

## Reconstruction of the Flagellar Apparatus and Microtubular Cytoskeleton in *Pyramimonas gelidicola* (Prasinophyceae, Chlorophyta)

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### Summary

The absolute configuration of the flagellar apparatus in *Pyramimonas gelidicola* MCFADDEN *et al.* has been determined and shows identity with *P. obovata*, indicating that they are closely related. Comparison with the flagellar apparatus of quadriflagellate zoospores from the more advanced *Chlorophyceae* suggest that *Pyramimonas* may be a primitive ancestral form. The microtubular cytoskeleton has been examined in detail and is shown to be unusual in that it does not attach to the flagellar apparatus. Cytoskeletal microtubules are nucleated individually, and this is interpreted as an adaptation to the methods of mitosis and scale deployment. In view of the primitive nature of these processes, it is proposed that this type of cytoskeletal organization may represent a less advanced condition than that of the flagellar root MTOCs (microtubule organizing centers) observed in the *Chlorophyceae*.

**Keywords:** Flagellar apparatus; Microtubular cytoskeleton; Phylogeny; *Prasinophyceae*; *Pyramimonas*.

### 1. Introduction

Evidence accumulated from comparative ultra-structure indicates that there are at least two main streams of evolution within the green algae: 1. the *Charophyceae*, which gave rise to the first land plants and 2. the *Chlorophyceae* (hereafter *sensu* STEWART and MATTOX 1978) containing many of the modern green algae (PICKETT-HEAPS 1975, STEWART and MATTOX 1975). It is now generally suggested that these and any further evolutionary streams diverged from the more

primitive, heterogeneous group of scaly green monads that comprise the *Prasinophyceae* Christensen ex Silva (MANTON 1965, NORRIS 1980, STEWART and MATTOX 1978, MOESTRUP and ETTL 1979, MELKONIAN 1982a, MOESTRUP 1982). Recently, the prasinophyte *Mesostigma viride* has been shown to have characteristics aligning it with both the *Charophyceae* (ROGERS *et al.* 1981, MELKONIAN 1983) and the *Chlorophyceae* (MELKONIAN 1983) indicating that it is probably similar to the ancestral flagellate from which the two major streams of evolution diverged. *Mesostigma* is closely related to another genus of the *Prasinophyceae*, *Pyramimonas* (MANTON and ETTL 1965), which could have arisen by a doubling of the two flagella in *Mesostigma* to become quadriflagellate like *Pyramimonas* (ROGERS *et al.* 1981). *Pyramimonas* could then have given rise to the *Chlorophyceae* by the loss of the scales and flagellar pit (characteristic of the *Prasinophyceae*) and the development of a cell wall and phycoplast cytokinesis (characteristic of the *Chlorophyceae*) (MELKONIAN 1982a, STEWART and MATTOX 1978).

A recent study of the flagellar apparatus in the quadriflagellate *Pyramimonas obovata* (MELKONIAN 1981) indicates that its structure shows identity with that of the quadriflagellate swimmers in the Chlorophycean alga, *Ulothrix belkae* (FLOYD *et al.* 1980). This identity of the flagellar apparatuses is interpreted as a direct phylogenetic link between *Pyramimonas* and certain *Chlorophyceae* that produce quadriflagellate swimmers. This hypothesis is

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supported by the fact that the flagellar apparatus in *Hafniomonas reticulata*—a quadriflagellate monad originally placed in the genus *Pyramimonas*, but now known to have Chlorophycean characteristics (ETTL and MOESTRUP 1980)—is highly comparable to both *U. belkae* and *P. obovata* and probably represents an intermediate form (MELKONIAN 1981).

While comparison of conservative features such as the structure of the flagellar apparatus and cytokinetic mechanisms has provided much information on phylogenetic linkages within the green algae, little attention has been given to the cytoskeleton. CAVALIER-SMITH (1982) has presented an hypothesis for the origin of microtubular roots in the original flagellate, but to our knowledge there have been no hypotheses on the origin of additional cytoskeletal elements. In quadriflagellate zoospores of several Chlorophycean algae (e.g., *Schizomeris*, BIRKBECH *et al.* 1974 and FLOYD and HOOPS 1980; *Fritschiella*, MELKONIAN 1975; *Stigeoclonium*, MANTON 1964; *Ulothrix belkae*, FLOYD, personal communication) there exists a sub-plasmalemmal microtubule array that originates from the microtubular flagellar roots and probably serves a cytoskeletal function (*cf.*, MELKONIAN *et al.* 1980). In *Polytomella*, a colorless Chlorophycean quadriflagellate, it has been shown that an accumulation of amorphous, osmiophilic matter on the flagellar roots acts as an MTOC (microtubule organizing center) exerting spatial control over the initiation of the microtubule array (BROWN *et al.* 1976, STEARNS and BROWN 1981).

In *Pyramimonas* a microtubular cytoskeleton has been reported in several species (MANTON 1966, 1968, MOESTRUP and THOMSEN 1974, NORRIS and PEARSON 1975, MARCHANT 1982) but there has been no detailed study on the origin, distribution and orientation of these microtubules. It did not seem likely that the microtubular cytoskeleton in *Pyramimonas* could be nucleated from an MTOC on the flagellar roots, as this system would portend difficulties during the processes of cytokinesis and scale deposition. The nature of the above processes in *Pyramimonas* suggested that the nucleation sites were more likely to show less inter-organization and perhaps be individual—not attached to a flagellar root. TUCKER (1979) has proposed that there may exist (in some unknown organism) individual, “free” nucleating elements (NEs) that generate a single microtubule. In this paper we describe the cytoskeleton in *Pyramimonas gelidicola* and report that each microtubule is nucleated individually. This phenomenon probably relates to the methods of

cytokinesis and scale production in this organism. Since comparison of the flagellar apparatuses indicates that *Pyramimonas* is antecedent to certain quadriflagellate Chlorophycean zoospores, we present reasons to suggest that the type of cytoskeleton observed in *Pyramimonas* represents a less advanced condition than that of some *Chlorophyceae*.

## 2. Materials and Methods

Cells of *Pyramimonas gelidicola* were grown, fixed and prepared for thin section electron microscopy as described by MCFADDEN *et al.* (1982). The absolute configuration was determined as outlined by FLOYD *et al.* 1980. Specimens for freeze-fracture were unfixed and without cryoprotectant, frozen in Freon 22 and then stored in liquid nitrogen prior to preparation in Balzer's 300 freeze-fracture apparatus. Specimens were fractured, but not etched, then shadowed with platinum/carbon from 45° and a carbon support film. Replicas were cleaned in concentrated sulphuric acid, followed by bleach and examined with a double tilt goniometer stage in a Siemens Elmiskop 102.

## 3. Results

The general ultrastructure of *Pyramimonas gelidicola* and details of the scale coverings has been previously reported (MCFADDEN *et al.* 1982, MCFADDEN and WETHERBEE 1982a). The number and spatial relationship of the cell organelles is consistent (MCFADDEN and WETHERBEE 1982b) and this allows for proper orientation when viewing random sections. We have adopted the definitions of cell symmetry and flagellar apparatus terminology proposed by MELKONIAN (1980, 1981) since they are appropriate to this study.

