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Ciliomyces spectabilis, nov. gen., nov. spec., a zoosporic fungus which parasitizes cysts of the ciliate *Kahliella simplex*

II. Asexual reproduction, life cycle and systematic account

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Abstract. The asexual reproduction, life cycle and systematic position of *Ciliomyces spectabilis*, nov. gen., nov. spec., is described. The terricolous, holocarpic and endobiotic fungus parasitizes cysts of the ciliate *Kahliella simplex*. The onecelled vegetative thallus is spherical, elongated or lobed. For sexual reproduction two thalli copulate and form one oospore of the Peronocean type. The zoosporangia are pyriform, the end of their discharge tube is conical. They are formed on thin, link-like and branched sporangiophorous hyphae which differ from the vegetative thalli in shape, size and fine structure. The zoosporangial cytoplasm is cleaved by vesicles. The zoospores are elongated and posteriorly tapered. A longitudinal groove starts below the subapical insertion point of the two flagella. Two bands of microtubules run along each side of the groove. Other vegetative thalli produce straight, thick hyphae which rupture the host cyst wall. They contain a large central vacuole and the cytoplasm is not condensed. These hyphae are supposed to reproduce asexually in the medium. Life cycle, morphological and ultrastructural data suggest that *Ciliomyces spectabilis* is a new genus within the Lagenidiaceae.

Introduction

This is the second part of a report about *Ciliomyces spectabilis*, which is parasitic on cysts of the hypotrich ciliate *Kahliella simplex*. The infection, vegetative growth and sexual reproduction have been treated in the previous paper (Foissner and Foissner 1986). Here we describe the asexual reproduction, the life cycle and the systematic position of this fungus.

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Material and methods

Culture conditions and methods for light and electron microscopy are given in Foissner and Foissner (1986).

Results

The sporangiophorous hyphae (Figs. 1–3)

Before the zoosporangium is formed the vegetative thalli (Foissner and Foissner 1986) transform into rather thin hyphae (diameter 2–6 μm), which are contorted and often link-like and occasionally branched. The hyphae contain small vacuoles, the cytoplasm is not condensed. The nuclei are pyriform with one nucleolus distal to the beaked end. Mastigonemes in ER cisternae along the mitochondria are abundant (Fig. 3, inset). The dictyosomes are located laterally at the nucleus. Blebs are frequently seen forming at the outer nuclear membrane and seem to be directly converted to the forming face of the dictyosomes (Fig. 2; compare Heath and Greenwood 1971; Grove and Bracker 1978). The angle between the centrioles at the nucleus beak is about 130°. They are connected by electron-dense material and – in later stages – also by a striated fibre. Numerous vesicles are present with granular content and a hyaline zone beneath the membrane (Fig. 3, inset; compare Fig. 37 in Lunney and Bland 1976a). Their diameter is about 400 nm. During growth of the sporangiophorous hyphae the innermost layer of the ciliate cyst wall (endocyst) disappears, suggesting that the hyphae produce lytic enzymes (Fig. 1).

The zoosporangium (Figs. 4–7)

