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# An ultrastructural comparison of the mitotic apparatus, feeding apparatus, flagellar apparatus and cytoskeleton in euglenoids and kinetoplastids

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**Summary.** The euglenoids and kinetoplastids form a diverse assemblage of organisms which show no obvious phylogenetic relationship with other flagellates. An ultrastructural examination and comparison of the flagellar apparatus, the feeding apparatus, and mitotic nucleus indicate a number of shared morphological features which support a common ancestry for the two groups. Of particular interest is the euglenoid, *Petalomonas cantuscygni*, which shares many of the ultrastructural features common to both groups. Based on the data presented, we hypothesize that a euglenoid with features similar to those now present in *P. cantuscygni* was ancestral to both the euglenoid and kinetoplastid lines.

**Keywords:** Bodonid; Crithidia; Cytoskeleton; Cytostome; *Diplonema; Euglena*; Feeding apparatus; Flagellar apparatus; Microtubular root; Mitosis; Pellicle; *Rhynchomonas; Trypanoplasma.*

**Abbrevation:** MTR complex of reinforcing microtubules.

## Introduction

It is well accepted that higher plants and animals are descended from ancestors in the kingdom Protoctista. Thus, the earliest branches of the evolutionary "tree" leading to higher eukaryotes are occupied by representative protists. This paper will focus on two specific groups of protists with a long, but uncertain evolutionary history, the kinetoplastid and euglenoid flagellates. At the molecular level, phylogenies inferred from 18 S-like rRNA sequences place the euglenoid flagellates closer to the base of the evolutionary tree than most other eukaryotes with the next branch of the tree being occupied by the trypanosomes (Sogin et al. 1986). It must be noted, however, that the molecular data also suggest that trypanosomes and euglenoids, though more closely related to each other than to other eukaryotes, have had a long and separate evolutionary history since the point of divergence. Sogin et al. (1986) note that "the genetic diversity in this collection of eukaryotes is seen to exceed that displayed within either the eubacterial or the archebacterial lines of descent".

The euglenoids and kinetoplastids form an extremely diversified, yet isolated group of protists occupying a variety of habitats and exhibiting numerous lifestyles. Our limited understanding of the phylogeny and relationships of these organisms with other algae and protists is evidenced by their past taxonomic histories. The kinetoplastids were at one time classified in the order Protomonadina along with other flagellates having from 1–4 flagella. In this classification, the bodonids were separated from the trypanosomes on the basis of flagellar number and arrangement and were grouped with other biflagellates. Hollande (1952) also recognized the trypanosomes and bodonids as two separate orders and included in his Bodonidea genera which did not possess kinetoplasts. The bodonids and trypanosomes were brought together into the single order Kinetoplastida by virtue of their possession of a kinetoplast by Honigberg (1963).

The taxonomic position of the euglenoids is similarly colored. In past phylogenetic schemes the euglenoids have had the distinction of occupying many different branches of the phylogenetic tree due to some char-

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acteristics they share with other groups. They have been placed near the green algae based on the presence of chlorophyll *b* in their plastids (Klebs 1883, Dougherty 1955). Flagellar hairs, although structurally different (Bouck et al. 1978) are present on euglenoid flagella and on the flagella of chlorophyll *a* and *c* containing organisms (Bouck 1971, 1972). The eyespot is free of the chloroplast as in the eustigmatophytes (Hibberd and Leedale 1970, 1971, 1972). A paraxial rod exists in the flagella of dinoflagellates and euglenoids (Cachon et al. 1988). None of these features by itself is sufficient to give us a clear indication as to the relationship of the Euglenophyta with other algae, a fact which has long been recognized (Klebs 1983; Senn 1900; Fritsch 1935; Dodge 1975; Leedale 1967, 1978). Since many euglenoids are phagotrophic they were claimed by protozoologists as well as phycologists. Their protozoological history is likewise confused with the euglenoids having a status ranging from an order, the Euglenida, in the class Phytomastigophorea (Honigberg et al. 1964) to a phylum Euglenida in the kingdom Protoctista (Margulis et al. 1989).

A number of investigators have suggested a common ancestry for the euglenoids and kinetoplastids (e.g., Mignot 1964; Schuster et al. 1968; Leedale 1970; Porter 1973; Vickerman and Preston 1976; Taylor 1976, 1980) and some have gone as far as to erect a new taxon the "Euglenozoa" (Cavalier-Smith 1981, Corliss 1984). This merger is supported in hypotheses presented by Kivic and Walne (1984) and Willey et al. (1988). These hypotheses focus on four primary ultrastructural features which are used to link the ancestry of euglenoids and kinetoplastids: (1) mitosis, (2) the feeding apparatus, (3) the flagellar apparatus, (4) the cytoskeleton. Suspected homologies in each of these four features are described and analyzed based on the available data. The addition of a substantial amount of new data concerning mitosis, the flagellar apparatus and feeding apparatus in the Euglenozoa warrant a review of the current hypotheses.

## Mitosis

Prior to 1985, ultrastructural features of mitosis had been described for only four genera of euglenoids: *Euglena* (Leedale 1968, 1982; Pickett-Heaps and Weik 1977; Gillott and Triemer 1978), *Astasia* (Sommer and Blum 1965, Chaley et al. 1977), *Phacus* (Pickett-Heaps and Weik 1977), and *Colacium* (Kugrens and Rosowski 1972) all belonging to the same taxonomic order, the Euglenales (sensu Leedale 1967). If ultrastructural features of mitosis are to be of any phylogenetic value they must be examined in a wide variety of euglenoids, including the colorless phagotrophic forms which are more likely to be closely related to the kinetoplastids than the photosynthetic forms. Mitosis has now been examined in four colorless, phagotrophic euglenoids: *Anisonema* sp. (Triemer 1985), *Ploeotia costata* (Triemer 1986, Triemer and Fritz 1988), *Entosiphon sulcatum* (Triemer 1988), and *Petalomonas cantuscygni* (Triemer and Farmer 1988). Furthermore, the fine structural features of nuclear division have been examined in *Diplonema ambulator* (syn. = *Isonema*; Triemer and Ott 1990) an unusual protist included in the larger taxon, Euglenozoa (Corliss 1984) and believed to be closely related to the euglenoids and kinetoplastids (Kivic and Walne 1984, Willey et al. 1988).

