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Original investigations

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

I. Infection, vegetative growth and sexual reproduction

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Abstract. The infection, vegetative growth and sexual reproduction of *Ciliomyces spectabilis*, nov. gen., nov. spec., a Lagenidiaceous fungus which parasitizes cysts of the ciliate *Kahliella simplex*, are described. Infection starts with encysted zoospores which perforate the ciliate cyst wall by their germ tubes. The tip of the tube enlarges spherically inside the host cyst and becomes surrounded by the host cell membrane. The germ tube is plugged with fungal cytoplasm from which the thallus detaches. The onecelled thalli which take up nutrients from the host are spherical or slightly lobed. Their cytoplasm is condensed and contains abundant reserve material. Adjacent thalli reproduce sexually by copulation via a simple porus. The brownish oospore has a central ooplast and a thick smooth wall. The periplasm and the residual male cytoplasm degenerate.

Introduction

A large number of fungi is known to parasitize ciliates or ciliate cysts (e.g. Kirby 1941; Gönnert 1935; Lubinsky 1955; Duddington 1955; Ball 1969; Barron and Szijarto 1982). Many of them are probably chytrids but the descriptions are often inadequate and then do not allow an unequivocal systematic classification. Stein (1854) even mistook a fungal infection in *Vorticella* cysts for a reproductive stage in the life cycle of the ciliate. Possibly the same problem exists with a sort of 'endocyst' described in amoeba (Chardez 1965; Couteaux 1976).

In the following we describe the infection, vegetative growth and sexual reproduction of a zoosporic fungus which parasitizes cysts of the hypotrich ciliate *Kahliella simplex*. The asexual reproduction, life cycle and systematic account are the subject of the next paper (Foissner and Foissner 1986).

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As far as we know, this is the first electron microscopical investigation of fungal parasitism in ciliates and only few electron microscopical reports exist from other representatives of the same family (Amerson and Bland 1973; Bland and Amerson 1973; Gotelli 1974a, b; and perhaps Manier 1976).

Material and methods

Soil was sampled from the top layer (0–2 cm) of a meadow near Salzburg (Schaming near Eugendorf). The soils of this area are loamy and slightly acid (pH 5–6). The sample was air-dried for some weeks and remoistened with distilled water. After 3 weeks this culture contained *Kahliella simplex* in abundance. The ciliates were isolated and transferred into another Petri dish without any food. They encysted there and 90% of the cysts became parasitized by a zoosporic fungus within a few days. Cysts of other ciliates were not infected but these were present only in low numbers. Attempts to recultivate both host and parasite on the same soil and under the same or similar conditions failed.

The light microscopic observations were performed with a Reichert microscope equipped with conventional and differential interference-contrast optics. Cells were processed for transmission electron microscopy following the procedure of Lynn (1980). Ultrathin sections were stained with aqueous uranyl acetate and lead citrate, and viewed on an AEI Corinth 500 and a Philips EM 400 T electron microscope.

