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The Fine Structure of the Resting Cysts of *Kahliella simplex* (Ciliata, Hypotrichida)

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Abstract

Resting cysts of *Kahliella simplex* were studied in the electron microscope. The fine structure is of the oxytrichid type which is characterized mainly by the absence of kinetosomes, a 4-layered cyst wall and fused macronuclei. Subpellicular microtubules are not disassembled. Thus their presence can not be used further as an euplotid character. Special features of the cysts of *Kahliella simplex* are epinuclear vesicles and regularly arranged subpellicular vesicles.

Introduction

Cysts of *Kahliella simplex*, considered as a "primitive" hypotrich (Fleury and Fryd-Versavel 1982) were investigated because of a remarkable infection by an oomycetous fungus, *Ciliomyces spectabilis*, which destroyed about 90% of the culture (Foissner and Foissner 1985, 1986a, b).

In this paper we describe the fine structure of the intact resting cyst, which seems to be an important criterion for establishing phylogenetic relationships, especially among hypotrich ciliates (Corliss and Esser 1974, Walker and Maugel 1980, Reid and John 1983).

Material and Techniques

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 (*p*H 5—6). The sample was air-dried for some weeks and remoistened with distilled water. After three 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 elec-

tron microscopy following the procedure of Lynn (1980). Ultrathin sections, most of them stained with aqueous uranyl acetate and lead citrate, were viewed on an AEI Corinth 500 and a Philips EM 400 T electron microscope.

Results

Light microscopy

The cysts of *Kahliella simplex* are spherical with a smooth surface and coarsely granular cytoplasm (Fig. 3). Its diameter is about 40 µm ($\bar{x} = 41.4$; $s = 2.6$; $S\bar{x} = 0.5$; $V = 6.3$; $n = 22$). The thickness of the yellowish wall amounts to 1.5 µm. The cyst is covered by a loose mucilaginous layer, which may be up to 40 µm thick and often joins two or more cysts. The mucus is slightly detached from the cyst wall, the inner border appears more refractive than the periphery. The space between cyst wall and mucus contains an agglomeration of nearly spherical, cristal-like particles (diameter about 1.4 µm) which were formerly located inside the cytoplasm of praecystonts. These particles disappear completely during the electron microscopical procedure. The mucus is fragile and sticky. Therefore the cysts often adhere to the Petri dish. Bacteria are frequently found on or inside the mucus (Fig. 2 and 3).

Electron microscopy

The condensed cytoplasm is densely filled with more or less regularly distributed ovoid granules of various size (up to 1.4 µm in length) which probably consist of paraglycogen (Verni et al. 1984). The mitochondria are spherical or slightly elongated (about 1 µm long) and mostly clustered. ER cisternae are thin and hardly visible because of the ribosome dense cytoplasm. Cristalline aggregates of ribosomes were not detected. Vacuoles which may reach the size of the mitochondria appear rather electron lucent with fainly stained fuzzy content. Electron dense autophagous vacuoles contain mostly degenerating mitochondria (Fig. 1 and 5).

The large lobed macronucleus (diameter up to 17 µm) occupies the center of the cyst (Fig. 1 and 5). Its chromatin is arranged in spherical bodies. The nuclear pores form a network in the double-membraned nuclear envelope. The meshes of the network consist mostly of nine pores (Matsusaka and Kimura 1981). The width of these meshes is then 260—300 nm. The center-to-center spacing of adjoining pores is about 90 nm. There exist also some regions with a denser, hexagonal pore distribution. The width of the pore amounts up to 40 nm, the outer diameter of the annulus is about 80 nm. A central granule is lacking. The chromatin beneath a nuclear pore complex forms a small pocket. The micronucleus (diameter 5 µm) appears uniformly fibro-granular and its surface is irregularly crenated. Pores were not detected. Nucleoli were found neither in

