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## On the Cytology and Taxonomic Position of *Nudispora biformis* N. G., N. Sp. (Microspora, Thelohaniidae), a Microsporidian Parasite of the Dragon Fly *Coenagrion hastulatum* in Sweden

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**ABSTRACT.** The microsporidium *Nudispora biformis* n. g., n. sp., a parasite of a larva of the damsel fly *Coenagrion hastulatum* in Sweden, is described based on light microscopic and ultrastructural characteristics. Merogonial stages and sporonts are diplokaryotic. Sporogony comprises meiotic and mitotic divisions, and finally eight monokaryotic sporoblasts are released from a lobed plasmodium. Sporophorous vesicles are not formed. The monokaryotic spores are oval, measuring 1.4-1.8 × 2.8-3.4 µm in living condition. The thick spore wall has a layered exospore, with a median double-layer. The polaroplast has two lamellar parts, with the closest packed lamellae anteriorly. The isofilar polar filament is arranged in 6 (to 7) coils in the posterior half of the spore. Laminar and tubular extracellular material of exospore construction is present in the proximity of sporogonial stages. In addition to normal spores teratological spores are produced. The microsporidium is compared to the microsporidia of the Odonata; its possible relations to the genus *Pseudothelohania* and to the *Thelohania*-like microsporidia are discussed. The new genus is provisionally included in the family Thelohaniidae.

**Key words.** *Pseudothelohania*, ultrastructure.

IN the summer of 1988 a diseased larva of the damsel fly *Coenagrion hastulatum* was given to me by my colleague Dr. Ulf Norling, Lund. The agent was a microsporidium, which in fresh smears appeared to have ungrouped spores. Further investigations made it clear that the species exhibited octosporoblastic sporogony, and the cytology was basically of the *Thelohania*-type. It differed only in one respect: sporophorous vesicles (pansporoblasts) were not formed. As the presence of persistent or subpersistent sporophorous vesicles is a crucial character for microsporidia of the family Thelohaniidae Hazard & Oldacre, 1975, the microsporidium could not be accommodated in an established genus of the family [2]. However, it might be related to the microsporidium for which the genus *Pseudothelohania* Codreanu & Codreanu-Balcescu, 1982 (*J. Protozool.*, **29**:301) was created. This genus was not placed in a family, and to my knowledge it was never described in the manner required by the International Code of Zoological Nomenclature [3].

The microsporidium, which is considered new to science, is briefly described. The taxonomic position is discussed, and a new genus is created.

### MATERIALS AND METHODS

A single infected larva was present in a sample of the damsel fly *Coenagrion hastulatum*, collected in a bog at Bökeberg, in southern Sweden, by Dr. Ulf Norling, University of Lund, on August 2, 1988.

Fresh squash preparations were made by the agar method of

Hostounský & Zížka (*J. Protozool.*, **26**:41A-42A), and studied using phase contrast microscopy and dark field illumination.

Permanent squash preparations were lightly air-dried and fixed in Bouin-Duboscq-Brasil solution overnight. For paraffin sectioning a part of the body was fixed in the same fixative overnight, washed and dehydrated in an ascending series of ethanol, cleared in butanol, and embedded in paraplast. Sections were cut longitudinally at 10 µm. Squash preparations and sections were stained using Giemsa solution and Heidenhain's iron haematoxylin. For details on the histological techniques used see the manual by Romeis [13]. All permanent preparations were mounted in DePeX. Measurements were made with an eye-piece micrometer at ×1,000.

For transmission electron microscopy infected segments were excised and fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4° C for 8 and 96 h. After washing in cacodylate buffer and post fixation in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4° C, the pieces were washed and dehydrated in an ascending series of buffer-acetone solutions to absolute acetone, and embedded in epon. Sections were stained with uranyl acetate and lead citrate.

### RESULTS

**Pathology.** The infected larva was recognized from an anomalous white colour of the entire body, including the head. The fat body was almost completely disintegrated (Fig. 1). The basal membrane of the lobes formed sacs, where parasite cells were floating among organelles of the host cells. In addition the hy-

