Euglenida

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Brian S. Leander, Gordon Lax, Anna Karnkowska, and Alastair G. B. Simpson

Abstract

Euglenids are a group of >1500 described species of single-celled flagellates with diverse modes of nutrition, including phagotrophy and photoautotrophy. The group also encompasses a clade of specialist "primary" osmotrophs (Aphagea) and, very likely, one group of phagotrophs that are ectosymbiont-supporting anaerobes (Symbiontida). Almost all euglenids are free-living. The (usually) one or two emergent flagella have thick paraxonemal (paraxial) rods and originate in a deep pocket/reservoir, while the cell surface is almost always supported by a pellicle of parallel proteinaceous strips underlain by microtubules. Cells with 4–12 strips are rigid; most of those with more strips (typically ~20–40) have them arranged helically and exhibit active cell deformation called "euglenid motion" or "metaboly." Most phagotrophic euglenids are surface-associated bacterivores or eukaryovores that employ a flagellar gliding motility; they are abundant in marine and freshwater sediments. Photoautotrophic species (Euglenophyceae) constitute a single subclade within euglenids and have a plastid (chloroplast) of secondary endosymbiotic origin, with three bounding membranes. The plastid is typically green, with chlorophylls *a* + *b*, and was derived from a chloroplastidan alga related to the Pyramimonadales. Photoautotrophic euglenids move primarily by swimming, and most (members of the taxon Euglenales, e.g., *Euglena*) have a single emergent flagellum and are generally restricted to fresh and brackish waters.

B.S. Leander (✉) • A. Karnkowska
The Departments of Botany and Zoology, University of British Columbia, Vancouver, BC, Canada
e-mail: bleander@mail.ubc.ca

G. Lax • A.G.B. Simpson
Department of Biology, Dalhousie University, Halifax, NS, Canada

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Cytoskeleton • Endosymbiosis • Euglenozoa • Evolution • Feeding apparatus • Pellicle • Phylogeny • Ultrastructure

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Summary Classification

●**Euglenida**
●●**Petalomonadida** (e.g., *Petalomonas, Notosolenus, Scytomonas, Sphenomonas*)
●●**"Ploeotiids"*** (e.g., *Ploeotia*, *Entosiphon*, *Keelungia*)
●●**Symbiontida** (*Bihospites, Calkinsia,* and *Postgaardi*)
●●**Spirocuta** (formerly "H clade" or "HP clade")
●●●**Aphagea** (e.g., *Rhabdomonas, Menoidium, Distigma, Astasia*)
●●●***Neometanema***
●●●**"Anisonemids"** (*Anisonema*, *Dinema*)
●●●**"Peranemids"*** (e.g., *Peranema, Jenningsia, Heteronema, Urceolus*)
●●●**Euglenophyceae**
●●●●***Rapaza***
●●●●**Eutreptiales** (e.g., *Eutreptia*, *Eutreptiella*)
●●●●**Euglenales** (= Euglenea)
●●●●●**Phacaceae** (*Lepocinclis, Phacus,* and *Discoplastis*)
●●●●●**Euglenaceae** (e.g., *Euglena*, *Colacium, Trachelomonas*)

* Both "ploeotiids" and "peranemids" are paraphyletic assemblages.

Introduction

Euglenids (sometimes referred to as "euglenoids") are a prominent group of free-living, aquatic flagellates, usually with one or two active flagella. Most of the >1500 descibed species are unicells that are 5–50 μm in length; a few are larger. Almost all are motile, either by swimming or by surface-associated gliding on the flagella or cell body.

Euglenida represents one of three major subgroups within the Euglenozoa, along with ▶ Kinetoplastea and Diplonemea, which they resemble in several conspicuous ways. For example, as in kinetoplastids, the flagella are inserted at the base of a deep pocket (also known as the reservoir), and active flagella are conspicuously thickenend due to the presence of paraxonemal (paraxial) rods. The mitochondrial cristae are also discoidal. However, euglenids are readily distinguishable by their cell surface architecture, which almost always is supported by a pellicle of abutting parallel strips of protein that lie directly under the cell membrane (Fig. 1). Cells with many helically arranged strips (>20) are often capable of a characteristic squirming or pulsing form of active cell deformation called "euglenid motion" or "metaboly," which is effected by sliding of adjacent strips.

Euglenids are notable for their diverse modes of nutrition, including phagotrophy (consumption of particles, especially other cells), osmotrophy (absorbtion of organic molecules), and photoautotrophy (photosynthesis) (Figs. 2, 3, 4, and 5). Among the phagotrophs, there is a convenient, if imperfect, distinction drawn between predominantly "bacterivorous" taxa, which have rigid pellicles with 12 or fewer strips and tend to be smaller in size, and predominantly "eukaryovorous" taxa that have pellicles with many strips, are usually flexible, and tend to be larger. The latter typically consume microbial eukaryotes, including unicellular algae. Meanwhile, some phototrophic forms are apparently also capable of pinocytosis, or even

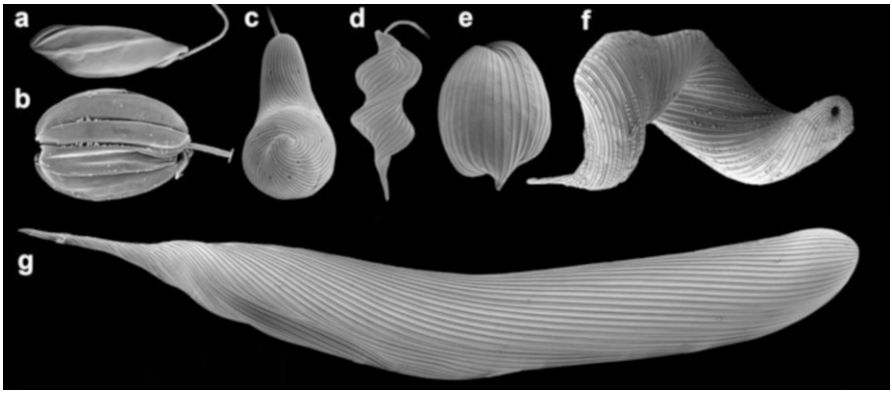

Fig. 1 Scanning electron micrographs showing the diversity of euglenids. (**a**) Petalomonad (phagotroph), (**b**) Ploeotiid (phagotroph), (**c**) *Euglena* (phototroph), (**d**) *Monomorphina* (phototroph), (**e**) *Phacus* (phototroph). (**f**–**g**) *Lepocinclis* (phototroph). Images not to scale; all cells between 10 and 100 μm

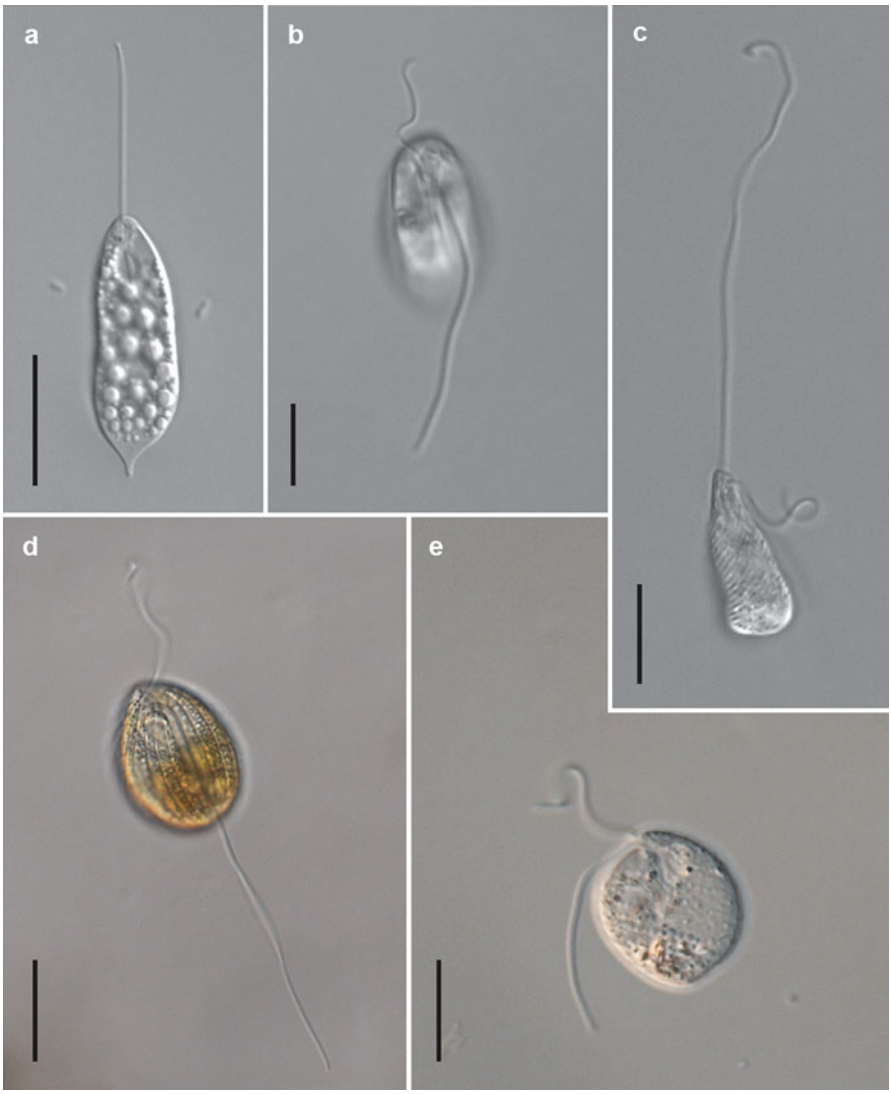

Fig. 2 Light micrographs (DIC) of phagotrophic euglenids, demonstrating various orientations of flagella and modes of locomotion. (**a**) *Petalomonas planus*, a rigid petalomonad. This species has only one flagellum, which is directed anteriorly. (**b**) *Ploeotia vitrea*, a "ploeotiid." While gliding on the posterior flagellum, the cell body is above the substrate, while the anterior flagellum beats from side to side. (**c**) *Heteronema globuliferum*, a flexible "peranemid" that glides on the anterior flagellum, with the posterior flagellum trailing under the cell during actual locomotion. (**d**) *Anisonema acinus*, an "anisonemid" gliding on its posterior flagellum, while the anterior flagellum beats anteriorly. (**e**) *Neometanema parovale* "skids" along surfaces, "skidding" being a form of swimming where the posterior flagellum is in loose contact with the substrate. The anterior flagellum beats freely. Scale bars are 20 μm for **a** and **d** and 10 μm for **b**, **c**, and **e**. Credit: **e**: Won Je Lee

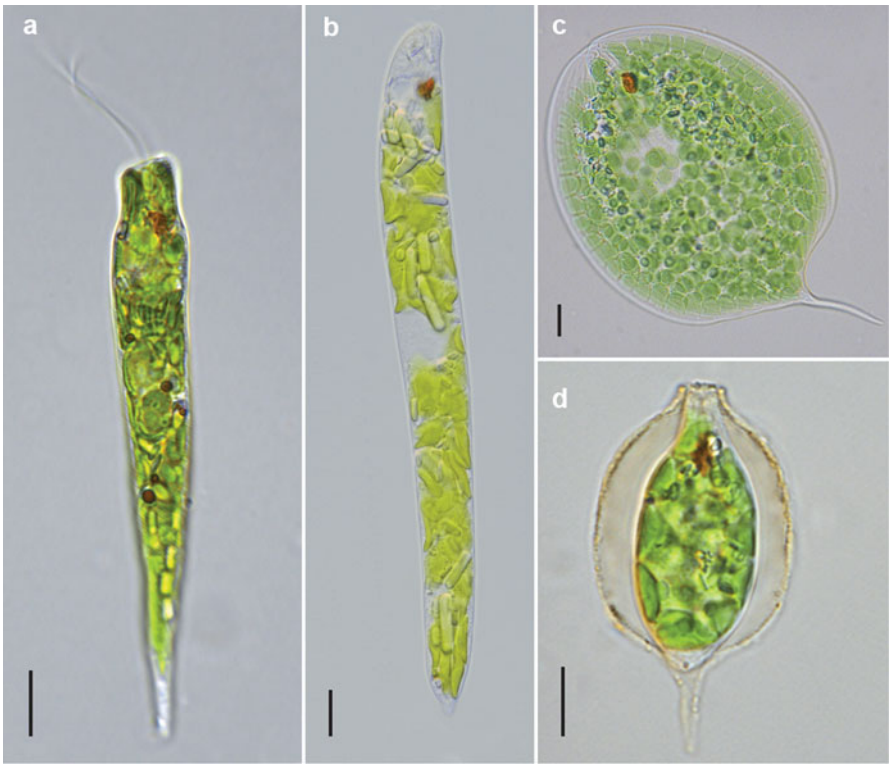

Fig. 3 Light micrographs (bright field) showing the diversity of photoautotrophic euglenids. (**a**) *Eutreptiella*, a marine cell showing two emergent flagella. (**b**) *Euglena*, a cell with shield-shaped plastids. (**c**) *Phacus*, rigid cell with small discoidal plastids. (**d**) *Strombomonas*, a cell enveloped by an organic lorica. Scale bars 10 μm. Credit: Bożena Zakryś

phagotrophy of eukaryotic algae in the case of the deep-branching phototroph *Rapaza* (Yamaguchi et al. 2012).