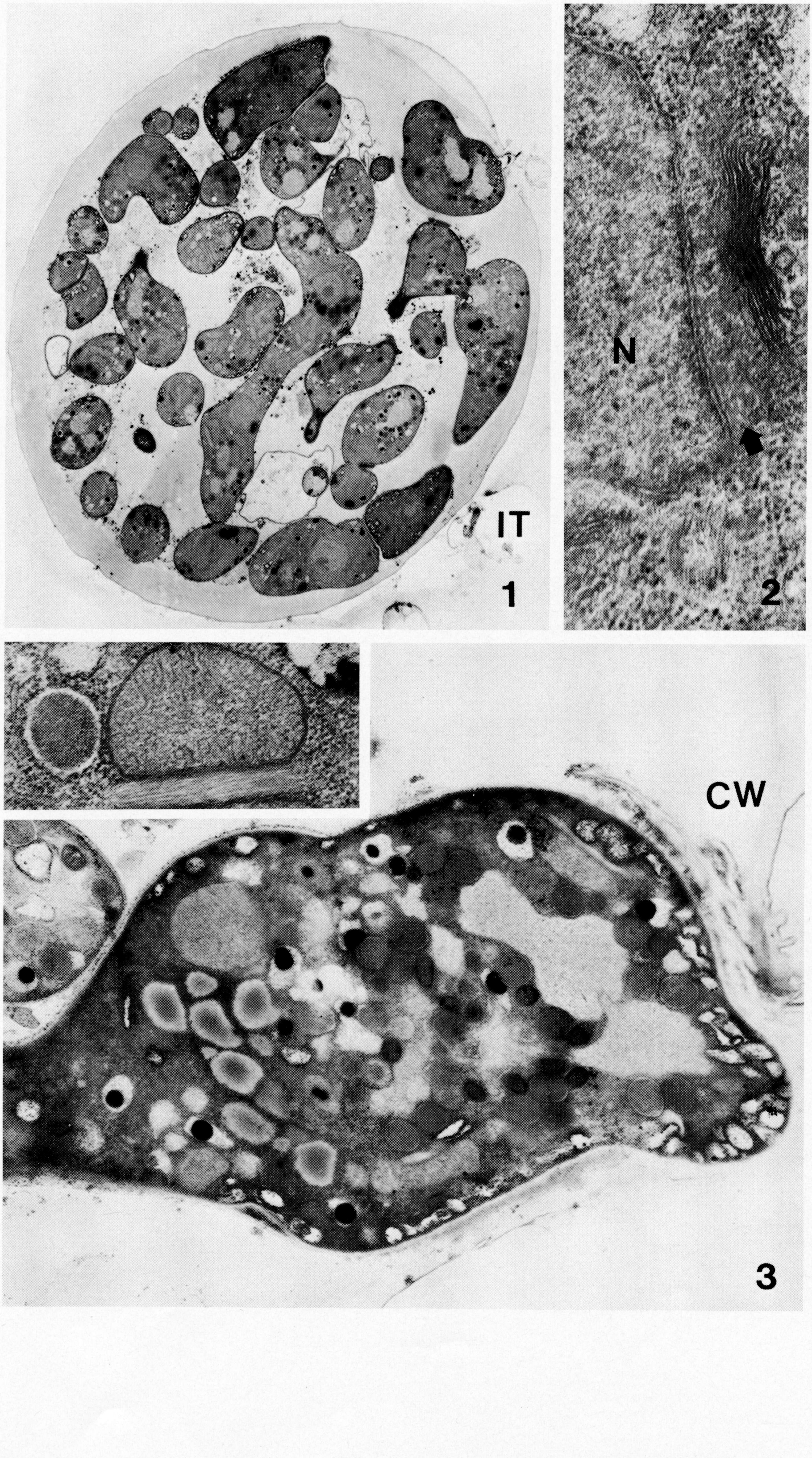
The apical tip of a hypha enlarges soon after it has penetrated the ciliate cyst wall (Fig. 3) and the whole cytoplasm of the sporangiophores accumulates therein. A basal septum is then formed, the thickness of which scarcely exceeds that of the adjacent zoosporangial wall (Fig. 6). The mature sporangium is pear-shaped, 22–20 × 30–44 μm. The wall consists of an inner electron-lucent layer and an outer component which is more electron-dense. The boundaries between the two layers are distinct only in the region of the apical papilla where the inner layer is thicker. The wall diameter (both layers) is about 200 nm at the base and up to 350 nm at the tube. The tube-like extension of the zoosporangium is about 4 μm in diameter and

Figs. 1–3. Sporangiophorous hyphae. Ciliate cyst wall (*CW*); infection tube (*IT*); nucleus (*N*)

Fig. 1. Ciliate cyst with sporangiophorous hyphae. × 2000

Fig. 2. Nucleus with centriole, microtubules (*arrows*) along the nuclear membrane, dictyosomes. × 40 000

