

## Chromista

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### Abstract

The concept of chromists, at its most expansive, includes the heterokonts (stramenopiles), alveolates, rhizarians, heliozoans, telonemians, haptophytes and cryptophytes. There is mounting evidence that this grouping is not valid. Even in the narrowest sense (the heterokonts), chromists include very diverse forms, exhibiting a great variety of trophic mechanisms. This great diversity in form and feeding make it difficult to identify any unifying features, but molecular phylogenetic studies have shown that this group of organisms is indeed monophyletic. The distribution of morphological characters over reconstructed trees allows for the identification of potential synapomorphic characters that have been secondarily lost or modified across the group. These include a combination of mitochondria with tubular cristae; the biflagellate heterokont condition; and, if photosynthetic, then with chlorophyll *c*, girdle lamellae and four membranes around the chloroplast, the outer continuous with the nuclear envelope. Heterotrophy appears to be ancestral but is also occasionally a derived state from autotrophic forms.

**Keywords:** bacillariophytes; Bigyra; chromalveolates; chrysophytes; Heterokonta; oomycetes; phaeophytes; phylogeny; Pseudofungi; silicoflagellates; stramenopiles

### Key Concepts:

- There is no consistency in the ranking of the various eukaryotic taxa, making reference to particular forms and their relationships awkward.
- Molecular studies, particularly over the last decade, indicate an ever-increasing delimitation of the Chromista to include very diverse forms.
- The chromalveolate theory, at the root of the concept of Chromista, has been fiercely debated and most recent evidence points to multiple independent events involving red algal endosymbionts in diverse eukaryotic hosts.
- Even the original grouping of Chromista (heterokonts, haptophytes and cryptophytes) is tenuous, making it more sensible to equate chromists with the heterokonts (= stramenopiles).
- Heterokonts enjoy robust support from molecular phylogenetic analyses, but there are no universal morphological and physiological characters.

- The most universal character is the biflagellate condition of swimming cells, with one tinsel (hairy) and one smooth flagellum. The hairy flagellum is invested with two opposite rows of tri-partite, tubular hairs that are responsible for reversing thrust.
- Plastids of autotrophic heterokonts have consistent features, including two additional surrounding membranes (the periplastidial membrane and the RER), girdle lamellae and thylakoids stacked in groups of three.
- The classification within the heterokonts also has a chequered history, but multigene phylogenetic analyses are providing a clearer idea of groupings.
- Heterotrophic forms are rooted deeply in phylogenetic trees reconstructed using molecular markers, but some are secondarily derived from autotrophic forms.
- Heterokonts play an important role both ecologically and economically.

### **Introduction**

The Chromista was first introduced as one of seven eukaryote kingdoms, itself comprised of the chromophytes (i.e. the heterokonts, bacillariophytes, eustigmatophytes and haptophytes) and the cryptophytes (Cavalier-Smith, 1981). The meaning of the word chromist has changed significantly since its early usage and its history is obfuscated by subjectivity in the concept of rank in the classification of eukaryotic protists (Patterson, 1999).

### **The kingdom Chromista: An evolving concept**

In the original scheme (Cavalier-Smith 1981), the Heterokonta (heterokonts) included the Chrysophyceae (inclusive of bicosoecids and silicoflagellates), Xanthophyceae, Phaeophyceae and Raphidophyceae in one subphylum (Chrysophytina) and the oomycetes, hyphochytridiomycetes, thraustochytrids and labyrinthulids in another (Phycomycotina). By 1998, Cavalier-Smith had reduced the number of eukaryote kingdoms to five, still with a kingdom Chromista, but now transferring the cryptophytes to the subkingdom Cryptista, and the heterokonts (now inclusive of the bacillariophytes and the eustigmatophytes) and the haptophytes to the subkingdom Chromobiota. Since then, and particularly over the last decade, there has been considerable interest in the deep rooting of the eukaryotes, with phylogenetic trees being generated from an assortment, and an increasing volume, of data. The consequence of this interest has been a progressive simplification in the deep division of eukaryotes into two principle lineages (see below).

The resultant 'evolution' of the concept Chromista is one of expansion, as the complexity of the deep rooting of the eukaryotes has progressively simplified to a single bifurcation into unikonts and bikonts/corticates (chromists being a member of the latter; see Cavalier-Smith, 2010). The first enlargement included the protozoan infrakingdom, Alveolata (including the apicomplexans, the ciliates and the dinoflagellates) in an informal "chromalveolates" (Cavalier-Smith, 1999; Cavalier-Smith and Chao, 2003). This supergroup was later termed the Chromalveolata, but the name remained informal rather than being touted as a formal kingdom name, so

as to avoid the problem of nesting – see below (Adl *et al.*, 2005). More recently, the Rhizaria (phyla Cercozoa - filose and reticulose amoebae - and Retaria – forams and radiolarians), despite being predominantly heterotrophic, were indicated as belonging to this supergroup (e.g. Hackett *et al.*, 2007) and the recognition of this expanded version introduced the use of the acronym ‘SAR’ (the monophyletic grouping of **s**tramenopiles (Heterokonta), **a**lveolates and **R**hizaria (Burki *et al.*, 2008)). The Chromista were formally expanded to include the SAR as well as the former protozoan group, Heliozoa (Cavalier-Smith, 2010). To accommodate this diversity, Cavalier-Smith (2010) erected two subkingdoms, namely the Harosa (comprising the heterokonts, alveolates and rhizarians), and the Hacrobia (comprising the haptophytes, heliozoans and cryptophytes). The Hacrobia, a taxon originally coined by Okamoto *et al.* (2009), should also include the telonemians (Schalchian-Tabrizi *et al.*, 2006; Burki *et al.*, 2009). It is further worth noting that the Chromista in this broad sense contains something in the order of 10 phyla and is seen as sister to the Kingdom Archaeplastida (Plantae in the broadest sense), which comprises the glaucophytes, green and red algae, as well as the embryophytes (or land plants) (Cavalier-Smith, 2010). A major problem that persists with this broadest vision of Chromista is that the Protozoa are still held as an ancestral kingdom, so that all advanced kingdoms, including the Chromista, are nested within it.

