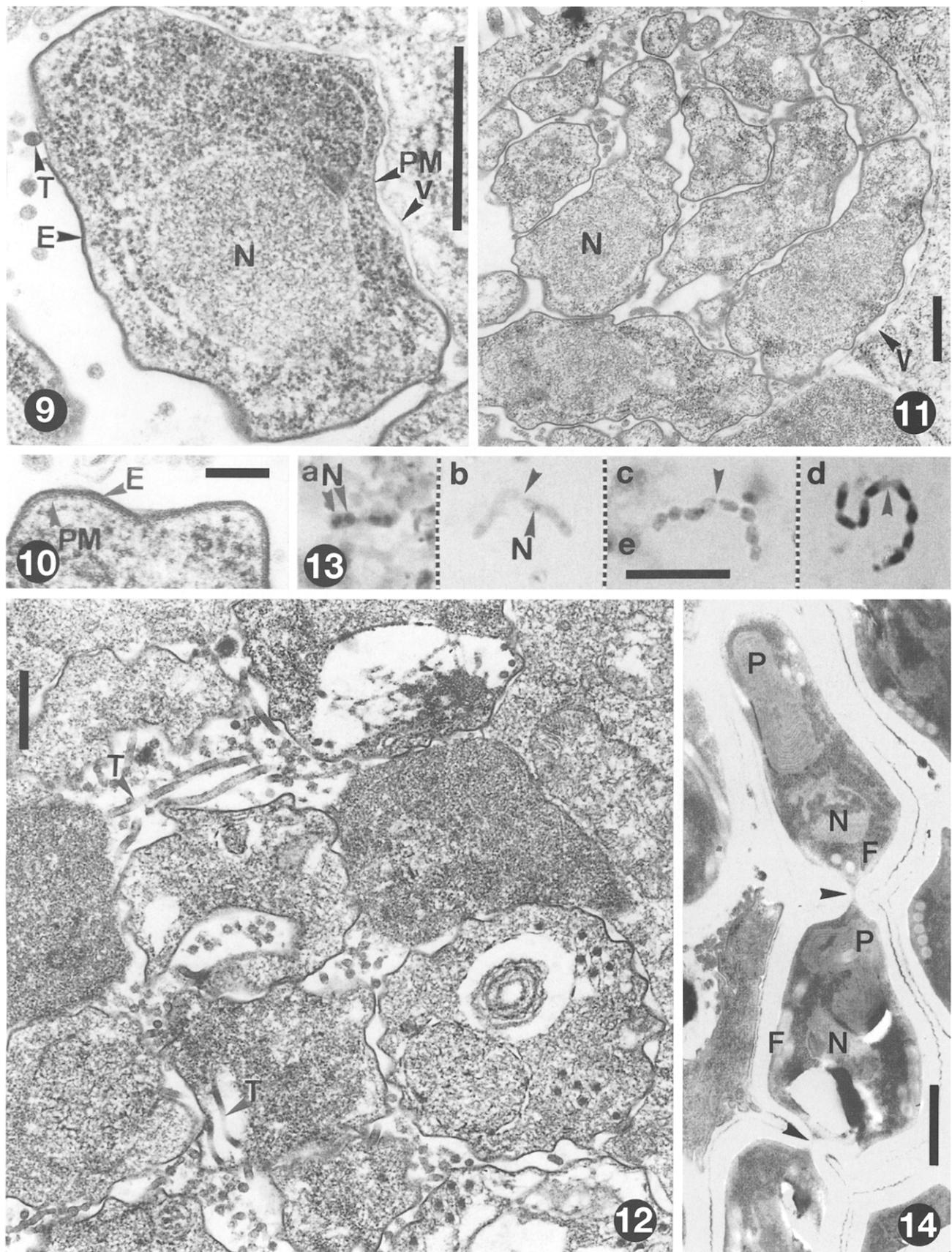
The polar filament was attached to a pad-like anchoring disc at the anterior pole of the spore (Figs. 17, 18, 22). The widest sectioned disc measured  $241 \text{ nm}$  in diameter. The filament was widest close to the disc, measuring up to  $150 \text{ nm}$  in diameter. The length of the wide region was  $1.5\text{--}2$  times the diameter of the filament (Fig. 18). The filament proceeded backwards in the centre of the spore for maximum  $1/3$  of the spore length (Figs. 18, 22), then turned sideways to almost touch the spore wall. The posterior part was arranged as  $5\text{--}6$  coils in one row of coils in the posterior half of the spore (Figs. 17, 18). The row of coils measured about  $1/4$  of the spore length. The angle of tilt of the anterior coil to the long axis of the spore was about  $50^\circ$ . The polar filament was isofilar with a coil diameter of  $82\text{--}85 \text{ nm}$ . Transverse sections revealed a distinct sequence of layers of differ-

