

Two Tetrasporoblastic Microsporidian Parasites of Caddis Flies (Trichoptera) with Description of the New Species *Episeptum invadens* sp. n. and *Episeptum circumscriptum* sp. n. (Microspora, Gurleyidae)

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Summary: The two microsporidia *Episeptum invadens* sp. n. and *Episeptum circumscriptum* sp. n. are described based on light microscopic and ultrastructural characteristics. All life cycle stages have isolated nuclei. The merogonial reproduction was revealed as plasmodia with a small number of nuclei and groups of merozoites. The sporogony yields four sporoblasts by rosette-like budding, enclosed in a sporophorous vesicle produced by the sporont. Spores are pyriform with pointed anterior pole. The exospore is plurilayered and the polar filament is lightly anisofilar. Unfixed spores of *E. invadens* measure 2.2–2.5×3.2–3.6 µm. The polar filament makes 5–7 coils, the 2–3 anterior coils are the widest (100–126 nm). The anterior polaroplast has wide lamellae, the posterior closely packed lamellae. The host is *Limnephilus fuscicornis* (fam. Limnephilidae). The fat body lobes are completely invaded by the parasite and there is a prominent lysis of the host cells. Unfixed spores of *E. circumscriptum* measure about 2.1×2.5–3.7 µm. The polar filament makes 4–5 coils, the 1–2 anterior are the widest (94–102 nm). The anterior region of the polaroplast has wide chambers, the posterior closely packed lamellae. The host is *Hydropsyche siltalai* (fam. Hydropsychidae). Infection is mostly restricted to the superficial layers of cells in the fat body lobes, and there is only slight lysis of infected cells. The taxonomic problems with the tetrasporoblastic microsporidia and the assignment to genus are briefly discussed.

Key Words: *Episeptum invadens*; *Episeptum circumscriptum*; Ultrastructure; Taxonomy; Pathological action; Larvae of Trichoptera.

Introduction

At least 17 species of microsporidia have been reported from Trichoptera. Five of them exhibit tetrasporoblastic sporogony: two species of the genus *Cougaudella* (WEISER 1965; BAUDOIN 1969; LARSSON 1989), and one species each of the genera *Episeptum* (LARSSON 1986), *Pyrotheca* (XIE & CANNING 1986) and *Gurleya* (MACKINNON 1911; LARSSON 1995). While the *Cougaudella* and *Pyrotheca* species produce spores of characteristic shapes, which can be identified already at the light microscopic level, the two species of *Episeptum* and *Gurleya* have spores of a short pyriform shape which is

shared by a number of other microsporidian genera. At the ultrastructural level *Episeptum inversum* exhibits a number of unique or unusual characteristics, while *Gurleya dorisae* has a cytology of the most common type found among microsporidia.

Tetrasporoblastic microsporidia with spores of similar type to *E. inversum* are fairly common among caddis fly larvae in Sweden (Fig. 1). Two species of this type, using caddis flies of different families, are briefly described herein. The species are new to science. Problems with cytological characters and the taxonomy of

the tetrasporoblastic microsporidia are briefly discussed.

Material and Methods

Hosts were larvae of caddis flies (Trichoptera) collected in the small river Klingavälsån, close to the village of Veberöd, in Scania, southern Sweden. In the first sample (microsporidium 1), collected on July (No. 850709-A, C, H), the hosts were four specimen of *Limnephilus fuscicornis* (RAMBUR, 1842) (fam. Limnephilidae). In the second sample (microsporidium 2), collected on July 8, 1986 (No. 860708-E), the hosts were three specimen of *Hydropsyche siltalai* DÖHLER, 1965 (fam. Hydropsychidae).

Fresh squash preparations were made by the agar method of HOSTOUNSKÝ & ŽIŽKA (1979) and studied using phase contrast microscopy and dark field illumination.

Permanent squash preparations were lightly air-dried and fixed in Bouin-Duboscq-Brasil (BDB) solution for at least one hour for staining with Heidenhain's iron haematoxylin or Giemsa's solution. For paraffin sectioning whole larvae or parts of larvae were fixed in BDB 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 longitudinally at 10 µm and stained with haematoxylin. For details on the histological techniques used see the manual by ROMEIS (1968). All permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd., Poole, England). Measurements of unfixed spores were made with an eye-piece micrometer at $\times 1.000$. Fixed spores were measured using an image analysis program (Micro Macro AB, Gothenburg, Sweden).

For transmission electron microscopy, pieces of infected tissues were excised and fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C. Microsporidium 1 was fixed for 24 and 40 hours, microsporidium 2 for 24 h. After washing in cacodylate buffer and post fixing in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4 °C, 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 (REYNOLDS 1963).

For scanning electron microscopy infected tissue was smeared on circular cover glasses, air dried lightly, and fixed in 2.5% glutaraldehyde in cacodylate buffer at 4 °C for 24 h. After washing in buffer and critical point drying the smears were covered with gold and palladium.

Abbreviations

A	anchoring disc	PA	anterior polaroplast
E	endospore	PM	plasma membrane
EX	exospore	PP	posterior polaroplast
F	polar filament	PS	polar sac
H	holotype	S	sporophorous vesicle
N	nucleus	V	posterior vacuole

Results

Prevalence and pathogenicity

All specimens infected with microsporidia were revealed by externally visible whitish discolouration. The specimens with microsporidium 1 were chalky white, coloured intensely and homogeneously, especially on the ventral side of the abdomen. Also microsporidium 2 gave a homogeneous white discolouration, but it was less intense.

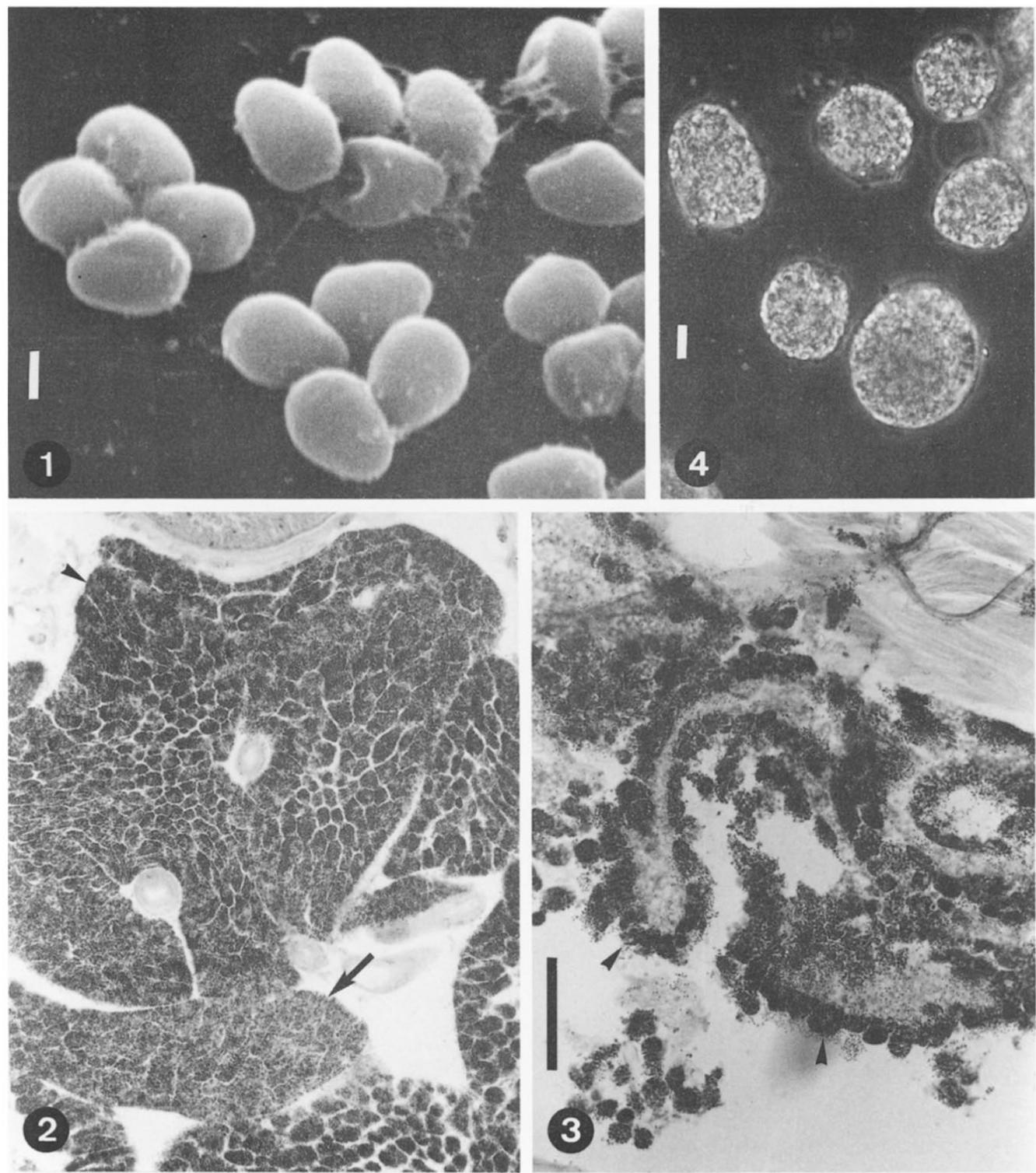
The development of both microsporidia was restricted to the adipose tissue. Microsporidium 1 invaded infected fat body lobes completely (Fig. 2), while the development of microsporidium 2 was nearly completely confined to the superficial layer of cells in the fat body lobe (Fig. 3). Both microsporidia caused hypertrophy of infected cells. Hypertrophy of host nuclei was not observed. In severely infected cells host nuclei were rarely observed, which might indicate that the host nuclei had been disintegrated.