The original characters used to define the Chromista are two-fold (Cavalier-Smith, 1981). Firstly, the presence of plastids (obviously limited to photosynthetic forms) that: (1) possess both chlorophyll a and c; (2) are surrounded by a periplastidial membrane; and (3) are additionally housed within the rough endoplasmic reticulum that is continuous with the nuclear envelope. Secondly, the presence of bi- or tripartite tubular hairs on one or both of their flagella. The major shift driving the subsequent increment in members was rooted in the **chromalveolate theory**, which holds that it is not parsimonious to infer that plastids of this nature were established by numerous independent endosymbiotic events in the various lineages in which they are found. This is because of the complexity involved in repeatedly establishing plastid-targeted proteins in the various host cell lineages (Cavalier-Smith, 1999). The history of the rationale behind the expansion of the concept of the Chromista is admirably provided by Keeling (2009) and the rationale behind the broadest circumscription is provided by Cavalier-Smith (2010); the reader is therefore referred to these papers for details. In short, however, the feature that unites the group (synapomorphy) is that all photosynthetic representatives have a red algal plastid ancestor. This means that the ultimate chromist ancestor was photosynthetic and that the multitude of non-autotrophic forms represents derived states that were repeatedly incurred in various divergent lineages. Despite the fact that the plastid of chromists *sensu lato* is generally accepted to be red algal in origin, analysis of phosphoribulokinase (PRK) genes in chromalveolate representatives showed them to have green algal affiliation (Petersen *et al.*, 2006). In addition, a complete genome analysis of certain diatoms revealed a far greater amount (> 70 %)

of green algal than red algal genes (Moustafa *et al.*, 2009). This pattern was also found in other heterokonts, like *Phytophthora* (an oomycete) and *Aureococcus* (a pelagophyte), and, to a much lesser extent, in haptophytes. These genes are, however, thought to represent a footprint of an earlier endosymbiotic event involving a picoprasinophyte that had been usurped by a later red algal endosymbiont (Moustafa *et al.*, 2009). This finding adds a further layer of complexity in trying to unravel deep branching in the eukaryotes tree, which is required to delimit the chromists. Interestingly, Baurain *et al.* (2010), in their subsequent analyses of chromists, found little or no evidence of a green algal genome present.

Much of the support for the chromalveolate theory comes from analyses of plastid-related gene sequences. Relationships based on such sequences are potentially misleading as they cannot differentiate between genes gained through vertical inheritance (the chromalveolate hypothesis) and those gained through lateral transfer (the serial endosymbiont hypothesis) (Kim and Graham, 2008; Baurain *et al.*, 2010). Thus more recent work turned towards analysis of nuclear sequences encoding cytosolic proteins that has progressively led to an undermining of a monophyletic Chromalveolata (e.g. Harper *et al.*, 2005; Kim and Graham, 2008). Parfrey *et al.* (2006) analysed the support for chromalveolates (excluding Rhizaria) and noted the absence of any clear synapomorphy. The supergroup was generally well-supported in analyses where it was targeted, but these were always using plastid genes and never using nuclear genes. Analysis of nuclear genes did, however, support a more restricted alveolate-heterokont clade, without cryptophytes and haptophytes (Harper *et al.*, 2005; Parfrey *et al.* 2006). The haptophytes were particularly singled out as having a highly variable sister group relationship (with rhizarians, with centrohelids and reds, or with cryptophytes) in various analyses, probably due to inadequate taxon sampling (Parfrey *et al.*, 2006). Analysis of the nuclear EEF2 (eukaryotic translation elongation factor 2) gene indicates that haptophytes and cryptophytes are affiliated more with katablepharids and green and red algae than with heterokonts and alveolates (Kim and Graham, 2008). In addition, and possibly even more damning are the inconsistencies, even within model (for the group) plastid-targeted genes (e.g. GAPDH) that have shaken the theory of a single Kingdom Chromista *sensu stricto* (i.e. heterokonts, haptophytes and cryptophytes) (Takashita *et al.*, 2009). Indeed, there is stronger evidence for ties between heterokonts and alveolates than between heterokonts and haptophytes and/or cryptophytes (e.g. Harper *et al.*, 2005). Most recently, Baurain *et al.* (2010) argued that the signal of support for chromalveolates derived from mitochondrial and nuclear genes should be as strong as the positive one they get from plastidial genes. Their analysis was carefully devised to be free of artifacts introduced by heterogeneities in evolution, and/or by models of phylogenetic inference used and/or by lateral gene transfer. It strongly suggests that the plastids of cryptophytes, alveolates, heterokonts and haptophytes are not acquired vertically from a single ancestor, but rather laterally by serial endosymbioses. This finding makes the validity of the supergroup highly questionable.

As the stability of the supergroup is seriously in question, for the purposes of this brief review, the chromists are restricted to the heterokonts. The traditional limits included the haptophytes and cryptophytes, but stronger ties having been demonstrated between heterokonts and alveolates than between heterokonts and haptophytes and/or cryptophytes (see above). This account will, however, exclude consideration of alveolates, rhizarians, heliozoans and telonemians for the reasons already provided above. A focus on members at the heart of this group (the heterokonts or stramenopiles) is deemed sensible as a common thread of all molecular studies is that they are monophyletic (e.g. Riisberg *et al.*, 2009), their clade incorporating heterotrophic forms that root deeply. This infers that the heterokonts represent a lineage isolated from other eukaryotes, one line of which became photosynthetic through an endosymbiotic event involving a red alga, and which radiated out into the photosynthetic forms of the group, the so-called ochrophytes. As a caution, however, Cavalier-Smith and Chao (2006) do counter-argue that early branching of heterotrophic forms from a recently-photosynthetic ancestor is to be expected before the organism becomes too tied into the physiology linked with the possession of a plastid (e.g. fatty acid synthesis).

