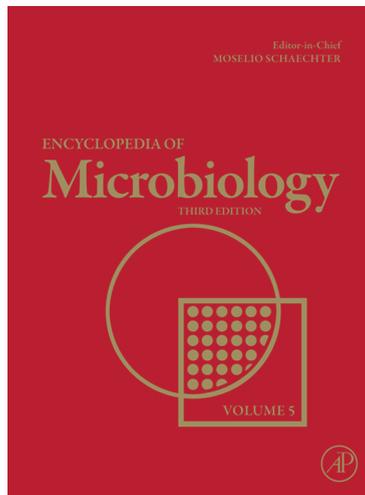


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Stramenopiles

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Defining Statement

Evolutionary History of the Stramenopiles

Diversity of the Stramenopiles

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molecular clock A technique based on DNA or protein sequence comparisons, used to estimate the evolutionary divergence time of two taxa. Fossil dates are used to constrain (calibrate) the divergence time estimation.

monophyly A taxonomic group that shares a single common ancestor.

phylogeny The evolutionary relationship of organisms, usually represented as a bifurcating tree diagram.

Abbreviations

CER chloroplast endoplasmic reticulum

ER endoplasmic reticulum

PER periplastidal endoplasmic reticulum

Defining Statement

The stramenopiles are a monophyletic group of eukaryotes that possess tripartite hairs along one flagellum. Twenty-one classes are recognized including five non-photosynthetic groups. Here, we review knowledge of the diversity and evolutionary history of the stramenopiles, which originated in the late Mesoproterozoic and arose to great ecological and economic importance.

Evolutionary History of the Stramenopiles

The stramenopiles (Latin, *stramen* – straw + *pila* – hairs) are a distinct, highly diverse, and yet, clearly monophyletic group of eukaryotes, all of whose swimming cells possess two different types of flagella (Figure 1). A long anteriorly directed flagellum has two rows of tripartite hairs (i.e., a basal section, a long tubular shaft, and terminal fibrils). The posteriorly directed flagellum is generally short and lacks these hairs. Because the two flagella act differently (i.e., beating pattern), the term heterokont was originally used for this lineage. Subsequently, the term Heterokontae was used over 100 years ago to name some algae now placed in the Xanthophyceae and Raphidophyceae. Approximately

40 years ago, the term Heterokontophyta was introduced for what is today's algal stramenopiles (stramenochromes). The terms chromophyte, Chromista, and ochrophyte are also sometimes used for these taxa, although the former two terms denote additional taxa not confirmed to be directly related to stramenopiles (haptophytes and cryptophytes) and the latter term only denotes a subset of species. Although the term stramenopiles is relatively new (1989), it has gained wide acceptance and is the most commonly used moniker to denote this lineage.

The stramenopiles comprise more than 100 000 species including very diverse life forms from single cells to large plasmodia to complex multicellular thalli. The best known members of the group are the colorless oomycetes (aquatic 'fungi'), diatoms, chrysophyte algae, and giant kelp seaweeds. Photosynthetic stramenopiles are the predominant eukaryotes in most aquatic environments, and they play an important role in ecosystems as major primary producers. The colorless groups include major plant pathogens for cultivated crops (oomycetes) and several groups of flagellate or amoeboid cells that are important in the microbial food web. Molecular evolutionary studies suggest that some, if not all, of the colorless stramenopiles are derived from the photosynthetic stramenopiles by loss of their plastids.

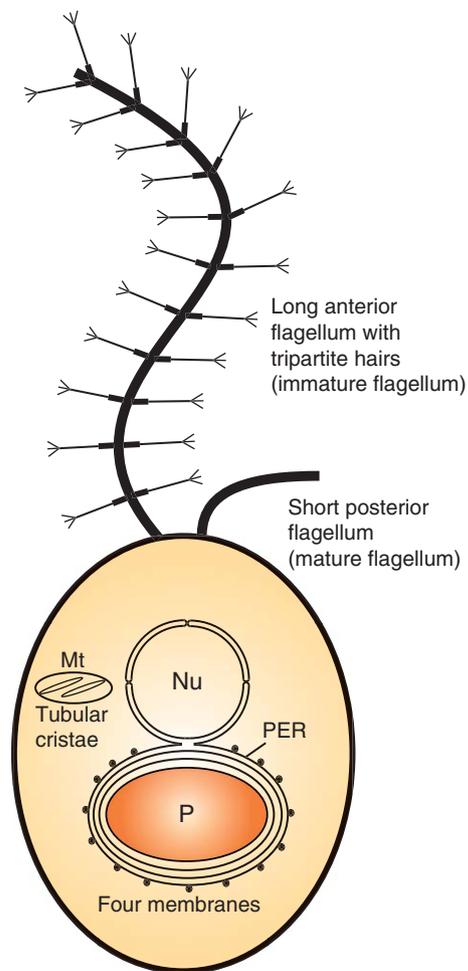


Figure 1 Simplified stramenopile cell. Stramenopiles possess two flagella: one, the long anterior flagellum, has two rows of tripartite hairs, each consisting of a basal section, a longer tubular shaft, and terminal fibrils; and a short and smooth posterior flagellum. The stramenopile plastid (P) is surrounded by four membranes that are located in the periplastidal endoplasmic reticulum (PER) lumen. Tubular cristae are found in the mitochondria (Mt).

Origin of the Stramenopiles

It is now widely accepted that the plastid of stramenopiles originated from a red alga through secondary endosymbiosis (cf. 'secondary endosymbiosis'). That is, a nonphotosynthetic protist engulfed a red alga but rather than digesting it as prey, the alga was maintained permanently. Over many generations, this red alga became a reduced and enslaved photosynthetic organelle; that is, it converted its new host from an animal-like organism into a plant-like organism. Evidence suggests that this event occurred early during the evolution of the chromalveolates, which include not only the stramenopiles, but also the cryptophytes, haptophytes, and alveolates (ciliates, apicomplexa, and dinoflagellates). Thus the chromalveolate hypothesis unites all chlorophyll *c*-containing algae

into a major taxon that also includes a large number of colorless relatives. This hypothesis has received considerable support from molecular phylogenetic studies of nuclear-encoded plastid-target genes and recently, from large multiprotein datasets. More recent phylogenetic studies suggest that chromalveolates may also include the supergroup Rhizaria, or even that Rhizaria may be embedded within what would then be referred to as the 'former chromalveolates' (Figure 2).