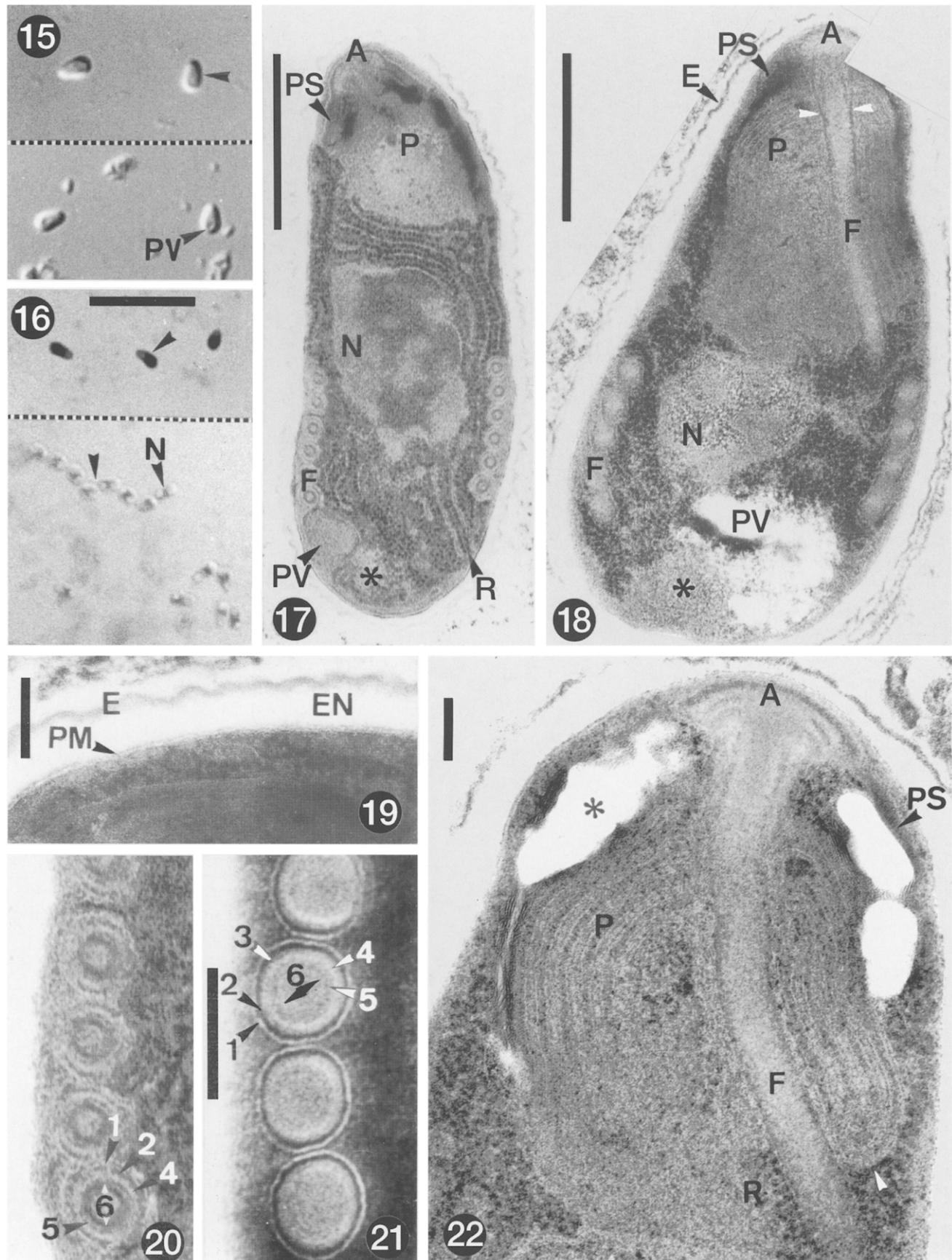
**Figs. 1–3.** Influence on the host by *Ordospora colligata* gen. et sp. n. 1 and 3. Semithinly sectioned gut epithelium with foci of developing microsporidia (black arrowheads) (toluidine and methylene blue); white arrowhead of Fig. 3 indicates a spore-filled epithelium cell shed into the gut lumen. 2. Ultrathin section revealing parasitophorous vacuoles with spores in the centre, presporal stages (\*) at the periphery. Scale bars: Figs. 1 and 3 =  $10 \mu\text{m}$ , Fig. 2 =  $1 \mu\text{m}$ .

