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# Did trypanosomatid parasites have photosynthetic ancestors?

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**Some molecular phylogenies of plastid-like genes suggest that chloroplasts (the structures responsible for photosynthesis in plants and algae) might have been secondarily lost in trypanosomatid parasites. Chloroplasts are present in some euglenids, which are closely related to trypanosomatids, and it has been argued that chloroplasts arose early in the diversification of the lineage Euglenozoa, to which trypanosomatids and euglenids belong (plastids-early hypothesis). This article reviews how euglenid ultrastructural systems are functionally integrated and phylogenetically correlated. I argue that chloroplast acquisition profoundly altered the structure of certain euglenids, and that the complete absence of these modifications in other euglenozoans is most consistent with their never having had a chloroplast. Ultrastructural evidence suggests that chloroplasts arose relatively recently within a specific subgroup of euglenids and that trypanosomatids are not secondarily non-photosynthetic (plastids-recent hypothesis).**

The past twenty years of molecular biology research have advanced phylogenetic research more quickly than at any other time in history, and most students of microbial diversity have a strong grasp of gene technologies, bioinformatics and molecular phylogenetic methods. Molecular sequence comparisons allow relatively straightforward assessments of homology that are simply unmatched in traditional approaches using morphological characters. However, today's molecular biologists can generate broad-scale phylogenetic trees irrespective of whether or not the researcher is versed in the basic biology of the organisms, and when the breadth of phylogenetic questions increases, so does the level of difficulty that is associated with trying to integrate all of the available information known about diverse groups. Consequently, as the focus of research programs relies more and more on gene technologies and sequence analyses, the significance of other organismal details tends to get lost into the background or reduced to a few key features. This is problematic because a general understanding of the historical correlations between different organismal characters at multiple levels of organization, from protein complexes to complex behaviors, is essential to avoid misguided inferences about character evolution.

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Understanding the significance of co-occurring characters is particularly important in comparative studies of eukaryotic microorganisms because of their relatively integrated ultrastructural systems, vast phylogenetic diversity and complicated history of acquiring intracellular prokaryotic symbionts (e.g. mitochondria and chloroplasts) [1–3]. Recent developments in the comparative genomics of euglenozoans have helped to demonstrate how parsimoniously mapping a single character onto a phylogenetic topology, without adequately considering the organismal context within which that character must function, can result in incongruous morphological data. This can occur because derived characters influence subsequent evolutionary trajectories and are often connected by historical relationships of cause and effect. Therefore, phylogenetic mapping of a single character cannot always be viewed as a simple exercise in parsimony-based reasoning. Seemingly independent characters should not be considered in isolation, but in the parsimony-based context of all other available characters. My aim here is to address this point by bringing into the forefront some of the morphological context that is necessary for future phylogenetic interpretations of genomic data from euglenozoans.

## Hypothetical origins of euglenozoan chloroplasts

The established sisterhood between kinetoplastids and euglenids has resulted in a rather counter-intuitive phylogenetic framework that closely links lethal human parasites (e.g. *Trypanosoma*) with innocuous free-living algae (e.g. *Euglena*) [4–7] (Box 1; Figure 1). However, the chloroplasts in phototrophic euglenids were ultimately derived from a SECONDARY ENDOSYMBIOSIS (see Glossary) with a green algal prey cell, an inference that is supported by biochemical, morphological and gene sequence data [8,9]. Currently, there is a good deal of discussion about when in euglenozoan history this endosymbiosis took place. Several studies using genome-based phylogenetic approaches have found plastid-like genes in distant relatives of phototrophic euglenids, namely trypanosomatids and HETEROLOBOSEANS, and have suggested that chloroplasts arose early in euglenozoan evolution (Figure 1) [10–12]. This is a tantalizing hypothesis because a relic plastid could be a potential target for therapeutic drugs in mammal-infecting trypanosomatids, an approach that has been explored extensively in malaria research [13,14].

