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Further observations on *Olisthodiscus luteus* (Raphidophyceae, Chromophyta): the flagellar apparatus ultrastructure

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The flagellar apparatus of the raphidophycean alga *Olisthodiscus luteus* Carter (Chromophyta) was examined. Basal bodies are overlapped in a counterclockwise orientation, and are connected by two striated fibers. A rhizoplast and a structure called the proximal plate link the basal bodies to the nucleus. There are four microtubular roots arranged in asymmetrical fashion. One of the anterior basal body-associated flagellar roots is a compound root, consisting of ca. 15 microtubules and carries an unusual layered structure. A microtubule organizing center is also associated with this root and cytoskeletal microtubules emanate laterally. Golgi-derived vesicles are located inside the loop-like path made by this root. The posterior basal body also carries a compound root consisting of five microtubules, a half-cylindrical band called a trough-shaped structure, and a proximally located lamellate structure. The homologies of the flagellar apparatus components of *O. luteus* and those of other chromophyte classes are discussed, and it is clear that the flagellar apparatus of *O. luteus* shares various features with that of other chromophytes, especially the Chrysophyceae. Similarities and differences of the flagellar apparatuses of *O. luteus* to those of other raphidophycean algae are also discussed and an isolated taxonomic position of *Olisthodiscus* in the Raphidophyceae is suggested. Despite the presence of considerable variations in flagellar apparatus structures, the Raphidophyceae should be accepted as a natural taxon.

Key Index Words: Chromophyta—Chrysophyceae—flagellar apparatus—layered structure—Olisthodiscus luteus—*Raphidophyceae—rhizostyle.*

The Raphidophyceae includes flagellates containing chlorophyll *a* and *c* and is generally placed in a supra-assemblage, the Chromophyta, together with some other algae such as chrysophytes, diatoms, xanthophytes, and brown algae (e.g. Christensen 1989).

Morphological features that delineate the raphidophytes from other chromophytan algae are multiple chloroplasts, a ring of Golgi bodies surrounding the anterior part of the nucleus and an absence of a photoreceptor apparatus (Hibberd 1986). The relationships between raphidophytes and other chromophytan algae are still not well understood. Some phycologists (Parke and Dixon 1976, Silva 1980) rank this algal group as a class, the Raphidophyceae. However Christensen (1980) divided the raphidophytes into two, placing some in the Chrysophyceae and some in the Xanthophyceae. Hibberd (1986) suggested that the key to solving this taxonomic problem lies in the structure of the flagellar root system. Possible affinities of the Raphidophyceae with other chromophyte algae was recently reviewed by Heywood (1989).

Vesk and Moestrup (1987) studied the flagellar apparatus of a marine raphidophyte *Heterosigma akashiwo* (Hada) Hada in an initial attempt to settle the dispute about this algal group. They described a unique layered structure associated with a band of microtubules that had not been observed in other groups of algae, and they suggested that it might be a suitable feature for delineating the Raphidophyceae. However, no detailed studies of the flagellar apparatus have been carried out on any other raphidophytes.

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In this paper, we describe the detailed structure of the flagellar apparatus of a raphidophyte alga *Olisthodiscus luteus* Carter (1937) and compare with other chromophytes in order to obtain evidences for determining the systematic position of the Raphidophyceae. Based on our observations, we propose that the Raphidophyceae should be recognized. Similarities and differences in the flagellar apparatus between *O. luteus* and other raphidophytes are also discussed.

Material and Methods

The culture strain of *Olisthodiscus luteus* used for this study is the same as that used for our previous investigation (Hara *et al.* 1985) which is now deposited in the Microbial Culture Collection of the National Institute for Environmental Studies (NIES), Tsukuba Japan, as strain no. NIES-15 (Watanabe *et al.* 1988).

The proceedure for transmission electron microscopy is also the same as Hara *et al.* (1985) except for the osmium fixation. Cells were collected by centrifugation and were fixed for 1 hr in 2% osmium tetroxide in 0.1 M phosphate buffer. The cells were rinsed in buffer twice, dehydrated with ethanol and embedded in Epon 812. Serial sections were cut with a diamond knife and collected on slot grids. The order and orientation of the sections were monitored during the whole procedure from sectioning to printing of micrographs. Absolute orientations of flagellar apparatus components were also determined by triplets' orientations of basal bodies. All sections were stained with uranyl acetate and lead citrate. Observations were made with the JEOL 100C and 100CXII transmission electron microscopes.

Results

General cell structure

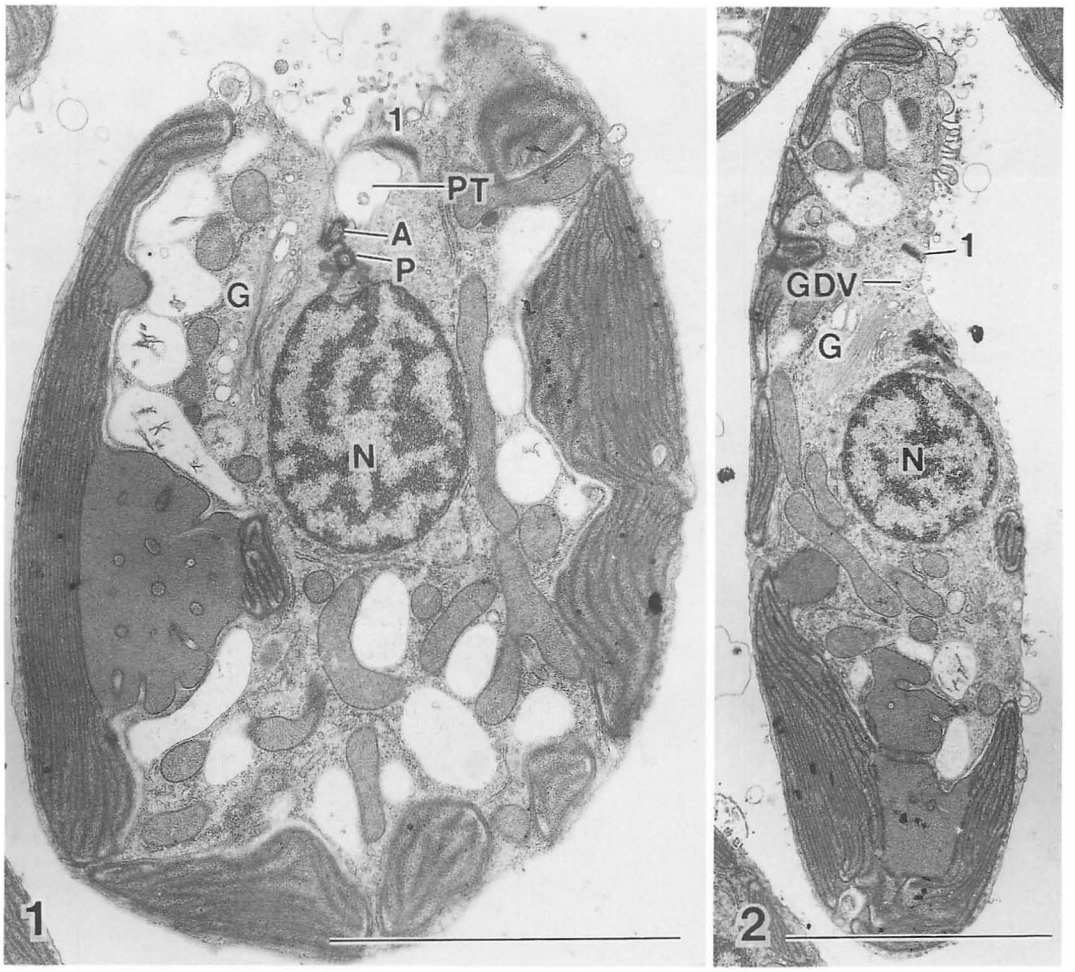
Figures 1 and 2 are longitudinal sections cut parallel and perpendicular to the dorsiventral plane, and they demonstrate the major cell organelles. Since we have already published the general features of *Olisthodiscus luteus* (Hara *et al.* 1985), we will describe only a few additional features here.