The "bacterivores" include the petalomonads (Petalomonadida), which glide with a forward-directed flagellum (e.g., *Petalomonas*, *Notosolenus*), and "ploeotiids," which glide on the posterior/ventral flagellum (e.g., *Ploeotia*, *Keelungia, Entosiphon*) (Fig. 2a, b). The eukaryovores include some taxa that glide primarily on a forward-directed anterior flagellum (i.e., similarly to petalomonads; Fig. 2c). An example is the well-known genus *Peranema*, and these organisms are referred to here as "peranemids." Other eukaryovores resemble ploeotiids in gliding on the posterior flagellum; the best known example is *Anisonema,* and these are referred to here as "anisonemids" (Fig. 2d). The unusual phagotroph *Neometanema* normally "skids" along surfaces rather than gliding (Fig. 2e). Ploeotiids and peranemids appear to be paraphyletic groups, the anisonemids may be as well.

Photoautotrophic euglenids are phylogenetically less diverse than phagotrophs, although more species have been described. Most are elongate, flexible cells that

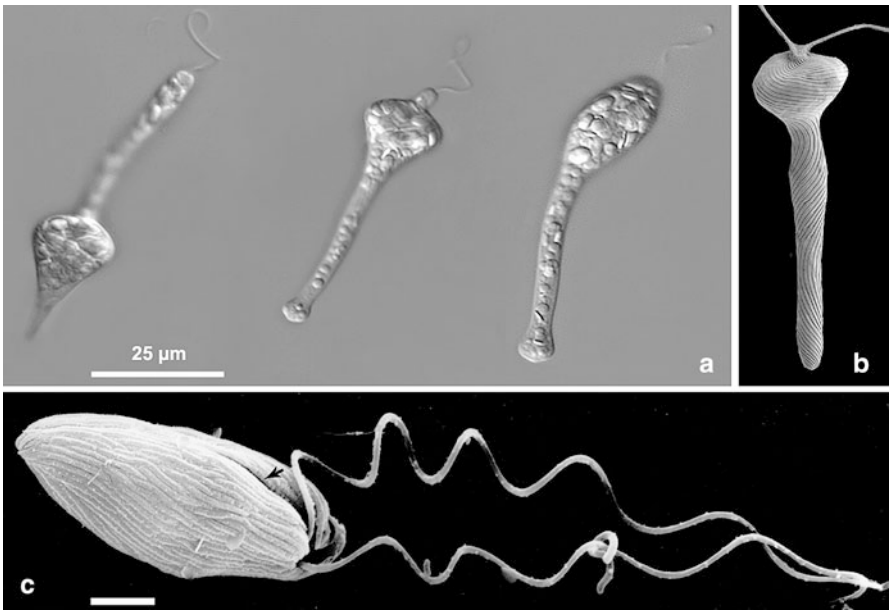

Fig. 4 Micrographs showing primary osmotrophs and symbiontids. (**a**) Three light micrographs (DIC) of a cell of *Astasia* sp. (primary osmotroph). This series also illustrates the process of metaboly ("euglenoid movement") in this particularly flexible euglenid. (**b**) Scanning electron micrograph of *Distigma* sp. (primary osmotroph), showing multiple distortions of the helical organization of the pellicle due to sliding of adjacent strips. (**c**) Scanning electron micrograph of *Postgaardi mariagerensis* (symbiontid) showing the epibiotic bacteria enveloping the cell. The arrow indicates a subtle ventral groove. Scale bars: **a**, 25 µm; **c**, 2 µm. Credit: **a**: William Bourland, **c**: modified from Simpson et al. 1997, reproduced with permission

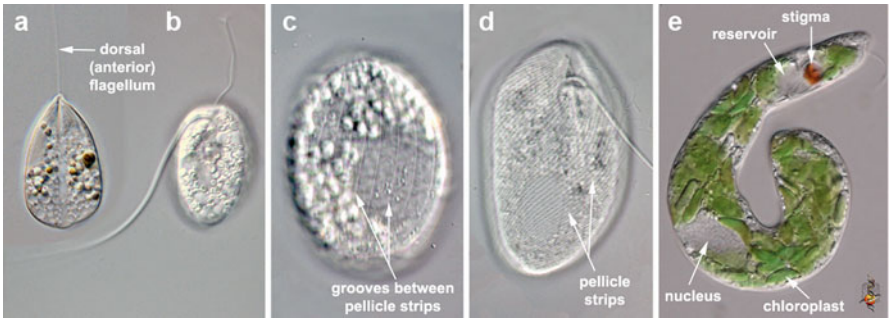

Fig. 5 Labeled light micrographs (DIC) showing several conspicuous traits in euglenids. (**a**) Petalomonad (phagotroph), (**b**) ploeotiid (phagotroph; note thickness of the ventral/posterior flagellum), (**c**, **d**) anisonemids (phagotrophs), (**e**) *Euglena* (phototroph). All cells between 20 and 50 µm. Credit: Linda Amaral Zettler and David Patterson

swim using one or (more rarely) two emergent flagella (e.g., *Eutreptia*, *Euglena*; Fig. 3a, b). Other commonly encountered forms are rigid cells with various cell shapes (e.g., *Phacus*; Fig. 3c) and cells that are enclosed in an extracellular lorica but are nonetheless capable of swimming (*Trachelomonas* and *Strombomonas*; Fig. 3d).

Among the osmotrophs, there are "primary osmotrophs" (the Aphagea, e.g., *Rhabdomonas*, *Distigma*, and *Astasia*; Fig. 4a, b), which descended from within eukaryovorous lineages, and "secondary osmotrophs," which are a polyphyletic collection of species and strains that descended from various photoautotrophs. Secondary osmotrophs now tend to be assigned to predominantly photoautotrophic genera, reflective of their evolutionary histories (see "Taxonomy").

The existence of both phagotrophic and photoautotrophic species led to euglenids being examined both as plant-like and animal-like life-forms. Among other things this resulted in competing classification schemes under the International Code of Botanical Nomenclature and the International Code of Zoological Nomenclature (i.e., they are "ambiregnal taxa" – see Patterson and Larsen 1992). Of course euglenids are neither plant nor animal, so the group does not fall neatly within the archaic plant-animal dichotomy. Photoautotrophic euglenids in fact acquired photosynthesis via a secondary endosymbiosis involving a chloroplastidan green alga (see below). The morphological and behavioral diversity of the group is also exceptional and provides compelling illustrations of major events in evolution, such as the punctuated effects of secondary endosymbiosis and changes in underlying developmental mechanisms (Leander et al. 2007; see "Evolutionary History").

Several photoautotrophic and osmotrophic species are bloom-formers in nutrient-rich conditions and are useful indicators of environmental pollution. Phagotrophic species are ubiquitous primary consumers and are likely to be important components of microbial food webs, especially in sediments. A few euglenids have been used as model systems for addressing a wide variety of questions in basic cell biology and physiology and as teaching aids. *Euglena gracilis*, for instance, is familiar to nearly every student who has taken a general biology course in high school, college, or university.

Literature and History of Knowledge

Knowledge of euglenids extends back to the late 1600s and involves several of the pioneers of eukaryotic microbiology. Photoautotrophic euglenids were among the organisms documented by Leeuwenhoek. In the eighteenth century, O.F. Mueller described some species, though he assigned them to non-euglenid genera. The first genera of photoautotrophs were introduced in the early nineteenth century by Ehrenberg, notably *Euglena* (1830), *Cryptoglena* (1832), *Colacium* (1835), and *Trachelomonas* (1835). By the mid-twentieth century, the number of described species had increased markedly, and several other important freshwater genera were introduced (*Phacus* Dujardin 1841, *Lepocinclis* Perty 1849, *Monomorphina* Mereschkowski 1877, *Strombomonas* Deflandre 1930) as well as two marine genera (*Eutreptia* Perty 1852 and *Eutreptiella* Da Cunha 1913). Accounts of osmotrophic

and phagotrophic euglenids accumulated in the nineteenth century, with genera introduced or regularized by Ehrenberg, Dujardin (1841; e.g., *Anisonema*, *Ploeotia*), Perty (1852), and Stein (1878), among others. In the mid-late twentieth century, monographic accounts of freshwater species, based on light microscopy, were produced by Gojdics (1953), Huber-Pestalozzi (1955), Pringsheim (1956), Popova (1966), Popova and Safonova (1976), Starmach (1983), and Tell and Conforti (1986). Those monographic studies were mainly focused on photoautotrophic species. Leedale's 1967 book "*Euglenoid flagellates*" summarized the ultrastructural and biochemical/cell physiological information available at the time for the group, and Buetov (1968) summarized research on *Euglena*.

Despite this long history, a considerable number of species and several genera have been described since the original publication of the *Handbook of Protoctista* (e.g., Larsen and Patterson 1990; Lee and Patterson 2000; Triemer et al. 2006; Linton et al. 2010; Bennett et al. 2014). Among the most important advances was the definition of the anaerobic Symbiontida, including the first descriptions of *Postgaardi* and *Bihospites* (Fenchel et al. 1995; Yubuki et al. 2009; Breglia et al. 2010; Fig. 4c), though as discussed below, the case that symbiontids are euglenids is not fully settled and some authors currently treat them as a separate group within the Euglenozoa (Cavalier-Smith 2016). As with most other groups of protists, the advent of molecular phylogenetics has resulted in considerable taxonomic and systematic changes, especially in the last ~15 years. Important syntheses and revisions of photoautotrophic euglenids include Marin et al. (2003), Linton et al. (2010), Kim et al. (2010), Karnkowska et al. (2015) and Kim et al. (2015), and Preisfeld et al. (2001) for osmotrophic euglenids. The phylogenetic relationships and systematics of phagotrophic euglenids remain much more poorly understood and are currently in a state of flux (Lax and Simpson 2013; Cavalier-Smith 2016; Cavalier-Smith et al. 2016).

Practical Importance

Euglenids are not known to cause disease in humans or livestock; rare possible cases of parasitism by euglenids involve noneconomic organisms such as tadpoles and gastrotrichs (Wenrich 1924; Brumpt and Lavier 1924; Kisielewska et al. 2015). However, some bloom-forming photoautotrophic euglenids have been shown to produce neurotoxins that can cause widespread fish die-offs in freshwater aquaculture facilities (Zimba et al. 2004, 2010).

A couple of species of euglenids have been exploited as model systems for biological research. For example, *Euglena gracilis* has been investigated for the production of important compounds (Krajčovič et al. 2015) such as vitamins A, C, and E (e.g., Takeyama et al. 1997; Fujita et al. 2008); polyunsaturated fatty acids (e.g., Korn 1964; Wallis and Browse 1999; Meyer et al. 2003); the carbohydrate paramylon (e.g., Santek et al. 2009; Rodríguez-Zavala et al. 2010; Shibakami et al. 2012); and wax esters (e.g., Inui et al. 1982; Teerawanichpan and Qiu 2010; Tucci et al. 2010; Dasgupta et al. 2012). *Euglena gracilis* can be grown in a wide range of

conditions: autotrophically or heterotrophically on various carbon sources (or both), under a broad range of pH values, and in high concentrations of cadmium, chromium, lead, mercury, and zinc. Therefore, it can be used for bioremediation of polluted waters (Krajčovič et al. 2015).