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## Glossary

**Bodonids:** refers to mostly free-living kinetoplastids with an anteriorly directed dorsal flagellum, a posteriorly directed ventral flagellum and a simple feeding apparatus.
**Euglenoid Movement:** a peculiar wriggling movement facilitated by the sliding of adjacent pellicle strips. Movements can range from subtle deformations in cell shape to highly coordinated cycles of peristalsis-like deformations. Euglenoid movement is a basic property of eukaryovorous euglenids, some phototrophic euglenids, and some primary osmotrophic euglenids.
**Eukaryovory:** a predatory mode whereby organisms can obtain and ingest nutrients directly from large eukaryotic prey.
**Heteroloboseans:** a phenotypically diverse group of heterotrophic amoeboflagellates with paddle-shaped mitochondrial cristae, eruptive pseudopodia and a flagellar apparatus consisting of parallel basal bodies (e.g. *Acrasis*, *Percolomonas*, *Tetramitus* and *Psalteriomonas*).
**Lorica:** a mineralized extracellular matrix impregnated with ferric and manganese compounds. Loricas form a hardened and often ornamented casing around some phototrophic euglenids (e.g. *Trachelomonas* and *Strombomonas*).
**Monophyletic:** a phylogenetic grouping of population lineages consisting of a common ancestor and all of its descendants.
**Morphostasis:** relative constancy in morphological characteristics through time. Unchanging morphological characters are inferred to result from external factors, such as stabilizing selection, and internal factors, such as the intrinsic constraints on systems of integrated characters.
**Myzocytosis:** a predatory mode whereby a cell can penetrate the cortex of a prey cell and draw in its cytoplasmic contents.
**Osmotrophy:** refers to the nutritional mode of heterotrophic flagellates that lack a feeding apparatus. Nutrients are assumed to be absorbed directly from the environment.
**Paraphyletic:** refers to a grouping of population lineages consisting of a common ancestor and some (not all) of its descendants.
**Paraxonemal Rod:** any lattice-like structure positioned within the flagellar membrane and running adjacent to the '9 + 2' microtubular axoneme (see Box 1 in main text). Paraxonemal rods mediate axonemal-bending forces and presumably facilitate substrate-mediated gliding motility (see Figure 2 in main text).
**Pellicle:** the proteinaceous network positioned beneath the plasma membrane of some unicellular eukaryotes (e.g. ciliates and euglenids).
**Phagotrophy:** a mode of nutrition whereby relatively large particles of food, such as entire prey cells, are completely ingested and contained within a membrane-bound vesicle.
**Phototaxis:** the ability to respond to the intensity or direction of light by changes in swimming behavior.
**Plesiomorphic:** refers to an ancestral characteristic as inferred from the most parsimonious distribution of character states on a specific cladogram.
**Primary Endosymbiosis:** refers to the acquisition of a plastid (e.g. chloroplast) by a phagotrophic eukaryote that has engulfed and intracellularly retained a photosynthetic prokaryote (e.g. a cyanobacterium). The plastids in red algae, green algae, land plants and glaucophytes originated by primary endosymbiosis.
**Secondary Endosymbiosis:** the acquisition of a plastid (e.g. chloroplast) by a phagotrophic eukaryote that has engulfed and retained the (primary) photosynthetic machinery (e.g. primary plastid) of its eukaryotic prey.
**Strip Projection:** proteinaceous structures that are continuous with and branch laterally from the main S-shaped body of the strips (see Figure 2 in main text). Strip projections from adjacent strips interconnect in the articulation zones between strips.
**Synapomorphies:** shared derived character states as inferred from the most parsimonious distribution of character states on a specific cladogram.

These views, which can be collectively referred to as the plastids-early hypothesis, have arisen from an overly simplified parsimony argument that can be paraphrased as follows: because the heterotrophic species within which plastid-like genes have been discovered are relatives of phototrophic euglenids, chloroplasts could have been acquired before the common ancestor of these lineages and subsequently retained in euglenids (for a more radical version of this view see Refs. [15,16]). This argument implies that the loss of photosynthesis and eventually the chloroplasts themselves occurred independently in several different lineages of heterotrophic euglenozoans (Figure 1). If widespread secondary loss of chloroplasts actually occurred in this group, it would be quite challenging to convincingly trace the endosymbiotic origin of these organelles with the aid of molecular phylogenetic topologies alone. Consequently, alternative explanations to the plastids-early hypothesis include discussions about horizontal gene transfer from food items [14], vestigial gene transfers from an ancient PRIMARY ENDOSYMBIOSIS [17] and misleading phylogenetic topologies of plastid-like genes as a result of methodological artifacts (e.g. taxon-sampling effects and long-branch attraction) [18]. Nonetheless, the most pertinent question is the following: can considerations of co-occurring morphological characters provide compelling insights into the validity of the plastids-early hypothesis?

### The diversification of bacterivores in euglenozoan evolution

The earliest stages of euglenozoan evolution were probably dominated by independent radiations of small bacterivores. Surveys of euglenozoan diversity indicate that (i) the majority of free-living kinetoplastids (all of which are BODONIDS) are very small bacterivores (~5 μm long) and (ii) the majority of phagotrophic euglenids are also small bacterivores. Although current molecular phylogenetic data are tenuous, bodonids, in the broad sense, occupy the earliest diverging positions in kinetoplastid phylogenies [19,20], and bacterivores (e.g. *Petalomonas*, *Entosiphon* and *Ploeotia*) tend to occupy the earliest diverging positions in euglenid phylogenies (Figure 1) [21,22]. Bodonids and many bacterivorous euglenids have several putative PLESIOMORPHIC features, including an unfolded peripheral cytoskeleton, two heteromorphic flagella, a relatively simple feeding apparatus, kinetoplast-like mitochondrial inclusions, and all of the features shared by the Euglenozoa (Box 1; Figure 1, step B). In other words, these lineages appear to exhibit strong degrees of MORPHOSTASIS [23]. More derived lineages have increased in complexity, particularly with respect to cytoskeletal organization (e.g. most other euglenids) and the molecular and biochemical sophistication associated with parasitic life cycles (e.g. trypanosomatids). Available data suggest that several existing lineages have retained intermediate characteristics within a transformation series that bridges small bacterivorous cells to cells with pronounced ultrastructural complexity, especially along the euglenid side of euglenozoan evolution [22,24].