Cells are 14–18 µm long with four scaly flagella, approximately 15 µm long, inserted in an apical pit (Fig. 1). The axoneme microtubules display the usual 9 + 2 configuration (Figs. 2 and 3), and there is a transition region where it connects to the basal bodies (Figs. 4–8). No twisting of the central pair was observed (Fig. 2). Details of the transition zone and basal bodies are, for the most part, in agreement with those described for *Pyramimonas parkeae* (NORRIS 1980). There is a transitional helix (coiled fiber) distal to the termination of the central pair (Figs. 4 and 8) and a stellate pattern proximally (Fig. 5). The basal body triplets show increasing imbrication proximally, eventually forming the cartwheel pattern (Figs. 6 and 7). We have observed a discontinuous osmiophilic core within the basal body, with fibrous spokes radiating to each triplet (Figs. 6 and 8).

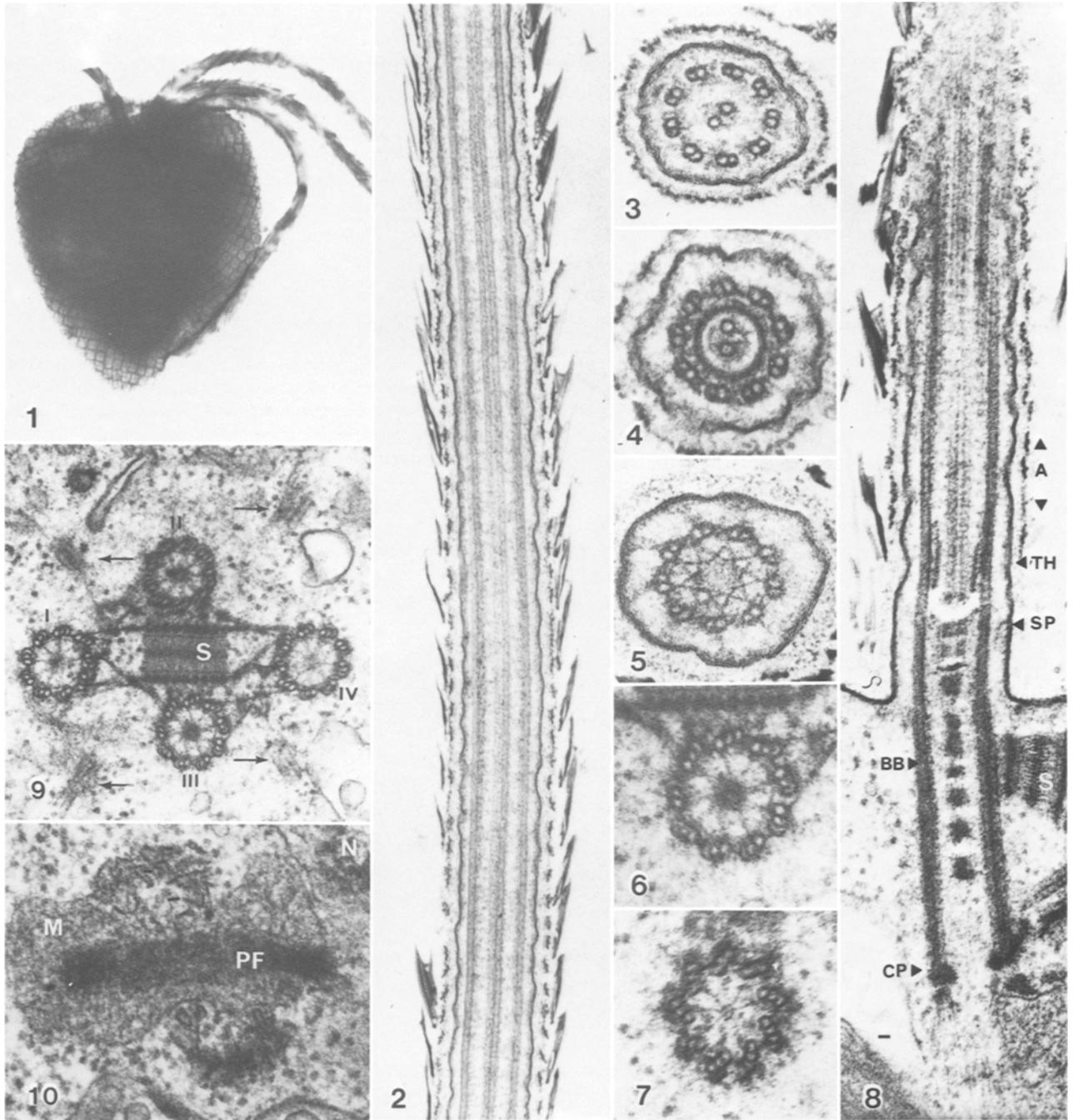


Fig. 1. Whole mount of *Pyramimonas gelidicola* stained in aqueous uranyl acetate, showing cell shape, four scaly flagella inserted in an apical pit and a periplast of box scales on the cell surface.  $\times 4,000$

Fig. 2. Longitudinal section (LS) of flagellar axoneme showing the arrangement of microtubules and the straight central pair.  $\times 40,000$

Fig. 3. Transverse section (TS) of axoneme showing 9 + 2 arrangement of flagellar tubules (corresponds to region A in Fig. 8).  $\times 93,000$

Fig. 4. TS of transitional helix or coiled fiber (corresponds to TH in Fig. 8).  $\times 112,000$

Fig. 5. TS of stellate pattern which corresponds to level SP in Fig. 8.  $\times 90,000$

Fig. 6. TS of basal body showing arrangement of triplets and osmiophilic core (corresponds to BB in Fig. 8).  $\times 88,000$

Fig. 7. TS of cartwheel pattern at most proximal end of basal body (see CP in Fig. 8).  $\times 88,000$

Fig. 8. LS of flagellum (II) showing cartwheel pattern (CP), basal body (BB) with synistosome (S) attached, stellate pattern (SP), transitional helix (TH) and axoneme (A).  $\times 60,000$

Fig. 9. TS of basal bodies (numbered) showing arrangement of connecting fibers including synistosome (S) and cruciate arrangement of four microtubular roots (arrows) (corresponds to level BB in Fig. 8).  $\times 53,000$

Fig. 10. TS at the most proximal end of basal bodies showing major proximal connecting fiber (PF), microbody (M) and nucleus (N).  $\times 60,000$