Fossil Record and Divergence Times for Stramenopiles

Stramenopiles have an old and extensive fossil record. The earliest known fossil that has been assigned to the stramenopiles is *Palaeovaucheria*, which was found in the upper Mesoproterozoic Lakhanda Formation (*c.*1000 million year ago, Ma) in eastern Siberia. This fossil was regarded as closely related to the modern xanthophycean genus *Vaucheria*. Another xanthophycean algal fossil, *Jacutianema*, was found in the early Neoproterozoic Svanbergfjellet Formation (*c.*750 Ma), Spitsbergen of Arctic Norwegian island, where the earliest known filamentous green algal fossil (*Proterocladus*) was also identified. These two *Vaucheria*-like fossils, however, may also be interpreted as filamentous green algae due to their simple morphology. Microfossils have also been reported from the upper Tindir Group (*c.*740 Ma) in northwestern Canada. On the basis of scale morphologies, the latter were first postulated to be chrysophyte algae but later were identified as scaled amoebae belonging to the Rhizaria. Other more compelling eukaryotic fossil remains were found from the late Neoproterozoic Doushantuo Formation (*c.*600 Ma), South China – *Miaobephyton* and *Konglingiphyton*. These were characterized as being regularly dichotomous, multicellular thalli, with apical and intercalary growth and conceptacles, suggesting an affinity with brown algae. It is noteworthy that there is a very well-preserved fucoid-like brown algal fossil, *Tbalassocystis*, from the Silurian of Michigan. The *Tbalassocystis* fossils are branched, and each branch terminates in an inflated structure that closely resembles the bladders of modern fucoid algae. There are still uncertainties as to the affinity of some stramenopiles fossils, but it is likely that the multicellular stramenopiles diverged prior to the late Neoproterozoic era.

It is sometimes possible to estimate evolutionary dates using a method referred to as a molecular clock analysis. This requires a reliable fossil record and an accurate molecular phylogeny for the group in question, in which case this method can provide a powerful approach to estimate divergence times for ancient evolutionary groups. In fact, the molecular clock method may provide, in some cases, the only possible approach because of the limited fossil data that are available for many ancient groups. However, modeling DNA sequence evolution is the most error prone in such

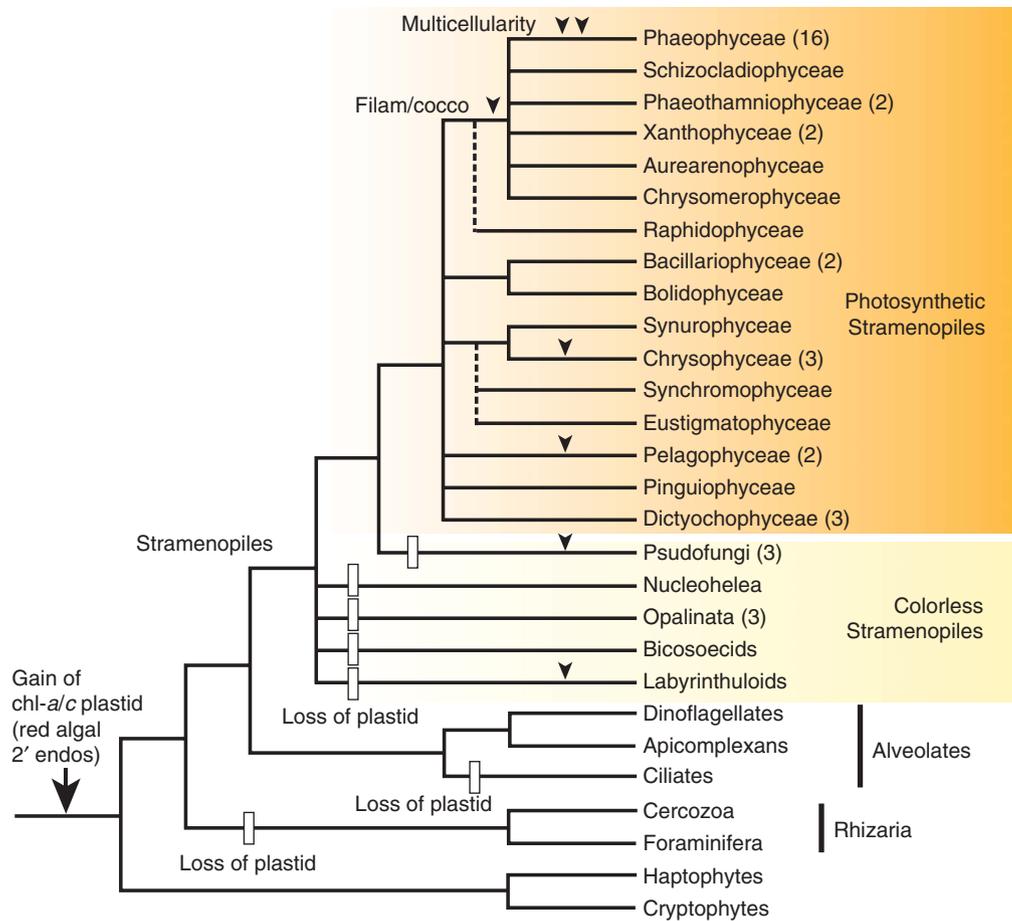


Figure 2 Evolutionary relationships among stramenopiles. An ancient red algal secondary endosymbiosis is thought to have occurred in a unique common ancestor of the chromalveolate + Rhizaria clade. This cell would then have given rise to the diverse chlorophyll *c*-containing protist groups including stramenopiles. Several plastid losses (marked as empty rectangles) occurred in the tree. The alveolates is the most closely related lineage to stramenopiles. The stramenopiles are composed of 21 classes or class-level of lineages including five colorless groups. The colorless pseudofungi and photosynthetic stramenopiles are sister groups of each other. The Phaeophyceae shows true multicellularity (double arrowhead), whereas some lineages have simple filamentous or coccoid forms (Filam/Cocco). Adapted from Adl *et al.* (2005), Andersen (2004), Cavalier-Smith and Chao (2006), Daugbjerg and Guillou (2001), Hackett *et al.* (2007), Horn *et al.* (2007), and Kai *et al.* (2008).

