Pl. Syst. Evol. [Suppl.] 11: 187–219 (1997)

—Plant—
Systematics
and
Evolution

Phylogenetic relationships of the 'golden algae' (haptophytes, heterokont chromophytes) and their plastids

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Key words: *Chrysophyceae, Haptophyceae.* – Actin, chloroplast, chromophyte, diatoms, heterokont, plastid, phylogeny, *rbc*L, stramenopiles, SSU rRNA, *tuf*A.

Abstract: The phylogenetic relationships of the "golden algae", like all algae, were rarely addressed before the advent of electron microscopy because, based upon light microscopy, each group was so distinct that shared characters were not apparent. Electron microscopy has provided many new characters that have initiated phylogenetic discussions about the relationships among the "golden algae". Consequently, new taxa have been described or old ones revised, many of which now include non-algal protists and fungi. The haptophytes were first placed in the class *Chrysophyceae* but ultrastructural data have provided evidence to classify them separately. Molecular studies have greatly enhanced phylogenetic analyses based on morphology and have led to the description of additional new taxa. We took available nucleotide sequence data for the nuclear-encoded SSU rRNA, fucoxanthin/chlorophyll photosystem I/II, and actin genes and the plastid-encoded SSU rRNA, *tuf*A, and *rbc*L genes and analysed these to evaluate phylogenetic relationships among the "golden algae", viz., the *Haptophyceae* (= *Prymnesiophyceae*) and the heterokont chromophytes (also known as chromophytes, heterokont algae, autotrophic stramenopiles). Using molecular clock calculations, we estimated the average and earliest probable time of origin of these two groups and their plastids. The origin of the haptophyte host-cell lineages appears to be more ancient than the origin of its plastid, suggesting that an endosymbiotic origin of plastids occurred late in the evolutionary history of this group. The pigmented heterokonts (heterokont chromophytes) also arose later, following an endosymbiotic event that led to the transfer of photosynthetic capacity to their heterotrophic ancestors. Photosynthetic haptophytes and heterokont chromophytes both appear to have arisen at or shortly before the Permian-Triassic boundary. Our data support the hypothesis that the haptophyte and heterokont chromophyte plastids have independent origins (i.e., two separate secondary endosymbioses) even though their plastids are similar in structure and pigmentation. Present evidence is insufficient to evaluate conclusively the possible monophyletic relationship of the haptophyte and heterokont protist host cells, even though haptophytes lack tripartite flagellar hairs. The molecular data, albeit weak, consistently fail to present the heterokont chromophytes and haptophytes as monophyletic. Phylogenetic resolution among all classes of heterokont chromophytes remains elusive even though molecular evidence has established the phylogenetic alliance of some classes (e.g., *Phaeophyceae* and *Xanthophyceae*).

The "golden" algae are today commonly referred to as chromophyte algae, heterokont algae or autotrophic stramenopiles, and historically have included the haptophyte algae. They range in size from minute picoplankton (1–2 µm) to the large kelps or brown seaweeds (40 m). Brown seaweeds were utilised by humans before recorded history, and almost certainly humans have cursed diatoms innumerable times as they slipped on rocks in streams. LINNAEUS (1753) described several genera, and of these, *Fucus* is still retained as a valid genus of brown seaweeds. Shortly after LINNAEUS' seminal publication, additional macroscopic algae were described (e.g., STACKHOUSE 1809, LAMOUROUX 1813, AGARDH 1820) that are now classified in the *Phaeophyceae*. LAMOUROUX (1813) and HARVEY (1836) made a major contribution to the classification of algae when they introduced the concept of colour, or pigmentation, as an important taxonomic feature for distinguishing major groups (viz., green, brown and red algae). The microscopic "golden" algae were discovered and reported by an entirely different group of workers, the early microscopists who were studying the *Infusoria* (microscopic organisms). For example, MÜLLER (1786), EHRENBERG (1838), RABENHORST (1853) and STEIN (1878) described microscopic organisms that are today considered relatives of the brown seaweeds. Unlike the macroalgae, which were clearly viewed as plants, the microalgae were frequently placed in the kingdom *Animalia* because they are often motile.

The evolutionary relationships among these algae have been controversial as documented in their long and complicated taxonomic history. The recognition of the relationship between the large, plant-like brown seaweeds and the small golden, yellow or brown microalgae first began with the works of KLEBS (1893) and BLACKMAN (1900). BLACKMAN (1900), who is best known for his ideas on the volvocine, tetrasporine and other green algal lineages, believed green algae gave rise to green plants via an ever-increasing degree of complexity and size, and proposed a similar scheme for "golden" algae. BLACKMAN placed the simple flagellate *Chromulina* at the base of the brown lineage and, with increasing size and complexity, the evolutionary lineage culminated with the brown seaweeds (see Chapter 12). In a separate but parallel scheme, he proposed an evolutionary tree for the yellow-green algae, which he considered to be distinct from, but related to, the brown lineage (BLACKMAN 1900). Beginning near the turn of the century, PASCHER began working extensively on "golden" algae, and he proposed a number of taxonomic changes and phylogenetic hypotheses that are relevant to this chapter. PASCHER (1913) combined the chrysomonads of KLEBS (1893), the heterokonts of LUTHER (1899) and the diatoms (e.g., KÜTZING 1834, RABENHORST 1853) into the single division *Chrysophyta*. The *Chrysophyta* stood as an equal taxonomic group to the division *Phaeophyta*, although it was still implicit, if not explicit, that the *Chrysophyta* gave rise to the evolutionarily advanced *Phaeophyta*. PASCHER (1910) also made another far-reaching taxonomic decision when he placed the haptophycean family *Isochrysidaceae* with the other chrysophytes having two equal flagella. This family united the organisms currently placed in the *Haptophyceae* (e.g., *Hymenomonas*) with certain other golden algae (e.g., *Synura, Syncrypta*). As more genera and species of haptophytes were described (e.g., LOHMANN 1913, LACKEY 1939), taxonomists followed PASCHER's classification and placed these haptophytes in the class *Chrysophyceae* (see BOURRELLY 1957).

Although the first half of the twentieth century brought the description of many species, the higher level taxonomic groups of algae were usually not treated in a phylogenetic sense. For example, FRITSCH (1945) states "The *Phaeophyceae* present no obvious affinities with any other class and are indeed in most respects so sharply circumscribed that little opportunity is afforded for speculations on their relationships.... On present evidence this class must be regarded as an altogether distinct evolutionary line (*Phaeophyta*)." Similarly, evolutionary relationships among the green and red algae, cryptomonads, dinoflagellates, etc., were rarely discussed during this time because shared characters were not obvious.

At the midpoint of the twentieth century, CHADEFAUD (1950) published a seminal paper in which he erected a new group, the *Chromophyceae*, based upon similarities of the flagella. This paper not only established the chromophytes sensu lato, but it also marked the first of many papers in the second half of the century that would address issues of algal phylogeny. CHADEFAUD combined the euglenoids, dinoflagellates, cryptophytes, chrysophytes, raphidophytes, brown algae and certain protozoans into a large group that was equal in stature to the "Blue-Green Algae", "Red Algae" and "Green Algae". Probably all modern workers exclude some of the organisms included by CHADEFAUD (viz., euglenoids, choanoflagellates), and many other workers exclude the cryptomonads and the dinoflagellates; however, the concept of a "chromophyte" group still exists. The group has been modified or renamed by several workers. For example, CHRISTENSEN (1962, 1989) proposed the division *Chromophyta* for algae lacking chlorophyll *b*; CAVALIER-SMITH (1986) proposed the kingdom *Chromista* for organisms having chlorophyll *c*, chloroplast endoplasmic reticulum (CER) and tripartite tubular hairs; PATTERSON (1989) proposed the stramenopiles for organisms having tripartite tubular hairs; and both VAN DEN HOEK (1978) and MOESTRUP (1992) expanded LUTHER'S name *Heterokonatae* to include not only the yellow-green algae (including freshwater raphidophytes) (sensu LUTHER 1899) but also all algae with tripartite tubular hairs. Conversely, CAVALIER-SMITH (1986) suggests that the haptophytes, heterokonts and cryptomonads are a monophyletic group (kingdom *Chromista*) that excludes the dinoflagellates.

Even today, there is no consensus on which organisms belong within this group, and no single name is in use. The rapid accumulation of ultrastructural, plastid pigment and molecular data have resulted in both the re-definition of old names and the creation of new names for the "golden algae." There are two reasons for this: (1) the group contains both pigmented and non-pigmented organisms so that "algae", "fungi" and "protozoa" must be contended with, and (2) there has been no unequivocal evidence that supports a single phylogenetic hypothesis. The two most widely used names, "chromophyte" and "heterokont", have changed in opposite ways: the chromophytes have become more restrictive by the removal of taxa from CHADEFAUD'S (1950) original definition, and the heterokonts have become more expansive by the addition of taxa to LUTHER'S (1899) original definition. CAVALIER-SMITH & CHAO (1996) have detailed much of the taxonomic nomenclature relating to these and other names. Thus, the "golden" algae discussed in this chapter are referred to in the literature by simple names, such as the chromophytes, chromists, heterokonts and stramenopiles, as well as by

compound names, such as the heterokont chromophytes or the pigmented stramenopiles. We use the name "heterokont chromophyte" because it acknowledges the two most commonly used names, and it indicates that most flagellate cells have "heterokont" flagella (= tripartite hairs, not heterodynamic flagellar beating) as well as "chromophyte" pigmentation (light-harvesting carotenoids, most with chlorophyll $a + c$). The haptophycean algae are included within the broad definition of the "golden algae" for this chapter, but the dinoflagellates and the cryptomonads are not.

Haptophyceae. The haptophyte algae were initially recognised as distinct from the *Chrysophyceae, Phaeophyceae* or *Xanthophyceae* with respect to ultrastructural featuree (PARKE & al. 1955, 1956, 1958). Ultimately, the class *Haptophyceae* was erected (CHRISTENSEN 1962) and its members were considered separate from, but related to, the *Chrysophyceae*. This decision was not met with universal support, as BOURRELLY (1968), STARMACH (1985) and others continued to place the haptophytes within the *Chrysophyceae*. However, after HIBBERD (1976) summarised the similarities and differences, he found little evidence for retaining the haptophyte taxa in the *Chrysophyceae*. Subsequently, most workers have considered the *Haptophyceae* to be distinct from the *Chrysophyceae* but often with some close, but undescribed, evolutionary relationship between the two classes. The unique or distinctive characters that separate the haptophytes from the *Chrysophyceae* and other heterokont chromophytes are: (1) haptonema, (2) flagellar transitional region and microtubular roots, (3) mitosis, (4) calcium carbonate biomineralisation (e.g., coccoliths in some representatives), (5) absence of tripartite flagellar hairs and (6) no plastid girdle lamellae (HIBBERD 1976, GREEN & al. 1989, GREEN & LEADBEATER 1994). Gene sequence data, which have been reported during the past few years, also suggest that the haptophytes are distantly related to the *Chrysophyceae* as well as to any other heterokont chromophytes (BHATTACHARYA & al. 1992, LEIPE & al. 1994, BHATTACHARYA & MEDLIN 1995, SAUNDERS & al. 1995, CARON & al. 1996, CAVALIER-SMITH & al. 1996, GREEN & DUNFORD 1996, MEDLIN & al. 1996a).