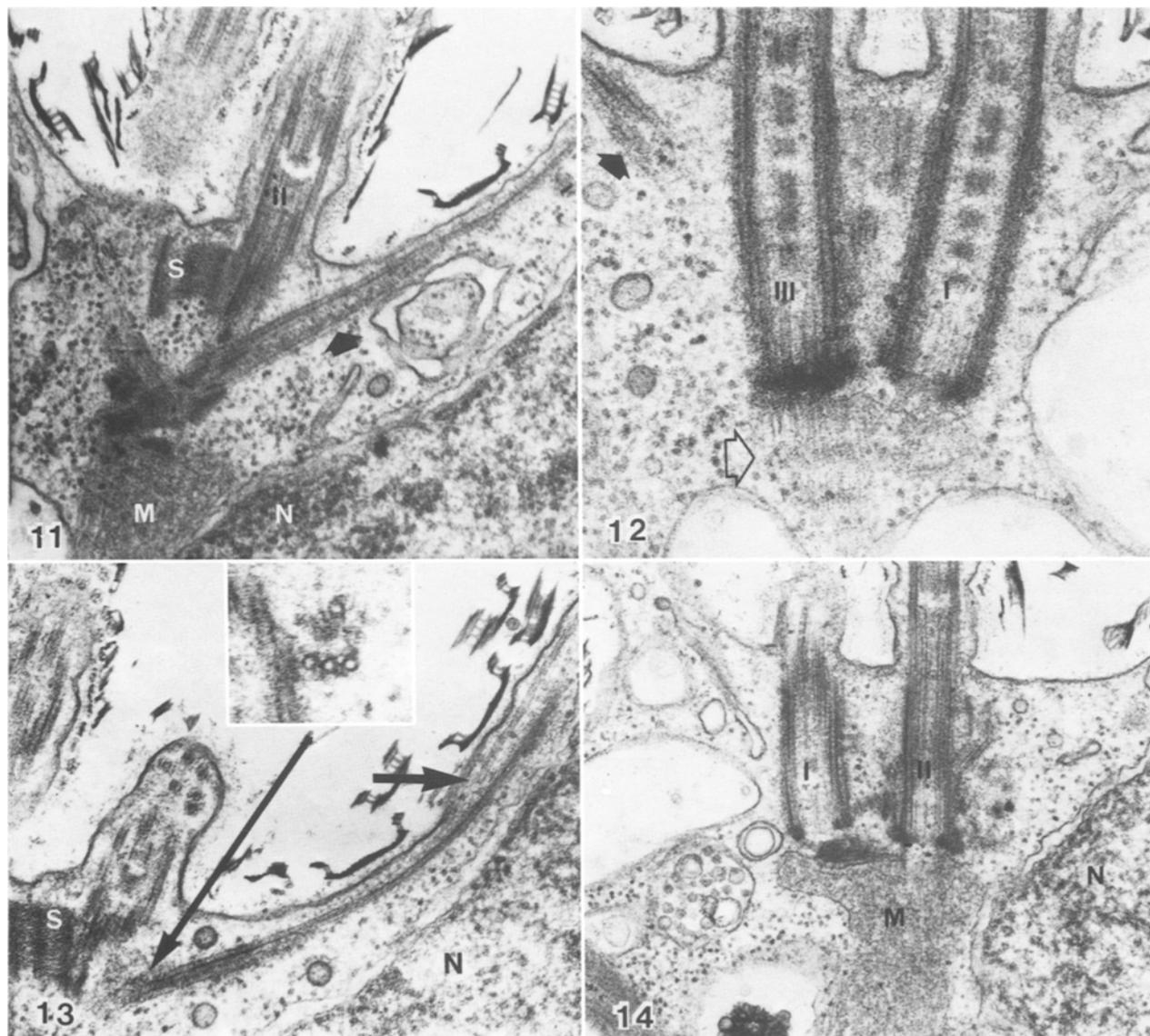


Fig. 11. Oblique LS through flagellum *II* showing synistosome (*S*), microbody (*M*), nucleus (*N*) and the right, ventral, four-stranded root (arrow).  $\times 47,000$

Fig. 12. LS of flagella *III* and *I* showing system II fibers (open arrow) attached to basal body *III*. The left, dorsal, four-stranded root is also visible (arrow).  $\times 65,000$

Fig. 13. LS of right, ventral, four-stranded root. A cross-section of this root is shown in the inset ( $\times 90,000$ ). One cytoskeletal microtubule can be seen originating remote from the root (short arrow).  $\times 50,000$

Fig. 14. LS of flagella *I* and *II* showing nucleus (*N*) and microbody (*m*) situated beneath the basal bodies.  $\times 35,000$

The four basal bodies are set in a trapezoidal pattern and two sets of fibers (proximal and distal) interconnect the basal bodies directly or indirectly (Fig. 9). The connecting fibers are very similar to those of *Pyramimonas obovata* (MELKONIAN 1981) and need not be described in detail here. Since they extend approximately 200 nm into the cell (Figs. 8, 11, and 13) the connecting fibers apparently take the form of sheets with the major part of the distal connecting fibers being

a block, termed the synistosome (NORRIS and PEARSON 1975). The synistosome measures some  $260 \times 175$  nm with a dark 30 nm band in the center (Fig. 9). There is a prominent proximal fiber connecting basal bodies *I* and *IV* with no visible striation pattern (Fig. 10).

The flagellar roots comprise a cruciate 4-2-4-2 microtubular system and two system II fibers (rhizoplasts) (Figs. 9, 11-13, and 15). System I fibers were not observed. The microtubular roots ascend from

between the basal bodies and underlie the cytoplasmic microtubules in the flagellar pit (Fig. 13). The four stranded roots display the usual 3 over 1 configuration proximally (Fig. 13, inset). All roots terminate distally in a small amorphous area of electron dense material within the flagellar pit region (Fig. 13).

The position of the roots relative to the other organelles is consistent. The left, ventral two stranded root shows an association with the opening of the scale reservoir. A fibrous band connects this root to a circular fibrous investment surrounding the reservoir opening. None of the microtubular roots was observed to contact the eyespot or chloroplast envelope.

The system II fibers (rhizoplasts) originate from the proximal ends of basal bodies II and III and ensheath the microbody (Figs. 11 and 12). The system II fibers are relatively fine and indistinct but display a striation periodicity of 100 nm at their proximal end (Fig. 12). The microbody is directly beneath basal bodies I and II with an anterior protrusion around basal body IV (Figs. 11 and 14). The proximal portion of the microbody is adjacent the nucleus (Fig. 14) and the distal portion projects into the pyrenoid, but no connections between these organelles were observed (*cf.*, Fig. 11, MCFADDEN *et al.* 1982). A diagrammatic reconstruction of the flagellar apparatus is given in Fig. 15.

The cytoplasmic microtubule network comprises between 70 and 80 microtubules that run roughly parallel (Figs. 16–24, 26, and 27). Each microtubule originates individually in a small amorphous mass of electron dense material (Figs. 16, 21–25). The microtubule endings do not display any inter-organization or connect to any cytoplasmic structure, although occasionally coated vesicles were observed close to microtubules. In the flagellar pit the microtubules are spaced at intervals of ca. 140 nm just beneath the plasmalemma (Fig. 16). The microtubules transcend the edge of the flagellar pit spreading to a distance of 200–250 nm at the anterior end (Fig. 17). The network curves around the rim of the pit and runs down the outside of the cell between the plasmalemma and chloroplast (Fig. 18) making a complete 180° turn in the space of 1 µm.

The number of microtubules on the outside exactly equals the number in the pit for each cell (excluding flagellar roots), and the ratio of outer and inner circumferences is approximately equal to the ratio of the distances between the microtubules in the two regions. In thin sections glancing the outside of the cell the microtubules are seen to be closely associated with

the chloroplast envelopes, lying in grooves in these membranes (Fig. 26). In freeze-fracture replicas of the outer chloroplast envelope, this groove can be seen clearly (Figs. 19, 20, 28, and 29). The grooves in the E fracture face are approximately 45 nm wide (Fig. 20) and the corresponding bumps in the P fracture face are approximately 35 nm wide (Fig. 19), which would account for the microtubular diameter and the membrane leaflet around it. The bumps in the P fracture face of the outer chloroplast membrane often exhibit a fibrillar substructure with 5–6 longitudinal fibers averaging 7 nm in thickness (Fig. 28). Fracture faces at the posterior end, where the cell tapers to become conical, show microtubules in this region converging and some microtubules terminate on or very near to others, thereby maintained the spacing between microtubules (Fig. 29). A pattern of large square impressions (ca. 300 nm square) from the external layer of box scales can be seen in most fracture faces (Figs. 19 and 20). Fracture faces of the plasmalemma, though rarely observed, showed square impressions of the box scales but no microtubular impressions.