Fig. 3. Apical tip which disrupts the ciliate cyst wall. × 11 400. *Inset:* mitochondria with adjoining mastigoneme cisterna and vesicle with granular content. × 28 800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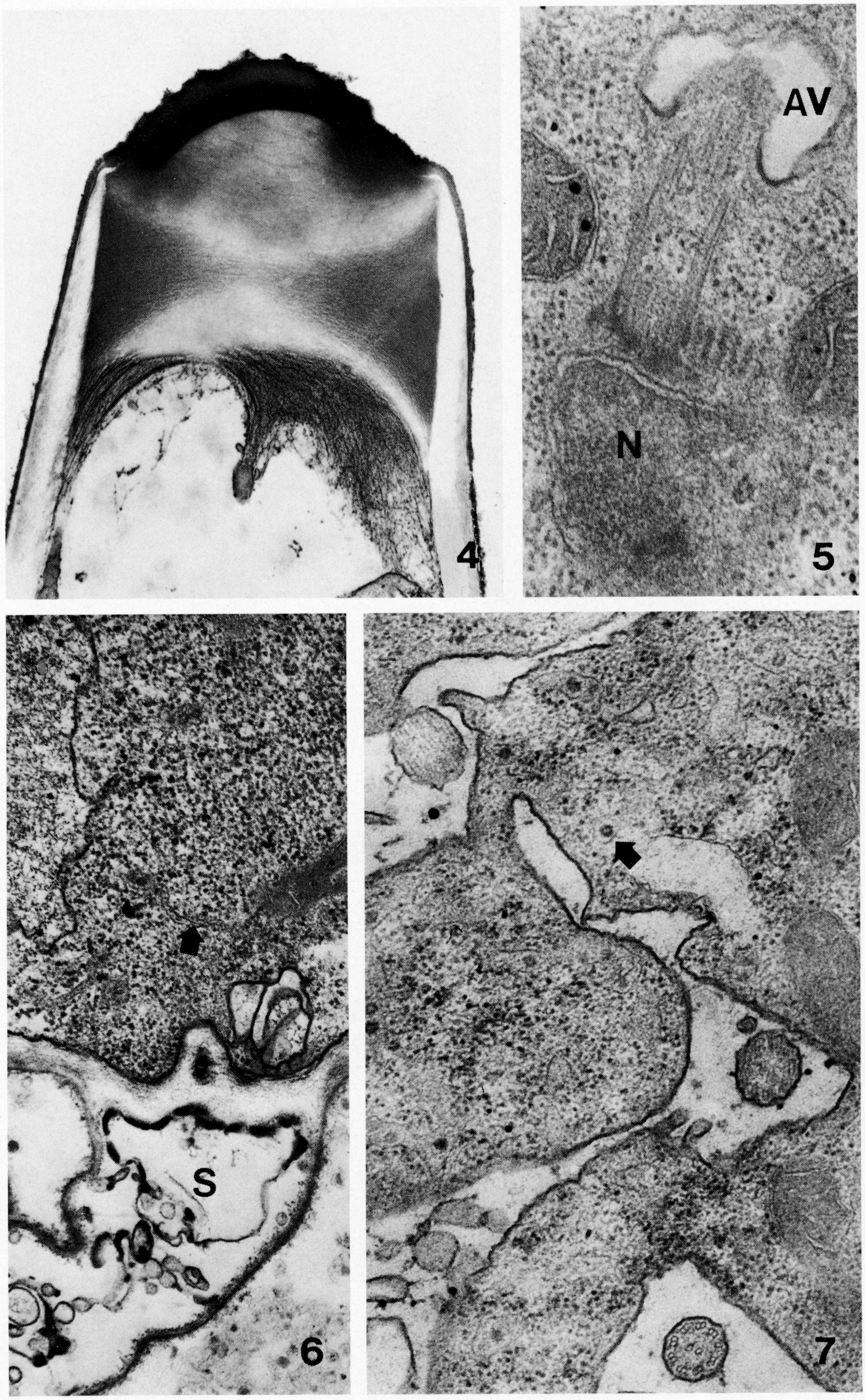
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4–8 μm in length. Its tip is slightly conical. The tube is plugged with filamentous material near the cytoplasm and more granular material near the tip where it forms a lens-like structure (Fig. 4).

The sporangial cytoplasm becomes partitioned by cleavage vesicles, no central vacuole is present (Fig. 7). The flagella are produced before cleavage is complete but not before cleavage as in *Lagenidium callinectes* (Gotelli 1974b). They develop in axonemal vesicles which fuse with the cleavage vesicles (Fig. 5). The angle between the kinetosomes is now 90° as in the free zoospore. They are connected by electron-denser material and a striated fibre. The kinetosomes (at least one of them) are embedded in a nuclear pocket. One flagellum, presumably the tinsel flagellum, develops before the other. The beaked end of the nucleus is osmiophilic and projects outwards mostly. The nucleolus has no fixed position but is frequently found behind the beak. Dictyosomes, which are so prominent in former and later stages, were absent in all zoosporangia examined, just as in resting *Pythium* sporangia (Heintz 1970). The mastigoneme cisternae are also no longer visible even when the flagella are not yet formed. Mastigonemes appear then at the flagellar surface but they are poorly preserved there. Membranous cisternae (ER?) contain fibrillar inclusions, the diameter of which is, however, far smaller (7 nm) than that of the mastigonemes (14.5 nm) (Fig. 6). Besides these filaments, they contain abundant electron-dense spherical inclusions with a fuzzy surface (diameter about 70 nm) which seem to be specific for the zoosporangial stage (Fig. 7).

The zoosporangia which we observed were always found partly inside the ciliate cyst, that is, only their distal part with the apical papilla had ruptured the wall. Sporangia which formed in the medium were not seen and release of zoospores was not observed.