Heterokont chromophytes. The heterokont chromophytes belong to a larger group of heterokont organisms (= stramenopiles) that can be characterised as follows: (1) two flagella are typically present and they are usually of distinctly different lengths, (2) the two flagella have different patterns of motion (stiff sinusoidal beat vs. irregular undulations), (3) one flagellum typically bears two rows of tripartite flagellar hairs (VLK 1938, DODGE 1975), (4) the flagellar hairs provide a reverse thrust to the flagellar beat and therefore pull the cell rather than push it (SLEIGH 1989), (5) presence of a girdle lamella (except in the *Eustigmatophyceae*) and (6) silica biomineralisation when mineralisation is present (viz., diatoms, silica-scaled chrysophytes and synurophytes, silicoflagellates). Three features, the β-1,3-linked glucan carbohydrate storage product (CRAIGIE 1974, WANG & BARTNICKI-GARCÍA 1974), the chloroplast endoplasmic recticulum (see review in GIBBS 1993) and tubular mitochondrial cristae (TAYLOR 1976, STEWART & MATTOX 1970), are shared with the *Haptophyta.*

Electron microscopic studies have contributed substantially to our understanding of this group, providing a suite of putatively homologous characters for algal systematics. These new observations have led to the description of several

new classes of heterokont chromophytes, viz., the *Eustigmatophyceae* (HIBBERD & LEEDALE 1971), the *Dictyochophyceae* (now including the *Pedinellophyceae*) (SILVA 1980), the *Synurophyceae* (ANDERSEN 1987), the *Coscinodiscophyceae* and *Fragilariophyceae* (ROUND & al. 1990) and the *Pelagophyceae* (ANDERSEN & al. 1993). However, ultrastructural data alone have been unable to resolve the phylogenetic relationships of the heterokont chromophytes (e.g., ANDERSEN 1991, WILLIAMS 1991). In contrast, molecular data have resolved a number of phylogenetic relationships in this group. For example, SSU rRNA data have shown a relationship between the *Phaeophyceae* and the *Xanthophyceae* (ARIZTIA & al. 1991, POTTER & al. 1997), between the *Chrysophyceae* and *Synurophyceae* (ARIZTIA & al. 1991, BHATTACHARYA & al. 1992), between the *Dictyochophyceae* and *Pelagophyceae* (SAUNDERS & al. 1995) and between the *Sarcinochrysidales* sensu stricto and the *Pelagophyceae* (SAUNDERS & al. 1997b). Nonetheless, these studies and others have failed to resolve unequivocally the relationships among the deeper branching heterokont chromophytes.

Hypotheses

Today, many questions remain unresolved regarding the phylogeny of the "golden" algae, but we will address two major issues in this chapter. The first is whether or not the haptophyte algae have any close evolutionary relationship with the heterokont chromophytes. The second question concerns the phylogenetic relationships among the heterokont chromophytes themselves. We have examined these relationships using new and/or existing molecular and morphological data. We proposed several hypotheses relative to these relationships and evaluated the data to determine if support for one hypothesis over another can be found. The hypotheses are outlined below.

Relationships between the haptophyte and heterokont algae. Relationships between these two groups are confounded by the questions of the monophyly of the host cells and the number of endosymbioses giving rise to their plastids. Thus, we can formulate four scenarios/hypotheses (1a–d) to explain their evolution.

Hypothesis 1a. The heterokonts and haptophytes form a monophyletic group that gained their plastids as the result of single secondary endosymbiotic event. That is, the host cells and the plastids from the two groups will have similar phylogenies because they share the same evolutionary history. Thus in the molecular analyses, both the two host cells and the two plastids should be each others' sister group, respectively.

Hypothesis 1b. The heterokonts and the haptophytes are a monophyletic group, but after their divergences, each acquired its plastid through independent endosymbioses. Thus, in the molecular analyses, the host cells are each others' sister group but their plastids are not.

Hypothesis 1c. The heterokonts and the haptophytes are not a monophyletic group. However, they both engulfed and retained a similar eukaryotic cell as their plastid. Thus in the molecular analyses, the host cells are not each others' sister group but their plastids are.

Hypothesis 1d. The heterokonts and the haptophytes are not a monophyletic group. Each gained their plastids from separate secondary endosymbiotic events. Therefore, neither the host cells nor the plastids for the two groups will have similar phylogenies because each has had an independent evolutionary history.

Hypotheses 1a, 1c, and 1d allow the possibility of plastid gain and loss in the heterokont lineage of major groups, e.g., the oomycetes.

Relationships between non-photosynthetic and photosynthetic heterokonts.

Hypothesis 2a. The heterokont chromophytes form a monophyletic group that does not include the major non-photosynthetic heterokont groups (i.e., oomycetes, hyphochytrids, thraustrochytrids).

Hypothesis 2b. The heterokont chromophytes are not a monophyletic group, i.e., one or more of the major non-photosynthetic heterokont lineages is included within the clade of heterokont chromophytes.

Relationships within the heterokont chromophytes.

Hypothesis 3a. The heterokont chromophytes contain two major monophyletic lineages: one with a well-developed flagellar apparatus and one with an often highly reduced flagellar apparatus. Those with a well-developed flagellar apparatus have microtubular roots, and the flagellar apparatus is typically distant from the nucleus, the two components often being connected via a striated rhizoplast. Those with a highly reduced flagellar apparatus typically lack microtubular roots, and the flagellar apparatus is often closely associated or directly in contact with the nuclear envelope.

Hypothesis 3b. The flagellar apparatus of heterokont chromophytes has been reduced two or more times independently, and the two lineages are not monophyletic.

Hypothesis 4a. The heterokont chromophytes contain two major monophyletic lineages: one has a diatoxanthin/diadinoxanthin-containing light-harvesting complex and the other has a violaxanthin-containing light-harvesting complex.

Hypothesis 4b. The pigmentation of heterokont chromophytes has evolved two or more times independently, and the two pigment groups are polyphyletic.

To evaluate these hypotheses, we have inferred phylogenies from both nuclear and plastid genes and compared these to other published phylogenies, where applicable. In each case we have focused on: (1) relationships between the major groups (first set of hypotheses) and (2) relationships within the heterokont group (second, third and fourth sets of hypotheses).

Our phylogenetic trees were constructed from nuclear and plastid ribosomal and protein-coding genes using the neighbor-joining method (SAITOU & NEI 1987, except for Figs. 1 & 2). Interpretations of bootstrap support for these trees is based on the analysis of bootstrap accuracy and repeatability by HILLIS & BULL (1993). With symmetrical phylogenies having an internodal change of $< 20\%$ and approximately equal rates of change, we interpret a bootstrap proportion of $\geqslant 70\%$ to indicate a $\geqslant 95\%$ probability that the recovered clade represents a true clade. We qualify our interpretation because readers may disagree with our interpretation of bootstrap support.

Nuclear genome

Among the nuclear genes we have selected for our analyses are the small subunit of the ribosomal cistron (e.g., BHATTACHARYA & al. 1992; LEIPE & al. 1994; SAUNDERS & al. 1995; CAVALIER-SMITH & al. 1996; MEDLIN & al. 1996b, c) and the multi-gene families of the fucoxanthin/chlorophyll photosystem I & II binding proteins (FCP) and the actin protein (LA ROCHE & al. 1994, CARON & al. 1996, BHATTACHARYA & EHLTING 1995, GREEN & DURNFORD 1996) (Figs. 1–3). The ribosomal genes occur in many, perhaps hundreds of copies per cell and are generally believed to evolve in a concerted fashion such that all copies are homogenised and should encode the same coding region. This effectively ensures that the ribosomal phylogenies more accurately represent species trees rather than gene trees (HILLIS & al. 1996). The phylogenies of the other two genes more likely reflect those of gene trees. It is generally believed that as more genes are compared, a better picture of the evolution of the group can be achieved as the phylogenies may converge upon one another.

In each of the three Figures presented for our analysis using nuclear genes, the heterokonts and the haptophytes are shown as separate, monophyletic groups and are never found as sister taxa. There is, however, an association of the heterokonts with the alveolates, which includes the dinoflagellates as their autotrophic members, in both the SSU rRNA analysis (Fig. 1) and in the FCP binding proteins (Fig. 2). Bootstrap support for this association is $< 50\%$ in the rRNA phylogeny (Fig. 1), but it is 100% in the FCP photosystem protein phylogeny (Fig. 2, taken from CARON & al. 1996; see also GREEN & DURNFORD 1996). Preliminary results using a combined SSU and LSU data set in a neighbor joining analysis show a strong association of the alveolates (*Prorocentrum, Toxoplasma, Tetrahymena*) with non-pigmented heterokonts (*Phytophthora*, hyphochytrids, bootstrap = 100%) (VAN DER AUWERA & DE WACHTER 1996). Similarly, the alpha tubulin gene shows a relationship between the alveolates and heterokonts (bootstrap = 50%, KEELING & DOOLITTLE 1996), with the haptophytes occupying a more distant relationship.

In the actin tree, the single haptophyte representative (*Emiliania huxleyi*) is separated from the heterokonts (Fig. 3). The short branch lengths with only moderate bootstrap support among the major groups indicate that the actin gene is unable to resolve the branching order of the eukaryotes. No dinoflagellate taxa, and only two heterokont chromophyte taxa, are included.

The molecular data do not support an affiliation of the haptophytes with the heterokonts or with any other eukaryotic group (see the absence of bootstrap support for the association of the haptophytes with any other lineage in the rRNA trees) (Fig. 1). This lack of clear bootstrap support for the haptophytes' nearest neighbor in the rRNA (and likely the actin) phylogeny has generally been assumed to be related to a very rapid evolution that occurred during the major radiation of the eukaryotic lineages, known as the crown radiation (KNOLL 1992, WAINRIGHT & al. 1993). The FCP binding protein phylogeny may help to resolve relationships among eukaryotes during this time frame; however only photosynthetic organisms can be compared, which may be misleading. Nevertheless, the most significant point to be gathered from the rRNA and FCP trees and other phylogenies is the recurrent association of the dinoflagellates with the heterokonts (with high