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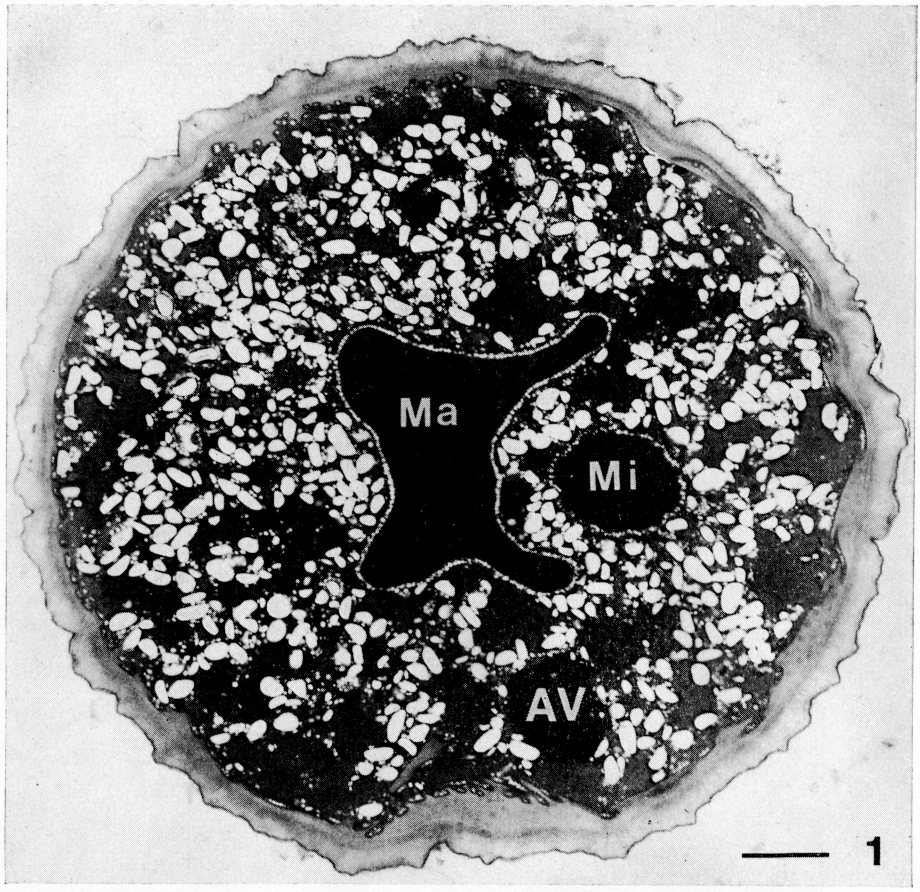
Fig. 1. Resting cyst of *Kahliella simplex*. Ma = macronucleus, Mi = micronucleus, AV = autophagous vacuole. Bar: 4 µm