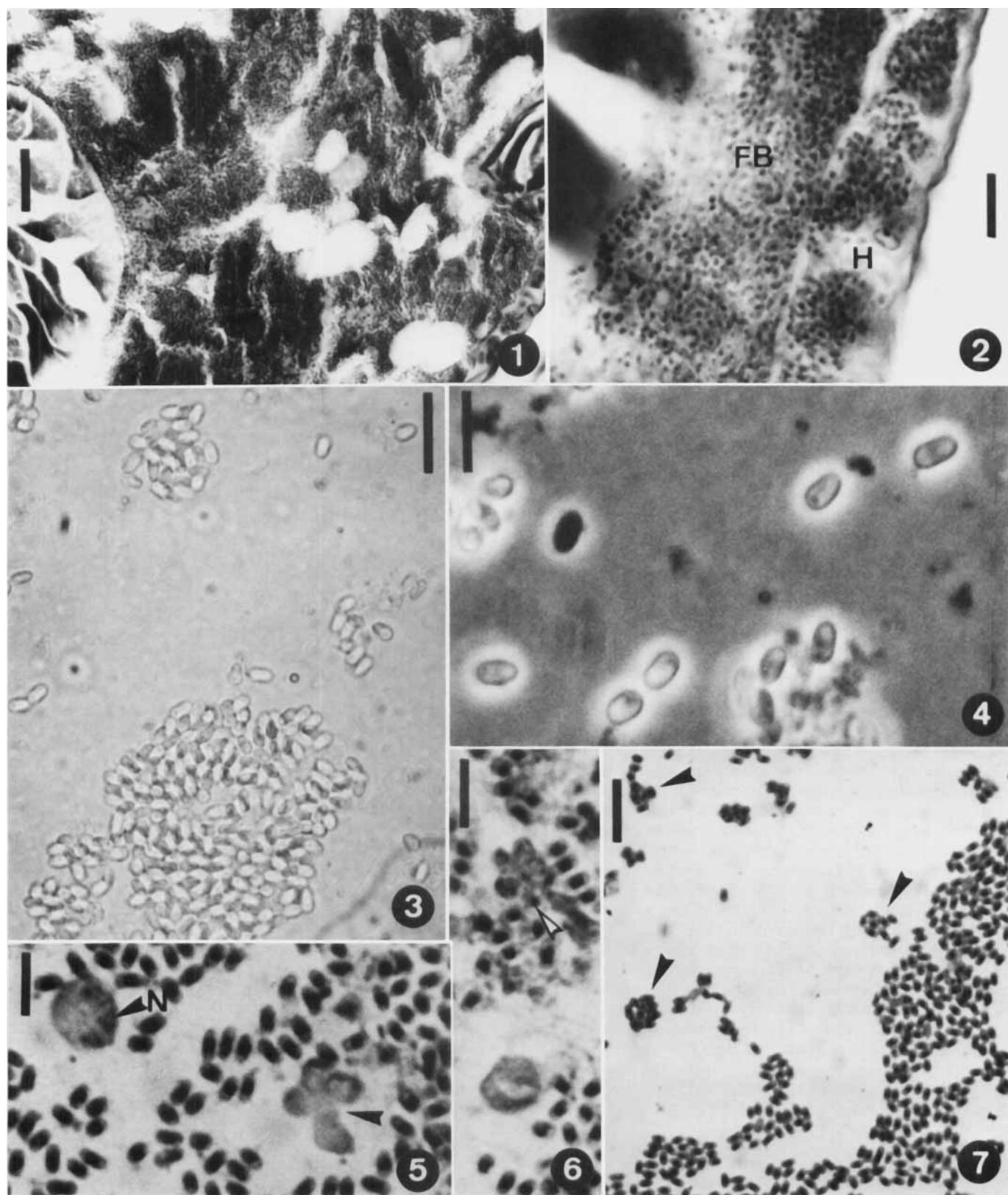


Fig. 1-7. Light microscopic appearance of *Nudispora biformis*. 1. Fat body of *Coenagrion hastulatum* completely filled with spores. Scale bar = 25  $\mu\text{m}$ . 2. The infected hypoderm (H) remains cellular, while the fat body (FB) disintegrates to a syncytium. Scale bar = 10  $\mu\text{m}$ . 3. Aggregations of living spores. Scale bar = 10  $\mu\text{m}$ . 4. Living spores, the lucent halo suggests the presence of gelatinous material. Scale bar = 5  $\mu\text{m}$ . 5. Undivided and lobed (arrowhead) sporogonial plasmodium with isolated nuclei (N), and mature spores. Scale bar = 5  $\mu\text{m}$ . 6. Sporogonial plasmodium (arrowhead) with seven lobes visible. Scale bar = 5  $\mu\text{m}$ . 7. Mature spores, arrowheads indicate octosporous groups. Scale bar = 10  $\mu\text{m}$ . 1-2, 5-7. Heidenhain's haematoxylin.

poderm was infected, but this tissue retained its cellular nature (Fig. 2). Nuclei of host cells were only lightly hypertrophic. Younger and older developmental stages of the microsporidium were mingled.

**Presporal stages and life cycle.** Sporogonial stages were dominant in squash preparations (Fig. 3-7). The interpretation of the merogony is based on ultrathin sections. Merogonial plasmodia of the last generation, with diplokaryotically arranged nuclei, was the earliest developmental stage observed (Fig. 8). Sectioned diplokarya measured up to 3.9  $\mu\text{m}$  in diameter. The wall of the plasmodium was a ca. 8 nm thick unit membrane. The plasmodia divided into diplokaryotic merozoites (Fig. 9). The bouts of merogony are unknown. The last generation of merozoites matured to sporonts, and external patches of electron-dense material appeared on the plasma membrane (Fig. 10). Initially ca. 25 nm thick and uniform, the patches rapidly differentiated into three-layered structures (Fig. 12). Their thickness increased to ca. 28 nm, and they successively became confluent to a nearly continuous cover on the plasma membrane (Fig. 11). The two external layers, a moderately dense layer of half the thickness of the cover, and a very dense, considerably thinner layer, were the primordia of the exospore. They were separated from the plasma membrane by a zone of translucent material, the future endospore (Fig. 12). Exospore material was also present in the proximity of the developing sporonts, visible either as perpendicular projections from the sporont wall or as more or less parallel layers in the host cell cytoplasm (Fig. 11, 12).

The late merogonial and early sporogonial stages had a uniform, granular cytoplasm with numerous free ribosomes (Fig. 8, 11). In the cytoplasm of merogonial and sporogonial plasmodia arrays of ca. 18 nm wide microtubules were present (Fig. 8, 13). In the sporogonial stages, with more complete thick sporont wall, ribosomes were to a greater extent associated with membranes to an endoplasmic reticulum.

At the beginning of the sporogony the two components of the diplokaryon moved apart. The 1st division was meiotic, revealed by 102-117 nm wide synaptonemal complexes, with distinct central and lateral elements (Fig. 10). It was followed by mitoses, resulting in four- and eight-nucleated, lobed plasmodia (Fig. 5, 6, 13, 15). Nuclei of four-nucleated plasmodia exhibited up to 156 nm wide, electron-dense centriolar plaques in depressions of the membranes of the nuclei, and a system of radiating, intranuclear, ca. 16 nm wide, mitotic spindle tubules (Fig. 13, 14). Plasmodia with more than eight lobes were not observed. Even the multilobed plasmodia had areas where the thick surface coat was discontinuous (Fig. 13, 15).

Sporoblasts and immature spores were grouped in sections. The configurations varied, but more than eight cells at the same stage of development were never closely associated (Fig. 16). The maturation of the spore followed the normal pattern for microsporidia: the electron-density of the cytoplasm increased, the polar filament was generated from Golgi vesicles in the posterior half of the sporoblast, and finally the electron lucent endospore layer increased in thickness (Fig. 16). The primordium of the anchoring disc reached the anterior pole at the two

filament coils stage (Fig. 16). The sporoblasts were neither joined by a sporophorous vesicle, nor by a parasitophorous vacuole.