instances due to the accumulation of superimposed mutations in molecular sequences. Using various corrections for these possible sources of error, a recent molecular clock study provided a hypothetical molecular timeline for the origin of photosynthetic eukaryotes using relaxed clock methods with several fossil constraints on a multigene phylogeny (Figure 3). This study estimated the date for the red algal secondary endosymbiosis that gave rise to the stramenopile plastid at $\approx 1261\text{--}1305$ Ma. This is after the split of the Cyanidiales from other red algae ($\approx 1350\text{--}1416$ Ma), that is, considerably after the origin of red algae. This study also placed the date for the primary eukaryotic plastid endosymbiosis (involving a cyanobacterium) at $\approx 1531\text{--}1602$ Ma. The stramenopiles and haptophytes appear to have split $\approx 1025\text{--}1077$ Ma after the cryptophyte's divergence ($\approx 1172\text{--}1219$ Ma) with each of these lineages radiating early in the Neoproterozoic (e.g., 791 Ma for haptophytes, 712 Ma for

stramenopiles, and 720 Ma for cryptophytes). These estimates are still quite preliminary as not all major lineages could be included (e.g., the dinoflagellates), and the addition of earlier diverging taxa could potentially push these dates further back into the Neoproterozoic. Also some parts of the molecular tree were not well resolved. The tree used plastid genes, and the relationship between these and host divergence times is still not clear. Nonetheless, this study suggests a late Mesoproterozoic origin of photosynthetic eukaryotes and a Neoproterozoic diversification of the major eukaryotic lineages. This estimate is consistent with the fossil record of eukaryotes based on biomarker data, such as steranes (2715–2600 Ma) from the Marra Mamba and Maddina Formations in Australia; *Taraxia* and *Chuarina* (1800–1900 Ma) from the Changzhougon Formation in China; acritarchs (1400–1500 Ma) from the Roper Group of Australia; and a reliable red algal *Bangiomorpha* fossil (1198 Ma) from the Hunting

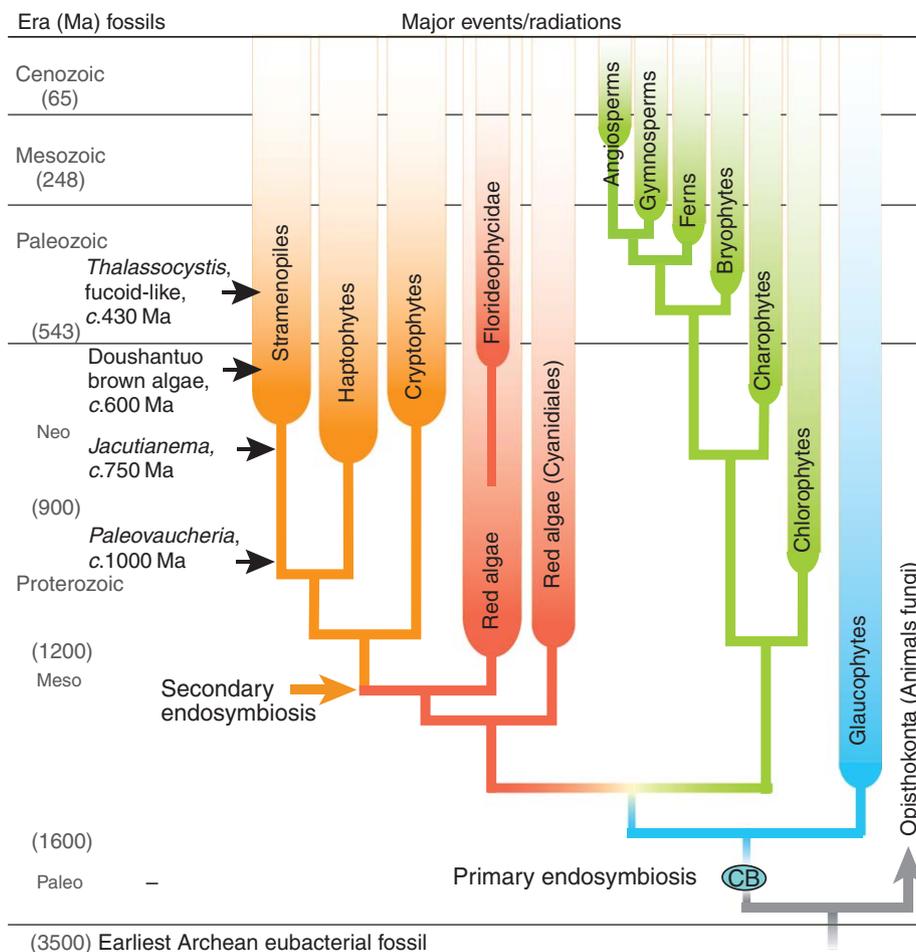


Figure 3 Molecular timeline for the origin of photosynthetic eukaryotes. The red, green, and glaucophyte algae diverged after primary endosymbiosis. Through secondary endosymbiosis, an ancestor of cryptophyte, haptophyte, and stramenopiles acquired its plastid from a red alga. The existence of Proterozoic fossils that are identified as stramenopiles are generally consistent with the divergence time of stramenopiles shown here. Modified from Yoon *et al.* (2004), with permission from Oxford University Press, Oxford, UK and adapted from Butterfield (2004), Allison and Hilgert (1986), and Xiao (1998) for fossil records.

Formation in arctic Canada. Thus, this molecular clock suggests the origin of stramenopiles to be around 1000 Ma followed by a major radiation at *c.*700 Ma.

Diversity of the Stramenopiles

The stramenopiles are one of the most diverse eukaryotic groups. Twenty-one class-level divisions are currently recognized, including five that are not photosynthetic. The stramenopiles encompass over 100 000 species, although some authors suggest there may be over 1 million species for the diatoms alone. (The diatoms are the ‘insects’ of the algal world, i.e., there are many species, yet their morphological diversity is constrained.) Regardless of the exact number of species that have been formally described, most scientists agree that there are many more undescribed species, probably including entire new classes (cf. ‘Picoeukaryotes’). Transmission electron

microscopy and molecular systematic studies have resulted in the description of 13 new class-level lineages of stramenopiles during the past several decades. That is, earlier classifications based on light microscopy did not recognize the details that became apparent when the internal cell structures were examined and genetic comparisons were made. We now know that the stramenopiles also include species previously classified as distinct kingdoms of algae, fungi, and protozoa 60 years ago.