Fig. 2. Cyst wall with bacterium trapped between mucus (mu) and ectocyst (ec). me = mesocyst, en = endocyst, gl = granular layer. Bar: 400 nm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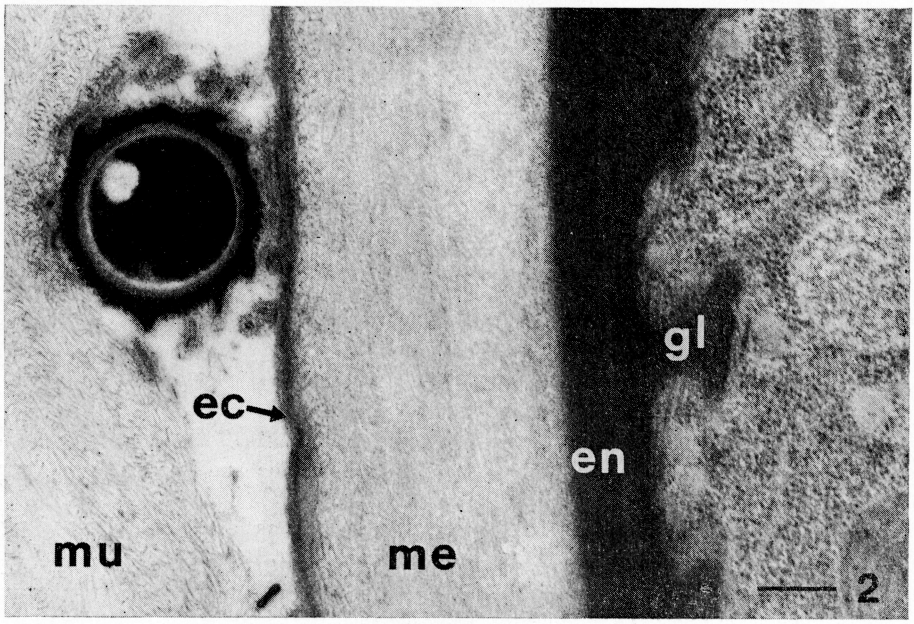
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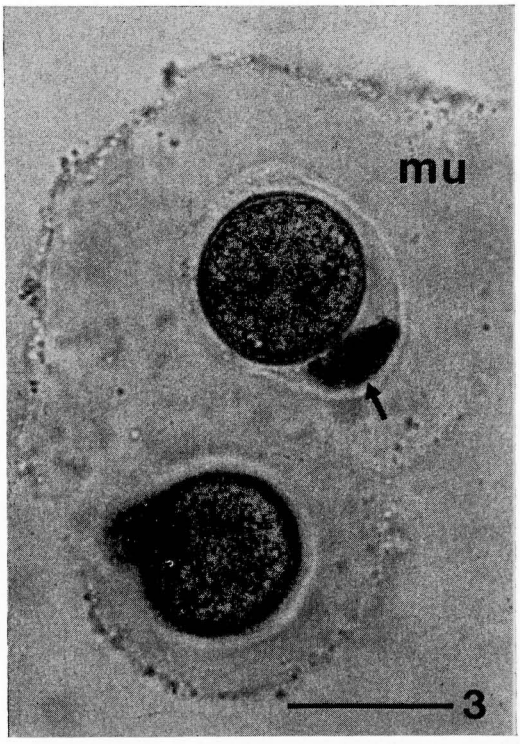
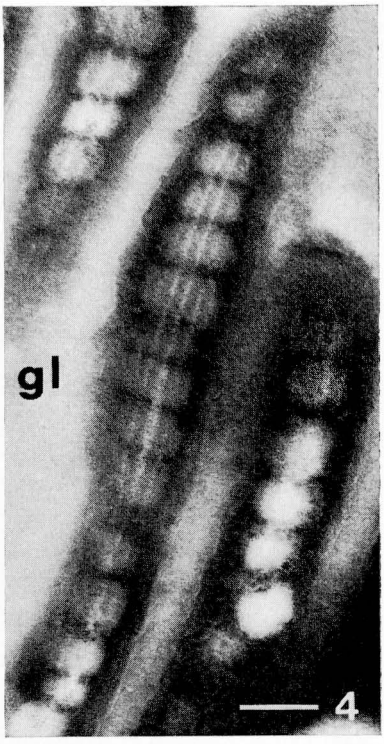