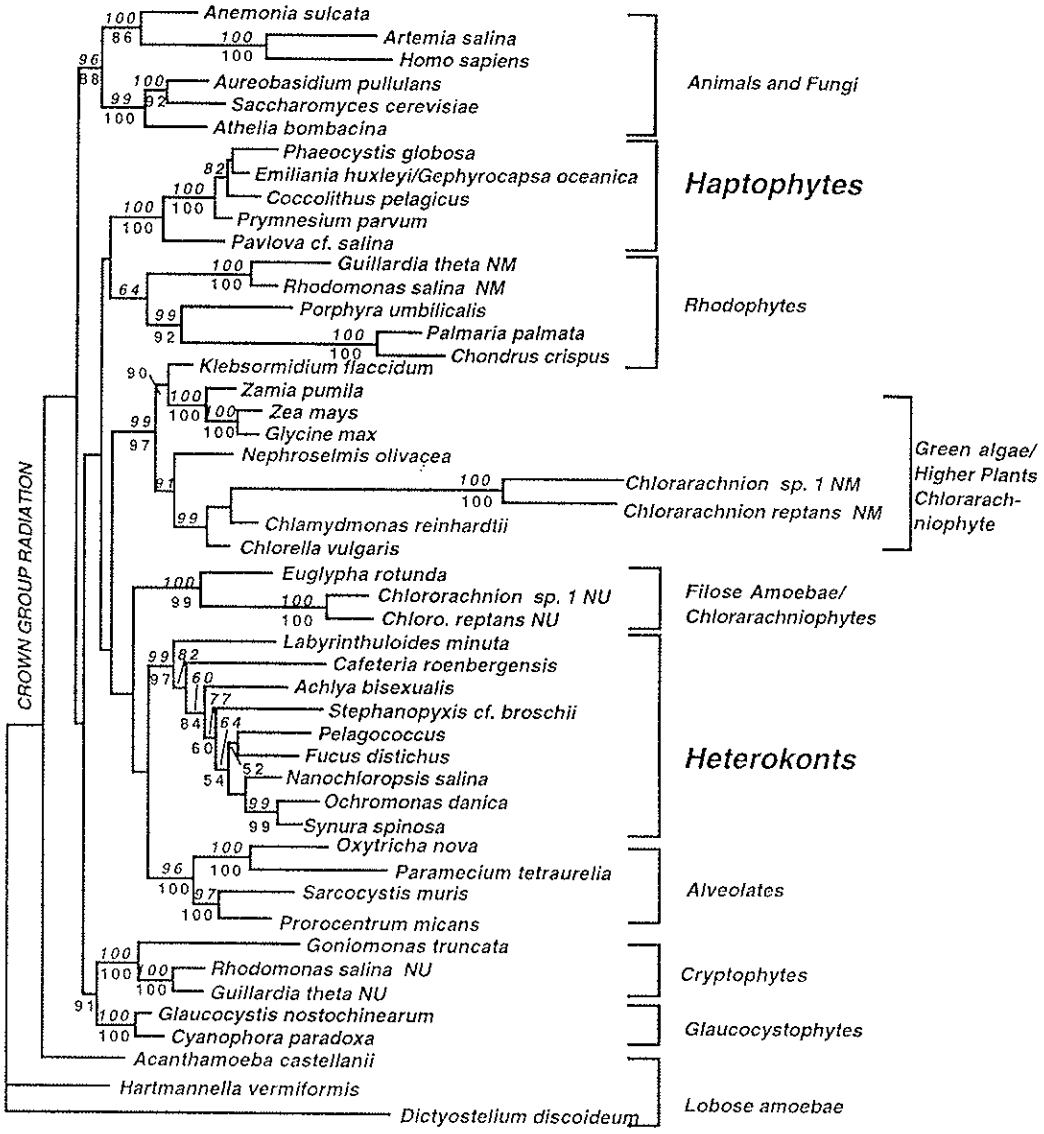


Fig. 1. Phylogenetic analysis of nuclear-encoded SSU rRNA genes from a selection of organisms belonging to the major radiation of eukaryotes using the maximum likelihood method. Representatives of all major eukaryotic groups, especially the algae, are included. Bootstrap values > 50% (100 replications, FELSENSTEIN 1985) from a neighbor-joining analysis (SAITOU & NEI 1987) of a KIMURA (1980) distance matrix and a PAUP (SWOFFORD 1993) weighted maximum parsimony analysis (MEDLIN & al. 1996b) are shown above and below the internal nodes, respectively. The positions of the haptophytes and the heterokonts are highlighted. NM refers to the gene from the nucleomorph or vestigial nucleus within the plastid; NU refers to the gene from the nucleus of the same organism. The tree is rooted within the branch leading to *Dictyostelium*. In addition to the plastid-containing groups named and bracketed in this Figure, two groups within the alveolates also contain plastids. These are the dinoflagellates (here represented by *Prorocentrum micans*) and the apicomplexans (here represented by *Sarcocystis muris*), which are thought to have acquired their plastids independently from one another (KOHLER & al. 1997, DELWICHE & PALMER 1996; see Chapter 3). Two plastid-containing groups that are not shown on this tree are the Euglenophytes, which branch well below the crown-group radiation in rRNA trees, and the enigmatic *Paulinell a chromatophora*, which branches with the filose amoeba *Euglypha rotunda* (BHATTACHARYA & al. 1995)

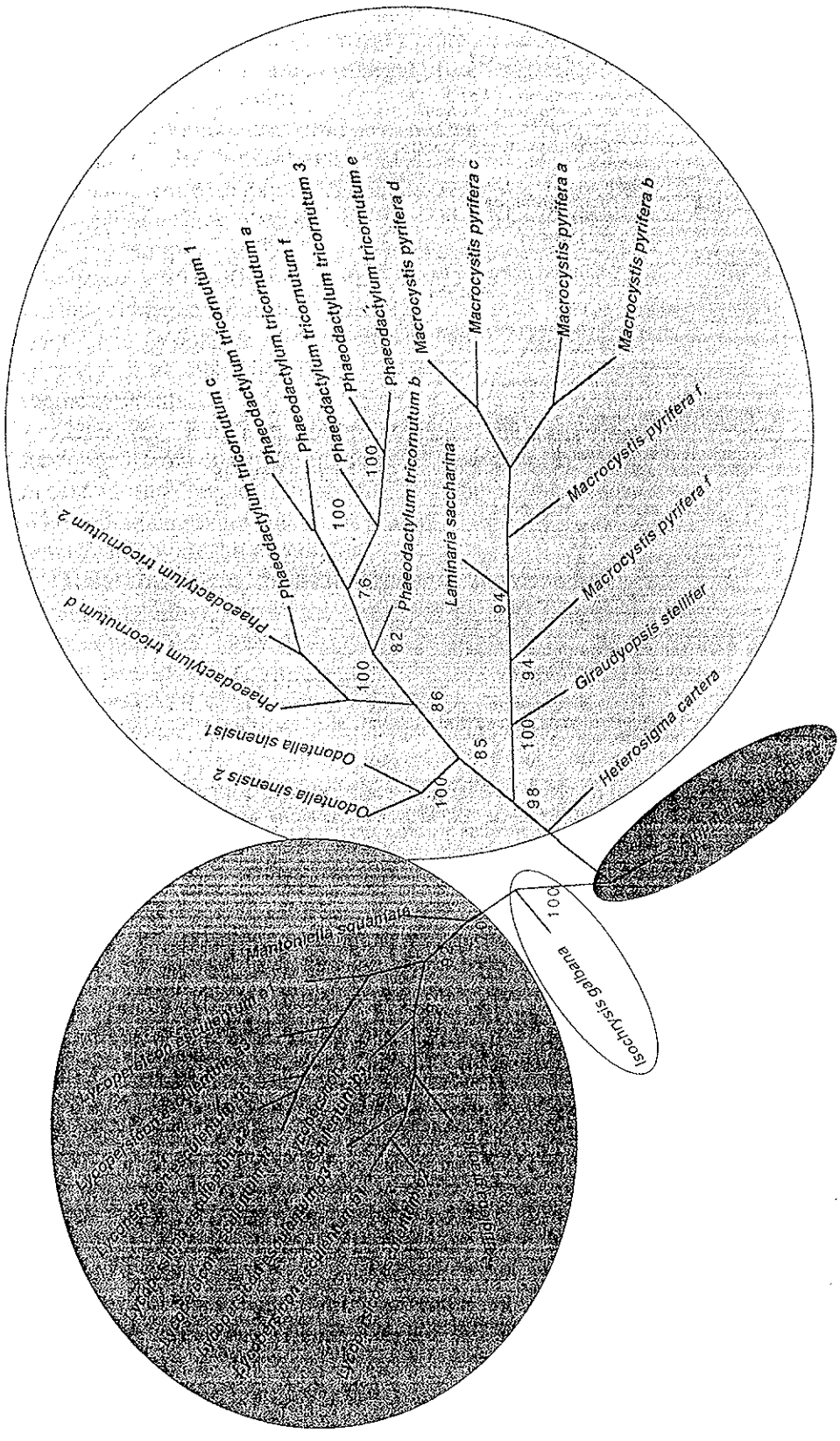


Fig. 2. Phylogenetic analysis of fucoxanthin/chlorophyll photosystem I & II-binding coding regions. This unrooted parsimony tree is redrawn from CARON & al. (1996). Bootstrap values > 90% (50 replications) are shown above the internal nodes. Major groups of algae/higher plants are highlighted as follows: clear haptophytes, light grey heterokonts, medium grey green algae and higher plants, dark grey dinoflagellates

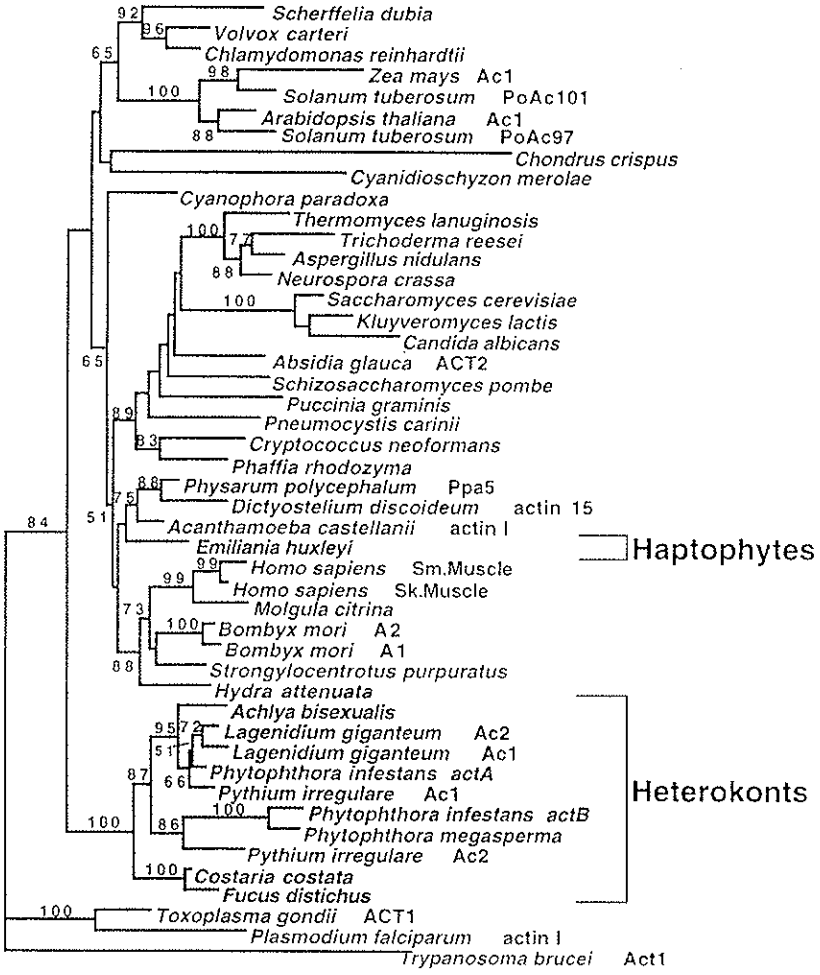


Fig. 3. Phylogenetic analysis of actin coding regions. The neighbor-joining method was used to infer the tree from a KIMURA (1980) distance matrix. Only first and second positions of actin codons were included in the analysis (702 nt). Bootstrap values (100 replications) are shown above the internal nodes. The positions of the haptophytes and the heterokonts are highlighted. The root of this phylogeny lies on the branch leading to the *Trypanosoma brucei* actin sequence

bootstrap support in some analyses) to the exclusion of the haptophytes. These data are consistent with hypotheses 1c and 1d.

Only in the large subunit rRNA phylogeny for major algal groups published to date (PERASSO & al. 1989), do the haptophytes group with the heterokonts, suggesting that hypotheses 1a and b are supported. However, taxon sampling is very low (*Ochromonas, Synura, Vacuolaria, Prymnesium, Cricosphaera*), and this may misrepresent the relationships among these and other groups. A similar relationship between the haptophytes and the heterokonts can be obtained with the SSU rRNA data set if taxon sampling is limited to only a few groups [see relationships of the *Haptophyta* to the *Heterokonta* in CAVALIER-SMITH & al. (1995),

