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The Fine Structure of the Zoospores of *Fritschiella tuberosa* Iyeng. *(Chaetophorineae, Chlorophyceae)* with Special Reference to the Flagellar Apparatus

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With 24 Figures

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Summary

The fine structure of the quadriflagellate zoospore of *Fritschiella tuberosa* Iyeng. (*Chlorophyceae*) is investigated. The zoospores have a typical ulotrichacean morphology, the flagellar apparatus has been examined in detail and is compared with that found in other filamentous green algae.

The basal bodies are joined together as two pairs in a mirror image relation. The four cruciately arranged flagellar roots are of two types: a two-stranded microtubular root with an associated striated fibril, and a multi-membered microtubular root with a maximum number of 7 to 8 tubules, the number and arrangement of tubules changing in different parts of the cell.

The eye-spot is closely associated with one of the multi-membered roots. The arrangement of root tubules is altered when approaching the eye-spot. The possible functional significance is briefly discussed.

At the flagellar tip the peripheral tubules probably prolong beyond the central tubules, their number decreasing to at least 4 single tubules.

The striking similarities of zoospores of *Fritschiella* and *Stigeoclonium* suggest that despite its highly developed thallus *Fritschiella* is closely related to the genera *Stigeoclonium* and *Draparnaldia* usually placed in the family *Chaetophoraceae*.

1. Introduction

There is a currently increasing interest in the fine structure of reproductive cells of filamentous green algae. The presence or absence of scales on the cell surface and the details of the flagellar apparatus are considered to be of phylogenetic importance (Manton 1965, Moestrup 1972, Mattox and Stewart 1973, Moestrup 1974).

The discovery of scales on the surface of some chaetophoralean swarmers (McBRIDE 1968, MATTOX and STEWART 1973, MOESTRUP 1974) and the presence of multilayered flagellar roots in *Coleochaete* (McBRIDE 1968), *Trentepohlia* (GRAHAM and McBRIDE 1974) and *Chaetosphaeridium* (MOESTRUP 1974) have again raised the question if chaetophoralean-type algae are ancestors of bryophytes and higher plants. However the number of thoroughly investigated zoospores is small.

Fritschiella tuberosa was once believed to be the prototype of a higher plant ancestor (BOWER 1935, FRITSCH 1945) because of its highly differentiated thallus (IYENGAR 1932) and its terrestrial habit. On the other hand cytokinesis and other ultrastructural details of the vegetative cell are very similar to ordinary *Chaetophoraceae* as *Stigeoclonium e.g.* (McBRIDE 1970, STEWART *et al.* 1973).

The present paper describes the fine structure of the quadriflagellate zoospores with special attention to the flagellar apparatus to elucidate the relation of *Fritschiella* to other chaetophoralean algae.

2. Material and Methods

A strain of *Fritschiella tuberosa* Iyeng. was kindly provided by Dr. KOCH (Institute of Plant Physiology, Göttingen). For several years the alga was cultured in a mineral medium in Hamburg (WEBER 1973). Methods for obtaining axenic cultures and mass induction of zoospores have been described elsewhere (MELKONIAN and WEBER 1975).

The material was fixed for one hour in a solution of 1% glutaraldehyde in culture medium at room temperature. The zoospores were then concentrated by mild centrifugation and washed several times in a sterile medium. They were postfixed in culture medium containing 1% osmium tetroxide (one hour). After washing they were embedded in a small drop of agar. Before dehydration by an ethanol series the zoospores where fixed for 20 minutes in a solution of 0.5% uranylacetate. After dehydration they were embedded in Epon.

Sections were cut with a diamond knife on a Reichert ultramicrotome OmU-2 and stained with uranylacetate and lead citrate. The micrographs were taken on the Siemens Elmiskop I.

3. Results

3.1. General

The general morphology of the cell is illustrated by Fig. 1 (longitudinal section) and Fig. 4 (transverse section).

Most of the structures are similar to those of other "ulotrichalean" swarmers (*e.g., Stigeoclonium;* MANTON 1964) and need not be described in detail.