A small depression or pit is present just anterior to the flagellar insertion (Figs. 1, 34). At the bottom of the pit are vesicles, termed Golgi-derived vesicles (GDVs), containing small round electron dense granules and rod-like granules (Figs. 2, 13, 34). The rod-like granules are referred to as scales since these are also found covering the cell surface in samples of one to three months old cultures (Figs. 3, 4). Those scales on the cell surface are more or less regularly arranged and interconnected with each other by fibrous material (Figs. 3, 4). The scales are cross-banded and have ill-defined outlines (Figs. 3-6). The scales are produced singly in Golgi cisternae (Figs. 6, 25). It seems that scale-containing vesicles merge with one another, resulting in GDVs (Fig. 5), and then scales are released outside the cell.

The structure of the pyrenoid canal is also worth noting. This structure was previously referred to as cytoplasmic canal (Hara *et al.* 1985), but it is actually a penetration of the chloroplast membranes (Figs. 7-10). Inside the canal is electron dense material which is also observed in the space between the chloroplast endoplasmic reticulum and the chloroplast membranes, the periplastidal compartment. This material is usually slender near the mouth of the canal (Figs. 7, 9). It broaden proximally and fills the bottom of the canal (Figs. 7, 10). The proximal broad region is always seen more electron dense than the distal slender region because the material is more densely packed in the proximal half of the canal (Fig. 7). When the canal is broad and shallow, the material is not slender near the mouth, however it is more electron lucent than that filling the bottom (Fig. 8).

Flagellar apparatus

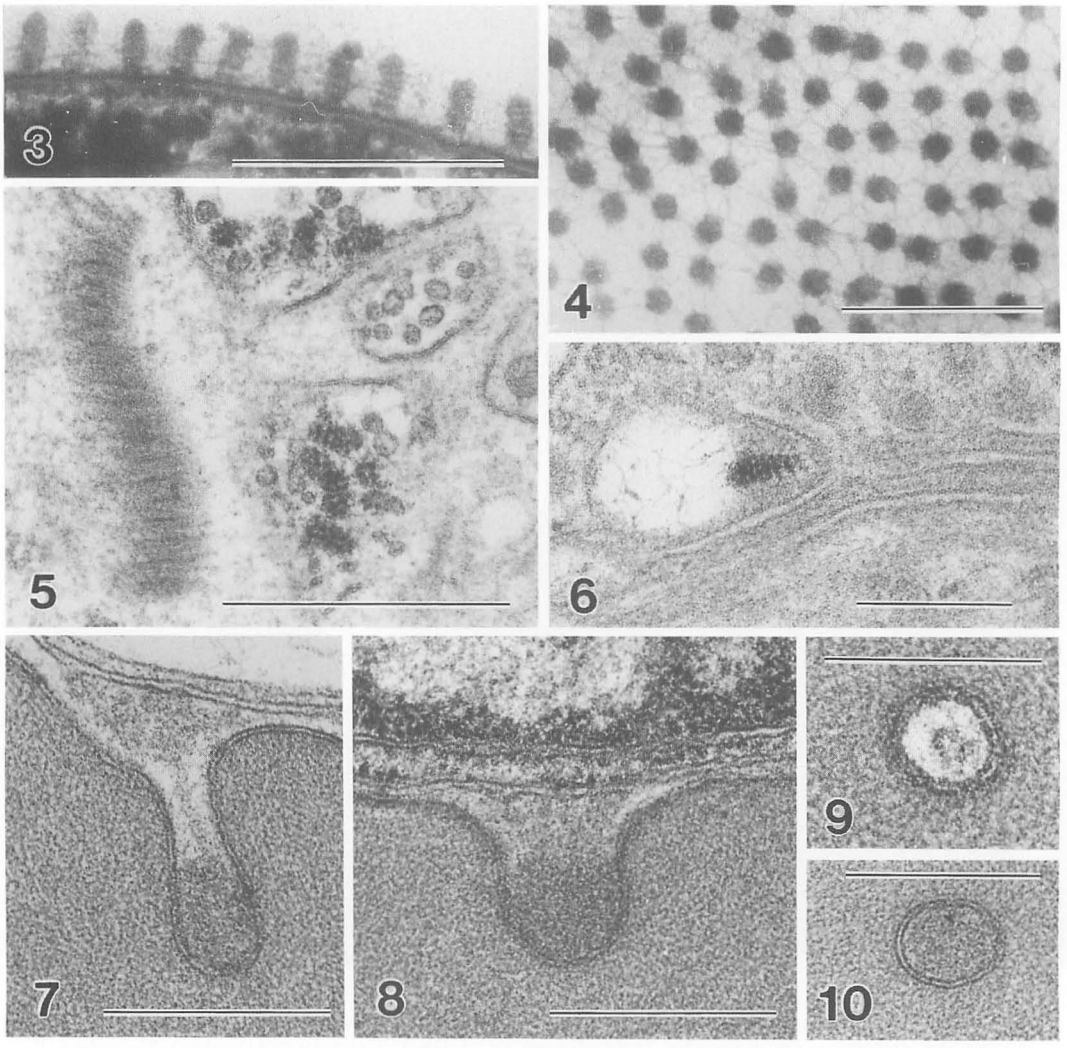
Swimming and trailing flagella are designated as the anterior and posterior flagella, respectively, and basal bodies of these are the anterior and posterior basal bodies. A transi-



Abbreviations used in figures
1, 2, 3, 4: microtubular flagellar roots R1, R2, R3 and R4. A: anterior basal body or anterior flagellum, CMT: cytoskeletal microtubules, DF: distal fiber, G: Golgi apparatus, GDV(s): Golgi-derived vesicle(s), N: nucleus, P: posterior basal body or posterior flagellum, PF: proximal fiber, PP: proximal-plate, PT: pit, R1LS: R1-associated layered structure, R3LS: R3-associated lamellate structure, RH: rhizoplast, TS: trough-shaped structure.

