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CELL DIVISION IN THE SCALY GREEN FLAGELLATE HETEROMASTIX ANGULATA AND ITS BEARING ON THE ORIGIN OF THE CHLOROPHYCEAE[1]

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ABSTRACT

H. angulata is a scale-covered, asymmetrical green unicell with two laterally attached, anisokont flagella. In recent years it has been classified in the Prasinophyceae. The flagellar apparatus replicates, and the cell begins to cleave at the side opposite the flagella before the nucleus can be perceived to be in prophase. The flagellar apparatuses separate, and the extranuclear development of the spindle occurs from the regions occupied by rhizoplasts. Rhizoplasts or partial rhizoplasts lie at the flat metaphase spindle poles. By metaphase, the cell has already elongated to the extent that it is nearly twice as long as at interphase. The spindle and the cell itself elongate greatly during anaphase with a concomitant further separation of the flagellar pairs. Although the interzonal spindle persists during cytokinesis as in charophycean algae, *H. angulata* is similar in flagellar scale morphology and other characteristics to the chlorophycean *Platymonas*, which has a collapsing interzonal spindle at telophase, a phycoplast, and a wall-like theca, which develops by the fusion of small stellate scales. It is hypothesized that the collapsing telophase spindle and phycoplast evolved in green flagellates similar to *Platymonas*, in which cell and spindle elongation became restricted by a cell wall that evolved from stellate scales similar to those in *Heteromastix*. Such walled flagellates are then visualized as having eventually given rise to *Chlamydomonas* and to the entire range of chlorophycean algae with phycoplasts. It is pointed out that the hypothesis has a number of implications by which its validity could be judged when sufficient information becomes available.

THE STATEMENT has often been made, even recently, that the motile cells of green algae are characterized by anteriorly inserted, isokont flagella. While this statement is at least approximately accurate for most genera and seems to provide an additional distinguishing criterion, it is an oversimplification that leads readers to underestimate the diversity of green algae. In the early sixties, Manton and Parke (Manton, 1959; Manton and Parke, 1960) showed that certain scaly or naked asymmetrical flagellates theretofore considered to belong to other algal groups are, in fact, green algae. The scale-covered *Heteromastix* Korshikov has always been considered a green alga, but it has laterally attached flagella which differ in length and manner of motion (see Manton et al., 1965).

In recent years, *Heteromastix* and other asymmetrical green flagellates have usually been classified in the Prasinophyceae, along with *Platymonas* G. S. West, *Prasinocladus* Kuckuck and *Pyramimonas* Schmarda, which also bear scales but differ conspicuously from *Heteromastix* in being nearly symmetrical and in having four anteriorly inserted, equal flagella. Even the Prasinophyceae are still occasionally characterized as having anterior flagella (e.g., Dodge, 1973, p. 16), presumably because genera such as *Prasinocladus* and *Pyramimonas* were among the earliest to be assigned to the class.

We have pointed out that the Prasinophyceae is not easily separated from other green algae by any set of characteristics (Mattox and Stewart, 1973; Birkbeck, Stewart, and Mattox, 1974; Stewart, Mattox, and Chandler, 1974; Stewart and Mattox, 1975a). There are, however, several presumably primitive characteristics that occur in various combinations in prasinophycean genera (Stewart and Mattox, 1975b). Cellular asymmetry, anisokonty, scales, and an interzonal spindle that persists throughout cytokinesis are features that might be considered primitive because they are more typical of other algal classes, certain fungi and protozoa than of most of the advanced green algal genera. More advanced, specialized, or less common characteristics such as a collapsing telophase spindle, a phycoplast, approximate radial symmetry, isokonty, and a wall-like theca are also found in prasinophycean genera. Scaly green flagellates could be the ex-

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tant remnants of early green algae which underwent extensive evolutionary diversification in cellular organization, symmetry, cell covering, and the details of cell division (cf. Stewart and Mattox, 1975a, b). The diversity found in cell division in the three scaly or naked green flagellates studied, *Platymonas* (Stewart et al., 1974), *Pedinomonas* (Pickett-Heaps and Ott, 1974) and *Pyramimonas* (Pearson and Norris, 1975), strongly support this view. The scaly green flagellates are clearly important to the understanding of the evolutionary origin of the different groups of higher green algae and land plants and perhaps even in assessing the relationship of green algae to other eucaryotic microorganisms. A minimal effort should include careful fine structural studies of at least one species of each of the small number of known genera.

MATERIALS AND METHODS—*Heteromastix angulata* Korshikov was obtained from the Indiana University Culture Collection of Algae (IUCC #1026, Starr, 1964). Stock cultures were maintained in soil-water bottles. Algae to be studied were grown in Fernbach Lo-Form culture flasks in a medium consisting of nine parts BBM (Nichols and Bold, 1965) and one part soil-water extract. The cultures were maintained for 12 days at 20 C and on a 16/8 lt/dk regime. A few dividing cells were present at all times, but there appeared to be a greater number from 2 to 4 h after the onset of darkness.

Cultures were killed with equal volumes of 2% glutaraldehyde and fresh growth medium. After centrifugation, the cells were rinsed in sterile medium and resuspended in 2% aqueous OsO_4. After OsO_4 treatment, the cells were rinsed with water, pelleted in BEEM capsules, and brought through dehydration to embedment in Spurr's Low Viscosity Medium.

RESULTS—*Cellular organization during interphase*—Interphase cells of *Heteromastix* have been described by Manton et al. (1965). We shall mention those aspects of cellular organization necessary for the clarity of later description and discussion. The cell seems best described as "bean-shaped" with the flagella inserted laterally in a groove which resembles the hilum of a bean seed (Fig. 1, 2). The shape of the cell results in its having two distinct aspects in median sections made through the basal bodies, depending on whether the section passes through the longer dimension of the cell (Fig. 1) or at a right angle to that plane in the shorter dimension (Fig. 2). The nucleus is eccentrically located and lies close to the plasmalemma at one side of the cell (Fig. 2). The narrow space between the nucleus and plasmalemma is occupied by numerous parallel microtubules, as can be seen most clearly in partially tangential sections (Fig. 3). These microtubules do not extend beyond the nucleus.

The flagellar apparatus includes two superficial microtubular roots, which start near the basal bodies and extend to either side of the flagellar apparatus parallel to the long axis of the cell. One root contains seven microtubules and the other two or three (Manton et al., 1965, and present results). A rhizoplast extends from the basal bodies inwardly through the cell to the vicinity of the chloroplast and makes contact with the nuclear envelope for much of its length (Fig. 9). The rhizoplast is narrow and delicate; it and its cross-striations stain lightly so that they are sometimes only faintly visible. At the point where the rhizoplast terminates near the chloroplast, it is attached to the cell's small, single microbody (Fig. 6). The microbody is often extensively lobed and sometimes appears, in a single section, to be a group of small microbodies (cf. Fig. 5, 7).

An unusual feature is the presence of a pyrenoid-like region at the outer periphery of the chloroplast (Fig. 1, 2), although a definite, more typical, pyrenoid with traversing thylakoids occurs elsewhere in the chloroplast (Fig. 1, 2).

Prophase—The first sign of the onset of mitosis is a slight cleavage in the chloroplast (Fig. 7, 10). The microbody lies in or near the chloroplast cleavage (Fig. 7, 10). The cell itself develops a conspicuous early cleavage before there is any indication that the nucleus has reached prophase (Fig. 10, 11).

By prophase, the flagellar apparatus has been replicated, but the two new flagella are noticeably shorter than the old ones and remain shorter until at least telophase. Each daughter cell receives one of the original flagella and one new one (Fig. 4; also see Manton et al., 1965). During prophase the two pairs of flagella begin to move apart (Fig. 8, 12). Early in prophase, therefore, a group of four flagella is observed close together near a shallow cleavage. A drawing of that stage in Manton et al. (1965) led us to believe (Stewart et al., 1974) that the basal bodies might continue to lie in the plane of the cleavage until the completion of cytokinesis and that *Heteromastix* might prove to have a phycoplast as occurs in *Platymonas*. However, as is described later, the two pairs of flagella have separated noticeably further by metaphase, and by telophase and the completion of cytokinesis, the two pairs are widely separated.