#### 4. Discussion

The absolute configuration of the flagellar apparatus in *Pyramimonas gelidicola* appears identical to that of *P. obovata* (MELKONIAN 1981) and *P. orientalis* (MOESTRUP and THOMSEN 1974) indicating that these species are closely related. Comparisons of organelle structure and fine details of the small scale varieties lead MELKONIAN and ROBENEK (1981) to propose that *P. obovata* and *P. orientalis* belong together in a subgroup of the genus, which should now include *P. gelidicola*. This proposal is supported by the fact that *P. obovata*, *P. gelidicola* (see MCFADDEN and WETHERBEE 1982 a) and *P. orientalis* (MCFADDEN, unpublished) all possess footprint scales. The connecting fibers between the four basal bodies of *Pyramimonas gelidicola* appear completely asymmetrical. The asymmetry of the flagellar apparatus is further compounded by the association of one microtubular root with the duct opening of the scale reservoir. While this association was not reported for *P. obovata* (MELKONIAN 1981), it was observed by MANTON (1966, 1968), in *P. amyliifera* and *P. tetrahyncus*, who suggested that the root may play some role in deploying the scale reservoir outlet. Although the significance of this association is still not entirely clear, it seems to be a further instance of a functional role for microtubular roots (*cf.*, MELKONIAN 1982 b).

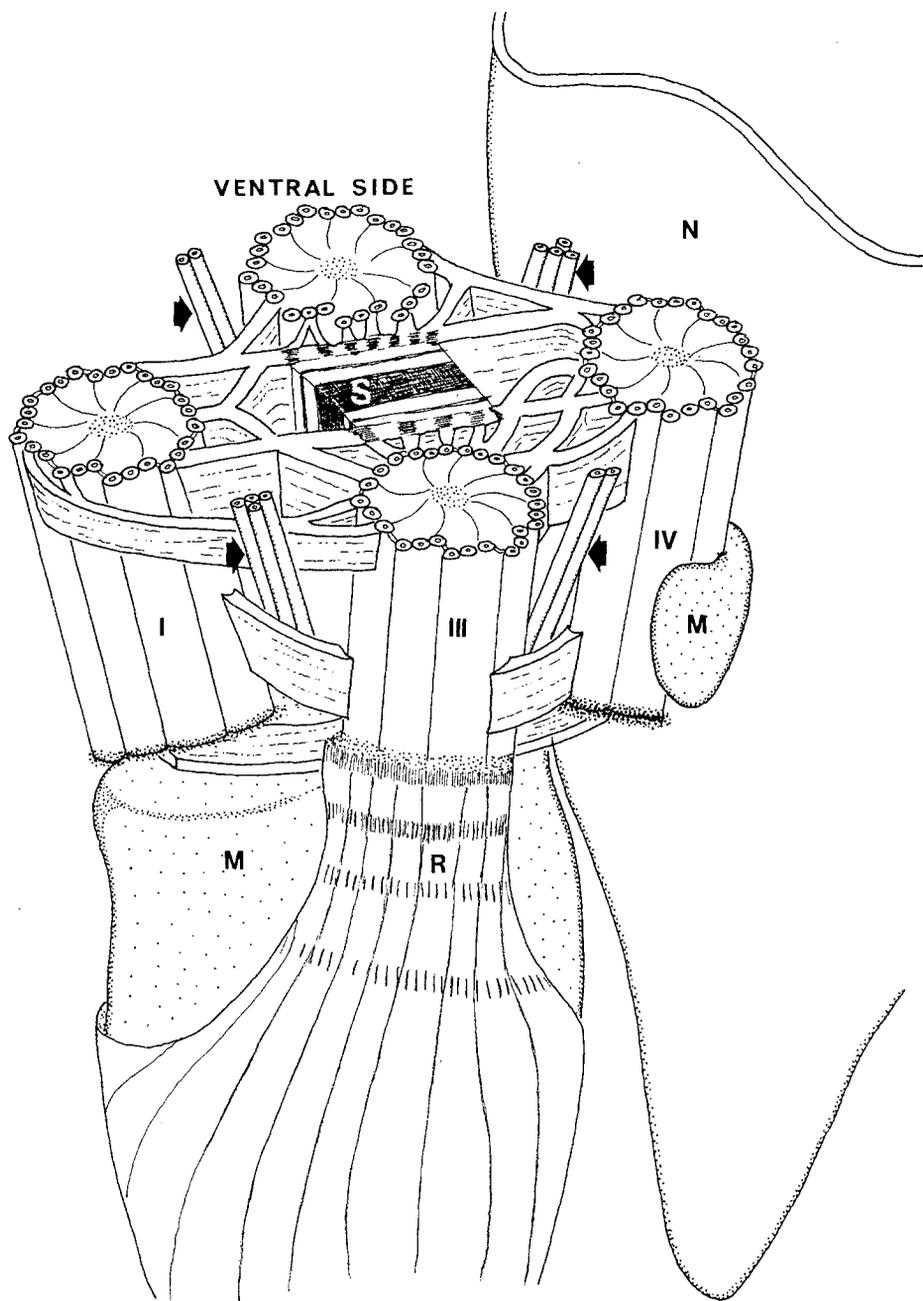


Fig. 15. Drawing showing arrangement of basal bodies (I—IV), 4-2-4-2 microtubular root system (arrows), synistosome (S), connecting fibers, microbody (M) and nucleus (N). The ventral side as defined by MELKONIAN (1981) is indicated (adapted from MELKONIAN 1981)

FLOYD and HOOPS (1980) and FLOYD *et al.* (1980) point out that quadriflagellate zoospores of Chlorophycean algae forming the *Chaetophorales* (*sensu* STEWART and MATTOX 1975) (*Ulothrix belkae*, *U. fimbriata*, *Schizomeris*, *Fritschella*, and *Stigeoclonium*) have an identical arrangement of flagellar apparatus components. There are cruciate microtubular roots and of the four basal bodies, two are connected by a large banded distal fiber, while a ring of peripheral fibers connects all basal bodies. This arrangement is

essentially similar to that of *P. gelidicola*, particularly if one considers the synistosome to be homologous to the distal connecting fiber as proposed by MELKONIAN (1980, 1981) and ROGERS *et al.* (1981) (see Fig. 30). We believe this identity to be indicative of a direct phylogenetic link between *P. gelidicola* and the *Chaetophorales* and support the proposal that *Pyramimonas* is antecedent to some quadriflagellate *Chlorophyceae* (MELKONIAN 1981). Cytoplasmic microtubules in *Pyramimonas gelidicola*