**The mature spore.** Mature spores were ovoid with blunt ends (Fig. 3, 4). Unfixed spores measured 1.4-1.8  $\times$  2.8-3.4  $\mu\text{m}$ , fixed and stained spores ca. 1.1  $\times$  1.9-2.3  $\mu\text{m}$  (measurements based on 25 spores). A lucent area around living spores suggested production of gelatinous material (Fig. 4). Stained spores had a dark centre and paler poles (Fig. 5-7). Spore groups with regular numbers were rare in squash preparations, but distinct octosporous groups were seen in peripheral, less dense positions (Fig. 7).

The spore wall was 119-170 nm thick, except for the anterior end where dimensions down to 57 nm were measured (Fig. 18). The wall had the normal three components: an internal ca. 8 nm thick plasma membrane; a structure-less endospore of variable thickness; and a 20-25 nm thick three-layered exospore, where a moderately dense substance was divided by a double-layer with more translucent centre (Fig. 22). In practically all spores the spore wall was deformed in certain areas, where the plasma membrane had broken away from the cytoplasm.

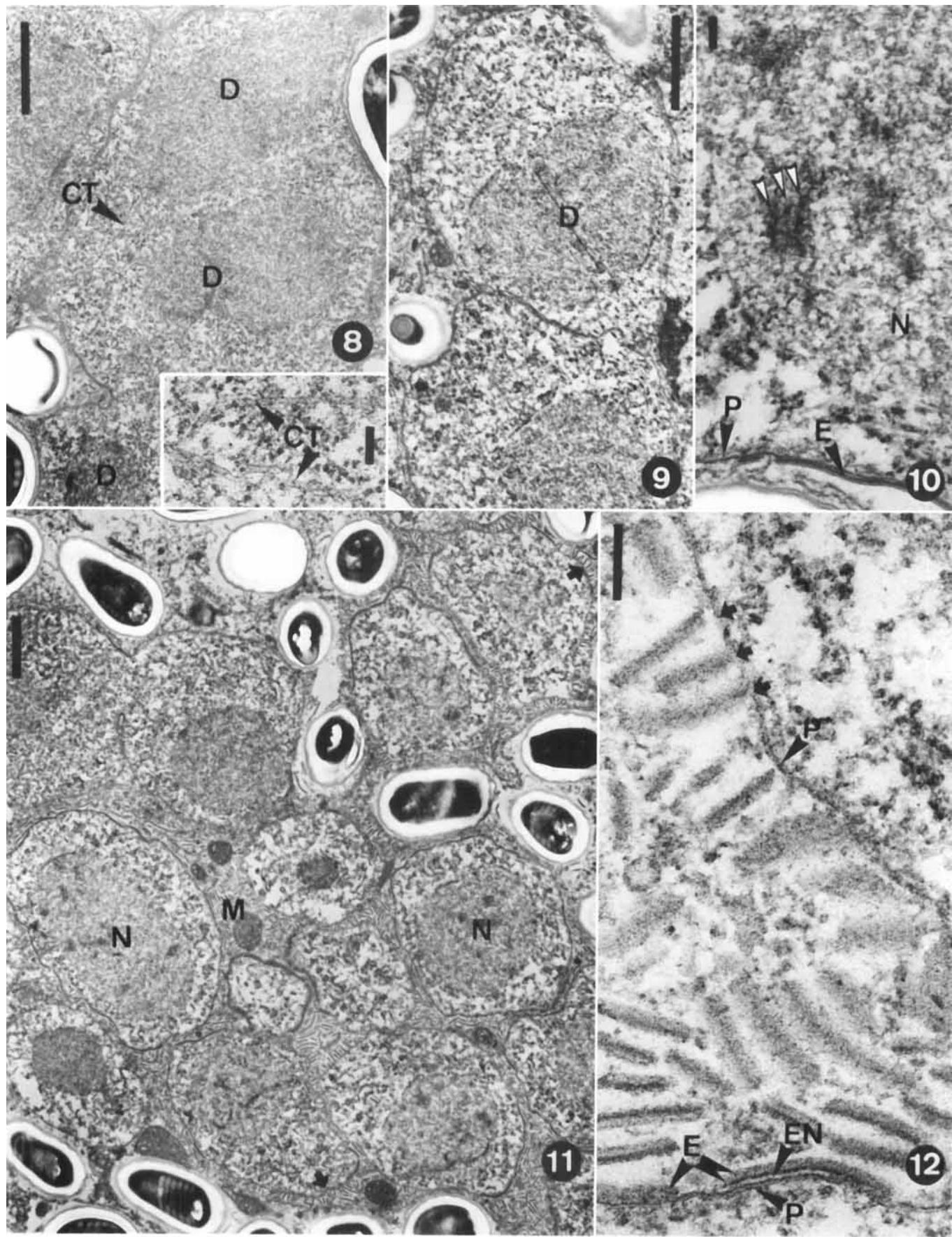
The polar filament was isofilar with 6 (to 7), 85.0-96.4 nm wide coils in a single layer close to the spore wall (Fig. 18). Neighbouring the up to 202 nm wide anchoring disc was a short, distinct, up to 135 nm wide attachment section (Fig. 17). From this point the filament tapered regularly towards the anterior filament coil in the mid-region of the spore. The angle of tilt of the anterior filament coil to the long axis of the spore was ca. 60°. The filament was constructed in the usual way, and transversely sectioned coils exhibited concentrical layers of variable thickness and electron density (Fig. 18). An external ca. 5 nm thick unit membrane was, with direction inwards, followed by a moderately dense layer of approximately the same thickness, a slightly wider translucent layer of fibrous texture, an indistinct zone of moderately dense material, and a fairly lucent centre with diffuse banding.