The rhizoplasts are replicated along with the rest of the flagellar apparatus. When the two pairs of flagellar basal bodies move apart later in prophase, a rhizoplast lies on each side of the nucleus (Fig. 8). At that time, microtubules are often seen lying parallel to the rhizoplasts (Fig. 12). By late prophase, microtubules radiate

from the vicinity of the rhizoplasts on each side of the nucleus; this is seen better when the rhizoplasts themselves are not included in the section (Fig. 13). A microbody lies near the end of each prophase rhizoplast (Fig. 8). Apparently the microbody splits during prophase and the two new microbodies move apart with the rhizoplasts. This positional relationship of the microbodies and rhizoplasts has also been observed in later stages of cell division. A definite attachment of rhizoplasts to microbodies has not been observed during mitosis as it has during interphase, but the delicate nature of the rhizoplast during mitosis (see below) would make such an attachment difficult to observe even if it exists.

Prometaphase—The two pairs of flagella have moved somewhat farther apart by prometaphase. The microtubules that radiate from the vicinity of the rhizoplast at late prophase have become organized into two half-spindles which sink into the nucleus. One such half-spindle partially inside the nucleus is shown in Fig. 14. The pole of the spindle is broad and flat.

Metaphase—The nuclear envelope becomes interrupted at metaphase, particularly at the poles. The spindle poles are still broad and flat (Fig. 18).

The two pairs of flagella are now well separated from each other, and one pair of basal bodies is associated with each spindle pole. The basal bodies are lateral to the spindle poles, however, and are attached to the rhizoplasts which lie directly at the flat spindle poles (Fig. 16). The rhizoplasts are very indistinct at metaphase (Fig. 16), but we cannot ascertain whether this is merely a lack of contrast or the result of partial dissolution of the rhizoplast during spindle formation similar to events in *Platymonas* (Stewart et al., 1974). In addition to the rhizoplasts, there is a thin layer of dense material associated with the polar ends of the spindle microtubules (Fig. 16, 19). The dense material has been observed at all stages from prometaphase to telophase, and it is often easily detected when the rhizoplast is only faintly visible.

Cell cleavage is in an advanced stage by metaphase, particularly at the side of the cell opposite the flagella (Fig. 13, 15, 16). The cell has also elongated further (Fig. 4, 6, 15). Extranuclear microtubules lie between the two pairs of basal bodies (Fig. 16) similar to those described in certain other organisms such as *Volvox* (Deason and Darden, 1971).

Anaphase—The spindle elongates greatly during anaphase, but there is little decrease in chromosome-to-pole distance (Fig. 19), at least until very late anaphase or early telophase. The spindle poles remain broad and flat. The entire cell elongates along with the extensive elongation of the spindle. The two pairs of basal bodies continue to move apart to remain lateral to the spindle poles.