In infections with microsporidium 1 severely infected fat cells were disintegrated already in the host (Fig. 2), and in squash preparations of living tissue no other spore groups were seen than the tetrasporous groups produced by each sporont (Figs. 1, 22). The cell membranes of fat cells infected with microsporidium 2 appeared more persistent. Even if some disintegration was observed in living hosts, whole infected fat cells were prominent in newly made squash preparations, visible as spherical aggregates filled with spores (Fig. 4). Outside the host the cells were rapidly disintegrated releasing the tetrasporous spore groups. If the two microsporidian species differed in their ability to provoke lysis of host cells or if the variation observed was an effect of the different hosts is unknown. When infected tissue was disintegrated also the basal membrane of the fat body lobes was destroyed. No syncytia were formed, and spores were released into the haemocoel.

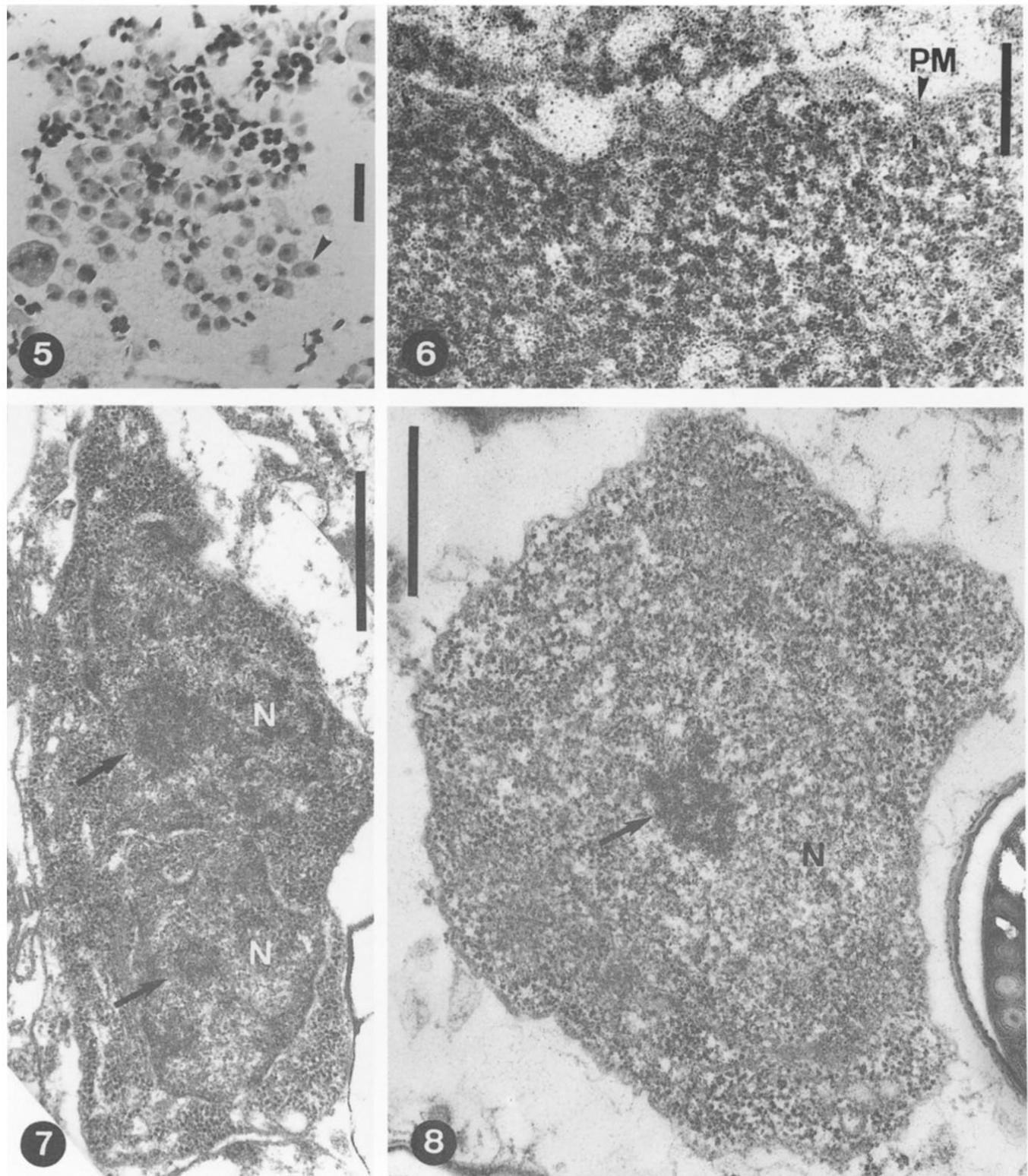
In the sample containing larvae with microsporidium 1 also a second microsporidium was present. Two larvae of the caddis fly *Polycentropus irroratus* (CURTIS 1835) (fam. Polycentropodidae) hosted the dimorphic microsporidium *Amblyospora callosa*. This infection appeared as dispersed islands in the fat body lobes, and it was externally visible as white spots, not as homogeneous discolouration.

Presporal stages and life cycle

The two microsporidia expressed similar cytology and identical life cycles. All life cycle stages of both microsporidia had isolated nuclei. In both species the merogonial reproduction was almost finished. Merogo-