**Figs. 4–8.** Merogony and early sporogony. 4. Two merogonial cells enclosed in a parasitophorous vacuole. 5. The border host-parasite at greater magnification. 6. Rounded merogonial plasmodium. 7. Elongated merogonial plasmodium; the two cells to the left are either part of another plasmodium or two merozoites. 8. Two joint sporonts, the one to the right with nearly complete exospore layer. Scale bars: Figs. 4 and 8 =  $0.5 \mu\text{m}$ . Fig. 5 =  $100 \text{ nm}$ , Figs. 6 and 7 =  $1 \mu\text{m}$ .

**Figs. 9–14.** Sporogony. 9. Sporont where half of the surface is covered by exospore-material. 10. The sporont wall at greater magnification. 11. Parasitophorous vacuole with curved chains of sporoblasts. 12. Sporoblasts connected by tubular bridges of exospore material. 13. Light microscopic aspects on the sporogony (Giemsa stain): a. Two joint binucleate sporoblast mother cells; b. Two probably four-nucleate sporoblast mother cells; c and d. Two associated chains each with four immature (c) or mature (d) spores (arrowheads indicate the point where the division products of the two sporoblast mother cells join). 14. Chain of three mature spores (arrowheads point at cytoplasmic bridges). Scale bars: Figs. 9, 11, 12 and 14 =  $0.5 \mu\text{m}$ , Fig. 10 =  $100 \text{ nm}$ , Fig. 13 =  $10 \mu\text{m}$ .







ent electron density, but with small differences in thickness. In the mature spores the following layers could be distinguished (Fig. 21, numbers 1–6); a c. 5 nm thick and membrane cover (1), two slightly thinner layers which were prominently electron-dense (2) and moderately dense (3), an about 5 nm thick lucent layer resembling transversely sectioned fibrils (4), a slightly thinner layer (5) of approximately the same density as layer 3, and the up to 40 nm wide, almost uniform centre (6). The transversely sectioned filament of the immature spore looked different: layer 2 was less electron dense, layer 3 was absent, and the external zone of the centre (6) was as dense as layer (Fig. 20).

The polaroplast surrounded the uncoiled part of the polar filament (Fig. 18). It was composed of about 28 nm wide lamellae arranged as a system of folds enclosing each other (Fig. 22). The construction was especially distinct posteriorly. The polaroplast was asymmetrical, slightly longer and distinctly wider to the convex side of the spore (Fig. 18). The polar sac enclosed the anchoring apparatus and proceeded backwards as an umbrella-shaped fold, which covered the anterior 1/3 of the polaroplast (Figs. 17, 18, 22). The polar sac, the polaroplast lamellae and the surface layer of the polar filament belonged to the same system of about 5 nm thick unit membranes. The polar sac was filled with uniform, fairly electron-dense material (Figs. 17, 20).