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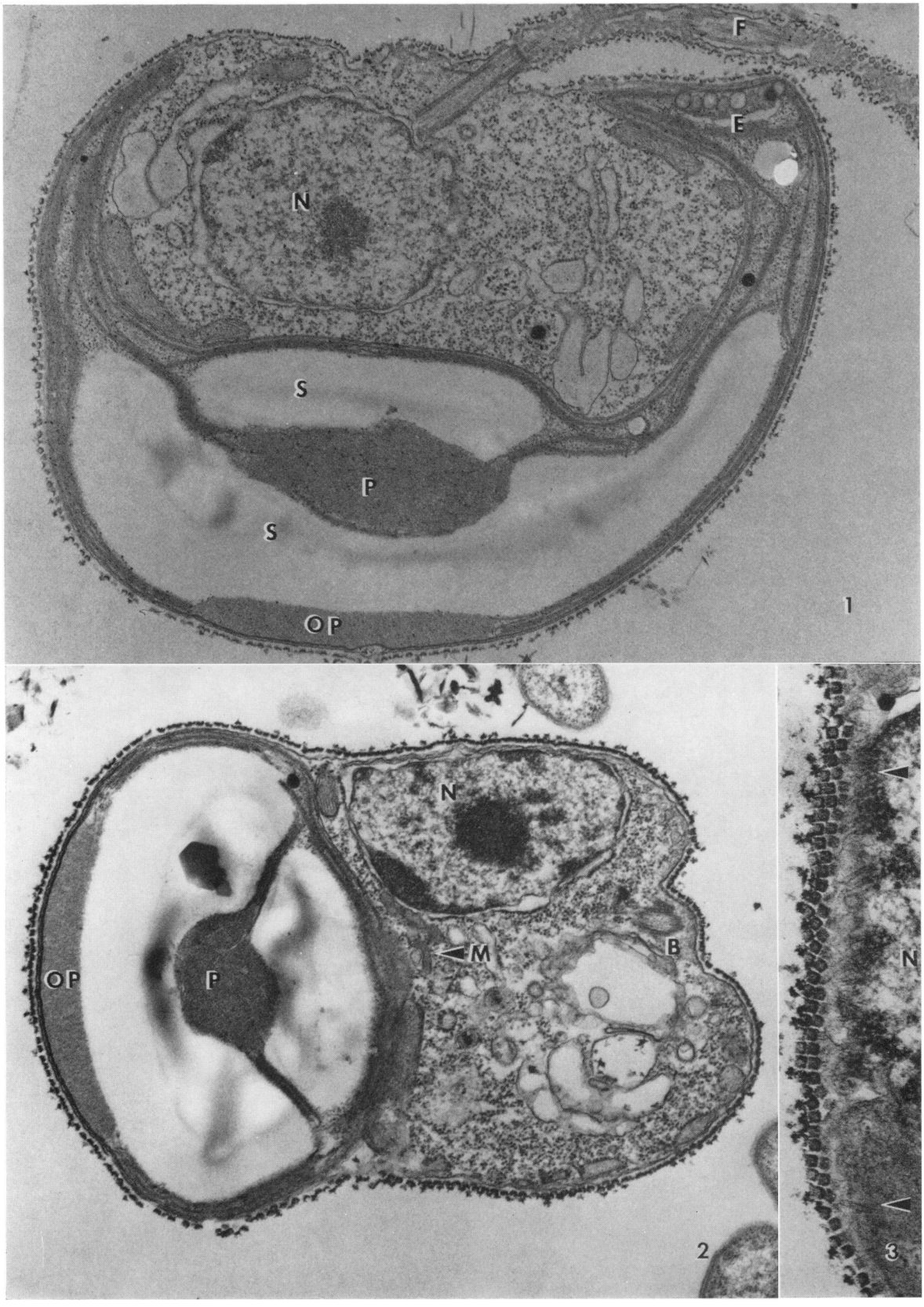
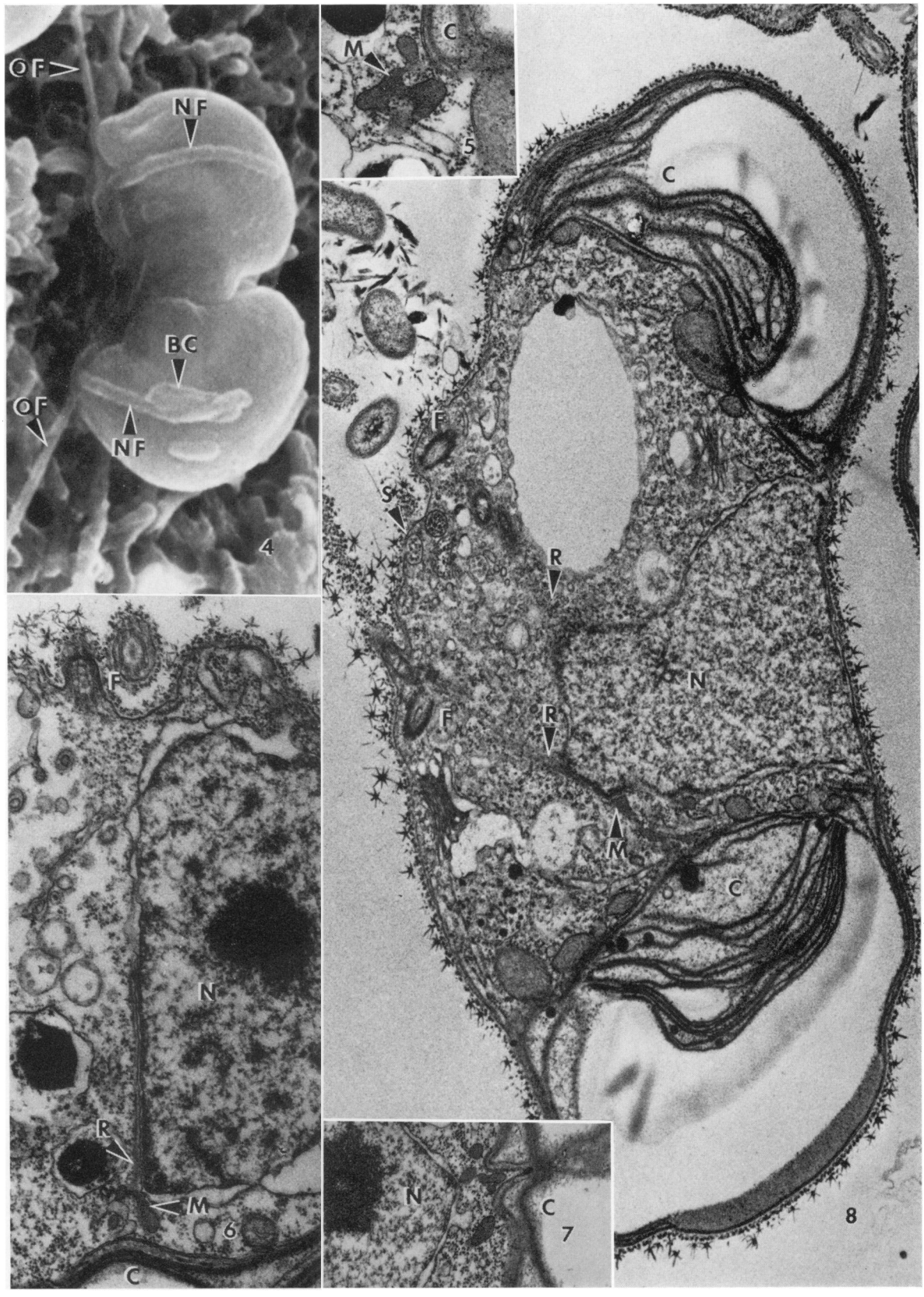
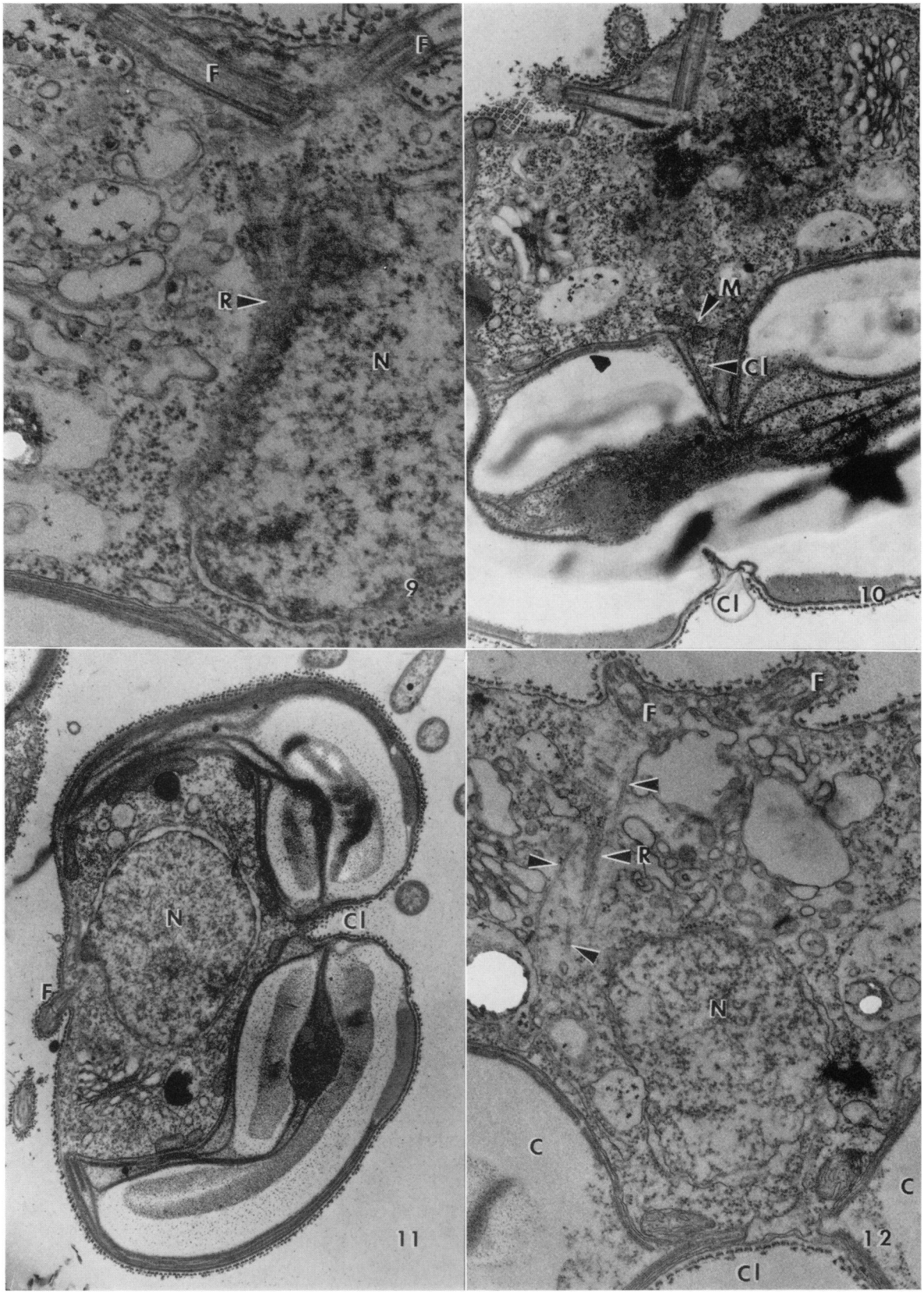
Fig. 1–3. **1.** Median section of the cell's longer dimension. One of the flagella is not included in the section. Since the flagella are lateral, the left and right sides of this section represent the posterior and anterior of the cell. Since the outer layer of stellate scales often falls off during processing, they are absent in this and in some of the other figures. E, eyespot; F, flagellum; N, nucleus; P, pyrenoid; S, starch; OP, superficial pyrenoid-like region of the chloroplast. × 23,500. **2.** Median section of cell's shorter dimension at right angle to the plane of Fig. 1. This micrograph must be turned 90° counterclockwise to be oriented in a similar fashion to the section in Fig. 1. Since the flagella are lateral, this section does not pass through the anterior or posterior of the cell. B, flagellar basal body; M, microbody; N, nucleus; P, pyrenoid; OP, superficial pyrenoid-like region of the chloroplast. × 26,500. **3.** Partially tangential section of region where the nucleus lies adjacent to the plasmalemma. Numerous short lengths of microtubules occur in the region between the two arrows. N, nucleus × 44,000.