Habitats and Ecology

Phagotrophic euglenids are widespread in marine, brackish, and freshwater sediments. These cells glide within the spaces between sand grains and within the narrow interface between mud and the water column. They can compose up to 85% of the biomass of bacterivorous flagellates in certain aerobic freshwater, marine, and brackish sediments and are presumably important predators in these ecosystems (Boenigk and Arndt 2002; Dietrich and Arndt 2000). Despite clear microscopical evidence of their presence, phagotrophic euglenids are suspiciously rare in many environmental sequencing datasets from sediments (e.g., Forster et al. 2016). Similarly phototrophic euglenids are poorly represented in freshwater environmental surveys (e.g., Simon et al. 2015). A possible reason for this contradiction might be that euglenids often have divergent and expanded SSU rRNA gene sequences, including the V4 region that is routinely used in environmental surveys. Divergence can result in "universal" primers not binding efficiently. Additionally, many euglenids exhibit such enlarged V4 regions that they cannot be fully sequenced using current high-throughput sequencing technology (Busse and Preisfeld 2002; Karnkowska-Ishikawa et al. 2013). To address these problems (in photoautotrophic euglenids at least), careful investigation of possible DNA barcodes was recently performed, and specific primers were proposed for the V2–V3 and V4 regions of the SSU rDNA (Łukomska-Kowalczyk et al. 2016).

Phagotrophic euglenids are mostly raptorial feeders on other microbial cells, although it is documented that some act as detritivores (e.g., consume cytoplasm and organelles from large ruptured cells), and at least one species, *Dolium sedentarium*, is a sessile "ambush" predator (Larsen and Patterson 1990). As discussed above, it has become common to divide phagotrophic euglenid taxa into "bacterivores" and "eukaryovores," based on morphological correlates of food preference and phylogenetic position. The bacterivores (petalomonads and ploeotiids) are rigid cells with few pellicular strips and tend to be relatively small (most are <25 μm long). The rigidity of the pellicle constrains them by gape limitation; thus they feed on small prey, primarily prokaryotes. The eukaryvores (e.g., "peranemids" and "anisonemids") are mostly slightly-to-highly flexible cells with unfused and more numerous pellicular strips, and they also tend to be larger (most are >20 μm long). As a consequence, they are typically capable of consuming larger prey items in both absolute and relative terms, such as large eukaryotic cells. For example, Chen (1950) documented that *Peranema trichophorum* can engulf whole *Euglena gracilis* cells, which are almost as large as themselves. Many eukaryovorous euglenids specialize in consuming benthic microalgae, especially pennate diatoms (e.g., Lee and Patterson 2000).

Despite the usefulness of this phylogenetic bacterivore/eukaryovore dichotomy, it is not a clear-cut autecological distinction. For example, it is documented that many rigid species that are phylogenetically grouped with "bacterivores" are quite capable of consuming eukaryotic cells; *Ploeotia*/*Serpenomonas costata* is known to eat yeast in culture (Linton and Triemer 1999), and large petalomonads and ploeotiids are not infrequently observed with food vacuoles containing the remains of algae (e.g., Larsen and Patterson 1990; Lax and Simpson 2013; see Fig. 5a). In fact, *Dolium*, a rigid cell with six pellicular strips, ingests whole pennate diatoms (Larsen and Patterson 1990).

Phototrophic lineages mainly inhabit the water column of freshwater environments. Extremely large and vermiform species have reduced flagella and often inhabit the interface between the sediment and water column (Leander and Farmer 2000b; Esson and Leander 2008) (Fig. 1g). Only a few phototrophs inhabit the marine plankton (e.g., the Eutreptiales), however, several species are found in brackish water and estuaries, either in sediments or in the water column. Some species migrate vertically in marine sand, in coordination with tidal and diurnal cycles (e.g., *Euglena rustica*). These species are usually found in high abundance and form easily visible green patches in marine sand during low tides (Brown et al. 2002).

The deep-branching euglenophycaean *Rapaza viridis* has an interesting, if little-studied autecology. The sole known isolate is a mixotroph that houses an apparently functional euglenid plastid (see below), but also feeds on cells of a particular strain of the chloroplastidan alga *Tetraselmis* (Yamaguchi et al. 2012). This feeding is extremely selective (other algae are rejected as prey, including other strains of *Tetraselmis*), but obligate; *R. viridis* could not survive in culture more than ~1 month without prey (Yamaguchi et al. 2012).

Characterization and Recognition

The following is a summary of characteristic morphological features of euglenids. Important systems are covered in more detail in later subsections.

- The best synapomorphy for the group is a pellicle consisting of proteinaceous strips beneath the plasma membrane, associated with microtubules. The pellicle strips are oriented longitudinally in bacterivorous euglenids and usually helically in eukaryovorous, photoautotrophic, and osmotrophic euglenids. The strips are secondarily longitudinal in some rigid photoautotrophs (e.g., *Phacus*) and primary osmotrophs (e.g., *Menoidium*).
- Cells usually have two heterodynamic flagella that originate within an anteriorly-directed flagellar pocket. One flagellum extends anteriorly or anterio-laterally, but historically has been called the "dorsal" flagellum; the other, the "ventral flagellum," bends to run posteriorly. In most photoautotrophs, most osmotrophs, and a

few phagotrophs, only the dorsal flagellum is emergent, while the ventral flagellum is reduced in length and confined to the flagellar pocket (or is absent altogether).

- The flagellar pocket in photoautotrophic species is modified into a "reservoir" (equivalent to the flagellar pocket sensu stricto) and a narrower cylindrical-shaped "canal" leading to the exterior of the cell.
- The flagella are thickened, sometimes extremely so, due to the presence of paraxonemal (paraxial) rods: As in other Euglenozoa, the rod in the dorsal flagellum has a tubular appearence when viewed in transverse section using TEM, while the rod in the ventral flagellum is a 3-dimensional lattice.
- The flagellar apparatus consists of two basal bodies (ventral and dorsal, representing basal bodies 1 and 2, respectively) and three microtubular roots: the dorsal root (R3), the ventral root (R2), and the intermediate root (R1), as in most other Euglenozoa (numbering for basal bodies and roots after Yubuki and Leander 2013).
- Freshwater lineages have contractile vacuoles associated with the reservoir.
- Photoautotrophic species have green plastids (chloroplasts) containing chlorophylls *a* and *b*. The plastids are surrounded by three membranes and have thylakoids in stacks of three. Pyrenoids are absent in the Phacaceae (*Discoplastis*, *Phacus,* and *Lepocinclis*) and *Euglenaformis*.
- Photoautotrophic species respond to the direction and intensity of light using a shading stigma ("eyespot") and a photosensory swelling at the base of the emergent flagellum.
- Cells have a feeding apparatus consisting of a tube or pocket reinforced longitudinally by microtubules. These originate ultimately from the ventral root, where traced (e.g., Surek and Melkonian 1986; Willey and Wibel 1987). The feeding apparatus in many phagotrophs is further elaborated by four or five electron-dense "vanes" and reinforced by two robust rods partly composed of microtubules. The feeding apparatus in photoautotrophic and osmotrophic species is highly reduced.
- Diverse and dynamic modes of motility are seen, including metaboly, substrate-mediated gliding, and swimming.
- Mitochondria have discoidal (paddle-shaped) cristae (as in other euglenozoans).
- The nucleus has permanently condensed chromosomes and a conspicuous nucleolus.
- The main storage polymer of most euglenids (perhaps all) is paramylon, a distinctive beta-1,3-glucan. Cytoplasmic paramylon granules may be small or extremely large (especially in some photoautotrophic species).
- Extrusomes (ejectile organelles) are common, almost always in the form of "typical" tubular extrusomes, mucocysts, or muciferous bodies.
- The Golgi bodies are usually elaborate, with a large number of cisternae (see Fig. 11a).
- Cytokinesis involves a longitudinal cleavage furrow.

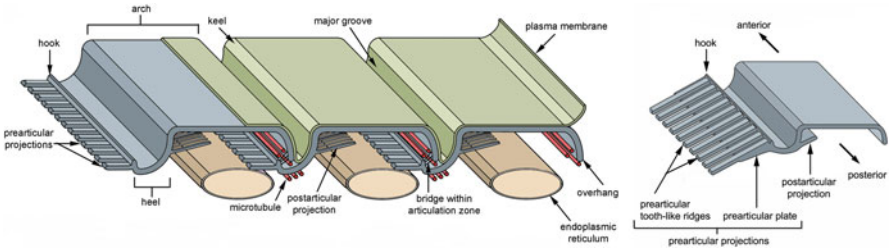

Fig. 6 Labeled illustrations showing the general organization of pellicle ultrastructure in flexible photoautotrophic euglenids. (*Left*) The configuration of three articulating strips and associated microtubules positioned beneath the plasma membrane and subtended by tubular cisternae of endoplasmic reticulum. (*Right*) A pellicle strip with robust toothlike prearticular projections and robust postarticular projections (e.g., some *Lepocinclis*)

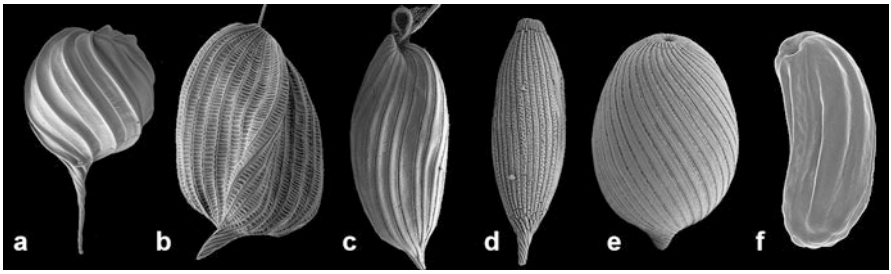

Fig. 7 Scanning electron micrographs showing the diversity of pellicle structure in rigid photoautotrophic euglenids (**a-e**) and primary osmotrophs (**f**). (**a**) *Monomorphina*. (**b**) *Phacus*. (**c**) *Phacus*. (**d**) *Lepocinclis*. (**e**) *Phacus*. (**f**) *Rhabdomonas*. All cells between 20 and 60 μm

Pellicle and Metaboly

The best synapomorphy for the Euglenida is a novel cytoskeleton comprised of parallel proteinaceous strips, underlain by microtubules, that run along the length of the cell (Leander 2004; Leander and Farmer 2000a, 2001a; Leander et al. 2001a, b) (Figs. 1, 5c, d, 6, 7). These elements are positioned immediately beneath the plasma membrane and are closely associated with cisternae of the endoplasmic reticulum. Collectively, this ultrastructural system is referred to as the euglenid pellicle.

The number of individual strips varies from 4 or 5 in some petalomonads to 120 in some very large euglenophytes (Esson and Leander 2008). Bacterivores (petalomonads and ploeotiids) have 12 or fewer longitudinal strips (often 8 or 10) that are fused to form a rigid pellicle. Eukaryovores (e.g., peranemids and anisonemids) have 20 to about 60 strips that are usually helically arranged and slide to allow metaboly; photoautotrophic (and secondarily osmotrophic) euglenids have 16–120 helically arranged strips, and most are capable of metaboly (Leander et al. 2007). However, some photoautotrophs (and secondary osmotrophs) have

secondarily become rigid and have longitudinally arranged strips. The earliest diverging primary osmotrophs (e.g., *Distigma*) have about 20 helically arranged strips and are metabolic; however, some lineages became rigid and now have fewer strips (e.g., 14) that run more longitudinally and that are often fused into a continuous proteinacous layer around the cell (e.g., *Menoidium* and *Rhabdomonas*; Leander et al. 2001b; Fig. 7f).

The strips are composed mostly of a family of proteins called "articulins" (Marrs and Bouck 1992). In general, the main frame of each pellicle strip is "S-shaped" in cross section and consists of an arch region and a heel region that defines a groove (Leander et al. 2007; Leander and Farmer 2001a) (Fig. 6). Adjacent strips articulate along their lateral margins; the strip arch overlaps with the heel of a neighboring strip, giving the surface of euglenid cells a striated appearance (Fig. 6).

The articulation zones between adjacent strips allow the dynamic changes in cell shape called "metaboly," "euglenoid motion," or "euglenoid movement" (Fig. 4a, b). They also facilitate cytoskeletal replication prior to cell division (i.e., cytokinesis). Metaboly is observed in most cells that have a large number of pellicle strips (16 or more). As well as serving a secondary locomotory role, metaboly is thought to facilitate the ingestion of large food particles, such as other eukaryotic cells, in eukaryovorous phagotrophs (Leander 2004; Leander et al. 2001, 2007; Yamaguchi et al. 2012).