### **The Phylum Heterokonta**

The heterokonts (also referred to informally as stramenopiles – for an overview of the abuse of this latter term, see Patterson (1999)) are a group that share a number of features or are derived from members that are thought to have once shared them. Thus there are no universally-present characters for the group, apart, perhaps, from having mitochondria with tubular cristae. However, this latter feature is not exclusive to the heterokonts and some forms are anaerobic (e.g. *Blastocystis*), which may influence the appearance of the mitochondria.

### ***Flagella and Flagellar Apparatus:***

Heterokonts are commonly biflagellate or have biflagellate stages, with one anteriorly-directed, tinsel (hairy) flagellum and one posteriorly-directed, smooth flagellum (Fig. 1). The hairs (or mastigonemes) are commonly tubular and tripartite (base, shaft and terminal fibril/s), glycoproteinaceous and are made in the endoplasmic reticulum or perinuclear space and modified in the Golgi (see Inouye, 1993). They are exported in vesicles to the cell surface and most often deposited on opposite sides of the anterior flagellum to reverse the thrust of its propulsive forces. In Opalinids, the hairs (or what are considered to be their homologues – now termed ‘somatonemes’) are instead found adorning the posterior half of the cell body (Hausman *et al.*, 2003). The flagella, as the name of the group suggests, are often different in length, attitude and behaviour, and often have a transitional helix (lacking in raphidophytes, phaeophytes, diatom sperm, bolidophytes and some others) and plate in their transition region (see overview in Guillou *et al.*, 1999). The longer, anterior, tinsel flagellum is developmentally younger and hence is labelled no. 2 (Beech *et al.*, 1991). There are at most four microtubular roots, two associated with each of the basal bodies. Those nucleated on the basal body of the smooth flagellum

(bb1) are labelled R1 and R2, while those on bb2 are R3 and R4 (relabelled by Moestrup, 2000). R2 often loops around and forms a feeding trough in heterotrophic and mixotrophic forms like thraustochytrids and *Epipyxis* (Inouye 1993). The R3 root often nucleates many cytoskeletal microtubules (see Inouye, 1993, but apply nomenclature of Moestrup, 2000). The basal bodies can be anchored to one another by fibrous bands and to the nucleus by a rhizoplast (e.g. *Ochromonas*) but lacking in others (e.g. pelagophytes, bolidophytes). In photosynthetic forms that have (most often intraplastidial) eyespots, the base of the axoneme of flagellum 1 can have a swelling held close to the eyespot, which is thought to function in light detection. This swelling and/or the entire flagellum 1 often autofluoresces when excited with blue-violet light (Kawai and Inouye, 1989). However, autofluorescence is absent in raphidophytes, *Vaucheria* and diatoms. In addition, autofluorescence is not specific to the heterokonts as it also is found sporadically in haptophytes (Kawai and Inouye, 1989).

However, there are many heterokonts with only one flagellum (no. 2, e.g. pelagophytes and sperm of centric diatoms), or with none (e.g. *Blastocystis* and the pennate diatoms, the latter of which have therefore resorted to amoeboid gamete transfer between aligned cells). Still other heterokonts (the Opalinids) have so many flagella that they were initially considered to be ciliates. Such variations obviously impact on the presence or absence of microtubular roots (e.g. *Pelagomonas*, but even biflagellate forms, especially if the basal bodies are parallel (Moestrup, 2000) can lack some (e.g. synurophytes that only have R3 nucleating an extraordinary number of cytoplasmic microtubules; Inouye, 1993), or even all, microtubular roots (e.g. pedinellids, silicoflagellates, pelagophytes and bolidophytes). Riisberg *et al.* (2009) suggest that, in ochrophytes, members of the Phaeista (see below) have the more complex or complete flagellar apparatus and members of the Khakista independently reduced their flagellar apparatus.

### **Photosynthetic Forms:**

The remaining other unifying feature is restricted to those members that are photosynthetic (or that have secondarily lost this ability); the so-called ochrophytes. The consensus is that the chloroplasts of this group are derived from red algae by secondary endosymbiosis (also referred to as eukaryote-eukaryote endosymbiosis or meta-algae). The chloroplasts are characterised by possessing water-soluble chlorophyll c (in one of three forms) in addition to the membrane-embedded chlorophyll a and being included within the rough endoplasmic reticulum (RER) (Fig. 2). The RER is variably also referred to as the periplastidial or chloroplast ER, the inner membrane of which is referred to as the periplastidial membrane (Cavalier-Smith, 1999). The RER may be contiguous with the nuclear envelope and its lumen can even be inflated to include further membranous vesicles (the periplastidial compartment). The result is that the thylakoids of the chloroplast are separated from the cytoplasm by four membranes. The chloroplast of many ochrophytes additionally has a girdle lamella (thylakoid) that completely encircles the underlying

thylakoids that, in turn, are generally stacked in groups of three. A variety of accessory pigments are found; either the yellow  $\beta$ -carotene, the brown fucoxanthin, the green-brown vaucheriaxanthin, or a variety of other xanthophylls (Graham and Wilcox, 2000). Most forms that have an eyespot (except the eustigmatophytes) have it in an intraplastidial location, associated with the swollen base of flagellum 1.

Photosynthetic forms are of great importance as they are the primary producers of many aquatic food webs, but also have an economic (e.g. the phaeophytes) or detrimental (e.g. toxic pelagophytes and raphidophytes) impact. As they have been implicated as significant contributors to oil deposits, they also are eyed as potential feed for biodiesel production.