The fine structural aspects of mitosis in kinetoplastids have been largely restricted to the trypanosomes (*Leishmania*, Bianchi et al. 1969, Croft 1979, Triemer et al. 1986; *Herpetomonas*, De Souza et al. 1976; *Leptomonas*, Souto-Padron et al. 1980; *Blastocrithidia*, Solari 1983) and focus on the genus *Trypanosoma* (Inoki and Ozeki 1969; Vickerman and Preston 1970; De Souza and Meyer 1974; Heywood and Weinman 1978; Solari 1980 a,b; Paterson and Woo 1984). As in the euglenoids, little information is available on mitosis in the organisms which are believed to be at the base of the evolutionary line, the bodonids. There is but a single short report on mitosis in *Trypanoplasma borelli* (Skarlato 1987). Despite the limitations on the available data noted above, some observations can be made.

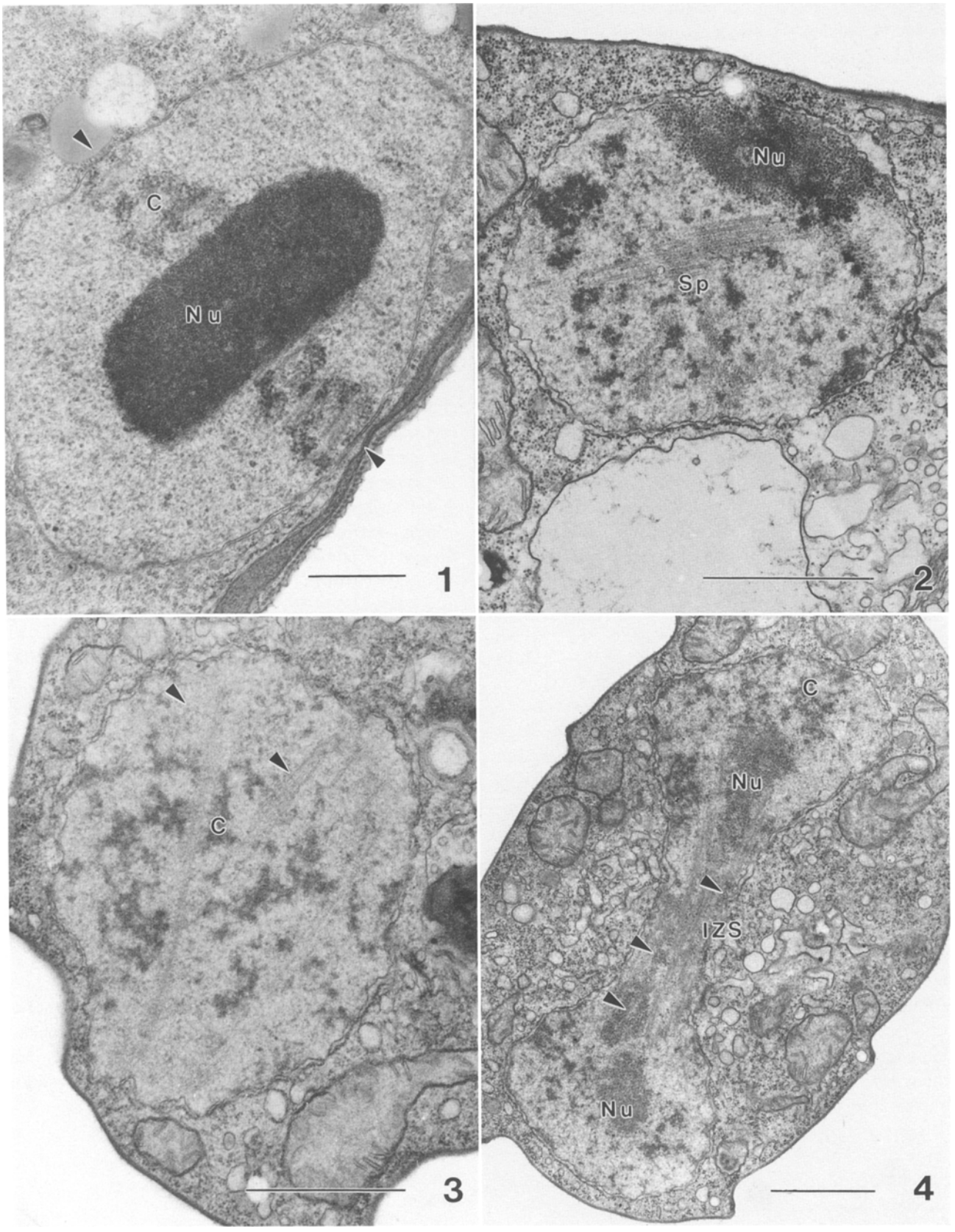
Kivic and Walne (1984:270) state that "Nuclear architecture and division in euglenoids... are essentially the same as in bodonids, trypanosomatids, and *Isonema*" (syn. *Diplonema*, Triemer and Ott 1990) " ... which have a totally closed mitosis, an intranuclear spindle, and no metaphase plate formation." Recent evidence contradicts this statement on a number of

**Fig. 1.** Dividing nucleus in *Diplonema ambulator* with persistent elongate nucleolus (*Nu*) and metaphase plate (▶). *C* Chromosome. Bar: 1 μm

**Fig. 2.** *Petalomonas cantuscygni*. Early division showing central spindle (*Sp*) and persistent nucleolus (*Nu*). Bar: 1 μm