Fig. 1. Longitudinal section through the mature zoospore of *Fritschiella tuberosa* displaying two contractile vacuoles (cV_1 and cV_2), cross sections through two of the four flagella (*f*), "hairy" vesicles (*hv*), mitochondrial profiles (*m*), one nucleus (*N*) with nucleolus (*no*), the large, reticulate chloroplast (*cp*), eyespot (*ey*), pyrenoid (*py*), starch grains (*s*), dictyosomes (*d*), large vacuoles (*v*), rough-surfaced ER (*ER*), sometimes continuous with the nuclear envelope (arrow) and lipid droplets (*L*). The cell boundary is the plasmalemma (*pl*). ×13,000

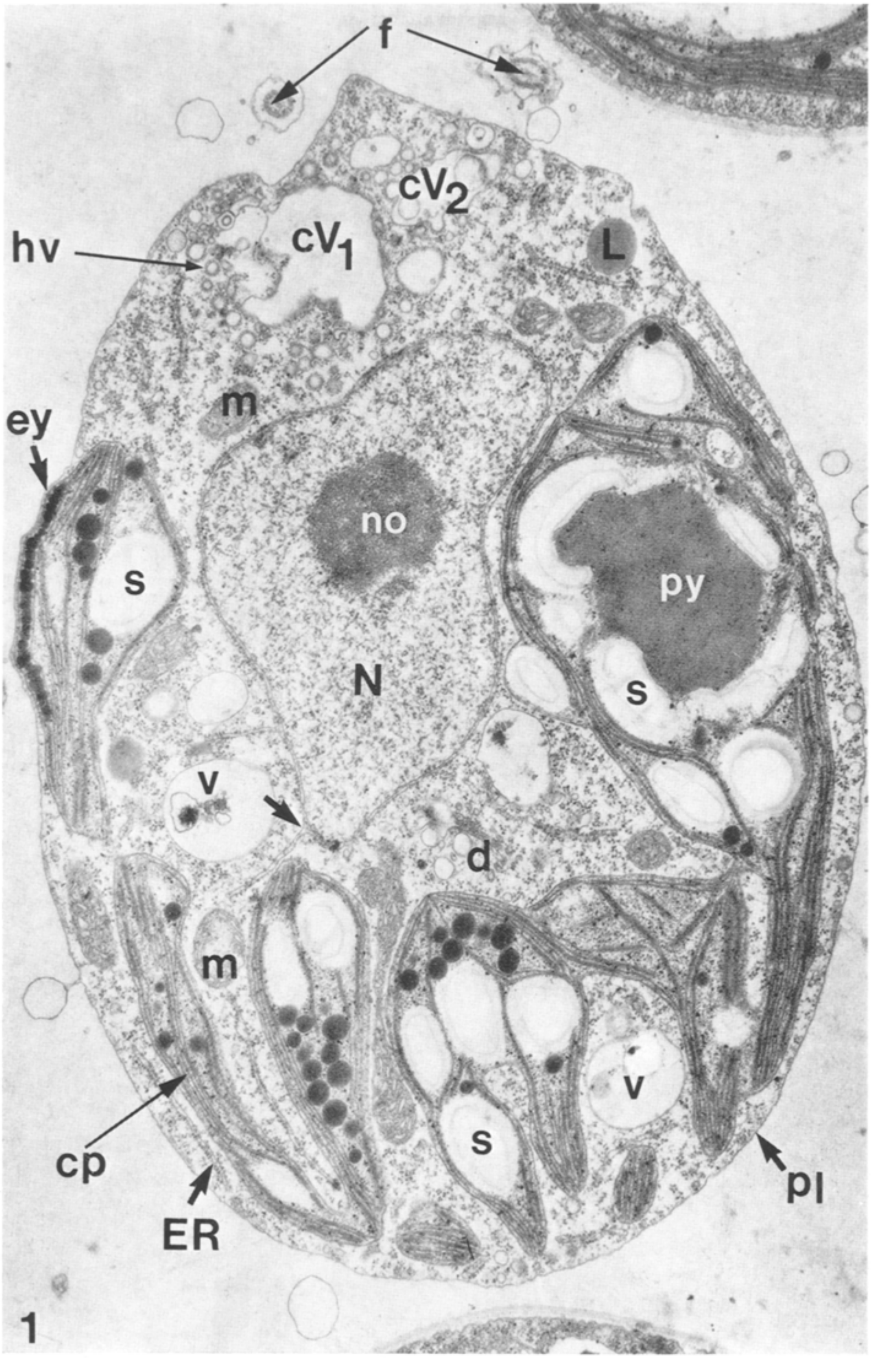


Fig. 1

The section in Fig. 1 is not median, but slightly oblique. Therefore the flagellar apparatus and its basal bodies are not to be seen. The anterior part of the cell contains two contractile vacuoles. They are surrounded by numerous "hairy" vesicles which apparently seem to fuse with the contractile vacuoles. A prominent nucleus containing one nucleolus is situated in the central part of the cell. The nuclear envelope is continuous with rough-surfaced ER, tangential sections through the nuclear envelope have shown a high frequency of nuclear pores.

The posterior part of the cell is dominated by one large, reticulate chloroplast. In a transverse section at the level of the nucleus, the chloroplast forms a peripheral cylinder (Fig. 4). It should be noted that the shape of the chloroplast in the vegetative cell is different depending upon the age of the cell. In young, growing filaments the chloroplast forms a curved plate-shaped body, in the older prostrate system it is reticulate.