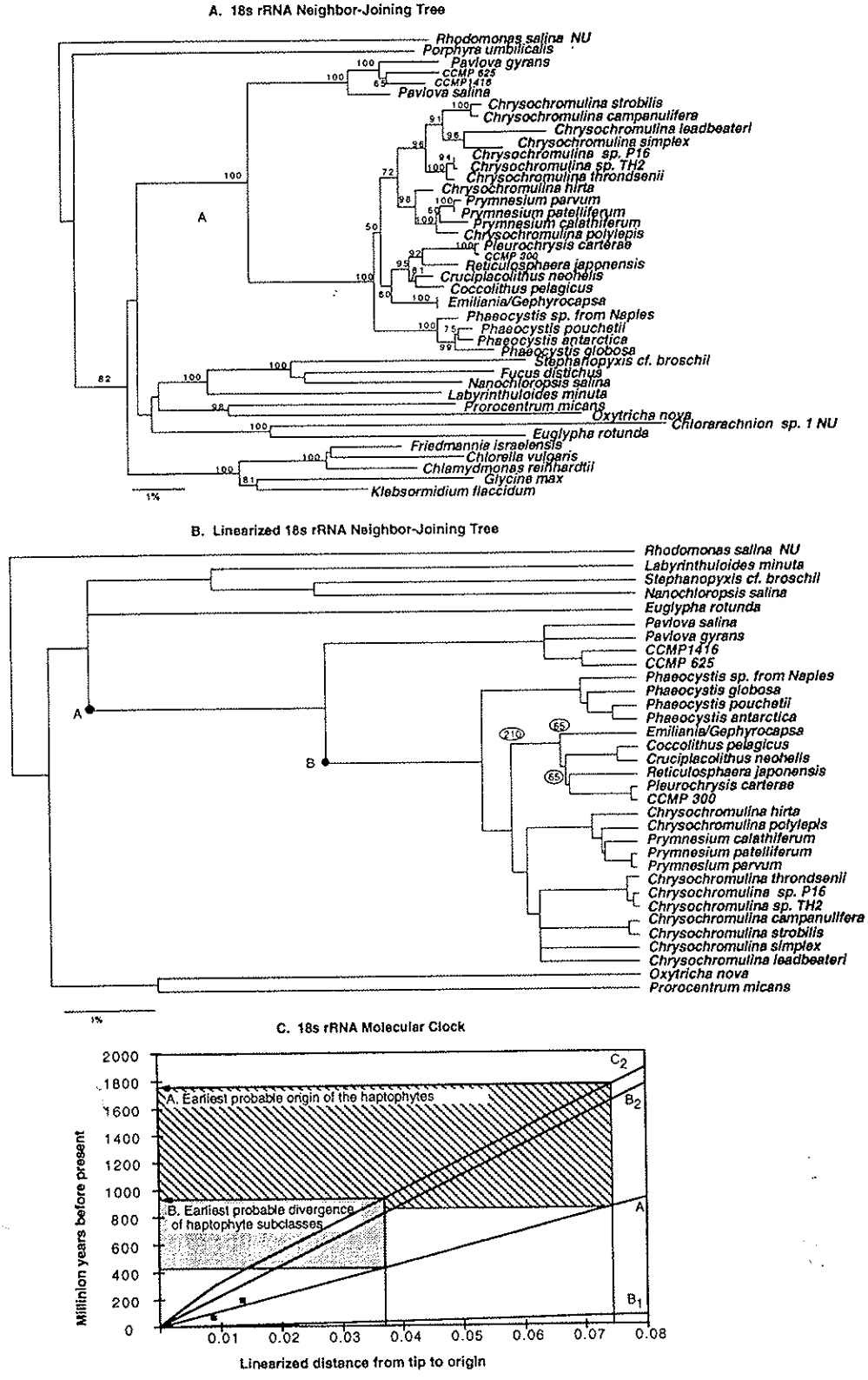
which is in contrast to their relationships in CAVALIER-SMITH & al. (1996) and CAVALIER-SMITH & CHAO (1996)]. A more extensive analysis using the entire LSU rRNA molecule for a variety of algal groups is presently being undertaken (G. VAN DER AUWERA, pers. comm.).

The cryptomonads, which according to CAVALIER-SMITH (1986) should be ancestral to the heterokonts and haptophytes in the monophyletic kingdom *Chromista*, are also phylogenetically removed from these two chromist groups. Cryptophytes show a strong relationship with the glaucocystophytes in our rRNA tree (bootstrap = 100%, Fig. 1), and cryptophytes are an independent clade (bootstrap = 99/100%) in another recent study (CAVALIER-SMITH & al. 1994, 1996). Further evidence from the GAPDH gene (LIAUD & al. 1997, bootstrap = 66%), the *sec*Y gene (VOGEL & al. 1996, bootstrap = 74%) and the stress-70 protein gene (RENSING & al. 1996, bootstrap =< 50%) also places the cryptophytes distant from the heterokonts.

Thus, the kingdom *Chromista* does not appear to be monophyletic, i.e., descended from a single endosymbiotic event that transformed its heterotrophic ancestors into "algae". In addition, the haptophytes appear to be a unique lineage with no clear sister taxon revealed. There is a recurrent association of the heterokonts with the alveolates.

Relationships within the *Haptophyta*. A moderate data set is now available for the haptophytes from the SSU rRNA genes (Fig. 4A, MEDLIN & al. 1996a and MEDLIN unpubl.). The haptophyte lineage is undifferentiated for some time after its origin (see point A on Fig. 4B) before it diverges into two groups, which correspond well with the two haptophyte subclasses, the *Pavlovophycidae* and the *Prymnesiophycidae* (JORDAN & GREEN 1994). This divergence is well supported in a bootstrap analysis, which is entirely consistent with the clear morphological differences between the subclasses (JORDAN & GREEN 1994). The *Pavlovophycidae* have unequal flagella with small tubular hairs and lack organic body scales. In contrast the *Prymnesiophycidae* have nearly equal flagella with no flagellar hairs but have organic body scales. In the taxonomic treatment put forth by JORDAN and GREEN, only a single order is retained in each subclass. The *Pavlovophycidae* contains extant species that can be traced back to earlier divergences in the rRNA tree than those in the other subclass. It contains both flagellate organisms plus an undescribed coccoid organism whose taxonomic affinities were only recognised through sequence analysis (POTTER & al. 1996). The remaining haptophytes are divided among three clades. Monophyletic groups within these three clades appear to reflect family level relationships in the *Haptophyta*. However, the genus *Chrysochromulina* is paraphyletic (see also FUJIWARA & al. 1995). It is clear that some key haptophytes (e.g., the *Isochrysidaceae*) are missing from the rRNA tree, and relationships within the tree are likely to change as more taxa are added (compare Fig. 4 with the interpretation of relationships within the haptophytes in rRNA tree in CAVALIER-SMITH & al. 1996). Significantly, all of the coccolithophorids form a monophyletic group with the family *Noelaerhabdaceae* (*Emiliania* and *Gephyrocapsa*) sister to the remainder of the lineage.

Relationships within the *Heterokonta*. Within the heterokont organisms we will discuss the rRNA-generated phylogenies, because these data are the most extensive. All recent rRNA analyses have shown the non-photosynthetic lineages


A. 18s rRNA Neighbor-Joining Tree
Rhodomonas salina NU
Porphyra umbilicalis
Pavlova gyrans
CCMP 625
CCMP1416
Pavlova salina
Chrysochromulina strobilis
Chrysochromulina campanulifera
Chrysochromulina leadbeateri
Chrysochromulina simplex
Chrysochromulina sp. P16
Chrysochromulina sp. TH2
Chrysochromulina throndsenii
Chrysochromulina hirta
Prymnesium parvum
Prymnesium patelliferum
Prymnesium calathiferum
Chrysochromulina polylepis
Pleurochrysis carterae
CCMP 300
Reticulosphaera japonensis
Cruciplacolithus neohelis
Coccolithus pelagicus
Emiliania/Gephyrocapsa
Phaeocystis sp. from Naples
Phaeocystis pouchetii
Phaeocystis antarctica
Phaeocystis globosa
Stephanopyxis cf. broschii
Fucus distichus
Nanochloropsis salina
Labyrinthuloides minuta
Prorocentrum micans
Oxytricha nova
Chlorarachnion sp. 1 NU
Euglypha rotunda
Friedmannia israelensis
Chlorella vulgaris
Chlamydmonas reinhardtii
Glycine max
Klebsormidium flaccidum
A
1%
B. Linearized 18s rRNA Neighbor-Joining Tree
Rhodomonas salina NU
Labyrinthuloides minuta
Stephanopyxis cf. broschii
Nanochloropsis salina
Euglypha rotunda
Pavlova salina
Pavlova gyrans
CCMP1416
CCMP 625
Phaeocystis sp. from Naples
Phaeocystis globosa
Phaeocystis pouchetii
Phaeocystis antarctica
Emiliania/Gephyrocapsa
Coccolithus pelagicus
Cruciplacolithus neohelis
Reticulosphaera japonensis
Pleurochrysis carterae
CCMP 300
Chrysochromulina hirta
Chrysochromulina polylepis
Prymnesium calathiferum
Prymnesium patelliferum
Prymnesium parvum
Chrysochromulina throndsenii
Chrysochromulina sp. P16
Chrysochromulina sp. TH2
Chrysochromulina campanulifera
Chrysochromulina strobilis
Chrysochromulina simplex
Chrysochromulina leadbeateri
Oxytricha nova
Prorocentrum micans
A
B
210
65
65
1%
C. 18s rRNA Molecular Clock
Millinion years before present
2000
1800
1600
1400
1200
1000
800
600
400
200
0
A. Earliest probable origin of the haptophytes
B. Earliest probable divergence of haptophyte subclasses
C2
B2
A
B1
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Linearized distance from tip to origin

as early divergences, whereas the photosynthetic algae emerge later as a monophyletic group. The presence of the oomycetes either outside or inside the autotrophic clade of the heterokonts has been controversial (CAVALIER-SMITH 1989, 1993; WILLIAMS 1991; LEIPE & al. 1994; CAVALIER-SMITH & al. 1996). This has fuelled speculations that the oomycetes, as well as all other heterotrophic heterokonts, may have secondarily lost their plastids. However, the monophyly for all the pigmented heterokonts has received stronger support as more pigmented taxa are included in the analyses (compare increases in bootstrap support in BHATTACHARYA & al. (1992), LEIPE & al. (1994), SAUNDERS & al. (1995), CAVALIER-SMITH & CHAO (1996), MEDLIN & al. (1997). Bootstrap support is generally lowered if many distant outgroups are included in the analyses. However, none of the recent analyses fail to present these algae as monophyletic. These data increasingly support hypothesis 2a.

Branching order within the heterokont algal groups is one of the most controversial issues concerning their phylogenetic analyses. Differences in the branching order within the pigmented heterokonts likely reflect differences in sequence alignments, analytical techniques, number of taxa and the subjective choice of the number of unambiguous nucleotides included in the analyses. In early studies, when using molecular data alone from six of the 12 or more classes of heterokont algae, the first divergence was between the diatoms and all remaining heterokont algae (BHATTACHARYA & al. 1992, LEIPE & al. 1994). However, a later cladistic analysis of 14 morphological/biochemical characters recovered a larger group within the heterokonts, which included the diatoms (SAUNDERS & al. 1995). This group could be defined morphologically as those algae containing a reduced flagellar apparatus; combined molecular and traditional data analysis further strengthened support for this clade (SAUNDERS & al. 1995).

Fig. 4. *A–C* Phylogenetic analysis of nuclear-encoded SSU rRNA genes from the *Haptophyta*. *A* Neighbor-joining tree inferred from a KIMURA (1980) distance matrix. Representatives of all subclasses/and or orders of the *Haptophyta* and the closely related groups are included. The tree was rooted on the branch leading to *Rhodomonas*. Bootstrap values (100 replications) are shown above the internal nodes. "A" marks the period of time before the *Haptophyta* diverge into their two subclasses. *B* Linearisation of the neighbor-joining tree in A according to TAKEZAKI & al. (1995) so that all rate variation in the molecule is eliminated. All significantly faster evolving taxa were excluded from the analysis. First appearances of coccolithophorid taxa from the fossil record are encircled and placed at the node where the taxa to the right are believed to have their first appearance. Point "A" marks the origin of the Haptophyta; point "B" is the divergence of its two subclasses. *C* Molecular clock constructed from *B*. Branch lengths from taxa in *B* with a fossil record were regressed against first appearance dates according to the molecular clock model in HILLIS & al. (1996). "A" is the regression line, constrained through the origin. Lines B_1 and B_2 are the 95% confidence limits around the regression line. Lines C_1 and C_2 are the 95% confidence limits for a new predicted value of time given the length of an undated node. Lower confidence limits, below zero, are reset at zero. C_1 is below the x axis and not shown. Blocks of time are shown for each group whose origin has been estimated from the molecular clock. The block spans the time of the average age of the group (from A) to the earliest probable time of origin based on the upper 95% confidence limit (C_2) of an undated node

The "reduced flagellar apparatus" group consists of the diatoms as a sister taxon to an assemblage containing the *Pelagophyceae* and other microalgae, which historically were loosely termed the "marine chrysophytes." The group is characterised by a flagellar transition region with two transitional plates and a small transitional helix below the major plate, a flagellar apparatus that lacks microtubular roots (see *Sarcinochrysidales* however, SAUNDERS & al. 1997b) and basal bodies positioned on or very near the nucleus. A paraxonemal rod, similar to that of dinoflagellates, is common in some members (paraxonemal rods of this type are absent in other heterokont chromophytes). Furthermore, there appears to be a tendency for a "sinking spindle" at the onset of mitosis (VESK & JEFFREY 1987, GREEN 1989, PICKETT-HEAPS & al. 1990), although few organisms other than diatoms have been examined in detail. The carotenoid pigments of this group are restricted to the diatoxanthin and diadinoxanthin types as well as fucoxanthin, 19′-butanoyloxyfucoxanthin and 19′-hexanoyloxyfucoxanthin (BJORNLAND & LIAAEN-JENSEN 1989); violaxanthin, anteraxanthin, zeaxanthin, heteroxanthin, vaucherioxanthin, etc. are not found in this group.