Stramenopiles thrive in many environments, from freshwater to seawater, on soils, in animals, within snow banks, as parasites on plants, and even growing as disease organisms within the guts of humans. They also have diverse life forms from typical autotrophic algae or heterotrophic flagellates and amoebae to mixotrophic organisms that are capable of both autotrophy (photosynthesis) and heterotrophy (herbivory, carnivory). The size and the shape of these organisms vary from tiny, microscopic single cells (two thousandths of a millimeter in diameter) to giant kelp

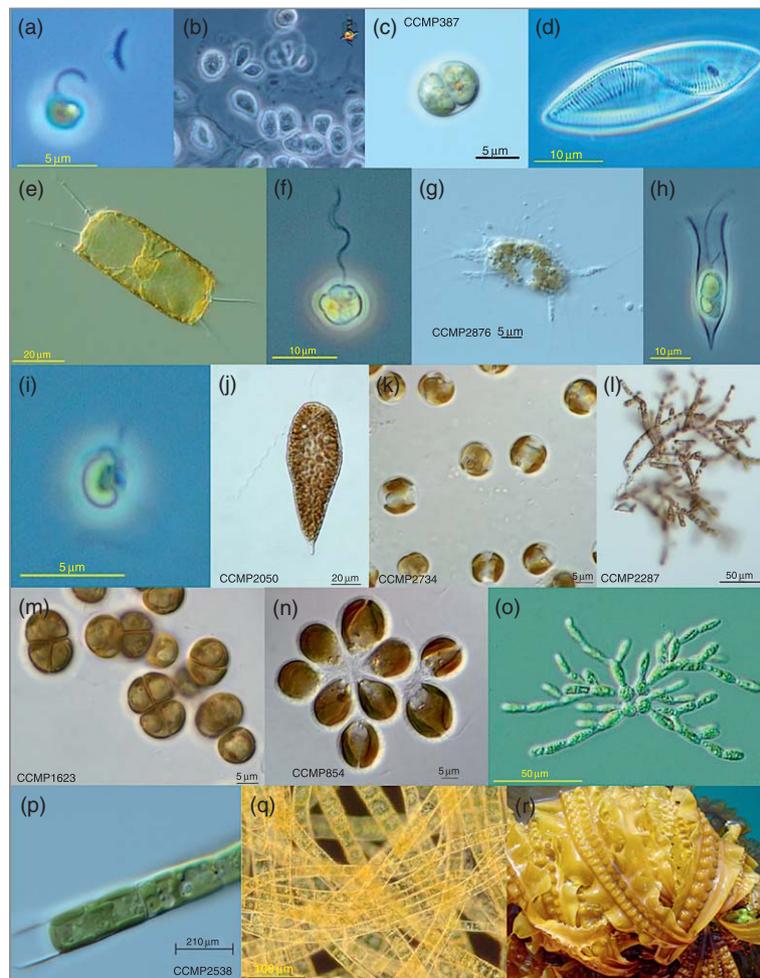


Figure 4 Examples of different stramenopile types. (a) *Symbiomonas* (Bicoecaceae); (b) *Labyrinthula* (labyrinthulids); (c) *Eustigmatos* (Eustigmatophyceae); (d) *Entomoneis* (Bacillariophyceae – pinnate diatom); (e) *Odontella* (Bacillariophyceae – Centric diatom); (f) *Pseudopedinella* (Dictyochophyceae); (g) *Synchronoma* (Synchromophyceae); (h) *Dinobryon* (Chrysophyceae); (i) *Bolidomonas* (Bolidophyceae); (j) *Chattonella* (Raphidophyceae); (k) *Chrysoreinhardia* (Pelagophyceae); (l) *Schizocladia* (Schizocladophyceae); (m) *Glossomastix* (Pinguiphyceae); (n) *Synura* (Synurophyceae); (o) *Phaeothamnion* (Phaeothamniophyceae); (p) *Tribonema* (Xanthophyceae); (q) *Pylaiella* (Phaeophyceae); (r) *Laminaria* (Phaeophyceae). All images are from the National Center for Culture Collection of Marine Phytoplankton of USA (CCMP), except (b) from Micro*scope and (r) from south coast of Korea taken by SMB.

seaweeds that often reach up to 60 m in length (Figure 4). Unicellularity is the most widely occurring life form, but colonies of cells are common, and the true multicellular seaweeds produce a complex thallus with rootlike, stemlike, and leaflike structures.

The Stramenopile Plastid

The plastid in photosynthetic stramenopiles is structurally distinctive in that it possesses four outer membranes, the outermost of which corresponds to the rough endoplasmic reticulum (ER) (Figure 1). This chloroplast endoplasmic reticulum (CER) or periplastidal endoplasmic reticulum (PER) is continuous with the outer nuclear membrane. The existence of four membranes appears to be a result of the secondary endosymbiosis that gave rise

to the stramenopile plastid. Thus, the inner three membranes are retained from the original red algal symbiont – the first, second, and third membrane corresponding to the cyanobacterial membrane, the primary host vacuolar membrane, and the primary host cytoplasmic membrane, respectively (for further information, see ‘Secondary endosymbiosis’).

Secondary acquisition of plastids is complicated by the fact that over 95% of the genes encoding plastid proteins are located in the host nucleus. This is a consequence of endosymbiotic gene transfer, where roughly 500–1000 formerly plastid-encoded genes have, over time, been relocated to the host nucleus. The nuclear-encoded plastid-target proteins have complex N-terminal extensions (transit peptides) to direct their posttranslational import into the plastid. Thus, secondary endosymbiosis

required not only the acquisition of a plastid, but also the acquisition of hundreds of nuclear genes required to service and reproduce these plastids. This is further complicated in the new (secondary) host by the requirement that the protein products of these genes must now be passaged through four plastid membranes. This is accomplished by the ribosomes attached to the outermost membranes of the PER lumen. Thus targeting into the PER membrane is achieved with a signal peptide that has an additional hydrophobic region. This bipartite leader sequence subsequently targets proteins across the inner two plastid membranes using a downstream transit peptide.

Stramenopile plastids have lamellae, each formed by three stacked or appressed thylakoids. In almost all species (excluding Eustigmatophyceae), a girdle lamella surrounds the other lamellae. Whereas the regular lamellae are more or less platelike, the girdle lamella is spheroidal, completely enclosing the other lamellae. The photosynthetic product is a β -1,3-linked glucan that is stored in a vacuole in the cytosol. Photosynthetic pigments vary in different lineages and are summarized in **Table 1**. The major photosynthetic pigments are chlorophylls *a*, *c*₁, *c*₂, *c*₃, and carotenoids (e.g., diatoxanthin, fucoxanthin, violaxanthin). The distribution of pigments is too variable to identify any clear evolutionary trend.

An eyespot is located within the plastid in most cases, except in the Eustigmatophyceae where it is located outside of the plastid. This eyespot is associated with the mature (generally shorter) flagellum, and together these are referred to as the photoreceptor apparatus. These structures detect the direction of the light source, which is used in turn to change the direction of cell movement (i.e., phototaxis).