In photoautotrophic and secondary osmotrophic lineages, the frame of each strip contains periodic arrays of projections that branch laterally from the heel (Leander et al. 2001b, 2007; Leander and Farmer 2001a). The projections of one strip articulate with the projections of an adjacent strip beneath the arches (Fig. 6). The projections that branch beneath the arch of an adjacent strip, so-called prearticular projections, and those that branch beneath the arch of the same strip, so-called postarticular projections, can vary considerably in robustness: some lineages possess delicate threadlike projections; some species possess more robust toothlike projections; and some species possess projections that form robust continuous plates (Fig. 6). Euglenid cells with more delicate strips tend to demonstrate more dramatic degrees of metaboly (Fig. 5); euglenid cells with robust strips tend to be rigid, or nearly so (Angeler et al. 1999; Leander 2004; Leander et al. 2001b, 2007; Leander and Farmer 2001a) (Fig. 7). Phagotrophic and primary osmotrophic euglenids lack strip projections altogether.

The euglenid pellicle is multigenerational; each strip or cohort of strips represents a different cytokinetic event in the history of any particular cell (Esson and Leander 2006, 2008; Leander et al. 2007; Yubuki and Leander 2012). Prior to cytokinesis, the number of pellicle strips around the cell periphery doubles. Each daughter cell (usually) inherits the same number of pellicle strips as the parent cell in a semiconservative manner. During strip doubling, new strips emerge within the articulation zones between mature strips. In the photoautotrophic euglenids, the newly produced pellicle strips do not extend to the posterior tip of the cell and consequently form whorled surface patterns of strip termination (Esson and Leander 2006, 2008; Leander and Farmer 2000a; Leander et al. 2001b). Strips that terminate before reaching the posterior tip of the cell occupy a relative position along the length of

the cell called a "whorl." The number of posterior whorls varies between different species, ranging from one to four. In some species, the whorls themselves can be dissociated into one or more subwhorls (Esson and Leander 2008; Leander and Farmer 2000a; Leander et al. 2001b; Yubuki and Leander 2012). Comparative analyses of the strip termination patterns in several different species have provided important insights into the developmental processes associated with the control and evolutionary diversification of the euglenid pellicle (Esson and Leander 2006, 2008; Leander and Farmer 2000a; Leander et al. 2001b; Yubuki and Leander 2012).

Variation in the number of strips within many species (though usually with a strong mode) indicates that strips are not necessarily distributed evenly during cell division. For instance, a parent cell with 40 strips doubles the number of strips to 80 prior to cell division. In most cases, the two daughter cells will each receive 40 strips and recover the number that was present in the parent cell. In other cases, the daughter cells might receive some other proportion, such as 38 and 42 or 36 and 44. It is also possible that strip duplication is not always faithful; for instance, a parent cell with 40 strips might only produce 39 new strips, in which case the daughter cells will receive 39 and 40 strips, respectively.

Permanent strip duplication events refer to cases where a cell duplicates its strips but fails to divide. Permanent strip halving events refer to cases where a cell divides without duplicating its strips. The distribution of strip numbers found in euglenids suggests that these events happened several times during the evolution of the group (see "Evolutionary History").

Flagella and Locomotion

Most euglenids possess two heterodynamic flagella that emerge from the flagellar pocket. A few lineages have more than two (e.g., some Eutreptiales have four flagella; McLachlan et al. 1994), and some have highly reduced flagella, giving the appearance of one or none when viewed with the light microscope (Figs. 2, 3, 4, and 5). Euglenids possess paraxonemal (paraxial) rods within the flagella that run alongside the 9 + 2 microtubular axoneme (Fig. 8a, b). The paraxonemal rods make euglenid flagella conspicuously thick when viewed under the light microscope (e.g., Fig. 5b); the thickest flagellum can approach or exceed 1 μm width in many larger cells, especially in large phagotrophic euglenids (e.g., Larsen and Patterson 1990). The paraxonemal rod in each flagellum has a different structure: the rod in the ventral flagellum has a latticelike structure, and the rod in the dorsal flagellum has, at core, a whorled structure that appears tubular in transverse sections (Fig. 8a, b). A major component of both structures are the paraxonemal rod proteins PAR1 and PAR2, whose genes arose through duplication prior to the divergence of euglenids and kinetoplastids (Talke and Preisfeld 2002).

Euglenid flagella characteristically have very thick investments of fine hairs, which generally emerge in horizontal (or shallowly helical) rows of tufts associated with the flagellar axoneme and/or paraxonemal rod (Bouck et al. 1978; Dawson and Walne 1991; Hilenski and Walne 1985; Mignot 1965, 1966) (Fig. 8). These hairs

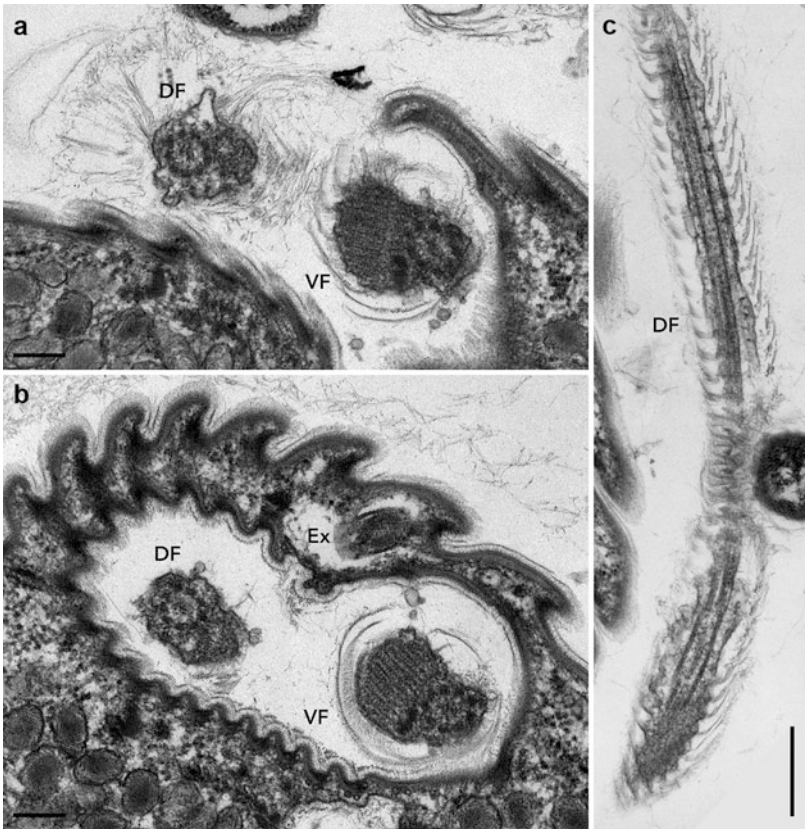

Fig. 8 Transmission electron micrographs of flagella. (**a**, **b**) Near-transverse sections of the proximal portions of the dorsal/anterior flagellum (*DF*) and ventral/posterior flagellum (*VF*), showing the paraxonemal rods (tubular in *DF*, latticed in *VF*) and the flagellar hairs. Note also the oblique section of an undischarged tubular extrusome (*Ex*); *Neometanema parovale*. (**c**) Longitudinal view of dorsal/anterior flagellum (distal end to top of page), showing flagellar hairs; *N. parovale*. Scale bars: **a**, 200 nm; **b**, 200 nm; **c**, 500 nm. Credit: **a**: courtesy of Won Je Lee, **b**, **c**: image by Won Je Lee, slightly modified from Lee and Simpson 2014a, reproduced with permission

typically lie oriented with their distal ends pointing toward the distal end of the flagellum (Fig. 8c). In addition, emergent flagella may have a single longitudinal row of bundles of larger hairs, which can be several micrometers long; these hairs are best studied in the sole emergent flagellum of Euglenean photoautotrophs such as *Euglena* (e.g., Leedale 1967; Bouck et al. 1978; Melkonian et al. 1982). The phototroph *Eutreptia*, which has two emergent flagella, has these rows of bundles of long hairs on both flagella (Dawson and Walne 1991), as, apparently, does the biflagellated primary osmotroph *Distigma proteus* (Leedale 1967), while the

peranemid eukaryotroph *Peranema* has them on the anterior flagellum only (Hilenski and Walne 1985). Petalomonads have sparser arrangements of flagellar hairs than other euglenids and/or finer hairs (though data is limited; Cann and Pennick 1986; Lee and Simpson 2014b), while flagellar hairs have not been reported at all in symbiontids (Yubuki et al. 2009).

In phagotrophic lineages, the flagella are heterodynamic, with one flagellum (i.e., the dorsal/anterior flagellum) held ahead of the cell, while the other flagellum (i.e., the ventral, recurrent, or posterior flagellum) bends backward and extends posteriorly from the cell, often within a ventral groove or sulcus (Figs. 2b–e, 5b). The hairs and paraxonemal rods of these flagella facilitate gliding motility across substrates (Saito et al. 2003). In petalomonads and peranemids, the dorsal/anterior flagellum is involved in gliding. During this gliding most of the anterior flagellum is held stiffly against the substrate, but the tip is in constant motion and functions as a sensory apparatus (Figs. 2a, c and 5a). In these cells the posterior/ventral flagellum is shorter and thinner than the anterior flagellum; in some cases it lacks a paraxonemal rod, does not emerge from the reservoir, or is completely absent (e.g., Cann and Pennick 1986; Lee and Simpson 2014b). In ploeotiids and anisonemids, only the posterior flagellum is involved in gliding, and the whole anterior flagellum sweeps from side to side; in these cells the anterior flagellum is almost always thinner and usually shorter than the posterior flagellum (Figs. 2b, d, e and 5b). Some phagotrophic euglenids also use the anterior flagellum like a hook to shovel prey cells into the feeding apparatus (Breglia et al. 2013).

Most osmotrophic and photoautotrophic euglenids primarily move using swimming motility (Leander 2004). They usually possess an emergent dorsal flagellum that extends from the canal and is highly dynamic, while the reduced ventral flagellum does not emerge from the canal and is inactive. The emergent flagellum beats in an organized and consistent pattern that takes the form of a "figure-eight" or a lasso. This beat pattern pulls the euglenid cell through the water column (Leander 2004). By contrast, eutreptialean photoautotrophs possess two emergent flagella that both beat during swimming (some primary osmotrophs also have two emergent flagella).

Although phagotrophic euglenids are usually poor swimmers, the symbiontid *Postgaardi* swims with a spiralling motion (Simpson et al. 1997), while the anisonemid-like *Neometanema* normally moves by rapidly "skidding" (i.e., swimming while maintaining physical contact with the substrate), powered by beating of the anterior/dorsal flagellum (Lee and Simpson 2014a; see Larsen and Patterson 1990, 2000). Conversely, gliding is seen in some photoautotrophic euglenids (Euglenophyceae), but gliding cells typically hold the cell body against the substrate, not the flagellum (which is often greatly shortened).

Flagellar Apparatus

The flagellar axonemes are anchored by basal bodies that are situated at the base of the flagellar pocket: the ventral flagellum originates from the ventral basal body, and

the dorsal flagellum originates from the dorsal basal body. A striated fiber connects both basal bodies. Three microtubular roots extend from the basal bodies: the dorsal root extends from the lateral side of the dorsal basal body, the ventral root extends from the lateral side of the ventral basal body, and an intermediate root extends from the medial side of the ventral basal body and thus lies initially between the basal bodies (Yubuki and Leander 2012). In the universal numbering system for the eukaryotic flagellar apparatus, the ventral basal body represents basal body 1, and the dorsal basal body is basal body 2 (i.e., the dorsal basal body is predicted to transform into the ventral basal body during cell division; Moestrup 2000). Meanwhile the dorsal root represents R3, the ventral root R2, and the intermediate root R1 (Yubuki and Leander 2013, noting that the identification of the roots in Moestrup 2000 was inaccurate). This flagellar apparatus constitutes the organizing center from which several other cytoskeletal elements arise – such as the microtubules associated with the cell surface (or pellicle), which originate in association with the dorsal root, and the central microtubules of the feeding apparatus, which, when traced, prove to originate from the ventral root (Belhadri et al. 1992; Belhadri and Brugerolle 1992; Farmer and Triemer 1988; Hilenski and Walne 1985; Leander 2004; Shin et al. 2001, 2002; Simpson 1997; Solomon et al. 1987; Surek and Melkonian 1986; Willey and Wibel 1985; Yubuki and Leander 2012).