This "reduced flagellar apparatus clade" is sister taxon to a clade containing the chrysophytes/synurophytes, the eustigmatophytes, the xanthophytes and the phaeophytes. If more taxa, such as the *Sarcinochrysidales* and the *Chrysomeridales* (SAUNDERS & al. 1997b) and the *Raphidophyceae* plus additional *Xanthophyceae* (POTTER & al. 1997), are added, then the diatoms emerge before all pigmented heterokonts in molecular phylogenies (Fig. 5). The reduced flagellar apparatus lineage appears intact only in combined molecular and morphological data sets (not shown), suggesting that insufficient data exist to place the diatom branch unequivocally either within the reduced flagellar apparatus clade or outside it. Therefore, we are unable to find conclusive support for either hypothesis 3a or 3b.

The remaining pigmented heterokonts diverge into three (possibly two) clades in both the molecular only and the combined analyses. One clade contains the *Xanthophyceae* and its sister group, the *Phaeophyceae*; the *Chrysomeridales* are sister to the remainder of this clade (SAUNDERS & al. 1997b and in Fig. 5A). There

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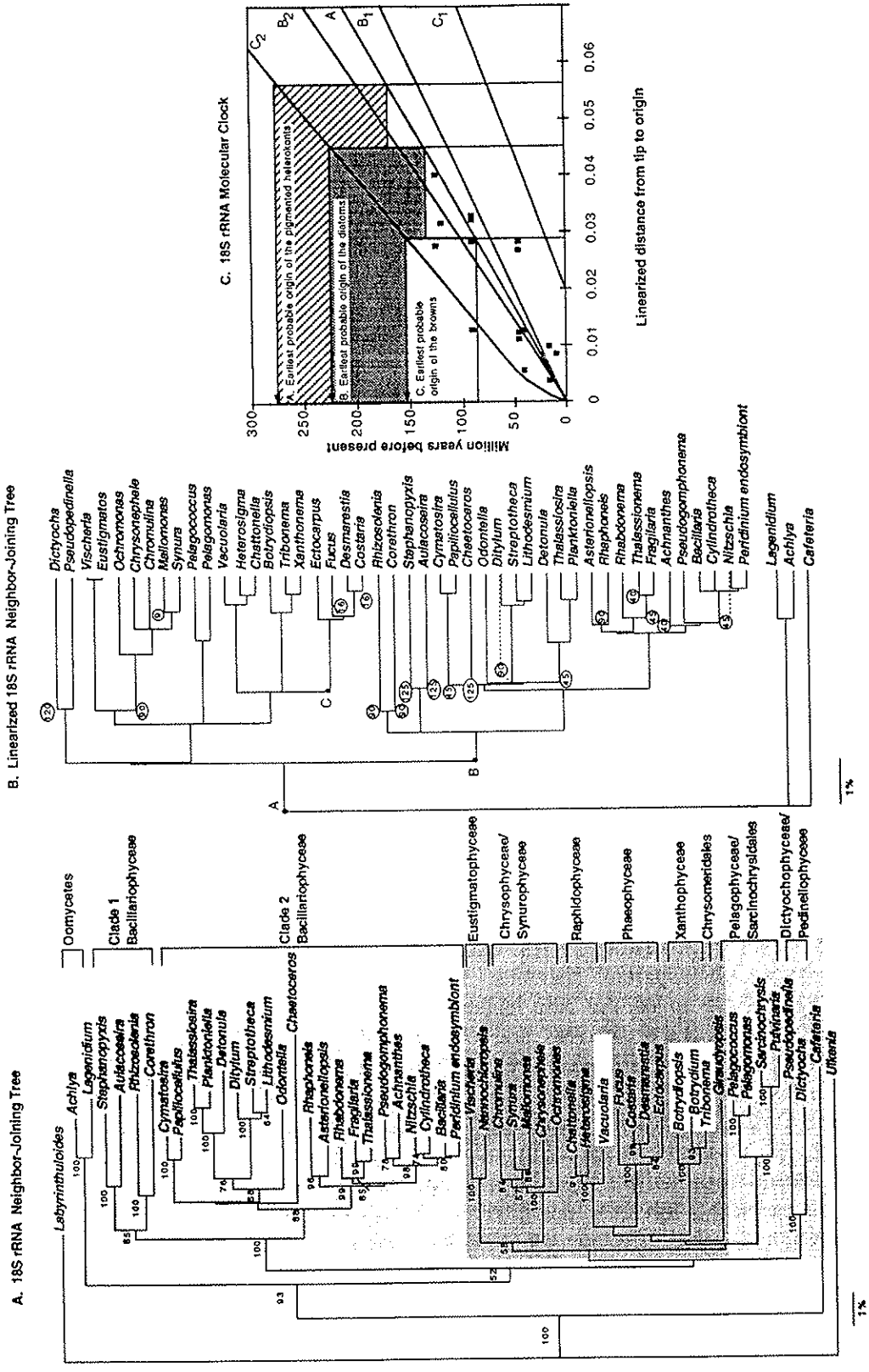
Fig. 5 *A–C*. Phylogenetic analysis of nuclear-encoded SSU rRNA genes from the *Heterokonta*. *A* Neighbor-joining tree inferred from a KIMURA (1980) distance matrix. Representatives of all classes/and or orders of the pigmented *Heterokonta* and the oomycetes are labelled on the tree, which was rooted on the branch leading to *Ulkenia*. Bootstrap values (100 replications) are shown above the internal nodes. The two light grey blocks contain algae with the diatoxanthin/diadinoxanthin-containing light-harvesting complex. The darker grey block contains algae with the violaxanthin-containing light-harvesting complex. Within this dark grey block are taxa highlighted in white that have a vaucherioxanthin (*Vacuolaria*) or heteroxanthin-containing light-harvesting complex (*Xanthophyceae*). *B* Linearisation of the neighbor-joining tree in *A* as in Fig. 4B. First appearances of diatom taxa from the fossil record are encircled and placed at the node where the taxa to the right are believed to have their first appearance. A hypothesis, which predates certain diatom taxa to have their origin before a major gap in the fossil record, i.e., at 125 Ma was used to predate three extant taxa. Point "A" marks the origin of the pigmented heterokonts; "B" is the origin of the diatoms; and "C" is the origin of the brown algae. *C* Molecular clock constructed from *B* as in Fig. 4C


A. 18S rRNA Neighbor-Joining Tree
Latrinthuloides
Achlya
Lagenidium
Stephanopyxis
Aulacoseira
Rhizosolenia
Corethron
Cymatosira
Papiliocellulus
Thalassiosira
Planktoniella
Detonula
Ditylum
Streptotheca
Lithodesmium
Odontella
Chaetoceros
Rhaphoneis
Asterionellopsis
Rhabdonema
Fragilaria
Thalassionema
Pseudogomphonema
Achnanthes
Nitzschia
Cylindrotheca
Bacillaria
Peridinium endosymbiont
Vischeria
Nannochloropsis
Chromulina
Synura
Mallomonas
Chrysonephele
Ochromonas
Chattonella
Heterosigma
Vacuolaria
Fucus
Cladosiphon
Desmarestia
Ectocarpus
Botrydiopsis
Botrydium
Tribonema
Glaucocystis
Pelagococcus
Pelagomonas
Sarcinochrysis
Pulvinaria
Pseudopedinella
Dictyocha
Cafeteria
Ulkenia
Oomycetes
Clade 1 Bacillariophyceae
Clade 2 Bacillariophyceae
Eustigmatophyceae
Chrysophyceae/ Synurophyceae
Raphidophyceae
Phaeophyceae
Xanthophyceae
Chrysomeridales
Pelagophyceae/ Sarcinochrysidales
Dictyochophyceae/ Pedinellophyceae
1%
B. Linearized 18S rRNA Neighbor-Joining Tree
Dictyocha
Pseudopedinella
Vischeria
Eustigmatos
Ochromonas
Chrysonephele
Chromulina
Mallomonas
Synura
Pelagococcus
Pelagomonas
Vacuolaria
Heterosigma
Chattonella
Botrydiopsis
Tribonema
Xanthonema
Ectocarpus
Fucus
Desmarestia
Costaria
Rhizosolenia
Corethron
Stephanopyxis
Aulacoseira
Cymatosira
Papiliocellulus
Chaetoceros
Odontella
Ditylum
Streptotheca
Lithodesmium
Detonula
Thalassiosira
Planktoniella
Asterionellopsis
Rhaphoneis
Rhabdonema
Thalassionema
Fragilaria
Achnanthes
Pseudogomphonema
Bacillaria
Cylindrotheca
Nitzschia
Peridinium endosymbiont
Lagenidium
Achlya
Cafeteria
A
B
C
1%
C. 18S rRNA Molecular Clock
A. Earliest probable origin of the pigmented heterokonts
B. Earliest probable origin of the diatoms
C. Earliest probable origin of the browns
Million years before present
Linearized distance from tip to origin
A
B1
B2
C1
C2

are few morphological and biochemical characters that unite this group. However, the zoospores of all taxa have an R_1 microtubular root that extends to the anterior of the cell, forming a beak-like projection. This accentuated anterior end places the flagellar insertion in a lateral, rather than apical, position. Other features, such as flagellar transitional region, mitosis and carotenoid pigmentation show no common thread, and for these reasons it was quite surprising to find molecular data supporting this relationship (e.g., ARIZTIA & al. 1991).

The second clade contains the *Chrysophyceae* and its sister group, the *Synurophyceae*; the *Eustigmatophyceae*, in turn is sister to these two classes (BHATTACHARYA & al. 1992, LEIPE & al. 1994; Fig. 5). This group is also difficult to characterise on the basis of ultrastructural and biochemical features. The flagellar apparatus is distinctly different in each group, and mitosis is variable where known. The carotenoid pigmentation shows some similarities (viz., violaxanthin), but the eustigmatophytes have vaucherioxanthin, a pigment not reported for chrysophytes and synurophytes. The *Chrysophyceae* appear paraphyletic in most analyses (SAUNDERS & al. 1995, 1997a, b). Presumably, better taxon sampling will resolve the possible paraphyly of the *Chrysophyceae.*

The third clade, contains both the freshwater and marine taxa of the *Raphidophyceae*. In our analysis (Fig. 5A) the *Raphidophyceae* form a sister relationship with the *Xanthophyceae* and *Phaeophyceae*, but in POTTER & al. (1997) the position of the *Raphidophyceae* was not consistently resolved, and in CAVALIER-SMITH & CHAO (1996) they are a sister taxon to the eustigmatophytes and chrysophytes/synurophytes. The raphidophytes are unusual in that the marine species have carotenoids that are similar to the chrysophytes, synurophytes and phaeophytes, whereas the freshwater species have carotenoids similar to the xanthophytes. The flagellar apparatus is distinct, showing no obvious relationship to other groups. Thus, no clear sister taxon relationship has been conclusively identified for this class of heterokont chromophytes.

The use of plastid pigmentation to delineate heterokont algal classes, as well as other algal groups, is generally accepted. Among the heterokont chromophytes, the *Raphidophyceae* is the only class that is a glaring exception (BJØRNLAND & LIAAEN-JENSEN 1989). However, when one tries to find congruence of pigment data and molecular data, the results are less clear. The xanthophyll-cycle pigments and the reduced flagellar apparatus characters have been plotted onto the SSU rRNA tree (Fig. 5A). There appears to be a tendency for early diverging lineages to possess 19′-fucoxanthin-like derivatives, and to a lesser degree, for diatoxanthin and diadinoxanthin to be restricted to early diverging lineages, weakly supporting hypothesis 4a. However, in part due to the lack of resolution in the branching patterns for the heterokont chromophytes, we are unable to state conclusively that support can be found for either hypothesis 4a or 4b.