The nucleus, which appeared slightly compressed, was located to the coil region below the equator (Figs. 16–18). The widest sectioned nucleus measured 0.41 µm in diameter. The vacuole region, at the posterior pole, was composed of one area of uniform moderately dense material, probably the remainders of the Golgi apparatus, and one cavity (Figs. 17, 18). The vacuole membrane was distinct in immature spores, but not preserved in mature spores (Fig. 18). The cytoplasm of immature and mature spores was rather dense (Figs. 17, 18). In immature spores strands of membrane-associated polyribosomes were prominent (Fig. 17), while the linear arrangement was less obvious in mature spores (Figs. 18, 22).

## Discussion

### Cytology

Cytologically the microsporidium treated herein conforms with the normal for microsporidia with the

exception of the polaroplast. This organelle is exceptional in two ways: the uniform construction and the folded lamellae. Most polaroplasts are composed of two, or sometimes three, structurally different regions. However, uniform lamellar polaroplasts are previously known from microsporidia, for example the weakly developed polaroplast of *Baculea daphniae* [21]. The arrangement of the components is special in that the lamellae are folded, enclosing each other (Fig. 22). Identical folding is characteristic for the posterior polaroplast region of the not related microsporidium *Cylindrospora fasciculata* (cf. Fig. 4 B in [16]). In the most commonly observed types of polaroplasts the lamellae are arranged like the petals of a flower, like in *Amblyospora bracteata* (cf. Fig. 9 in [19]).

## Taxonomy

Previously, four microsporidian parasites of the gut epithelium of cladocerans were known: *Baculea daphniae* Loubès and Akbarieh, 1978, *Glugoides intestinalis* (Chatton, 1907) Larsson et al., 1996, *Nosemoides simocephali* Loubès and Akbarieh, 1977, and *Perezia diaphanosomae* (Voronin, 1977) Voronin, 1988. All of them differ from the microsporidium treated herein.

*Baculea daphniae* has narrow rod-shaped spores [21]. *G. intestinalis* has polysporoblastic sporogony in sporophorous vesicles [17]. *N. simocephali* invades both the cytoplasm and nuclei of the gut epithelium of *Simocephalus vetulus*, where it develops in close contact with the host cell [20]. Parasitophorous vacuoles or sporophorous vesicles are not formed. Further differences are the oval shape of the spores and the slightly longer polar filament. *Perezia diaphanosomae* was reported from two tissues: ovary [33] and gut epithelium [34]. It is polysporoblastic, devoid of sporophorous vesicles and parasitophorous vacuoles, and sporulates as ribbon-like, or more rarely oval, sporogonial plasmodia with 6–8 nuclei. The merogonial reproduction is diplokaryotic and the polaroplast has closely packed lamellae anteriorly, wider lamellae posteriorly [34]. We must conclude that the microsporidium described herein is a new species.

Nearly half of the microsporidian genera share a life cycle where the nuclei are isolated in all developmental stages. If we restrict the comparison to genera sporulating in the absence of a sporophorous vesicle and pro-

Figs. 15–22. The mature spore. 15–16. Light microscopy of living (15, DIC) and Giemsa stained (16) spores (arrowheads indicate the convex surface). 17–18. Longitudinally sectioned immature (17) and mature (18) spores (\* indicates the remainders of the Golgi apparatus). 19. Detail of the spore wall. 20–21. Transversely sectioned coils of immature (20) and mature (21) polar filament (the layers are indicated 1–6). 22. Longitudinally sectioned anterior end of the spore exhibiting the construction of the polaroplast (arrowhead points at the folding of the lamellae, \* indicates an artifact). Scale bars: Figs. 15–16 (with common bar on 16) = 10 µm, Figs. 17–18 = 0.5 µm, Figs. 19, 20–21 (with common bar on 21) and 22 = 100 nm.

ducing more than two sporoblasts per sporont, the following genera remain: *Baculea*, *Endoreticulatus*, *Microfilm*, *Microgemma*, *Nosemoides*, *Orthosomella*, *Pleistosporidium* and *Tetramicra*.