Zoospore formation is usually confined to the cells of the prostrate system only, the zooid chloroplast retaining the reticulate shape. When zoospores germinate to form a slender filamentous cell the chloroplast undergoes considerable rearrangement. It is quite interesting that chloroplast microtubules —although present in young growing cells (McBRIDE 1970)—have never been observed in mature zoospores, but were easily identified as soon as the zoospore settled and formed a cell wall.

Constituents of the zoospore chloroplast include a large eyespot (Fig. 1), one large pyrenoid with starch shell and appressed thylakoids, other starch grains distributed throughout the stroma, and osmiophilic globules.

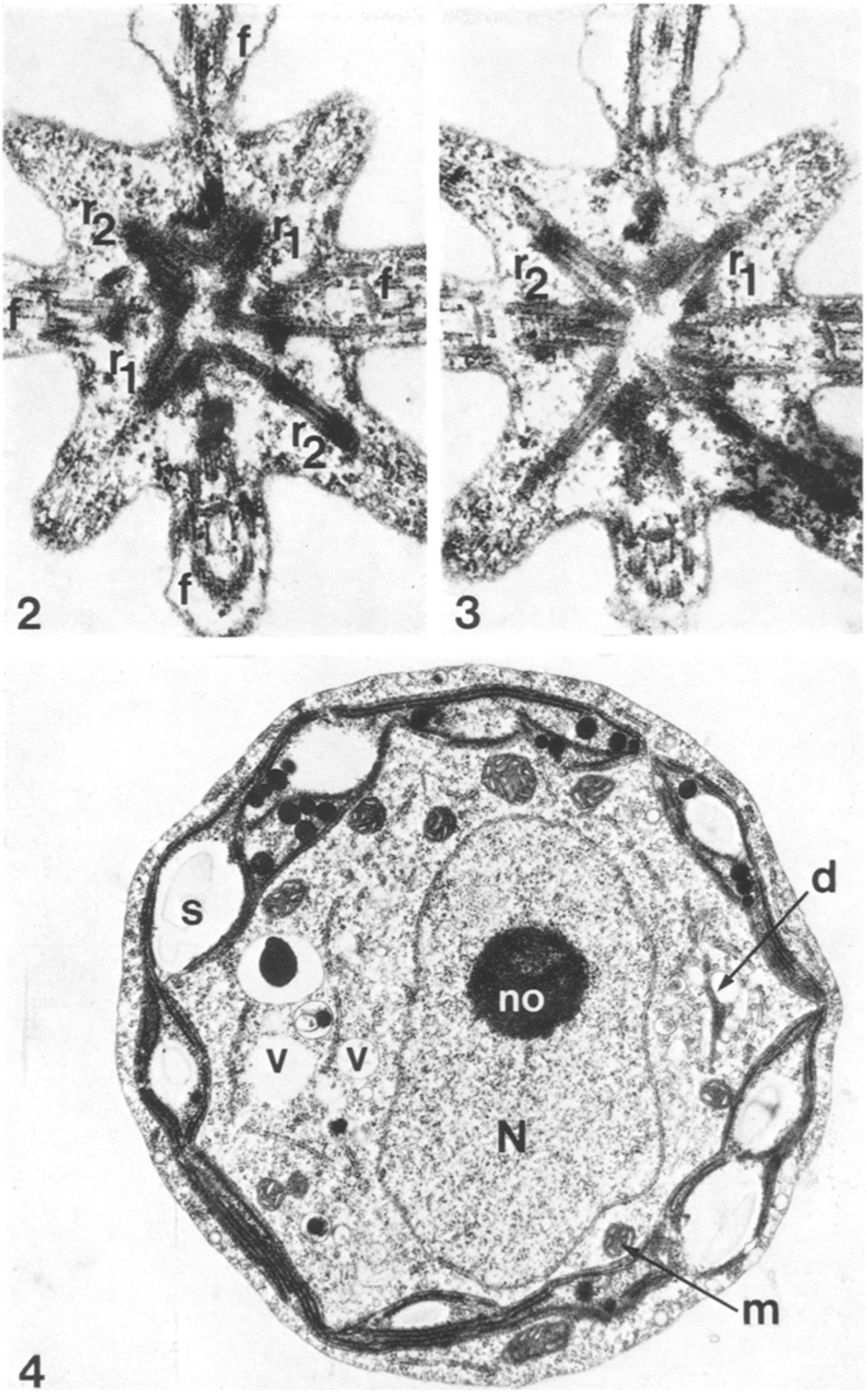
Mention should be made on diverse types of vesicles and small vacuoles. At the tip of the cell vesicles with granular contents probably function in adhesion (Fig. 14). Centrally located near a dictyosome-ER-complex are small vacuoles with dark staining material (Fig. 4).