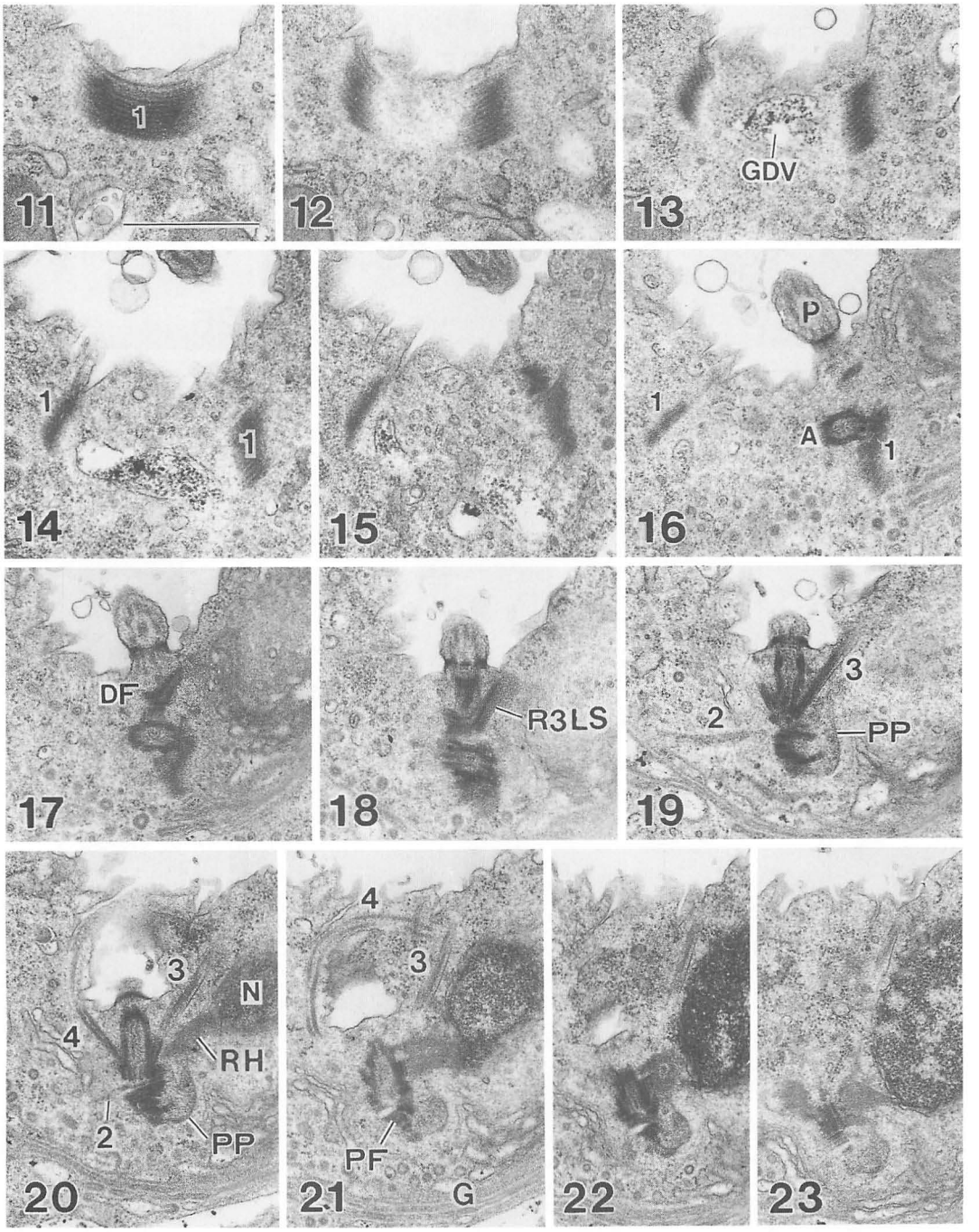
Figs. 1–2. *Olisthodiscus luteus*. Figs. 1, 2. Longitudinal sections of cell cut perpendicular (Fig. 1) and parallel (Fig. 2) to the dorsi-ventral axis. Configuration of major organelles is demonstrated. Basal bodies are situated just anterior to the nucleus. The R1 is associated with the pit and a Golgi-derived vesicle. Scale bars = 5 μm.

tional helix is absent (Fig. 24) as in other raphidophytes so far investigated (Hibberd 1979, Moestrup 1982, Heywood 1989). The basal bodies make an angle of about 90 degree and overlap their proximal ends in a counter-clockwise orientation (as defined for green algae, see O'Kelly and Floyd 1983, 1984a). They are interconnected by two striated fibers. The distal fiber links the distal end of the basal bodies (Figs. 16–18, 24, 25). This fiber is thicker and wider in the middle, and striations are conspicuous at this point (Fig. 25). The proximal fiber seems to be striated evenly, and attaches laterally to both the basal bodies at their overlapping region (Figs. 24, 31, 35). There is an electron transparent space in the middle of this fiber clearly separating it in half (Figs. 21, 22, 24, 31, 35). The half of the fiber associated with the anterior basal body juxtaposes with the most proximal