There are five major colorless groups of stramenopiles, and, assuming the ancestral stramenopile was photosynthetic, this means that plastid loss has occurred, possibly several times independently. Since transfers of plastid genes to the nucleus are common in plastid-containing cells, some of these transferred plastid genes may remain in the genomes of taxa that have secondarily lost the plastid. There are two possible examples of this in colorless stramenopiles. Glutamine synthetase, an essential gene in glutamine biosynthesis and ammonium assimilation, has three gene family members (GSI, GSII, and GSIII). A recent study indicated that the GSII gene from two oomycetes (colorless stramenopiles) shows a strongly supported monophyletic relationship with the GSII genes of photosynthetic diatoms and green and red algae. This suggests that the GSII gene of oomycetes evolved through endosymbiotic gene transfer from a red algal plastid, presumably early in stramenopile evolution. Similarly, a 6-phosphogluconate dehydrogenase gene (*gnd*), in members of the oomycete genus *Phytophthora*, shows a cyanobacterial origin and groups together with homologues found in photosynthetic stramenopiles and red and green algae. Thus, while GSII and *gnd* are not directly involved in photosynthesis, their presence strongly suggests that 'footprints' of a red algal endosymbiosis are still detectable in the colorless oomycetes. This hypothesis was recently substantiated with the finding of at least 30 genes of putative cyanobacterial and algal (i.e., endosymbiotic) origin in the completely sequenced nuclear genomes of two oomycete species (*Phytophthora* spp.).

In addition to the exclusively heterotrophic stramenopiles (e.g., oomycetes, thraustochytrids), there are some

Table 1 Plastid pigmentation and cell coverings of the stramenopiles

Class	Chlorophyll	Carotenoid	Cell covering
Aurearenophyceae	<i>a</i>	fuc, dia, vio	Cell walls
Bacillariophyceae	<i>a</i> , <i>c</i> _{1,2} (<i>c</i> ₃)	fuc, hex, dia	Siliceous frustules
Bolidophyceae	<i>a</i> , <i>c</i> ₁₋₃	fuc, hex, dia	Naked flagellates
Chrysomerothryceae	<i>a</i> , <i>c</i> _{1,2}	fuc, vio	Cell walls
Chrysophyceae	<i>a</i> , <i>c</i> _{1,2}	fuc, vio	Cell walls, organic loricas, organic or silica scale cases, gelatinous coverings, naked cells
Dictyochophyceae	<i>a</i> , <i>c</i> _{1,2}	fuc, dia	Silica skeletons, organic scales, or naked cells
Eustigmatophyceae	<i>a</i>	vio, vau	Cell walls
Pelagophyceae	<i>a</i> , <i>c</i> _{1,2}	fuc, (hex), but, dia	Cell walls, thecae, gelatinous coverings, naked cells
Phaeophyceae	<i>a</i> , <i>c</i> _{1,2}	fuc, vio	Cellulosic cell walls
Phaeothamniophyceae	<i>a</i> , <i>c</i> _{1,2}	fuc, dia, het	Cell walls
Pinguiphyceae	<i>a</i> , <i>c</i> _{1,2}	fuc, vio	Mineralized loricas, gelatinous coverings, or naked cells
Raphidophyceae-FW	<i>a</i> , <i>c</i> _{1,2}	fuc±, dia, het, vau	Naked cells
Raphidophyceae-Mar	<i>a</i> , <i>c</i> _{1,2}	fuc±, vio	Naked cells
Schizocladophyceae	<i>a</i> , <i>c</i> (type ?)	fuc, (?)	Cell walls without cellulose
Synchromophyceae	<i>a</i> , <i>c</i> ₂	fuc, vio	Lorica
Synurophyceae	<i>a</i> , <i>c</i> ₁	fuc, vio	Silica scales
Xanthophyceae	<i>a</i> , <i>c</i> _{1,2}	vio, het, vau	Cell walls

fuc, fucoxanthin; hex, 19'-hexanoyloxyfucoxanthin; but, 19'-butanoyloxyfucoxanthin; dia, pigments of the diatoxanthin and diadinoxanthin cycle; vio, pigments of the violaxanthin, antheraxanthin, zeaxanthin cycle; het, heteroxanthin; vau, vaucherioxanthin; ±, present or absent; (?), unknown.

Modified from Andersen (2004), Horn *et al.* (2007), and Kai *et al.* (2008).

colorless stramenopiles reported among the photosynthetic lineages. These are most certainly due to the secondary loss or reduction of the ancestral plastid. For example, *Pteridomonas danica* and *Ciliophrys infusionum* are colorless species of the Dictyochophyceae, a class that consists largely of photosynthetic members. These two species, however, retain an unpigmented plastid (leucoplast) that still encodes the *rbcL* gene. This indicates that the loss of photosynthetic ability occurred relatively recently, and apparently also independently in these two species. A remnant plastid is also found in some chrysophycean algae (e.g., *Spumella*, *Paraphysomonas*, and *Anthophyssa*) as well as in certain diatom species.

Cell Covering

The cell coverings found among stramenopiles are very diverse (Table 1). The cell wall is the most common covering, but there are several types. Probably the most striking are the diatoms, which have cell walls (frustules) made of opaline glass, identical to that found in windowpanes. Organic cell walls are found in the brown algae, oomycetes, yellow-green algae, and certain species in many other classes. Oomycetes generally have a cellulosic wall, but some species have chitinous walls and others have walls based on β -1,3-linked glucans. Meanwhile, brown algae have a cellulosic wall that is impregnated with alginate and silicate, but Schizocladiphyceae have a cellulosic wall without alginate. For other classes, the composition of walls is still not known (e.g., Pelagophyceae and Phaeothamniophyceae).

Scales are the second most common cell covering. Silica scales are found on all Synurophyceae and some Chrysophyceae. Organic scales cover the cells of some Chrysophyceae, some Dictyochophyceae, some labyrinthulids, and organic scales cover the flagella in the Synurophyceae. Loricas, wall-like coverings with at least one opening, are common among the Bicosoeciophyceae and Chrysophyceae. Loricas may be composed of an intertwining network of cellulose-like fibrils, or of numerous organic scales or sometimes the lorica is impregnated with mineral deposits (e.g., iron, manganese). Naked cells are also widespread among some of the stramenopile groups (e.g., Bolidophyceae, Chrysophyceae, and labyrinthulids), whereas other naked cells are surrounded by only a gelatinous matrix. The silicoflagellates have a silica skeleton, which is neither a wall nor a scale.