Results

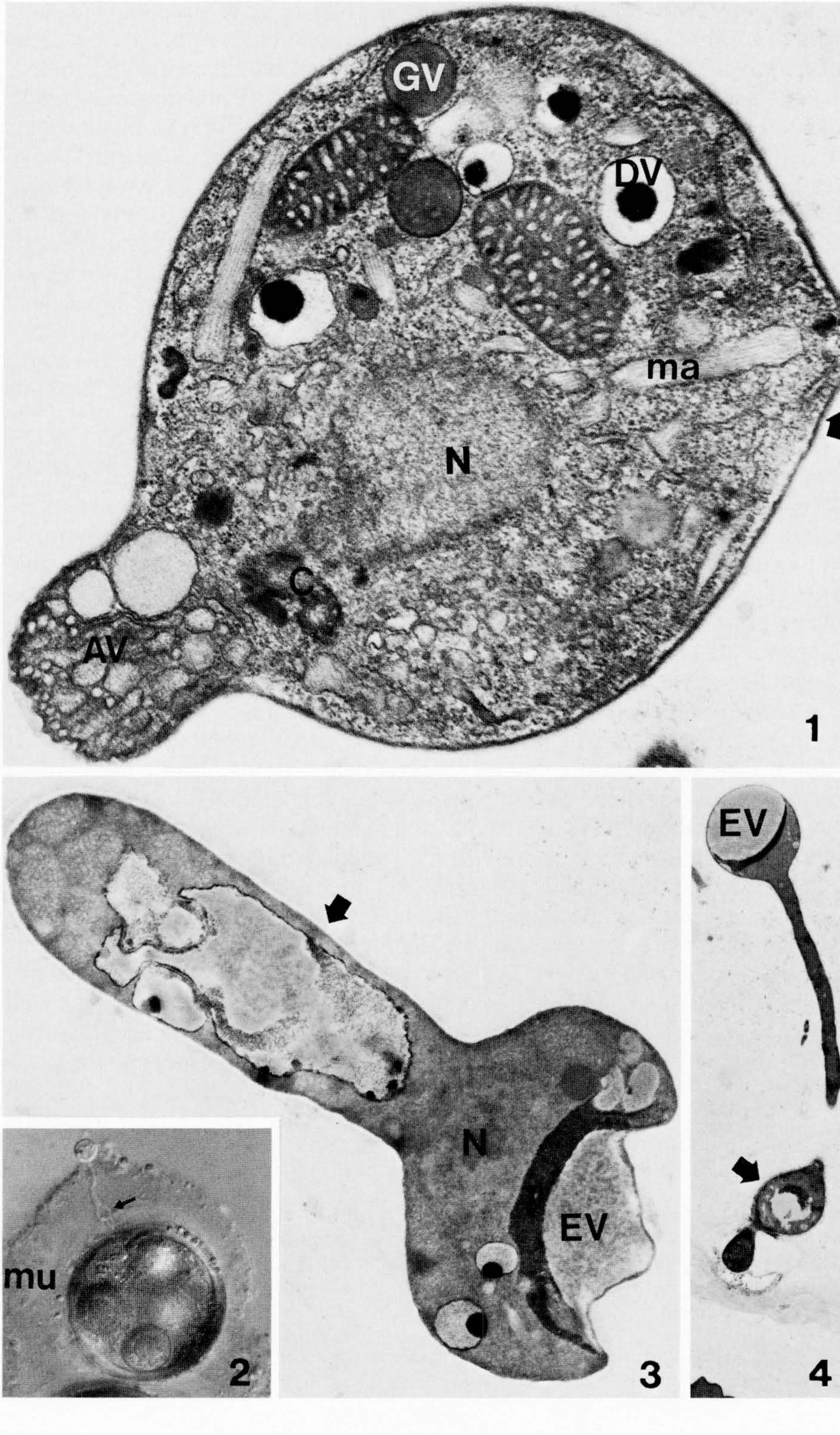
The infection (Figs. 1–6)

The cysts of Kahliella simplex. The cysts of the ciliate are spherical and about 40 µm in diameter. The yellow wall is ca. 1.5 µm thick and appears smooth. The cyst is covered by a loose mucilagous layer which may be up to 40 µm thick (Fig. 2). The fine structure of the cyst (Figs. 5 and 6) corresponds to that of other representatives of the same group, e.g. *Oxytricha* (Verni et al. 1984) or *Gastrostyla* (Walker et al. 1980). The cyst wall consists of the inner granular layer filling the rims of the cytoplasmic surface, the endocyst (about 170 nm), the filamentous mesocyst (800–1,000 nm) and the electron-dense ectocyst (50–100 nm).

Infection. One to several (mostly 3–5) zoospores encyst on or slightly in the mucilagous layer of the host cyst (Figs. 1–4). The fungal cysts are roughly spherical with a diameter of about 4 µm. Some of them have a short

Figs. 1–4. Germinating cysts of *Ciliomyces spectabilis*. Apical vesicles (*AV*); centrioles (*C*); dense body vesicles (*DV*); expulsion vacuole (*EV*); granular vesicle (*GV*); mastigonemes (*ma*); mucus (*mu*); nucleus (*N*)