Sometimes lipid droplets are seen in the anterior part of the cell (Fig. 19). In vegetative senescent cells they are numerous (McBRIDE 1970) and possibly contain secondary carotenoids (WEBER 1975).

The zoospores are naked, body scales have not been observed either in developing or mature zoospores. A microtubular cytoskelet underlies the plasmalemma. The tubules are arranged parallel to the long axis of the cell. From transverse sections through the central part of the cell their number was estimated to be about 70.

Figs. 2 and 3. Transverse sections through the anterior part of the cell. The four flagella (*f*) emerge from gaps between the four projecting ribs, all four flagellar roots are visible, two root types (r_1 and r_2) can be distinguished. ×45,000

Fig. 4. Transverse section through the central part of the cell. v = small vacuoles with dark staining material. For explanation of abbreviations not specially mentioned see legend to Fig. 1. ×13,000



Figs. 2–4

3.2. *Flagella*

The four flagella are of equal length and not covered by scales or hair-like projections. Cross sections of the flagellum show the typical 9 + 2—arrangement of tubules, but in Fig. 6, towards the tip, merely 4 single tubules are found. Serial sections show that the two central tubules terminate earlier than the peripheral doublets. The number of peripheral tubules gradually decreases in the tip region.
The transitional region between the flagellum and its basal body is essentially the same as in most other green algae. The presence of a stellate pattern in the transitional region and a cartwheel pattern at the proximal end of the basal body is confirmed.

3.3. *Flagellar Roots*

The arrangement of flagellar bases and roots can best be seen in transverse sections of the tip of the cell (Figs. 2 and 3).
The flagella emerge from gaps between the four projecting ribs which form a cruciform papilla in the anterior part of the cell. The flagellar bases are two L-shaped pairs joined together in mirror image (Fig. 5) as in other quadriflagellate zoospores (MANTON 1964). Inside the four projecting ribs run 4 flagellar roots alternating with the flagella and therefore cruciately arranged (Fig. 3).
The flagellar bases and roots are connected with each other by electron-dense material, the whole apparatus being continuous. Transverse sections show that the roots are of two kinds (a broad and a two-stranded root; Fig. 3), although their exact nature can only be evaluated by consideration of cross-sections.

Fig. 5. Transverse section through the anterior part of the cell showing the cruciform papilla and the four flagellar bases as two pairs in a mirror image relation. ×45,000

Figs. 6 *a* and *b*. Cross sections through the tip of the flagellum, 4 single tubules are present. ×125,000

Figs. 7–12. Cross sections through proximal and distal parts of the multi-membered root

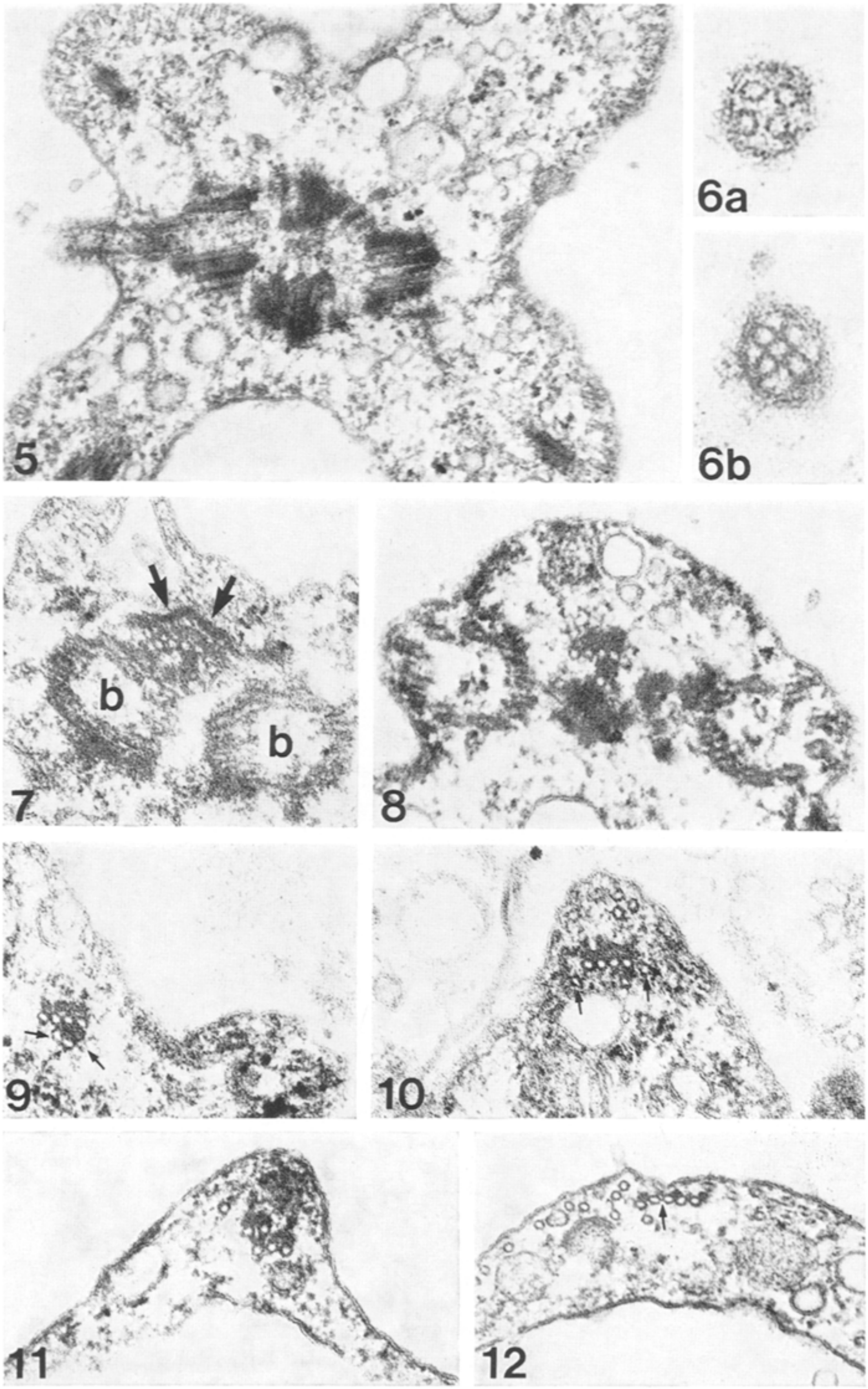
Fig. 7. Origin of multi-membered root between two basal bodies (*b*). At least 7 tubules lying in one layer; above the root tubules "capping material" (arrows). ×67,000