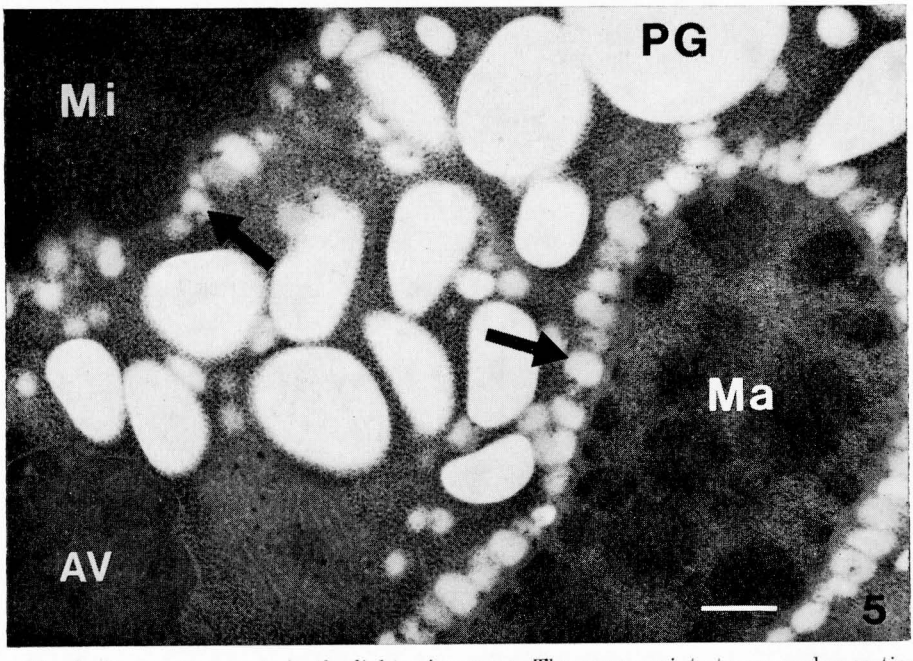


Fig. 3. Two resting cysts in the light microscope. The arrow points to an agglomeration of cristal-like particles. mu = mucus. Bar: 40 μm

Fig. 4. Tangentially sectioned surface grooves. Subpellicular vesicles and superficially aligned microtubules are visible. gl = granular layer. Bar: 180 nm

Fig. 5. Micro- (Mi) and macronucleus (Ma) covered with vesicles (arrows). AV = autophagous vacuole, PG = paraglycogen granule. Bar: 380 nm

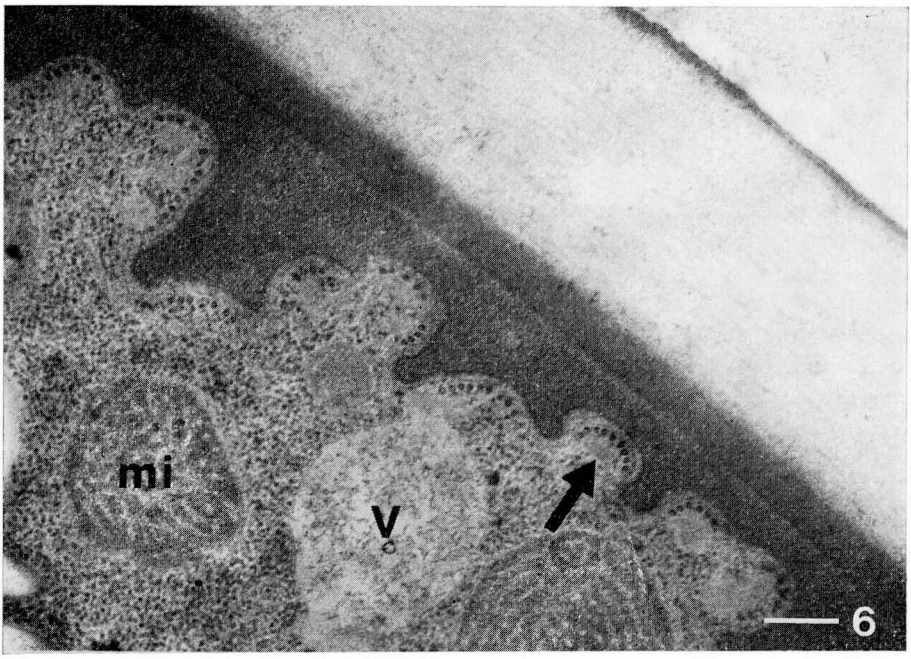
macro- nor in micronuclei. Both nuclei are densely covered by vesicles of low electron density, which have a diameter of about 150 nm. Their shape is often irregular because of the dense packing.

The cytoplasmic surface consists of a single unit membrane which is more or less regularly wrinkled (Fig. 2, 4 and 6). These surface grooves were always found to radiate or converge spirally (Hashimoto 1963, Grimes 1973). Ribbons made of 5—10 microtubules each are exclusively located beneath the cytoplasmic protrusions. They are underlined by rows of subpellicular vesicles. Their shape, size and structure are identical to the epinuclear vesicles described above. A vesicular substructure is sometimes seen in unstained sections (Fig. 7). The vesicles are joined to each other by small protrusions which indent the surface of the adjacent vesicle. Some of these vesicles are found also in the residual cytoplasm but they are hardly distinguishable from small paraglycogen granules, especially in stained sections.