Feeding Apparatus

Phagotrophic euglenids have feeding apparatuses that range from relatively simple microtubule-reinforced pockets or tubes (MtR pockets) to highly complex systems of rods and vanes (Leander et al. 2007; Triemer and Farmer 1991a, b). One major group of bacterivorous euglenids, petalomonads, have MtR pockets, with some of the reinforcing microtubules likely derived from the ventral root of the flagellar apparatus via the MtR structure (although this connection has not been proved yet in any species; Lee and Simpson 2014b). Ploeotiids (e.g., *Ploeotia* and *Entosiphon)* and eukaryovorus euglenids possess feeding apparatuses that are much more complex. These include two robust rods composed of ordered arrays of microtubules embedded within an amorphous matrix (Triemer and Farmer 1991a; Linton and Triemer 1999). In *Entosiphon*, one of the rods bifurcates near the anterior end of the cell and gives the impression of three feeding rods in transverse view (Triemer and Fritz 1987; Leander et al. 2007). The feeding rods in ploeotiids typically extend the entire length of the cell, as do those of some eukaryovorous euglenids, like *Dinema*. By contrast, the feeding rods are confined to the anterior third of the cell in eukaryovorous euglenids that are capable of extreme metaboly, such as *Peranema*, *Urceolus*, and *Jenningsia*. A smaller "accessory rod" is sometimes positioned along the lateral margin of each feeding rod in both bacterivorous and eukaryovorous euglenids (Nisbet 1974; Breglia et al. 2013). Between the two feeding rods are four to five plicate or lamellar vanes, depending on the species.

The action of the feeding apparatus has been studied in some detail in *Entosiphon*; when a prey cell is about to be ingested, the rods of the feeding

apparatus protrude from the anterior end of the cell, and the vanes twist open like the blades of a pinwheel (Triemer and Fritz 1987). When the feeding apparatus retracts, the vanes twist back into their original position, gripping and internalizing the prey in the process. Although most phagotrophic euglenids ingest their prey whole, some euglenids (e.g., *Peranema*) can also feed by myzocytosis (Triemer 1997). This mode of feeding is vampire-like, in that the feeding rods pierce the prey cell, allowing the cell contents to be sucked into a phagosomal vacuole within the euglenid. The feeding apparatuses present in photoautotrophic and osmotrophic euglenids are highly reduced, corresponding to the switch from predominantly phagotrophic modes of nutrition to photoautotrophy and surface absorption, respectively (Leander et al. 2001a; Shin et al. 2002; Surek and Melkonian 1986; Willey and Wibel 1985).

Plastids (Chloroplasts)

Photoautotrophic euglenids (Euglenophyceae) evolved once from eukaryovorous euglenid ancestors that established a secondary endosymbiosis with green algal prey cells (Gibbs 1978; Leander 2004). These algae were related to the prasinophyte *Pyramimonas* (Turmel et al. 2009; Hrdá et al. 2012). The chloroplasts of euglenophytes are themselves green, are surrounded by three membranes, and possess thylakoids in stacks of three (Fig. 9). Most euglenid plastids contain a conspicuous pyrenoid (a region containing RuBisCO protein), although the small disc-shaped plastids of *Discoplastis*, *Lepocinclis*, *Phacus*, and *Euglenaformis* lack pyrenoids altogether (Figs. 3, 9a, c). Carbohydrate storage in the form of paramylon granules is also often associated with the pyrenoids, but is also distributed throughout the cytoplasm (Fig. 9a). Plastids with conspicuous paramylon caps on both sides

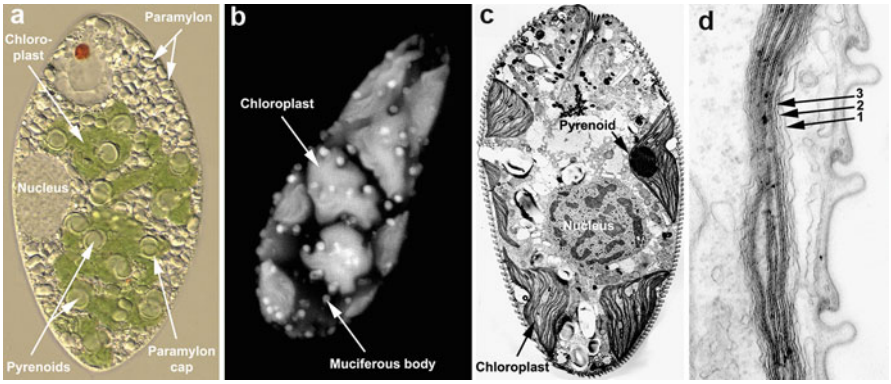

Fig. 9 Light and electron micrographs showing the general ultrastructure of chloroplasts (i.e., plastids) in *Euglena*. (**a**) Light micrograph showing paramylon, pyrenoids, chloroplasts, and the nucleus. (**b**) Confocal micrograph showing autofluorescence and the spatial distribution of chloroplasts and muciferous bodies. (**c**) Low magnification transmission electron micrograph showing the nucleus, pyrenoid, and chloroplasts. (**d**) High magnification transmission electron micrograph showing three membranes surrounding the chloroplast

of the pyrenoid are referred to as being "diplopyrenoidal" and on the one side as "haplopyrenoidal" (Brown et al. 2003; Monfils et al. 2011).

The number and morphology of euglenid plastids are very diverse (e.g., shield-shaped, disc-shaped, and star-shaped) and reflect evolutionary relationships, different stages in cell development and environmental conditions. Some photoautotrophic euglenids are known to switch nutritional modes and survive in the dark, whereby the plastids become "bleached" over time. Several different groups of photoautotrophic euglenids include species that have independently lost photosynthesis (e.g., *Euglena quartana*, *Euglena longa*, and *Lepocinclis cyclidiopsis;* Triemer and Farmer 2007; Bennett and Triemer 2014). Plastids with reduced genomes still exist in at least some of these secondary osmotrophs (e.g., *Euglena longa*, Hachtel 1998; see below).

The plastid genome of the model species *Euglena gracilis* was sequenced more than 20 years ago (Hallick et al. 1993). The genome is surprisingly large (~143 kb) but not because of the gene repertoire, which is relatively small (97 predicted genes), but due to extensive noncoding DNA sequence, including an enormous number of introns (~150). For comparison, the representative prasinophyte *Pyramimonas parkeae* has only one intron (Turmel et al. 2009). The next sequenced plastid genome was that of the secondarily non-photosynthetic species *Euglena longa* (Gockel and Hachtel 2000). This is about half the size of the *E. gracilis* plastid genome due to the loss of all genes encoding photosynthesis-related proteins, except for the *rbc*L gene encoding the large subunit of RuBisCO. The *E. longa* plastid genome is required for cell growth and viability (Gockel et al. 1994; Gockel and Hachtel 2000; Hadariová et al. 2016).

Fifteen more plastid genomes have been sequenced since 2010, covering most of the genera of Euglenophyceae (Bennett et al. 2012, 2014; Hrdá et al. 2012; Wiegert et al. 2012, 2013; Pombert et al. 2012; Bennett and Triemer 2015; Dabbagh and Preisfeld 2017; Kasiborski et al. 2016). Comparative studies revealed that they have very similar complements of protein-coding genes; however, there have been major changes in gene arrangement. The most striking differences are the numbers of introns. Two early-diverging Eutreptiales have few introns (7–23; Hrdá et al. 2012; Pombert et al. 2012; Wiegert et al. 2012), the only sequenced representative of Phacaceae (*Phacus orbicularis*) has 67, and representatives of Euglenaceae have 53–150. The pattern of intron proliferation observed in the Euglenophyceae corresponds with the number of identified maturases (Eutreptiales, 1; Phacaceae, 2; Euglenaceae, 3), which are possibly involved in intron mobility (Kasiborski et al. 2016).

Photoreception

Euglenophytes (and most secondary osmotrophs) can respond to the intensity and direction of light and orient themselves in the water column accordingly (Kuznicki et al. 1990). Photoreception is accomplished by an apparatus consisting of a photosensory swelling at the base of the emergent dorsal flagellum and a closely

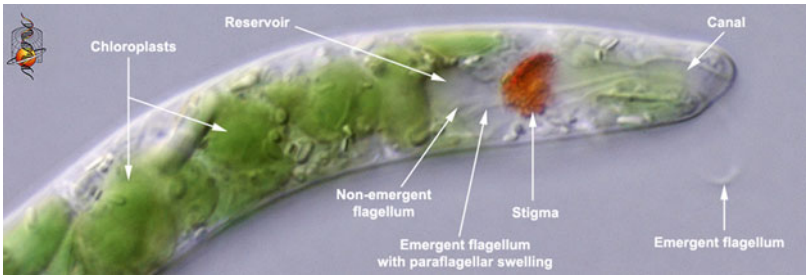

Fig. 10 Labeled light micrograph showing the photoreceptor apparatus in a photoautotrophic euglenid, consisting of an expanded reservoir, a canal, a paraflagellar swelling near the base of the emergent flagellum, and a shading stigma (i.e., eyespot). Credit: Linda Amaral Zettler and David Patterson

associated shading structure composed of orange or red carotenoids, called the "stigma" or "eyespot" (Fig. 10). The stigma of euglenids is positioned near the base of the flagellar pocket/reservoir. Interestingly, it lies in the cytoplasm, instead of being embedded within the plastid as in most other photosensory algae (e.g., within the green algae, dinoflagellates, and chrysophyceans). The stigma shades one side of the flagellar swelling; as the cell rotates through the water, the swelling can detect the direction of the most intense light source. The behavior of the swimming flagellum will then respond in a way that allows the cell to maintain a position in the water column that is optimal for photosynthesis.

There is evidence of a photosensory swelling and stigma in the phagotrophic eukaryovorous euglenid *Urceolus* (Leander et al. 2001a). This putative photoreception apparatus in *Urceolus* might enable it to maintain a position in light regimes favoured by its algal prey. There is no evidence that *Urceolus* has or had plastids, but it was inferred to be a close relative of Euglenophyceae in analyses of morphological data (Leander et al. 2001a), and thus it is possible that its putative photoreception apparatus is homologous to that of photoautotrophic euglenids.

Mitochondria

The mitochondria are distinctive in having stalked, paddle-shaped cristae, usually referred to as "discoidal" cristae (Fig. 11b). They are homologous to the discoidal cristae of ▶ Kinetoplastea and probably those of ▶ Heterolobosea. The mitochondrion of *Euglena gracilis* forms a large reticulated network (Pellegrini 1980). This conformation may be widespread among euglenids, although numerous separate elongated mitochondria are reported in some taxa (e.g., *Peranema*; Roy et al. 2007; Leedale 1967). The anaerobic symbiontids retain conspicuous mitochondria-related organelles; these have a homogeneous matrix, and profiles through them generally lack cristae altogether (Simpson et al. 1997; Yubuki et al. 2009; Fig. 11g). Nonetheless, a few flattened crista-like structures have been seen in *Bihospites bacati* (Breglia et al. 2010).