Fig. 1. Young stage with short infection tube and appendix (*arrow*, partly cut off). ×24000
Figs. 2 and 4. Infection tubes penetrating the mucus of the ciliate cyst. The cyst in Fig. 2 already contains oospores of the parasite. The arrows mark germ tube vacuoles. ×600 and ×2400
Fig. 3. Germling with collapsed extension vacuole and germ tube vacuole, tip of infection tube not sectioned. ×9300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appendix opposite to the future infection tube (Fig. 1, arrow). The cyst wall is thin (about 25 nm), the outer layer, the cyst coat, appears flocculent (compare e.g. Grove and Bracker 1978). The nucleus is centrally located, pear-shaped (2.5×2 μm) and contains one nucleolus. Two centrioles which are connected by electron-dense material are situated at the beaked end of the nucleus at an angle of about 160° to each other. Axonemes have never been observed in the cysts. The mitochondria (about 1×0.6 μm) have a dense matrix and tubular cristae with fine filamentous or tubular inclusions. Their surface is smooth without tubules or particles (compare Heath 1976). Each cyst contains characteristically shaped ER cisternae with presumed mastigoneme precursors but there is no evidence for a secondary swarmer. The distal cisternae of the dictyosomes appear more electron-dense than the proximal ones. The ribosomes are evenly distributed, the ER more peripherally. Vacuoles with granular content (diameter about 400 nm) and microbodies are present (Fig. 1).

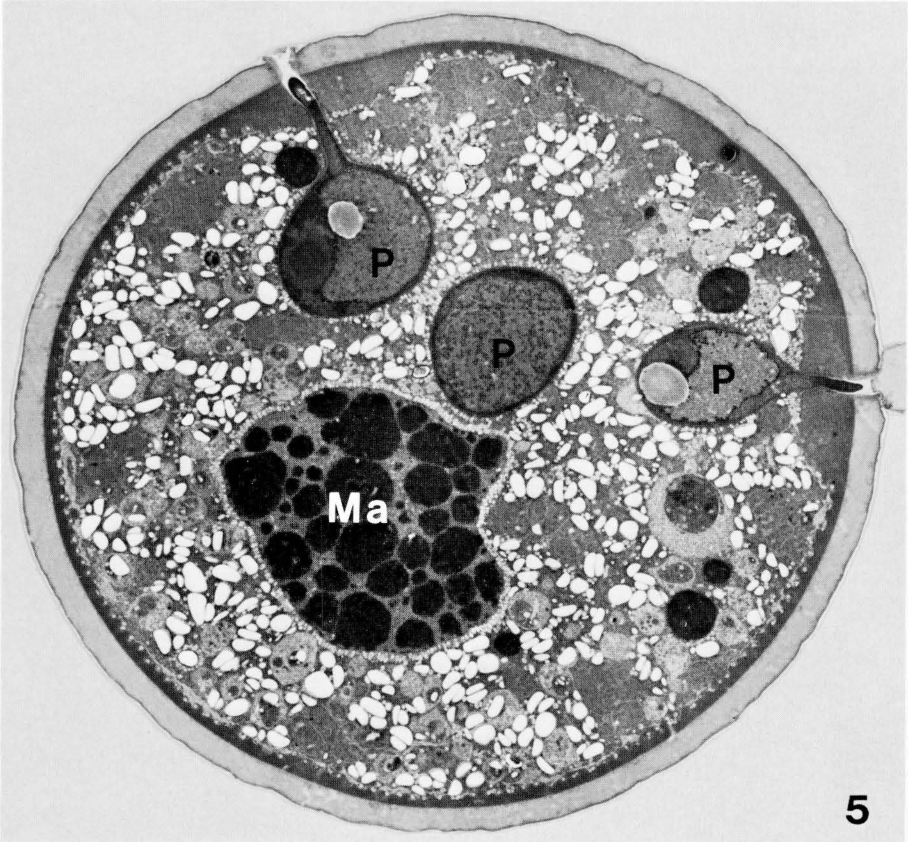
Germ tube formation is preceded by the accumulation of apical vesicles. Germ tubes are also produced on ciliate cysts which are already parasitized or even empty (Figs. 2 and 14). The wall of the germ tube seems to be continuous with the cyst wall. The elongation of the germ tube is accompanied by vacuole formation distal to the apex (Figs. 3 and 4). The cytoplasmic site of the vacuole is covered by granular-fibrillar material. When the vacuole collapses, the germling has a characteristic wrench-like shape (Fig. 3). Similar vacuoles appear also in the germ tube, which is then characteristically swollen at these sites (Figs. 2 and 4). The germ tube is thus not uniform in thickness and its diameter ranges from 1 to 4 μm. It rarely appears straight but often tortuous and may attain a length of up to 50 μm. The fibrils of the mucus appear more tightly packed near the infection tube (Fig. 4) and the mesocyst is more electron-dense (Fig. 6).