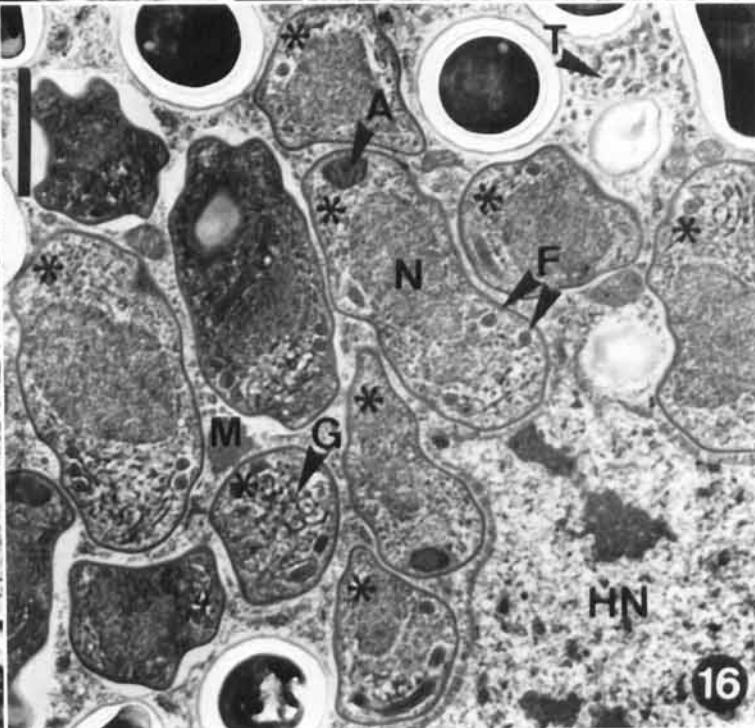
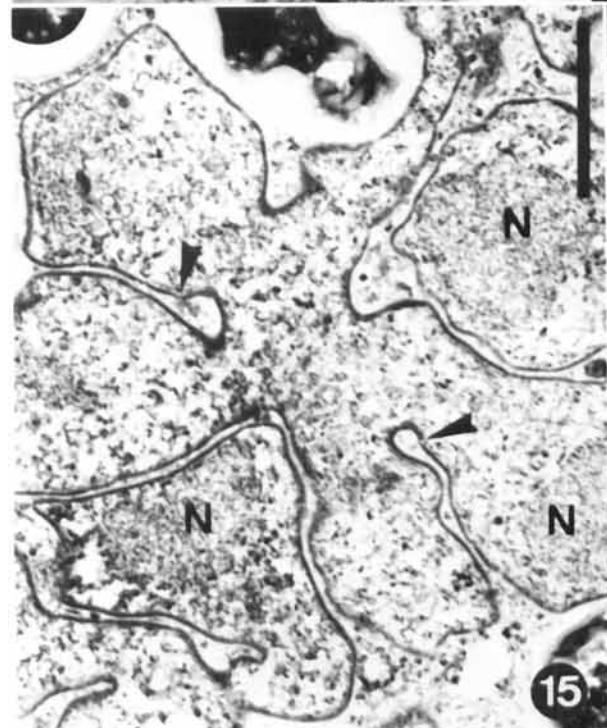
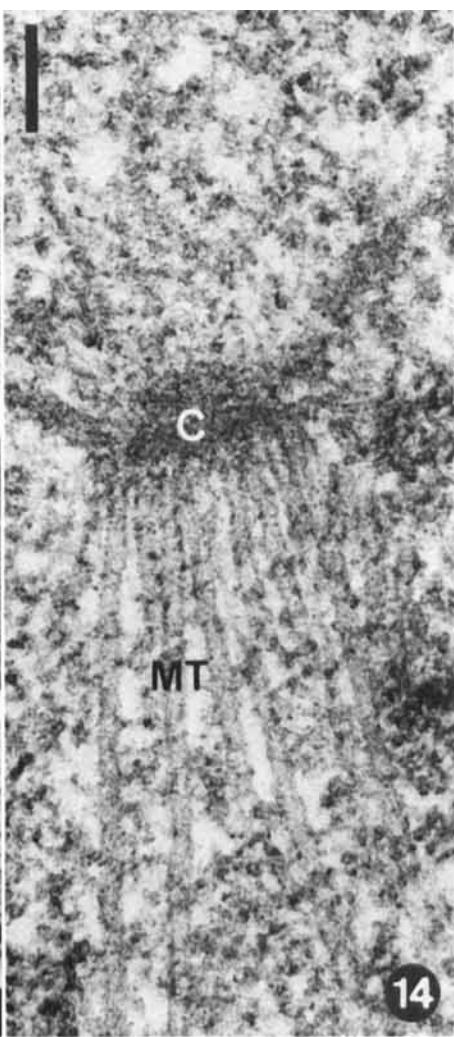
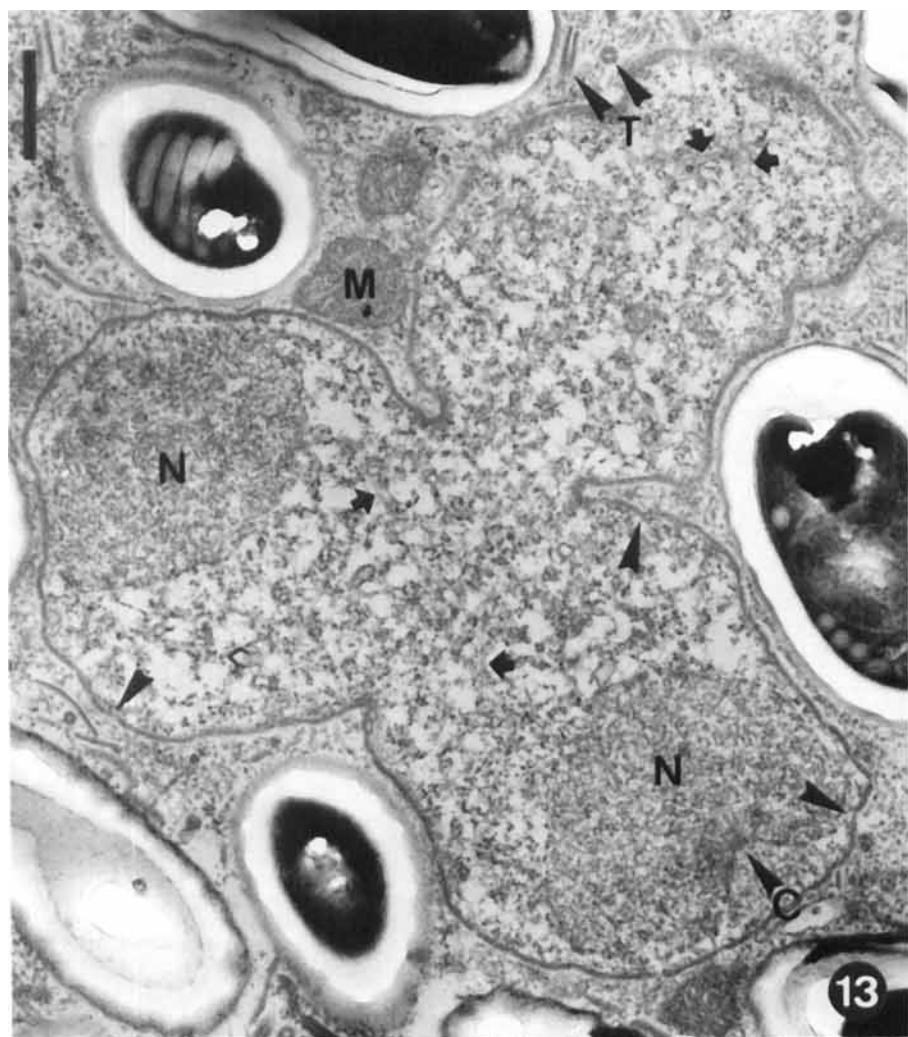
The polaroplast had two regions with regularly arranged lamellae, delimited by ca. 5 nm thick unit membranes, identical to the cover of the polar filament (Fig. 17). The lamellae were folded in concentrical layers around the polar filament. In the anterior region the lamellae were so closely packed that they apparently lacked a lumen. This part was attached to the polar filament for 85-100 nm, and the folds extended backwards for about one third of the spore length (Fig. 18). The 27-54 nm wide lamellae of the posterior part were associated with the filament to the level of the anterior coil. The fairly short polar sac enclosed approximately the anterior half of the narrow polaroplast in an umbrella-like manner.