Three of the genera, *Microfilm*, *Microgemma* and *Tetramicra*, are exclusively parasites of fish. As there seems to be a host specificity border between microsporidia of invertebrate and vertebrate hosts – there is at least so far no clear documentation of a microsporidian genus combining vertebrate and invertebrate parasites – these genera are unlikely to include the present microsporidium. However, there are also cytological differences. *Microfilm* has the unusual type of polar filament characteristic for the Mrazekidae [12, 18]. The spores of *Microgemma* are oval, the sporogony involves distinct budding processes, and the infection provokes production of xenomas [25]. *Tetramicra* has tetrasporoblastic sporogony by rosette-like budding and xenomas are formed [24]. As neither the description of *Microgemma* nor of *Tetramicra* seems to illustrate fully mature spores, we can hardly compare details of the sporal cytology. However, the illustrations suggest that both genera have polaroplasts composed of two different regions.

We can further exclude *Baculea* and *Pleistosporidium*. *Baculea* has rod-shaped spores of unusual cytology [21]. The genus *Pleistosporidium* was established in a congress abstract without illustrations which makes detailed comparison impossible, but the tetrasporoblastic sporogony by rosette-like budding makes the difference clear [8].

The genus *Nosemoides* has been used for microsporidia of gregarines (the type host) [31], cladocerans [20] and fish [28, 13]. The diagnosis of the genus is rather short: microsporidia with isolated nuclei in all stages, development in direct contact with the cytoplasm of the host cell, absence of plasmodial stages, and sporoblasts produced in rosette-like formations or in groups [31]. The type species *Nosemoides vivieri* is dimorphic, producing elongated oval microspores and almost rod-shaped macrospores [32]. The two fish parasites attributed to the same genus are obviously both monomorphic and produce pyriform spores [13, 28]. Recently it was proven that one of them, *N. tilapiæ*, expressed diplokaryotic nuclei in the merogonic part of the life cycle, and the new genus *Neonosemoides* was established for this species [14]. *N. simocephali*, the parasite of the gut epithelium of a cladoceran, conforms with the characteristics of the genus, except for the absence of macrospores [20]. All species attributed to the genus have polaroplasts with two distinct regions. The genus *Nosemoides* still appears to be heterogeneous, and it is obvious that the type species, *N. vivieri*, and the species treated herein cannot belong to the same genus.

Characteristic for *Orthosomella* are the ribbon-like sporogonial plasmodia generating chains of associated sporoblasts [1, 7]. Spores are not associated [7]. The spores of the type species, *O. operophterae* are almost rod-shaped, exhibiting a wide variation in size [7], the spores of *O. lambdinae* are not so elongated [1]. Superficially the species treated herein seems to fit well in *Orthosomella*, but there are two significant differences: the pyriform spores and the undivided polaroplast. Ultrastructural details can best be compared with *O. lambdinae* which has a polaroplast with two distinct lamellar regions [1].

*Endoreticulatus* was established for a species of the *Pleistophora*-complex [3]. Characteristics for the genus include the isolated nuclei, the development of meronts and sporonts in independent parasitophorous vacuoles (i.e. in vacuoles in which either meronts or sporonts but not the mixture of them occur), and the multisporous sporogony by multiple fission [3]. At the onset of sporogony the sporogonial plasmodium withdraws the plasma membrane from the parasitophorous vacuole in a characteristic manner (Fig. 21 in [3], Fig. 2d in [5]). The type species, *E. fidelis* [3], and the second species, *E. schubergi* [5], are parasites of the gut epithelium of insects, while the third species, *E. durforti*, invades the gut epithelium of the aquatic crustacean *Artemia* sp. [23].