As soon as the hyphal tip reaches the granular layer beneath the endocyst, the host cytoplasm responds to the fungal attack by forming a 'callosity' (e.g. Aist and Williams 1971; Hoch and Fuller 1977), which consists of the same granular material as the layer beneath the endocyst and is deposited in the region around the incoming germ tube. The regularly wrinkled cytoplasmic surface is then lost and the underlying vesicles are irregularly distributed (Figs. 5 and 6). In the host cytoplasm the hyphal tip forms a balloon-like inflation in which the fungal cytoplasm accumulates. The nucleus appears roughly spherical and is peripherally located with two centrioles, which are, however, hardly discernable. The center of the balloon is occupied by a large vacuole with granular content. An electron-transpar-

Figs. 5 and 6. Accumulation of fungal cytoplasm in the host. Autophagic vacuole (*AV*); ectocyst (*Ec*); endocyst (*En*); granular layer (*GL*); infection tube (*IT*); presumed lipid body (*L*); macronucleus of the host (*Ma*); mesocyst (*Me*); nucleus of the parasite (*N*); parasite (*P*)

Fig. 5. Ciliate cyst with three parasites. $\times 2800$

Fig. 6. Enlarged detail of Fig. 6. Callosity around incoming infection tube. $\times 16000$



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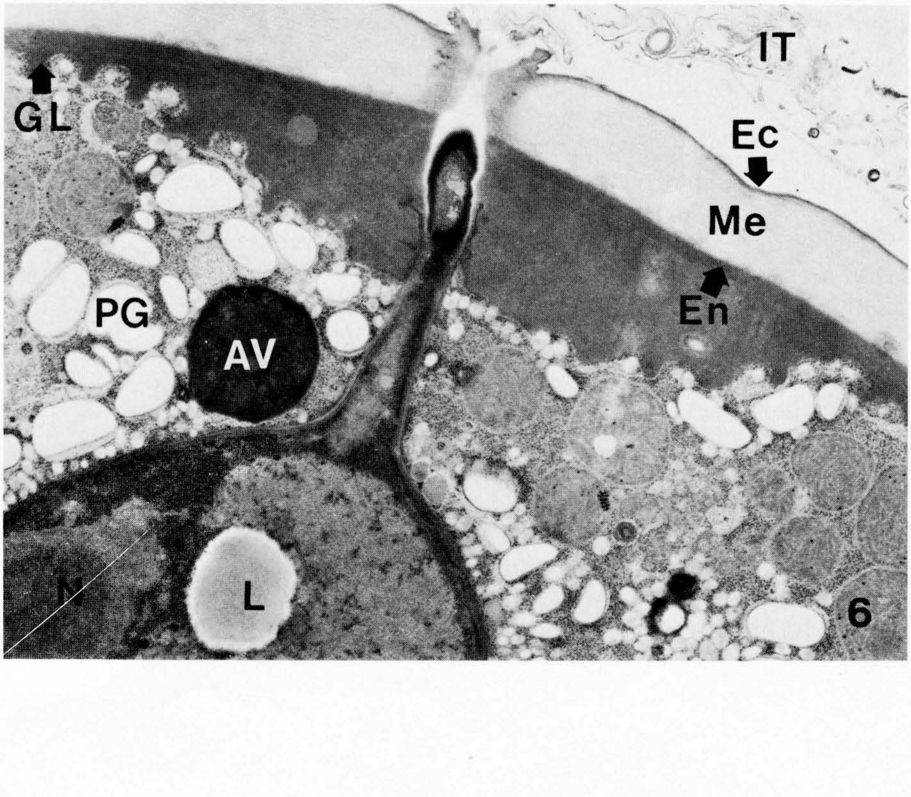