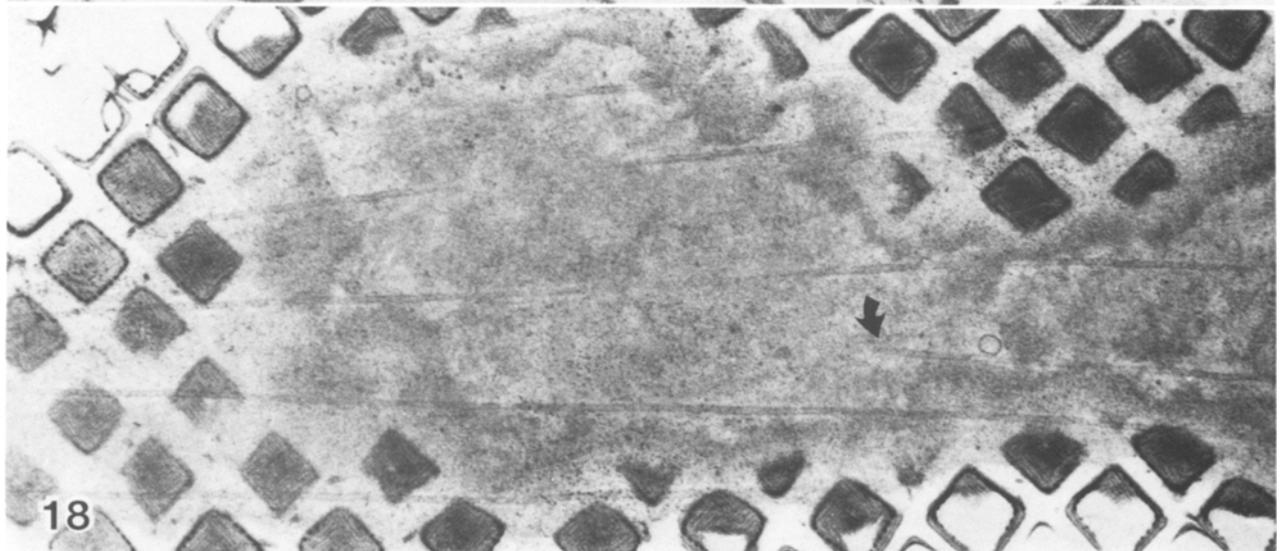
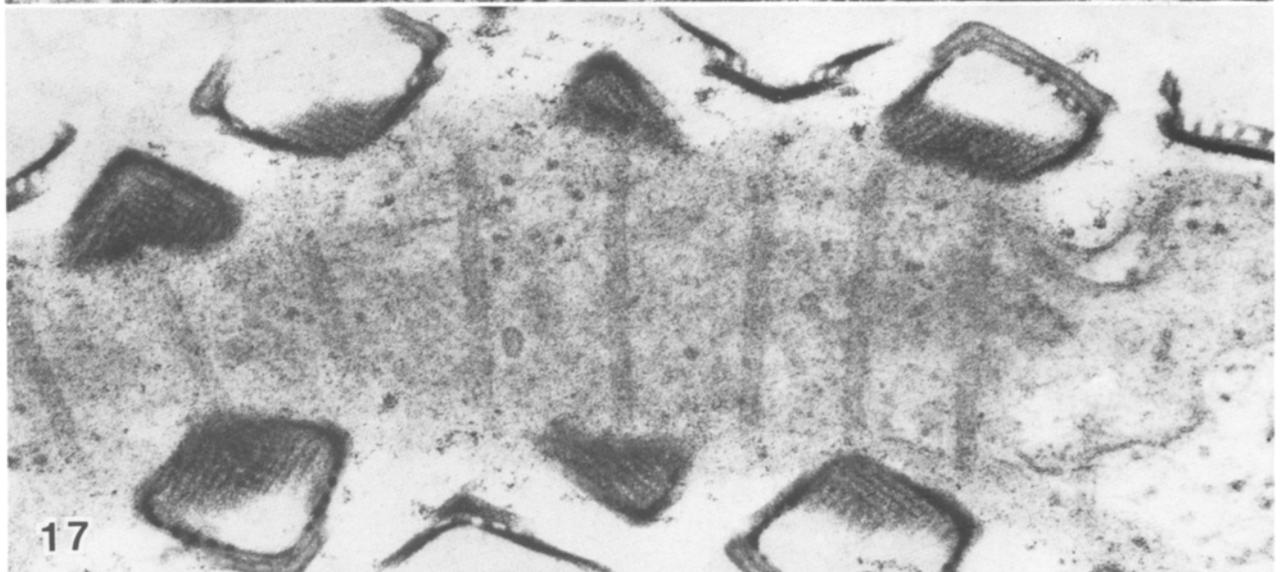
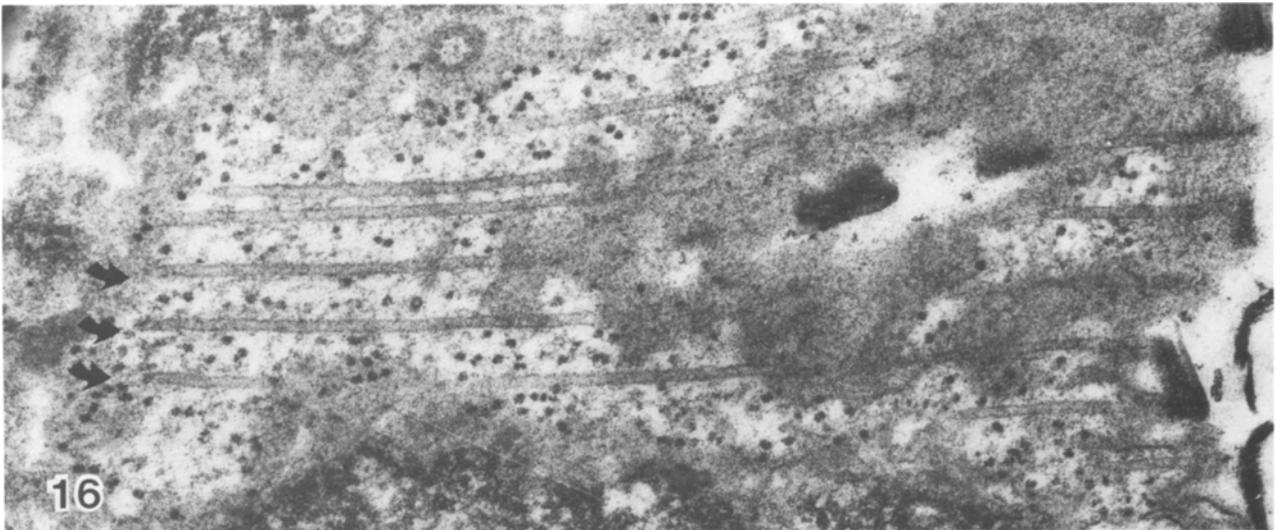


Fig. 16. One of a series of glancing sections of the flagellar pit showing the origin of several cytoskeletal microtubules at the base of the pit on the left hand side of the micrograph (arrows).  $\times 47,000$

Fig. 17. Cytoskeletal microtubules transcending the rim of the flagellar pit at the anterior of the cell.  $\times 105,000$

Fig. 18. One of a series of glancing sections of the cell exterior showing microtubules converging toward posterior end (left hand side of the micrograph). One microtubule (arrow) ends, maintaining the spacing between microtubules.  $\times 33,000$