Concerning the cyst wall components, we follow the actual terminology as used for instance by Grimes (1973), Walker et al. (1980), and Verni et al. (1984). Accordingly the wall consists of the inner granular layer, filling the rims of the cytoplasmic surface, the endocyst (about 170 nm), the filamentous mesocyst (800—1000 nm) and the thin ectocyst (Fig. 2, 6—8). The granular layer appears more coarsely granular than the endocyst. Both layers are separated by a thin electron lucent line. Unstained sections reveal that the granular layer consists of an electron dense inner and an electron lucent outer region. Furthermore, the endocyst is more prominent because it appears electron denser than the granular layer (Fig. 7). The filaments of the mesocyst are delicate. It is thus difficult to recognize a special pattern. The outer border of the mesocyst is electron dense both in stained and unstained sections and similar to the ectocyst, which is very thin (about 20 nm) and probably consists of only one lamella. The thickness of the ectocyst can only be evaluated from appropriate cross-sections (Fig. 8). The fibrils of the mucus are more tightly packed near the cyst wall than at the periphery, which probably accounts for the refractive layer visible in the light microscope (Fig. 2 and 3). The cyst wall and the cytoplasmic surface often appear folded, which is probably due to shrinkage during the electron microscopical procedure because the cysts are always spherical when viewed in the light microscope. A schematic representation of the cyst fine structure is given in Fig. 9.

Discussion

Walker and Maugel (1980) suggested two general classifications for hypotrich cysts. Oxytrichid hypotrichs have kinetosome resorbing (KR) and euplotid hypotrichs non kinetosome resorbing (NKR) cysts. These classifications were based on the investigation of the cysts of *Oxytricha fallax* (Grimes 1973), *Stylonychia mytilus* (Walker et al. 1975), *Gastrostyla steinii* (Walker et al. 1980) and *Diophrys scutum* (Walker and Maugel 1980). The latter ciliate is the only representative of the euplotids in this group. Since then, KR cysts of *Laurentiella acuminata* (Gutierrez et al. 1983a, b, Gutierrez and Perez-Silva 1983), *Histriculus muscorum* (Matsusaka 1979, Matsusaka and Kimura 1981, Matsusaka and Hongo 1984), *Histriculus similis* (Calvo et al. 1983) and *Oxytricha bifaria* (Rosati et al. 1984, Verni et al. 1984, Ricci et al. 1985) have been studied in the electron microscope. *Pleurotricha* sp. was already investigated in 1976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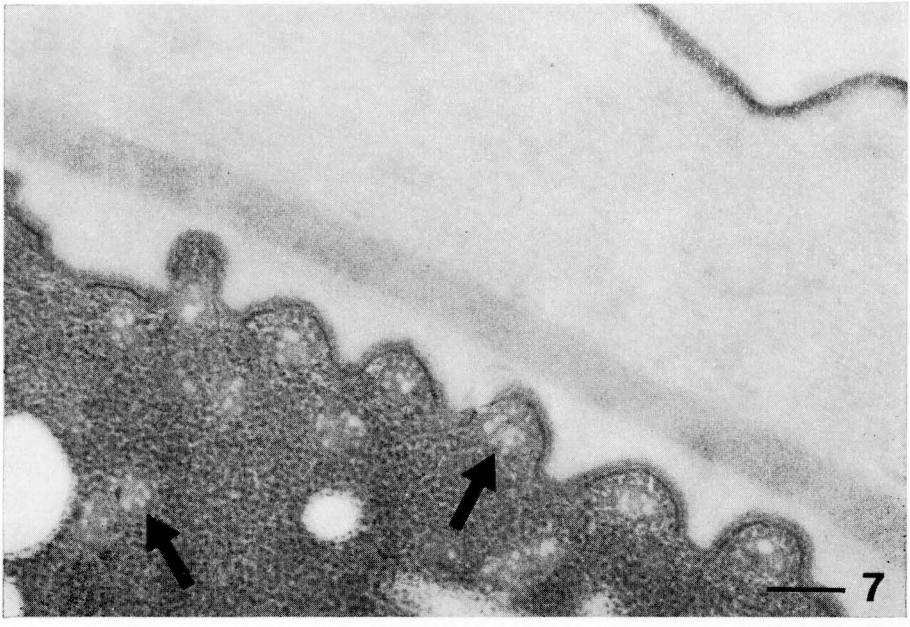
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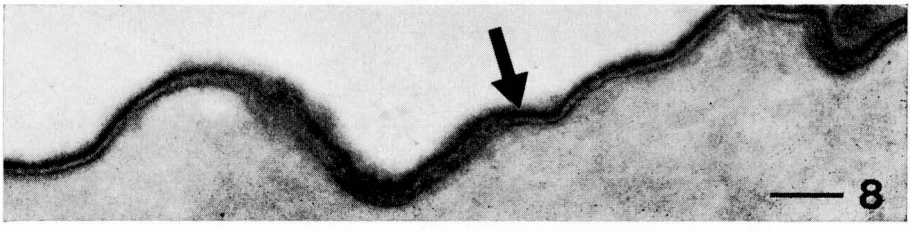
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(Matsusaka). Only one abstract exists of the NKR cyst of *Euplotes iliffei* (Hill et al. 1985). *Pseudourostyla cristata* is considered as a phylogenetic intermediate because the cysts have characters of both groups (Grim and Manganaro 1985).