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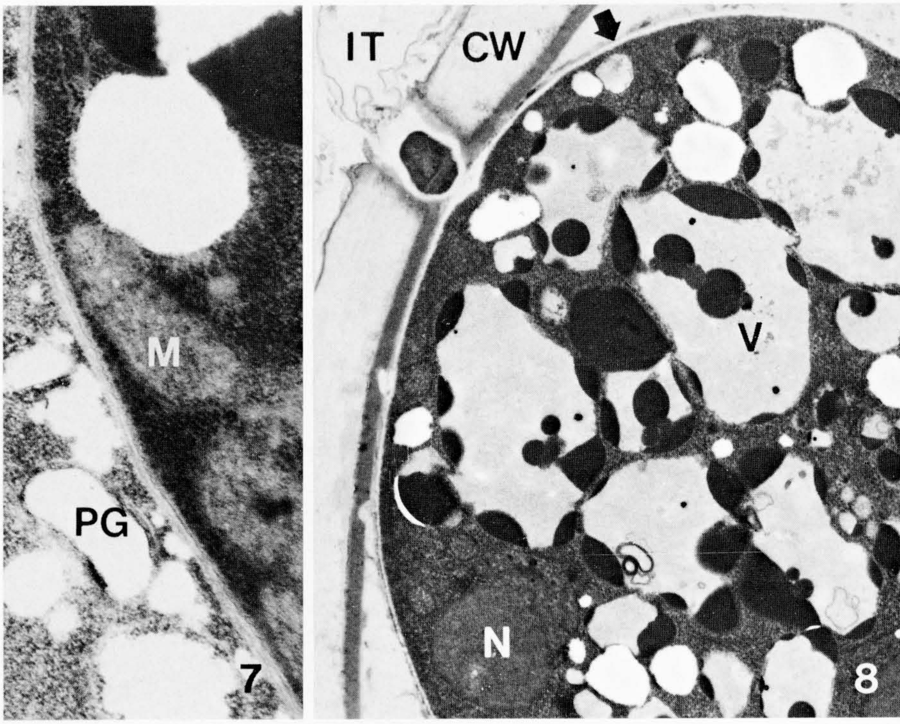


Figs. 7 and 8. Vegetative thallus. Ciliate cyst wall (*CW*); infection tube (*IT*); mitochondrion (*M*); nucleus (*N*); paraglycogen granule (*PG*); vacuole with peripheral appositions (*V*)

Fig. 7. Host (*left*) and parasite (*right*) separated by two membranes. ×27600

Fig. 8. Host cytoplasm degenerated, perforated ciliate cyst wall sealed by a plug. Note the thin cell wall (*arrow*). ×6900