### The first euglenids

The origin of the Euglenida is demarcated by the emergence of PELLICLE strips (Figures 1 and 2, step 1), which are cytoskeletal structures that are S-shaped in transverse section and composed mostly of a novel family of proteins called articulins [25–27]. Pellicle strips run beneath the plasma membrane from anterior to posterior and articulate along their lateral margins (Figure 2). Although only a few species have been studied using electron microscopy, the earliest diverging euglenids have a few broad strips (4 to 12) that are extremely thin, longitudinally arranged and fused along their articulation zones [22,26]. These strip properties are associated with rigid cells that are, for the most part, limited to

**Box 1. What are euglenozoans?**

The Euglenozoa consists of a heterogeneous group of single-celled flagellates, most of which are members of two major subgroups: kinetoplastids and euglenids (see Figure 1 in the main text). Kinetoplastids are morphologically defined by highly structured mitochondrial inclusions of DNA called kinetoplasts, and euglenids are morphologically defined by the presence of proteinaceous pellicle strips that subtend the plasma membrane and run parallel to one another, from anterior to posterior, over the cell surface (see Figure 2 in the main text) [25,49]. Several other euglenozoan lineages lack these diagnostic features and have unclear phylogenetic positions within the clade (e.g. *Postgaardi*, *Calkinsia* and diplonemids; Figure I).

Nonetheless, euglenozoans as a whole share several plesiomorphic characteristics, such as closed mitosis with an intranuclear spindle, paddle-shaped mitochondrial cristae (see step A in Figure 1 in the main text), and two flagella consisting of an anteriorly directed dorsal flagellum and a posteriorly directed ventral flagellum (Figure Ic; see step B in Figure 1 in the main text). The best SYNAPOMORPHIES for the group are perhaps a microtubule-reinforced ventral feeding apparatus (Figure Id–f), a distinct tripartite flagellar root system (Box 2) and heteromorphic paraxonemal rods (Figure Ig,h).

Euglenozoans have diverse modes of nutrition, including predation, osmotrophy, parasitism and phototrophy. Predatory euglenozoans are phylogenetically widespread within the group and tend to have diverse feeding apparatuses, feeding strategies and prey preferences. For instance, some predatory species are limited to small prey, such as bacteria (e.g. *Bodo* and *Entosiphon*), whereas other species frequently consume larger prey, such as other eukaryotic cells, by either MYZOCYTOSIS or true phagotrophy (e.g. *Peranema* and *Rhynchopus*). Osmotrophic euglenozoans are heterotrophs that lack a feeding apparatus and are, therefore, assumed to absorb nutrients directly from their aquatic environments (e.g. *Distigma* and *Rhabdomonas*). Parasitic (and commensalistic) euglenozoans appear to have evolved independently several times within kinetoplastids [20], and some species, specifically some trypanosomatids, cause important human illnesses, such as sleeping sickness and Chagas' disease.

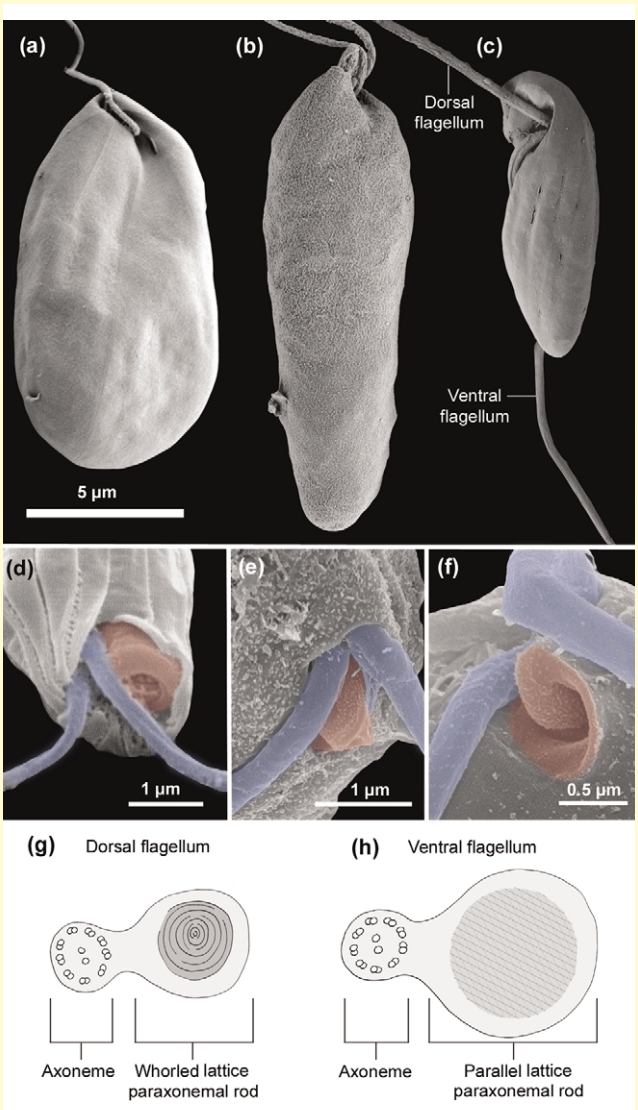


**Figure I.** General features of euglenozoans. Scanning electron micrographs of **(a)** the putative euglenid *Notosolenus*, **(b)** *Diplonema papillatum*, **(c)** an unidentified euglenid from anaerobic sediments, **(d)** the anterior end of the euglenid *Entosiphon sulcatum* and **(e,f)** the anterior end of the kinetoplastid *Bodo caudatus*; pink highlights the feeding apparatus and blue highlights the flagella. Illustrations of transverse sections through **(g)** the dorsal flagellum showing the whorled lattice paraxonemal rod and **(h)** the ventral flagellum showing the parallel lattice paraxonemal rod [4,5,49,50]. (d) Reproduced, with permission, from the Journal of Eukaryotic Microbiology [26].

bacterivorous or OSMOTROPHIC modes of nutrition. These features co-occur with a novel form of substrate-mediated gliding motility that is made possible by molecular motors on the flagellar surface (e.g. flagellar hairs) and presumably by the PARAXONEMAL RODS (Box 1) [28]. Accordingly, the first euglenids have a distinct flagellar configuration, whereby the dorsal (anterior) flagellum is held straight forward and the ventral (posterior) flagellum is bent backwards beneath the ventral surface of the cell (Figures 1 and 2, step 1).