Relationships within the diatoms. Among the diatoms, the centric and the araphid pennate forms are paraphyletic (MEDLIN & al. 1996b,c). The diatoms diverge into two clades (clade 1 + 2). Each clade can be defined by the position of specialised tubes (termed labiate processes) in the cell wall and the arrangement of the Golgi bodies (see references in MEDLIN & al. 1996b,c). The traditional features of the morphology of the silica cell wall are only valuable in defining younger branches in the tree.

Plastid genome

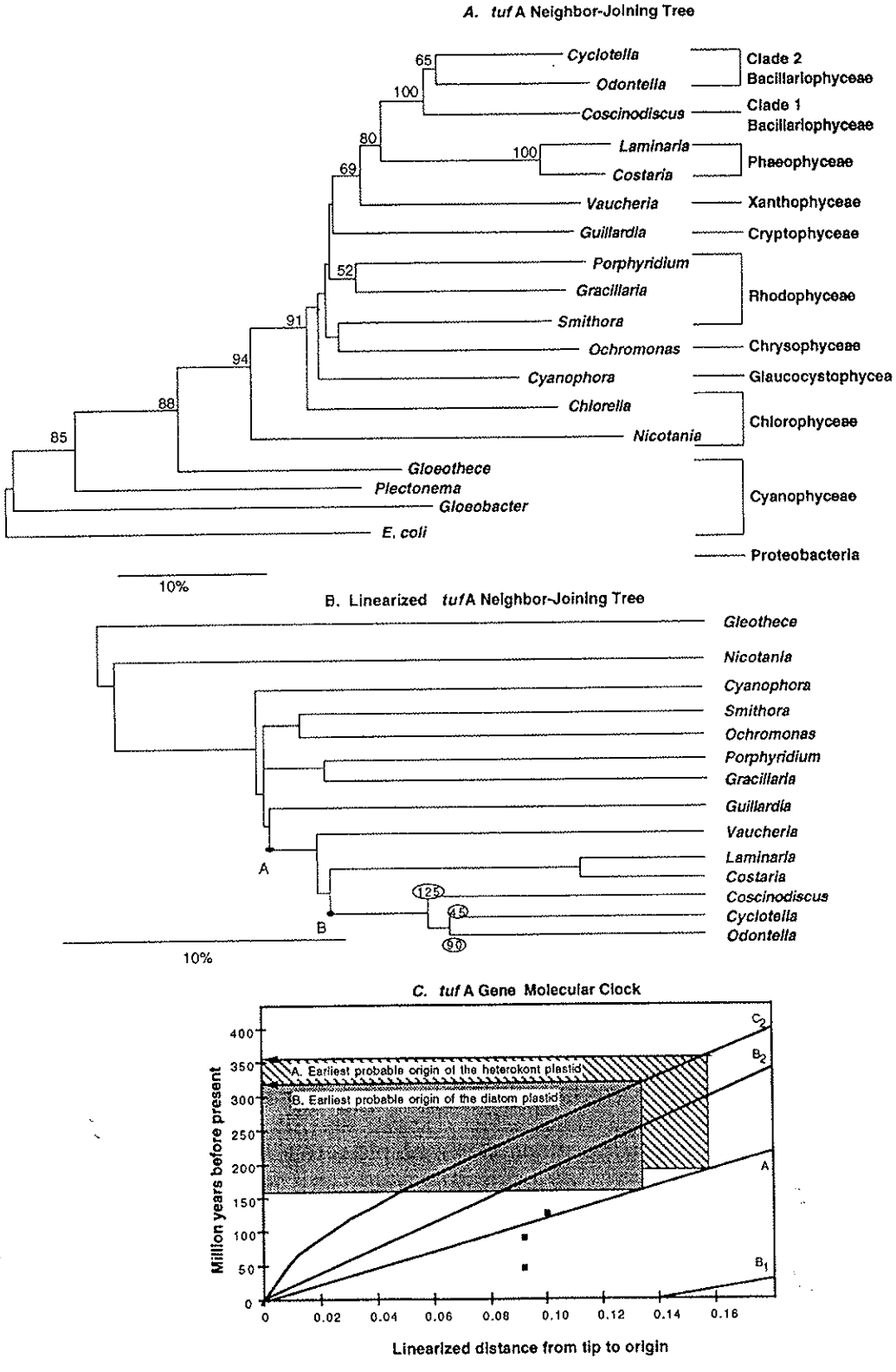
Endosymbiosis. The endosymbiotic hypothesis of plastid evolution maintains that plastids were acquired by primitive eukaryotic heterotrophs through the engulfment and maintenance of photosynthetic prokaryotes (SCHIMPER 1883, MERESCHKOWSKY 1905, RAVEN 1970, MARGULIS 1981). This hypothesis was once opposed by those who argued that the plastid arose directly without endosymbiosis during the evolution of the first eukaryotes (KLEIN & CRONQUIST 1967, CAVALIER-SMITH 1975), but this view is no longer supported by evolutionary biologists.

The plastids of the rhodophyte, chlorophyte and glaucocystophyte algae and the higher plants have only two membraned-plastids and are assumed to have resulted from a primary endosymbiotic event in which a eukaryotic host engulfed a prokaryotic cell. The host organisms associated with the primary endosymbiosis appear to arise as independent plastid-bearing lineages within the crown group radiation of the eukaryotes (Fig. 1, BHATTACHARYA & MEDLIN 1995).

The algae with 3–4 membraned plastids are hypothesised to have arisen through a secondary endosymbiotic event(s) in which a heterotrophic eukaryote host engulfed and reduced a photosynthetic eukaryote cell to a plastid. The additional membranes surrounding the plastid are remnants of the endosymbiosis (i.e., the host cell vacuole and the plasmalemma of the endosymbiont, see review in GIBBS 1993). Algae resulting from the secondary endosymbiosis include the euglenophytes and the chlorarachniophytes, which contain chlorophyll $a + b$, as well as the heterokont chromophytes, haptophytes, dinoflagellates and cryptophytes, most of which contain chlorophyll $a + c$ (GIBBS 1978, 1981; CAVALIER-SMITH 1989; JEFFREY 1989; ROWAN 1989; KOWALLIK 1992; VALENTIN & al. 1992).

Whereas current evidence from molecular and morphological/biochemical data suggests that the primary endosymbiotic event occurred only once, the secondary endosymbiotic event may have occurred several times (see review in BHATTACHARYA & MEDLIN 1995 and DELWICHE & PALMER 1996). The host organisms associated with the secondary endosymbioses, (viz, the euglenoids, cryptomonads, chlorarachniophytes, dinoflagellates haptophytes, and heterokont chromophytes, with 3–4 membraned plastids,) do not share a common ancestry, and thus a more likely hypothesis for their emergence as pigmented lineages is that each lineage has acquired its plastid through an independent secondary endosymbiosis, i.e. multiple secondary endosymbioses rather than through a single event (see CAVALIER-SMITH 1982). The identification of the vestigial nucleus (nucleomorph) in the plastids of the cryptophytes and chlorarachniophytes as being associated with the red algae and the green algae, respectively (Fig. 1), provides direct evidence using the nuclear genome of the endosymbiont that multiple secondary endosymbioses have occurred.

Many of the host lineages believed to have arisen from secondary endosymbiosis event(s) also have heterotrophic taxa as sister groups or as early divergences in their lineages (viz., heterokonts, cryptomonads, euglenoids, alveolates, chlorarachniophytes, see Fig. 1 and BHATTACHARYA & MEDLIN 1995). Either these lineages were originally photosynthetic and these heterotrophic taxa lost their plastids, or the lineages gained their plastids through secondary endosymbioses later in their evolution. The phylogenies of the plastids arising from


A. tufA Neighbor-Joining Tree
Cyclotella
Odontella
Coscinodiscus
Laminaria
Costaria
Vaucheria
Guillardia
Porphyridium
Gracillaria
Smithora
Ochromonas
Cyanophora
Chlorella
Nicotania
Gloeothece
Plectonema
Gloeobacter
E. coli
65
100
80
100
69
52
91
94
88
85
Clade 2 Bacillariophyceae
Clade 1 Bacillariophyceae
Phaeophyceae
Xanthophyceae
Cryptophyceae
Rhodophyceae
Chrysophyceae
Glaucocystophycea
Chlorophyceae
Cyanophyceae
Proteobacteria
10%
B. Linearized tufA Neighbor-Joining Tree
Gleothece
Nicotania
Cyanophora
Smithora
Ochromonas
Porphyridium
Gracillaria
Guillardia
Vaucheria
Laminaria
Costaria
Coscinodiscus
Cyclotella
Odontella
A
B
125
45
90
10%
C. tufA Gene Molecular Clock
A. Earliest probable origin of the heterokont plastid
B. Earliest probable origin of the diatom plastid
Million years before present
Linearized distance from tip to origin
C2
B2
A
B1
0
50
100
150
200
250
300
350
400
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16

secondary endosymbioses must be examined and compared with those of their host cells to infer the likely source of the photosynthetic eukaryote that was transformed into the plastids.

There is a moderate to extensive heterokont chromophyte, haptophyte and cryptophyte data set available for several plastid genes: the *tuf*A gene (DELWICHE & al. 1995), the large subunit of the RUBISCO operon (FUJIWARA & al. 1995, CHESNICK & al. 1996) and the small subunit of the ribosomal operon (BHATTACHARYA & MEDLIN 1995, MEDLIN & al. 1995 and unpubl.) (Figs. 6–8). In each case, we will compare the plastid phylogenies with those of the host lineages to infer the likely source of the taxa transformed into a plastid.

In the *tuf*A phylogeny (Fig. 6A), we have selected representatives from the larger data set published by DELWICHE & al. (1995) for our analysis. There are no haptophyte sequences available; however the heterokonts are well represented, and there is one cryptophyte in the tree. The *tuf*A gene suggests that plastids are monophyletic and originate from the cyanobacteria. The *tuf*A gene of 2-membraned plastids of the green algae/higher plants, the glaucocystophytes and the red algae are the first divergences from the cyanobacterial *tuf*A gene. The anomalous position of the chrysophyte *Ochromonas* among the red algae in the *tuf*A phylogeny has been discussed by DELWICHE & al. (1995) as being either a contaminant or an evolutionary novelty. The *tuf*A gene of 4-membraned plastids, represented in this tree by the heterokonts and cryptomonads, are later divergences and, fall within the red algae. Among the diatoms, *Coscinodiscus*, a centric diatom belonging to clade 1 diatoms as inferred from the nuclear-encoded SSU rRNA tree, is sister to two other centric diatoms of clade 2. The position of the cryptophyte *Guillardia* is not supported in the *tuf*A phylogeny and likely represents a problem of taxon sampling.

Although there is no bootstrap support for the clade containing the red and the chromophyte algae in the *tuf*A phylogeny, the separation of a "green lineage" from a "red plus golden lineage" is congruent with that found in the phylogenetic reconstructions from other plastid genes (see below). The lack of bootstrap support for the lineages in this phylogeny may in part reflect the asymmetry of the tree and internodal differences closer to 20% (see HILLIS & BULL 1993).