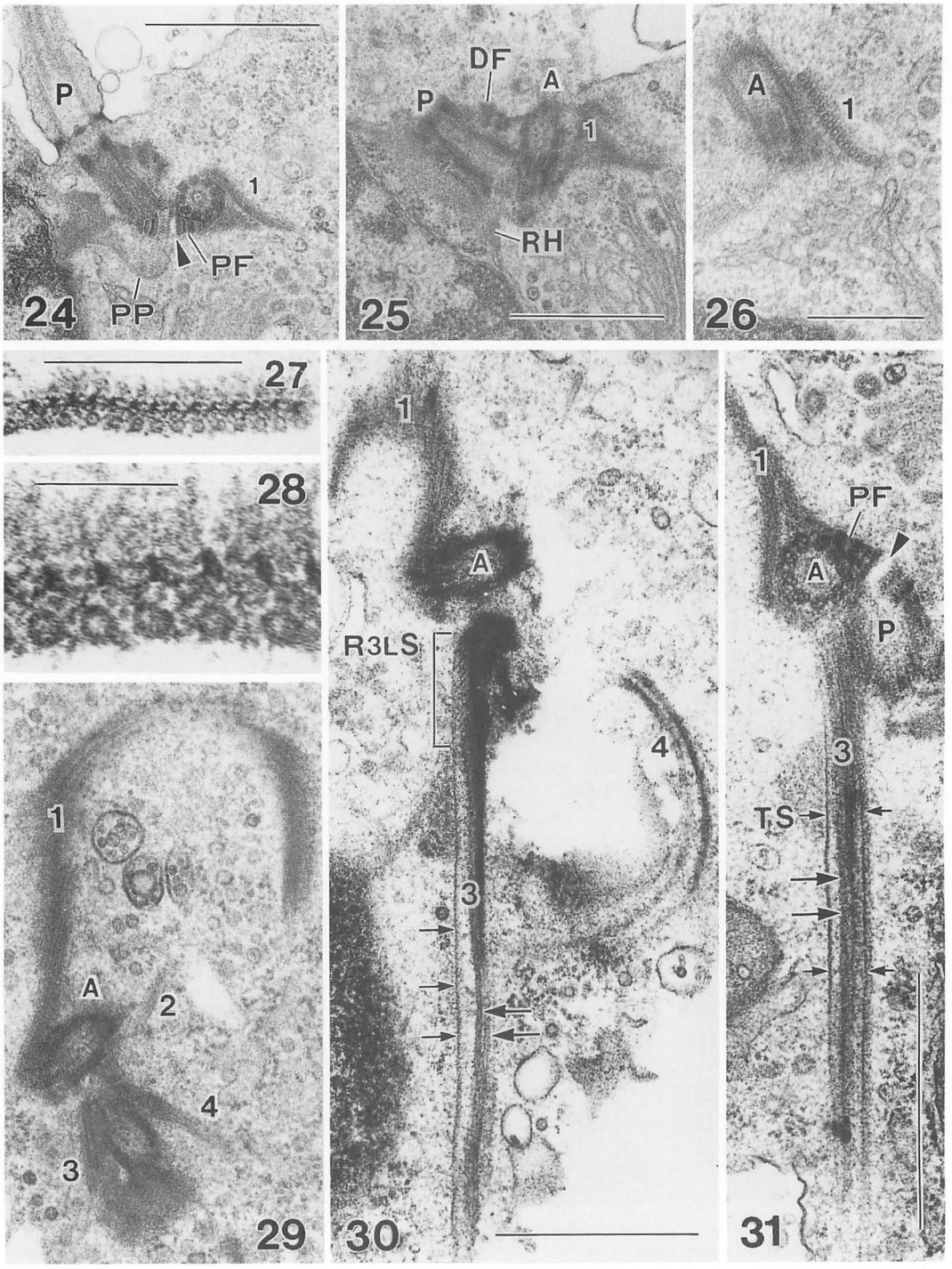


Figs. 3–10. *Olisthodiscus luteus*. Fig. 3. Osmium fixation. Scales with a cross-banded pattern are situated on the cell surface and are interconnected to one another by fibrous material. Fig. 4. Osmium fixation. A transverse section of scales arranged more or less regularly and interconnected by fibrous material. Fig. 5. Golgi-derived vesicles containing scales. Fig. 6. A part of Golgi cisternae containing scales. Fig. 7, 8. A pyrenoid canal containing electron dense material. Fig. 9. Transverse section of pyrenoid canal cut near the mouth. Fig. 10. The same as Fig. 9 cut near the bottom. Scale bars=0.5 μm (Figs. 3–5, 7, 8, 10), 0.25 μm (Figs. 6, 9).

region of a broad microtubular root, R1 (Figs. 24, 31). The basal body complex is linked to the nucleus by a broad fibrous root, the rhizoplast (Figs. 20-23, 25), which is short, very wide and often non-striated (Figs. 21, 25, 33), although its striated nature has been demonstrated previously (fig. 18, Hara *et al.* 1985). The rhizoplast is attached along the entire length of the posterior side of the posterior basal body (Fig. 25). It widens and extends to the concave anterior surface of the nucleus (Figs. 25, 34, 35). A peculiar electron-dense plate, referred to as the proximal-plate, arises from the basal body complex (Figs. 19-23, 24, 35). The proximal plate has a well-defined sigmoid edge (Fig. 24), and is attached anteriorly either to the proximal end of the anterior basal body (Figs. 19-23) or to the proximal fiber (Figs. 24, 35). Posteriorly, it joins with the rhizoplast and they extend



Figs. 11–23. Consecutive serial sections of the flagellar apparatus of *Olisthodiscus luteus*. Micrographs show images viewed from the anterior side of the cell. Configuration of most components of the flagellar apparatus can be traced. The R3-associated lamellate structure is seen in Fig. 18. Two microtubules composing the R4 are seen in Figs. 20, 21. Scale bar=1.0 μm. Scale bar in Fig. 11 is also applicable to Figs. 12–23.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together towards the nucleus (Figs. 22-24).

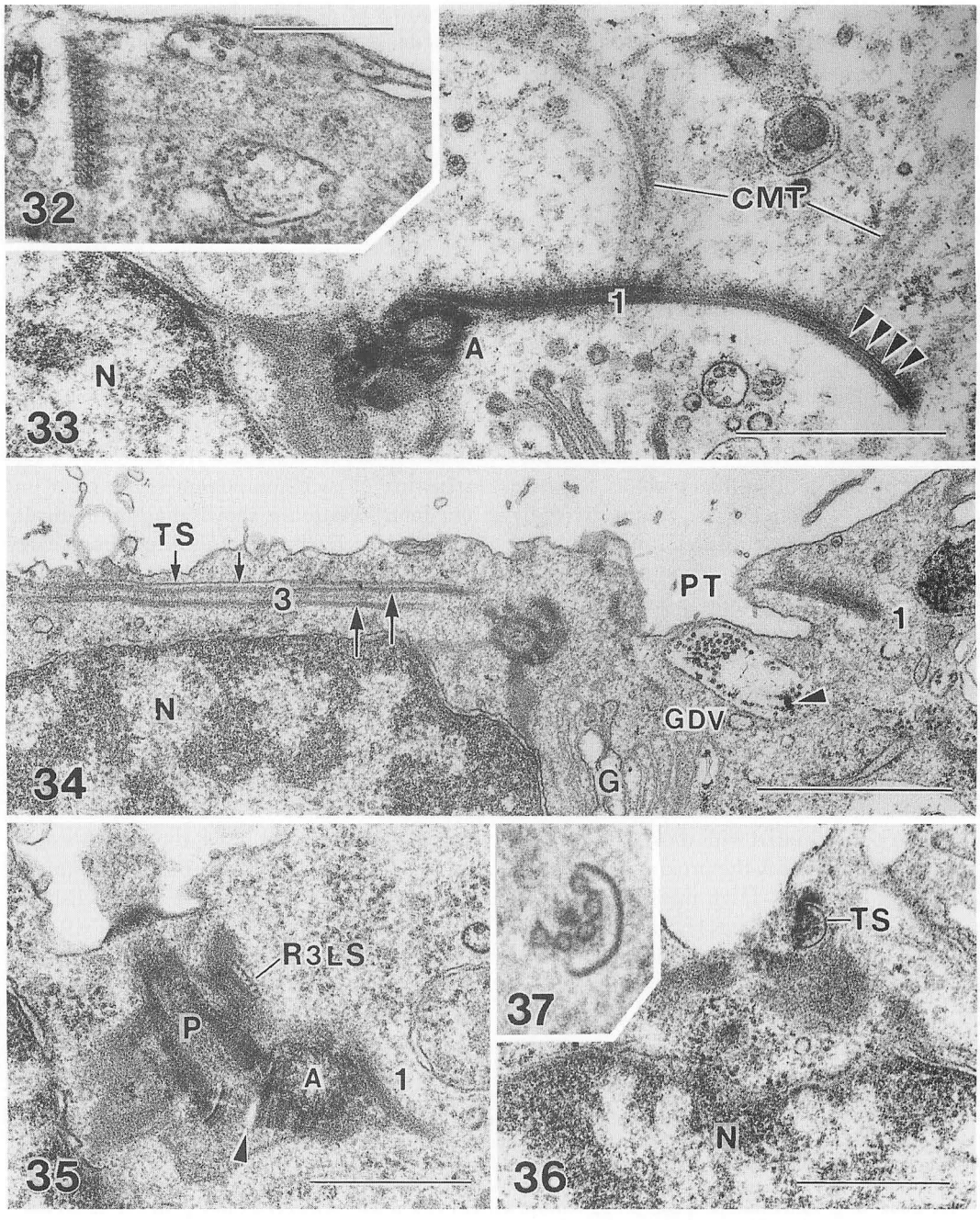
Four microtubular flagellar roots were observed, two per each basal body (called R1 and R2 for those associated with the anterior basal body, and R3 and R4 for those associated with the posterior basal body).