**Fig. 3.** *Petalomonas cantuscygni*. Mid division nucleus with chromosomes (*C*) on subspindles (▶). Bar: 1 μm

**Fig. 4.** *Petalomonas cantuscygni*. Late anaphase nucleus showing nucleolus (*Nu*) partially dispersed (▶) over interzonal spindle (*IZS*). Bar: 1 μm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points. First, closed intranuclear spindles are not restricted to euglenoids and kinetoplastids but are found in some fungi, other protozoans, and some algae (for review, see Raikow 1982). Second, *Diplonema* has a distinct metaphase plate (Fig. 1). In euglenoids, a distinct metaphase plate with compacted chromosomes is not present. However, the chromosomes can assemble in a loose equatorial plate at metaphase in some genera (Triemer 1988, Triemer and Fritz 1988). Likewise, kinetoplastid nuclei contain a number of dense plaques arranged loosely about the equatorial plane during mitosis (Solari 1980 a, b, 1983; Triemer et al. 1986; Skarlato 1987). The exact nature of these plaques is unknown but it has been suggested that they function as kinetochores (Solari 1980 a, b). In any case, *Diplonema* has a definite metaphase plate and euglenoids and kinetoplastids do align kinetochores or plaques at the equatorial plane at some point in mitosis. Third, the types of spindles formed differ. The spindle of the trypanosomes consists of a large central bundle of microtubules surrounded by radial bundles which apparently attach to the chromosomal plaques. During mitosis the plaques first move to the poles followed by elongation of the central spindle (Solari 1980 a, b). The spindle in the bodonid, *Trypanoplasma*, consists of three to four bundles of about forty microtubules each, which attach to kinetochore-like plaques. Since some of the chromatin remains associated with the nuclear envelope it has been hypothesized that mitosis includes both a primitive division mechanism involving segregation of chromosomes attached to the nuclear membrane and a microtubular spindle utilizing kinetochore-like structures (Skarlato 1987). The euglenoid spindle is composed of a number of independent subspindles each of which contains chromosomal and non-chromosomal microtubules. Chromosomal segregation is concomitant with nuclear elongation and does not appear to rely on shortening of the chromosomal microtubules as occurs in the kinetoplastids. The distance between the chromosomes and the nuclear envelope remains about the same until late in anaphase (Triemer 1985). In effect, anaphase A and B appear to be reversed in the euglenoids examined to date. Therefore, at least three different spindle types and mechanisms of chromosomal separation are present in euglenoids and kinetoplastids. A spindle with multiple subspindles and reversed anaphase A/B sequence is characteristic of euglenoids. A central spindle with radial bundles of microtubules characterizes trypanosomes. The bodonids, represented only by *Trypanoplasma*, utilize three to four large bundles of microtubules and make use of the nuclear envelope in segregating chromosomes (Skarlato 1987). Furthermore, chromosomal separation in *Diplonema* appears to follow the anaphase A, B sequence characteristic of most eukaryotes (Triemer unpubl.) unlike that of euglenoids and has a spindle similar to that found in higher plants and animals. Fourth, nucleolar behavior varies during mitosis. In euglenoids the nucleolus remains intact, elongates, becomes dumbbell-shaped and eventually pinches in two. In kinetoplastids, the nucleolar material fragments and disperses over the spindle. Lastly, the chromosomes in kinetoplastids are not permanently condensed as they are in the euglenoids but undergo condensation and decondensation during the cell cycle and unwind into thin fibers at mitosis. Based on the previous discussion it appears that the fine structural features of mitosis cited previously (Kivic and Walne 1984) do not support the statement that "Nuclear architecture and division in euglenoids... are essentially the same as in bodonids, trypanosomatids, and *Isonema* ... " There are many features which are distinctly separate and only a single unifying feature, the closed nuclear envelope, which is present in a number of other protists as well. However, before the features of mitosis are eliminated from phylogenetic considerations it may be useful to examine a few more bodonids and euglenoids which are believed to have retained features ancestral to the entire phagotrophic line. For this reason we have studied mitosis in *Petalomonas cantuscygni*. This euglenoid has a rigid cell surface with few pellicle strips and has a feeding apparatus which is less structurally complex than that found in most euglenoid phagotrophs. These features lead us to propose that this organism is more closely related to the ancestral euglenoid than other phagotrophs. Preliminary studies of mitosis show that *P. cantuscygni* may develop a central spindle early in mitosis (Fig. 2), similar to the trypanosomes. Later it forms a number of sub-spindles as in other euglenoids (Fig. 3). The nucleolus also undergoes some degree of fragmentation as it separates on the central spindle into the two daughter nuclei (Fig. 4). These features temptingly suggest a common ancestry with the kinetoplastids but until such studies are completed and mitosis is examined in detail in more bodonids, one cannot conclude that the similarities that do exist between euglenoid and kinetoplastid mitoses are the result of synapomorphies.

**Feeding apparatus**

The ultrastructural features which are most likely to provide key information for determining the phylogeny

of the euglenoids and their relationships to the kinetoplastids are to be derived from the feeding apparatus. Information is available on the feeding apparatus for a number of bodonid genera, including *Bodo* (Brooker 1971, Burzell 1975, Eyden 1977, Brugerolle et al. 1979), *Cryptobia* (Brugerolle et al. 1979, Nohynkova 1984), *Cephalothamnion* (Hitchen 1974), *Trypanoplasma* (Brugerolle et al. 1979), and *Rhynchomonas* (Burzell 1973, Swale 1973). In all cases the feeding apparatus consists of a pocket originating at the cell's anterior end adjacent to the flagellar opening. The pocket is supported by interconnected microtubules running along its length. Brugerolle et al. (1979) termed this complex of reinforcing microtubules the "MTR". The number and arrangement of microtubules varies between organisms and within genera. Most bodonids have one or more small bands of microtubules associated with the pocket. However, a few genera (e.g., *Cephalothamnion cyclopum*, Hitchen 1974; *B. curvifilus*, Burzell 1975; *Bodo designis*, Eyden 1977; *Phyllomitus apiculatus*, Mylnikov 1986) have supporting microtubules arranged in a small rod-like bundle which represents the limit of supporting rod complexity found in bodonids. The membrane adjacent to the MTR is usually denser and somewhat thicker than the rest of the cytostomal membrane.

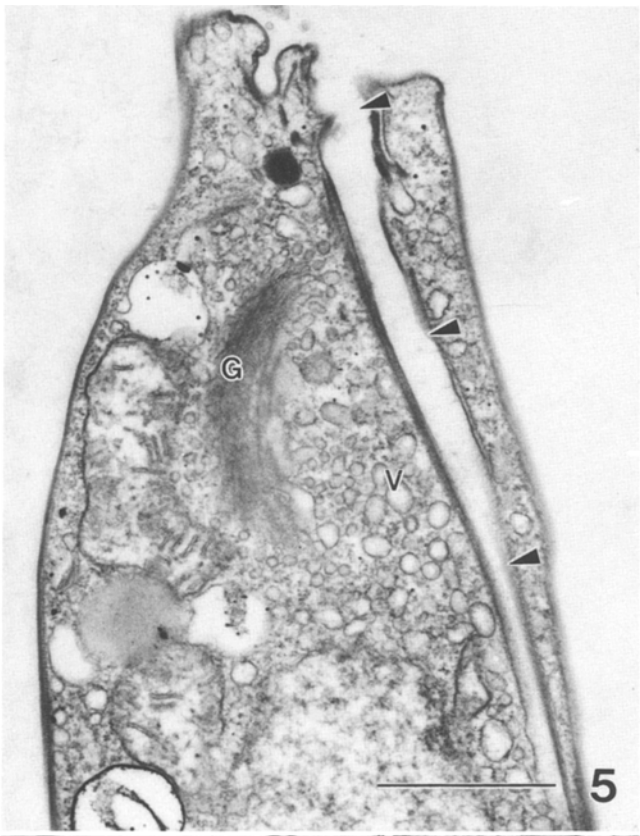
The presence of a cytostomal complex is not restricted to the bodonids but also exists in some trypanosomes where it functions in pinocytosis rather than phagocytosis (e.g., *Trypanosoma mega*, Steinert and Novikoff 1960; *Trypanosoma raiae*, Preston 1969; *Crithidia fasciculata*, Brooker 1971). Furthermore, the cytostome may open into the flagellar pocket rather than directly to the cell surface (Brooker 1971). Feeding apparatuses with multiple supporting rods and/or vanes have not been reported in any kinetoplastid.