Fig. 8. Root tubules arranged in two layers of 5 + 2. ×62,000

Figs. 9 and 10. Two serial sections through the proximal part of the multi-membered root, the arrangement of tubules changes from 4 + 3 to a 4 + 1 pattern. Two tubules of the second layer leave the root (small arrows). ×60,000

Fig. 11. More distal part of the multi-membered root with 2 + 3 pattern of root tubules. ×62,000

Fig. 12. Arrangement of 5 tubules in one layer, cross bridges linking the tubules can sometimes be seen (small arrow). ×52,000



Figs. 5–12

The broad root shows different numbers and arrangements of microtubules along its length (Figs. 7–12). The root starts between the two basal bodies with 7 or 8 (number varying) tubules accompanied by electron-dense material and forming one row (Fig. 7). Above this row "capping material" connects the two basal bodies. Serial sections through several cells in this region have shown the following general patterns of microtubular arrangement: The tubules arrange themselves in two layers. During this process or after the establishment of two layers the number of tubules decreases to 6 or 5. The following microtubular numbers in the two layers have been observed: 5 + 2 (Fig. 8), 4 + 2, 4 + 3 (Fig. 9), and 4 + 1 (Fig. 10). The fate of the 1–3 tubules being lost remains uncertain, possibly they join the superficial body microtubules. Since Figs. 9 and 10 are serial sections it can be supposed that the two outermost tubules of the second layer are withdrawn from the root. If 5 tubules remain, in the distal part of the cruciform papilla they are often arranged in a 2 + 3 pattern (Fig. 11).

At the transition between papilla and the rest of the cell the tubules take a superficial position and further rearrangements take place. The 2 + 3 (or 4 + 2) arrangement is lost, the 2 tubules of one layer join the other 3 (or 4). Eventually 5 or 6 tubules lie close together in one layer just beneath the plasmalemma (Fig. 12). In transverse section through the anterior part of the cell this multi-membered root can easily be detected. The further fate of this root will be traced in connection with the description of the eyespot.

The two-stranded root consists of a pair of microtubules covered by over- and underlying electron-dense material (Fig. 16). The underlying material is cross-banded when viewed in longitudinal section (Fig. 13). This striated fibril bifurcates at the basal bodies (Fig. 13), each part being connected with

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Figs. 13–19. Various aspects of the two-stranded flagellar root

Fig. 13. Slightly oblique section through the anterior part of the cell, both root types (r_1 = two-stranded root, r_2 = multi-membered root) can be seen, the striated fibril (*sf*) of the two-stranded root bifurcates at the basal bodies (arrow). *mt* = superficial body microtubules as part of the cytoskelet. ×36,000