According to this classification, the *Kahliella simplex* cysts belong to the KR (oxytrichid) type, which is further characterized by for example a 4-layered cyst wall, absence of food vacuole precursors, clustered mitochondria and fused macronuclei. The cyst volume: cell volume ratio of 0.2 and the cyst wall thickness: cyst radius ratio of 0.08 are similar to those given for oxytrichid cysts (0.07—0.15; 0.11 ± 0.02).

A feature which is not in accordance is the presence of subpellicular microtubules. Subpellicular microtubules are also visible in *Oxytricha bifaria* (Verni et al. 1984) and *Pseudourostyla cristata* (Grim and Manganaro 1985). Thus their presence should not be used further as an euplotid character, the more so because microtubules are often difficult to preserve or to detect and could have been overlooked in other oxytrichid cysts. Another oxytrichid feature, the agglomeration of mitochondria was not observed in the cysts of *Laurentiella acuminata* (Gutierrez and Perez-Silva 1983), but this could have been due to the investigation of too young cysts (Verni et al. 1984). Concerning the phylogenetic value of euplotid cyst characters we have to await further studies. The three-layered cyst wall of *Diophrys scutum* (Walker and Maugel 1980) is for example not shared by *Euplotes iliffei* whose cyst wall is said to be composed of only two layers (Hill et al. 1985).

Rather unusual — at least for hypotrich cysts — is the presence of regularly arranged subcortical vesicles and epinuclear vesicles in *Kahliella*. Whether the similarity in size, shape and structure implies also similar functions has to be investigated. The subpellicular vesicles in *Kahliella* are probably not involved in wall formation (Matsusaka 1976, Rosati et al. 1984) because fusion with the cell membrane was never observed during thickening of the granular layer (Foissner and Foissner 1986a).

A release of particles prior to cyst wall formation has also been reported for e.g. *Oxytricha fallax* (Hashimoto 1962) or *Pleurotricha* sp. (Matsusaka 1976). Their disappearance during preparation for electron microscopy suggests that their constituent substances are easily soluble in water and/or alcohol even after or during fixation. These granules are very probably not homologous with those described e.g. in *Stylonychia histrio* where they lie between cytoplasm and cyst wall (Hashimoto 1954).