Fig. 4–8. **4.** SEM view of dividing cell which was probably at or near metaphase as judged by the distance separating the flagellar pairs (cf. Fig. 15). Note that the cell had already elongated to the extent that it is nearly double the length of an interphase cell. Also note that the two new flagella (NF) are shorter than the two original flagella (OF). BC, bacterial cell. × 6,000. **5.** Note the branched nature of the microbody (M). C, chloroplast. × 27,500. **6.** From median section of cell in the shorter dimension, similar to that shown in Fig. 2. A short segment of the rhizoplast (R) is present. Note that the rhizoplast terminates at and appears to be attached to the microbody (M). C, chloroplast; F, flagella. × 25,000. **7.** The branched microbody between the nucleus (N) and the chloroplast (C) appears in this section to be several smaller microbodies. Note that the microbody lies in the cleavage of the chloroplast which had just begun to divide. × 20,000. **8.** Cell at prophase. The flagellar apparatus had replicated and the flagellar pairs (F) had begun to separate. The rhizoplast had also replicated or split so that rhizoplasts (R) lie at both sides of the nucleus (N). A microbody (M) lies near the point where one of the rhizoplasts appears to terminate. This section is nearly median to the developing mitotic apparatus but is very oblique to the cell. The nucleus remains at one side of the cell so that a section that passes through both the center of the nucleus and the two rhizoplasts can never be median to the cell itself (refer to Fig. 2). A vesicle (S) containing scales appears to have been releasing the scales in the area between the separating flagellar pairs, one site where the plasmalemma presumably grows during cell elongation. C, chloroplasts. × 17,000.







Telophase and cytokinesis—As telophase is reached and cleavage progresses, the spindle and cell have elongated together, the cell eventually reaching a length as great or greater than twice that of an interphase cell (cf. Fig. 19, 20). The nuclei are distantly separated as cytokinesis progresses (Fig. 17). Microtubules of the interzonal spindle persist until the completion of cytokinesis (Fig. 20). The nuclear envelope reforms first on the interzonal side of each chromosome mass (Fig. 19) so that there is not a tightly arranged interzonal spindle surrounded by nuclear envelope as described in *Pedinomonas* (Pickett-Heaps and Ott, 1974). The cell halves also do not rotate in relation to each other as in *Pedinomonas*. In late anaphase or in early telophase, the chromosomal microtubules finally begin to shorten as the outer edge of each chromosome mass becomes curved toward the spindle pole (Fig. 19).

DISCUSSION—Cell division in *Heteromastix angulata* Korshikov, the type species of the genus, is different in a number of respects from that in *Platymonas subcordiformis* (Stewart et al., 1974), *Pedinomonas minor* (Pickett-Heaps and Ott, 1974) and *Pyramimonas parkeae* (Pearson and Norris, 1975), the other three "prasinophycean" species in which the fine structural details of cell division have been studied. *H. angulata* also differs in pyrenoid structure from those species of *Heteromastix* for which Manton et al. (1965) published electron micrographs of thin sections. Other details by which interphase cells of *H. angulata* differ from those species of *Heteromastix* described previously—the microtubules between the nucleus and plasmalemma (Fig. 3) and the attachment of the rhizoplast to the microbody (Fig. 6)—are possibly the result of improved fixation procedures.

We believe that a comparison of cell division in *Heteromastix* with that of other green algae, combined with a consideration of other important features such as cell symmetry, cell covering and the flagellar apparatus, has important implications concerning the evolutionary origin of the Chlorophyceae. At present, Chlorophyceae (sensu Stewart and Mattox, 1975a) contains the majority of familiar green algal genera, and with the exception of the Ulvales, can be characterized as having an interzonal spindle which collapses before the completion of cytokinesis and is replaced by a phycoplast which consists of microtobules lying in the plane of cytokinesis. Although the collapsing spindle and the phycoplast occur in many genera of green algae, they clearly represent specialized condition, because they are absent in some green algae and are apparently absent in all other algae and eucaryotic organisms. The Charophyceae, which have an interzonal spindle which persists until the completion of cytokinesis, are therefore more primitive or generalized in that regard. Since the interzonal spindle of *Heteromastix* persists until the completion of cytokinesis, it would seem at first consideration that its affinities lie more closely with the Charophyceae and such "prasinophytes" as *Pedinomonas* and *Pyramimonas* than with the Chlorophyceae and such "prasinophytes" as *Platymonas*, which has a collapsing telophase spindle and a phycoplast. Other features, however, indicate that the relationships are more complex. For example, the attachment of the microbody to the rhizoplast might be regarded as a similarity between *Heteromastix* and the charophycean alga *Klebsormidium*, in which the microbody is attached close to the centrioles (Pickett-Heaps, 1972a), but the microbody in *Heteromastix* lies in the chloroplast cleavage at a distance from the centrioles, as it does in some filamentous, non-charophycean algae (e.g., *Pseudendoclonium*, Mattox and Stewart, 1974, and *Gloeotilopsis*, Kao, unpublished results).

There is, furthermore, strong evidence, based on scale morphology, that *Platymonas* and *Heteromastix* are related. The flagella of *Platymonas* and the closely similar genus *Prasinocladus* are strikingly similar to those of *Heteromastix* in the nature of their scaly covering. In all three genera, the plasmalemma of the flagella is coated by small diamond-shaped scales, which are found in a number of green algae and are not by themselves