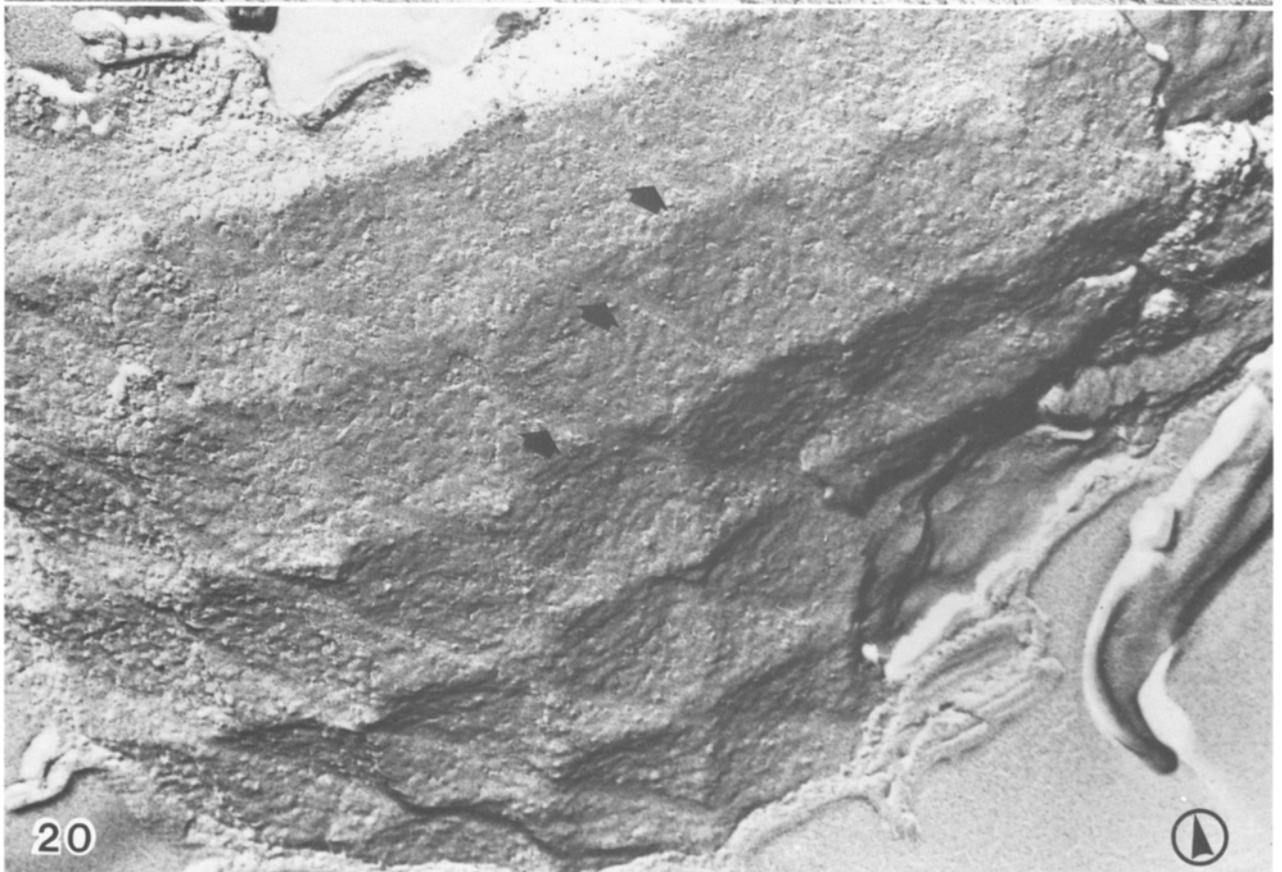
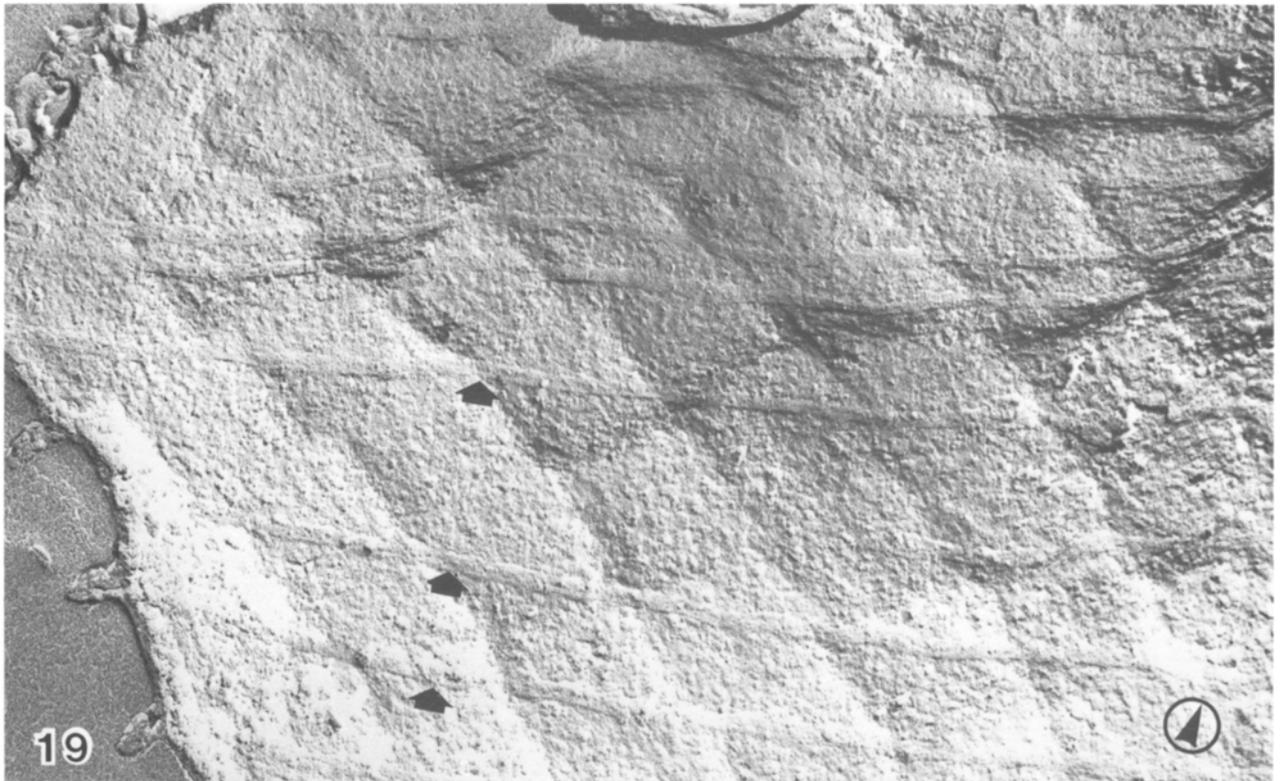


Fig. 19. Freeze-fracture of outer chloroplast envelope (*PF*) showing square impressions of scale periplast and longitudinal impressions of cytoskeletal microtubules (arrows). In this, and subsequent freeze-fracture micrographs, an encircled arrow-head indicates the direction of shadowing.  $\times 67,000$

Fig. 20. Freeze-fracture of the outer chloroplast envelope (*EF*) showing corresponding impressions of scales and microtubules (arrows).  $\times 64,000$

follow the cell contour, forming a precise parallel array directly beneath the plasmalemma. Preliminary experiments using the microtubule depolymerizing agents colchicine, oryzalin and hydrostatic pressure indicate that the microtubules act as a cytoskeleton. The characteristic cell shape of *P. gelidicola* is lost in cells observed to have lost their cytoplasmic microtubules (MCFADDEN, unpublished).

Microtubule grooves in the chloroplast envelopes were observed in both thin sections and freeze-fractures and may represent the effect of cell turgor pressing the chloroplast envelopes against the cytoskeleton in regions where the chloroplast envelopes are adjacent the plasmalemma (see Fig. 31). The fibrils observed in the microtubule impressions are of approximately the same dimensions as tubulin molecules composing protofilaments (AMOS 1979). That an impression as small as a protofilament can be observed in a membrane leaflet seems quite remarkable and a specific microtubule/membrane association cannot be ruled out. It is possible that some molecular specialization occurs, such as a parallel organization of membrane proteins, or alternatively, that a modification of the lipid layer in the zone of microtubule/membrane contact allows visualization of the protofilaments in freeze-fracture. A microtubule/membrane association would serve in anchoring the cytoskeleton, which is otherwise unattached, and would maintain spatial relationships of internal components. Effective experimentation on the cytoskeleton was prevented by the variable response of cells to anti-microtubule agents. Using the microtubule depolymerizing techniques outlined by BROWN and BOUCK (1973) and STEARNS and BROWN (1981), a maximum of only 30% of cells in a population of *P. gelidicola* lost the cytoskeleton (MCFADDEN, unpublished). This result is at variance with that of BURCH and MARCHANT (1983) but we can offer no explanation for this at present. The variability was apparently not related to cell cycle as

cells in various stages of cytokinesis may or may not round up when exposed to colchicine (MCFADDEN, unpublished). This variable response has prevented us from determining if the cytoskeleton is re-established after removal of the depolymerizing agents.