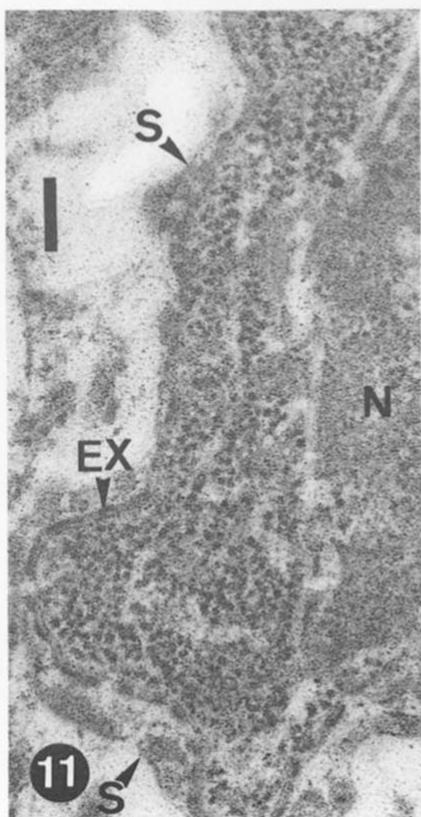
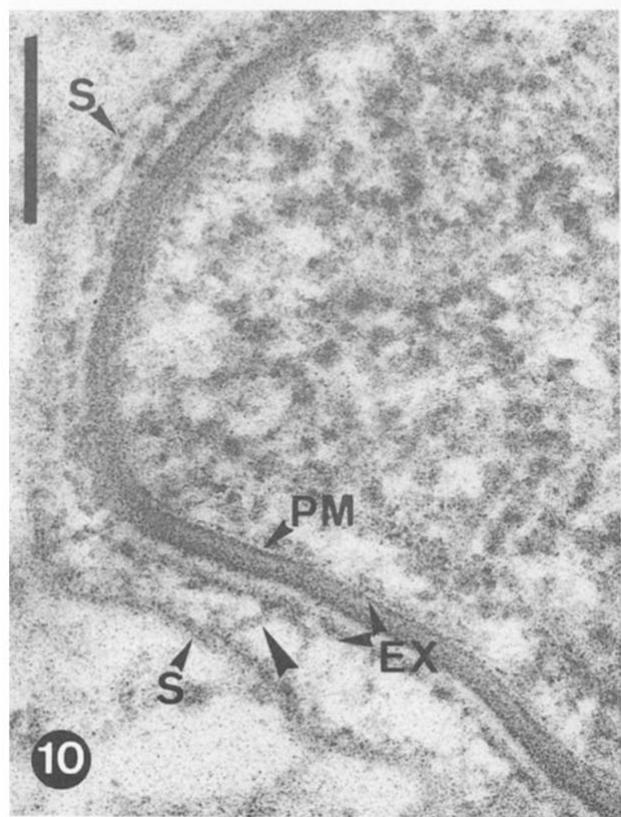
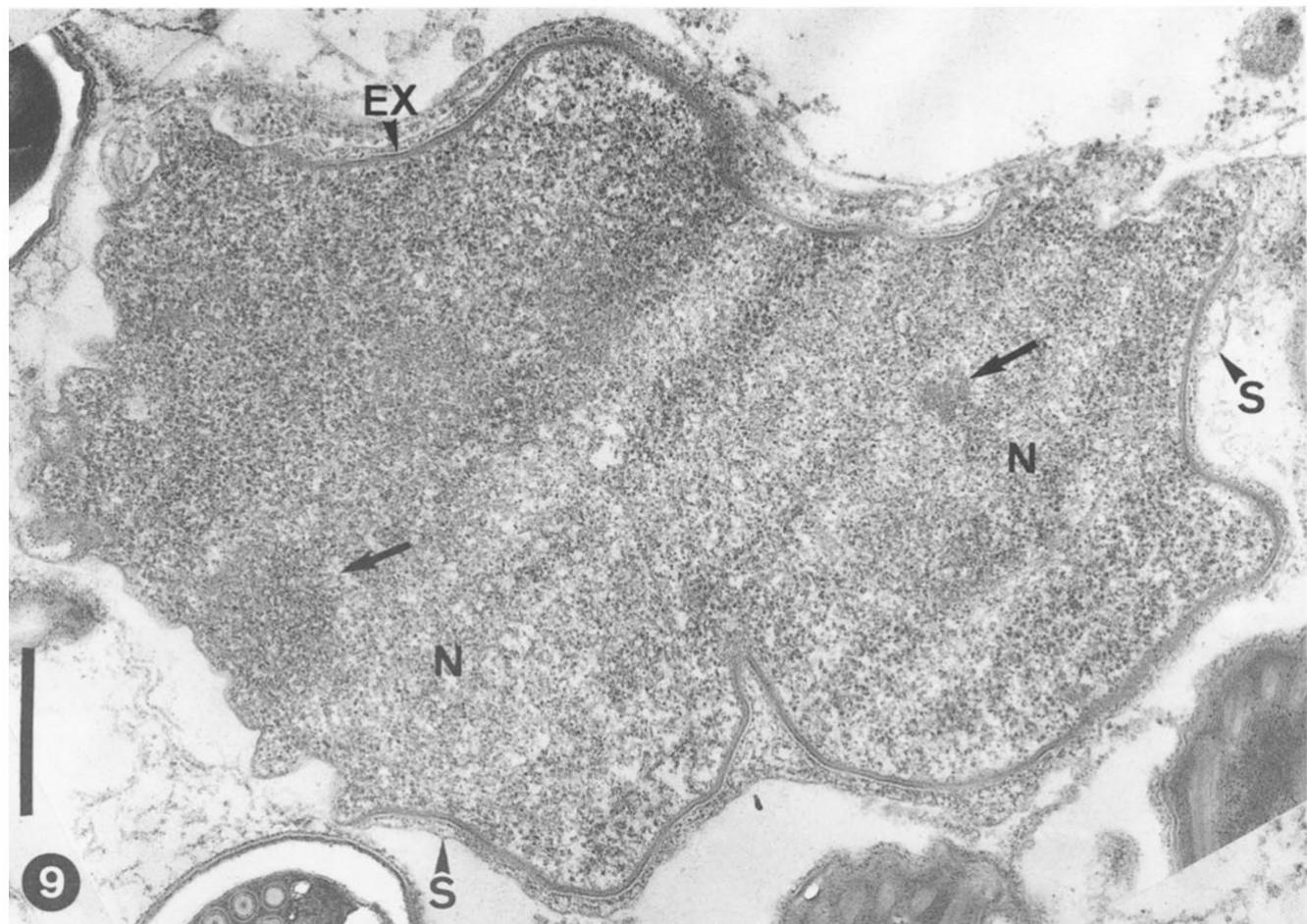


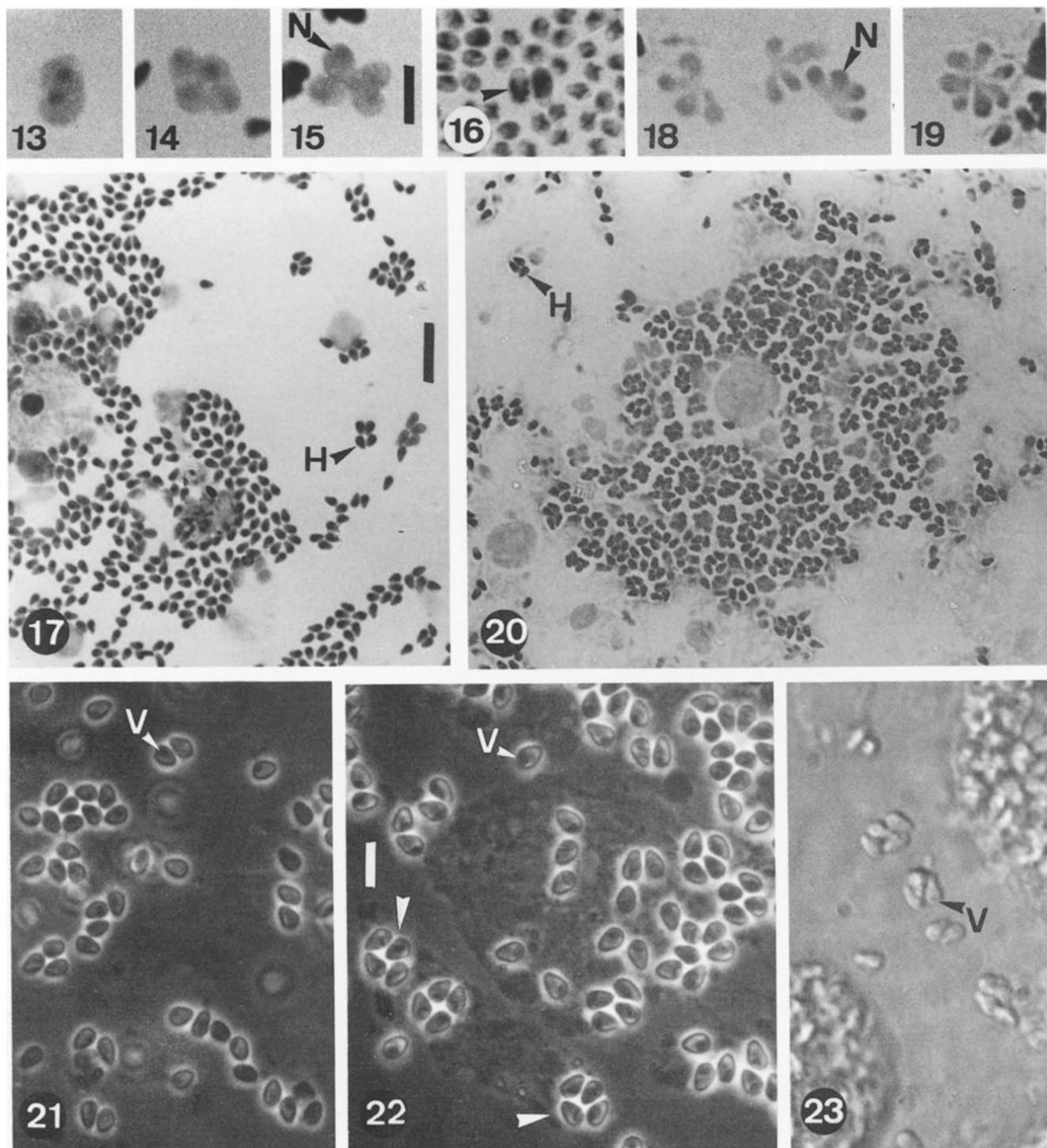
Figs. 1–4. Sporophorous vesicles and pathogenic action of microsporidium 1 (*Episeptum invadens* sp. n.; Figs. 1–2) and microsporidium 2 (*Episeptum circumscriptum* sp. n.; Figs. 3–4). 1. Tetrasporous sporophorous vesicles. 2. Fat body lobes completely filled with microsporidia (arrow-head), beginning degradation of the infected tissue (arrow). 3. Fat body lobes were the surface layer is completely invaded (arrow-heads), while the central parts of the lobes are unaffected. 4. Fat cells filled with microsporidia are released when the fat body lobes are squashed. Fig. 1 SEM, 2–3 haematoxylin stain, 4 dark field. Scale bars: 1 = 1 μ m; 2–3 (with common bar on 3) = 100 nm; 4 = 10 μ m.