The zoospore (Figs. 8–10)

The zoospore is elongated, about 6×3 μm, and posteriorly slightly tapered. Cross-sections of the middle region are reniform because of a longitudinal groove which starts below the protuberance where the flagella are inserted. This insertion site is located in the upper third of the cell (subapically). The flagella are of the whiplash and the tinsel type respectively. Unfortunately, we have no information about their length and the mode of zoospore movement, because the zoospores were examined in the electron microscope

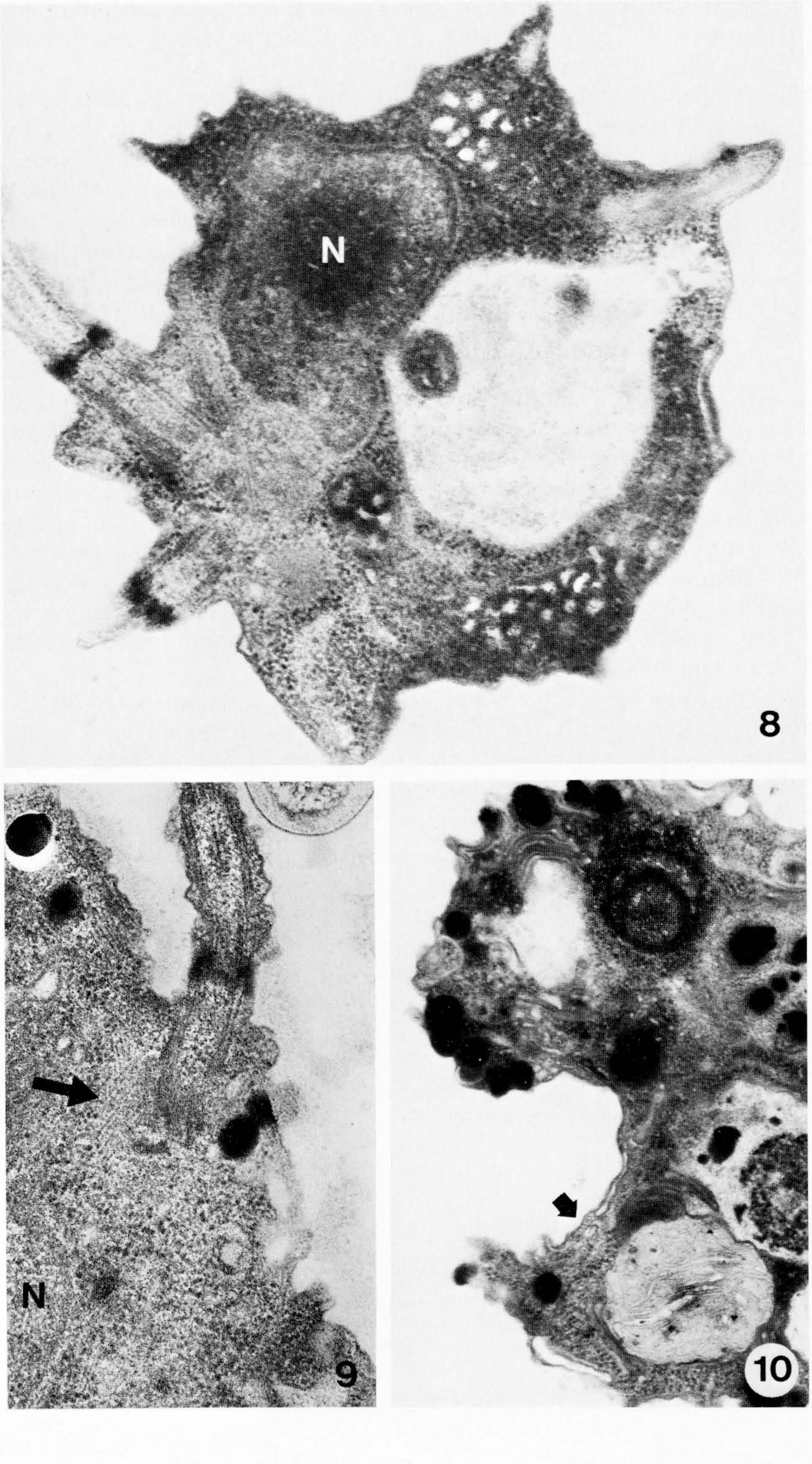
Figs. 4–7. Zoosporangium. Axonemal vesicle (*AV*); nucleus (*N*); sporangiophorous hypha (*S*)

Fig. 4. Apical papilla with fibrillar material that was ruptured during fixation. $\times 15000$

Fig. 5. Nucleus beak with kinetosome and striated fibre. $\times 56000$

Fig. 6. Basal plug with unusual finger-like protrusion. ER cisternae with filamentous inclusions (*arrow*). $\times 26400$

Fig. 7. ER cisternae with granular inclusions (*arrow*). $\times 39000$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only. The kinetosomes form an angle of about 90° to each other (Fig. 8) and are connected by electron-dense material. The striate fibre is less distinct than in the sporangium probably because it is contracted. Microtubules radiate from the proximal kinetosomal bases towards the nucleus and the anterior and posterior cell periphery (Fig. 9). The posteriorly directed microtubules form two flagellar roots consisting of at least four microtubules each of which run at both sides of the rim (Fig. 10). Microtubules which originate at right angles from these roots were not observed.