A phylogeny of the SSU rRNA gene has been constructed with the LogDet transformation [to avoid base compositional bias that can distort the relationships in this gene (LOCKHART & al. 1994)] and with the neighbor-joining analysis. The branching order of the major lineages are identical, and we present the neighbor-joining tree (Fig. 7A), which we will use below for our molecular clock

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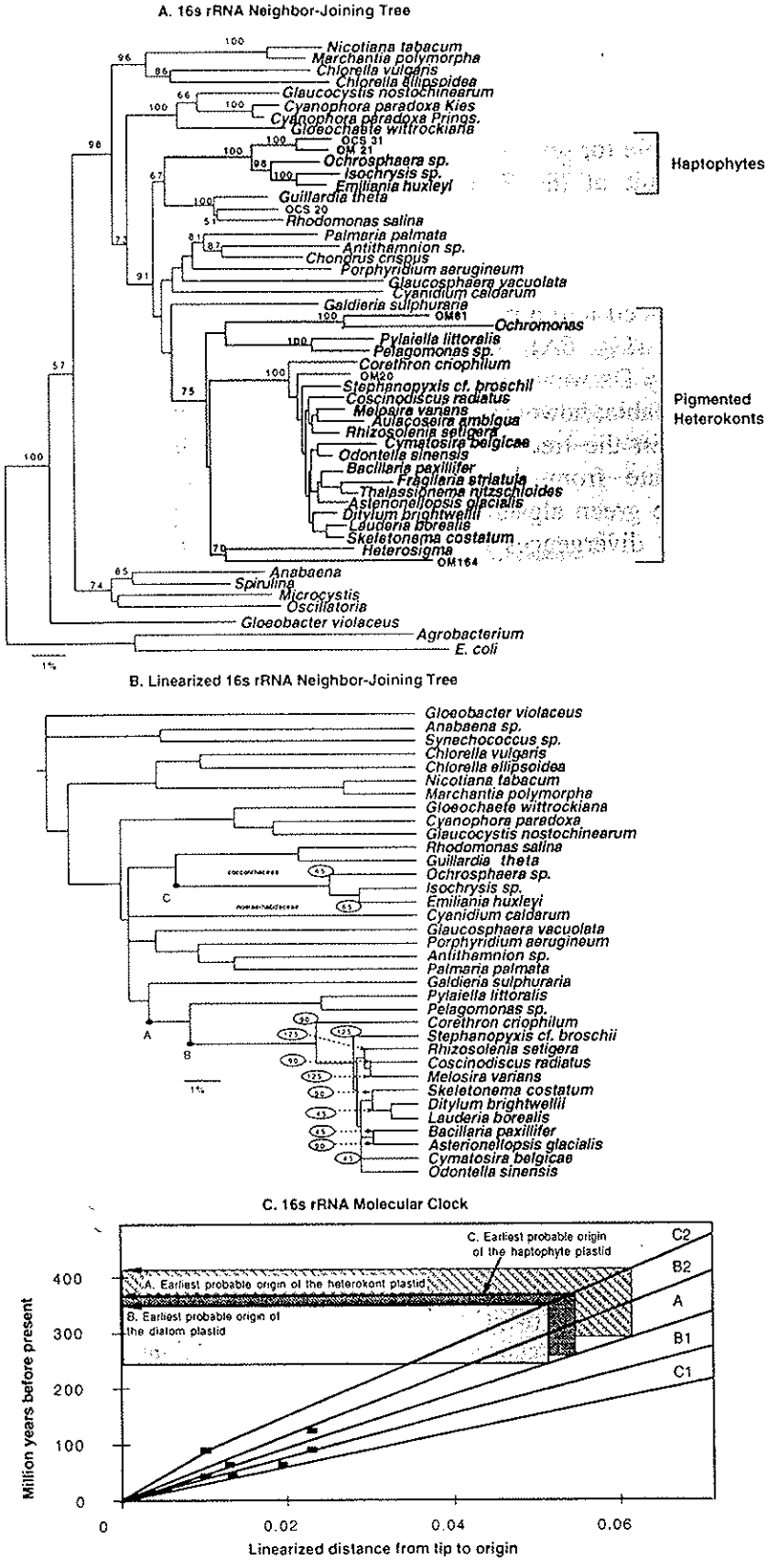
Fig. 6 *A–C*. Phylogenetic analysis of *tuf*A coding regions. *A* Neighbor-joining tree inferred from a gamma-weighted distance matrix (MEGA, KUMAR & al. 1993, a= 2) using all three codon positions. Bootstrap values (100 replications) are shown above the internal nodes. The tree was rooted on the branch leading to *E. coli*. *B* Linearisation of the neighbor-joining tree in *A* as in Fig. 4B. First appearances of diatom taxa from the fossil record are encircled and placed at the node where the taxa to the right are believed to have their first appearance. Point "A" marks the origin of the heterokont plastid; "B" is the origin of the diatom plastid. *C* Molecular clock constructed from *B* as in Fig. 4C. C_1 is below is the x axis and not shown

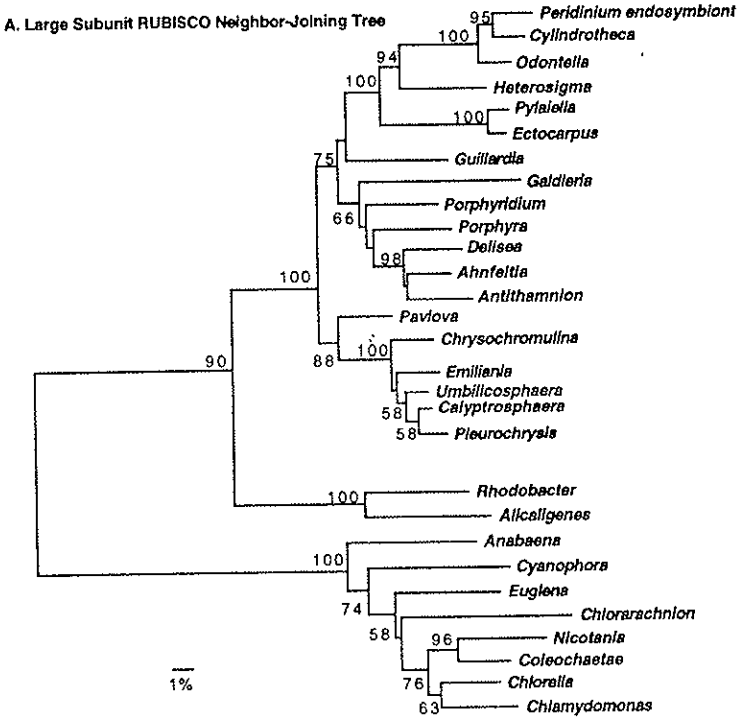

A. 16s rRNA Neighbor-Joining Tree
Nicotiana tabacum
Marchantia polymorpha
Chlorella vulgaris
Chlorella ellipsoidea
Glaucocystis nostochinearum
Cyanophora paradoxa Kies
Cyanophora paradoxa Prings.
Gloeochaete wittrockiana
OCS 31
OM 21
Ochrosphaera sp.
Isochrysis sp.
Emiliania huxleyi
Guillardia theta
OCS 29
Rhodomonas salina
Haptophytes
Palmaria palmata
Antithamnion sp.
Chondrus crispus
Porphyridium aerugineum
Glaucosphaera vacuolata
Cyanidium caldarum
Galdieria sulphuraria
OM61
Ochromonas
Pylaiella littoralis
Pelagomonas sp.
Corethron criophilum
OM20
Stephanopyxis cf. broschii
Coscinodiscus radiatus
Melosira varians
Aulacoseira ambigua
Rhizosolenia setigera
Cymatosira belgicae
Odontella sinensis
Bacillaria paxillifer
Fragilaria striatula
Thalassionema nitzschioides
Asterionellopsis glacialis
Ditylum brightwellii
Lauderia borealis
Skeletonema costatum
Heterosigma
OM164
Pigmented Heterokonts
Anabaena
Spirulina
Microcystis
Oscillatoria
Gloeobacter violaceus
Agrobacterium
E. coli
1%
B. Linearized 16s rRNA Neighbor-Joining Tree
Gloeobacter violaceus
Anabaena sp.
Synechococcus sp.
Chlorella vulgaris
Chlorella ellipsoidea
Nicotiana tabacum
Marchantia polymorpha
Gloeochaete wittrockiana
Cyanophora paradoxa
Glaucocystis nostochinearum
Rhodomonas salina
Guillardia theta
coccolithaceae
Ochrosphaera sp.
Isochrysis sp.
Emiliania huxleyi
C
noelaerhabdaceae
Cyanidium caldarum
Glaucosphaera vacuolata
Porphyridium aerugineum
Antithamnion sp.
Palmaria palmata
Galdieria sulphuraria
Pylaiella littoralis
Pelagomonas sp.
Corethron criophilum
A
B
Stephanopyxis cf. broschii
Rhizosolenia setigera
Coscinodiscus radiatus
Melosira varians
Skeletonema costatum
Ditylum brightwellii
Lauderia borealis
Bacillaria paxillifer
Asterionellopsis glacialis
Cymatosira belgicae
Odontella sinensis
1%
C. 16s rRNA Molecular Clock
C. Earliest probable origin of the haptophyte plastid
A. Earliest probable origin of the heterokont plastid
B. Earliest probable origin of the diatom plastid
C2
B2
A
B1
C1
Million years before present
400
300
200
100
0
0
0.02
0.04
0.06
Linearized distance from tip to origin

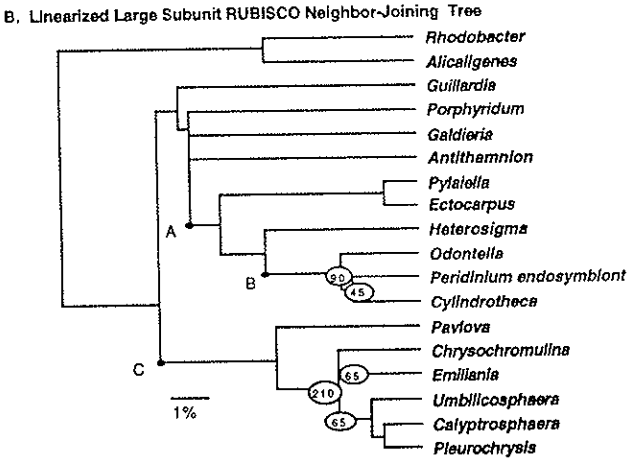
calculations. Branch lengths in the LogDet transformation are not linear using small data sets (< 2000 aa) and cannot be used for clock calculations unless they are corrected (Gu & Li 1996).

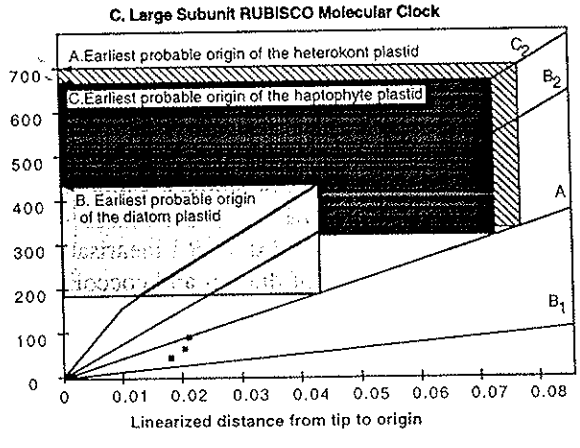
The 16S rRNA phylogeny also suggests that plastids are monophyletic (bootstrap = 98%) and originate from the cyanobacteria. The 2-membraned plastids of the green algal/higher plants, the glaucocystophytes and the red algae diverge as independent monophyletic lineages. The 4-membraned plastids of heterokont chromophytes, haptophytes and cryptophytes share a recent evolutionary history with the red algae (bootstrap = 91%). However, their hosts do not (Fig. 1), and this is supportive evidence that these lineages obtained their plastids from a red algae or a red algal-like ancestor via endosymbiosis. The haptophytes and cryptomonads are moderately supported (bootstrap = 67%) and are sister taxa to a largely unresolved assemblage of red algae and the heterokont chromophytes. The primitive unicellular reds contribute substantially to the problems of unresolved branching order within this lineage. The advanced red algae are well supported (boostrap = 81%) and are later divergences in the tree. The heterokont chromophytes are well supported (bootstrap = 75%) and branch from within the red algal lineage. Because the haptophytes and cryptomonads are held outside the true red algal lineage by moderate bootstrap support, one possible interpretation of these data is that the heterotrophic ancestors of the haptophytes and cryptomonads engulfed and retained a red algal-like ancestor, whereas the heterotrophic heterokonts are more likely to have engulfed a primitive red algae. Further taxon sampling among the primitive reds may help to improve support for the branching order among these taxa. The LogDet tree also supports the position of the haptophytes and cryptomonads outside the red algal lineage (tree not shown, but see Medlin & al. 1995).