The square impressions in the membrane layers appear to result from cell turgor pressing against the box scale layer (see Fig. 31). The scales remain closely appressed to one another indicating that there is some cohesive force between individual box scales allowing them to act, to a limited extent, as an exoskeleton. The nature of the scale interconnections is unknown, but possibly similar to that observed in *Tetraselmis* where divalent cations interconnect scales on the flagella (MELKONIAN *et al.* 1981, MELKONIAN *et al.* 1983). The box scales are known to form an almost continuous covering on the plasmalemma in *P. gelidicola* and a protective function has been suggested (MCFADDEN and WETHERBEE 1982a) but this is the first evidence for an exoskeletal function.

Cytoplasmic microtubules in *Pyramimonas gelidicola* do not show any association with the flagellar roots, and initiation sites are individually located around the flagellar pit. This statement is supported by the observation that in those cells susceptible to microtubule depolymerizing agents, the flagellar pit is the last part of the characteristic shape to be lost (MCFADDEN, unpublished). We propose that the small osmiophylic area observed at the end of each microtubule in the flagellar pit is an example of the hypothetical individual nucleating elements (NEs) postulated by TUCKER (1979). Studying serial thin sections, we have not been able to visualize any inter-organization or structuring of these NEs nor any affiliations with cell organelles. The NEs are evenly spaced in a ring encircling the base of the flagellar pit. Perhaps some organization of the nucleating elements might be visible by other forms of analysis such as high voltage EM (see PALEVITZ 1981) or possibly the NEs

Figs. 21–24. Serial sections glancing the base of the flagellar pit showing the origin of several cytoskeletal microtubules. Three tubules are marked with arrow-heads in consecutive sections to assist in tracing the separate points of origin.  $\times 34,000$

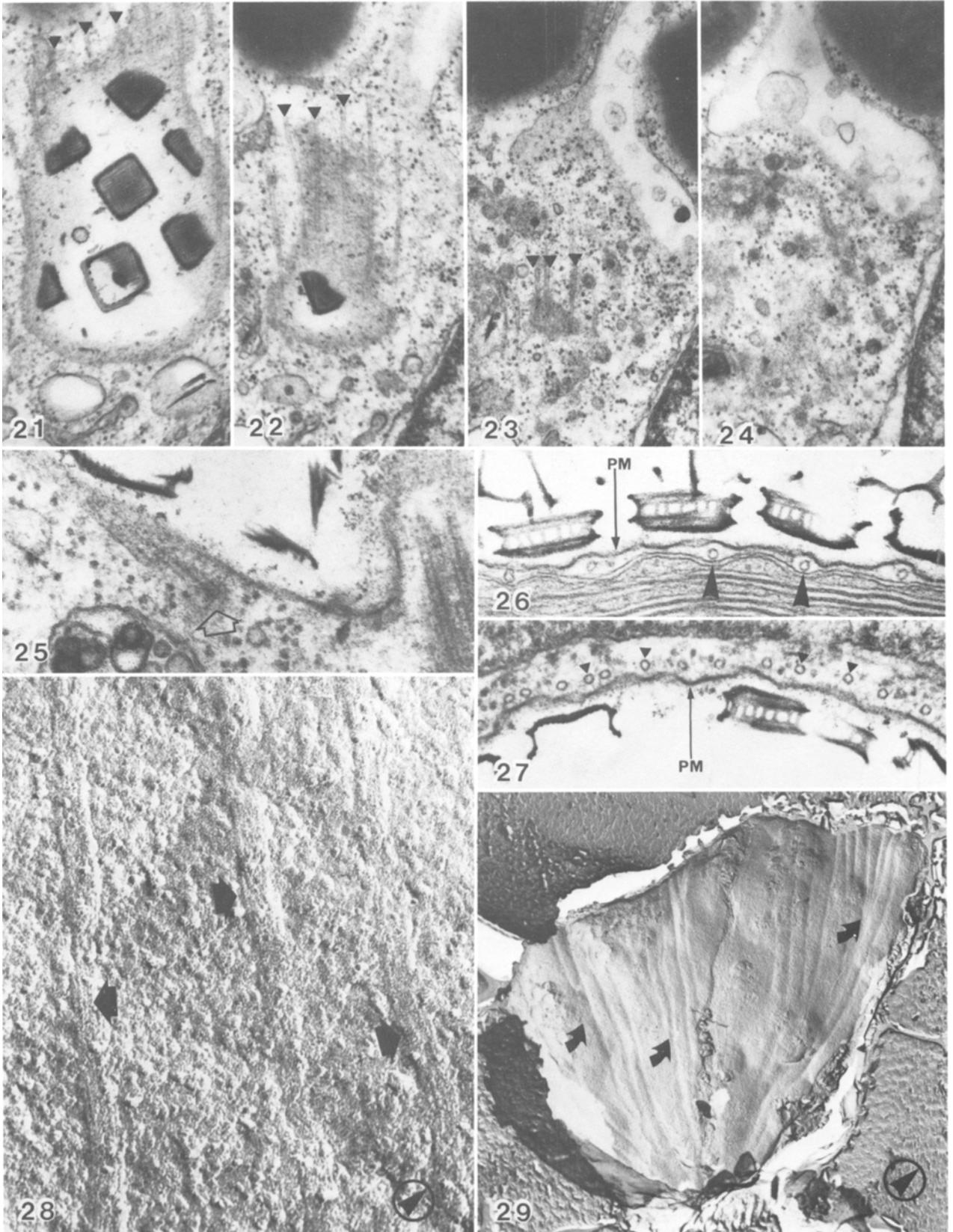
Fig. 25. Section at the base of the flagellar pit showing one flagellum and the origin of a cytoskeletal microtubule in an amorphous osmiophilic region (arrow).  $\times 111,000$

Fig. 26. Vertical section of cell exterior showing box scales, plasmalemma (PM), cytoskeletal microtubule profiles, and the chloroplast envelopes which are closely appressed to the microtubules (arrow-heads).  $\times 67,000$

Fig. 27. Vertical section of cell surface within the pit showing scales, plasmalemma (PM) and cytoskeletal microtubules (arrow-heads).  $\times 62,000$

Fig. 28. Freeze-fracture of the outer chloroplast envelope (PF) showing fibrillar sub-units of microtubule impressions (arrows).  $\times 160,000$

Fig. 29. Freeze-fracture of outer chloroplast envelope (EF) at the posterior of the cell showing impressions of cytoskeletal microtubules converging and terminating (arrows).  $\times 15,000$