Figs. 5–8. Merogonial reproduction of microsporidium 1 (Figs. 6, 8) and microsporidium 2 (Figs. 5, 7). **5.** Group of merozoites (arrow-head). **6.** Periphery of a merogonial plasmodium. **7.** Binucleate merogonial plasmodium (arrows in Figs. 7–8 indicate the nucleolus). **8.** Merozoite. Scale bars: 5 = 10 µm, 6 = 100 nm; 7–8 = 0.5 µm.

Figs. 9–12. Early sporogony. **9.** Sporogonial plasmodium of microsporidium 1, partially enclosed in the primordial sporophorous vesicle (arrows indicate nucleoli). **10–12.** Periphery of the sporogonial plasmodium of microsporidium 1 (10; arrow-head indicates fibrous material), microsporidium 2 (11), and *Episeptum inversum* (12). Note that Figs. 10 and 12 are shown at the same magnification. Scale bars: 9 = 0.5 µm; 10–12 = 100 nm.





Figs. 13–23. Light microscopic aspect of the sporogony (Figs. 13–17, 22: microsporidium 1; 18–20, 23: microsporidium 2; 21: *Episeptum inversum*). **13–15.** Binucleate and tetranucleate sporongonal plasmodia. **16.** Normal spores and macrospores (arrow-head). **17.** Mature normal spores, with the holotype indicated (slide No. 850709-A-15). **18.** Four-lobed sporongonal plasmodia. **19.** Sporongonal plasmodium with 7 lobes. **20.** Mature spores with the holotype indicated (slide No. 860708-E-5). **21–23.** Living mature spores of *Episeptum inversum* (21), microsporidium 1 (22; arrow-heads point at tetrasporous groups), and microsporidium 2 (23). Figs. 13–20 haematoxylin stain, 21–22 phase contrast, 23 interference phase contrast. Scale bars: 13–16, 18–19 (with common bar on 15) = 5 µm; 17, 20 (with common bar on 17) = 10 µm; 21–23 (with common bar on 22) = 5 µm.

nial plasmodia with a few nuclei and small groups of merozoites were seen both in light and electron microscopic preparations (Figs. 5, 7). In permanent preparation merogonial stages were more intensely stained than sporogonial stages, and the dimensions were slightly greater. It is unknown if there is more than one sequence of merogonial divisions.

Merogonial plasmodia and merozoites of both species were delimited by an approximately 8 nm thick plasma membrane, a unit membrane without external reinforcements (Fig. 6). Numerous free ribosomes made the cytoplasm densely granular. There were no signs of an endoplasmic reticulum. Also the nuclei were prominently granular with dispersed isles of chromatin. Characteristically a distinct electron-dense, up to 300 nm wide, nucleolus was visible (Figs. 7–8). Nuclear envelopes were of normal type: two unit membranes separated by a perinuclear space and with pores. The widest sectioned merozoites of both species measured about 2.3 µm, the widest sectioned nuclei were 1.4 µm wide.

The last generation of merozoites matured to sporonts. Concentrical layers of endoplasmic reticulum around the nuclei of sporogonial stages, which normally are characteristic for sporulating microsporidia, were never seen. The envelope of a sporophorous vesicle and the primordium of the exospore were initiated simultaneously (Figs. 9–12). The primordial exospore grew directly to an electron-dense layered structure, covered by less dense episporal material (Figs. 10–12). The exospore primordium of microsporidium 1 (except for the episporal material) measured 13–14 nm, the primordium of microsporidium 2 was 10–11 nm thick. The construction of the primordium was almost identical in the different species.

The nucleus of the sporont divided mitotically twice to produce tetranucleate plasmodia before the sporoblasts were released by rosette-like budding (Figs. 13–15, 18). In microsporidium 2 occasionally plasmodia with more than 4 lobes were seen (Fig. 19). The transformation from sporoblast to spore (the initiation and morphogenesis of the sporal organelles) equalled the normal for microsporidia.