In comparison to the data available on feeding apparatus ultrastructure in the kinetoplastids there are published data on only two genera of euglenoids, in which sufficient details have been presented to warrant any useful comparisions (*Entosiphon*, Mignot 1966, Triemer and Fritz 1986; *Peranema*, Mignot 1966, Nisbet 1974). We have therefore engaged in an extensive study of euglenoid feeding apparatuses and have serially sectioned through eight phagotrophic euglenoid genera and the purportedly related genus *Diplonema*.

Scanning electron microscopy has proven useful in giving an overall view of the feeding apparatus and demonstrating that the feeding apparatus may be directly associated with and arise as a fold in the pellicle as in *Ploeotia* (Triemer 1986) or alternatively, that the feeding apparatus is separate from the pellicle as in *Entosiphon* (Triemer and Fritz 1986). Internally there are also a number of structural variations. The feeding apparatus may be supported by a few microtubules or by bundles of microtubules forming two or possibly three rods. Even within the rods there is variation in the number and organization of the microtubules. The cytostome may be a simple sac or may be surrounded by a diaphragm-like set of vanes. Striated fibers are associated with some of the feeding apparatuses (e.g., *Peranema*, Mignot 1966). The feeding apparatuses found in the euglenoids can be grouped into one of four types, the MTR/pocket type (Type I) as found in *Petalomonas cantuscygni*, the plicate type (Type II) as found in *Ploeotia costata*, the short extensible type (Type III) as found in *Peranema trichophorum*, and the siphon type (Type IV) as found in *Entosiphon sulcatum*.

The Type I feeding apparatus has the least structural complexity and is present in *Petalomonas cantuscygni* and *Calycimonas* sp. It consists of a cytoplasmic pocket which extends posteriorly for roughly one half to three quarters the length of the cell (Fig. 5). At its anterior end the feeding apparatus merges with the flagellar pocket. The feeding pocket is lined by microtubules which extend along its entire length (Figs. 6 and 7). These microtubules extend from the site where the feeding apparatuses and flagellar pocket merge and appear to be derived in part from one of the microtubular flagellar rootlets. A number of small vesicles border one side of the feeding apparatus. Type I feeding apparatuses are restricted to bacteriotrophic euglenoids and *Diplonema*.

The Type II feeding apparatus is found in *Ploeotia costata* (syn. *Serpenomonas costata*, Triemer 1986), *P. vitrea* (Farmer and Triemer 1988 b), and in the three species of *Diplonema* examined (Schuster et al. 1968, Porter 1973, Triemer and Ott 1990). In this type of apparatus the cytostome is supported by two rods which extend into the cytoplasm and a series of plicate folds, the vanes. The supporting rods contain few microtubules, but may possess a dense armorphous matrix. At the anterior end of the apparatus the vanes surround the membrane invagination which forms the cytostome. For most of the length of the apparatus a portion of the vane complex remains closely appressed to the supporting rods. At the base of the feeding apparatus the rods and vanes are intimately associated with one another forming a single complex. Individual microtubules may be associated with the vanes in some, if not all, Type II feeding apparatuses. As is the case for the Type I apparatus, all of the organisms known to have a Type II feeding apparatus are bacteriotrophs.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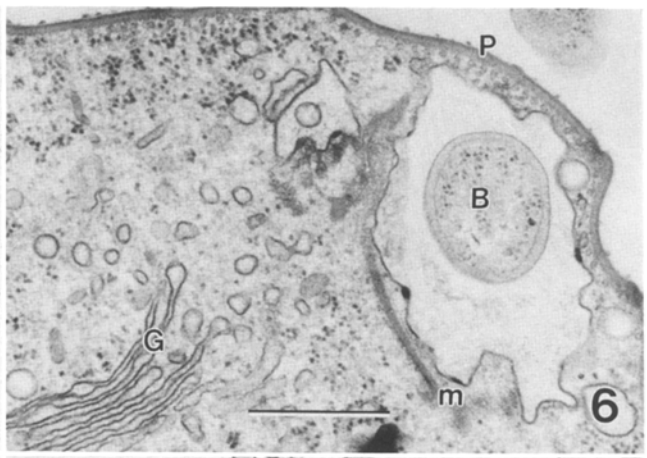
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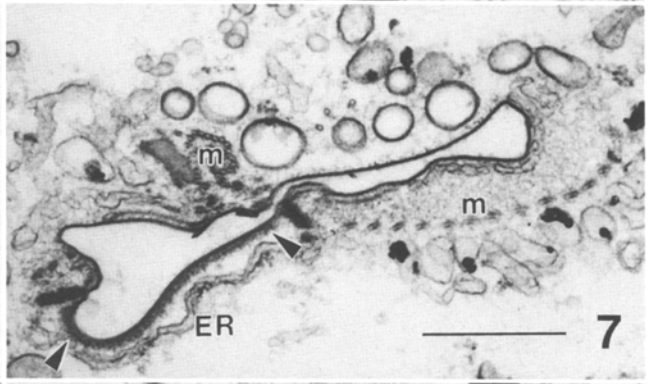
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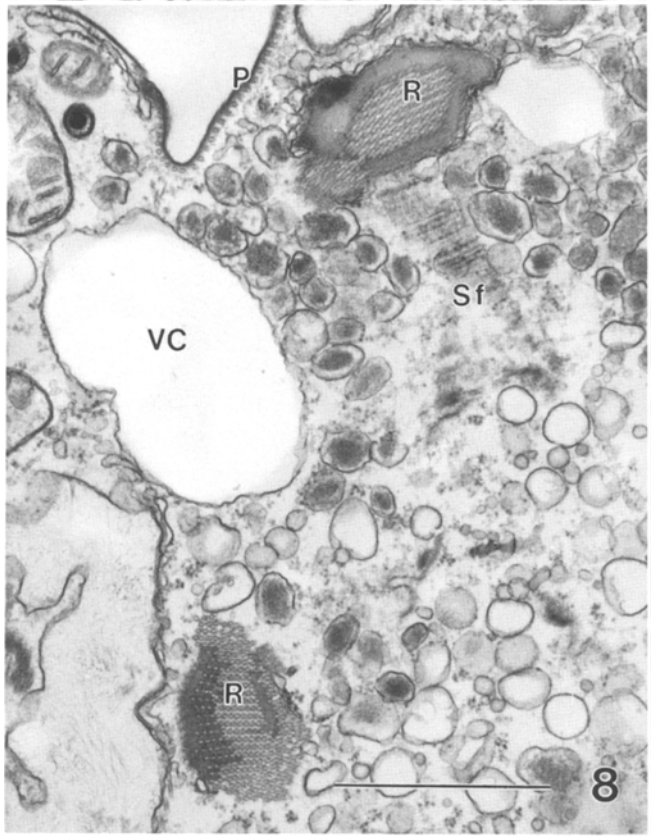
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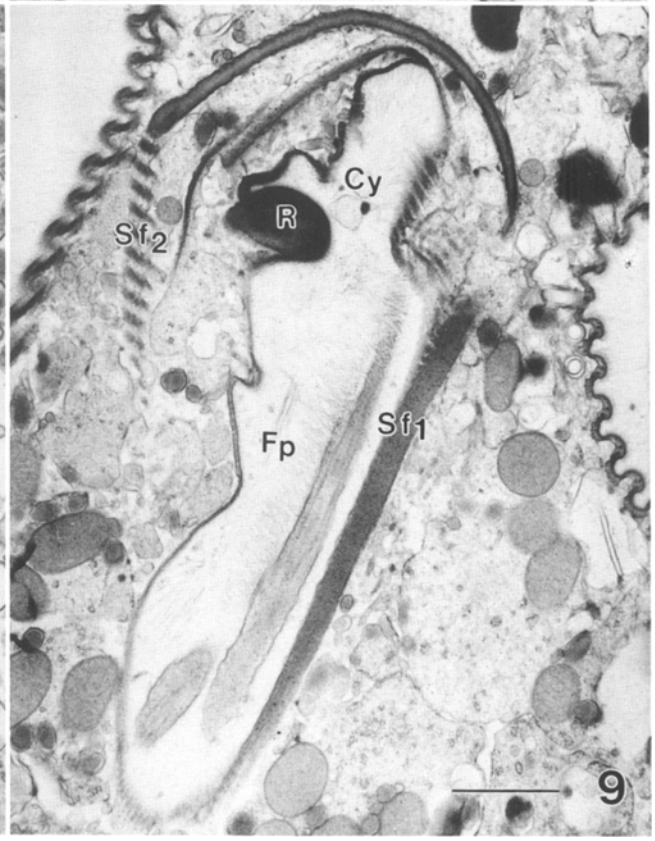
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The type III feeding apparatus is found in *Peranema trichophorum* (Nisbet 1974), *Dinema sulcatum* (Farmer and Triemer 1988 a), *Urceolus cyclostomus* (Farmer 1988), and *Anisonema* sp. (Triemer 1985). All of these organisms are capable of engulfing eukaryotic prey. In the Type III apparatus the cytostome is surrounded by vanes and supported by two rods. In contrast to the Type II apparatus, the supporting rods are composed primarily of microtubules and the vanes do not remain appressed to the rods. The number and arrangement of microtubules in the rods varies by genus. In *Urceolus cyclostomus*, the rods are composed of a central cylinder of loosely packed microtubules surrounded by a matrix which itself is completely encircled by microtubules (Farmer 1988). A similar rod organization with more central microtubules and less matrix is present in *Peranema* (Nisbet 1974). Lastly, the rods found in *Anisonema* and *Dinema* are composed of a solid mass of microtubules with only a small amount of matrix material associated with them and located primarily at the anterior end (Fig. 8). Four centrally located vanes associate with the supporting rods for most of their length. The vanes arise from and are embedded in the rods at the base of the feeding apparatus and diverge from the rods towards the anterior of the apparatus. In addition, several fibrillar components can be associated with the Type III feeding apparatus. For example, in *Dinema* distinct striated fibers extend from the rods toward the vanes near the anterior end of the apparatus (Fig. 8) and in *Peranema* large striated fibers are associated with the cytostome (Fig. 9).