The feeding apparatus in many of these euglenids (e.g. *Petalomonas* and *Notosolenus*) is a relatively simple pocket that is reinforced by a small band of microtubules and positioned ventrally, below the flagellar pocket (Figure 2; Box 2). However, the complexity of the feeding apparatus increased dramatically during the diversification of bacterivorous euglenids, giving rise to vanes that behave like the blades of a pinwheel and microtubule-reinforced feeding rods that extend the entire length of the cell (Figure 2) [29–31]. Cladistic analyses of morphological characters have suggested that a rod-and-vane-based feeding apparatus was a precursor to more derived modes of feeding, namely EUKARYOVORY [22,29,32]. However, before this type of feeding could occur with any regularity in euglenids, the rod-and-vane-based feeding apparatus

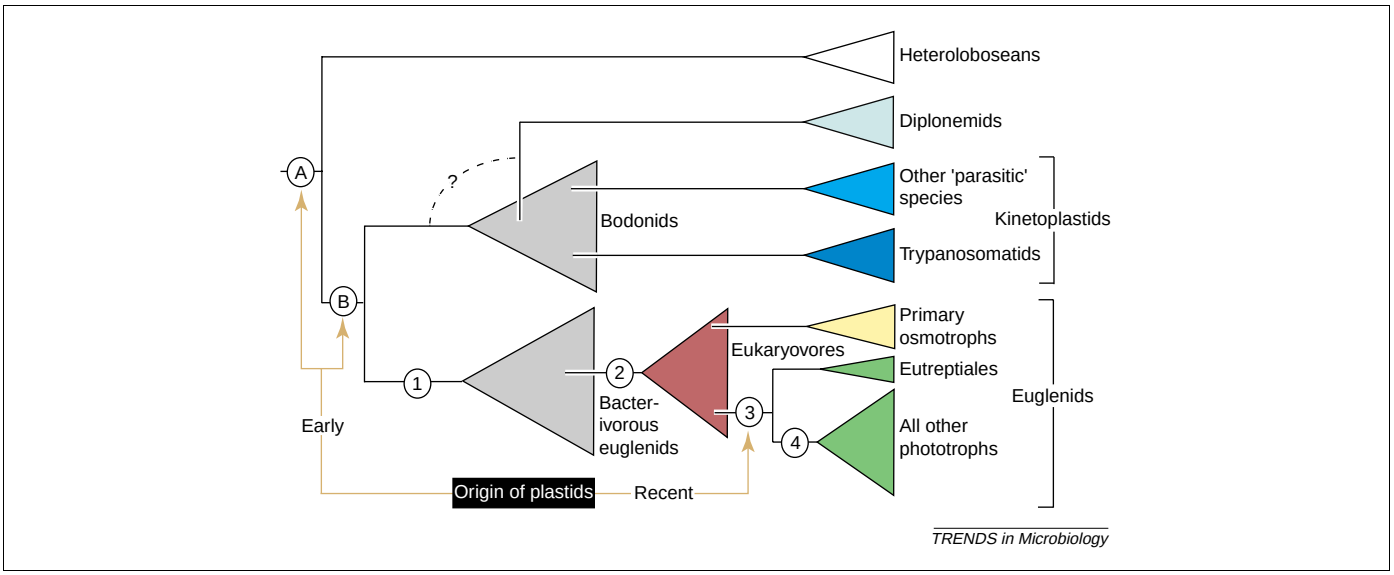


**Figure 1**. An illustration of euglenozoan relationships, emphasizing the diverse modes of nutrition present in the group. This general framework reflects the current state of knowledge about euglenozoan phylogeny. It is a synthetic hypothesis based primarily on comparative morphology (cladistic analysis) and secondarily on the limited amount of available molecular phylogenetic data. Molecular phylogenies suggest that heterolobosean are the nearest outgroup to the Euglenozoa, which consists of diplonemids, kinetoplastids and euglenids. Colored triangles indicate putative radiations of organisms with distinct nutritional modes: grey, bacterivory; red, eukaryovory; yellow, primary OSMOTROPHY; green, phototrophy; medium and dark blue, parasitism; light blue and white, mixed modes of heterotrophy. The placement of diplonemids relative to other kinetoplastids is uncertain, and although only two lineages are shown, parasitic kinetoplastids have multiple independent origins. For illustrative purposes, PARAPHYLETIC radiations (e.g. bodonids, bacterivorous euglenids and eukaryotrophic euglenids) are positioned to the left of nested monophyletic groups. Letters in circles denote pre-euglenid evolutionary events. Numbers in circles denote derived characters in euglenid evolution. Alternative hypotheses for the endosymbiotic origin of chloroplasts are noted, namely the plastids-early hypothesis and the plastids-recent hypothesis.

had to become integrated with a very different kind of pellicle and associated cell motility system.