### ***Heterotrophic Forms:***

Heterotrophy is a condition thought by some to be a derived condition, so that all the variety of heterokonts without plastids represents independently-derived forms (the basic premise of the chromalveolate theory; see above). However, the counter-argument, which is gaining in momentum, is that the ancestral heterokont was heterotrophic. There are a few heterotrophs (e.g. some chrysophytes), however, that are considered to be derived states of photosynthetic ochrophytes. Heterotrophs (and mixotrophic ochrophytes) have the ability to phagocytose whole particles (even assisting the process by using their flagella, e.g. *Cafeteria* and *Spumella*; Hausman *et al.*, 2003), or are restricted to pinocytosis (e.g. *Proteromonas*).

Some heterotrophic forms, such as the oomycetes, are not restricted to an aquatic habitat. Many heterotrophs are important as they are either gut commensals (e.g. opalinids), saprotrophs or parasites (e.g. oomycetes, thraustochytrids and labyrinthulids). They have even led to catastrophic events, such as the Irish famine of the 1840s which occurred because of the outbreak of late potato blight (*Phytophthora*).

### ***Phylogeny of the Heterokonts:***

The history of systematics within the heterokonts has also been quite fluid, but the most recent multigene phylogenetic analyses of the group confirms their monophyly (e.g. Cavalier-Smith and Chao, 2006; Riisberg *et al.*, 2009). These analyses also suggest an early divergence of the heterokonts into heterotrophic forms and the phylum Ochrophyta, the latter of which would include some secondarily heterotrophic forms (e.g. *Spumella* and some pedinellids).

The heterotrophs are further bifurcated into the phyla Pseudofungi and Bigyra. The Pseudofungi include the Oomycetes (e.g. *Phytophthora* and water molds like *Saprolegnia*), hyphochytrids (a relatively small group of saprophytes, necrophytes and parasites in fresh and marine waters) and bigyromonads (*Developayella*, another marine parasite). The Bigyra include the opalozoans (proteromonads and opalineans, both gut commensals of amphibians, and the potentially-pathogenic blastocystans), bicoecians (including familiar forms such as *Cafeteria* and *Bicosoeca*) and sagenistans (thraustochytrids and labyrinthulids, which are mostly

marine organisms that exist as ectoplasmic nets associated with decaying vegetation and detritus, and capable of ‘wasting disease’ of seagrasses).

The Ochrophyta are divided into two subphyla, the larger Phaeista and the smaller Khakista. The Phaeista in turn are divided into those organisms mostly found in freshwater environments, the infraphylum Limnista, and the largely marine infraphylum, Marista. The Limnista comprise the classes Eustigmatophyceae, Chrysomonadea (= Chrysophyceae, including the orders Chromulinales, Paraphysomonadales, Ochromonadales, Hibberdiales and Synurales) and a new class, Picophagea (Cavalier-Smith and Chao, 2006). Groups affiliated with the Marista include the pelagophytes, sarcinochrysidaleans, silicoflagellates (dictyochophytes), pedinellids, pinguiophytes, raphidophytes, chrysomerophytes, phaeothamniophytes, xanthophytes and the phaeophytes. The Khakista comprise the bolidophytes and the bacillariophytes (Diatomae). However, the phylogenetic analysis of Riisberg *et al.* (2009) led them to conclude that the dictyochophytes and pelagophytes should be transferred from the Phaeista to Khakista.

### **Selected Chromist (Heterokont) Subtaxa**

The full diversity of the Heterokonts cannot be presented in this short overview. However, the following groups hopefully provide some insight.

#### **Bigyra**

All molecular phylogenetic analyses point to the phylum Bigyra as being a basal group of the heterokonts, although they may represent a long-branch attraction artifact. However, it appears to be robust as attempts to reduce the effects of this do little to alter the topology of its branching (Riisberg *et al.*, 2009). The phylum is divided into three subphyla, the Opalozoa, Bicoecia (often referred to as the Bicosoecids; see Cavalier-Smith and Chao, 2006) and the Sagenista, the last taxon previously considered to be a superphylum and phylum (Cavalier-Smith, 1998), but the branching order is not robustly resolved (Cavalier-Smith and Chao, 2006).

The Opalozoa have two superclasses, the Nucleohelea and the Opalinata (Cavalier-Smith and Chao, 2006). The Nucleohelea has the order Actinophyrales, a group of freshwater or marine heliozoan amoebae (e.g. *Actinophrys*) with stiffly-held axopodia that radiate out of their spherical cell bodies and which result in the common name for these organisms, the ‘sun animalcules’. The axopodia are supported by microtubules that originate from the nuclear envelope in two intermingled spirals. The Opalinata have three classes, the Proteromonadea, Opalineae and Blastocystea. Proteromonadeans live as commensals in the alimentary tract of amphibians. They have one or two pairs of apically-inserted heterokont, but naked flagella. The rhizoplast attaches the basal bodies to the nucleus after passing through a typical circular Golgi body and the tapering cell body is covered by a helical pellicle subtended by microtubules and the posterior exterior surface of which is covered by the previously described somatonemes. Opalineans also are alimentary commensals in amphibians and are covered by a myriad of short cilia arranged in

dense, helical rows, resulting in their original classification as ciliates. They feed solely by pinocytosis. The blastocystans are aflagellate and potential disease-formers in mammals.

The Bicoecia comprise a small collection of heterotrophic unicells, some (*Bicosoeca*) with a chitinous lorica in which they anchor themselves by their posterior flagellum. The lorica itself can be attached to a substrate or the organism can be free-swimming, but the emergent tinsel flagellum functions to draw water and prey to the cell body. Other commonly-known, but lorica-less, genera found here are *Cafeteria* and *Wobblia* (see additional reading).

The Sagenista are comprised of the thraustochytrids and labyrinthulids. These organisms (more commonly known as slime nets), also have flagellated stages in their life cycle that possess the typical heterokont-type flagella. As said before they feed on detritus, but are also known to parasitise algae and seagrasses. In their trophic stage, they are unique in their ability to produce or secrete an anastomosing slime net which represents a membrane-bound, pseudopodia-like ectoplasm through which individual cells migrate. Each cell is itself surrounded by a double membrane with pores called sagenogens or bothrosomes. The pores allow the cells to directly communicate with each other and the colony's common membrane and thus interface with external stimuli.