The dense cytoplasm contained numerous, but not always distinctly membrane-associated, ribosomes. The single nucleus in the centre of the spore was delimited by double unit membranes (Fig. 18). The widest section nucleus measured 837 nm. The up to 567 nm wide, distinctly membrane-lined, vacuole at the posterior pole was filled with a dense heterogeneous material.

Most of the linear exospore material, abundant in the proximity of sporonts (Fig. 12), disappeared during sporoblastogenesis (Fig. 16). A 2nd type of structure, 60-74 nm wide tubules

Fig. 8-12. Merogony and sporonts. 8. Merogonial plasmodium with diplokarya (D) and with tubular structures (CT) in the cytoplasm. Scale bar = 0.5  $\mu\text{m}$ . Inset shows more magnified tubules. Scale bar = 100 nm. 9. Merozoites with diplokarya (D) and a thin plasma membrane. Scale bar = 1  $\mu\text{m}$ . 10. The periphery of a sporont nucleus (N), arrowheads indicate the central and lateral elements of a synaptonemal complex; exospore material (E) is produced on the plasma membrane (P). Scale bar = 100 nm. 11. Sporonts with almost complete thick cell wall; arrows indicate strands of projecting exospore material. M = host cell mitochondria, N = sporont nucleus. Scale bar = 1  $\mu\text{m}$ . 12. The periphery of two sporonts at higher magnification; exospore (E) and endospore (EN) material are visible outside the plasma membrane (P); exospore projections indicated by arrows. Scale bar = 100 nm.





with a wall displaying the layers of the exospore, was associated with spores (Fig. 13, 23). Whether they originated from surplus material from the sporoblastogenesis or were formed by reorganization of the linear material associated with sporonts is unclear.

Groups of teratological spores were present at the periphery of sectioned host cells and also observed in stained squash preparations (Fig. 19, 20). They were approximately 10% greater than normal spores and had distinctly broader shape. In smears they could be erroneously taken for emptied spores. However, the wall of ultrathinly sectioned macrospores was never ruptured, and the interior was not empty, but filled with a cytoplasm-like substance, containing ribosomes. They had an identical exospore to normal spores, even if the layers were slightly less distinct, a thinner endospore, and lacked the plasma membrane. The cytoplasm-like material was traversed by more or less regularly arranged strands of exospore construction. At the broadest pole of the spore was a membrane-lined compartment with denser texture, containing another membrane-lined structure (Fig. 21). This structure might be a deformed sporoplasm: the distribution of dense and translucent material in the internal compartment suggested that it might be a nucleus.

## DISCUSSION

**Cytology.** Cytoplasmic microtubules are a characteristic component in the cytoplasm of certain groups of protozoa, like flagellates of the order Trichomonadida and the Heliocozida, but there are few observations from microsporidia. Ishihara found cytoplasmic microtubules in merogonial stages of *Nosema bombycis* [4], and Vivier & Schrevel observed 20–25 nm wide cytoplasmic tubules in the vegetative development of *Metchnikovella hovassei* [14]. These microtubules resemble those of the present species (Fig. 8) and are arranged in a similar way. Their function is unknown.

In a number of microsporidia mature spores have plurilayered exospores, where one of the layers is a double-layer, resembling a unit membrane. When initiated the layer is uniformly electron dense, but it is reorganized during the maturation of the spore. It is probably not a unit membrane. The double-layer was originally observed in microsporidia of *Theholohania* [8] and related genera, like *Toxoglugea* [6], *Cylindrospora* [9] and *Systenostrema* [10], and it was believed to be characteristic for the *Theholohania*-like microsporidia [8]. However, later investigations have revealed similar exospore double-layers in microsporidia, like *Cougourdella polycentropi* [11], which at the present time are not considered to be closely related to the *Theholohania*-like microsporidia.