The last point to be mentioned here is the relatively thick mucus of the cyst of *Kahliella simplex*. Obviously it has the same function (grouping of the cysts, attachment to a substrate) as the jelly ectocyst in e.g. *Oxytricha bifaria* (Ricci et al. 1985). This could also be an explanation for the very thin ectocyst in *Kahliella*.

Fig. 6. Cytoplasmic surface in cross-section. Protrusions are underlined by microtubules (arrow). mi = mitochondrion, V = vacuole with fuzzy content. Bar: 380 nm

Fig. 7. Cytoplasmic surface in non-stained cross-section. Note the substructure of the vesicles (arrows) and the electron dense endocyst. Bar: 270 nm

Fig. 8. Electron dense outer region of the mesocyst and thin ectocyst (arrow). Bar: 310 nm

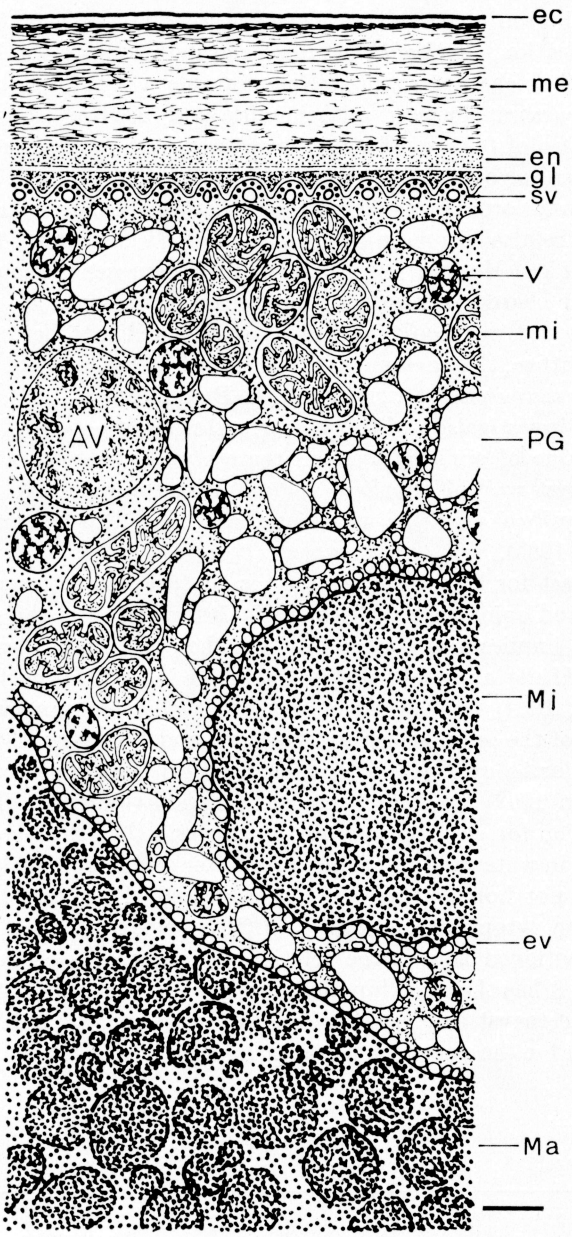

Fig. 9. Schematic representation of the fine structure of the resting cyst of *Kahliella simplex*. ec = ectocyst, en = endocyst, ev = epinuclear vesicles, gl = granular layer, Ma = macronucleus, me = mesocyst, mi = mitochondrion, Mi = micronucleus, sv = subpellicular vesicles. Bar: 500 nm

Zusammenfassung

Die Ruhecysten von *Kahliella simplex* gehören zum oxytrichiden Typ, der vor allem durch die Abwesenheit von Kinetosomen, eine 4schichtige Cystenwand und fusionierte Makronuclei gekennzeichnet ist. Ein abweichendes Merkmal ist die Anwesenheit von subpellikulären Mikrotubuli, das deshalb zur Charakterisierung von euplotiden Cysten nicht mehr verwendet werden darf. Ungewöhnlich ist die Ansammlung von Vesikeln an der Kernoberfläche. Subpelliculäre Vesikel sind in regelmäßigen Reihen angeordnet.

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