### **Oomycetes**

The class Oomycetes, or 'egg fungi' (because of the large round oogonia containing the double walled zygotes) is a member of the Pseudofungi. The majority are filamentous and multicellular, but with few septa and thus mycelial (forming a branched filamentous coenocyte) in nature. Some are unicellular and elaborate (e.g. *Haptoglossa*) infecting nematodes using an elaborate gun cell). The Oomycetes are commonly known as water moulds and downy mildews and many are important plant (e.g. *Phytophthora*) and fish (e.g. *Saprolegnia*) pathogens. The Oomycota have been classified as chromists because their free-swimming zoospores possess the heterokont-type flagella. Furthermore, in oomycetes food is stored in the form of mycolaminarin, an energy storage molecule similar to that found in diatoms and brown algae. Their walls are cellulosic rather than the chitinous form of fungal cell walls and their dominant life cycle form is diploid rather than the haploid form of true fungi.

### **Bacillariophyceae (Diatomae)**

Commonly known as diatoms, the bacillariophytes are unicellular or colonial, but are never organized into more complex thalli. Only the male gamete of some centric diatoms is uni-flagellated with the flagellum (no. 1) typically of the heterokont-type and with no root system. The chief distinguishing feature of this group is the cell wall, which is siliceous and composed of two overlapping halves, rather similar to a shoebox or Petri dish. This type of cell wall is termed a frustule.

This group was traditionally always divided into the pennate (bilaterally symmetrical) and centric (radially symmetrical) diatoms. However, analyses using molecular data indicate that centrics are paraphyletic and that the primary split of the group (division in their system) Bacillariophyta (Medlin and Kazmarska, 2004) is between the Coscinodiscophytina (centrics with peripheral linkers and radially-symmetrical patterning) and the Bacillariophytina (centric and pennate diatoms that are bi- or multipolar). The Bacillariophytina are further divided into the two classes, the Mediophyceae (centrics with central connectives) and Bacillariophyceae (bipolar pennates with or without raphes) (Medlin and Kazmarska, 2004). Only raphid diatoms are capable of movement (gliding). The raphe is an elongate slit, right through the depth of the frustule, along the surface of each valve. Each of two raphes runs from a pole to terminate near the centre where they form a break called the central nodule and, at the poles, each raphe terminates in the polar nodules. Mucilage is extruded through the raphe near their nodular extremities and is used to achieve the gliding movement. The mucilage is also used statically for attachment or protection from abrasion. There are a myriad of features present on the surface of the frustule, many of which are best resolved using the scanning electron microscope and which are useful as taxonomic characters.

### **Silicoflagellata (=Dictyochales)**

The silicoflagellates are generally uncommon members of the marine plankton, but can form blooms, particularly in colder waters. They are unicells with numerous chloroplasts per cell and have two stages, a naked stage and an exoskeleton-bearing phase. The naked stage is derived from the skeleton phase by the simple 'excretion' of the siliceous skeleton with a concomitant thickening of the periplast (van Valkenburg and Norris, 1970). Silicoflagellates are biflagellate, but flagellum 1 is very short (naked stage) or reduced to a basal body (skeleton stage) (Moestrup, 1995). Flagellum 2 has the typical mastigonemes, but additionally has a paraxonemal rod. Transitional helices are absent in the flagellar transition region. Silicoflagellates are photosynthetic and, despite having 'pseudopodia' supported by microtubules (much like the bigyan, *Actinophrys*; see above), they are not thought to be mixotrophic.

The nearest relatives to the silicoflagellates are the pedinellids and the rhizochromulinids, and then the pelagophytes and the sarcinochrysidaleans (Cavalier-Smith and Chao, 2006). This group as a whole has an underdeveloped flagellar apparatus.

### **Chrysomonadea (= Chrysophyceae)**

This principally freshwater group, named after the gold colouring of their cells, used to be far wider in its delimitation. It is considered as displaying the archetypal heterokont organisation, with *Ochromonas* considered the model, but variation here is high, from unicellular flagellates to amoeboid forms and colonies. The covering of the cell in this group is extremely diverse from naked, to being covered by siliceous scales, organic loricas, and mucilage. Many have siliceous-walled, asexual or sexual

resting cysts called stomatocysts. Mixotrophy is common in this group. Indeed, there are several examples here of secondary heterotrophy (e.g. *Spumella* and *Paraphysomonas*).

Presently, five orders are grouped here, the Chromulinales, Paraphysomonadales, Ochromonadales, Hibberdiales and Synurales (Cavalier-Smith and Chao, 2006), although the last have often been considered as a separate class, the Synurophyceae. The Eustigmatophyceae and Picophagea are considered close relatives to the Chrysophyceae (Cavalier-Smith and Chao, 2006).

### **Phaeophyceae**

These are the brown algae. They only occur in multicellular forms, from filamentous species (e.g. *Ectocarpus*) to complex parenchymatous species reaching up to 60 m or more in length (e.g. the giant kelps like *Macrocystis* and *Laminaria*). They have cellulosic cell walls, supplemented by alginic acid (a substance of considerable economic significance as it is used as a gelling and emulsifying agent in a large number of industries) and sulphated polysaccharides. Brown algae commonly store polyphenolics, that may serve an anti-herbivory role, and their photosynthetic reserve is laminarin. Another unique feature of the phaeophytes is a stalked pyrenoid that protrudes from the chloroplast.

It is the organisation of their zoospores and gametes (often restricted to spermatia) that attest to their heterokont affiliations. However, some of these have a shorter anterior flagellum (no. 2) and in others flagellum 1 is lacking. The flagella here are unique in that they are laterally rather than apically inserted (Fig. 1).