## Origin of euglenid eukaryovory

Somatic plasticity is a basic requirement for any relatively small predator attempting to ingest large incompressible food items. Some euglenid predators are able to accommodate consumed prey cells that are close to their own size. Some species of *Dinema*, for instance, are relatively small euglenids with a rod-and-vane-based feeding apparatus that extends the length of the cell, as is the case in many bacterivores (Figure 2), but is capable of completely devouring very large and unyielding prey organisms, such as diatoms. There appear to be good reasons why *Dinema* can accomplish these remarkable feats of eukaryovory, whereas most bacterivorous euglenids cannot; *Dinema* species acquired EUGLENOID MOVEMENT (Figures 1 and 2, step 2).

This form of cell plasticity not only accommodates the ingestion of large prey but might also provide the capacity for drawing it into the cell. Euglenoid movement is achieved by the relative sliding of adjacent strips at the zones of lateral articulation, although the motor behind this mechanism is unknown. The ability to produce euglenoid movement is directly correlated with a significant increase in the helical pitch and also the total number of pellicle strips around the cell periphery (Figures 1 and 2, step 2) [22,24,25,29]. As the number of strips increases so does the number of articulation zones between strips, which provides more unrestricted regions for cell deformation. Other *Dinema*-like eukaryovores, such as species of *Anisonema* and *Metanema*, show different degrees of euglenoid movement, which provides context for inferring the intermediate character states that must have occurred in the evolution of euglenoid movement from rigid ancestors. However, determining whether these particular species evolved from more flexible or more rigid ancestors requires new sources of molecular phylogenetic data.

The number of strips around the cell periphery is an excellent indicator of phylogenetic relationships and general modes of nutrition [22,24,25]. For instance, rigid bacterivores usually have approximately 10 strips or less, whereas the number of strips in *Dinema sulcatum* ranges from 20 to 24. Distinctly different eukaryovorous euglenids, such as the *Peranema*-like species, have ~50 strips around the cell periphery [24]. The large discontinuities in total strip number between bacterivores (~10 strips), *Dinema*-like eukaryovores (~20 strips) and *Peranema*-like eukaryovores (>40 strips) suggest that a series of permanent strip duplication events occurred throughout euglenid evolution. This interpretation is consistent with euglenid cytokinesis, where the number of strips around the cell periphery doubles in preparation for cell division. Each daughter cell receives half of the newly duplicated strips, bringing the total number of strips in the daughter cell back in line with the original number in the parent cell [33,34]. If the pre-divisional cell fails to divide, then the result is a cell with twice the original number of strips. Although this macroevolutionary mechanism is plausible, it is difficult to comprehend how pellicle strips are able to double independently of the flagellar and feeding apparatus (research on euglenids with four or more flagella, such as *Tetreutreptia* spp. and *Hegneria* spp., might provide important insights into this putative mechanism; Box 2). Nonetheless, it is tempting to entertain the possibility that the origin of the strip-based pellicle in euglenids arose by the same process, whereby an ancestral 1-stripped pellicle gave rise to a 2-stripped pellicle that

**Box 2. Cytoskeletal integration in euglenozoans**

The flagellar apparatus, feeding apparatus and cortical cytoskeleton of euglenozoans are reinforced by microtubules that are structurally integrated with one another (Figure Ia) [42,50,51]. The dorsal and ventral flagella anchor to basal bodies that are positioned at the base of the flagellar pocket. The basal bodies form the microtubular organizing center for the distinctive euglenozoan flagellar apparatus, which consists of three microtubular roots: a dorsal root originating from the dorsal basal body (Figure Ia, purple), a ventral root originating from the ventral basal body (Figure Ia, yellow), and an intermediate root also originating from the ventral basal body (Figure Ia, green). The dorsal root yields a dorsal band of microtubules (Figure Ia, red) that lines the flagellar pocket and continues superficially to support most of the cell cortex (i.e. subtends the pellicle strips in euglenids). The ventral root gives rise to microtubules that reinforce the ventral feeding apparatus (Figure Ia, orange). The intermediate root gives rise to an intermediate ventral band of microtubules (Figure Ia, blue), which might line the flagellar pocket in a similar manner to the dorsal band, and continue superficially to support specific regions of the cell cortex.

Cell division is preceded by duplication of the basal bodies and the associated microtubular root system. Both the parent ventral and dorsal flagella become associated with a new daughter dorsal flagellum. However, the parent dorsal flagellum then becomes completely transformed and acquires the cytoskeletal properties of a ventral flagellum. In this way, each daughter cell inherits the heteromorphic configuration of flagella present in the parent cell. The feeding apparatus duplicates in coordination with the morphological transformation of the parent dorsal flagellum into a ventral flagellum [30]. Following these microtubular reconfigurations, the pellicle strips of euglenids double in number. Nascent pellicle strips emerge between existing strips near the opening of the flagellar pocket and migrate posteriorly over the cell surface and into the flagellar pocket (Figure Ib,c). During strip duplication, the flagellar pocket is partitioned, beginning from its posterior base and ending at its anterior opening [34]. Cytokinesis then proceeds longitudinally from the anterior end of the cell to the posterior end, where each daughter cell inherits an equal number of new strips intercalated between old strips [33].