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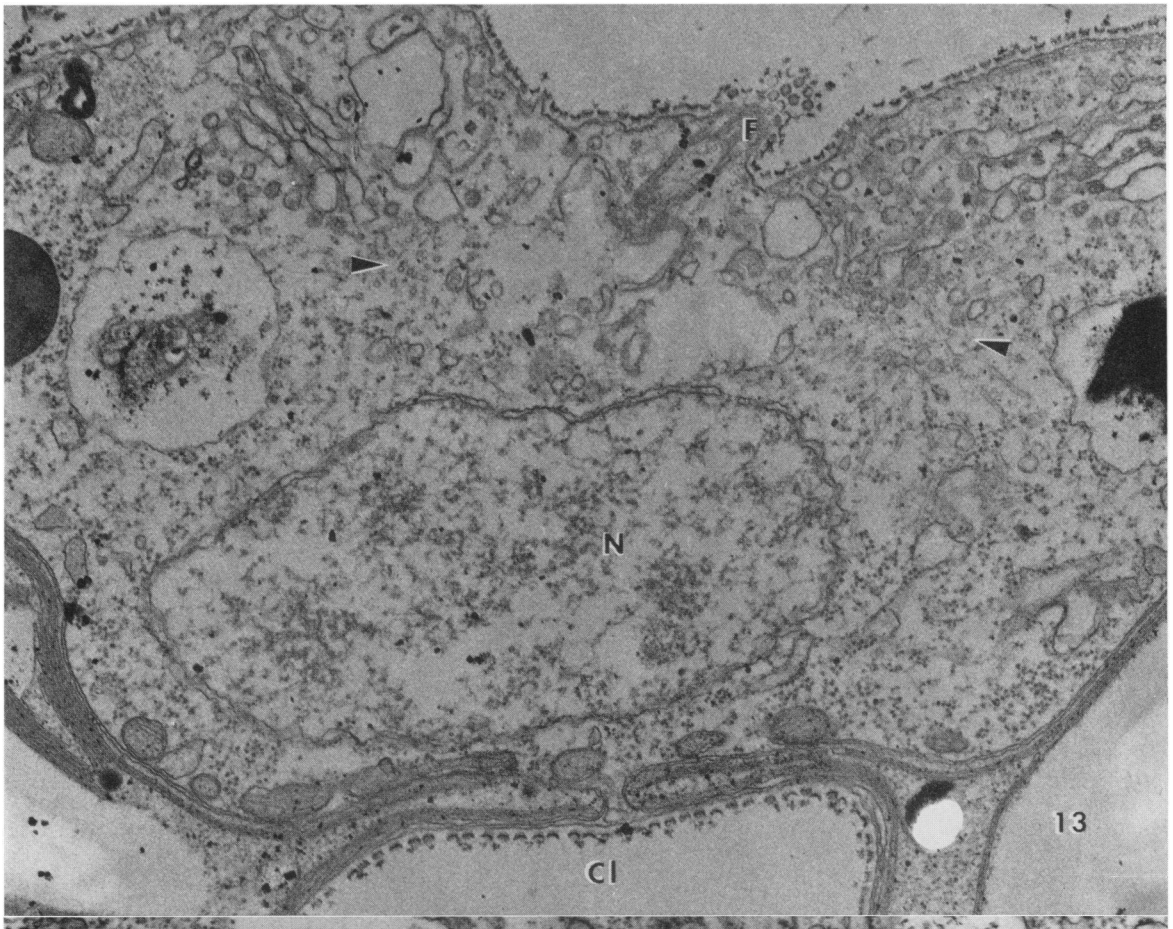
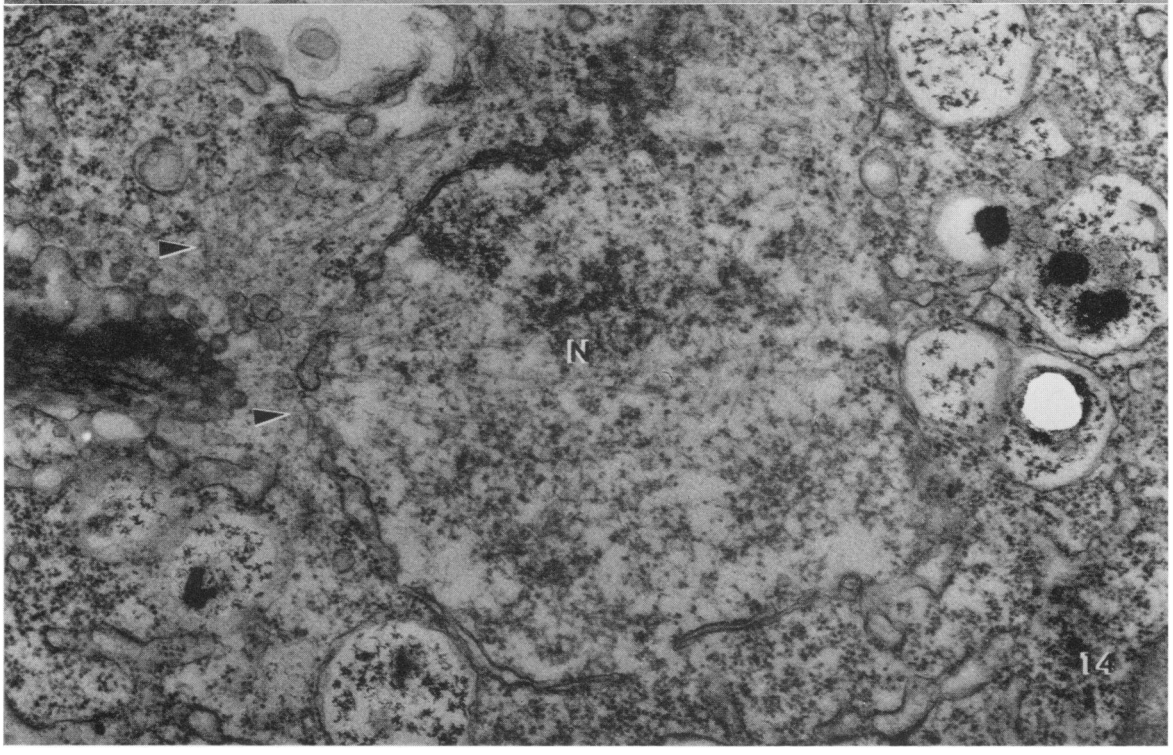
Fig. 9–12. **9.** Interphase cell. A rhizoplast (R) is attached to the flagellar basal bodies (F) and extends along the side of the nucleus (N). × 34,000. **10.** Early stage of cell division. The flagellar apparatus had replicated; note parts of three flagella at top of micrograph. The chloroplast had begun to cleave (Cl) at both its inner and outer surfaces. A microbody (M) lies in the chloroplast cleavage. The nucleus still appears to be in interphase in this and even in later stages; see Fig. 11. × 22,000. **11.** Early stage of cell division but a little later than Fig. 10. The nucleus (N) still resembles an interphase nucleus. The chloroplast had completed its division and the cell itself had already developed a deep cleavage (Cl) along the side opposite the flagella (F). × 13,000. **12.** Prophase. Although only two flagella (F) are visible, the flagella apparatus had replicated by this time, and the two pairs of flagella had become slightly separated. One of the two rhizoplasts present at this time is included in the section. Note that a number of microtubules (unlabeled arrows) parallel the rhizoplast (R). Also note that the striations in this rhizoplast are very distinct just below the flagellar apparatus, but the rhizoplast is very indistinct at its distal extremity. Cl, cell cleavage; N, nucleus. × 32,000.





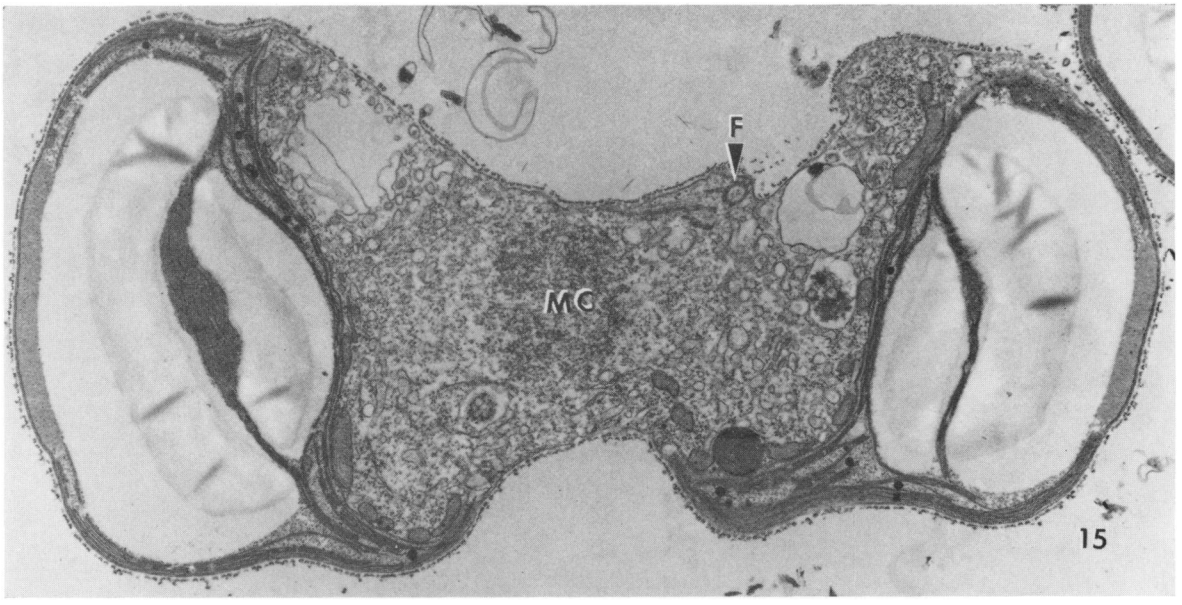


Fig. 15. Metaphase. The cell had already elongated considerably by this time. The two pairs of flagella had separated and lie lateral to the spindle poles; one of the pairs (F) is included in the section. Compare this internal view with the SEM view (Fig. 4) of a metaphase or early anaphase cell. MC, metaphase chromosome plate. × 14,000.

a reliable indicator of close relationship. However, stellate scales of a type not known outside these three genera lie over the diamond-shaped scales and are attached at the junctures of the diamond-shaped scales (Parke and Manton, 1965). *Heteromastix*, *Prasinocladus* and *Platymonas*, therefore, have an unique feature in common, and Parke and Manton (1965) point out that the similarity of the flagella in the three genera is so great ". . . that an isolated flagellum of *Prasinocladus* could only be distinguished from the equivalent of *Heteromastix* by the fact that in the latter the stellate scales over the junctures are isodiametric, whereas they are elongated in *Prasinocladus*." Parke and Manton (1965) go on to say, "This degree of resemblance can only be interpreted in terms of close phyletic affinity."