Spores

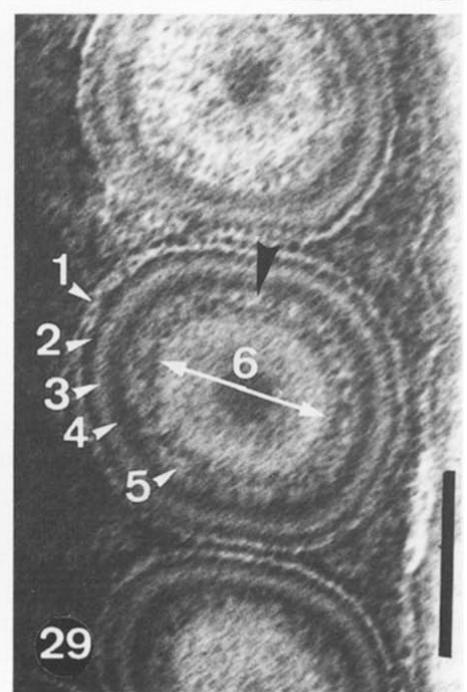
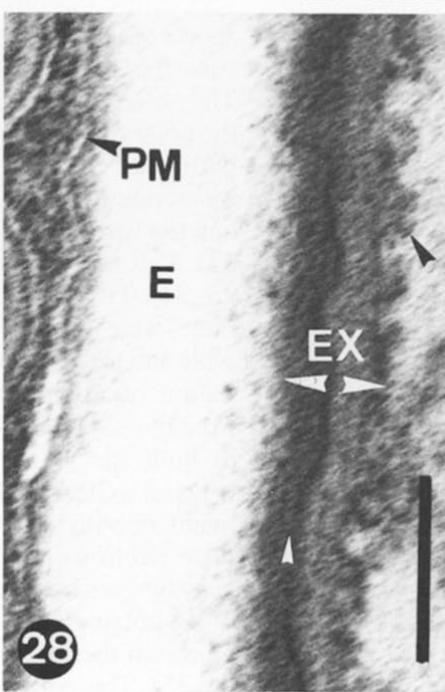
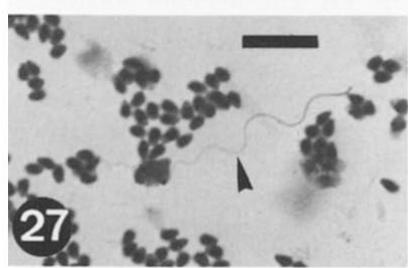
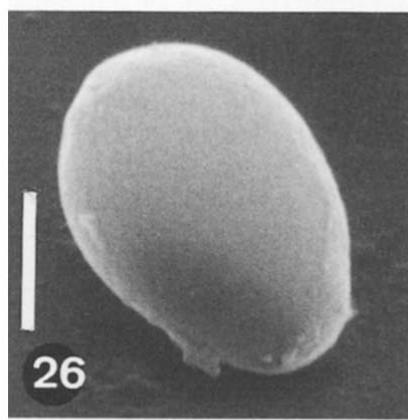
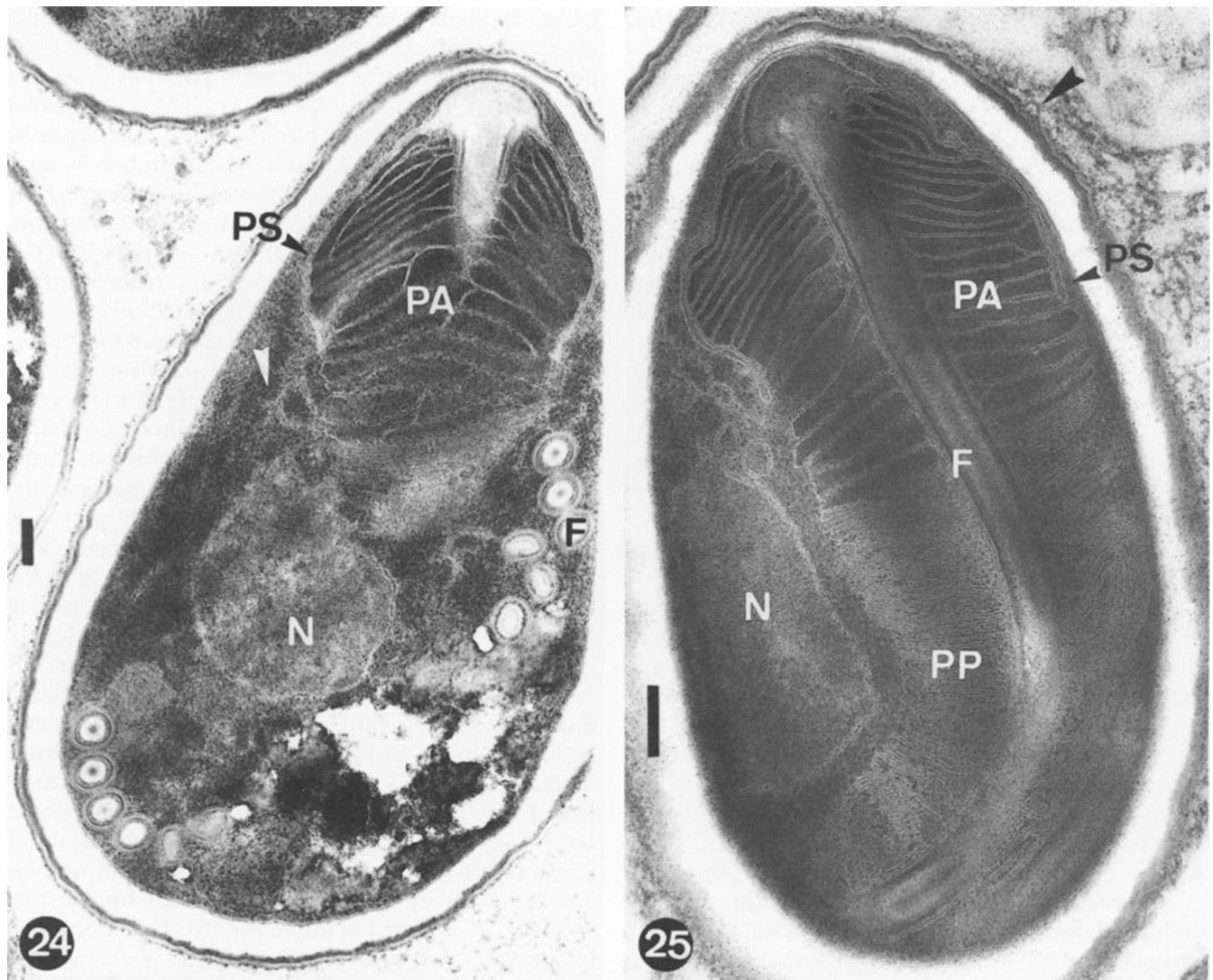
The general features, shared by both species, will be covered here, while the details, including measurements of the various structures, will be presented in the descriptive part.

Mature spores of both species were pyriform, with pointed anterior pole (Figs. 22–23, 26). Living spores exhibited a distinct vacuole in oblique position close to the posterior pole (Figs. 22, 23). The spore wall had the normal three subdivisions: the internal plasma mem-

brane, an about 8 nm thick unit membrane, a median electron lucent endospore of slightly variable thickness, prominently thinner at the anterior pole of the spores, and a layered exospore (Figs. 28, 31). The internal layer of the exospore was fairly electron dense. The border to the surface layer appeared as an about 5 nm thick double strand of electron dense material. The external zone was moderately electron dense. The surface was covered by a blister-like episporal material (Figs. 28, 31), which was the remainder of the thick foamy surface cover of the primordial exospore (Figs. 10–12).

The polar filament was anteriorly connected to a biconvex anchoring disc (Figs. 25, 30, 34–35). The filament was slightly wider close to the anchoring disc (Figs. 25, 30). The uncoiled part of the filament proceeded backwards along the central axis of the spore and, in the mid-region of the spore, turned lightly towards the spore wall (Figs. 25, 30). The posterior part was arranged in one layer of anteriorly directed coils close to the spore wall (Figs. 24, 30). For both species the angle of tilt between the most anterior coil and the long axis of the spore was 40–45°. The filament was lightly anisofilar (Figs. 24, 33). The transversely sectioned filament exhibited a distinct series of layers, in direction inwards (Figs. 29, 32: 1–6): a c. 5 nm thick unit membrane (1), followed by a 4–5 nm wide electron-dense layer (2), a slightly wider, 6–7 nm, moderately dense layer (3), a 4–6 nm dense layer (4), a moderately dense layer, 8–9 nm wide, composed of fibrous material (5). These layers had the same dimensions in both species. The variation in coil diameter recorded for the two species was confined to the centre (6). This region had only traces of stratification, and in the wide coils the very centre was electron-dense (Figs. 24, 29, 33). Some spores ejected the polar filament spontaneously when squash preparations were made (Fig. 27).

The polaroplast, which surrounded the uncoiled part of the polar filament, was divided into two regions of compartments limited by approximately 5 nm thick unit membranes (Figs. 25, 30). In microsporidium 1 the anterior part was shorter than the posterior one (Fig. 25). This region was composed of wide lamellae (Figs. 25, 34). The width and shape was fairly irregular, and there were spores where a few lamellae were short, wide and triangular in sections. The anterior polaroplast region of microsporidium 2 was composed of wide irregular chambers (Fig. 30). The posterior polaroplast of both species had closely packed narrow lamellae (Figs. 25, 30, 34). In both microsporidia the compartments of both polaroplast regions were filled with uniform electron dense material (Figs. 25, 30). There were anastomoses between the lamellae of the compartments of the polaroplast (Figs. 30, 34) and between the polaroplast and the surface layer of the polar filament (Figs. 34–35). The polar sac enclosed the anchoring appara-



tus, and proceeded backwards as a narrow fold which surrounded the anterior part of the polaroplast. In microsporidium 1 about 2/3 of the anterior polaroplast was surrounded by the polar sac (Fig. 25). The polar sac of microsporidium 2 was shorter and covered only the anterior surface of the polaroplast (Fig. 30). In both species the polar sac was filled with a moderately electron-dense material.