About 2000 species are known at present, assigned to approximately 265 genera, with the vast majority restricted to the marine environment. The Phaeophyceae are currently grouped with the Xanthophyceae and Chrysomerophyceae (Cavalier-Smith and Chao, 2006).

### **Legends for Figures:**

**Figure 1:** A typical heterokont flagellate. Most heterokont motiles have apical or near-apical insertion of their flagella whereas this diagram shows the lateral insertion typical of phaeophyte gametes and spores. The tinsel flagellum is anteriorly-directed and the smooth flagellum trails the cell during swimming.

**Figure 2:** The heterokont chloroplast has the normal plastidial double membrane envelope, but surrounded by a periplastidial membrane and by rough endoplasmic reticulum which may be confluent with the outer membrane of the nuclear envelope. The periplastidial membrane is considered to be the remnants of the eukaryotic endosymbiont's (a red alga) plasmalemma. The chloroplast interior is occupied by thylakoids, the outer one/s of which is/are continuous and just beneath the envelope - the girdle lamella.

### **References**

Adl SM, Simpson AGB, Farmer MA, Anderson RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup Ø, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW and Taylor MFJR (2005) The higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* **52**: 399-451.

Beech PL, Heimann K and Melkonian M (1991) Development of the flagellar apparatus during the cell cycle in unicellular algae. *Protoplasma* **164**: 23-37.

Burain D, Brinkmann H, Petersen J, Rodríguez-Ezpeleta N, Stechman A, Demoulin V, Roger AJ, Burger G, Lang BF and Philippe H (2010) Phylogenomic evidence for separate acquisition of plastids in cryptophytes, haptophytes, and stramenopiles. *Molecular Biology and Evolution* **27**: 1698-1709.

Burki F, Shlachian-Tabrizi K and Pawlowski J (2008) Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. *Biology Letters* **4**: 366-369.

Burki F, Inagaki Y, Bråte J, Archibald JM, Keeling PJ, Cavalier-Smith T, Sakaguchi M, Hashimoto T, Horak A, Kumar S, Klaveness D, Jakobsen KS, Pawlowski J and Shlachian-Tabrizi K (2009) Large-scale phylogenomic analyses reveal that two enigmatic protest lineages, Telonemia and Centroheliozoa, are related to photosynthetic chromalveolates. *Genome Biology and Evolution* **1**: 231-238.

Cavalier-Smith T (1981) Eukaryote kingdoms: seven or nine? *Biosystems* **14**: 461-481.

Cavalier-Smith T (1998) A revised six-kingdom system of life. *Biological Reviews* **73**: 203-266.

Cavalier-Smith T (1999) Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. *Journal of Eukaryotic Microbiology* **46**: 347-366.

Cavalier-Smith T (2010) Kingdoms Protozoa and Chromista and the eozoan root of the eukaryotic tree. *Biology Letters* **6**: 432-345.

Cavalier-Smith T and Chao EE (1996) 18S rRNA sequence of *Heterosigma carterae* (Raphidophyceae), and the phylogeny of heterokont algae (Ochrophyta). *Phycologia* **35**: 500-510.

Cavalier-Smith T and Chao EE-Y (2003) Phylogeny of Choanozoa, Apusozoa, and other protozoa and early eukaryote megaevolution. *Journal of Molecular Evolution* **56**: 540-563.

Cavalier-Smith T and Chao EE-Y (2006) Phylogeny and megasystematics of phagotrophic heterokonts (kingdom Chromista). *Journal of Molecular Evolution*. **62**: 388-420.

Guillou L, Chrétiennot-Dinet M-J, Medlin L, Claustre H, Loiseaux-de Goër S and Vaultot D (1999) *Bolidomonas*: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *Journal of Phycology* **35**: 368-381.

Hackett JD, Yoon HS, Li S, Reyes-Prieto A, Rümmele SE and Bhattacharya D (2007) Phylogenomic analysis supports the monophyly of cryptophytes and haptophytes and the association of Rhizaria with chromalveolates. *Molecular Biology and Evolution* **24**: 1702-1713.

Harper JT, Waanders E and Keeling PJ (2005) On the monophyly of chromalveolates using a six-protein phylogeny of eukaryotes. *International Journal of Systematic and Evolutionary Microbiology* **55**: 487-496.

Hausman K, Hülsmann N and Radek R (2003) *Protistology* (3<sup>rd</sup> Edition). E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. 379pp. ISBN 3-510-65209-6.

Inouye I (1993) Flagella and flagellar apparatuses of algae. In. *Ultrastructure of Microalgae*. Berner, T. (Ed.). Boca Raton, CRC Press. pp. 99-133.

Kawai H and Inouye I (1989). Flagellar autofluorescence in forty-four chlorophyll c-containing algae. *Phycologia* **28**:222-227.

Keeling PJ (2009) Chromalveolates and the evolution of plastids by secondary endosymbiosis. *Journal of Eukaryotic Microbiology* **56**: 1-8.

Kim E and Graham LE (2008) EEF2 analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS ONE* **3**: e2621.

Medlin LK and Kaczmarska I (2004) Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia* **43**: 245-270.

Moestrup Ø (1995) Current status of chrysophyte 'splinter groups': synurophytes, pedinellids, silicoflagellates. In. *Chrysophyte Algae. Ecology, Phylogeny and Development*. Sandgen CD, Smol JP and Kristiansen J (Eds.) Cambridge, Cambridge University Press. pp.75-91.

Moestrup Ø (2000) The flagellate cytoskeleton. Introduction of a general terminology for microtubular roots in protists. In. *The Flagellates*. The Systematics Association Special Volume 59. London, Taylor and Francis. pp. 69-94.

Moustafa A, Beszteri B, Maier UG, Bowler C, Valentin K and Battacharya D (2009) Genomic footprints of a cryptic plastid endosymbiosis in diatoms. *Science* **324**: 1724-1726.