R1 is a broad and compound root comprising c.15 microtubules aligned in a row (Fig. 26) and an associated layered-structure (abbreviated as R1LS) (Fig. 27, 28, 32). The R1 microtubules arise along the anterior basal body on the side away from the posterior basal body (Figs. 16, 17). It extends anteriorly bending clockwise when viewed from above the flagellar insertion. The R1 returns to the vicinity of the basal bodies so that it has a loop-like path as a whole (Figs. 1, 29). The R1 is also characterized by the nucleation of cytoskeletal microtubules which emanate laterally (Figs. 32, 33), indicating that a microtubule organizing center (MTOC) is associated with this root. Cytoskeletal microtubules have been observed in many cells but not in all since these are not well preserved in fixed samples used in this study. The R1LS is situated at one side of R1 microtubules, the side where cytoskeletal microtubules arise (Figs. 32). It seems to be associated with most of the length of the R1 but not with the proximal part of the root (Figs. 24, 26). High magnification of a transverse section of the R1LS and associated root microtubules is given in Figs. 27 and 28, but it is difficult to show clearly its detailed structure. Figure 38 is a tentative reconstruction of the R1LS which may help to clarify the structure of R1LS. In transverse section, two dense layers are visible above the R1 microtubules and less electron dense amorphous material overlies these layers (Figs. 27, 28). The R1LS seems to be composed of repeating units, electron dense triangular components and several other components of ill-defined outline (Figs. 28, 38). The latter are less electron dense than the triangular component. Adjacent triangular components are interconnected by these ill-defined components at both the distal and proximal regions so that the R1LS appears as two layers in transverse and tangential sections (Figs. 27, 33, 38). The R1LS seems to be linked to the R1 microtubules (Fig. 28). The structure of each component is not clear but our interpretation is shown diagrammatically in Fig. 38. Each microtubule seems to carry a short arm on the side away from the R1LS (Figs. 28, 38). Cytoskeletal microtubules probably arise from amorphous layer overlying the R1LS (Figs. 27, 28), suggesting that it may have a MTOC role (Fig. 32).

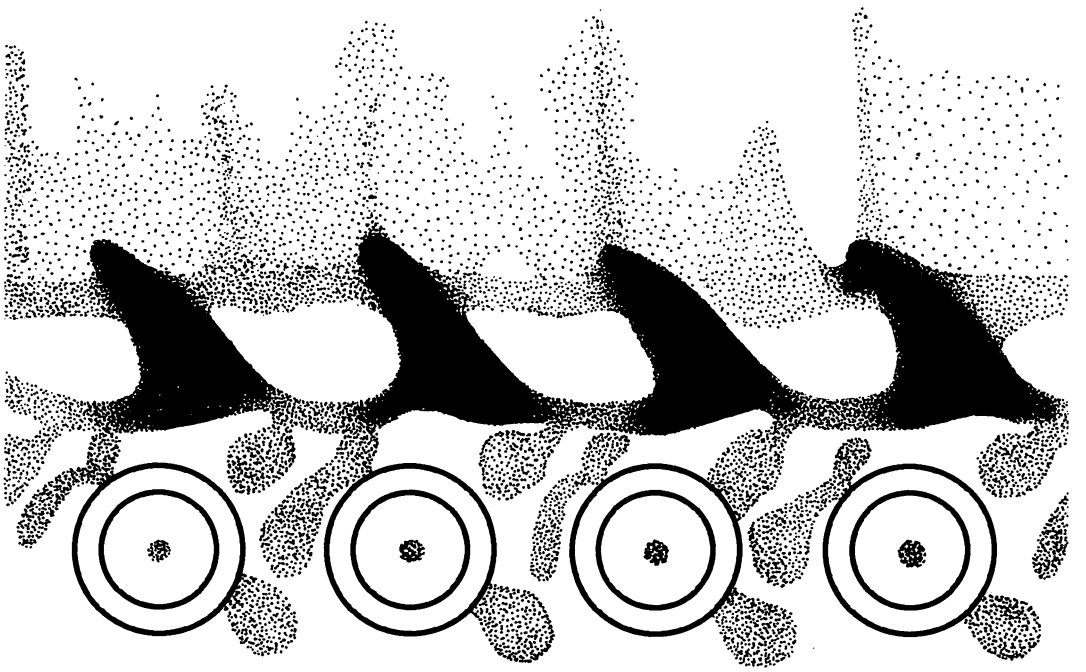
The R2, another microtubular root associated with the anterior basal body, arises from the opposite side of the R1 and extends anteriorly (Figs. 19, 20, 29). It is much less conspicuous than all other roots of this alga, and is probably composed of a single microtubule which terminates a short distance inside the path made by the R1 (Figs. 19, 20).

The R3 is a compound root that extends directly along the ventral side of the nuclear surface in a posterior direction and beneath the plasmalemma (Figs. 34, 36). It is com-

Figs. 24–31. Flagellar apparatus of *Olisthodiscus luteus*. Fig. 24. A section illustrating a counterclockwise orientation of the basal bodies. The striated proximal fiber with an electron-transparent gap at the middle (arrowhead), the proximal plate that interconnects the flagellar apparatus and the nucleus and R1 attaching to the anterior basal body are seen. Note that transitional helix is absent in the transition region of the posterior flagellum. Fig. 25. A rhizoplast connecting concave anterior end of the nucleus and entire posterior side of the posterior basal body. The distal fiber and the R1 are also visible. Fig. 26. Transverse section of R1 at its most proximal part. Note that no R1-associated layered structure is visible. Fig. 27. Transverse section of R1-associated layered structure. Note two electron dense layers and overlying amorphous material. Fig. 28. High magnification of the R1-associated layered structure. Fig. 29. The clockwise path of the R1. Orientations of other three roots can also be traced. Fig. 30. Paths and structure of the R3 and R4. The R3-associated lamellate structure situated at the proximal part of the R3 (indicated by a bracket), trough-shaped structure (small arrows) and R3 microtubules (large arrows) are seen. Clockwise path of the R4 is also seen. Fig. 31. Similar section to Fig. 30, but of slightly different angle. Both edges of the trough-shaped structure (small arrows) are seen sandwiching microtubular components (large arrows). Proximal fiber with a gap is also visible (arrowhead). Scale bars=1.0 μm (Figs. 24, 25, 29, 30, 31), 0.5 μm (Fig. 26, 27), 0.1 μm (Fig. 28).



posed of five microtubules, a thin half-cylindrical band called a trough-shaped structure and an associated lamellate structure called R3LS (Figs. 17-23, 30, 31, 34, 35). The microtubules are along the concave surface of the trough-shaped structure (Figs. 30, 31) and appear as an arc in transverse section (Figs. 36, 37). The trough-shaped structure does not attach directly to the basal bodies, but arises from a region some distance distal to the posterior basal body, and extends posteriorly (Figs. 30, 31, 34). The R3LS is



Figs. 38. A tentative reconstruction of a possible structure of the R1-associated layered structure of *Olisthodiscus luteus*. See text for explanation. Not to scale.

seen only at the proximal region of the R3 (Figs. 18, 30). It is made up of an electron dense plate and several lamellae which are regularly arranged and projecting towards the basal body (Fig. 35). At the proximal region of the R3, dense material lies on the trough-shaped structure and R3LS (Figs. 30, 35, 36). It continues posteriorly as a dense bar situated in the concave surface of microtubules (Fig. 37). R4 is a simple root, probably made up of two microtubules (Figs. 20, 21, 30), and arises from the opposite side of the R3, extends posteriorly, bends clockwise when viewed from the flagellar insertion, and its distal end is close to the R3 (Figs. 21, 30).