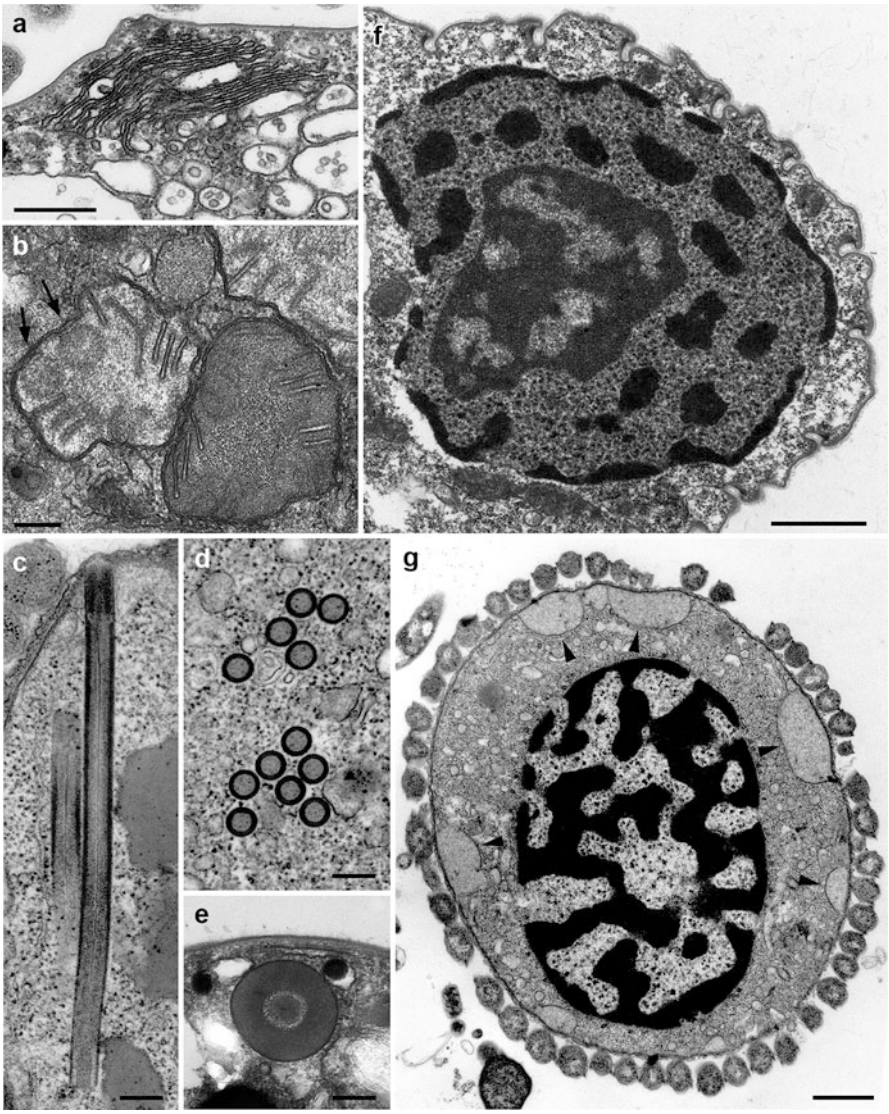

Fig. 11 Transmission electron micrographs of major organelles. (**a**) Golgi apparatus; *Notosolenus urceolatus*. (**b**) Profiles of mitochondria with rigid, discoidal mitochondrial cristae. Note two cristae lying parallel to the plane of section, thus showing discoidal profile (*arrows*); *N. urceolatus.* (**c**) Two tubular extrusomes, one viewed in longitudinal section; *Postgaardi mariagerensis*. (**d**) Transverse sections of a dozen tubular extrusomes; *P. mariagerensis*. (**e**) Globular presumptive extrusome of a petalomonad, shown at the same scale as the tubular extrusosomes in d and e; *N. urceolatus.* (**f**) Nucleus, showing extensive permanently condensed chromatin; *Neometanema parovale*. (**g**) Transverse section through a symbiontid (*P. mariagerensis*). Note the large nucleus with extensive condensed chromatin, the mitochondrion-related organelles that lack cristae (*arrowheads*), and the epibiotic bacteria (>40 cut transversally in this section). Scale bars: **a**, 500 nm; **b**–**e**, 200 nm; **f**, **g**, 1 μm. Credit: **a**, **b**, **e**: courtesy of Won Je Lee. **c**, **d**, **e**; modified from Simpson et al. 1997, reproduced with permission. **f**: image by Won Je Lee, modified from Lee and Simpson 2014a, reproduced with permission

The mitochondrial genome of euglenids is not well understood. For example, it is only recently that the mitochondrial genome of *E. gracilis* was fully sequenced (Dobakova et al. 2015). This consists of a heterogeneous population of DNA molecules roughly 1–10 kb long (Spencer and Gray 2010; Dobakova et al. 2015) and houses just seven protein-coding genes, while the mitochondrial ribosomal RNAs are encoded as multiple fragments (Spencer and Gray 2010; Dobakova et al. 2015). No evidence of kinetoplastid-type RNA editing or RNA-editing machinery was found in *Euglena* (Dobakova et al. 2015). Nonetheless, transmission electron micrographs of the petalomonads *Petalomonas cantuscygni* and *Notosolenus urceolatus* show fibrous compacted inclusions within the mitochondria that are similar in appearance to the kDNA inclusions present in kinetoplastids (Leander et al. 2001a; Lee and Simpson 2014b). This fibrous nature was not seen in a subsequent study of *P. cantuscygni* (perhaps due to fixation differences); however, some small circular DNA molecules were observed in electron micrographs of mtDNA preparations, along with many larger linear molecules (Roy et al. 2007). Therefore, it is currently unclear whether the mitochondrial genomes of some deep-branching euglenids might contain "minicircles" (encoding "guide rRNA" genes) like those found in ▶ Kinetoplastea.

Extrusomes

Some typical bacterivorous euglenids display thick-walled "tubular extrusomes," often with cruciate central filaments, that are similar to those present in a few diplonemids and free-living kinetoplastids (Brugerolle 1985; Schuster et al. 1968). These extrusomes were studied in detail in the ploeotiid *Entosiphon sulcatum* (Mignot 1966; Mignot and Hovasse 1973). They are also present in all described symbiontids; *Bihospites,* Postgaardi, and *Calkinsia* (Breglia et al. 2010; Simpson 1997; Yubuki et al. 2009; Fig. 11c, d). Where well documented, these extrusomes are highly elongated in the undischarged state (>2 μm; see Fig. 11c) and expand in length during discharge into an open lattice structure (Breglia et al. 2010; Mignot 1966; Simpson et al. 1997). Their function has not been studied directly, but presumably they operate in predation or in protection from predation. A homologous but modified form is seen in some eukaryovorous euglenids. These are usually shorter, have a dense central region when in the undischarged state (Lee and Simpson 2014a; see Fig. 8b), and exhibit less length expansion upon discharge, where known (Hilenski and Walne 1983). They have been found in *Teloprocta*/*Heteronema scaphurum*, in *Neometanema parovale*, and in *Peranema trichophorum*, where they are called mucocysts (Breglia et al. 2013; Hilenski and Walne 1983; Lee and Simpson 2014a; Mignot 1966).

Mucilaginous bodies called "mucocysts" are present in two subclades within *Euglena* and might be homologous to the extrusomes of phagotrophic euglenids. Mucocysts of photoautotrophic euglenids sit beneath pores positioned in rows within the articulation zones between the pellicle strips (Leander et al. 2001b; Esson and Leander 2008). The number of strips between the rows of mucosyst pores is variable,

which makes them suitable diagnostic characters at the species level (Leander et al. 2001b; Kosmala et al. 2009).

Tubular extrusomes have not been observed in petalomonads, instead various globular membrane-bounded bodies have been imaged or illustrated in ultrastructural reports (see Lee and Simpson 2014b). The only detailed study is in *Notosolenus urceolatus*, where the bodies are pill-shaped or rounded, about 0.5 μm in diameter and have a dense axial core (Fig. 11e). Typically, several are present in the anterior portion of the cell. It was proposed that these organelles represent a class of extrusome that is not homologous to the tubular extrusomes of other euglenozoans, but discharge has not been observed (Lee and Simpson 2014b).

The symbiontid *Bihospites* possesses ejectile ectosymbionts, known as epixenosomes, in addition to tubular extrusomes (Breglia et al. 2010). These epixenosomes are verrucomicrobial bacteria and are closely related to similar defensive symbionts reported earlier in certain ciliates (Petroni et al. 2000). In *Bihospites* the epixenosomes lie in rows between the rod-shaped epibiotic bacteria (see "Habitats and Ecology") and discharge by rapidly unwinding a central filament structure (Breglia et al. 2010).

Extracellular Structures

Conspicuous extracellular structures enclosing the main cell body are rare in euglenids. A strikingly thickened glycocalyx is present in several taxa, including *Neometanema* and several osmotrophs (Lee and Simpson 2014a). Most spectacularly, a group of photoautotrophic euglenids comprising *Trachelomonas* and *Strombomonas* produce a globular organic lorica that may be smooth or decorated with spines. The lorica has a single opening for the flagellum, and the cells locomote by swimming. The primary component of the lorica is mucus (Hilenski and Walne 1983; Mignot 1966), and during its development, the lorica slowly becomes thicker and ornamented. Iron and manganese are the main nutrients necessary for the lorica formation (e.g., Pringsheim 1953; Singh 1956). Differences in lorica formation between *Trachelomonas* and *Strombomonas* (Brosnan et al. 2005) are concordant with molecular phylogenetic data showing two distinct genera of loricate euglenids (e.g., Brosnan et al. 2005; Ciugulea et al. 2008). The sister group to the loricates is *Colacium*, which also has the ability to produce copious amounts of mucus, but instead forms mucilaginous stalks and dendroid colonies (Leedale 1967).

The Nucleus, Reproduction, and Cytokinesis

Euglenids cells usually have a single, large nucleus during interphase. The nucleus typically has a conspicuous subcentral nucleolus and large amounts of permanently condensed chromatin. This chromatin may give the nuclear material a lumpy appearance when viewed by light microscopy (Fig. 5e) and appears electron dense in transmission electron micrographs (Fig. 11f, g; see also Fig. 9c).

The nuclear genome organization of euglenids exhibits some bizarre features; rRNA genes are extrachromosomal, circular molecules, with thousands of copies per cell (Cook and Roxby 1985; Ravel-Chapuis 1988). Moreover, three types of introns are present in euglenid genomes; in addition to conventional spliceosomal introns, both noncanonical introns (for which a splicing mechanism is unknown) and so-called intermediate introns have been documented (Canaday et al. 2001; Milanowski et al. 2014). All euglenid species studied so far add a noncoding capped spliced-leader (SL) RNA to nucleus-encoded mRNAs via spliceosome-dependent *trans*-splicing (Frantz et al. 2000; Kuo et al. 2013), a process also reported in the other groups of Euglenozoa: kinetoplastids (Walder et al. 1986) and diplonemids (Sturm et al. 2001, Gawryluk et al. 2016). Full sequencing of the nuclear genome of *Euglena gracilis* is in progress but has been hindered by the genome size (approximately 2 Gb) and the high percentage of repetitive regions (O'Neill et al. 2015; see also EuglenaDB https://sites.dundee.ac.uk/euglenadb/). Furthermore, the nuclear DNA contains the unusual base "J," which makes up approximately 0.2% of all the bases (Dooijes et al. 2000) and hampers sequencing.

Asexual reproduction in euglenids occurs by mitosis followed by cytokinesis. The basal bodies and associated flagellar root system replicate first, followed by the feeding apparatus (if present) and then the pellicle. In many species the probasal bodies form early in interphase, such that they are present alongside the flagellated basal bodies in most cells within a population (e.g., *Entosiphon*; Solomon et al. 1987; *Peranema*; Hilenski and Walne 1985).

The mechanics of mitosis in euglenids was summarized at the level of light microscopy by Leedale (1967) and at the ultrastructural level by Triemer and Farmer (1991a). As with many protists, the nuclear envelope persists throughout mitosis, and the nucleolus does not break down but elongates and divides (in a few species, there are multiple nucleoli that divide separately; Leedale 1967; Zakryś 1986). The chromosomes are usually reported as permanently condensed (see above) but attached to the nuclear envelope prior to mitosis; they detach to assemble loosely at the division plane during metaphase (though spindle microtubules connect to the chromosomes before detachment in *Anisonema*; Triemer 1985). The relative timing of this assembly on one hand, and the process of chromosome replication through the separation of sister chromatids on the other, reportedly varies from species to species (Leedale 1967). The mitotic spindle system is intranuclear, with microtubules originating against the nuclear envelope. Almost all accounts indicate the presence of multiple subspindles originating from different foci around each pole of the dividing nucleus (Triemer and Farmer 1991a). Separation of the chromosomes is initially due to elongation of the nucleus rather than shortening of the spindle microtubules, which only happens near the end of anaphase; Triemer and Farmer (1991a) refer to this pattern as a "reversed anaphase A/B sequence." There is normally an association of the poles of the dividing nucleus and the replicated flagellar apparatus, but not always; in *Anisonema* the flagellar apparatus completes replication and begins segregation only after mitosis is well advanced (Triemer 1985).

After the nucleus and cytoskeleon have duplicated, a cleavage furrow forms at the base of the flagellar pocket near the basal bodies and migrates toward the anterior opening, forming two flagellar pockets within the cell. The cleavage furrow subsequently migrates posteriorly down the longitudinal axis of the cell; the posterior tip of the cell is the last part to become cleaved. The cleavage furrow forms between a (mature) parent strip and a newly generated (nascent) strip on two sides of the cell (Esson and Leander 2006). Each daughter cell (usually) contains the same number of pellicle strips as the parent cell (Yubuki and Leander 2012); however, an unequal distribution of strips can also occur during cytokinesis (see above).