We are, then, presented with two flagellates, *Platymonas* and *Heteromastix*, which on the one hand show a "close phyletic affinity" but on the other differ in the process of cell division by those features which characterize the evolutionary divergence of two large green algal classes, Charophyceae and Chlorophyceae (cf. Stewart and Mattox, 1975a). The situation is not surprising in theoretical terms; it has been postulated that the interzonal spindle which persists until the completion of cytokinesis is the primitive or general condition (Pickett-Heaps, 1972b), and it has been assumed that green algae with a persistent interzonal spindle lie at the base of both the charophycean and chlorophycean lines (Stewart and Mattox, 1975a). It is somewhat surprising, however, that enough green algae of sufficient diversity to make such lines of evolution possible to study have remained extant. The affinity of *Heteromastix* and *Platymonas* not only demonstrates that the two types of cell division can occur in related primitive monads, but that a comparison of other features of the two genera provides some insight as to how and why the collapsing telophase spindle and the phycoplast evolved. In *Heteromastix*, the extensive elongation of the spindle at anaphase is accompanied by an equal elongation of the cell itself so that the nuclei are widely separated at the time of

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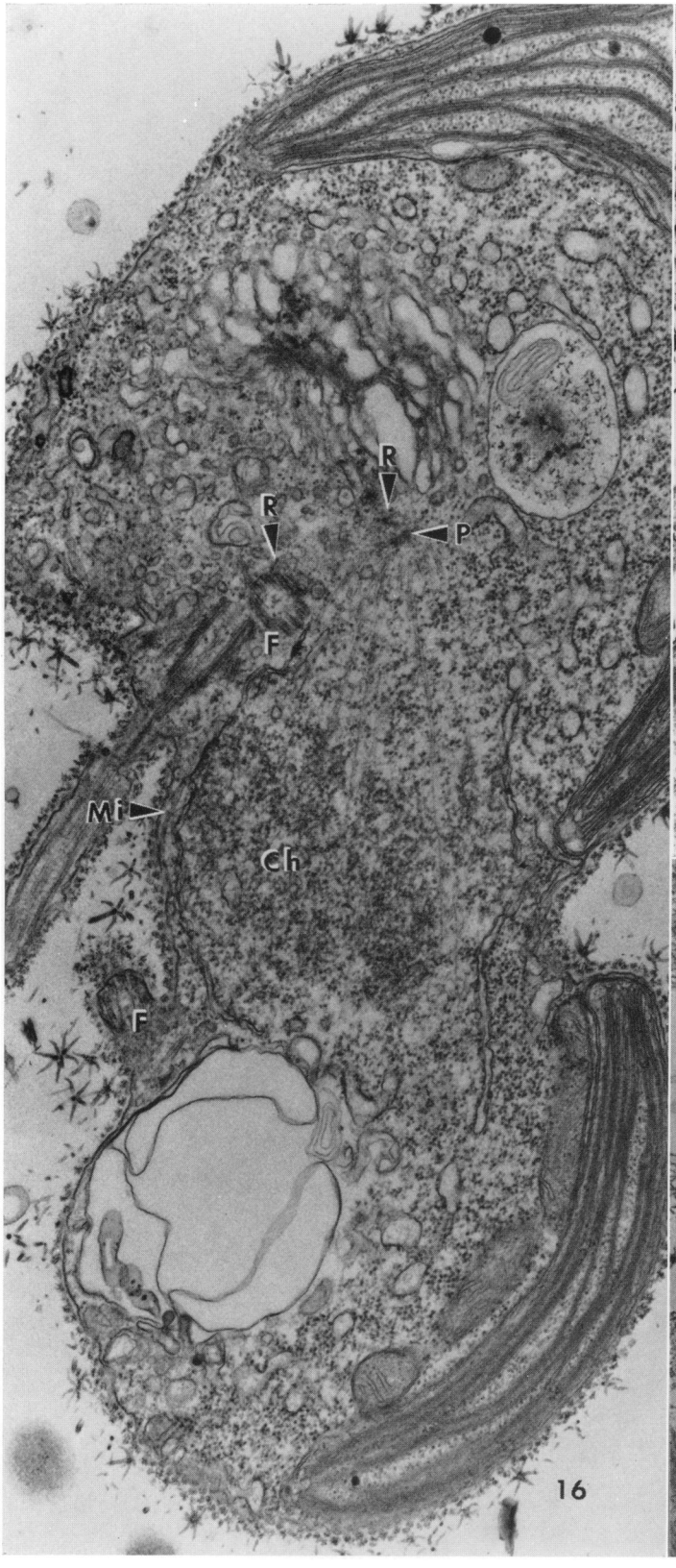
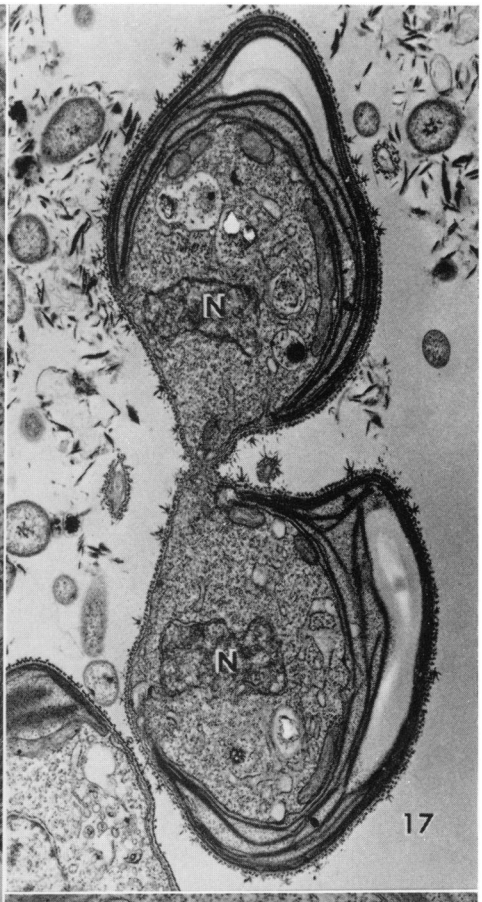
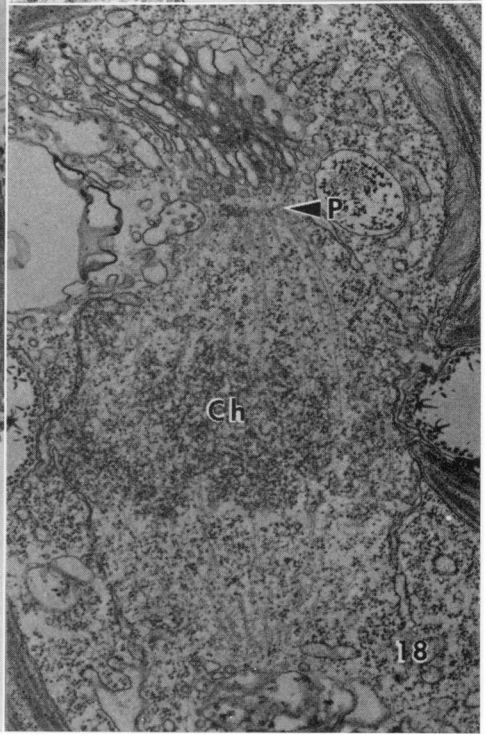
Fig. 13, 14. **13.** Prophase. Later than Fig. 12. The cell cleavage (Cl) opposite the flagella (at F) had become wider and deeper. Numerous microtubules (a group at each arrow) radiate from the regions occupied by the two rhizoplasts at this stage. The rhizoplasts themselves are not included in the section. N, nucleus. × 36,000. **14.** Prometaphase. An oblique section showing only one pole of the developing spindle. The region between the two arrows is the flat pole of the half-spindle. Some of the microtubules of the half-spindle had penetrated the nucleus (N) while others remain in the cytoplasm (just above the nucleus on the left side). The position of the flagellar apparatuses would be above the nucleus in this micrograph. × 40,000.







cytokinesis. Presumably the scaly covering of *Heteromastix* is either extensible enough to accommodate such elongation or new scales are deposited on those areas of the plasmalemma undergoing elongation (cf. legend for Fig. 8). In contrast, the body of the cells of *Platymonas* is covered by a wall-like theca (Manton and Parke, 1965). The theca expands somewhat during cell division but apparently lacks the extensibility of the scaly covering in *Heteromastix*. The spindle of *Platymonas* cannot elongate to the degree observed in *Heteromastix*, and, as a result, the daughter nuclei lie close together at the time of cytokinesis (cf. Fig. 17, 19, 20 of this paper to Fig. 13 of telophase in *Platymonas* in Stewart et al., 1974). It is also possibly significant in this regard that chromosome separation at anaphase in *Platymonas* is apparently largely accompanied by a shortening of chromosome-to-spindle pole distance rather than by spindle elongation as in *Heteromastix*.