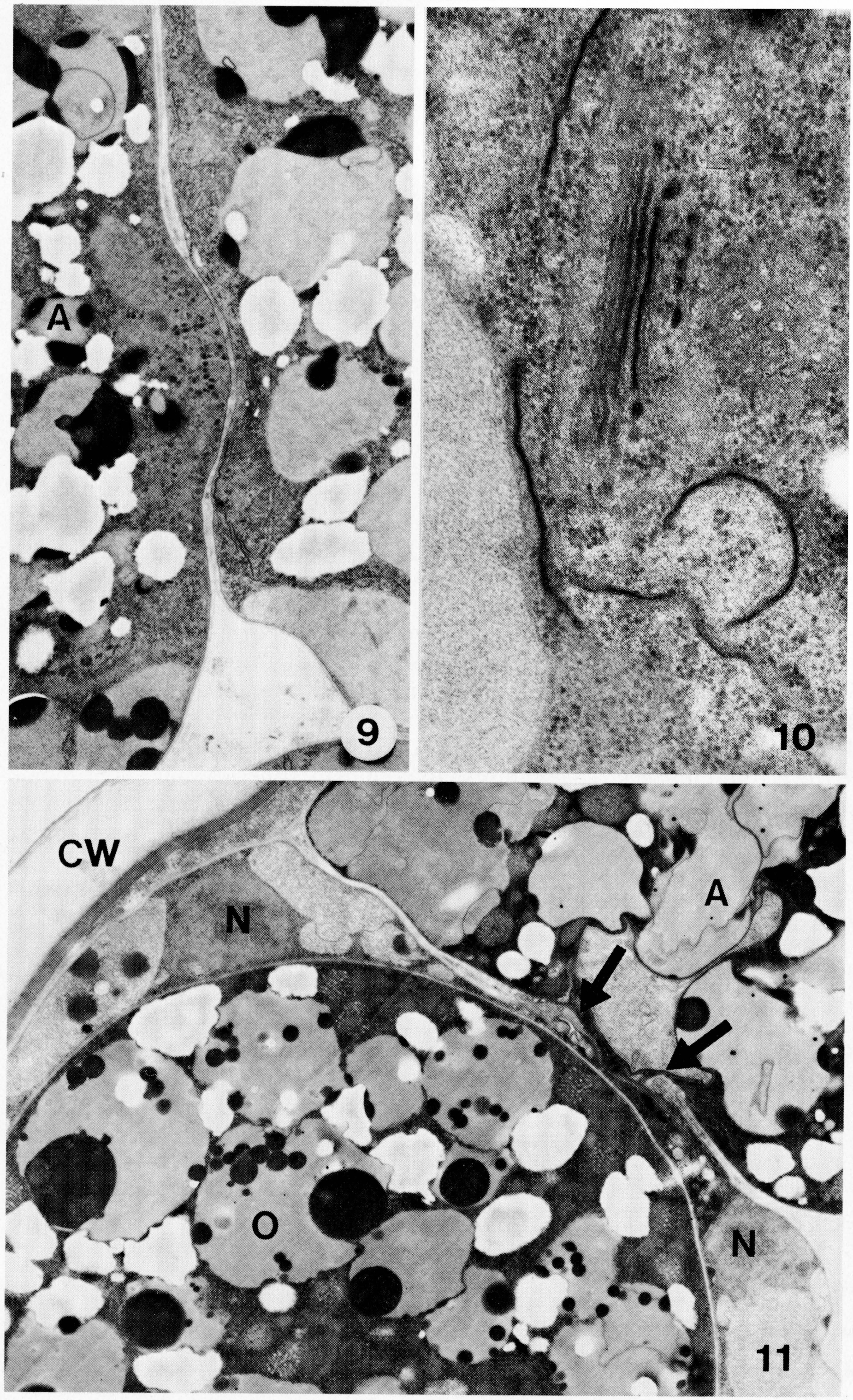
ent inclusion (diameter about 1 μm) which indents the vacuole could be the first structural evidence for the accumulation of reserve material at the expense of the host cytoplasm. The ribosomes of the parasite are densely packed except for very small channels which are presumed to be ER cisternae, although a membrane is not clearly visible. The mitochondria are less

Figs. 9–16. Sexual reproduction. Male thallus (*A*); ciliate cyst wall (*CW*); nuclei in periplasm (*N*); oosphere (*O*)

Fig. 9. Accumulation of vesicles in the male thallus (*left*) prior to porus formation. The female thallus (*right*) has already been fertilized by another cell, the periplasm becomes delimited by an electron-dense membrane. ×10600

Fig. 10. Cisterna which separates the periplasm (*left*) from the future oosphere (*right*) and adjacent dictyosome. ×48400

Fig. 11. Gametangial fusion via a porus (*arrows*). Female thallus with oosphere and periplasm. ×8100


A
9
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CW
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O
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11

distinct than in the cyst, thinner and without a clear difference in the electron densities of matrix and cristal space. Mastigoneme vesicles disappear while the cytoplasm passes the germ tube. They reappear later in the life cycle when hyphae are produced. A part of the fungal cytoplasm forms a sort of plug in the infection tube and detaches later from the thallus (Fig. 8). The cell membrane of the parasite becomes covered by the host cell membrane except for the distal part of the plug. The clefts between the callosity and the proximal part of the plug are also coated with host cytoplasm and cell membrane, which – in this region only – is underlined by very fine electron-dense granules or filaments. The rest of the host membrane which encloses the parasite is covered with electron-lucent vesicles just as the ciliate membrane underneath the wall or the membranes of the ciliate nuclei. The cyst wall and the infection tube remain persistent – although somewhat collapsed – and are full of membraneous remains.

Vegetative growth (Figs. 7 and 8)

The central vacuole of the incoming parasite is replaced by or changes to several vacuoles which obviously store reserve material. They are of medium electron density with characteristic peripheral appositions of electron-dense material and seem to fuse with more electron-transparent inclusions (lipid?) which increase in number. The Golgi apparatus is reduced. Only few cisternae are visible with electron-dense content. Nuclei and mitochondria are barely recognizable because of the dense packing of ribosomes. The diameter of the thalli increases and the number of paraglycogen granules in the host diminishes. The thallus shape is mostly spherical but may become elongated or irregularly lobed. The host cytoplasm appears degenerated only in late stages, when the thalli nearly fill the whole cyst. The granular layer of the ciliate cyst wall and the callosities are also digested. The endocyst remains at first intact. Then the host membrane, which has previously surrounded the whole parasite, is dissolved and the fungus forms a thin electron-transparent wall.