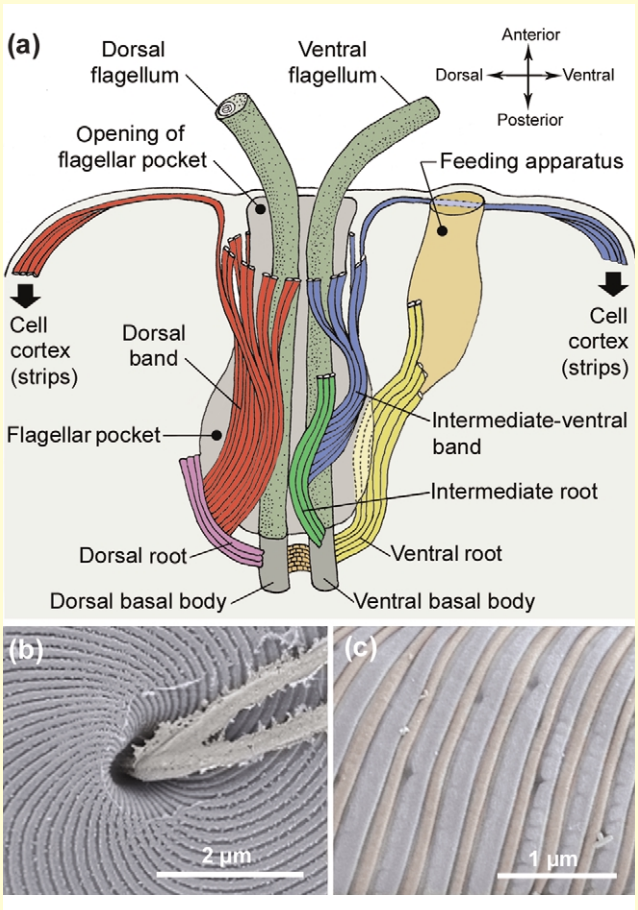


**Figure I.** General features of the euglenid cytoskeleton. **(a)** An illustration showing the microtubular integration associated with the flagellar apparatus, feeding apparatus and cell cortex (pellicle). Scanning electron micrographs of **(b)** the anterior end of a *Eutreptia* species showing the opening of the flagellar pocket and **(c)** the cell surface of a dividing *Eutreptiella* cell showing nascent strips (pink) positioned between old strips (blue).

gave rise to a 4-stripped pellicle that gave rise to an 8-stripped pellicle and so forth.

*Peranema*-like eukaryovores (e.g. *Peranema trichophorum*) tend to be larger than *Dinema* species (e.g. *D. sulcatum*) and bacterivorous euglenids, and have a rod-and-vane-based feeding apparatus that is localized to the anterior third of the cell (Figure 2). Phagotrophic euglenids, regardless of being bacterivorous or eukaryovorous, use the gliding motility system associated with an anteriorly directed dorsal flagellum and a posteriorly directed ventral flagellum (Figures 1 and 2, step 1). The increase in cell size and in the number of strips in *Peranema*-like eukaryovores directly correlates with increased cell plasticity and, presumably, the range of potential prey organisms. *Peranema*-like euglenids possess the ability to ingest eukaryotic (photosynthetic) prey by true PHAGOTROPHY and, therefore, possess the fundamental characteristics that are necessary for establishing a secondary endosymbiosis.

## The evolutionary radiation of phototrophic euglenids

Molecular phylogenies consistently place phototrophic euglenids in a MONOPHYLETIC group that is nested within the Euglenozoa (Figure 1) [21,35–40]. Moreover, it appears more than coincidental that the eukaryovore *P. trichophorum* is almost always a close sister lineage to phototrophic euglenids in molecular phylogenies [36–38,40]. This sisterhood is entirely congruent with comparative morphological data [22,24]. For instance, the earliest diverging phototrophic euglenids, namely several species of Eutreptiales and *Euglena* (Figures 1 and 2), have cell sizes and pellicle features that are very similar to *P. trichophorum*: euglenoid movement is pronounced, the total number of helically arranged strips is over 40, and the transverse width and shape of the S-shaped strips are essentially identical (Figure 2) [24]. In addition, all phototrophs examined so far possess a highly reduced feeding apparatus (Figures 1 and 2, step 3) [41,42]. In this context, it is clear that many phototrophic euglenids possess several plesiomorphic features, suggesting that the presence of euglenoid movement and reduced feeding apparatus in this group are relics of a eukaryovorous ancestry.

However, as expected, the newfound presence of integrated chloroplasts in a eukaryovorous host introduced a