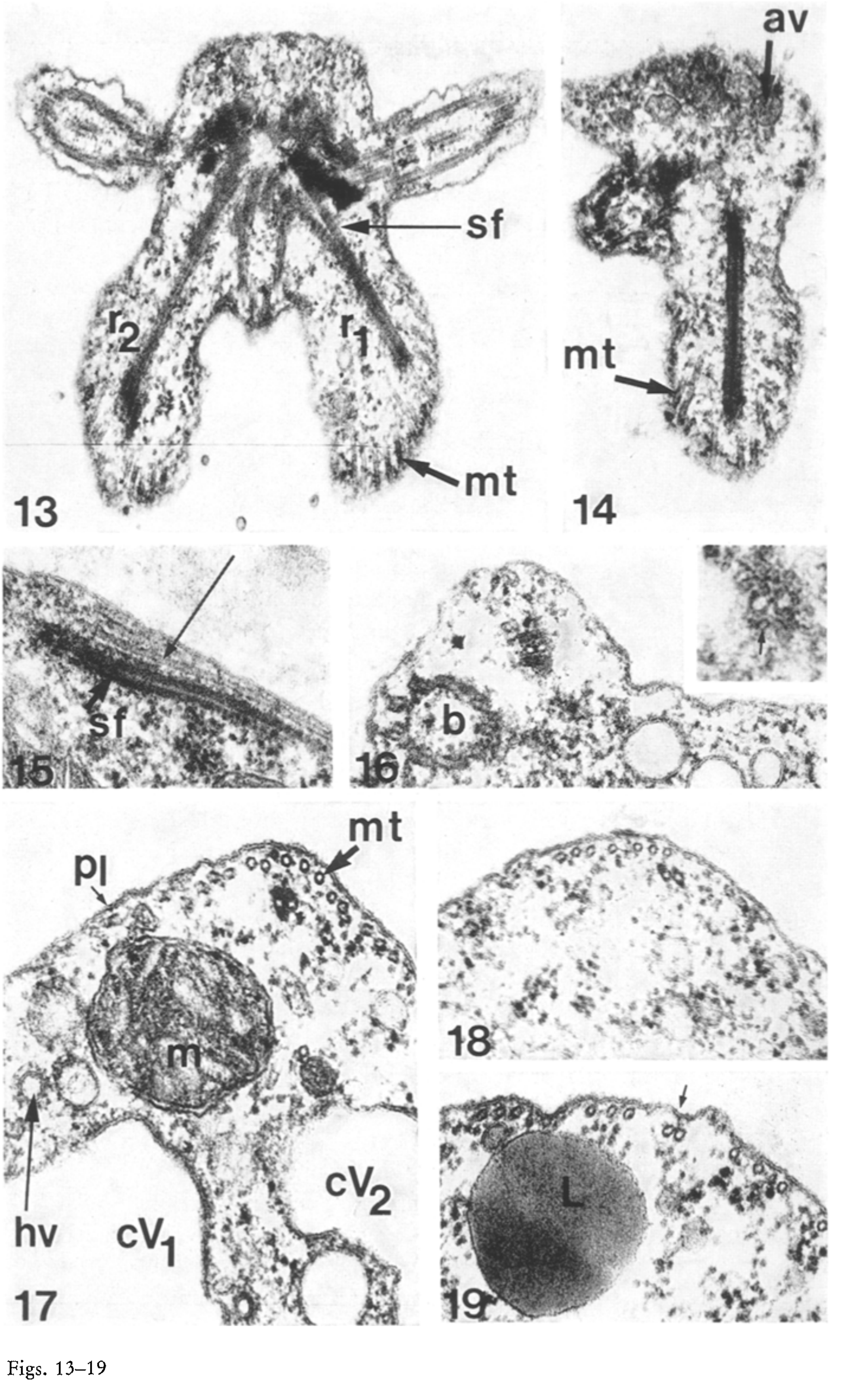
Fig. 14. Another oblique section showing the microtubular pair of the two-stranded root. *av* = "adhesive" vesicle. ×40,000

Fig. 15. Longitudinal section through the two-stranded root in a plane perpendicular to that of Figs. 13 and 14. The root consists of a striated fibril (*sf*), which is perhaps two-layered and the microtubular pair (arrow). ×70,000

Figs. 16–19. Cross sections through the two-stranded root in different parts of the cell

Fig. 16. Cross section through the root near basal body (*b*), the inset shows details of the striated fibril in cross section (arrow). ×63,000 and ×120,000

Figs. 17–19. Represent serial sections through the distal extremities of the two-stranded root, in Fig. 19 the root tubules are almost indistinguishable (arrow) from the superficial body tubules. ×60,000



Figs. 13–19

either of the basal bodies. Distally the striated fibril joins the tubules for a considerable length and then gradually disappears (Fig. 15).