Sexual reproduction (Figs. 9–16)

Sexual reproduction occurs in the host cyst by copulation of equally sized thalli which act as gametangia as soon as a thin cell wall is formed. Adjacent

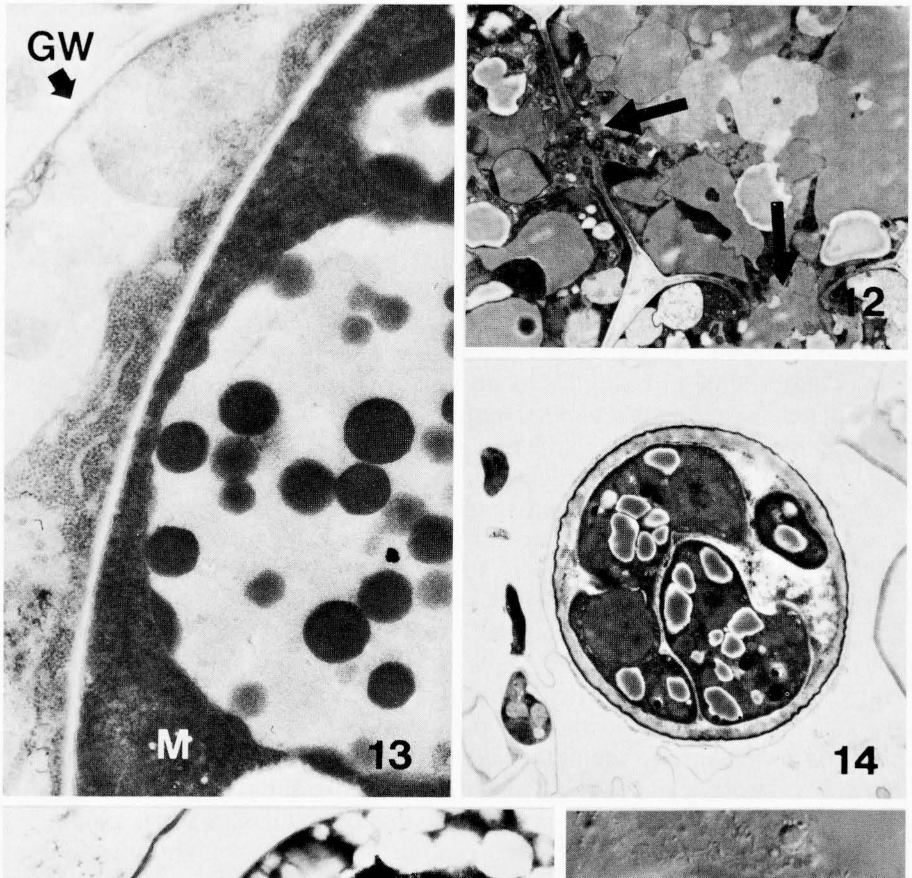
Fig. 12. Pori (*arrows*) between three copulating thalli. Endospore (*En*); exospore (*Ex*); gametangial wall (*GW*); lipid body (*L*); mitochondrion (*M*); ooplast (*OP*). × 5000

Fig. 13. Degenerating periplasm (*left*) and oosphere (*right*) separated by two membranes. × 29000

Fig. 14. Oospore with developing swarmers and infection tube of a germling (left). × 3600

Fig. 15. Ripe oospore with ooplast inclusion. × 13250

Fig. 16. Ciliate cysts with vegetative hypha (*arrow*), ripe (*arrow head*) and developing oospores. × 600


GW
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cells fuse via a simple porus (Fig. 11). One fungus was observed to copulate with two neighbouring cells (Fig. 12). Perforation of the cell walls seems to be achieved by small vesicles (diameter 50–100 nm) with dense content which accumulate in the male thallus (Fig. 9, compare Hemmes and Ribeiro 1977). Resting spore formation without gametangial fusion did not occur.