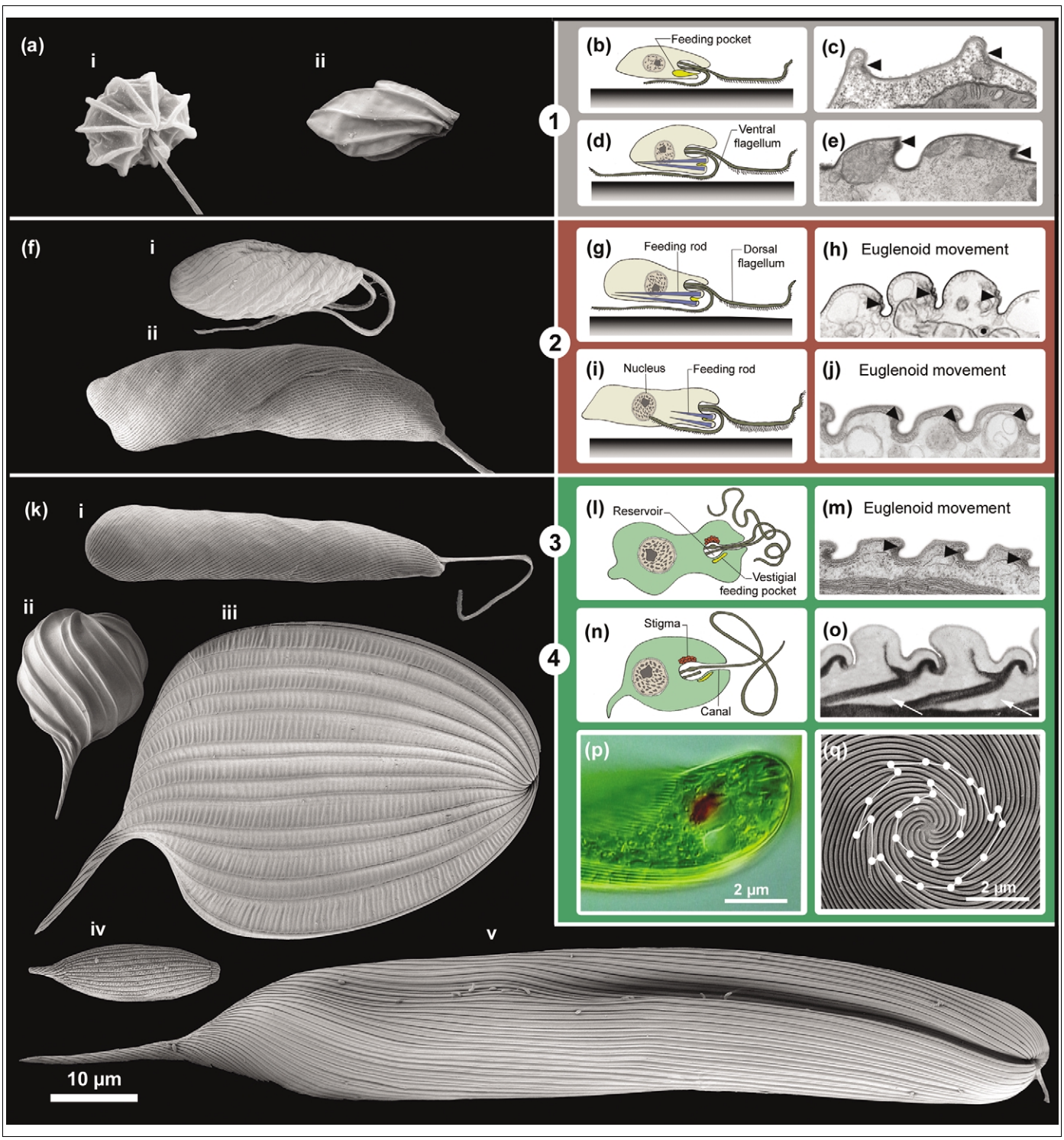


**Figure 2**. The characteristics present in three categories of euglenids based on nutritional mode. Background colors in the right hand boxes correspond to the following: grey, bacterivory; red, eukaryovory; green, phototrophy. The left hand boxes include scanning electron micrographs of **(a)** bacterivorous euglenids, (i) *Ploeotia vitrea* and (ii) *Petalomonas cantuscygni*; **(f)** eukaryovorous euglenids, (i) *Dinema sulcatum* and (ii) *Peranema trichophorum*; and **(k)** phototrophic euglenids, (i) *Euglena geniculata*, (ii) *Monomorphina ovata*, (iii) *Phacus* sp., (iv) *Lepocinclis* sp. and (v) *Lepocinclis oxyuris* (all images at same scale). **(b,d)** Illustrations of the substrate-mediated gliding motility and different feeding apparatuses present in bacterivorous euglenids. **(c,e)** Transmission electron micrographs showing the thin, broad strips of *Petalomonas cantuscygni* and *Entosiphon sulcatum*, respectively. **(g,i)** Illustrations of the substrate-mediated gliding motility and different rod-and-vane-based feeding apparatuses present in eukaryovorous euglenids. **(h,j)** Transmission electron micrographs showing the thin, S-shaped strips of *Dinema sulcatum* and *P. trichophorum*, respectively. **(l,n)** Illustrations of the swimming motility, photoreception apparatus and vestigial feeding apparatus present in phototrophic euglenids. Transmission electron micrographs showing the thin, S-shaped strips of *Euglena terricola* **(m)** and the thick strips and strip projections (arrows) of *Euglena helicoideus* **(o)**. **(c,e,h,j,m)** Arrowheads mark articulation zones between strips. **(p)** Light micrograph of *Euglena helicoideus* showing the orange stigma near the flagellar pocket. **(q)** Scanning electron micrograph of *Euglena myxocylindracea* showing the whorled pattern of strip reduction near the posterior end of phototrophic cells. The numbers in circles represent suites of co-occurring characters at significant positions in euglenid phylogeny: (1) gliding motility, $<12$ broad strips; (2) $>20$ strips, euglenoid movement; (3) photoreception apparatus, reservoir, vestigial feeding pocket, swimming motility; whorls of strip reduction (4) thick strips, strip projections, figure-eight flagellar beat pattern. (a,c,f,h) Reproduced, with permission, from the European Journal of Protistology [22]. (e,m,o) Reproduced, with permission, from the Journal of Eukaryotic Microbiology [26].