While merogonial and sporogonial stages of the two terrestrial *Endoreticulatus* species are located in independent parasitophorous vacuoles, the division products of the merogony and sporogony of *E. durforti* occur in the same vacuole with spores in the centre and immature stages at the periphery (Fig. 12 in [23]). This is identical to the arrangement observed by us (Fig. 2), and further similarities are found in the ultrastructure. Both *E. durforti* and the species treated by us have uniform thin exospores (cf. Fig. 19 with Fig. 34 in [23]), and fairly weakly developed undivided polaroplasts where the external lamellar folds enclose the internal ones (cf. Fig. 22 with Fig. 32 in [23]). The shape of the spores is obviously also the same (cf. Fig. 18 with Fig. 30 in [23]). Also the illustrations of the merogonial plasmodia resemble each other (cf. Fig. 7 with Fig. 13 in [23]). The sporogony of *E. durforti* was described to be by rosette-like division, but rosettes are not visible in the ultrathin sections. There is no light microscopic documentation of the reproduction. It seems obvious that *E. durforti* and the species treated herein belong in the same genus, but it is more doubtful if this genus is *Endoreticulatus*.

The parasitophorous vacuole is believed to be a production of the host, and hence it is unclear how much weight it has as taxonomic character. However, the typical *Endoreticulatus* species from insects differ from the two more dubious microsporidia from aquatic

crustaceans not only in the development of the parasitophorous vacuole but also in the shape of the spore and the mode of division in the sporogony. The spores of *E. fidelis* and *E. schubergi* appear more blunt, and the sporogonial reproduction is by fragmentation of multi-nucleate, rounded sporogonial plasmodia [2, 5]. Neither of the two recent publications on these species [2, 5] illustrate mature spores sectioned so the construction of the polaroplast is visible. However, both species have a short section of closely packed lamellae, identical to the anterior region of the most common type of polaroplast, and some kind of posterior material that might be a second polaroplast region (Fig. 26 in [2], Fig. 4b in [5]). If we neglect differences related to the parasitophorous vacuole, spore shape and sporogonial reproduction, we also remove the barrier between *Orthosomella* and *Endoreticulatus*, which would make *Endoreticulatus* a junior synonym of *Orthosomella*. That cannot be sound taxonomy. If we retain them as important characters that will exclude the microsporidium treated by us from both genera. Consequently a new genus must be created to accomodate the species treated herein together with *E. durforti*.

The family position is also problematic. In the recent taxonomic survey of the microsporidia Sprague and colleagues introduced the new character "interfacial envelope" which was used in the diagnoses of the families [30]. "Interfacial envelope" was defined as "A structure of any kind or origin that encloses the parasite" [30]. As this definition does not distinguish between host derived envelopes (parasitophorous vacuoles) and envelopes produced by the microsporidium (merontogenetic sporophorous vesicles, sporontogenetic sporophorous vesicles or exospore-derived envelopes) this character is useless for taxonomic purposes, and consequently useless in the diagnosis of family. In the following discussion the families are treated according to the original descriptions, disregarding the modified diagnoses in the recent monograph [30].

If we combine a development with isolated nuclei in all stages of the life cycle, uninucleate spores, sporal cytology of the basic type for microsporidia (which means disregarding microsporidia of the *Metchnikovella-Chytridiopsis*-type), and monomorphic sporogony with the absence of microsporidia-derived envelopes, the following selection of families remain: Encephalitozoonidae Voronin, 1989, Enterocytozoonidae Call & Owen, 1990, Microfilidae Sprague et al., 1992, Tetramicridae Matthews and Matthews, 1980, and Unikaryonidae Sprague, 1977.

The Encephalitozoonidae [35] are disporoblastic. The Tetramicridae [24] and Microfilidae [30] are tetrasporoblastic, sporulating by rosette-like budding, and in addition the Microfilidae have a specialized type of polar filament. The Enterocytozoonidae, with charac-

teristic early initiation of the polar filament [4], and the Unikaryonidae [29] lack parasitophorous vacuoles and sporulate in direct contact with the host cytoplasm. All of them can be disregarded.