The nucleus was situated in the region of coils, laterally to the polaroplast, in the middle (microsporidium 1, Figs. 24–25) or in the posterior half of the spore (microsporidium 2, Fig. 30). Close to the posterior pole was a membrane-lined vacuole filled with amorphous material of varying electron density (Fig. 33). The cytoplasm was dense with polyribosomes in prominent layers around the nucleus and the polar filament-polaroplast complex (Figs. 24, 30).

The sporophorous vesicle

The sporophorous vesicle was initiated at the beginning of the sporogony simultaneously with the development of the exospore layer (Figs. 9–12). The two layers grew along a vast area, in sectioned sporonts seen along one or two sides of the sporont wall (Fig. 9). The envelope was not released in the blister-like manner most commonly seen in microsporidia. The envelope of the sporophorous vesicle was an about 6 nm thick coat of electron dense material. The episporontal space was filled with a fibrous material of approximately the same thickness, uniting the exospore with the envelope (Fig. 10). The fibres were persistent and remained also in vesicles with mature spores (Figs. 25, 30). When filled with mature spores the sporophorous vesicle formed a tight envelope around the group of spores, and sporophorous vesicles had no particular shape (Figs. 1, 17, 20, 22–23). The envelope of the vesicles was not visible in stained smears, and the presence of vesicles could only be deduced from the tetrasporous groups observed (Figs. 17, 20).

Descriptions

Microsporidium 1: *Episeptum invadens* sp. n.

Merogony: Plasmodia with a small number of nuclei and groups of uninucleate merozoites observed. The

number of merozoites per meront, and the bouts of merogony unknown.

Sporogony: Four sporoblasts are produced by rosette-like dividing plasmodia.

Spores: Pyriform with pointed anterior pole. Unfixed spores measure 2.2–2.5×3.2–3.6 µm, fixed and stained spores 1.12–1.42×1.90–2.83 µm. Macrospores, measuring approximately 3.5 µm when fixed and stained, were rarely seen. The spore wall is 128–139 nm thick, with 25–26 nm thick exospore. The polar filament is arranged in 5–7 coils in a single row of coils close to the spore wall. The angle of tilt is 40–45°. In spores sectioned longitudinally the anterior group of sectioned coils is found in the middle of the spore, the posterior group of coils close to the posterior pole (Fig. 24). The anterior part of the polar filament is arranged in 2–3, 100–128 nm wide coils; the posterior part in 3–5, 95–96 nm wide coils. Sectioned anchoring discs measured up to 260 nm wide. Polaroplast with two lamellar parts: wide (43–128 nm) lamellae (or sometimes triangular chambers) anteriorly, narrow (about 11 nm) lamellae posteriorly. Anterior part of the polaroplast slightly shorter than the posterior part, which ends close to the polar filament coils near the posterior pole. Nucleus (sectioned) up to 0.8 µm wide, in the centre of the spore.

Sporophorous vesicle: Envelope electron-dense, about 6 nm thick, and fairly persistent. Episporontal space with thin fibrous inclusions.

Host tissues involved: Fat body cells. Entire lobes are invaded, disintegration visible already in the living host. Infected cells are hypertrophic. No production of syncytia.

Type host: *Limnephilus fuscicornis* (RAMBUR, 1842) (Trichoptera, Limnephilidae), larvae.

Type locality: The river Klingavälsån, close to the village of Veberöd, Scania, Southern Sweden.

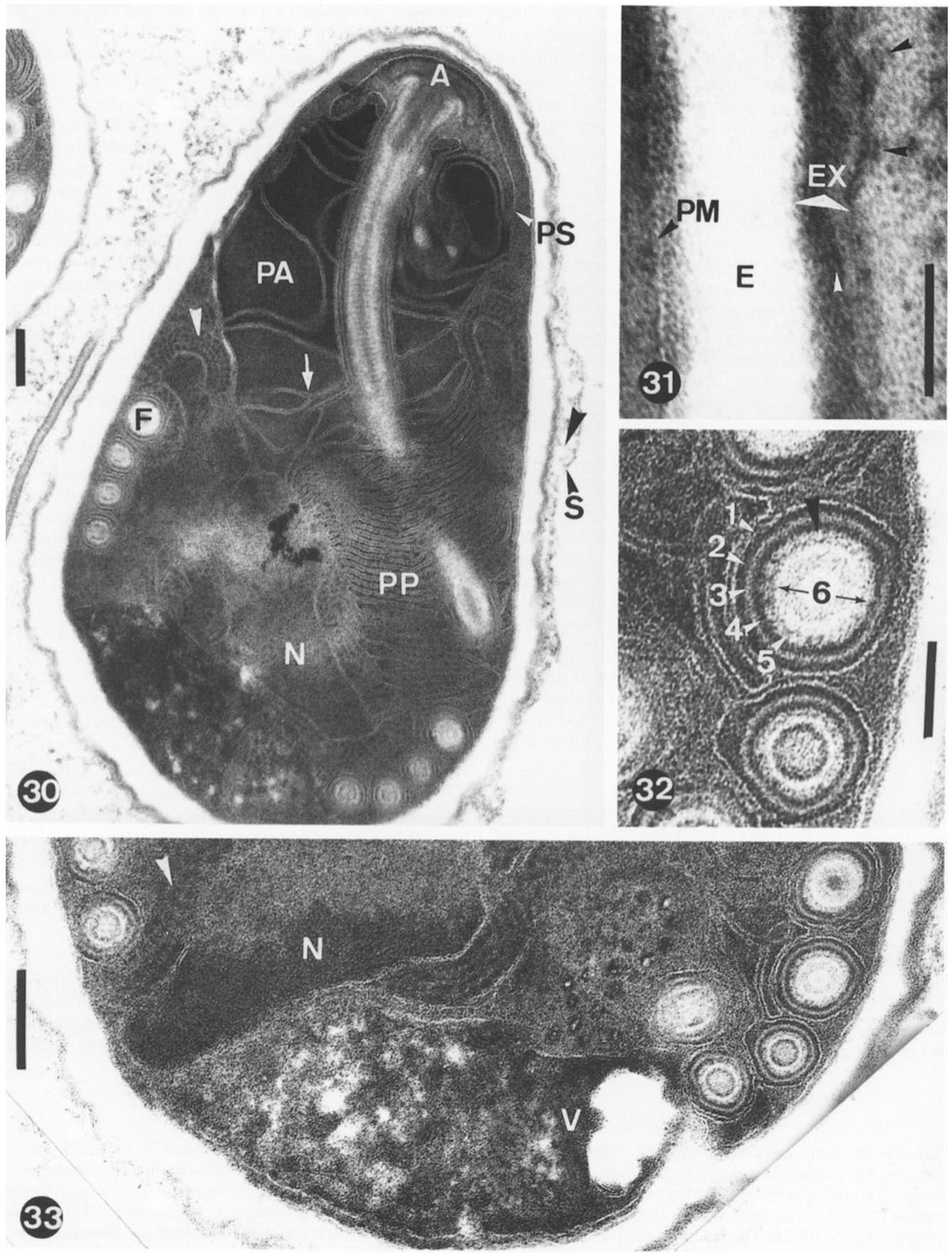
Types: Holotype (Fig. 17) on slide No. 850709-A-15, paratypes on slides No. 850709-A-(1-31).

Etymology: Species name alluding to the pathogenic action upon the host tissue.

Microsporidium 2: *Episeptum circumscriptum* sp. n.

Merogony: Plasmodia with a small number of nuclei produce uninucleate merozoites. The number of mero-

Figs. 24–29. Mature spores of microsporidium 1, *Episeptum invadens* sp. n. **24–25.** Longitudinally sectioned spores revealing the ultrastructural cytology (white arrow-head indicates polyribosomes; black arrow-head fibrous material of the episporontal space). **26.** SEM picture of a spore. **27.** An ejected polar filament (arrow-head) in a smear stained by haematoxylin. **28.** The layers of the spore wall (white arrow-head points at the double layer, black arrow-head shows the episporal material). **29.** The layers of the polar filament (numbered 1–6; black arrow-head indicates the fibrous component). Scale bars: 24–25 = 100 nm; 26 = 1 µm; 27 = 10 µm; 28–29 = 50 nm.



zoites per meront and the bouts of merogony unknown.