Since two of the major differences between *Heteromastix* and *Platymonas* are the presence of an outer body covering of stellate scales and a persistent interzonal spindle in the former and the occurrence of a wall-like theca and a collapsing telophase spindle/phycoplast in the latter, a reasonable inference is that the evolution of the theca and the collapsing telophase spindle/phycoplast are related. The idea is clearly attractive when it is recalled that Fig. 10, 11, 13 in Stewart et al. (1974) indicate that spindle elongation is severely restricted in *Platymonas* and that the daughter nuclei are never greatly separated. The comparison strongly supports the idea that the collasping telophase spindle and the phycoplast evolved in green flagellates with the function of insuring that the cleavage furrow passes accurately between two daughter nuclei whose degree of separation is restricted by a cell wall or theca. Although there has been no previous morphological evidence that the phycoplast evolved under those circumstances, Pickett-Heaps (1975) has speculated that it may have evolved under some special circumstances where there was a need for cell division to occur within restricted space.

A close or direct relationship between *Heteromastix* and *Platymonas* is indicated not only by the similarity in the scaly covering of their flagella, but by the fact that the theca of *Platymonas* shows a relationship to the outer layer of stellate scales on the body of *Heteromastix*. The theca of *Platymonas* is formed by tiny stellate scale-like structures which are synthesized in the cell, transported to the outside, and are then fused to form a solid wall-like structure, in which the individual stellate particles are no longer discernible (Manton and Parke, 1965). In addition to larger stellate scales as shown in Fig. 16, *Heteromastix* possesses smaller stellate scales (see other figures and Manton et al., 1965), some approaching the size of those which fuse to form the theca in *Platymonas*. Manton and Parke noted that the finished theca bears a resemblance to a true wall but pointed out that its relationship to the cell walls of other green algae was not clear because Lewin (1958) had shown that residues from the theca contain amino acids and some sugars but no glucose, indicating an absence of cellulose (see also Gooday, 1971).

The relationship of the theca of *Platymonas* to the walls of other green algae is a matter of great importance to this discussion. If the phycoplast evolved in green flagellates similar to *Platymonas*, one would suppose that such flagellates gave rise to *Chlamydomonas*, further to other related flagellates with phycoplasts, and eventually to a vast array of algae now included in the Chlorophyceae. Fortunately, some recent and surprising research on the nature of the cell wall of *Chlamydomonas* and related monads (Miller et al., 1974; Roberts, 1974) has shown that their walls also have no cellulose but do have glycoproteins which give residues similar to those of the theca of *Platymonas*. There is, then, no apparent barrier to deriving the walls of some typical chloropyhcean algae from structures similar to the theca of *Platymonas*.

The evidence that the phycoplast evolved as a result of the evolution of a cell wall in such green flagellates as *Platymonas* raises a question as to why a collapsing telophase spindle and a phycoplast, or similar mechanisms, do not occur in other green algae or in other algal phyla. The answer seems to be that flagellated unicellular stages with a complete one-piece wall have not evolved among any group of eucaryotic microorganisms except chlorophycean flagellates.

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Fig. 16–18. **16.** Metaphase. Slightly oblique section through spindle but with the structure of one of the poles shown clearly. The flagellar basal bodies (F) lie lateral to the spindle poles. A very faint segment of a rhizoplast (between the two arrows labeled R) lies over the spindle pole. A thin layer of dark substance (P) is often seen overlying the ends of the microtubules at the spindle pole. A microtubule (Mi) is seen in the cytoplasm between the basal bodies. Ch, metaphase chromosomes. × 27,000. **17.** Oblique section of cell in telophase. Note that the daughter nuclei (N) are widely separated at time when cytokinesis is near completion. × 6,500. **18.** Metaphase spindle. This spindle is from a cell sectioned in the long dimension but at right angles to that which would include the basal bodies and rhizoplasts. The broad flat spindle pole (P) is shown better here than in the other figures. Ch, metaphase chromosomes. × 18,000.

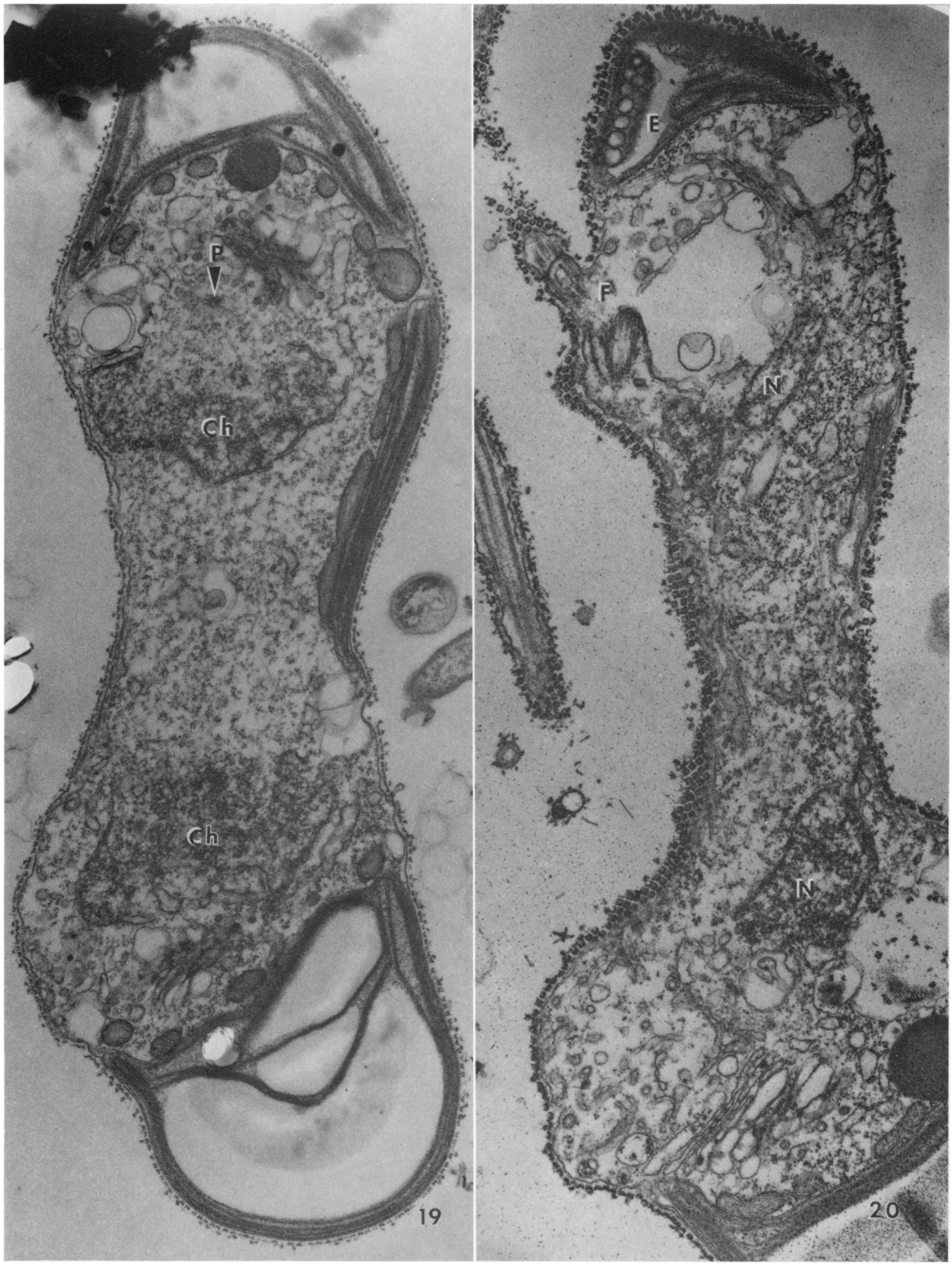

Fig. 19, 20. **19.** Anaphase. The cell had become greatly elongated. The section is through the middle of the spindle pole in the upper part of the micrograph. A comparison of the upper spindle pole with the metaphase spindles in Fig. 16 and 18 shows that there is little decrease in spindle pole-to-chromosome distance by the time anaphase has reached this stage. In the upper spindle pole the outer edge of the chromosome mass (Ch) had be-