The last category of feeding apparatus, Type IV, is found only in the bacteriotroph, *Entosiphon sulcatum* (Mignot 1966, Triemer and Fritz 1986). It is similar to a Type III apparatus in that it has supporting rods composed of closely packed microtubules and four centrally located vanes surrounding the cytostome. However, near the base of the feeding apparatus one of the rods bifurcates giving rise to a total of three rods which extend nearly the length of the cell. In cross-section the feeding apparatus appears C-shaped, with the sides of the "C" being formed by the three roughly triangular bundles of microtubules. About one-third the distance down the apparatus (from the anterior end) the number of microtubules per rod increases dramatically and then decreases in number toward the base. This tapering gives the apparatus the overall appearance of a cone with one side open. Numerous vesicles are adjacent to the open side of the cone. Unlike the Type III, this feeding apparatus is usually in motion, extending toward the anterior of the cell and then with-drawing down into the cell for a distance of 3–5 μm. It is primarily the presence of the third supporting rod and the mechanism of movement which separates this feeding apparatus from the Type III apparatus.

In addition to these four types of feeding apparatuses, there exists another organelle which must be placed in this category. In 1985, Willey and Wibel (1985 a) described the existence of a pocket formed from the reservoir membrane in *Colacium*. A band of microtubules (MTR) lined the pocket and a dense fibrillar mesh was associated with the membrane. The similarity between this structure and the cytostome of colorless euglenoids (Type I) and bodonids raised the possibility that this pocket was a cytostomal homologue (Willey and Wibel 1985 b). Surek and Melkonian (1986) then demonstrated that similar pockets are present in three species of *Euglena*. By tracing the origin of the MTR in serial sections they discovered that it was continuous with, and in fact the same as, the ventral flagellar root. By comparing published information on bodonid cytostomes, colorless euglenoid cytostomes, and the newly discovered reservoir pockets, they proposed that the three structures were homologous, indicating that photosynthetic euglenoids arose from phagotrophic ancestors. Since that time the MTR/pocket complex has been reported in yet another green euglenoid, *Cryptoglena pigra* (Owens et al. 1988). In spite of the supporting data, the case for homology between reservoir pockets and cytostomes is not as clear as it may at first

**Fig. 5.** *Petalomonas cantuscygni.* Longitudinal section through MTR/pocket (▶). Vesicles (*V*) line the feeding apparatus. *G* Golgi. Bar: 1 μm

**Fig. 6.** *Petalomonas cantuscygni.* Cross-section through feeding apparatus adjacent to pellicle (*P*) ingested bacterium (*B*). *m* Microtubules. Bar: 0.5 μm

**Fig. 7.** *Calycimonas* sp. Cross-section through feeding apparatus showing thickened region of membrane (▶) and associated microtubules (*m*). Endoplasmic reticulum (*ER*) is adjacent to the apparatus. Bar: 0.5 μm