A part of the male cytoplasm, including at least one nucleus, enters the female thallus. At that time the boundaries of the single oosphere are defined by electron-dense cisternae which probably derive from the dictyosomes (Fig. 10; compare McKeen 1975). The periplasm contains several nuclei and degenerates together with the residual male cytoplasm. Their storage material is probably taken up by the oosphere. Before the periplasm is completely degenerated two membranes are seen, one lining the oosphere and a thinner one lining the adjacent periplasm (Fig. 13).

The final oospore wall is smooth or slightly folded and consists of an outer electron-opaque zone (exospore) which is up to 200 nm thick, and a thicker (1–1.5 μm), more electron-transparent layer (endospore) (Figs. 15 and 16). The oospore diameter is 10–15 μm. The oospore is brownish in colour and surrounded by the periplasmatic space and the gametangial walls.

The oosporal cytoplasm changes after wall formation and consists then of a central membrane-bound reserve globule (diameter 3.5–7 μm) with darkly stained granular content. The storage vesicles of the feeding parasite have disappeared suggesting that they are involved in formation of the central ooplast. An intermediate stage shows vacuoles with smaller globular inclusions. The nucleus and other organelles are embedded in numerous lipid vesicles which seem to derive from the larger ones in the gametangia. The nucleus corresponds probably to the pellucid inclusion seen in the light microscope. One oospore was observed with a thinner endospore and less condensed cytoplasm that contained several pear-shaped nuclei (Fig. 14).

Discussion

The zoospores presumably recognize their host by the mucilagous layer by a process independent of the presence of a digestable cytoplasm (Held 1973). The structure of the encysted zoospore and the mode of germination are essentially similar to those of other oomycetes (Hemmes and Hohl 1971; Bimpong and Hickman 1975; Heath 1976; Grove and Bracker 1978; Schnepf et al. 1978). The position of the apex seems to be determined by that of the nucleus or the centrioles since the apex was always located in front of the nucleus beak. The host wall seems to be disrupted both mechanically (mucus) and enzymatically (proper cyst wall) as shown in other cases (Hoch and Fuller 1977). In contrast, the penetration of the ciliate cyst wall by outgrowing hyphae appears to be predominantly mechanical, probably because of their larger size (Foissner and Foissner 1986).

Inside the ciliate cyst the fungus becomes surrounded by the host cell membrane as, for example, in *Lagenisma* (Schnepf et al. 1978) or *Plasmodiophora* (Aist and Williams 1971). A compound membrane as in *Aphelidium* is not formed (Schnepf et al. 1971). The affinity of the host cell membrane for a certain type of vesicle is apparently not lost during its extension by the parasite. The late degeneration of the host cytoplasm and the diminution of the paraglycogen granules indicate that the parasite first uses the dispensable reserve material. This could be facilitated by the adjacent host cell membrane which is only indented but not destroyed by the fungus. Pinocytosis of host cytoplasm was not observed. Thus we assume that host material is ingested in a soluble form at the molecular level (compare e.g. Schnepf et al. 1978; Foissner and Foissner 1986). The condensation of cytoplasm and storage material is rather unusual during vegetative growth. The thalli are therefore small and several cells may parasitize one cyst which promotes sexual reproduction.

The sexual reproduction of *Ciliomyces spectabilis* by copulation of equally sized thalli is typical of many holocarpic unicellular oomycetes (Alexopoulos 1966). The resulting oospore and the mode of formation is of the Peronosporacean type (Sparrow 1976). The changes in the cytoplasm during oospore formation are less drastic than in other fungi because the thalli are full of storage material even before fusion (Hemmes and Bartnicki-Garcia 1975; McKeen 1975; Bartnicki-Garcia and Hemmes 1976; Haskins et al. 1976). The presumed lipid vesicles of the oospore are derived from larger ones and not formed by coalescence of small vesicles (McKeen 1975). The periplasm is delimited by a Golgi-derived cisterna. ER cisternae which are said to serve this function e.g. in *Pythium* (Haskins et al. 1976) are structurally different, they are also not very prominent in the periplasm (McKeen 1975). The cisterna forms the membranes of the oosphere and the adjacent periplasm which are seen in later stages. The oospore wall is secreted in a rather short time because transition stages are infrequent. The cytoplasm of the oospore with the thinner wall is assumed to develop into swarmers. Akai et al. (1976) also noted that the wall is partly reused during oospore germination.

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