Flagella

The stramenopiles usually have two flagella (Figure 1), a long anteriorly directed flagellum (immature flagellum) that bears two rows of tripartite hairs (mastigonemes), and a (usually) short posteriorly directed flagellum (mature flagellum). The flagellar hairs on the flagellum reverse

the swimming direction, that is, the same flagellar action causes the cell to be pushed backward when the hairs are lacking, but the cell is pulled forward when the hairs are present. A small number of stramenopiles have only one flagellum, specifically diatom sperm cells, *Pelagomonas* species (Pelagophyceae), some species of *Mallomonas* (Synurophyceae), and the zoospores of Hyphochtridiomycetes. Nonetheless, this single flagellum in all cases still has the tripartite hairs of the distinctive stramenopile type. The flagellum axoneme typically has the 9 + 2 microtubular arrangement, but the diatom sperm flagellum is an exception because it lacks the central pair of microtubules (9 + 0 arrangement). Stramenopile flagella are inserted subapically or laterally and are usually supported by four microtubule roots that are arranged in a distinctive pattern. However, a number of groups have a reduced flagellar apparatus with few or no microtubular roots (e.g., diatom sperm, Pelagomonadales, and Dictyotales). Other features of the flagellar apparatus include a green autofluorescent substance in many organisms, the presence or absence of a transitional helix, and the occasional presence of a paraflagellar rod (Table 2).

Phylogeny and Classification of the Stramenopiles

Stramenopiles constitute at least 21 major classes. Five colorless lineages, which are presumed to have lost their plastid secondarily, are positioned at the base of the group (Figure 2). Phylogenetic relationships among these classes are still mostly unresolved (Figure 2), probably due to the insufficient phylogenetic information from current molecular phylogenies, which are based on single gene datasets (i.e., SSU or *rbcL*). Multigene studies are likely to solve this problem but these are currently extremely limited in taxon sampling within stramenopiles, often only including a single class. Beginning in 2007, the National Science Foundation, USA, funded a tree of life (ATOL) project on the 'Algal Heterokont Tree of Life' (<http://ccmp.bigelow.org/>), which will generate sequences for 7 genes from 300 stramenopiles species and plastid genomes from representatives of 30 genera. As with similar multigene studies of major eukaryotic taxa, it is expected that phylogenetic analyses of these data will resolve most if not all class-level relationships among stramenopiles at the end of this 5 year project.

Colorless Stramenopiles

Five colorless stramenopile class-level lineages are recognized. These appear to diverge at the base of the clade, although relationships among these lineages are not yet resolved. Nonetheless, a few tentative conclusions can be drawn at this time. The Opalinata, which includes the

Table 2 Features of the flagellar apparatus of the stramenopiles

Class	Tri-H	# flagella	TH	Flagella beat	Lateral hairs	Mt roots	Green flagellum	Paraflagella rod	Striated root
Bacillariophyceae	+	1,0	0	Pulls	–	0	–	?	–
Bolidophyceae	+	2	0	Pulls	–	0	?	–	–
Chrysomerophyceae	+	2	6↑	Pulls	–	4	?	–	?
Chrysophyceae	+	2	4–6↑	Pulls	+	2–4	+	–	+
Dictyochophyceae	+	1	0–2↓	Pulls	–	0	–	+	–
Eustigmatophyceae	+	2,0	6↑	Pulls	–	4	–	–	+
Pelagophyceae	±	2,1,0	0–2↓	Pulls	–	0	–	+	–
Phaeophyceae	+	2	0	Pulls	–	4	+	–	–
Phaeothamniophyceae	+	2	6↑	Pulls	–	4	+	–	+
Pinguiphyceae	±	2,1,0	0,2↓	Pulls, ?	–	3,4	±	–	+
Raphidophyceae	+	2	0	Pulls	–	?	–	–	?
Schizocladophyceae	+	2	6↑	Pulls	–	?	+	–	?
Synurophyceae	+	2,1	6–9↑	Pulls	+	2	+	–	+
Xanthophyceae	+	2,0	2 × 6↑	Pulls	–	4	+	–	+

Tri-H, tripartite tubular hairs; TH, transitional helix; mt roots, microtubular roots. Arrows (↑; ↓) indicate the position (distal, proximal) of the transitional helix with respect to the major transitional plate. +, present; –, absent; ±, present or absent; ?, unknown.

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

Opalina, Proteromonadea, and Blastocystea, shows a monophyletic relationship with the Nucleohelea (Figure 5). The genus *Opalina* is unusual because it occurs only in the cloaca of frogs and other amphibians. The labyrinthulids (Figure 4(b)) form a monophyletic group, whereas relationships of the Bicoecia are unresolved. *Labyrinthula* spp. typically grow on coastal seaweeds, where they form networks of slime along which the cells glide. The genus *Bicosoeca* (Figure 4(a)) is made up of naked or loricate flagellates. In these species, the mature flagellum is used to anchor the cell to a substrate, and the immature (hairy) flagellum draws bacteria to the anterior of the cell, where these prey are ingested by phagocytosis. In addition to the 21 recognized classes, a number of class-level stramenopile assemblages have recently been identified from environmental surveys. Since the organisms corresponding to these sequences have yet to be identified and isolated, they are still known only from partial 18S rRNA sequences (phylotypes). Therefore, these environmental samples are generally depicted as independent lineages at the base of the clade. Among other things, it is not even unknown if these sequences are from pigmented (photosynthetic) or unpigmented organisms.

An informal grouping, the pseudofungi appears to be the sister group to the photosynthetic stramenopiles. The monophyly of pseudofungi is uncertain, but it appears to include the oomycetes, the hyphochytrids, and the Bigyromonadea (certain zooflagellates like *Developiella*). The Oomycetes (water molds), which used to be classified as fungi, include some of the most important known plant pathogens. For example, *Phytophthora infestans* causes late blight of potato, which led to the Great Irish potato famine. Other *Phytophthora* species are the main causes of some of the most important diseases of plants, such as

dieback, sudden oak death, rhododendron root rot, and ink disease in the American chestnut tree. Various species of *Pythium* cause diseases such as seed rot and ‘damping off’ in young plant seedlings. A freshwater mold, *Saprolegnia*, causes tail rot on fish such as salmon and trout. Therefore, these colorless stramenopiles are important both in economic and in scientific terms. For these reasons, genome sequencing projects are completed or underway for three *Phytophthora* species (*P. infestans* T30-4, *Phytophthora ramorum*, *Phytophthora sojae*).

Photosynthetic Stramenopiles

Sixteen photosynthetic classes of stramenopiles (including two recently described classes, the Synchronophyceae and the Aurearenophyceae) are currently known. As with the nonphotosynthetic taxa, the phylogenetic relationships among the photosynthetic classes are still largely unresolved (Figure 2). However, there is some indication that Phaeophyceae, Schizocladophyceae, Phaeothamniophyceae, Xanthophyceae, Aurearenophyceae, and Chrysomerophyceae may form a clade (PSPXAC clade). These six PSPXAC classes also share common morphological characters, such as the presence of coccoid, filamentous, or true multicellular thalli (see arrowhead in Figure 2). The Xanthophyceae (yellow-green algae; Figure 4(p)) are a diverse class, with many morphological types. The filamentous forms may be siphonous or composed of cells with cell walls. These cell walls may be entire or they may be formed from two H-shaped pieces. The single cells are mostly coccoid, but capsoid, amoeboid, and flagellate cells have been described. Colonies of various types are also known. The siphonous genus *Vaucheria* grows on wet soils where it forms a velvet-like mat.