Sporogony: Normally four sporoblasts are formed by rosettelike budding. Rarely rosettes with up to 8 lobes were seen.

Spores: Pyriform with pointed anterior pole. Unfixed spores measure approximately $2.1 \times 2.5\text{--}3.7 \mu\text{m}$, fixed and stained spores $1.15\text{--}1.65 \times 1.92\text{--}2.37 \mu\text{m}$. Macrospores were not observed. The spore wall is up to 106 nm thick, with a 19–21 nm wide exospore. The uncoiled part of the polar filament touches the spore wall approximately in the middle of the spore. The posterior part forms 3–4 coils in a single layer. The angle of tilt is 40–45°. In spores sectioned longitudinally the anterior group of sectioned coils is found immediately below the equator of the spore, the posterior group of coils close to the posterior pole (Fig. 30). The 1–2 anterior coils are 94–102 nm wide, the posterior 2–3 coils measure 77–90 nm. Sectioned anchoring discs were up to 247 nm wide. Polaroplast with an anterior part with up to 276 nm wide chambers, and a posterior part with closely arranged, about 11 nm wide, lamellae. Anterior part of the polaroplast slightly longer than the posterior part, which ends close to the polar filament coils near the posterior pole of the spore. Nucleus (sectioned) up to 0.9 μm wide, in the posterior half of the spore.

Sporophorous vesicle: Envelope electron-dense, about 6 nm thick. Episporontal space with thin fibrous projections.

Host tissues involved: Infection restricted to the fat body, and normally only the superficial layer of cells is invaded. Infected cells are hypertrophic. No production of syncytia.

Host: *Hydropsyche siltalai* DÖHLER, 1965 (Trichoptera, Hydropsychidae), larvae.

Type locality: The river Klingavälsån, close to the village of Veberöd, Scania, Southern Sweden.

Types: Holotype on slide No. 860708-E-5 (Fig. 20), paratypes on slides No. 860708-E-(1-28).

Etymology: Species name alluding to the characteristic utilization of the fat body lobes, invading only the superficial layer of cells.

Deposition of types: The slides with the holotypes in the International Protozoan Type Slide Collection at Smithsonian Institution, Washington, DC. Paratypes in the collections of Dr. JAROSLAV WEISER, Charles University, Prague, Czech Republic, and in the collection of the author.

Discussion

When viewed under the light microscope the two species described herein are strikingly similar to *Episeptum inversum*, the type species and so far the only species of the genus (Figs. 21–23). However, the spores of microsporidium 1 (Fig. 22) are slightly greater than the spores of *E. inversum* (Fig. 21), and the spores of microsporidium 2 (Fig. 23) look more slender. If the comparison is extended also to include ultrastructural characters, it becomes obvious that the species differ in a number of characteristics, for example the dimensions of the primordia of the sporont wall, the episporal layer of mature spores, the anterior polaroplast region, and in numerical characters of the polar filament (Table 1). These distinct cytological differences between the two microsporidia, and between them and *E. inversum*, and the different species of caddis fly larvae used as hosts, clearly show that they are distinct species.

Hydropsyche siltalai is previously known to host a tetrasporoblastic microsporidium: *Pyrotheca hydropsycheae* (see XIE & CANNING 1986). The spores of this species are nearly three times longer than the spores of the *Episeptum* species, the shape is different, and they exhibit different cytology. The distinction between *P. hydropsycheae* and the *Episeptum* species is clear, and the cytology tells that they cannot be congeneric.

The taxonomy of the tetrasporoblastic microsporidia will remain problematic until a new investigation reveals the characteristics of *Gurleya tetraspora*, the type species of the oldest tetrasporous genus (DOFLEIN 1898). *Gurleya* is normally used as a temporary repository for tetrasporoblastic microsporidia, sporulating in sporophorous vesicles, and exhibiting a cytology of the normal type for microsporidia. The caddis fly parasite *Gurleya dorisae* was handled according to this principle (LARSSON 1995). When the unique cytology of *Episeptum inversum* was revealed, *Gurleya*, as it was characterized at that time and still is, was not a suitable genus. There appeared to be no alternative to establish a new genus (LARSSON 1986).

The two new species described herein are clearly not microsporidia exhibiting an ultrastructure of the most common type for microsporidia. *Gurleya* is not a suitable genus for them. *Episeptum* is a possible alternative, but there are two divergences from the type species, *E. inversum*.

Figs. 30–33. Mature spores of microsporidium 2, *Episeptum circumscripum* sp. n. **30.** Longitudinally sectioned spore (white arrow-head indicates polyribosomes; black arrow-head fibrous material; arrow anastomoses between polaroplast membranes). **31.** The layers of the spore wall (white arrow-head points at the double-layer, black arrow-head shows the episporal material). **32.** The layers of the polar filament (numbered 1–6; black arrow-head indicates the fibrous component). **33.** Posterior region of the spore, revealing the posterior vacuole. Scale bars: 30, 33 = 100 nm; 31–32 = 50 nm.