Okamoto N, Chantagsi C, Horák A, Leander BS and Keeling PJ (2009) Molecular phylogeny and description of the novel katablepharid *Roombia truncate* gen. et sp. nov., and establishment of the Hacrobia taxon nov. *PLoS ONE* **4**: e7080.

Parfrey LW, Barbero E, Lasser E, Dunthorn M, Bhattacharya D, Patterson DJ and Katz LA (2006) Evaluating support for the current classification of eukaryotic diversity. *PLoS Genet* **2**: e220

Patterson DJ (1999) The diversity of eukaryotes. *American Naturalist* **154**: S96-S124.

Petersen J, Teich R, Brinkman H and Cerff R (2006) A 'green' phosphoribulokinase in complex algae with red plastids; evidence for a single secondary endosymbiosis leading to haptophytes, cryptophytes, heterokonts, and dinoflagellates. *Journal of Molecular Evolution*. **62**: 143-157.

Riisberg I, Orr RJS, Kluge R, Shalchina-Tabrizi K, Bowers HA, Patil V, Edvardsen B and Jakobsen KS (2009) Seven gene phylogeny of heterokonts. *Protist* **160**: 191-204.

Shalchian-Tabrizi K, Eikrem W, Klaveness D, Vaulot D, Minge MA, Le Gall F, Romari K, Thronsen J, Botnen A, Massana R, Thomsen HA and Jakobsen KS (2006) Telonemia, a new protist phylum with affinity to chromist lineages. *Proceedings of the Royal Society B* **273**: 1833-1842.

Takashita K, Yamaguchi H, Maruyama T and Inagaki Y (2009) A hypothesis for the evolution of nuclear-encoded, plastid-targeted glyceraldehydes-3-phosphate dehydrogenase genes in "chromalveolate" members. *PLoS ONE* **4**: e4737.

Van Valkenburg SD and Norris RE (1970) The growth and morphology of the silicoflagellate *Dictyocha fibula* Ehrenberg in culture. *Journal of Phycology* **6**: 48-54.

### **Further reading**

Archibald JM (2009) The puzzle of plastid evolution. *Current Biology* **19**: R81-R88.

Anderson RA (2004) Biology and systematics of heterokont and haptophyte algae. *American Journal of Botany* **91**: 1508–1522.

Battacharya D, Yoon HS, Hedges SB and Hackett JD Eukaryotes (Eukaryota). In. *The Timetree of Life*. Hedges SB and Kumar S (eds.) Oxford, Oxford University Press. pp. 116 -120.

Karpov SA, Sogin ML and Silberman JD (2001) Rootlet homology, taxonomy, and phylogeny of bicosoecids based on 18S rRNA gene sequences. *Protistology* **2**: 34–47.

Margulis L, Corliss JO, Melkonian M and Chapman DJ (eds.) (1990) *Handbook of Protoctista*. Boston, Jones and Bartlett. 914 pp.

Moriya M, Nakayama T and Inouye I (2000) Ultrastructure and 18S rDNA sequence analysis of *Wobblia lunata* gen. et sp. nov., a new heterotrophic flagellate (stramenopiles, *incertae sedis*) *Protist* **151**: 41–55.

Moriya M, Nakayama T and Inouye I (2002) A new class of the stramenopiles, Placididea classis nova: description of *Placidia cafeteriopsis* gen. et sp. nov. *Protist* **153**: 143–156.

Nozaki H (2005) A new scenario of plastid evolution: plastid primary endosymbiosis before the divergence of the “Plantae,” emended. *Journal of Plant Research* **118**: 247-255.

Sekiguchi H, Moriya M, Nakayama T and Inouye I (2002) Vestigial chloroplasts in heterotrophic stramenopiles *Pteridomonas danica* and *Ciliophrys infusionum* (Dictyochophyceae) *Protist* **153**: 157–167.

## **Glossary**

### **Lorica**

An extracellular, protective case.

**Microtubular roots**

Microtubules nucleated on the basal bodies of flagella and diverging from them to run superficially under the cell membrane. They are thought to act as additional anchors for the flagella and to position certain organelles in the cell accurately relative to the flagella (e.g. the eyespot).

**Mixotrophy**

Nutrition via both autotrophy (obtaining energy by photosynthesis) and heterotrophy (obtaining energy by digesting organic material).

**Monophyletic (holophyletic)**

On a phylogeny, a monophyletic group may be traced back to a single ancestral species. In addition, this group should include all of the ancestor's descendants. It is recognized by a homologous character state (synapomorphy) in all of its members (cf. paraphyletic, polyphyletic).

**Paraphyletic**

A paraphyletic group originates from a single common ancestor, but all the descendants of this ancestor are not included in the group (cf. monophyly, polyphyly). Its members share only ancestral character states (symplesiomorphies); they do not uniquely share any synapomorphies.

**Phylogeny**

The unique historical relationship (resulting from evolution) among terminal taxa, represented as a tree.

**Pinocytosis**

'Cell drinking' as apposed to 'cell eating' (phagocytosis). The process by which liquid or dissolved material is taken up by a cell.