The nucleus is roughly pyriform (2.3 × 1.2 μm) with one central nucleolus. The beaked end points towards the kinetosomes. The apical region of the cell is full of stacked cisternae, which probably correspond to the water expulsion vesicle. The cytoplasm appears in general dense because of abundant ribosomes. The mitochondrial cristae are somewhat compressed and sometimes triangular in cross-section. They bear fine filamentous inclusions. A large vacuole with fibrillar and granular content but nonetheless relatively electron transparent is located besides the nucleus. Some cells contained vacuoles with apparently membranous inclusions (Fig. 10). None of these vacuoles has been observed in encysted zoospores. The cell surface appears irregular because of many protuberances. Peripheral vesicles obviously produce the cyst coat (e.g. Hemmes and Hohl 1971; Bland and Amerson 1973; Lunney and Bland 1976b; Grove and Bracker 1978). Flattened vesicles are also present.

The vacuolated hyphae (Figs. 11–13, Fig. 16 in Foissner and Foissner 1986)

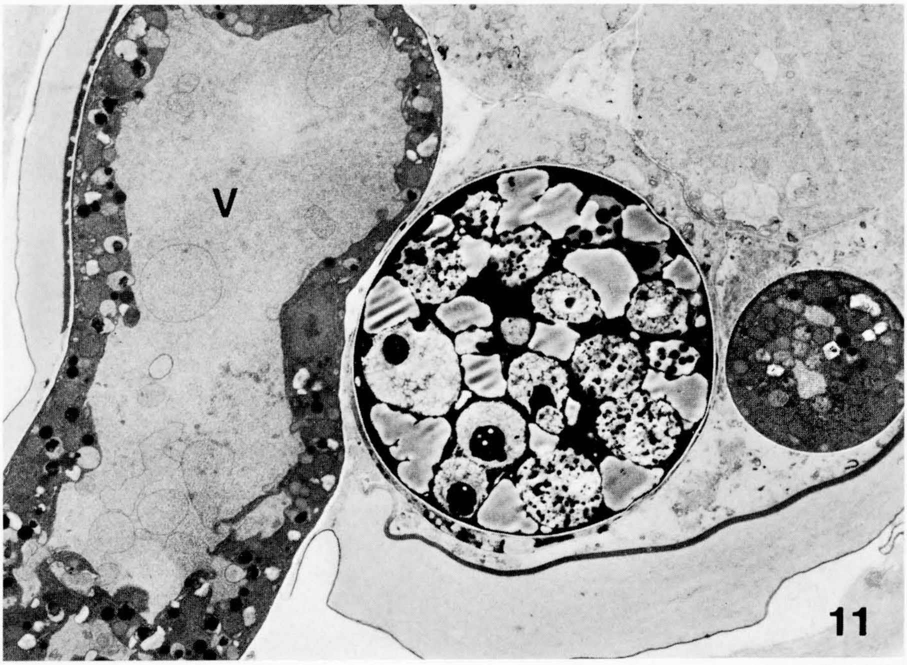
Those thalli which do not undergo sexual reproduction or asexual formation of zoosporangia inside the ciliate cyst protrude a straight hypha into the surrounding medium and leave the ciliate cyst. The ciliate cyst wall is forcibly ruptured and deformed. The hyphae are never branched or septated, although they may reach a length of up to 70 μm. Their diameter is mostly about 15 μm, but is often larger. Numerous nuclei are present with distinct centrioles which are positioned at an angle of about 180° to each other, and covered by Golgi cisternae (Fig. 12). The two or three distal ones are more electron dense than the proximal ones (compare Heath and Greenwood 1971; Hemmes and Ribeiro 1977; Schnepf et al. 1978). Microtubules have been observed to radiate from the bases of the centrioles along the nuclear envelope. Mastigoneme precursors (diameter 14.5 nm) are produced in numerous ER cisternae (Fig. 13) which are often orientated along the