**Fig. 8.** *Dinema sulcatum.* Oblique section near cell anterior showing microtubular supporting rods (*R*) and associated striated fiber (*Sf*). The vestibular cavity (*VC*) which leads to the cytostome, is positioned between the supporting rods. *P* Pellicle. Bar: 1 μm

**Fig. 9.** *Peranema trichophorum.* Oblique section near cell anterior showing cytostome (*Cy*) and flagellar pocket (*Fp*). One of the two supporting rods (*R*) and two striated fibers ($Sf_1$, $Sf_2$) associated with the feeding apparatus are visible. Bar: 1 μm

seem. We have identified MTR/pockets in two phagotrophic euglenoids, *Ploeotia* and *Dinema*, and in *Diplonema* (Triemer and Ott 1990). *Ploeotia*, in addition to having a Type II feeding apparatus has an invagination of the reservoir lined with microtubules and surrounded by a dense fibrillar material. The microtubules can be traced back to the ventral basal body of the flagellar apparatus. A similar pocket exists in *Diplonema* which also has a Type II apparatus and in *Dinema*, which has a Type III apparatus. If these MTR/pockets are homologous with those of *Colacium* and *Euglena*, it would be difficult to explain why they are found in genera which already have a second, well-developed feeding apparatus composed of supporting rods and vanes. The implication is that some genera have developed a more elaborate feeding apparatus while still retaining a Type I apparatus derived from some ancestral form. Perhaps the MTR/pocket is retained for pinocytotic functions as in trypanosomes while the supporting rod/vane feeding apparatus is utilized primarily for phagocytosis. The need for retaining two feeding apparatuses remains enigmatic.

## Flagellar apparatus

The arrangement of components in the flagellar apparatuses of both euglenoids and kinetoplastids is remarkably similar. The basic configuration of two basal bodies and three asymmetrically distributed microtubular roots is found in the majority of euglenoid and kinetoplastid species (Fig. 10). In addition to these structures, a striated connecting fiber is often associated with the two basal bodies (Fig. 11). This fiber is most prominent in those taxa whose flagella move heterodynamically. In some trypanosomes and euglenoids the second flagellum is so reduced that its basal body is little more than a barren stub or plate, thus making the cells functionally uniflagellate (Farmer and Triemer 1988 a, Sherwin and Gull 1989). Despite these modifications the basic architecture of the flagellar apparatus is recognizable throughout the group. The specific configuration of the microtubular flagellar roots and the role each root plays in the construction of the cytoskeleton also seems to be nearly identical in the euglenoids and kinetoplastids (see below).

Other flagellar features that link the euglenoids and kinetoplastids include the presence of a paraxial rod (syn. paraflagellar rod) and flagellar hairs. Although paraxial rods are apparently absent from some members of the Kinetoplastida (Freymuller and Camargo 1981) they are present in the vast majority of euglenoids and kinetoplastids. Biochemical studies have shown that the paraxial rod of *Trypanosoma brucei* is composed of a single protein (Schlaeppi et al. 1989). That this protein can assume two different conformations explains why two distinct bands of approximately 70 kDa are observed in SDS-gel electrophoresis of paraxial rod proteins from euglenoids (Hyams 1982) and kinetoplastids (Russell et al. 1983, Cuhna et al. 1984). Despite the slight disparity in molecular weights and other differences in architecture of paraxial rods in the two groups (De Souza et al. 1980, Souto-Pardon et al. 1980, Hyams 1982, Farina et al. 1986), immunological studies show that the paraxial rod protein of *Euglena* is related to the paraxial rod protein of trypanosomes (Gallo and Schrevel 1985). Paraxial rods are present in other protists (e.g., dinoflagellates) but the lack of biochemical data prevent further speculation regarding the evolutionary relatedness of this structure in other groups (Cachon et al. 1988). Flagellar hairs when present consist of thin nontubular structures that are unilaterally arranged.

One feature that has been cited as an indicator of evolutionary relatedness of the euglenoids and the kinetoplastids is the flagellar transition zone (Kivic and Walne 1984). The hollow flagellar transition zone between the axoneme and basal body of *Euglena* was long thought to be characteristic of the euglenoids (Leedale 1967, Moestrup 1982). While the type of transition zone may prove to be useful in grouping certain taxa into families, a great deal of variability exists among different euglenoid species (Farmer and Triemer 1988 a). This variability warrants caution in using the ultrastructure of the flagellar transition zone as proof that the euglenoids and kinetoplastids form a monophyletic group.

## Cytoskeleton

Overall cell morphology is perhaps the oldest and most commonly used criterion by which the taxonomic relationships (phenetic) between protists are assessed. Superficially, the euglenoids and kinetoplastids resemble one another. Both are principally flagellate unicells that lack elaborate cell walls or plates and whose cell membranes are underlain by subpellicular microtubules. Unfortunately these features are shared by other protistans as well (e.g., Retortomonads, Brugerolle 1973, 1977; Proteromonads, Brugerolle and Joyon 1975; *Hemimastix*, Foissner et al. 1988). The one cytoskeletal feature that distinguishes the euglenoids from all other