**Polyphyletic**

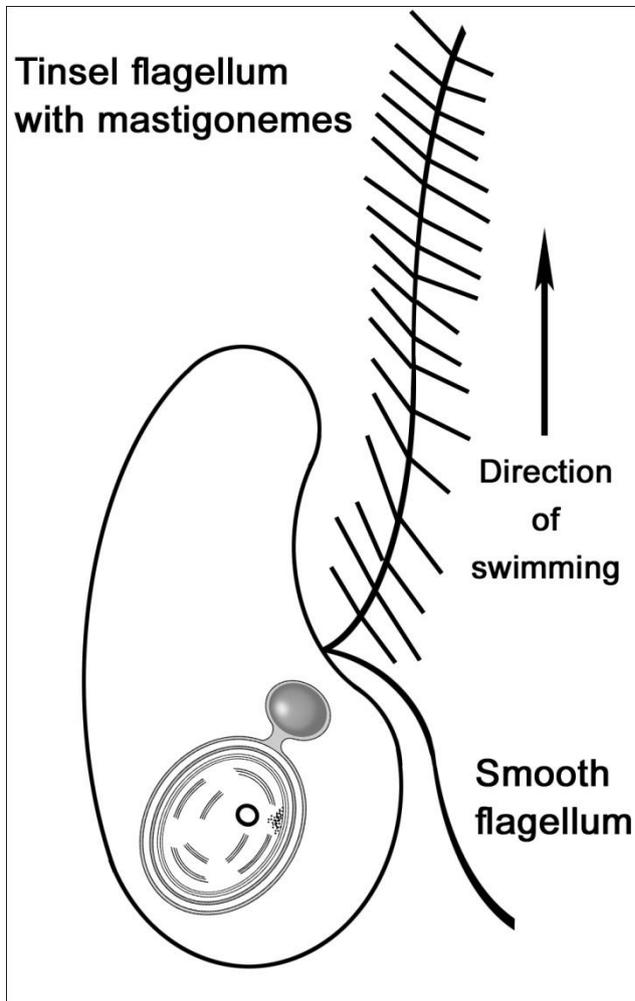
A polyphyletic group has more than one common ancestor, i.e. it has multiple evolutionary origins. This concept is best restricted to groups of hybrid origin, e.g. eukaryotes.

**Rhizoplast**

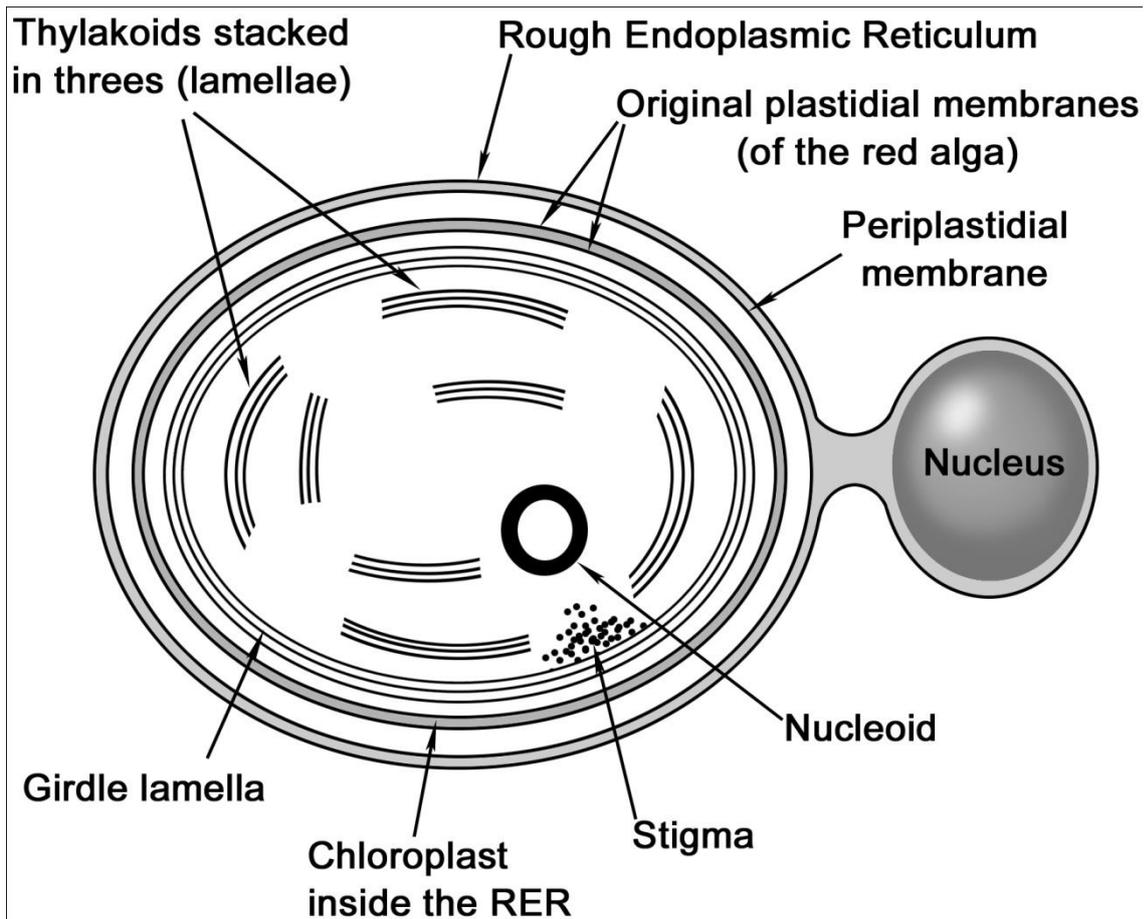
A deep root system, as opposed to the superficial microtubular roots, anchoring the basal bodies of flagella to the nuclear surface.

**Transitional Helix**

A spiral structure found in the transitional region of the flagellum (i.e. the part where the flagellum enters the cell body), usually found external to the microtubular doublets of the flagellum.



**Figure 1:** A typical heterokont flagellate. Most heterokont motiles have apical or near-apical insertion of their flagella whereas this diagram shows the lateral insertion typical of phaeophyte gametes and spores. The tinsel flagellum is anteriorly-directed and the smooth flagellum trails the cell during swimming.



**Figure 2:** The heterokont chloroplast has the normal plastidial double membrane envelope, but surrounded by a periplastidial membrane and by rough endoplasmic reticulum which may be confluent with the outer membrane of the nuclear envelope. The periplastidial membrane is considered to be the remnants of the eukaryotic endosymbiont's (a red alga) plasmalemma. The chloroplast interior is occupied by thylakoids, the outer one/s of which is/are continuous and just beneath the envelope - the girdle lamella.