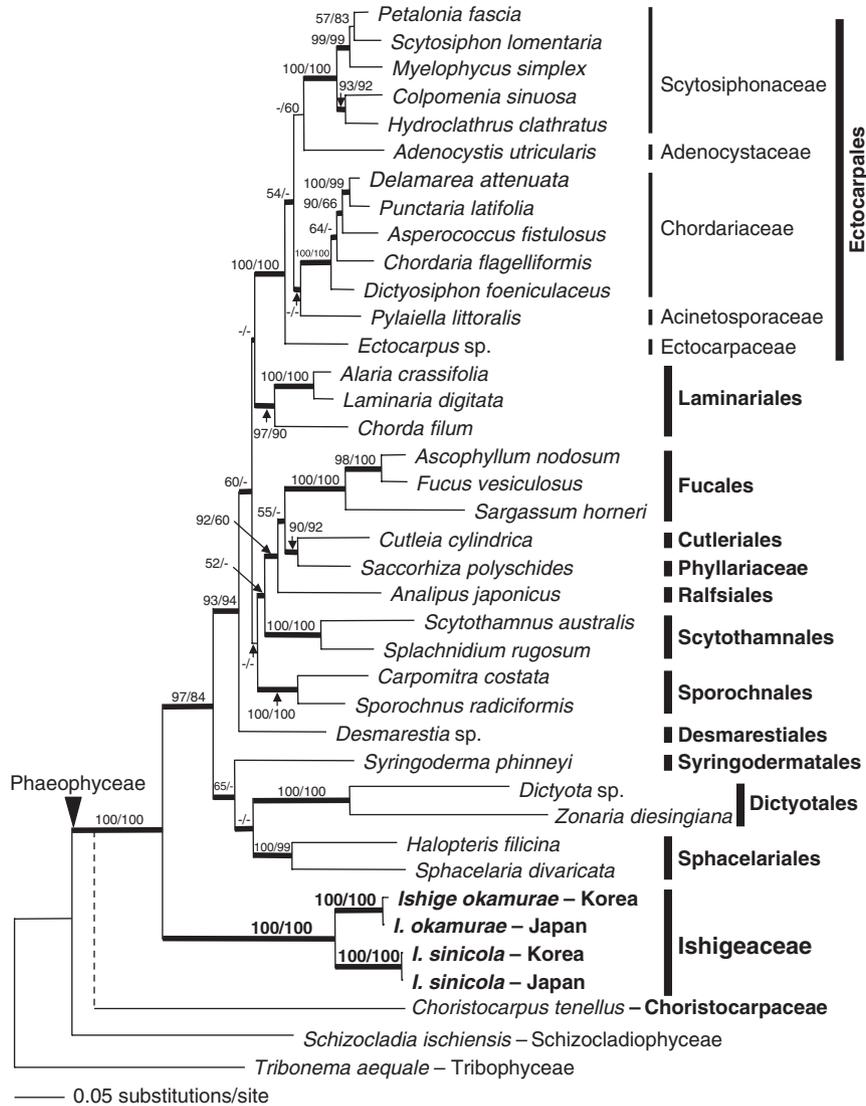


Figure 5 Maximum likelihood (ML) tree of the Phaeophyceae using a concatenated *rbcL*, *psaA*, and *psbA* dataset. The bootstrap values shown above the branches are from maximum likelihood/maximum parsimony methods and dashes indicate <50% bootstrap support. The thick branches indicate Bayesian posterior probabilities >0.9. The dashed line for the Christocarpaceae was based on a *rbcL* phylogeny in the same study. Modified from Cho GY, Lee SH, and Boo SM (2004) A new brown algal order, Ishigeales (Phaeophyceae), established on the basis of plastid protein-coding *rbcL*, *psaA*, and *psbA* region comparisons. *Journal of Phycology* 40: 921–936.

The Phaeothamniophyceae (Figure 4(o)) are also diverse, with unicellular, colonial, and filamentous species occurring in freshwater and marine environments. *Phaeothamnion* is a branched filament that occurs in freshwater ponds, where it grows attached to pondweeds, other algae, and rocks. The Schizocladiphyceae (Figure 4(l)) contain only a single filamentous species, which is similar to the Chrysomerophyceae. The unusual *Schizocladia* has been reported only from the Bay of Naples, Italy. The recently characterized class Aurearenophyceae includes only coccoid species. *Aurearena* is known only from sandy beaches in Japan; the coccoid cell is unusual because inside the cell wall resides the living cell with two fully formed flagella.

The Phaeophyceae (brown algae; Figures 4(q) and 4(r)) are filamentous or parenchymatous in form. Some members of the class have true multicellularity with different tissue types and different organ-like structures including a holdfast, stipe, and blade that are structurally analogous to the root, stem, and leaf of land plants. In addition, these phaeophytes possess complex male and female reproductive structures, phloem sieve cell-like tissue for conductance, and plasmodesmata for communication between cells. This is the only group among the stramenopiles in which true multicellularity has arisen (see double arrowhead in Figure 2), although filaments and colonies of multiple cells are found in many

different stramenopile groups. The Raphidophyceae (Figure 4(j)) may also belong to this lineage. This class has marine members that are brown in color but freshwater species that are bright green. Species of the marine genus *Heterosigma* have caused massive salmon mortalities when blooms of the alga occur near salmon farms.

A second major clade within the photosynthetic stramenopiles is the strongly supported CSSE clade, including the Chrysophyceae, Synurophyceae, Synchronophyceae, and Eustigmatophyceae. The Chrysophyceae (golden-brown algae; Figure 4(h)) are an old taxonomic class, which originally contained numerous organisms that are now placed in other classes. Most members are freshwater flagellates, including single cells, colonies, loricate cells, and scaled cells. *Uroglenopsis* may grow abundantly in reservoirs, and blooms of this alga cause taste and odor problems when the reservoir water is used as a human drinking water source. The Synurophyceae (silica-scaled algae; Figure 4(n)) are mostly freshwater single-celled or colonial flagellates that bear bilaterally symmetrical silica scales. *Synura* and *Mallomonas* are common in unpolluted freshwater, and *Synura* may cause taste and odor problems in drinking water. The Synchronophyceae (Figure 4(g)) contain one described species, which is an unusual marine amoeba with 20 or more chloroplasts per cell. *Synchroma* is known only from the Canary Islands where it grows on basaltic rock in tide pools. The Eustigmatophyceae (Figure 4(c)) are a class of almost exclusively coccoid microalgae that occur in a variety of habitats, including freshwater, seawater, and soils. The genus *Nannochloropsis* is used widely as a food for shellfish, and more recently it has been grown as a source of lipids in efforts to produce biodiesel.