In the cruciate papilla the cytoskeletal microtubules are present in the ribs only (Figs. 13 and 14), they originate at the tip of the cell and take a more superficial position in the ribs than the roots. In the transitional region between the cruciate papilla and the main cell body the root tubules lie just beneath the plasmalemma (Fig. 19) and although the two tubules are joined together they are virtually indistinguishable from the cytoskeletal tubules (Fig. 19). Figs. 17–19 show serial cross-sections through the distal part of the two-stranded root.

3.4. Eyespot

The large ellipsoid eyespot of the *Fritschiella* zoospore consists of one row of globules arranged in hexagonal close packing (Fig. 21). The number of globules per eyespot is about 150–200. Some of the globules of one eyespot do not stain as strong as others, a phenomenon which has been observed in other algae as well. The eyespot protrudes from the cell surface, the plasmalemma overlying the eyespot does not show modifications (Figs. 20 and 24).

The most interesting observation regarding the eyespot is its clearly defined position in the cell. It is always associated with the multi-membered root (Fig. 23). As serial section show the 5 or sometimes 6 tubules lying near the eyespot are the distal extremities of the multi-membered root. A structural contact between the root tubules and the outer plastid membrane is not ruled out, although difficult to prove (Fig. 23).

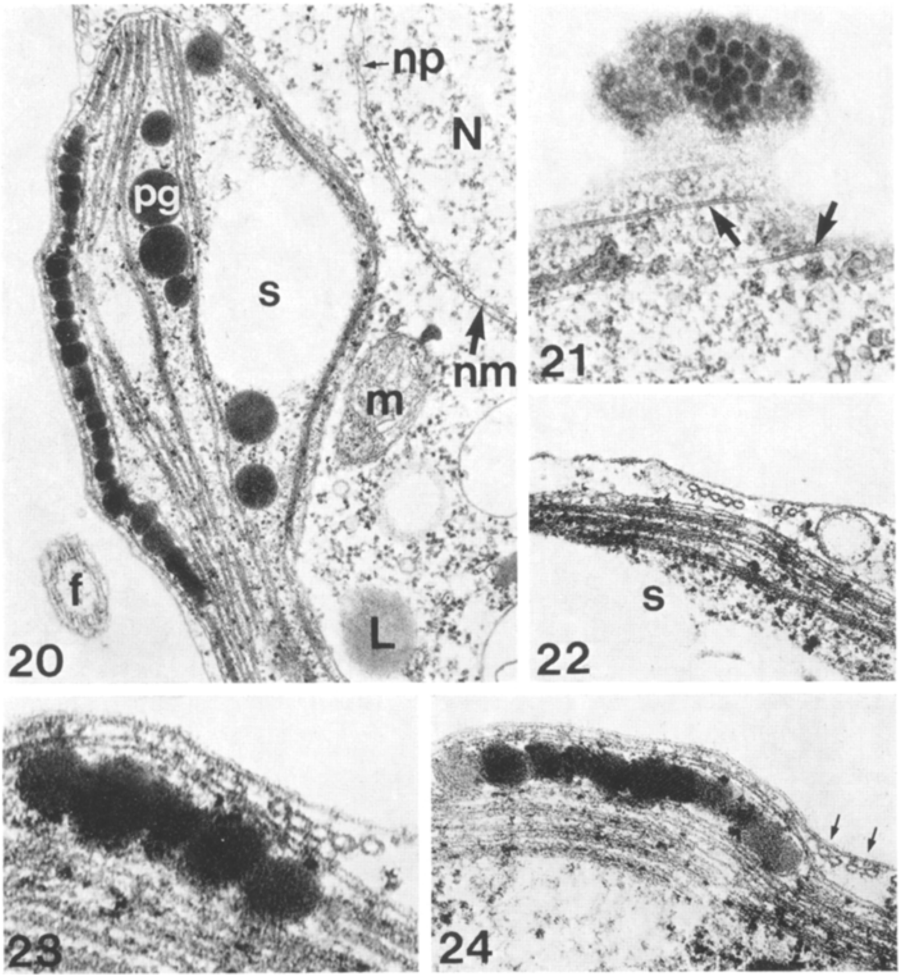
The multi-membered root is disorganized distally from the eyespot. The 5 or 6 tubules dismember, they often reveal their former arrangement when two and three tubules remain together (Fig. 24). At least only single tubules appear in cross sections.

4. Discussion

It has recently become evident that filamentous green algae belong to two evolutionary series, and probably evolved from two distinct types of motile unicellular ancestors (for review see PICKETT-HEAPS 1972, PICKETT-HEAPS and MARCHANT 1972).

Motile cells of both series are mainly distinguished by the type of flagellar root they possess. One series shows a complex multilayered root (the so called multi-layered-structure [MLS] or "Vierergruppe"), the other one has four cruciately arranged microtubular roots.