Figs. 21-29

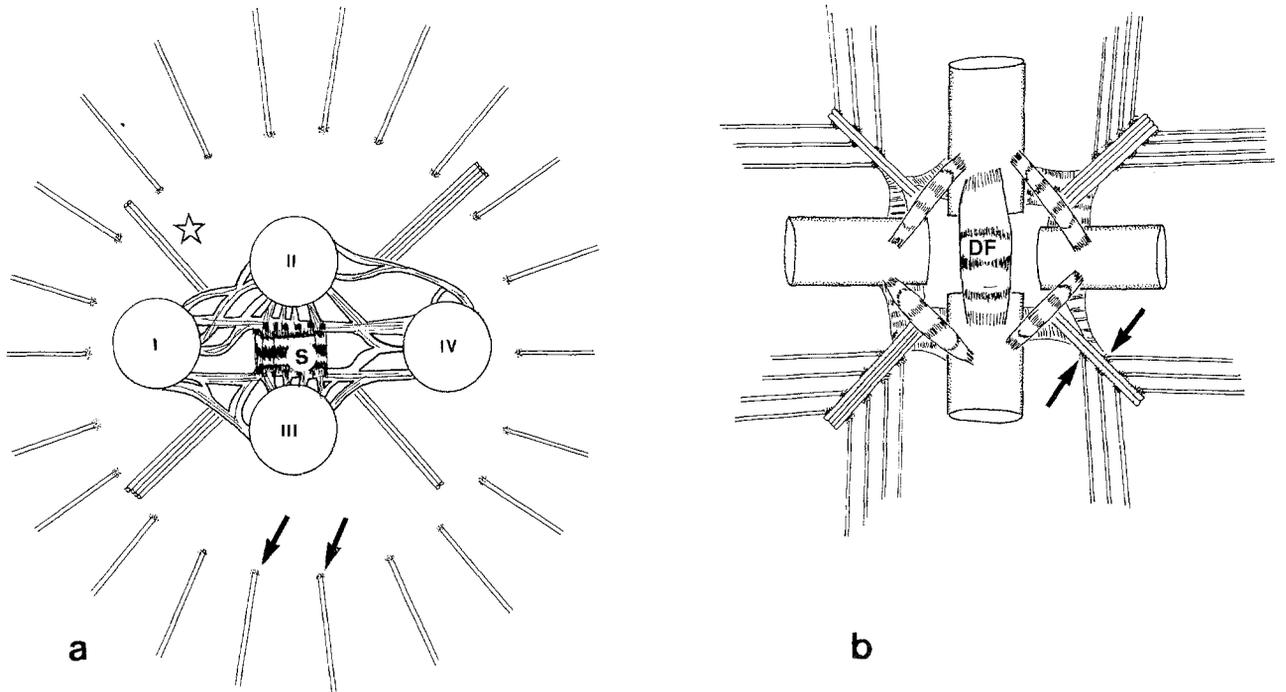


Fig. 30. Diagrammatic representations of the absolute configurations of the flagellar apparatuses and cytoskeletons in *Pyramimonas* (a) and the quadriflagellate zoospores of Chaetophoralean algae (b) (after FLOYD *et al.* 1980, FLOYD and HOOPS 1980, FLOYD, personal communication) allowing comparison of possible homologues [*i.e.*, the synstosome (S) in *Pyramimonas* and the distal connecting fiber (DF) in the *Chlorophyceae*]. We support the proposal that the Chaetophoralean zoospores could have developed by the modification of the flagella to project laterally and the roots posteriorly (MELKONIAN 1981) and suggest that at this time the nucleating elements for cytoskeletal microtubules (arrows) became organized onto the microtubular flagellar roots. A star marks the region, void of cytoskeletal microtubules, where the scale reservoir opens into the flagellar pit in *Pyramimonas*. (Not all cytoskeletal microtubules shown)

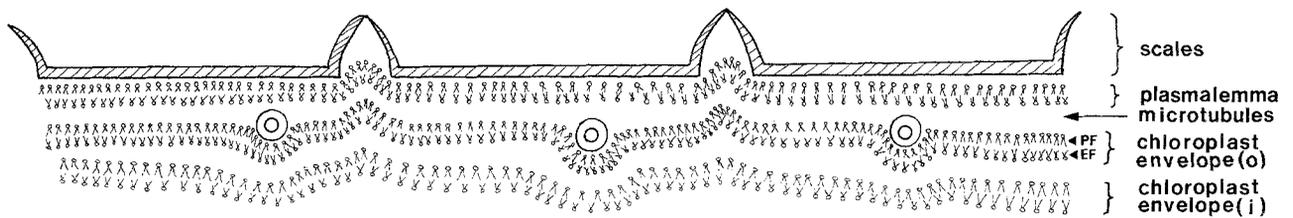


Fig. 31. Diagrammatic representation of the cell surface layers in *Pyramimonas*, showing how impressions of the scale periplast and cytoskeleton might be formed by turgor pressure on both the inner (i) and outer (o) chloroplast envelopes where they are adjacent the plasmalemma

dissociate from a cytoplasmic structure after establishment of the cytoskeleton. In *Pyramimonas* the cytoskeleton is retained throughout cytokinesis (PEARSON and NORRIS 1975, WOODS and TRIEMER 1981) and a study of the nucleating elements during cytokinesis should provide information on the establishment of this system.

We believe that a cytoskeleton of this type is present in *Pyramimonas* due to both the methods of cytokinesis and scale deposition. Firstly, *Pyramimonas* cells only reproduce by longitudinal fission (NORRIS 1980) while

retaining their motility and cytoskeleton throughout division (WOODS and TRIEMER 1981, PEARSON and NORRIS 1975). It seems unlikely that a cytoskeleton nucleated from the microtubular roots could remain intact during the process of division as the four new basal bodies are formed *de novo* during prophase, each daughter cell acquiring two new basal bodies and two old (BELCHER 1969). Secondly, a cytoskeleton nucleated from the flagellar roots would effectively bar the release of scales to the cell surface, since box and crown scales exceed the width of the spaces between microtubules.

The scales, manufactured internally, are known to be released via a duct opening into the base of the flagellar pit (MANTON 1966, MANTON 1968, MOESTRUP and THOMSEN 1974). Our observations show that the cytoskeleton in *Pyramimonas* is nucleated above this region leaving this small ring of surface area available for scale release (see Fig. 30).

It now becomes relevant to ask why the *Chlorophyceae* have a flagellar root MTOC. MATTOX and STEWART (1977) believe that the development of the cell wall in the *Chlorophyceae* induced the evolution of phycoplast cytokinesis. Since most of the *Chlorophyceae* dismantle their cytoskeleton to divide (possibly so that the tubulin may be used in the phycoplast) an MTOC seems highly suitable, particularly in re-establishing the cytoskeleton when swimmers are produced. Although members of the *Chlorophyceae* do not produce scales, some quadriflagellate zoospores of the *Ulvophyceae*, *sensu* STEWART and MATTOX (1978)—which produce scales but lack a phycoplast—also possess flagellar root MTOCs (*Ulothrix zonata*, SLUIMAN *et al.* 1980; *Ulva*, MELKONIAN 1979; *Enteromorpha*, EVANS and CHRISTIE 1970). However, nothing is known of the deposition method for these very small scales, making it difficult to rationalize this observation in terms of the above hypothesis.

This leads us to the question of whether the cytoskeleton of the *Chlorophyceae* could have arisen from that of a *Pyramimonas*-like organism. TUCKER (1977) and BROWN *et al.* (1976) have proposed that an MTOC represents the organization of nucleating elements onto an existing cytoplasmic structure (*e.g.*, centriole, rhizoplast, microtubular root) to give ordered initiating sites able to generate pre-patterned microtubular arrays. We suggest that during the development of the *Chlorophyceae* from a pyramimonad ancestor, the requirements of the cytoskeleton changed due to the loss of the pit and scales and the development of a wall and phycoplast. The individual nucleating elements became organized onto the flagellar roots, probably in conjunction with the loss of the flagellar pit when the flagellar roots were modified to project posteriorly (see Fig. 30). This hypothesis could be tested by an examination of the cytoskeleton in *Mesostigma* which appears to be similar to *Pyramimonas* (*cf.*, Fig. 3, ROGERS *et al.* 1981). Studies of the cytoskeleton in organisms such as *Hafniomonas* (a possible intermediary form between *Pyramimonas* and the *Chlorophyceae*—see Introduction) or *Tetraselmis* (a scale producing prasinophyte with a wall and phycoplast) will also be useful in validating this hypothesis.

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