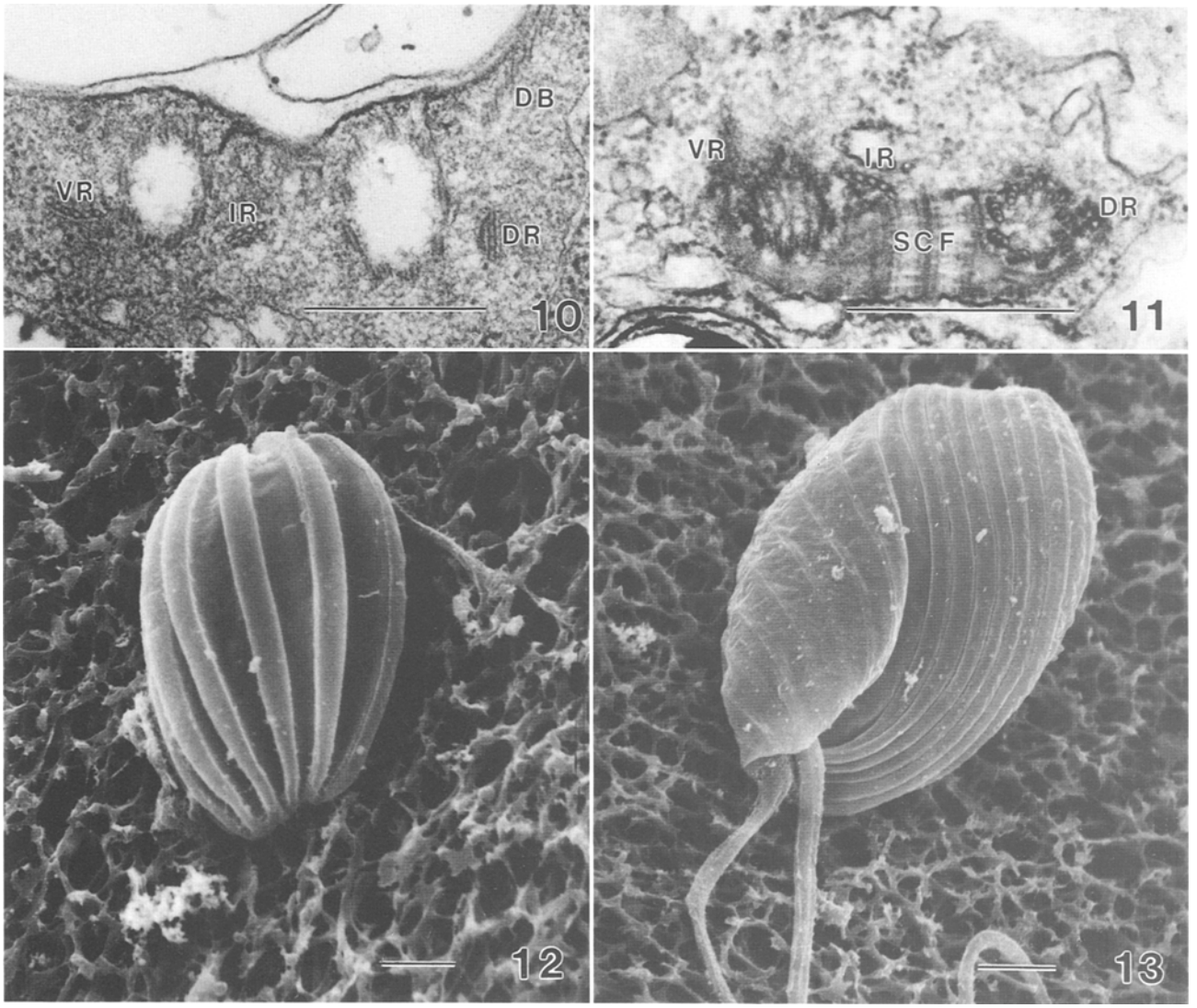


**Fig. 10.** Flagellar apparatus of *Euglena gracilis* showing the distribution of ventral (*VR*) and intermediate (*IR*) microtubular roots with the ventral basal body and the dorsal basal body with its associated dorsal root (*DR*) which gives rise to the dorsal band (*DB*) of microtubules. Bar: 0.5 μm

**Fig. 11.** Flagellar apparatus of *Ploeotia costata* showing large striated connecting fiber (*SCF*) between the two basal bodies and asymmetrical arrangement of microtubular roots. Bar: 0.5 μm

**Fig. 12.** SEM of *Entosiphon applanatum*, a species with an aplastic pellicle composed of ten longitudinally arranged pellicular strips. Bar: 5 μm

**Fig. 13.** SEM of *Dinema sulcatum*, a species with a plastic pellicle composed of multiple strips arranged in a helical fashion. Bar: 5 μm

protists is the presence of a pellicle composed of four or more strips that extend the length of the cell.

The euglenoid pellicle is believed to be responsible for allowing for euglenoid movement (i.e., "metaboly") in genera such as *Euglena*. Many euglenoids such as *Entosiphon, Petalomonas*, and *Ploeotia* have only a few pellicular strips that are arranged longitudinally (Fig. 12). Unlike *Euglena*, these euglenoids are completely rigid and are not capable of metaboly. In contrast, many species that have a pellicle composed of helically arranged strips are capable of altering their cell shape (Fig. 13). Based on these and other factors the euglenoids can be divided into two major groups; those possessing plastic pellicles (numerous helically arranged strips) and those with aplastic pellicles (fewer longitudinally arranged strips). Although aplastic spe-

cies are incapable of altering their cell shape, metaboly alone can not be used as a distinguishing feature between the two pellicular types. Many euglenoids that have plastic pellicles are also essentially rigid (e.g., *Phacus, Cryptoglena*). Genera with plastic pellicles that do not undergo metaboly (e.g., *Euglena spirogyra, Phacus longicauda*) often have secondary elaborations or thickenings associated with their pellicles which may be responsible for their inability to change shape significantly.

The array of microtubules that underlies the cell membrane of the kinetoplastids has been cited as further evidence that the two groups are more closely related to one another than either is to other protistan groups (Kivic and Walne 1984, Willey et al. 1988). A comparison of the microtubular flagellar roots and the origin of the microtubules of the cytoskeleton suggests that the euglenoids and kinetoplastids do indeed have very similar architectures. The microtubules that lie beneath each of the pellicular ridges in euglenoids are continuous with those that line the flagellar canal and extend into the region of the flagellar reservoir (Willey and Wibel 1985 a, b). This group of microtubules has been termed the dorsal band (Willey and Wibel 1985 a, b, 1987) (Fig. 10) and appears to nucleate tangentially from one of the three microtubular flagellar roots (Willey and Wibel 1987, Owens et al. 1988). Development and replication of the pellicular microtubules in the euglenoid *Cyclidiopsis* is intimately related to the replication and separation of the microtubular flagellar roots (Mignot et al. 1987).

Likewise in some bodonids the majority of the cytoskeletal microtubules appear to be derived from a large band of microtubules that originate at the anterior end of the cell in the region of the flagellar opening. Like the dorsal band found in the euglenoids, this group of microtubules does not appear to be continuous with any of the microtubular flagellar roots. As in the euglenoids, the microtubules of this cytoskeletal band seem to nucleate adjacent to a small microtubular root that emanates from one of the two basal bodies (Brugerolle et al. 1979). Cell body deformations similar to those described for euglenoid metaboly have been observed in some bodonids (Swale 1973, Vickerman 1977) and may be a further indicator that the cytoskeletons of both groups are derived from a common ancestor.

The other major group of kinetoplastid cytoskeletal microtubules is the MTR (see section on feeding apparatus). As in the euglenoids (Willey and Wibel 1985 a, 1987; Surek and Melkonian 1986; Owens et al. 1988) the microtubules of the kinetoplastid MTR originate adjacent to one of the two basal bodies (Brugerolle et al. 1979, Nohynkova 1984). A third group of cytoskeletal microtubules that are continuous with one of the flagellar roots is found in the region of the flagellar opening in bodonids (Brugerolle et al. 1979, Nohynkova 1984).