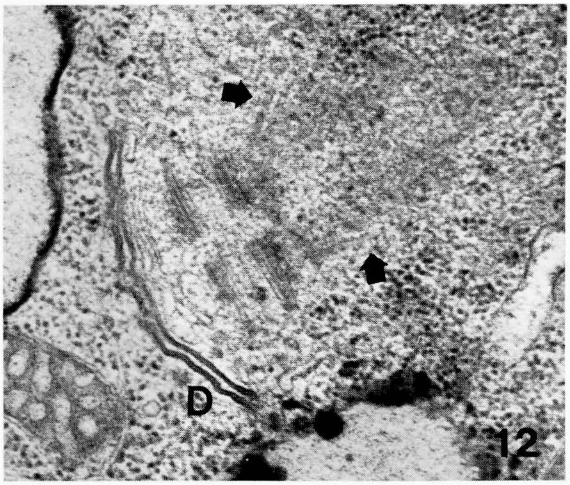
Figs. 8–10. Zoospore. Nucleus (*N*)

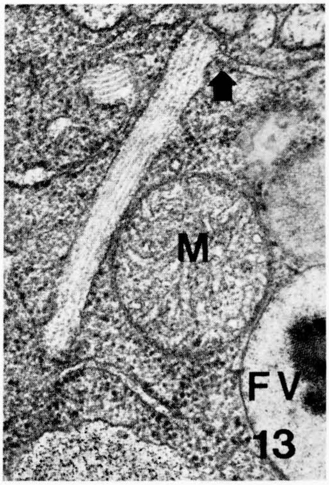
Fig. 8. Cross-section through the upper third of the cell with flagellar insertion. × 40 500

Fig. 9. Longitudinal section showing the anterior flagellum, the kinetosome and microtubules (*arrow*) running anteriorly and towards the nucleus. × 38 900

Fig. 10. Cross-section through the longitudinal groove. One of the microtubular roots is clearly visible (*arrow*). × 24 300







Figs. 11–13. Vacuolated hyphae. Dictyosome (*D*); finger print vacuole (*FV*); mitochondrion (*M*); central vacuole (*V*)

Fig. 11. Developing oospore between two vacuolated hyphae. × 3360

Fig. 12. Centrioles with dictyosome; microtubules along the nuclear envelope (*arrows*). × 47000

Fig. 13. Vesicles with mastigoneme precursors. Note continuity with the ER (*arrow*). × 36300