The phylogenetic affinities of the remaining classes of photosynthetic stramenopiles, Pelagophyceae, Pinguiphyceae, and Dictyochophyceae, remain uncertain. The Pelagophyceae (Figure 4(k)) are predominately marine microalgae, and *Pelagomonas* and *Pelagococcus* are important components in the open-ocean phytoplankton (open oceans cover 66% of the earth's surface). The Pinguiphyceae (Figure 4(m)) are a marine microalgal group that is famous for its high content of omega-3 fatty acids. For example, *Pinguicoccus* forms large vacuoles full of eicosapentaenoic acid, which is known to provide human health benefits by reducing heart disease, breast cancer, and rheumatism. The Dictyochophyceae (Figure 4(f)) are flagellate and amoeboid algae found in oceans and freshwater lakes and ponds. The group is named after the silicoflagellates, which are flagellate cells that each occupy an elaborate siliceous skeleton or basket. Conversely, the Bacillariophyceae and Bolidophyceae form a strong monophyletic group in all phylogenetic analyses. The Bolidophyceae (Figure 4(i)) are a small group of naked biflagellate cells that occur in the open oceans, especially in tropical waters. The Bacillariophyceae (diatoms; Figures

4(d) and 4(e)) are the most species-rich class of stramenopiles, and they comprise more than 200 genera and at least 100 000 species.

Diatoms are characterized by a silica frustule with two parts fitting one within the other like a glass box. This structure encases the cell through most of its life cycle. Traditionally, diatom species are classified based on the shape of the frustule into the centric diatoms (radially symmetrical; Figure 4(e)) and pennate diatoms (bilaterally symmetrical; Figure 4(d)), although this classification is now rejected by molecular phylogenetic studies. Because of these elaborate and beautiful silica frustules, diatoms have been known and studied for a long time and are popular in applied fields, such as architectural science and design. Diatoms thrive in almost all environments from freshwater and oceans to wet soils. Therefore, they are important primary producers in all water bodies, contributing about 45% of total eukaryotic marine primary production. Diatoms also play an important role in the global silicate cycle, and silicate sediments are frequently important in the geological record. Diatoms are also used in water quality assessment, as different species are characteristic of different trophic conditions. Because of their ecological importance, genome sequencing projects have been completed for two diatom species, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*, and a number of others are under way (e.g., *Pseudo-nitzschia*).

Phaeophyceae

The Phaeophyceae (brown algae) include 16 orders with approximately 285 genera and about 1800 species. Morphologies range from simple microscopic filaments to giant kelps that may reach 60 m in length. The large multicellular Phaeophyceae, or giant kelps, play important roles in coastal marine environments. These are truly multicellular organisms, consisting of a variety of differentiated multicellular structures including a holdfast, stipe, and blade(s). The latter use specialized conductive cells (called trumpet hyphae based on their shape) for translocation of metabolites. Giant kelps produce a large biomass with high growth rates and form marine forests that serve as habitats for a diversity of organisms. Many brown algal species are also important as human food sources, and brown algal-based industries have existed for thousands of years in Asia. Aquaculture of kelp species is very popular in Korea and Japan with a significant economic impact. Bioremediation using kelp cultivation is also under development.

Early studies of brown algae morphological traits (i.e., thallus organization, mode of growth, and type of sexual reproduction and life history), led to the suggestion that the simple filamentous Ectocarpales were the earliest diverging group, whereas the morphologically complex

Fucales and Durvilleales were the most derived. This 'simple is primitive' concept was challenged by molecular phylogenetic studies. Phylogenies based on rDNA and/or *rbcL* sequences of brown algae suggested that reevaluation of the traditional classification system was needed. For example, the concept of the Ectocarpales was changed to include the Chordariales, Dictyosiphonales, Punctariales, and Scytosiphonales, while the Durvilleales was synonymized with the Fucales. The Laminariales now contain primitive kelps such as Pseudochordaceae and Akkeshiphycaceae, and it excludes the Phyllariaceae.

A recent, detailed multigene study using a fairly taxonomically broad sampling of species (Figure 5) provides an overall picture of brown algal phylogeny. Although much remains to be resolved, it is clear that the Choristocarpaceae are the earliest diverging lineage, followed by a newly established order, Ishigeales, which is in turn followed by a clade, including Sphacelariales, Dictyotales, and Syringodermatales. The remaining taxa (e.g., Fucales, Laminariales) cluster together in a monophyletic 'crown' group, within which the branching orders remain unresolved. Interestingly, the simple filamentous Ectocarpales are not located at the base but rather at the tip of the phylogeny, suggesting that ectocarpalean morphology represents a secondarily derived simplification. Taken together, contemporary taxa belonging to the basal group of brown algae occur mostly in tropical to warm water, whereas most brown algae that exhibit the greatest diversity in terms of species and morphology occur in cold waters.

An extreme level of conflict between morphological and molecular concepts for genera is shown in *Colpomenia*, *Petalonia*, and *Scytosiphon*. These taxa are found in most intertidal areas from tropical to temperate regions, and cryptic species and misapplied names are common. Because convergent evolution is a common theme in brown algae, robust multigene phylogenies are greatly needed in order to reevaluate brown algal diversity and the evolution of their ultrastructural and life history traits.

Conclusion

The stramenopiles, which include diatoms, brown algae, and oomycetes, are a well-known and important group of aquatic organisms and plant pathogens. Major advances in understanding their origin and evolution have occurred with the development of advanced molecular and ultrastructural techniques. However, many questions remain,

including many of the deep phylogenetic relationships within the group. Multigene analyses from a broad taxon sampling are essential in our efforts to unravel the evolutionary history of this group, and this is the aim of the Heterokont Tree of Life project. In addition, several genome projects, including sequences from the oomycetes *P. sojae* and *P. ramorum*, the diatoms *T. pseudonana* and *P. tricornutum*, and the brown alga *Ectocarpus* sp., are contributing significant new knowledge to our understanding of the stramenopiles.

See also: Plant Pathogens: Oomycetes (water mold); Paleontology, Microbial; Picoeukaryotes; Secondary Endosymbiosis

Further Reading

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Relevant Website

<http://ccmp.bigelow.org/> – National Center for Culture of Marine Phytoplankton