The strongest argument in favor of all three microtubular flagellar roots and their derived cytoskeletal microtubules of bodonids being homologous to similar structures in the euglenoids is the fact that the distribution of these microtubules is identical in the two groups. The three microtubular flagellar roots are arranged asymmetrically. Of these three roots two are associated with one basal body while the third root emanates from the other basal body. In both the euglenoids and the bodonids it is this single microtubular root that gives rise to (but is not continuous with) the major group of cytoskeletal microtubules. Likewise the flagellar root that ultimately becomes the MTR of both euglenoids and bodonids is one of the two roots associated with the first basal body.

A number of studies have shown that many protists undergo a complex developmental process during cell division in which one parental basal body and its associated microtubular roots matures into a basal body that is identical to the alternate type of parental basal body (Melkonian et al. 1987; Moestrup and Hori 1989; Heimann et al. 1989 a, b). A similar process has been suggested for the euglenoids in which the basal body that has only one microtubular root associated with it develops into the type with two roots (Farmer and Triemer 1988 a) Although such developmental studies have not yet been done on any of the bodonids, it seems likely that a similar mechanism exists. If this proves to be the case the microtubular flagellar roots and the cytoskeletal microtubules that they ultimately give rise to would be structurally, positionally, functionally, and developmentally identical in both groups.

While similarities in cytoskeletal arrangement might suggest that euglenoids and bodonids are members of a monophyletic assemblage, the issue is less clear when one considers the trypanosomes. Unlike the bodonids and the majority of euglenoids, the trypanosomes are uniflagellate. Furthermore, discerning which group of microtubules gives rise to the cytoskeletal microtubules and which group is responsible for lining the cytostome remains difficult (Willey et al. 1988). Despite these discrepancies, the basic architecture of an encircling corset of cytoskeletal microtubules that are directly or indirectly derived from the flagellar microtubular roots is consistent with what is found in the euglenoids and

bodonids. Immunocytochemical studies indicate that proteins that localize with isolated corset microtubules of trypanosomes may be responsible for the cross binding of the microtubules and determination of the cell architecture (Balaban et al. 1989, Bramblett et al. 1989). If the antibodies raised against these proteins prove to be cross reactive with the microtubular cytoskeleton of euglenoids and bodonids but not other protists, it would provide an additional link between these groups. The presence of a second barren basal body in all trypanosomes (Vickerman 1989) suggests that they are evolved from a biflagellate ancestor. Ultrastructural studies of trypanosome basal body replication (Paulin 1969) coupled with the fact that the single flagellum is homologous with the anterior flagellum of bodonids (Vickerman 1989) suggest that basal body development is identical to that observed in euglenoids. In both groups the basal body that has two microtubular roots represents the terminal or mature state. Together, the distribution of cytoskeletal microtubules and the unique arrangement of basal bodies and their microtubular roots strongly support the hypothesis that euglenoids and kinetoplastids are derived from a single common ancestor.

## Conclusions

The data now available continue to provide support for the hypothesis that euglenoids and kinetoplastids are more closely related to each other than they are to other protists. However, the details of this relationship remain for the most part speculative. With only a single exception, mitosis in euglenoids is not similar to that of kinetoplastids in terms of spindle formation, chromosome organization and segregation, and nuclear division. Yet at least one euglenoid, *Petalomonas cantuscygni*, does undergo nucleolar dispersion and appears to form a central spindle at some point during mitosis.

The MTR/pocket (Type I) feeding apparatus can be found in both kinetoplastids and euglenoids. In the kinetoplastids this appears to be the limit of feeding apparatus development, whereas in the euglenoids the MTR/pocket is the least complex of the feeding apparatus types. Interestingly, this type of feeding apparatus is found in *Petalomonas cantuscygni*, the euglenoid with a mitosis most like that of kinetoplastids. The reduced MTR/pocket present in many photosynthetic euglenoids provides evidence for ancestry with a colorless phagotroph.

In both groups of organisms cytoskeletal microtubules underlie the plasma membrane forming a continuous or discontinuous supporting corset. In the euglenoids an additional glycoprotein layer is appressed to the inner surface of the plasma membrane generating the characteristic ridge and groove pattern of the pellicle. The rigid euglenoid phagotrophs have few pellicular ridges while the photosynthetic genera such as *Euglena* have many. Among those genera with the fewest ridges is *Petalomonas cantuscygni*.

The basic configuration of the flagellar apparatus with two basal bodies and three asymmetrically distributed microtubular roots is found in the majority of euglenoid and kinetoplastid species. In addition, both groups have paraxial rods which contain proteins of similar electrophoretic mobility and which exhibit some degree of immunological cross reactivity. Thin non-tubular flagellar hairs are also characteristic of both groups. The flagellar transition zones of some kinetoplastids are morphologically similar to those found in some phagotrophic euglenoids, yet the differences noted in the structure of the transition zones within the entire group of euglenoids is very diverse (Farmer and Triemer 1988 a) and provides evidence for a long and separate evolutionary history.

In summary, while we agree with Vickerman (1989) and others that the trypanosomes are descended from the bodonids, we do not believe that bodonids were ancestral to the euglenoids (Kivic and Walne 1984) but hypothesize that the euglenoids and bodonids both diverged from a common ancestor with yet a separate branch giving rise to *Diplonema*. A long and separate evolutionary history would explain why mitotic features and mechanisms differ substantially between the two groups. Furthermore, most bodonids have a Type I feeding apparatus supported by a few microtubules. This is also true for the euglenoid *Petalomonas cantuscygni*. However, some bodonids (e.g., *Bodo designis*, Eyden 1977) do form a small microtubular supporting rod and nearly all phagotrophic euglenoids have microtubular supporting rods. Therefore, we can trace the evolution of the feeding apparatus in bodonids from a Type I apparatus with few microtubules to one with a single supporting rod and no vanes. In the phagotrophic euglenoids we can present a similar, albeit more lengthy scenario moving from the Type I apparatus in *Petalomonas cantuscygni* to the Type IV apparatus. We interpret these patterns of supporting rod formation as representing homoplasies rather than evidence for direct descendence. We hypothesize that the ancestral form to both euglenoids and kinetoplastids was a heterodynamic phagotroph with a Type I feeding appa-

ratus supported by a few microtubules, possessing two basal bodies with three asymmetrically distributed microtubular rootlets. Mitosis would occur within a closed nuclear envelope using a central spindle and the nucleolus would fragment either partially or completely. At present the kinetoplastid with features closest to this hypothetical ancestor would lie in the genus *Bodo* (Vickerman 1989) and within the euglenoids the organism which best represents the ancestral form is *Petalomonas cantuscygni*.

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