**Microsporidia of the Odonata.** Ten species of microsporidia have been described from larvae of dragonflies. Five of them have octosporous sporogony in sporophorous vesicles: *Resiemeira odonatae* [9], *Systenostrema alba* [10], *Systenostrema canadensis* [10], *Theholohania limbata* [12], and *Toxoglugea tillargi* [5]. Two of these species have been identified to genus by light microscopic characters alone, but there are no obvious reasons to doubt that they at least belong in the family Thelohaniidae.

*Gurleya aeschneae* [1] has tetrasporoblastic sporogony in sporophorous vesicles. These species are clearly different from the species described here.

Three species have ungrouped spores when viewed under the light microscope. *Nosema limbata* [12], and the species originally described under the name *Perezia aeschneae* [1] are diplokaryotic in all stages of development, including the spores. *Tuzetia verdandiae* [7] has been investigated using electron microscopy, and it has been verified that the spores possess the individual sporophorous vesicles characteristic of *Tuzetia*. Also these species are different from the one treated here.

The last species was described under the name *Plistophora calopterygis* [15], but was later transferred to the genus *Stempellia* by the describer [16]. The microsporidium produced spores of two types:  $1.7 \times 3.5 \mu\text{m}$  great microspores, and  $3-3.5 \times 5-6 \mu\text{m}$  large macrospores. The macrospores were more intensely stained and gave the impression of degenerated stages [16]. The sporogony yielded a variable number of sporoblasts in sporophorous vesicles. All developmental stages had isolated nuclei. If the dimensions refer to living spores, the microspores of *P. calopterygis* are of similar size to normal spores of the present species, but the macrospores are considerably greater. If stained spores were measured, which seems most probable, the Swedish microsporidium is much smaller. Further the aberrant spores of the two microsporidia stain differently, and the aberrant spores of the microsporidium treated herein hardly take stain at all (Fig. 21). *Plistophora calopterygis* was never investigated using electron microscopy. Even if the cytology of *P. calopterygis* and the Swedish microsporidium could not be compared in detail, the obvious differences are great enough to indicate that they are different species.

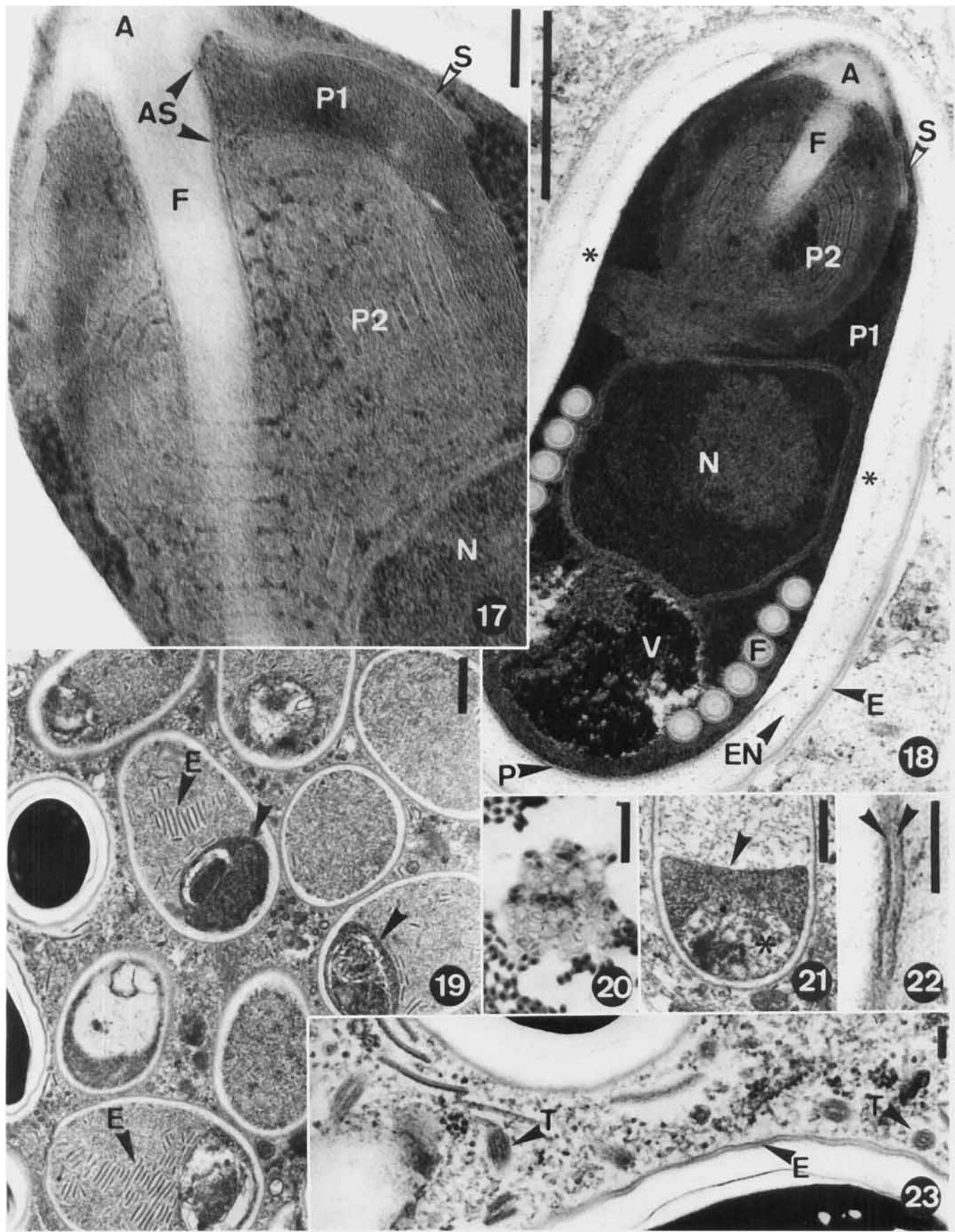
**The genus *Pseudothelohania*.** In 1982 Codreanu & Codreanu-Balcescu (*J. Protozool.*, 29:301) established the new genus *Pseudothelohania* for an octosporoblastic microsporidium of blackfly larvae. The light and electron microscopic cytology, the characteristics of the nuclei, and the life cycle stages were all of the *Theholohania*-type, but sporophorous vesicles were not formed. The new genus was mentioned at a congress and only published as an abstract. To my knowledge no detailed and valid description has appeared. No type species was selected, no other species was indicated to belong in the genus, and the host and type locality were not specified further than "Les larves de *Simulium des Carpates Méridionales . . .*" According to Article 13 (b) of the International Code of Zoological Nomenclature [3], a new genus name published after 1930 must be accompanied by the fixation of a type species. Consequently this is not a valid description, and the name *Pseudothelohania* is not available.