Sexuality is almost unknown in euglenids, but Mignot (1962) gave a light microscopy account of a small petalomonad, *Scytomonas pusilla*, that included normal-looking cells behaving as isogametes and undergoing syngamy (i.e., pairs of cells fused, and then their nuclei fused).

Taxonomy

About 1500 species of euglenids are recognized, with the majority being photoautotrophs. The taxonomy of the photoautotrophs was extensively scrutinized over the last 20 years based on molecular and morphological data, and the current assignment of species to genera largely follows phylogeny. Notably, a large number of species that were formerly placed within *Euglena*, but are not closely related, have been given new generic assignments. Conversely, certain genera of secondary osmotrophs have been suppressed on phylogenetic grounds (e.g., *Hyalophacus* – Marin et al. 2003; *Cyclidiopsis* – Bennett and Triemer 2014). The traditional genus *Astasia* turned out to include species of both primary and secondary osmotrophs, but now includes only primary osmotrophs.

Photoautotrophic euglenids, or Euglenophyceae, are a monophyletic group (Marin et al. 2003; Karnkowska et al. 2015; Kim et al. 2015; Cavalier-Smith 2016) comprising the basal monotypic genus *Rapaza* (Yamaguchi et al. 2012), Eutreptiales and Euglenales (here and elsewhere we use the dominant botanical-tradition naming for higher taxa of Euglenophyceae; see Cavalier-Smith 2016 for a recent but particular "zoological" taxonomy for photoautotrophic euglenids). Eutreptiales comprise two predominantly marine genera – *Eutreptia* and *Eutreptiella* – while a third genus, *Tetraeutreptia,* was subsumed within *Eutreptiella* (Marin et al. 2003; Cavalier-Smith et al. 2016). Due to the limited number of taxa and genes used for phylogenetic reconstructions, the genus-level taxonomy is not well resolved within the Eutreptiales and *Eutreptiella* might be paraphyletic (Marin et al. 2003; Cavalier-Smith 2016). The Euglenales is much more diverse and is subdivided into two clades: Phacaceae (with three genera) and Euglenaceae (with eight genera).

The Phacaceae contains *Discoplastis*, *Lepocinclis*, and *Phacus* (Kim et al. 2010; Linton et al. 2010; Karnkowska et al. 2015; Kim et al. 2015). *Discoplastis* was erected to accomodate two species previously classified in the genus *Euglena* (Triemer et al. 2006). *Phacus* and *Lepocinclis* are closely related (Kim et al. 2010;

Linton et al. 2010; Karnkowska et al. 2015; Kim et al. 2015). Both genera have been intensively studied over the last 15 years, with several species transferred from other genera to either *Phacus* (Linton et al. 2010) or *Lepocinclis* (Marin et al. 2003; Kosmala et al. 2005; Bennett and Triemer 2012; Bennett and Triemer 2014). Those taxonomic changes resulted in the loss of morphological characters distinguishing those two genera (Linton et al. 2010). Some phylogenetic analyses have indicated paraphyly of the genus *Phacus* (Kim and Shin 2014; Karnkowska et al. 2015); however, the most comprehensive study (Kim et al. 2015) supported the division into two genera.

The Euglenaceae contains seven monophyletic genera (*Euglenaria*, *Euglenaformis*, *Colacium*, *Cryptoglena*, *Monomorphina*, *Strombomonas*, and *Trachelomonas*) and the paraphyletic *Euglena* (Kim et al. 2010; Linton et al. 2010; Karnkowska et al. 2015; Kim et al. 2015). The earliest branching lineage is the recently established genus *Euglenaformis* (Bennett et al. 2014) with one species, *Euglenaformis proxima* (formerly *Euglena proxima*). The remaining genera form two sister clades. One clade includes *Colacium* and the closely related loricate genera *Trachelomonas* and *Strombomonas*. Marin et al. (2003) proposed merging *Trachelomonas* and *Strombomonas*, but all recent phylogenetic analyses have supported their phylogenetic distinction (Brosnan et al. 2005; Triemer et al. 2006; Ciugulea et al. 2008; Kim and Shin 2008; Kim et al. 2010; Linton et al. 2010; Karnkowska et al. 2015; Kim et al. 2015). The second clade includes the closely related rigid genera *Monomorphina* and *Cryptoglena,* together with *Euglena* and *Euglenaria* (Karnkowska et al. 2015; Kim et al. 2010, 2015; Linton et al. 2010). *Euglenaria* was erected to accommodate three *Euglena* species placed outside the main clade of *Euglena* (Linton et al. 2010). *Euglenaria* is sister to *Monomorphina* and *Cryptoglena* in most phylogenetic analyses (Linton et al. 2010; Kim et al. 2010; Karnkowska et al. 2015), but branched as sister to *Euglena* in one recent study (Kim et al. 2015). The taxonomy of the genus *Euglena* is the most problematic because species which did not fit morphologically into other genera were assigned to it, resulting in an amalgam of species. Currently, two species of *Euglena* fall outside the main well supported *Euglena* clade: *E. archaeoplastidiata* (Kim and Shin 2008; Kim et al. 2010; Karnkowska et al. 2015; Kim et al. 2015) and *E. velata* (Karnkowska-Ishikawa et al. 2012; Kim et al. 2015).

The genera *Euglenamorpha* and *Hegneria*, which were originally observed in tadpole guts, are not represented in any molecular phylogenetic trees. Therefore, their validity and phylogenetic positions are questionable.

Most of the species of phototrophic euglenids were described in the nineteenth and twentieth centuries based solely on morphology. Thousands of taxa have been described (~3000 including forms and varieties according to AlgaeBase: http://www.algaebase.org) because of the great morphological diversity of euglenid cells. The species-level taxonomy of the group is riddled with duplications and re-descriptions, as well as formulations of artificial classification schemes. The advent of DNA sequencing combined with careful morphological investigation allowed some of the taxonomic confusions to be resolved. Many species have been verified, and new taxa have been described to accommodate the observed

molecular and morphological diversity (Bennett and Triemer 2012; Kosmala et al. 2005, 2007a, 2007b, 2009; Karnkowska-Ishikawa et al. 2010, 2011, 2012, 2013, 2014; Kim et al. 2013a, b, 2014; Kim et al. 2016; Linton et al. 2010; Łukomska-Kowalczyk et al. 2015; Shin and Triemer 2004; Zakryś 1997; Zakryś et al. 2002, 2004, 2013).

The primary osmotrophs (Aphagea) are a phlyogenetically cohesive group that includes the Rhabdomonadales (*Menoidium*, *Rhabdomonas*, *Gyropaigne*, *Parmidium*, and *Rhabdospira*) plus *Distigma* and *Astasia* (see above). Both *Distigma* and *Astasia* appear to be paraphyletic at present (Preisfeld et al. 2001; Muellner et al. 2001; Cavalier-Smith 2016).

The taxonomy of phagotrophs is far less well organized than that of photoautotrophic euglenids, partly because sequence information is sparse. Current genus-level taxonomy is a mix of traditional systems that emphasize a few conspicuous morphological characters on one hand (e.g., flagellar number and lengths, degree of flexibility, visibility of the feeding apparatus) and molecular phylogenetic information derived from very few species on the other (plus a small amount of ultrastructural data). The genus-level taxonomy is covered here using the four informal assemblages introduced earlier. Throughout we will use the the predominant "zoological" genus names (but see below).

Petalomonads (Petalomonadida) are probably monophyletic, and this group contains several dozen species assigned to the genera *Petalomonas*, *Notosolenus*, *Calycimonas*, *Sphenomonas*, *Scytomonas*, *Tropidoscyphus*, *Atraktomonas*, the recently created *Biundula*, and perhaps *Dolium* and *Dylakosoma* (Lee and Simpson 2014b; Cavalier-Smith 2016). The boundaries among many of these genera are highly uncertain; the morphological differences between them are often subtle, and some are known to currently represent non-monophyletic groupings (e.g., *Notosolenus*; Lee and Simpson 2014b). Much better DNA sequence coverage of genera and species (including type species) is needed, and it is likely to precipitate considerable changes to the genus-level taxonomy.

Most ploeotiid species, with the exception of *Entosiphon* spp., were descibed within the last 30 years, and most have been included at some point in the genus *Ploeotia* (Larsen and Patterson 1990). However recent phylogenies inferred from SSU rDNA sequences indicate that ploeotiids are genetically diverse and not monophyletic (Lax and Simpson 2013; Chan et al. 2013; Cavalier-Smith 2016; Cavalier-Smith et al. 2016), and the current trend is to recognize several genera in addition to *Ploeotia* (and *Entosiphon*), namely, *Decastava, Keelungia*, *Lentomonas*, and *Serpenomonas* (see Chan et al. 2013; Cavalier-Smith 2016). These are a mix of new taxa and genera that were previously considered as synonyms of *Ploeotia*. The rational distribution of most ploeotiid species to genera awaits further molecular sequence data (e.g., from the type species of *Ploeotia, P. vitrea*).

Peranemids include several genera, namely *Peranema*, *Chasmostoma*, *Urceolus*, and *Jenningsia* (and *Peranemopsis*, a synonym of *Jenningsia* according to Lee et al. 1999), as well as most but not all of the organisms that have typically been assigned to *Heteronema* (see below), including that assigned to the newly proposed *Teloprocta* (Cavalier-Smith et al. 2016). Peranemids in this broad sense

are probably not monophyletic (note that the taxa Peranemia, Peranemida, and Peranemidae have all recently been used to encompass just *Peranema*, *Urceolus, Jenningsia*, and *Peranemopsis*; Cavalier-Smith 2016; but this more restricted "peranemid" assemblage is likely not monophyletic either). As of late 2016, there are sequences available from just two species from this entire assemblage, so the phylogenetic appropriateness of the genus-level taxonomy is difficult to evaluate at present.

The anisonemid assemblage includes *Dinema* and *Anisonema* and, almost certainly, some species currently assigned to *Heteronema* (see below). It is unclear at present whether anisonemids are monophyletic (compare Lax and Simpson 2013; Lee and Simpson 2014a; Cavalier-Smith 2016). The assignment of species to *Anisonema* and *Dinema* is problematic; molecular phylogenies usually recover *Dinema* as non-monophyletic (Lee and Simpson 2014a; Cavalier-Smith 2016), while *Anisonema* has a very diffuse circumscription, to the extent that some species are probably actually ploeotiids.

Neometanema and the taxonomic entity *Semihia* are related to (and possibly derived from) anisonemids, from which they differ by having a distinctive "skidding" motility, although they also retain a supplementary ability to glide (Lee and Simpson 2014a). *Neometanema* and *Semihia* collectively absorb all the euglenid species previously assigned to *Metanema* (e.g., Larsen 1987), which has a zoological homonym, as well as a couple of species of *Heteronema* (see below; Lee and Simpson 2014a). Molecular phylogenetic analysis shows with moderate support that *Neometanema* is closely related to Aphagea (Lax and Simpson 2013; Lee and Simpson 2014a; Cavalier-Smith 2016), and the taxon name Natomonadida has recently been proposed for this grouping, based on the frequent use of swimming locomotion (Cavalier-Smith 2016).

The genus *Heteronema* is particularly problematic. At present it mainly includes "peranemids," but also includes a small number of "anisonemids" (see Larsen and Patterson 1990) even after the recent transfer of species to *Neometanema* (Lee and Simpson 2014a). Although the first described *Heteronema, H. marina* (Dujardin 1841), was some kind of anisonemid (as defined here), the modern concept of the genus comes from Stein (1878) and is based on peranemid species. This switch has long been recognized and tolerated (Larsen and Patterson 1990). Cavalier-Smith (2016) recently proposed returning to Dujardin's earlier concept, but we advocate overlooking this proposal, which is potentially destabilizing for no real gain (and if carried to a logical conclusion, could dramatically affect the application of the genera *Dinema* and/or *Anisonema* as well as *Heteronema)*.

The Symbiontida (synonym Postgaardea – see Cavalier-Smith et al. 2016) encompasses the three genera *Calkinsia*, *Postgaardi*, and *Bihospites*. Each includes a single described species at present (Yubuki et al. 2009; Breglia et al. 2010).