*Endoreticulatus* was classified among the Pleistophoridae although typical merontogenetic sporophorous vesicles were lacking [3]. *Orthosomella* was not classified in a family when the genus was established, but in the description of the second species the genus was placed in the Unikaryonidae [1], which for reasons stated above cannot be used for the microsporidium treated herein. The present microsporidium cannot belong to the Pleistophoridae as merontogenetic sporophorous vesicles with thick envelopes, a prominent feature of the typical pleistophorid genera *Pleistophora* and *Vavraia* [6], are lacking. Consequently a new family must be established for the new microsporidium of *D. magna*.

## Taxonomic Summary and Description

### Ordosporidae fam. nov.

All life cycle stages with isolated nuclei. Merogonial and sporogonial stages appear together in a parasitophorous vacuole of host cell origin. Sporogony tetra- or polysporoblastic in ribbon-like configurations.

### Ordospora gen. nov.

In addition to the characters of the family: spores pyriform, exospore uniform, polaroplast uniform and lamellar, polar filament isofilar.

**Etymology:** Prefix derived from the Latin noun *ordo* = line of similar specimen.

### Species

#### 1. *O. colligata* sp. nov., type species

**Merogony:** Rounded four-nucleated plasmodia transform into ribbon-like stages, releasing four merozoites. The bouts of merogony unknown.

**Sporogony:** The sporont generates two sporoblast mother cells, each giving rise to four sporoblasts. Chains of four or 8 sporoblasts and spores.

**Spores:** Pyriform with pointed anterior pole. Unfixed spores measure  $1.33-2.29 \times 2.32-3.69 \mu\text{m}$ , fixed and stained spores  $1.12-1.84 \times 2.08-3.19 \mu\text{m}$ . The spore wall is 106–132 nm thick with an about 11 nm thick uniform exospore layer. The isofilar polar filament is arranged in 5–6 coils, 82–85 nm wide, in a single row of coils, close to the spore wall. The row of coils measures about 1/4 of the spore length. In spores sectioned longitudinally the anterior group of coils is located immedi-

ately below the middle of the spore, the posterior group of coils close to the posterior pole. The angle of tilt is about 50°. Sectioned anchoring discs measure up to 241 nm in diameter. Polaroplast uniform with about 28 nm wide lamellar components, folded around each other. Nucleus (sectioned) up to 0.41 µm wide, immediately below the equator of the spore.

**Sporophorous vesicle:** Absent.

**Host tissues involved:** Gut epithelium. Merogonial and sporogonial stages occur together in a parasitophorous vacuole of host cell origin, with presporal stages at the periphery, mature spores in the centre. Persistent, 63–65 nm wide tubules, with a wall exhibiting the construction of the sporont wall, are present from early sporogony.

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

**Type locality:** North Oxfordshire, close to the village Ambrosden, UK, Lat. 51° 52.80' North. Long. 1° 7.20 West.

**Types:** Syntypes on slides No. 950727-P3-(1-2)

**Deposition of types:** Slide No. 950727-P3-1 in the International Protozoal Type Slide Collection at Smithsonian Institution, Washington, DC, the second slide in the collection of RL.

**Etymology:** After the Latin verb colligo (= bind together), alluding to the connected sporoblasts and spores.

## 2. *O. durforti* (Martinez, Vivarès & Bouix, 1993)

In the description [23] named *Endoreticulatus durforti* Martinez, Vivarès & Bouix, 1993.

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**Address for correspondence:** Ronny Larsson, Department of Zoology, University of Lund, Helgonav. 3, S - 223 62 Lund, Sweden; Dieter Ebert, Zoologisches Institut, Universität Basel, Rheinsprung 9, CH - 4051 Basel, Switzerland; Jiří Vávra, Charles University, Department of Parasitology, Vinična 7, 128 44 Prague 2, Czech Republic.