The similarities between the unnamed *Pseudothelohania* species and the microsporidium of *Coenagrion hastulatum* are obvious, although size and host differences clearly show that they are different species. It is probable, although not proven, that they belong in the same genus.

**The family Thelohaniidae.** The great and diverse family Thelohaniidae Hazard & Oldacre, 1975 contains microsporidia with a life cycle comprising octosporous sporogony in sporophorous

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Fig. 13–16. Sporogony. 13. Four-lobed sporogonial plasmodium with three lobes visible, a centriolar plaque (C) is present in a depression of one nucleus (N); arrowheads show positions where the thick sporont wall is incomplete, arrows indicate cytoplasmic microtubules. M = mitochondrion of the host cell, T = tubules of exospore material in the proximity of mature spores. Scale bar = 0.5  $\mu\text{m}$ . 14. An electron-dense centriolar plaque (C) with radiating intranuclear mitotic spindle tubules (MT). Scale bar = 100 nm. 15. Eight-lobed sporogonial plasmodium with five lobes visible, arrowheads indicate positions with still incomplete sporont wall. N = nucleus. Scale bar = 1  $\mu\text{m}$ . 16. Sporoblasts of two different ages close to a host nucleus (HN), \* indicates eight sporoblasts of the same age, possibly the daughter cells of one sporont. The primordium of the anchoring disc (A), the 1st coils of the polar filament (F), the Golgi vesicles (G), and the single nucleus (N) are visible in the sporoblasts. M = mitochondrion of the host cell, T = tubules of exospore material. Scale bar = 1  $\mu\text{m}$ .



vesicles [2]. The merogonial stages are diplokaryotic and the sporont is diplokaryotic, but the spores are monokaryotic. For a number of genera it has been proven that the sporogony starts with a meiotic division. The octospores have a characteristic layered exospore, with a double-layer resembling a unit membrane [8]. During sporogony surplus material accumulates in the episporal space, forming different kinds of inclusions, the most common type is tubules with walls organized identical to the exospore [2, 9, 10].

The microsporidium of *Coenagrion hastulatum* has a life cycle identical to the microsporidia of Thelohaniidae, including a reductional division in the transition from the diplokaryotic to the monokaryotic parts. The exospore is of the basic type of Thelohaniidae. Sporophorous vesicles are not formed, but tubular "inclusions" of the normal type seen in Thelohaniidae are formed during sporoblastogenesis. In all essential characters but one it conforms to the diagnosis of the family.

Obviously the new genus is closely related to the genera of the family Thelohaniidae. If it should be accepted as an aberrant genus of the family or of a new family is a matter of taste. We are now beginning to realize that the simple and convenient characters used for the taxonomy of the microsporidia must be re-evaluated. Complex polymorphic life cycles are revealed, the cytology of the spore has proven more variable than previously expected, and spore-containing envelopes are not necessarily sporophorous vesicles (pansporoblasts), but may be of various origins. It is obvious that the sporophorous vesicle in the future will lose importance as a taxonomic character. In this situation it seems better to include the new genus provisionally in the family Thelohaniidae than to establish a new family.

#### Taxonomic Summary and Descriptions

##### *Nudispora* n. g.

**Diagnosis.** Merogony diplokaryotic. Merogonial plasmodium divides into numerous merozoites. Diplokaryotic sporont divides meiotically. Eight monokaryotic sporoblasts bud off simultaneously. Spores with oval shape. Exospore three-layered, with a median double-layer. Polaroplast with two lamellar parts, anterior lamellae more closely arranged. Polar filament isofilar. Sporogony associated with production of laminar and tubular extracellular structures, composed of exospore material. Sporophorous vesicles not produced. Only one sporogonial sequence observed.

**Etymology.** Alluding to the not enveloped spore.

##### *N. biformis* n. sp.

**Merogony.** As for the genus. The bouts of merogony unknown.

**Sporogony.** As for the genus. Sporoblasts formed by rosette-like budding. 60–74 nm wide tubules appear in the proximity of sporoblasts and spores.

**Spores.** Unfixed spores measure 1.4–1.8 × 2.8–3.4 µm, fixed and stained spores ca. 1.1 × 1.9–2.3 µm. The spore wall is 119–170 nm thick, with a 20–25 nm thick exospore. The polar filament is 85–96 nm wide, arranged in 6 (to 7) coils in a single layer close the spore wall in

the posterior half is the spore. The angle of tilt is ca. 60°. The regularly arranged anterior polaroplast lamellae are tightly compressed. The posterior regularly arranged lamellae are 27–54 nm wide. The polaroplast ends close to the anterior filament coil. A single nucleus in the centre of the spore. Teratological sporogony produces macrospores.

**Host tissues involved.** Fat cells, which are disintegrated to a syncytium, and hypoderm.

**Type host.** *Coenagrion hastulatum* (Charpentier, 1825) (Odonata, Coenagrionidae), larva.

**Type locality.** A bog at Bökeberg, Scandia, in the south of Sweden.

**Types.** Syntypes on slides No. 880802-A-(1-27) RL.

**Deposition of types.** In the International Protozoan Type Slide Collection at Smithsonian Institution (Washington, DC), in the collection of Dr. J. Weiser, Prague (Czechoslovakia), and in the collection of the author.