The flagellar apparatus as a whole has an extremely asymmetrical architecture, but all the components are arranged in a single absolute configuration. This is summarized diagrammatically in Fig. 39 which also incorporates some data from a previous paper (Hara *et al.* 1985).

Discussion

Observations obtained in this study make it possible to consider homologous relationships between the flagellar apparatus of *Olisthodiscus*

Figs. 32–37. Flagellar apparatus of *Olisthodiscus luteus*. Fig. 32. R1 and associated cytoskeletal microtubules. Note that cytoskeletal microtubules arise from electron-dense material. Fig. 33. R1 and cytoskeletal microtubules viewed from different angle. The R1-associated layered structure is seen as two dense lines (arrowheads). Fig. 34. A section cut in similar plane as Fig. 2 but viewed from opposite direction. Positional relationships between the pit, R1 and Golgi-derived vesicle is illustrated. A trough-shaped structure (small arrows) and microtubular components (large arrows) comprising R3 are extending posteriorly above the nucleus and beneath the plasmalemma. A scale is visible in the Golgi-derived vesicle (arrowhead). Fig. 35. A section from the same series of serial sections as Fig. 24. Transverse view of the R3-associated lamellate structure is seen as an thin plate and associated lamellae projecting inward. Electron-transparent gap of the proximal fiber (arrowhead) is also seen. Fig. 36. Transverse view of the trough-shaped structure and associated electron dense material. Fig. 37. High magnification of transverse section of trough-shaped structure and R3 microtubules. Scale bars=0.5 μm (Figs. 32, 35, 36), 1.0 μm (Figs. 33, 34), 0.25 μm (Fig. 37).

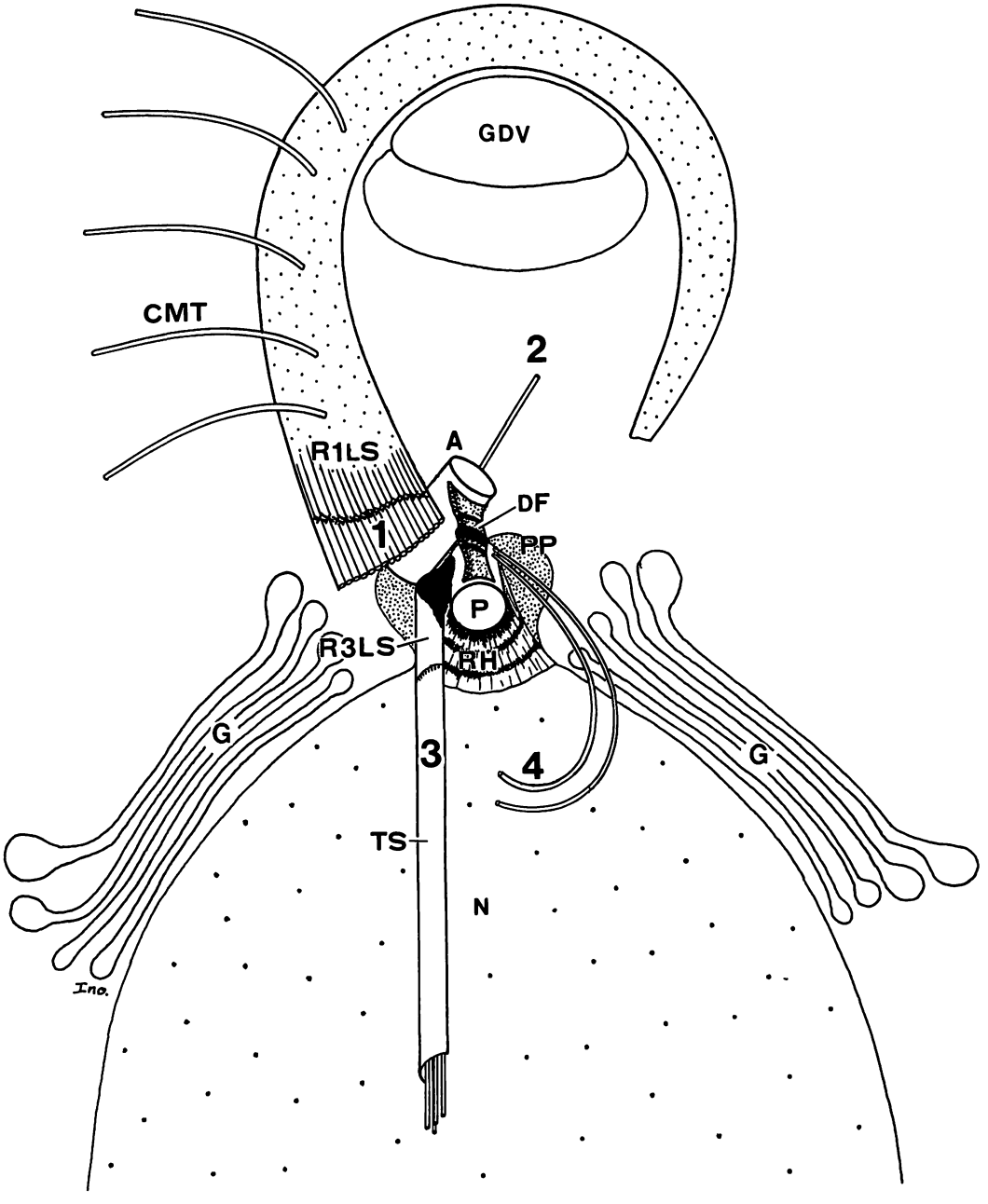


Fig. 39. Reconstruction of the flagellar apparatus of *Olisthodiscus luteus*. Not to scale.

luteus and that of other chromophyte classes. The anterior and posterior flagella of the raphidophytes are homologous to those of the other chromophyte classes based upon the occurrence of mastigonemes and the configuration of the basal bodies. In Fig. 40, diagrammatic reconstructions of the flagellar apparatuses of chrysophytes, brown algae and *O. luteus* are shown based on the available data (Bouck and Brown 1973, Andersen 1987, 1990 and Owen *et al.* 1990a for chrysophytes, Henry and Cole 1982, O'Kelly and Floyd 1984b, O'Kelly 1989a for brown algae). Each basal body has two microtubular