It is important to note that several genera of phagotrophic euglenids have homonyms in botanical taxonomy, and alternative botanical names have been

proposed: *Dinema* = *Dinematomonas*; *Entosiphon* = *Entosiphonomonas*; and *Peranema* = *Pseudoperanema*.

Evolutionary History

The phylogeny of euglenids has been addressed most extensively using nucleotide sequences amplified from ribosomal genes (i.e., small and large subunit rRNA genes) (Brosnan et al. 2003, 2005; Busse et al. 2003; Ciugulea et al. 2008; Karnkowska et al. 2015; Kim and Shin 2008; Kim et al. 2015; Linton et al. 1999, 2000, 2010; Marin et al. 2003; Milanowski et al. 2001, 2006; Montegut-Felkner and Triemer 1997; Müllner et al. 2001; Nudelman et al. 2003; Preisfeld et al. 2001; Triemer et al. 2006; von der Heyden et al. 2004; Zakryś et al. 2002). Although these genes have been helpful in resolving the phylogeny of photoautotrophic euglenids, they do not provide satisfactory phylogenetic signal at deeper levels in the phylogeny (e.g., among the bacterivorous euglenids). Additional evidence for deep-level phylogenetic relationships of euglenids comes from comparative analyses of morphological data and some nucleus-encoded protein genes (e.g., heat shock protein 90), although the latter are still very sparse (Breglia et al. 2007; Leander et al. 2001a, b; Karnkowska et al. 2015; Montegut-Felkner and Triemer 1997; Simpson et al. 2002; Simpson and Roger 2004; Talke and Preisfeld 2002; Cavalier-Smith et al. 2016). These data also confirm the placement of euglenids within the Euglenozoa, as sister to kinetoplastids and diplonemids. Multigene molecular phylogenetic analyses also strongly support the placement of the Euglenozoa as a whole within a clade, the Discoba, that also includes Heterolobosea, Jakobida, and *Tsukubamonas* (e.g., Hampl et al. 2009; Kamikawa et al. 2014).

The following summarizes the current state of knowledge about phylogenetic relationships among euglenids (see also Fig. 12).

- Photoautotrophic euglenids (Euglenophyceae or euglenophytes) are a monophyletic subgroup nested within a paraphyletic assemblage of phagotrophic lineages.
- Euglenophytes with one emergent flagellum (Euglenales) are monophyletic; the Eutreptiales, with two emergent flagella (or rarely more), are their closest relatives. The recently described mixotroph *Rapaza* (also with two emergent flagella) is the deepest branch within euglenophytes.
- *Phacus* and *Lepocinclis* are each probably monophyletic and together form a more inclusive monophyletic group within the euglenophytes; these lineages tend to have 32 pellicle strips, are rigid, show great diversity in cell shape, and possess many small disc-shaped chloroplasts without pyrenoids and dimorphic paramylon grains.

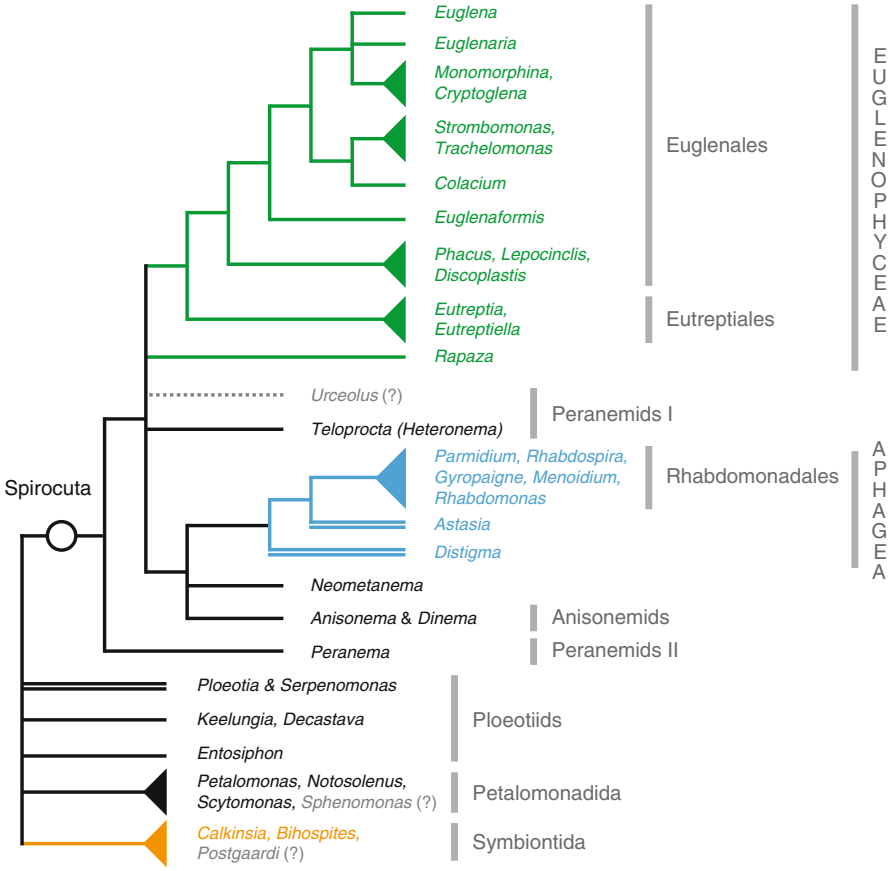

Fig. 12 Current knowledge of the evolutionary tree of euglenids, based primarily on SSU rRNA gene phylogenies. Photoautotrophic taxa are shown in green, primary osmotrophs in blue, "typical" phagotrophic taxa in black, and symbiontids in orange. Selected higher taxa are depicted to the right, though Spirocuta (= "H" or "HP" clade) is marked with a circle on its basal branch. Polytomies indicate regions of the tree that are poorly supported and/or resolved differently in various recent analyses. Genera shown in gray are important taxa whose positions are inferred from morphological information alone, since no molecular data are currently available (genera for which there are relatively limited data are not shown). Double lines on a branch denote paraphyletic groups (note also that both "peranemids" and "ploeotiids" appear to be paraphyletic). *Ploeotia* is probably paraphyletic at present, but it is also unclear whether *Serpenomonas* and *Ploeotia* are phylogenetically distinct (sequence information is awaited from *Ploeotia vitrea*, the type species of *Ploeotia*). The clade containing *Phacus*, *Lepocinclis*, and *Discoplastis* represents the taxon Phacaceae; the clade containing all other genera within Euglenales corresponds to the taxon Euglenaceae (see text)

- *Discoplastis* is the monophyletic sister group to *Phacus* and *Lepocinclis* and shares several morphological features with them (e.g., disc-shaped plastids without pyrenoids and 32 pellicle strips); however, these cells undergo dynamic metaboly.

- The monotypic genus *Euglenaformis* branches at the base of the assemblage of Euglenaceae.
- The loricate taxa *Trachelomonas* and *Strombomonas* are each monophyletic and together form a monophyletic group.
- The nearest sister group to the loricates is *Colacium*, which forms mucilaginous stalks.
- The rigid euglenophytes *Monomorphina* and *Cryptoglena* form a monophyletic group; these lineages have only one plastid and a relatively small number of broad pellicle strips (around 16–20).
- *Euglenaria* is monophyletic and possess morphological features similar to those of some representatives of *Euglena* (lobate plastids with diplopyrenoids), but distinct molecular signatures in nuclear SSU rDNA sequences. The molecular phylogenetic position of that lineage is not well resolved.
- The modern (revised) version of the genus *Euglena* is monophyletic with two known exceptions (see above); *Euglena* species often have 40 pellicle strips, undergo metaboly, and show great diversity in cell shape and plastid morphology (e.g., shield-shaped, stellate, lobed, spherical).
- Photosynthesis was lost several times independently within the euglenophytes (e.g., *Euglena longa* and *Euglena quartana* – previously assigned to "*Astasia*" and "*Khawkinea,*" respectively).
- The nearest sister lineages to euglenophytes are certain eukaryovorous euglenids, possibly *Teloprocta* (formerly *Heteronema*) and/or *Urceolus*.
- Primary osmotrophic euglenids (Aphagea, e.g., *Distigma*, *Rhabdomonas, Astasia*) are monophyletic and diverged from eukaryovorous ancestors independently from euglenophytes.
- Euglenophytes, primary osmotrophs, and eukaryovorous euglenids form a monophyletic group (Spirocuta; formerly the "H" or "HP" clade).
- Eukaryovorous euglenids are paraphyletic because they gave rise, independently, to both primary osmotrophs and euglenophytes – see above.
- Bacterivorous euglenids are probably paraphyletic.
- One clade of bacterivorous euglenids, petalomonads (Petalomonadida), has retained several possibly ancestral characters, such as few pellicle strips (10 or fewer), an MtR pocket, kDNA-like mitochondrial inclusions, and bacterivorous modes of nutrition; however, phylogenetic evidence that petalomonads are a particularly deep branch within euglenids is equivocal at best.
- Other bacterivorous euglenids ("ploeotiids," including *Entosiphon*) have unclear molecular phylogenetic positions vis-à-vis each other and petalomonads and symbiontids. These lineages have rigid pellicles with 12 or fewer strips (usually 10), somewhat similar to petalomonads, but have complex feeding apparatuses, including rods and vanes, similar to eukaryovorous euglenids.
- Symbiontids are a monophyletic group of anaerobes that lack pellicular strips, but usually branch among bacterivorous euglenids in molecular phylogenies, albeit with weak statistical support. They likely descended from "classical" bacterivorous euglenids, and secondarily lost pellicular strips, perhaps as a consequence of entering into symbioses with epibiotic bacteria. However,

transverse sections through the cell surface of *Bihospites* show many S-shaped profiles that are reminsicent of pellicle strips.
- Knowledge about the overall diversity and phylogenetic relationships of bacterivorous and eukaryovorous euglenids is still very poor.

Morphological Evolution, Especially the Pellicle

The euglenid pellicle is very diverse, and comparative analyses have demonstrated a great array of intermediate states for several cytoskeletal characters. This diversity placed in a molecular phylogenetic context demonstrates many large-scale evolutionary trends within the group (Leander et al. 2007).

The evolution of strip number involved at least three mechanisms associated with cytoskeletal replication and cell division: (1) asymmetrical segregation of strips to daughter cells, (2) permanent strip doubling events, and (3) permanent strip halving events (Esson and Leander 2006; Leander 2004; Leander et al. 2001a, b, 2007; Yubuki and Leander 2012). Permanent strip duplication events refer to a cell that duplicates its strips but fails to divide. Permanent strip halving events refer to a cell that divides without first duplicating its strips. The distribution of strip numbers found in euglenids suggests that these mechanisms collectively happened several times during the evolution of the group; there is evidence for the following events: four strips to eight strips (or vice versa) in petalomonads, 10 strips to 20 strips coincident the emergence of Spirocuta (the HP clade), and 20 strips to 40 strips near the origin of the Euglenales (Esson and Leander 2006; Leander et al. 2001a, b, 2007; Leander 2004).

The ancestral state for the number of strips in phototrophic euglenids is between 40 and 50; strip numbers that are significantly higher or lower than 40–50 are inferred to represent derived states. For instance, some relatively enormous species have either doubled or tripled this number of strips (e.g., 80 strips in *Lepocinclis helicoideus* and 120 strips in *Euglena obtusa*) (Esson and Leander 2008; Leander and Farmer 2000b). The phototrophic lineages that have lost metaboly, such as *Phacus* and *Lepocinclis*, tend to have 32 strips, which is the inferred ancestral state for the more inclusive clade consisting of these two genera plus *Discoplastis*. A subgroup of *Phacus* reduced the number of strips even further to about 20; these cells are among the smallest of all known photoautotrophic euglenids (Fig. 6). The strip-halving process (see above) helps explain the reduction of strips during the evolution of the rigid photoautotrophic lineage *Monomorphina* (32 strips to 16 strips; Leander and Farmer 2001b) (Figs. 1 and 6).

Fossil Record

Euglenid fossils are sparse. Aside from the loricas of *Trachelomonas* and *Strombomonas*, euglenids do not secrete hard parts that would promote fossilization.

However, some photoautotrophic euglenids have exceedingly thick proteinaceous strips, which could presumably fossilize. *Moyeria* is an enigmatic fossil with euglenid-like features (e.g., strips and a canal opening) that was discovered in Silurian deposits (Gray and Boucot 1989). The size, shape, and surface morphology of these fossils are reminiscent of some phototrophic euglenids in the genus *Monomorphina*.

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