**Etymology.** Alluding to the production of normal and anomalous spores.

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Fig. 17–23. Spores. 17. Anterior end of a mature spore displaying the anchoring disc (A), the attachment section (AS) and the obliquely sectioned anterior straight part of the polar filament (F). The narrow lamellae of the anterior polaroplast region (P1) are partly enclosed by the polar sac (S); they are closely associated with the filament for a short distance and are directly followed by the wider lamellae of the posterior polaroplast region (P2), which are seen to be continuous around the filament. N = nucleus. Scale bar = 100 nm. 18. Longitudinal section of a mature spore showing the anchoring disc (A), the polar sac (S), the straight and coiled parts of the polar filament (F), with visible concentrical layers, the two polaroplast regions (P1, P2), the nucleus (N), and the posterior vacuole (V). In the spore wall the plasma membrane (P), the endospore (EN) and the exospore (E) are visible, \* indicates artifacts. Scale bar = 0.5 µm. 19. Teratological spores with a posterior membrane-lined compartment (arrowheads) and internal strands of exospore material (E). Scale bar = 0.5 µm. 20. Haematoxylin stained squash preparation exhibiting practically unstained teratological spores. Scale bar = 10 µm. 21. Posterior end of a teratological spore, arrowhead indicates the supposed plasma membrane; \* indicates a membrane-lined compartment resembling a nucleus. Scale bar = 0.5 µm. 22. Exospore of a mature spore, arrowheads indicate the double-layer. Scale bar = 100 nm. 23. Tubules (T) with walls of exospore material in the proximity of mature spores; E = exospore. Scale bar = 100 nm.

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## Strombidium inclinatum n. sp. and a Reassessment of Strombidium sulcatum Claparède and Lachmann (Ciliophora)

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**ABSTRACT.** *Strombidium sulcatum* is the type species for the genus *Strombidium* and has been repeatedly referred to over the last 130 yr. However, there are several taxonomic problems associated with it. We discuss why the original description of *S. sulcatum* lacks resolution to describe a single species. We conclude that: (1) the description of *S. sulcatum* sensu Fauré-Fremiet, 1912 be used to diagnose the species; (2) there are ambiguities in several redescriptions of *S. sulcatum*; and (3) *S. sulcatum* sensu Lynn et al., 1988 is *Strombidium emergens* (Leegaard, 1915) Kahl, 1932. From this analysis we present a description for *Strombidium inclinatum* n. sp. (previously *S. sulcatum* sensu Fenchel and Jonsson, 1988).

**Key words.** Oligotrich, planktonic ciliates, *Strombidium sulcatum*.

C LAPARÈDE & Lachmann described the marine ciliate *Strombidion sulcatum* (Pl. 8, Fig. 6a, b [5] and our Fig. 1; diagnosis [6]) as the type for a new genus. The name *Strombidion* apparently referred to the ciliate being like a fast or small top (Gr. *strombos*: a top or a turban; Gr. *ion*: "to go" or the diminutive suffix). They also described a 2nd species: *Strombidium turbo*; changing the Greek diminutive suffix "ion" to the Latin equivalent, "ium." Corliss [7], the 1st revisor to consider both *Strombidium* and *Strombidion*, fixed *Strombidium* as the spelling of the genus.

Over the past 130 yr there have been numerous reports of *S. sulcatum* from various localities and habitats (see references below). Strombidiid ciliates were extensively investigated at the turn of the century and more recently have been recognized as an important component of the plankton. Consequently, there has been a resurgence of interest in their trophic status and their diversity. This growing awareness has generated a more critical analysis of their taxonomy [e.g. 19-21, 26].

We are concerned with two problems associated with the taxonomic status of *S. sulcatum*. First, the original description lacks sufficient resolution to identify a single species. Second, later descriptions of ciliates identified as *S. sulcatum* are not consistent in their use of diagnostic criteria; in fact, several of these are not of *S. sulcatum*.

In this study we propose means to stabilize *S. sulcatum*: (1) we suggest that the description of *S. sulcatum* sensu Fauré-Fremiet, 1912 be used to diagnose the species; (2) we indicate ambiguities in several redescriptions of *S. sulcatum*; (3) we sug-

gest that *S. sulcatum* sensu Lynn et al., 1988 is *Strombidium emergens* (Leegaard, 1915) Kahl, 1932; and (4) we present a description for *Strombidium inclinatum* n. sp. (previously *S. sulcatum* sensu Fenchel & Jonsson, 1988).

### METHODS

We have examined the different descriptions and reports of *S. sulcatum* Calparède & Lachmann, 1858 [1-4, 8-12, 14-18, 20-24, 27-31, Gold, K. 1982. *J. Protozool.*, **29**:A67]. When data were not presented in the text they were determined from figures.

We examined specimens of *S. sulcatum* sensu Fenchel & Jonsson, 1988 that were isolated from Limfjorden, Denmark (strain CCAP 1680/1, Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology, The Windermere Laboratory, Far Sawrey, Ambleside, Cumbria A22OLP, UK). Live specimens were observed and photographed using Nomarski microscopy. Bouin's fixed material was protargol stained [25] and the appropriate meristics and morphometrics were obtained from 30 specimens. We followed the recommendations of Montagnes & Lynn [26] for description.

### RESULTS AND DISCUSSION

Our initial goal was to determine the characters that defined *S. sulcatum* in order to assess which of the many subsequent identifications of *S. sulcatum* were correct. However, a more fundamental problem arose. In the original description of *S. sulcatum* [5, 6], which is the type for the genus, Fig. 6a and 6b which accompany the description are of two different species that reside in different genera, families and orders [see 13, 32]. We have accepted Fig. 6a and ignored Fig. 6b [5]. As a result the number of oral polykinetids, depicted in 6b, cannot be used

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