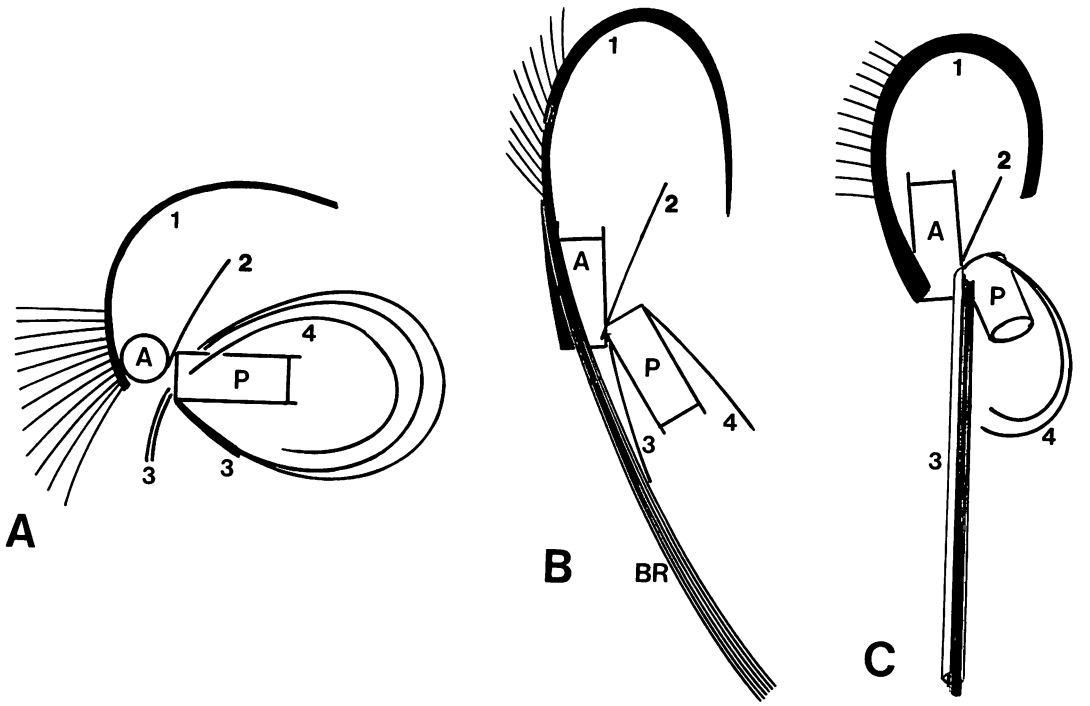


Fig. 40. Diagrammatic representation of flagellar apparatuses (viewed from above the flagellar insertion) of a typical chrysophyte (A) (based on Andersen 1990 and Owen *et al.* 1990a), brown algae (B) (based on O'Kelly and Floyd 1984, O'Kelly 1989a), and *Olisthodiscus luteus* (C). Structures labeled by the same characters or numbers are considered homologous. See text for explanation. Not to scale.

roots and these roots arise from the same sides of homologous basal bodies, so that the roots labeled by the same numbers in Fig. 40 are homologous to one another. This is supported by observations that one of the microtubular roots associated with the anterior flagellum, R1, is responsible for nucleating cytoskeletal microtubules (Bouck and Brown 1973, Schnepf *et al.* 1977, Henry and Cole 1982, O'Kelly and Floyd 1984b, Andersen 1987, 1991, Preisig 1989), and that, when viewed from above the flagellar insertion, it has a clockwise path. This is also the case for the R1 of *Heterococcus tectiformis* (O'Kelly 1989b), an only xanthophyte alga of which flagellar apparatus has been studied in enough detail. The R1 is thus not only positionally but also, at least partially, functionally homologous in all algal groups concerned here. The clockwise path is also found in a eustigmatophyte *Vischeria stellata* (Santos and Leedale 1991). Although R1-associated cytoskeleton is absent in this alga, there is no question about their homology to R1 of other chromophytes. Homologous relationships of basal bodies and R1 in chromophyte algae lead us to further considerations of positional homologies of other roots.

The features of the R2 root are also held in common in many chromophyte algae: it is simple, most typically made up of a single microtubule, extends straight only a short distance, and terminates inside the R1 path. *Olisthodiscus* also resembles many chromophytes in this respect. A eustigmatophyte *Vischeria stellata* is once again exceptional, where R2 is made up of two microtubules and forms an antiparallel loops together with R1 (Santos and Leedale 1991).

The position where the R3 is situated in typical chromophyte flagellar apparatus is occupied by a structure called a rhizostyle. In *Heterosigma akashiwo*, the rhizostyle passes between two basal bodies and reaches anteriorly

to the flagellar groove and extends posteriorly between the plasmalemma and nuclear surface (Vesk and Moestrup 1987). The R3 of *Olisthodiscus* occupies the same position in relation to basal bodies, but it does not extends anteriorly beyond the basal bodies. The components of the rhizostyle has not been documented in enough detail. In *H. akashiwo*, the rhizostyle is rather fibrillar than microtubular, and in other raphidophytes it may contain microtubules in addition to fibrillar components (Vesk and Moestrup 1987). The R3 or rhizostyle of *O. luteus* consists of five microtubules, a trough-shaped structure and R3LS and obviously associated with the posterior basal body. The arrangement pattern of microtubules is particularly interesting when we consider homologous relationship of this root and that of other chromophytes. In *O. luteus*, five microtubules form a trough-like structure along the concave side of the trough-shaped structure. In some chrysophytes such as *Dinobryon cylindricum* Imhof. (Owen *et al.* 1990a), *Uroglena americana* Calkins (Owen *et al.* 1990b), *Chrysosphaerella brevispina* (Andersen 1990) and *Epipyxis pulchra* (Andersen and Wetherbee 1992), the R3 consists of six microtubules and these are organized in a trough-like structure at the proximal region. Four of these microtubules are rather short and terminate in some distance, and the rest two microtubules extend further and form a loop. In *C. brevispina*, a thin band is associated with these loop-forming two microtubules (Andersen 1990). This band resembles the trough-shaped structure of *O. luteus* in its position and thickness (about one third of microtubule diameter) (Fig. 37, see also fig. 12, 16 in Andersen 1990), suggesting that these structure are homologous to each other. All these similarities support homologous relationship of the R3 of *O. luteus* and R3 of the chrysophytes.

All chrysophyte algae mentioned above are mixotrophic organisms and are able to feed prey particles such as bacteria, small blue green algae and organic detritus. It has recently been clearly demonstrated in *Epipyxis pulchra* that R3 plays an important role in a food-cup formation during phagotrophy (Andersen and Wetherbee 1992, Wetherbee and Andersen 1992). In contrast, *Olisthodiscus luteus* is not mixotrophic and, as far as we know, there has been no record of phagotrophy in raphidophytes which possess chloroplasts, and there is no evidence to include the chloroplast-less (therefore assumed to be phagotrophic) genera in the Raphidophyceae (Heywood 1980).

Another functional role of the R3 in many chrysophytes seems to be determining the position of the eyespot beneath the flagellar swelling of the short flagellum (=posterior flagellum). In brown algae, R3 (usually referred to as major posterior rootlet, MPR) occurs in many species which possess an eyespot (Motomura 1989), suggesting that R3 contributes to determine the position of the eyespot perhaps together with so-called bypassing root. The flagellar swelling and eyespot probably act together as a photoreceptor apparatus and play an important role in phototactic response as has been revealed in brown algal swarmers (Kawai *et al.* 1990, Kawai 1992). Flavin-like autofluorescence substance is present in the flagellar swelling and it probably acts as photoreceptor (Coleman 1988, Kawai 1988, Müller *et al.* 1988). The eyespot reflects and focuses light on the flagellar swelling (Kawai *et al.* 1990, Kreimer *et al.* 1991). Raphidophytes have neither the eyespot nor the flagellar swelling, and no autofluorescence is detected in the posterior flagellum (Coleman 1988, Kawai and Inouye 1988).