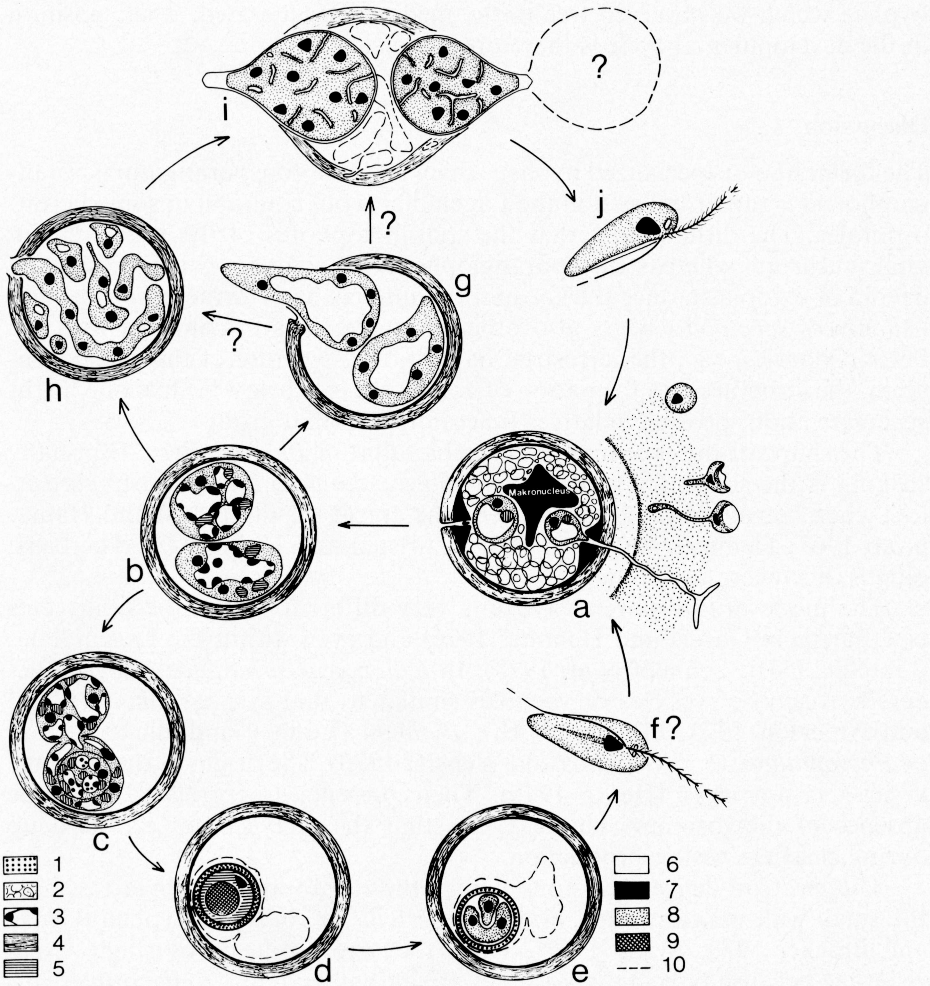


Fig. 14a–j. Life cycle of *Ciliomyces spectabilis*. *a*, infection; *b*, vegetative thalli; *c–f*, sexual reproduction; *g–j*, asexual reproduction. Unknown stages or transitions are indicated by a question mark. The diplophase is probably restricted to the oospore. All stages are shown in optical sections. Magnification: about × 560, except zoospores: × 2800 Symbols: *1*, oospore wall; *2*, ciliate cytoplasm; *3*, vacuole with peripheral appositions; *4*, ciliate cyst wall; *5*, lipid; *6*, vacuole; *7*, nucleus; *8*, cytoplasm/mucus; *9*, reserve globule; *10*, empty cell wall/zoosporangial vesicle

mitochondria. Wall vesicles are present underneath the hyphal apex. Some apices were found with thickened cell walls and retracted cytoplasm.

The cytoplasm of the vacuolated hyphae differs from that of the sporangiophorous hyphae mainly in the less pyriform shape of the nuclei, the larger angle between the shorter centrioles, the position of the dictyosomes and the absence of granular vesicles with a hyaline zone. The differences at the light microscopical level are the thicker diameter of the hyphae, their shape and the presence of a large central vacuole. The vacuolated hyphae which we observed free in the medium degenerated. Their position in the developmental cycle is therefore not clear.

Discussion

The formation of specialized hyphae which bear the zoosporangium (sporangiophores) is rather unusual in the Lagenidiales but common in some Peronosporales. The difference is that the sporangiophores of the latter remain vital (eucarpic) whereas the sporangiophores of *Ciliomyces* are completely devoid of cytoplasm once the zoosporangium has been formed (holocarpic). *Ciliomyces spectabilis* bears also other characteristics in common with the Peronosporales, e.g., the terrestrial habitat, the structure of the zoosporangium, the structure and formation of zoospores (see below). Thus one might speculate about possible relatives (ancestors?) in that group.

The shape of the sporangium resembles that of *Phytophthora*. Especially striking is the similarity with *Phytophthora palmivora* (erroneously *parasitica*) when considering the structure of the apical papilla (Hohl and Hamamoto 1967; Hemmes and Hohl 1969; Christen and Hohl 1972). The basal plug is, however, far thinner.

The mode of zoospore cleavage is very different within the oomycetes (see Bartnicki-Garcia and Hemmes 1976) and even within the Lagenidiales (Gotelli 1974b; Schnepf et al. 1978). In *Ciliomyces spectabilis* cleavage occurs by fusion of vesicles or vacuoles similar to that in *Lagenidium* (Bland and Amerson 1973; Gotelli 1974b), *Pythium* (Lunney and Bland 1976a) or *Phytophthora* (e.g. Williams and Webster 1970). The origin of the cleavage vesicles is not clear (Heath 1976). Their presence is correlated with the absence of dictyosomes. This suggests that the dictyosomes are used up during cleavage vesicle production.

The electron-dense inclusions within the membrane cisternae resemble the small wall vesicles found in e.g. *Gilbertella* or *Rhizopus* hyphae (Grove and Bracker 1970; Syrop 1973). There they are produced by single Golgi cisternae because typical dictyosomes are absent in the Zygomycotina. This corresponds to the situation found in late stages of zoosporogenesis in *Ciliomyces spectabilis*. Thus these inclusions could have the same origin and function, i.e. they could represent precursors of wall vesicles found in mature zoospores.

A further problem is the disappearance of the mastigoneme cisternae during early stages of zoosporangium formation. We did not observe mastigoneme precursors in Golgi cisternae (Bouck 1971) because these were also absent. In nearly mature zoospores they are found at the flagellar membrane, but badly preserved (compare Schnepf et al. 1978). Thus the transport form of the mastigoneme precursors is either not recognizable as such or they are soon released to the inside of cleavage or axonemal vesicles where they are hardly visible. In *Lagenidium callinectes* the mastigoneme vesicles persist until late cleavage (Bland and Amerson 1973).