MLS's have been observed in gymnosperms, ferns, bryophytes, *Charophyceae* and *Chaetophorineae* with considerable differences regarding numbers of layers and tubular numbers.



Figs. 20–24. Eyespot ultrastructure and association between root tubules and eyespot

Fig. 20. Longitudinal section through eyespot. *pg* = Plastoglobuli, *nm* = nuclear membrane, *np* = nuclear pore. ×38,000

Fig. 21. Tangential section of eyespot showing hexagonal close packing of eyespot globules and superficial microtubules (arrows). ×34,000

Fig. 22. Transverse section through the cell at the level of the chloroplast. Root tubules of the multimembered root can easily be detected. ×50,000

Figs. 23 and 24. Cross sections through eyespot displaying close relationships between eyespot and multi-membered flagellar root

Fig. 24. Cross section through posterior part of the eyespot. The root tubules start to dismember, 2 and 3 respectively are still linked together (arrows). ×60,000

In filamentous algae with cruciately arranged roots there are further differences especially concerning the microtubular number. Some of these algae apparently have four equally numbered roots [*Schizomeris* (BIRKBECK *et al.* 1974), *Urospora* (KRISTIANSEN 1974)], others have two types of roots with unequal numbers of tubules [*Draparnaldia*, *Chaetomorpha* (MANTON *et al.* 1955), *Stigeoclonium* (MANTON 1964), *Ulothrix* (MANTON 1965), *Microthamnion* (WATSON and ARNOTT 1973)]. In the latter group one root type is always two-stranded, while the other one has more than two tubules, their number varying from 3 (*Chaetomorpha*) to 6 (*Microthamnion*). In the *Microthamnion* zoospore, however, there is a striated fibril not associated with a root, together with other characters of the vegetative cell the fact just mentioned separates *Microthamnion* from other *Chaetophorineae*.

The *Fritschiella* zoospore shows a flagellar root-type identical with that found in *Stigeoclonium* and *Draparnaldia*. The two-stranded root is accompanied by a striated fibril, which seems to be a constant feature of this root-type, although its presence has only been proved in the *Stigeoclonium* zoospore (MANTON 1964).

The number of tubules in the multi-membered root of the *Fritschiella* zoospore is variable depending upon the part of the cell under consideration. The designation of a definite tubular number to the multi-membered root at least in *Fritschiella* is questionable and this may prove to be true in other algae of the same root-type. On the other hand a definite pattern of tubular arrangement in the multi-membered root can be stated, while an explanation to this phenomenon cannot be given at present.

An ultrastructural comparison of zoospores shows that despite its highly developed thallus *Fritschiella tuberosa* has close affinities to algae like *Stigeoclonium;* therefore, the suggestion of STEWART *et al.* (1973) to place *Fritschiella* together with *Stigeoclonium*, *Draparnaldia*, *Chaetophora* and others in the family *Chaetophoraceae* may probably be accepted.

The internal structure of the flagellar tip in motile algal cells has received very little attention, although a probable phylogenetic importance was already suggested (MANTON 1965). In the unicellular algae studied in this respect the central tubules of the flagellum project into a tip, while the peripheral tubules terminate earlier with gradually decreasing number (MANTON 1959, RINGO 1967). In the *Fritschiella* zoospore probably the reverse is true, the peripheral fibers are prolonged beyond the central strands, their number gradually decreases.

A similar type of flagellar tip has recently been observed in the *Vaucheria* zoospore (OTT and BROWN 1974), and perhaps is as well present in spermatozoids of mosses (MANTON 1965). So far a great diversity of flagellar tip structure is displayed, and further studies are needed in a phylogenetic approach.

The function of the eyespot in the *Chlorophyceae* sensu CHRISTENSEN is not known. There is no obvious structural relation to the flagellar apparatus as in several other algal classes (for review see DODGE 1969).

In the *Fritschiella* zoospore the position of the eyespot in the cell can be related to one of the flagellar roots, the multi-membered root. Although a structural contact between the root tubules and the outer plastid membrane could not be established this possibility cannot be discarded since both structures closely approach each other. In this respect it is important to notice that the root tubules are characteristically arranged in the proximity of the eyespot—they lie in one layer of 5 or 6 tubules linked together, an arrangement not present in the cruciform papilla and immediately given up in the posterior part of the cell. In cross sections through the anterior part of the cell only one of the multi-membered roots can be seen in this band-like arrangement.

While electron microscopy taken by itself will certainly not solve the question of eyespot function, the possible involvement of flagellar roots either in transmitting a stimulus from the eyespot to the basal bodies or simply in determining eyespot position during zoosporogenesis should be taken into consideration.

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