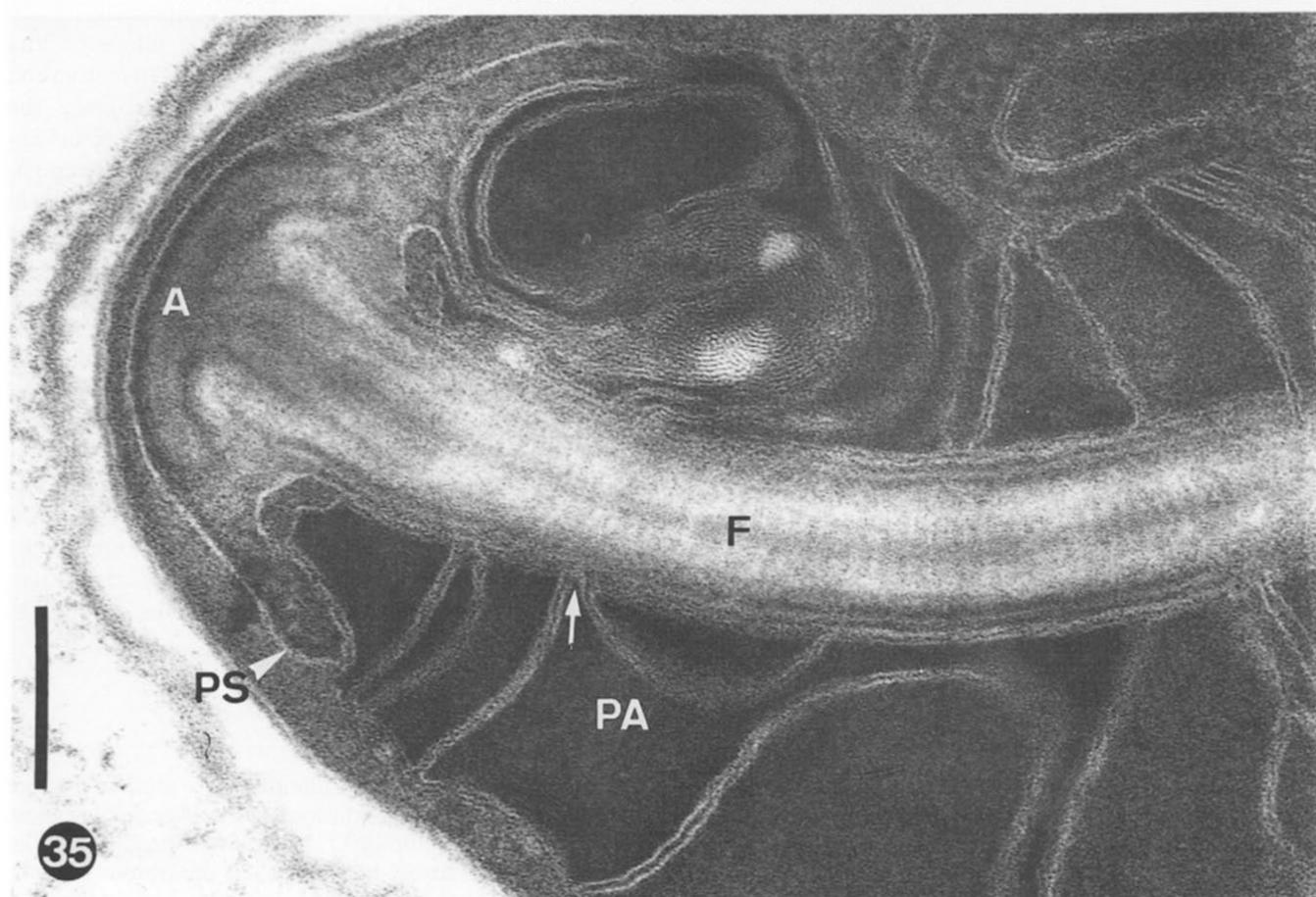
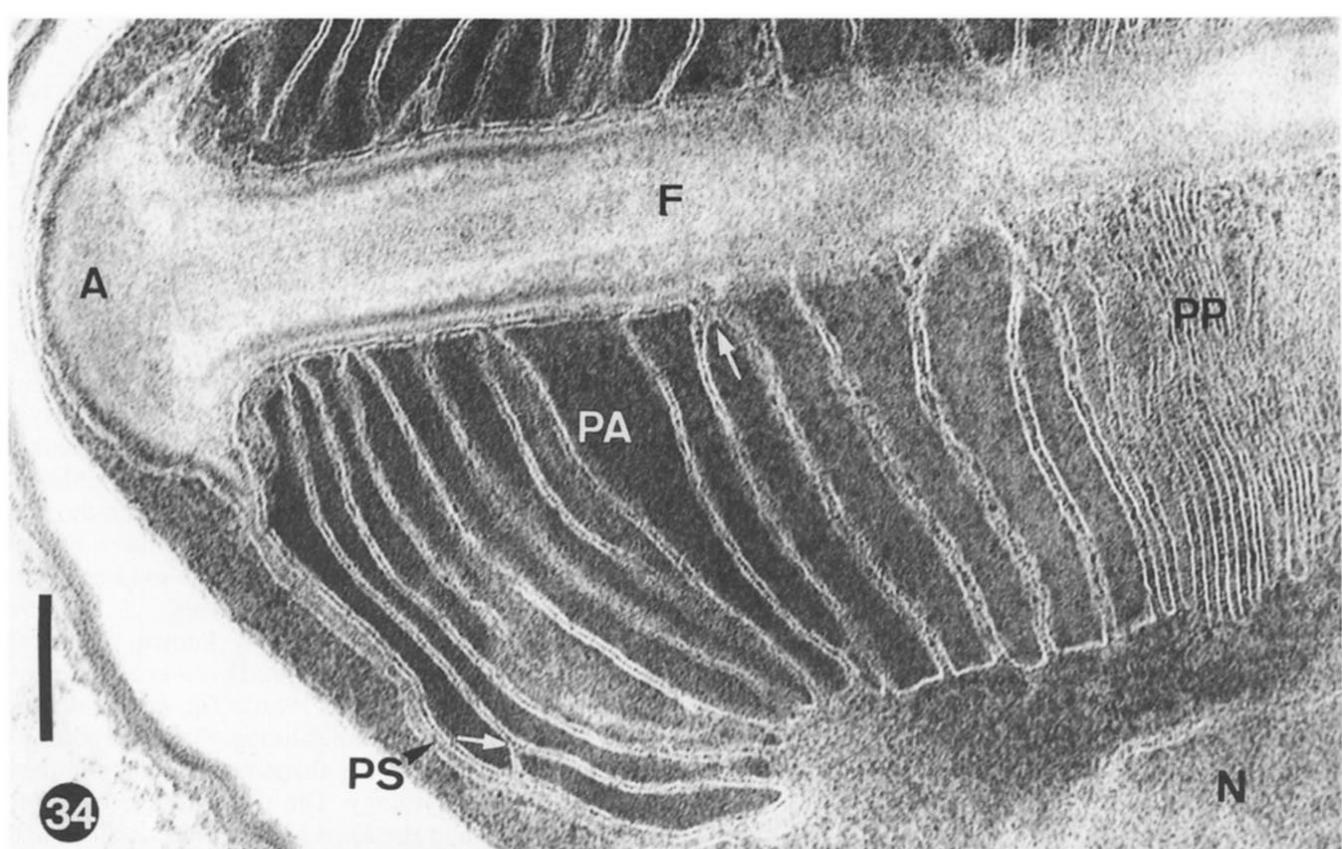


Table 1. Distinctive characters of the three *Episeptum* species.

	<i>Episeptum inversum</i> LARSSON, 1985	<i>Episeptum invadens</i> sp. n.	<i>Episeptum circumscripum</i> sp. n.
Spore size, unfixed (μm)	c. $2.1 \times 2.5\text{--}3.2^1)$	$2.2\text{--}2.5 \times 3.2\text{--}3.6$	$c. 2.1 \times 2.5\text{--}3.7$
Spore size (fixed) (μm)	$1.28\text{--}1.77 \times 1.98\text{--}2.45^2)$	$1.12\text{--}1.42 \times 1.90\text{--}2.83$	$1.15\text{--}1.65 \times 1.92\text{--}2.37$
Macrospores	not observed	rare	not observed
Primordium of sporont wall (plasma membrane and episporal layer excluded) (nm)	$28\text{--}30^2)$	13–14	10–11
Exospore (episporal layer excluded) (nm)	24–27 ²⁾	25–26	19–21
Episporal layer	wide, chambered	thin, irregular	thin, irregular
Polar filament coils:			
total number	5–6	5–7	4–5
wide (nm)	3 (130–140)	2–3 (100–128)	1–2 (94–102)
narrow (nm)	2–3 (108–113)	3–5 (95–96)	2–3 (77–90)
Anterior polaroplast region	wide lamellae	wide lamellae (chambers)	chambers
Type host	<i>Holocentropus picicornis</i> (Polycentropodidae)	<i>Limnephilus fuscicornis</i> (Limnophilidae)	<i>Hydropsyche siltalai</i> (Hydropsychidae)
Habitat	pond	stream	stream

¹⁾ Taken from LARSSON (1986). ²⁾ New measurement made on type material.

The episporal coat of *E. inversum* remains unchanged from the initiation (Fig. 12), persisting as a thick cover on the mature spore (LARSSON 1986). The mature spores of the two species described herein are only covered by a thin layer of episporal material (Figs. 28, 31). However, the initiation of the layer is of the *Episeptum* type (Figs. 10–12).

The second difference is found in the polaroplast. In *E. inversum* the polaroplast has wide lamellae anteriorly, narrow lamellae posteriorly (LARSSON 1986). Microsporidium 1 (*E. invadens* sp. n.) exhibits a polaroplast of this type (Fig. 34), even if a few wide chambers occasionally might be present in the anterior part of the polaroplast. The anterior polaroplast region of microsporidium 2 (*E. circumscripum* sp. n.) is different: the compartments are not lamellae but irregular chambers (Fig. 35). If we scrutinize the species belonging to well characterized microsporidian genera, we are not likely to find great differences in the construction of

the polaroplast. However, in this particular case we are facing a variation in the development of the polaroplast, with *E. inversum* and microsporidium 2 as the opposite poles, and microsporidium 1 exhibiting the intermediate condition.

Gurleya is excluded for the new microsporidia, and the choice is to rank in *Episeptum* or to establish a new genus. Even if the two species express a cytology that is not in accordance with the cytology of *E. inversum* in all minute details, nevertheless a classification in *Episeptum* appears to be the best choice.

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Figs. 34–35. The longitudinally sectioned polaroplast of microsporidium 1 (34) and microsporidium 2 (35) (anastomoses between membranes of the polaroplast and polar filament indicated by arrows). Scale bars: 100 nm.

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