Unicellular flagellated forms in other groups are naked or covered with plates or scales, and in other algal groups walls appear to have evolved only in connection with the origin of coccoid or filamentous species or stages. The evolution of complete, one-piece walls in flagellated unicells was probably a more intricate process than the evolution of walls in non-motile forms in which the cells can gradually double in size to accommodate the development of the spindle and division into daughter cells. Although the walls of *Chlamydomonas* grow gradually as the cell matures, *Chlamydomonas* does not rapidly double in cell width during cell division like *Heteromastix*, and a gradual doubling beyond mature cell width, as occurs in filamentous forms, would probably be hydrodynamically disavantageous. Furthermore, the theca of *Platymonas*, which may be the primitive form of chlorophycean cell wall, does not appear to possess the degree of extensibility found in the wall of the more advanced *Chlamydomonas*. In contrast to *Platymonas*, most species of *Chlamydomonas* may produce small daughter cells within the old cell wall. Each daughter cell is able to grow to mature size with the accompanying growth of its cell wall. The theca of *Platymonas* extends only slightly during cell division, but since only two daughter cells are produced, they do not undergo the degree of growth exhibited by the young cells of *Chlamydomonas*.

The restriction of cell elongation during cell division was probably not the only complexity involved with the evolution of true cell walls in green flagellates. *Chlamydomonas* and related walled genera drop or resorb the old flagella prior to cell division, whereas scaly or naked algal flagellates retain the old flagella. Apparently, the necessity for new flagella to be produced in the proper position so that the new walls are secreted around their bases requires the loss of the old flagella in walled flagellates. We believe that the foregoing discussion not only helps to explain the evolution of the phycoplast and collapsing telophase spindle in chlorophycean algae but also provides some basis for understanding why walled flagellates did not evolve independently in a number of algal groups.

In a brief preliminary discussion of the idea that the phycoplast and collapsing telophase spindle evolved in connection with the evolution of cell walls in green flagellates (Stewart and Mattox, 1975b), we pointed out that the idea has certain implications that might provide some opportunity for judging its validity. One obvious implication is that all walled green flagellates will be found to have phycoplasts. Another, not quite so obvious, is that no filamentous green algae with phycoplasts and early-collapsing interzonal spindles will be found to have body scales on the zoospores, because the hypothesis assumes that the flagellated unicellular ancestors of such filaments had already evolved cell walls and had lost body scales. It should be emphasized that the absence of scaly zoospores in advanced green algae with phycoplasts should not be taken as evidence that these algae do not have scaly ancestors; the presence of both a phycoplast and scaly flagella in *Platymonas* clearly establishes the relationship of the Chlorophyceae to scaly green flagellates. One other implication that logically follows, but which was not mentioned in the earlier discussion (Stewart and Mattox, 1975b), is that all green flagellates with body scales will prove to have a persistent interzonal spindle and to lack a phycoplast. It is not implied, however, that naked green flagellates will necessarily have persistent interzonal spindles and lack phycoplasts; some naked green flagellates may be primitively wall-less (e.g., probably *Pedinomonas*, cf. Pickett-Heaps and Ott, 1974) and lack a phycoplast, while others could have lost the wall in the course of evolution but retained the phycoplast (e.g., probably *Asteromonas*, Floyd and Salisbury, 1976, and pers. comm.).

In summary, the preceding discussion presents evidence and rationale for a concept that the distinctive features of the phycoplast-containing Chlorophyceae are the result of an unique evolution of a cell wall in the flagellated ancestors of such chlorophycean algae. Further reasons are given for suspecting that the origin of the cell wall in those ancestral flagellates involved the extracellular fusion of modified stellate scales similar to those in *Heteromastix*. Finally, the hypotheses are justified by a discussion of certain implications which allow a test of their predictive merits. Although the discussion might provide an explanation for a number of cytological phenomena in the green algae, it also brings attention to several questions and problems. For one example, what was the nature of the evolutionary origin of those filamentous ulvacean al-

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come curved toward the spindle pole (P). A dark mass is present at the pole as in the metaphase spindle of Fig. 16. × 19,000. **20.** Off-median section of telophase. Note that the cell has elongated to the extent that it is at least twice as long as an interphase cell. Also note that microtubules are still present in the interzone between the daughter nuclei (N). The presence in the section of one (F) of the two flagellar pairs shows that they have continued to move apart during cell elongation and have remained associated with each group of separating chromosomes. E, eyespot. × 27,000.

gae (e.g., *Pseudendoclonium* and *Trichosarcina*, Mattox and Stewart, 1973, 1974; and *Monostroma*, Jonsson and Chesnoy, 1974) and charophycean algae (e.g., *Coleochete*, Marchant and Pickett-Heaps, 1973; and *Chaetosphaeridium*, Moestrup, 1974; see also Thompson, 1969) that do produce motile reproductive cells with body scales? Those algae do not have phycoplasts and therefore support the ideas discussed above. Their scaly zoospores and gametes clearly indicate that their ancestors were scaly rather than walled and that cell walls evolved in non-flagellated stages of the ancestral scaly flagellates. We have mentioned before (Stewart and Mattox, 1975a, b) that the scaly ancestors of these filamentous algae might have clung together to form primitive, scaly filament similar to the plate-covered cells of filamentous dinoflagellates, but a more likely explanation might lie with stages similar to the walled, non-motile "*Halosphaera*-stage" of the scaly green flagellate *Pyramimonas* (Manton, Oates, and Parke, 1963; Parke and den Hartog-Adams, 1965). *Halosphaera* can cleave to form flagellated, scaly unicells reminiscent of the production of scaly zoospores in the genera mentioned above. A *Halosphaera*-like stage could give rise to a walled filament which would retain the ability to produce flagellated reproductive cells with body scales. The presence of cell walls in the non-motile stages of chrysophytes (e.g., Leadbeater, 1970) also suggests that cell walls in filamentous or parenchymatous Chromophyta similarly had their origin with non-motile stages of unicellular flagellates and further emphasizes the uniqueness of *Platymonas*, *Chlamydomonas*, and related genera. It should also be mentioned in this regard that there is no evidence that the cell wall in filamentous charophycean algae evolved from scales as is apparently the case in chlorophycean algae. It is possible, therefore, that the wall of the two classes may be different in origin and at least to some degree in structure and composition. Further research on the nature of the wall in chlorophycean algae with phycoplasts is greatly needed.

The presence of scaly zoospores in the Ulvaceae raises another serious question with regard to the earlier discussion. The structure of the flagellar apparatus and certain features of cell division in ulvacean genera have been interpreted in the past as an indication that the Ulvaceae is closely related to those chlorophycean algae with phycoplasts (Mattox and Stewart, 1974; Pickett-Heaps and McDonald, 1975; Pickett-Heaps, 1975), but if the ideas discussed earlier in this paper are valid, the presence of scaly zoospores along with the absence of a phycoplast in the Ulvaceae indicates that their flagellated ancestors had body scales and therefore lacked phycoplasts also, unlike *Platymonas*, *Chlamydomonas*, and the ancestors of the Chlorophyceae. We have stated before that the Ulvales are a problematical group (Stewart and Mattox, 1975a, b); it is possible that those organisms represent a third line of evolution distinct from the Chlorophyceae and Charophyceae.

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