Despite of various morphological similarities, functional roles of the R3 of raphidophytes are obviously different from those of the chrysophytes. This seems to indicate that morphological features of R3 are rather conservative and would be useful for delineating higher taxa. The R3 or rhizostyle in raphidophytes seems to act as a backborn of the cell as it extends posteriorly along the longitudinal axis of the cell. Anchoring the flagellar apparatus in the proper position may be another function of R3.

The path of R4 is interesting in that it has

a clockwise path in both chrysophytes and *O. luteus*. A similar path is also found in R4 (=minor posterior root) of brown algae and in the chrysophyte *Giraudyopsis stelifer* which is thought to be closely related to the Phaeophyceae (O'Kelly and Floyd 1985), although it is more or less straight and the radius of curvature is much smaller than that of chrysophytes and *O. luteus*.

All these flagellar root homologies listed above adequately support a close affinity between *O. luteus* and other chromophyte classes, especially chrysophyte algae, and the flagellar apparatus of *Olisthodiscus* falls into the same category to which many chromophyte algae also belong.

Despite of similarities of general cell morphology mentioned in the introduction, *O. luteus* has many features in the flagellar apparatus distinct from other raphidophytes. The flagellar apparatus of *O. luteus* is different in various respects from that of *Heterosigma akashiwo*, the only other raphidophyte of which flagellar apparatus has been described in detail (Vesk and Moestrup 1987). Comparisons of the flagellar apparatus components of *O. luteus*, *H. akashiwo* and other raphidophytes suggest that the raphidophytes are highly diverse as far as flagellar apparatus structure is concerned. For example, the R2 and R4, distal connecting fiber and proximal-plate found in *Olisthodiscus* have not been found in other raphidophytes. The presence of a connecting fiber was suggested for *H. akashiwo*, but this is situated at the proximal side of the basal bodies (Fig. 8c in Vesk and Moestrup 1987) and it should be regarded as a proximal fiber. There are some other differences between the flagellar apparatuses of these two raphidophytes. The R1 of *O. luteus* and the homologous broad root of *H. akashiwo* have a layered-structure, but its position and structure is different between these two species. In *H. akashiwo*, the layered structure is on the side facing the basal bodies at the proximal end of the microtubules, whereas in *O. luteus* the R1LS is situated on the opposite side of the basal bodies at some distance away from basal body complex, and continues some distance along the root microtubules. In the region of the layered structure, each microtubule of *H. akashiwo* has a stalk that extends towards the basal bodies, broadening to form the intermediate layer of the layered structure, then forking into two filaments that sit on the electron-dense base plate (Vesk and Moestrup 1987). In addition, the intermediate layer and the base plate are closely connected to some triplets of the anterior basal body, and the striated root is also in contact with the base plate. These features are distinctly different from the R1LS of *O. luteus* where the base plate is lacking, and there is no contact either with basal body triplets or the striated root. Similarities of the layered structure of *H. akashiwo* and that of other raphidophytes such as *Chattonella subsalsa* Biecheler (see Figs. 12, 13 in Mignot 1976), *Gonyostomum semen* (Ehrenberg) Diesing (pl. IV-6 in Mignot 1976) and *Vacuolaria virescens* (Heywood 1972) have been suggested (Moestrup 1982). In these species, the layered structure covers on one side of the sheet of microtubular root. A homologous relationship between the layered structure of these raphidophytes and *Olisthodiscus* is thus unlikely. The R1LS seems to be a structure unique to *Olisthodiscus*.

The path of the R1 is also considerably different, that is, it extends straight anteriorly in *H. akashiwo*, whereas it has a loop-like appearance in *O. luteus* and has GDVs inside the loop.

A flagellar root probably homologous to the R3 of *O. luteus* was observed in other raphidophytes. It is called as rhizostyle and has been thought to be a unique feature to raphidophytes (Preisig 1989). The rhizostyle of *H. akashiwo* may contain microtubules in addition to fibrillar components (Vesk and Moestrup 1987). A root called a rhizostyle is found in *Chattonella subsalsa* (Mignot 1976), *Gonyostomum semen* (Mignot 1967) and *Vacuolaria virescens* (Heywood 1980). This root seems to be composed of microtubules and fibrous or membranous elements (see fig. 16 in Mignot 1976, fig. 4 in Heywood 1980) and runs posteriorly along the nuclear surface. A structure similar to the trough-shaped struc-

ture is probably present in *G. semen* (pl. IV-7 in Mignot 1967). In *C. subsalsa*, this root also extends anteriorly over the basal body complex as it does in *H. akashiwo*. In all these raphidophytes the rhizostyle is present and may be consisting of similar components to the R3 of *O. luteus*, although these should be reinvestigated in detail.

The presence of such differences between raphidophytes suggests that the raphidophytes are relatively far from one another in terms of evolutionary relationships, and *Olisthodiscus* must have a discrete position. It does not mean that they have polyphyletic origins, however; the class Raphidophyceae may include organisms which are diverse in flagellar apparatus components, but we consider that they are derived from a common ancestor because the layered structure of *Heterosigma*-type and the rhizostyle are widely distributed in this class and has not been found in other algal classes. Features previously proposed in the circumscription of the Raphidophyceae (Hibberd 1986) probably support this. Taxonomic treatments separating the raphidophytes into two based on photosynthetic pigment composition such as that proposed by Christensen (1980) are, in our opinion, of dubious value. Detailed analysis of the flagellar apparatuses of other raphidophyte genera may provide further supporting evidence.

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井上　勲・原　慶明・千原光雄：ラフィド藻 *Olisthodiscus luteus* の鞭毛装置の微細構造

ラフィド藻 *Olisthodiscus luteus* Carter の鞭毛装置を観察した。基底小体は反時計回りに配置され，互いに 2 個の結合繊維で結ばれる。基底小体は，さらにリゾプラストと proximal plate と名づけた構造で核に連結する。微小管性の鞭毛根は 4 個で，非対称に配列する。前鞭毛の基底小体から生じる鞭毛根の一つは約15本の微小管からなり，層状構造と骨格微小管が付随する。この鞭毛根は時計回りの配行を示し，ループを形成する。ループの内部にゴルジ体由来の小胞がみられる。後鞭毛から細胞後方に伸びる鞭毛根は 5 本の微小管と樋状構造からなり，基部には薄膜構造が付随する。鞭毛根の配行と部分要素の構造を他の黄色植物のそれと比較したところ，*O. luteus* のそれは黄金色藻に最も類似していることが明らかになった。ラフィド藻の他の種との比較から，*Olisthodiscus* はラフィド藻としての性質を示しながらも，綱のなかで特異な位置を占めることが示唆された。(305 茨城県つくば市天王台1-1-1　筑波大学生物科学系)