The zoospore of *Ciliomyces* resembles the secondary type (Holloway and Heath 1977), although it is not typically reniform. It differs from that of *Lagenidium* not only in its shape but also in the insertion site of the flagella. The root system is *Pythium*-like (Bland and Amerson 1973; Gotelli 1974b; Lunney and Bland 1976b; Grove and Bracker 1978) with two bands of microtubules along each side of the groove. Perpendicular microtubules are, however, lacking. Rather unusual for zoospores of the secondary type is the subapical insertion of the flagella which is similar to the *Blastulidium* zoospore (Manier 1976), the systematic position of which has still to be clarified. The 90° angle between the kinetosomes corresponds to that of the primary zoospores of *Lagenisma coscinodisci* (Schnepf et al. 1978) or *Lagenidium callinectes* (Gotelli 1974b) but for the latter organism it has also been described as being 130° (Bland and Amerson 1973). These different values could be due to different fixation procedures or different metabolic states and would then have no systematic value. The large vacuoles with membranous content, which were occasionally seen, resemble autophagic vacuoles.

The further development of the vacuolated hyphae which leave the host cyst remains to be elucidated. We suppose that they reproduce asexually in the medium under more favourable conditions because the cytoplasmic organization suggests a close affinity to the sporangiophorous hyphae. A direct transformation of the vacuolated hyphae into zoosporangia must be considered but seems to be unlikely because zoosporangia were exclusively found at the tip of sporangiophorous hyphae.

Life cycle and systematic account

The life cycle of *Ciliomyces spectabilis* is depicted in Fig. 14. Infection starts with one to several encysted zoospores which produce a germ tube toward the ciliate cyst (a). In the host cytoplasm the hyphal tip forms a balloon-like inflation in which the fungal cytoplasm accumulates. The vegetative thalli which take up nutrients from the host are mostly spherical (b). Sexual reproduction occurs by copulation of equally sized thalli via a simple porus (c). The degenerating periplasm contains several nuclei (d). The mature, single oospore has a thick, smooth wall and a central ooplast (e). During the development of swarmers the oosporangial wall becomes thinner (f). Pyriform zoosporangia are formed holocarpically on thin, contorted and occasionally branched hyphae (h–i) which develop from the vegetative thalli. The zoospores are elongated and posteriorly tapered with subapically inserted flagella and a longitudinal groove (j). The thalli which do not reproduce sexually or asexually inside the host cyst protrude a straight hypha with a central vacuole into the medium and leave the ciliate cyst (g). Most probably these thalli reproduce asexually in the medium under favourable conditions.

Ciliomyces nov. gen.

Lagenidiaceae. Zoosporangia solitaria orta in hyphis sporangiophoris. Flagellae zoosporarum subapicaliter insertae sunt.
Species typica: *Ciliomyces spectabilis* nov. spec.

Ciliomyces spectabilis nov. spec.

Ciliomyces cum sphaerico vel elongatio thallo vegetativo, consistente ex una cella (usque ad 20 μm diametro, usque ad 40 μm longitudine). Pyriforme sporangium (22–30 × 30–44 μm) cum una papilla (4 × 4–8 μm), ortum in hypha sporangiophora tenui (2–6 μm), constricta et convoluta, quae raro ramosa est. Fines papillae (4 × 4–8 μm) similis metae est. Zoosporae pyriformes, 6 × 3 μm, flagellis a latere prope anteriorem partem insertis in initio canalis elongati. Copulatio thallorum magnitudine aequali. Oospora bruneola, 10–15 μm diametro. Paries oosporae levis, usque ad 1.5 μm latitudine; cum uno globulo 3.5–7 μm diametro. Parasiticus in spora perdurante ciliophori *Kahliella simplex* (Hypotrichida).
Locus typicus: Stratum superius soli prati prope Salzburg (Austria)
Type specimens: Embedded material as used for electron microscopy is deposited in the upper Austrian Museum in Linz (Austria).

Comparison with related taxa. The fungus described here is a holocarpic, endobiotic organism. The zoospores bear two laterally (although subapically) inserted flagella. The oospore contains a central ooplast and is surrounded by a degenerating periplasm. The zoospore cyst and the infection tube persist and sexual reproduction occurs by copulation of thalli. The fungus is therefore considered as a member of the Lagenidiaceae (Sparrow 1960, 1965–1968, 1976; Alexopoulos 1966). This assignment is, however, preliminary until the release of zoospores has been observed.

Three genera have been described up to now in this family. *Lagena* is parasitic on cereal roots and has a special adhesion structure, *Myzocytium* has strongly constricted cross-walls and *Lagenidium*, which is the only genus so far examined in the electron microscope (Amerson and Bland 1973; Bland and Amerson 1973; Gotelli 1974a, b), has the typical Lagenidiaceous zoospores that are reniform with laterally inserted flagella. Another feature which distinguishes our organism from all of the above cited genera is the formation of specialized hyphae prior to zoosporangium formation which are therefore called sporangiophorous hyphae. In our opinion, these differences merit a genus of its own, as diagnosed above.

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