brand new set of selective pressures that led to a large suite of co-occurring innovations. For instance, to maintain their position in the photic zone, phototrophic euglenids replaced substrate-mediated gliding motility with swimming motility. In the vast majority of phototrophs, this was accomplished by drastically shortening the ventral flagellum and converting the dorsal flagellum into an anterior propeller that beats with a controlled figure-eight configuration (Figures 1 and 2, step 4). All phototrophs possess a photoreception apparatus, which is used in PHOTOTAXIS and consists of a carotenoid-based shading structure called the stigma, an expanded flagellar pocket called the reservoir and a photosensory swelling at the base of the dorsal flagellum (Figures 1 and 2, step 3) [43]. For unclear reasons, every phototrophic euglenid examined so far, to the exclusion of all other euglenids, possesses distinct whorls of strip reduction on their posterior cell surfaces (Figures 1 and 2, step 3) [22,24,25,44,45]. This pellicle feature is not only diagnostic of phototrophs, but unambiguously distinguishes primary osmotrophs (e.g. *Distigma*) from secondary osmotrophs (e.g. *Astasia longa* and *Cyclidiopsis acus*) and provides an inferential tool for recognizing phototrophy in putative euglenid fossils (e.g. *Moyeria*) [22,24,46].

The acquisition of chloroplasts also caused the ancestral *Peranema*-like cytoskeleton to become significantly modified in similar ways along different lineages. For instance, there is a trend toward the convergent evolution of pellicle rigidity (e.g. *Phacus*, *Lepocinclis* and *Monomorphina*), which is directly correlated with a reduction in the total number of pellicle strips, the thickening of strips and the advent of interlocking STRIP PROJECTIONS (Figure 2) [24,26,35,44]. These projections essentially close-off strip articulation zones by forming crosshatched interconnections. The thickening of strips and their projections might limit or prevent sliding between adjacent strips, which could be an adaptation to eliminate the unneeded capability for euglenoid movement and its associated energetic costs (afterall, phototrophs have abandoned eukaryovory). However, other functions might also explain the origin of strip projections and relative strip thickness in phototrophs, including a role in diffusing the light that is used in photosynthesis and in protection from invasion by MYZOCYTOTIC predators, including eukaryovorous euglenids. The extracellular LORICAS of *Trachelomonas* and *Strombomonas* probably represent an alternative strategy for counteracting the same environmental pressures. There are also dramatic increases in cell size and flattened cell shapes in several phototrophic lineages that presumably influence light harvesting, sedimentation rates and predator avoidance, as larger cells cannot be phagocytized by smaller predators (some phototrophic euglenids resemble giant solar panels; Figure 2k, part iii). Overall, the pellicle properties of phototrophic euglenids changed with clear evolutionary polarities; the earliest diverging phototrophic lineages have thin strips, fine (thread-like) strip projections and pronounced euglenoid movement, whereas more derived lineages have thick strips, thick strip projections and pellicle rigidity [24].

## Concluding remarks and morphology-based implications

Although the plastids-early and plastids-recent hypotheses are both valid frameworks for future research, in my opinion, the overall pattern of morphological change in euglenids undermines the parsimony argument that is associated with the plastids-early hypothesis and favors a plastids-recent hypothesis in euglenozoan evolution (Figure 1). In addition, phagotrophic lineages are never intermixed with phototrophic lineages in molecular phylogenetic analyses, which would be an expectation of the plastids-early hypothesis, but instead consistently diverge before a well-supported clade of phototrophic euglenids. However, this framework needs to be significantly reinforced with increased taxon sampling and phylogenies that are derived from several different nucleus-encoded protein genes, particularly from phagotrophic euglenids [6,21,22,47,48].

Nevertheless, if the last common ancestor of euglenozoans was a phototrophic organism, then much more than rampant plastid loss must be accounted for. For instance, on what grounds could one assume that this hypothetical ancestor was significantly different from existing phototrophs? If this ancestor did not possess a photoreception apparatus, a reservoir, a dorsal flagellum configured for swimming above substrates, more than 40 pellicle strips, interconnecting strip projections and whorls of strip reduction, then how does one explain the co-occurrence of these features in a distinct clade of deeply nested phototrophs? Why is it that all members of this clade suddenly possess a highly reduced feeding apparatus? Is it just coincidence or is one willing to infer that the elaborate rod-and-vane-based feeding apparatus and associated substrate-mediated gliding motility evolved after the origin of secondary plastids?

Before eukaryotes evolved ways to feed on large prey cells, bacterivory must have preceded eukaryovory. In my opinion, it is more than coincidental that bacterivores tend to diverge near the nexus of early euglenozoan evolution. The subsequent origin of euglenoid movement and associated pellicle modifications (e.g. numerous strips) allowed euglenid phagotrophs to accommodate the internalization of large prey and opened the door to a new predatory niche based on eukaryovory. This ultimately provided the necessary organismal context for chloroplast acquisition by secondary endosymbiosis. This scenario is not only consistent with available molecular data, but provides the best backdrop for comprehending the selective forces that must have been involved in shaping the co-occurring innovations and evolutionary trends associated with the radiation of phototrophic euglenids.

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