Within the heterokonts, several lineages are recovered. These correspond to the major classes of the heterokont algae (except for the clade comprising the *Pelagophyceae* and *Phaeophyceae*), but support for the branching order is not strong. Within the diatoms, clade 1 diatoms are broken into separate lineages, however clade 2 diatoms remain intact. There are not enough identified taxa sampled in the haptophyte and cryptophyte lineages to comment on their branching order, but each is a monophyletic lineage (bootstrap = 100%).

Fig. 7 *A–C*. Phylogenetic analysis of plastid-encoded SSU rRNA genes. *A* Neighbor-joining tree inferred from a Kimura (1980) distance matrix. Representatives from all of the algae are included except for the *Chlorarachniophyta* and the *Euglenophyta*. Bootstrap values (100 replications) are shown above the internal nodes. The *Haptophyta* and the *Heterokonta* are labelled on the tree. The tree was rooted on the branch leading to *E. coli*. Terminal taxa in the tree represented by codes (e.g., OM81) are unidentified sequences from a 16S rRNA clone library provided courtesy of Dr. M. Rappé. *B* Linearisation of the neighbor-joining tree in *A* as in Fig. 4B. First appearances of diatom and coccolithophorid taxa from the fossil record are encircled and placed at the node where the taxa to the right are believed to have their first appearance. Point "A" marks the origin of the heterokont plastid; "B" is the origin of the diatom plastid; "C" is the origin of the haptophyte plastid. *C* Molecular clock constructed from *B* as in Fig. 4C


A. Large Subunit RUBISCO Neighbor-Joining Tree
Peridinium endosymbiont
Cylindrotheca
Odontella
Heterosigma
Pytaiella
Ectocarpus
Guillardia
Galdieria
Porphyridium
Porphyra
Delisea
Ahnfeltia
Antithamnion
Pavlova
Chrysochromulina
Emiliania
Umbilicosphaera
Calyptrosphaera
Pleurochrysis
Rhodobacter
Alicaligenes
Anabaena
Cyanophora
Euglena
Chlorarachnion
Nicotania
Coleochaetae
Chlorella
Chlamydomonas
1%


B. Linearized Large Subunit RUBISCO Neighbor-Joining Tree
Rhodobacter
Alicaligenes
Guillardia
Porphyridum
Galdieria
Antithamnion
Pytaiella
Ectocarpus
Heterosigma
Odontella
Peridinium endosymbiont
Cylindrotheca
Pavlova
Chrysochromulina
Emiliania
Umbilicosphaera
Calyptrosphaera
Pleurochrysis
A
B
C
90
45
65
210
65
1%


C. Large Subunit RUBISCO Molecular Clock
A.Earliest probable origin of the heterokont plastid
C.Earliest probable origin of the haptophyte plastid
B. Earliest probable origin of the diatom plastid
C2
B2
A
B1
700
600
500
400
300
200
100
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Linearized distance from tip to origin

The RUBISCO gene is the only single-copy gene whose molecular analysis indicates a polyphyletic origin for the plastids (see review in PALMER 1993, LOISEAUX DE GOËR 1994). The phylogenetic relationships reconstructed from the RUBISCO tree are far more complicated than those presented here in Fig. 8 (see DELWICHE & PALMER 1996). We show only the major lineages containing the "red" and "green" type of Form I of the large subunit of RUBISCO. The two lineages are so divergent that it is not incorrect to root one lineage with the other as we have done in Fig. 8A (see DELWICHE & PALMER 1996).

The RUBISCO genes from the chlorophyll *a* + *b* lineage, containing the green algae/higher plants, glaucocystophytes, chlorarachniophytes and euglenoids, originate from cyanobacterial genes (Fig. 8A). In contrast, the non-green algal lineages (excluding the dinoflagellates, which have an entirely different RUBISCO origin, see PALMER 1993) originate from *Proteobacteria* (Fig. 8A). Historically, the branching order among the major groups has changed as more taxa have been added to the phylogenetic analyses (compare LOISEAUX DE GOËR 1994, FUJIWARA & al. 1995, MCFADDEN & al. 1995, and Fig. 8A). However, it is clear that the RUBISCO gene of the heterokont chromophytes and cryptophytes in this lineage (all of which apparently obtained their plastids through a secondary endosymbiosis) share a common ancestry with the red algal lineage (bootstrap = 100%). Our tree would support the hypothesis that the heterokont chromophytes, haptophytes and cryptophytes obtained their plastids by engulfing a red algae or an ancestor that gave rise to the red algae. As with the other plastid genes, the host organism genes of the heterokont chromophytes, haptophytes and cryptophytes do not share a recent evolutionary history with the red algae.

As in the 16S rRNA tree, the haptophytes (a monophyletic group in 88% of bootstrap replicates) are recognised as a discrete sister lineage (bootstrap = 75%) to the red algae plus other chlorophyll *a* + *c* algae. However, if fewer primitive red algae are included in the analysis, the cryptophytes will group with the haptophytes (bootstrap = 64%, data not shown) as sister group to the red algae plus heterokont chromophytes. In no cases are the haptophytes and heterokont chromophytes sister taxa. Lineages corresponding to the diatoms, raphidophytes and phaeophytes are recovered within the heterokont chromophytes, but taxon sampling is too low to comment on their branching order. Among the haptophytes, the divergence of the two subclasses are confirmed. The family *Noelaerhabdaceae* (*Emilinia huxleyi*)

Fig. 8 *A–C*. Phylogenetic analysis of the large subunit of RUBISCO coding regions. *A* Neighbor-joining tree inferred from a gamma-weighted distance matrix (MEGA, KUMAR & al. 1993, a = 1) using all three codon positions. Bootstrap values (100 replications) are shown above the internal nodes. The tree was rooted on the branch leading to the green algae/higher plants. *B* Linearisation of the neighbor-joining tree in *A* as in Fig. 4B. The entire green algal/higher plant lineage was evolving too fast and was eliminated from the linearisation. First appearances of diatom and coccolithophorid taxa from the fossil record are encircled and placed at the node where the taxa to the right are believed to have their first appearance. Point "A" marks the origin of the heterokont plastid; "B" is the origin of the diatom plastid; "C" is the origin of the haptophyte plastid. *C* Molecular clock constructed from *B* as in Fig. 4C. C_1 is below the x axis and not shown

is sister to the other coccolithophorids, which is congruent with the host tree (Fig. 5A).

Plastid genes from haptophytes and heterokont chromophytes were never sister taxa in any of our analyses, and the heterokont chromophyte plastid genes were always embedded within the red algal plastid lineage. The convergence of the 16S rRNA and RUBISCO plastid gene phylogenies suggests that the heterokont chromophytes likely engulfed a primitive red algae, whereas the cryptophytes and the haptophytes are more likely to have engulfed an ancestor of the red algae. This suggests that the *Chromista* are not monophyletic and that haptophyte, heterokont chromophytes and cryptophyte plastids arose from separate endosymbiotic events. These data support hypotheses 1c and 1d.

Molecular clock calculations

Molecular data are normally used to reconstruct the phylogenetic history of extant organisms. Ideally, as organisms diverge, their genomes accumulate base substitutions in a stochastic, but clock-like manner. It is now widely recognised that a universal molecular clock does not exist and that the base substitution rate varies within lineages and genes. Nevertheless, if potential errors are identified with a relative rate test and corrected by eliminating the significantly fast and slow taxa and by linearising the rate of evolution, it is then possible to use molecular data to estimate divergence times.

Using the method of HILLIS & MORITZ (1990), we have estimated from the nuclear and plastid genes the time of origin (1) for the diatoms, (2) for the heterokont chromophytes, (3) for the haptophytes and the divergence of their two subclasses and (4) for the timing of the secondary endosymbiotic events for the pigmented heterokonts and the haptophytes. We initially calculated a relative rate or branch length test in which the evolutionary rate of all pair-wise combinations of taxa was compared to several outgroups (WU & LI 1985, TAKEZAKI & al. 1995). In this manner we identified taxa not evolving within a stochastic model of base substitution. We then selected a range of taxa with varying degrees of distance from one another to be used for the construction of a linearised neighbor-joining tree in which rate variation between the taxa was assumed to be eliminated (TAKEZAKI & al. 1995). A linearised neighbor joining (NJ) tree was constructed from the nuclear-encoded SSU rRNA genes and from the plastid-encoded SSU rRNA, *rbc*L and the *tuf*A genes (Figs. 4–8B). First appearance dates of diatom and coccolithophorid taxa with a fossil record were regressed against estimated branch lengths of lineages in each tree to construct a molecular clock for each gene or group of organisms (Figs. 4–8C). First appearance dates of taxa immediately after a gap in the diatom fossil record were predated to the middle of the gap or before, it, if potential ancestors of the extant taxa could be identified in well-preserved diatom deposits before the gap (GERSONDE & HARWOOD 1990).

For each linearised tree, we estimated an average age of the clade and its earliest probable age ($p = 95\%$). The average age of any undated node was determined by multiplying the length of its median or average lineage by the regression coefficient. The earliest probable age for any undated node was taken

from the upper 95% confidence limit around the age estimate given the length of its median or average lineage.

Fossil dates may seriously underestimate the first appearance date of any lineage (WRAY & al. 1996). Thus, time estimates based on the average age for the lineage, given that the fossil dates may be later than first appearances, are also likely to underestimate the origin of groups. The HILLIS & MORITZ (1990) model for the calculation of a molecular clock provides an estimate for an upper and lower 95% confidence limit for the origin of any undated node. Thus, using this calculation, the actual time of origin of any undated node in the tree, should realistically lie somewhere between the average age determined from the regression line and the earliest probable age determined from the upper 95% confidence limit. Presumed dates of origin between the lower 95% confidence limit and the regression line would be nullified by fossil taxa present during this time. With few fossil dating points, the 95% confidence interval can be quite broad, pushing the earliest probable age farther back in time.

From the SSU rRNA clock calculated for the pigmented heterokonts, we have estimated the average age of the brown algae, the diatoms and the pigmented heterokonts (see also KOOISTRA & MEDLIN 1996). The recent appearance of the brown algae is well in agreement with other molecular, morphological and biogeographic evidence (see SAUNDERS & DRUEHL 1992), but contrasts with some earlier putative brown algal fossils (TAGGART & PARKER 1976). The average age of the diatoms is very close to their first fossil record (MEDLIN & al. 1996c). Using our average and earliest probable dates for the origin of the pigmented heterokonts (170–270 Ma), we conclude that this group is unlikely to have existed much before the Permian-Triassic boundary.

In contrast, the SSU rRNA molecular clock for the haptophyte lineage indicates that they are a much older group. Their average age is 850 Ma; their earliest probable age is c. 1800 Ma. These dates may be greatly overestimated because the haptophyte SSU rRNA clock is based only on three divergence times from the coccolithophorid fossil record. Nevertheless, rate variation in the SSU rRNA gene for this group is minimal (data not shown), so we feel that our predictions of time of divergences are reasonable. Interestingly, the mean of the average age of the divergence of the two subclasses of the haptophytes (299 Ma) is closer to the average age of the pigmented heterokonts (248 Ma). These figures are determined by averaging both the nuclear and plastid age estimates.

By constructing a molecular clock from our plastid gene sequence data, we can date the timing of the endosymbiotic event leading to the transfer of photosynthetic capacity to the heterokont and haptophyte lineages. Thus, the ages derived from plastid genes can be compared to those of the origins of their host cells. If hypothesis 1a is true, then the dates for the divergence of the haptophyte and heterokont algae should be younger that the dates for the endosymbiotic event leading to the transfer of photosynthetic capacity to the haptophyte and heterokont lineages.

Hypotheses 1a, 1c and 1d can support the possibility that the early heterotrophic divergences in the heterokont lineage are the result of plastid loss. If that is true, then the endosymbioses should predate the origin of the hosts. If the converse is true, then the origin of the heterokont algae should coincide with the timing of

Table 1. Estimated average time of origin (in millions of years) of the host cells and their plastids from the heterokont chromophytes, diatoms and haptophytes

Algal groups	Host cells 18S rRNA	Plastids 16S rRNA	Plastids *tuf*A	Plastids RUBISCO	Plastids Mean
Heterokont chromophytes	170	293	190	337	274
Diatoms	135	249	160	190	200
Haptophytes					
Group origin	850				
Subclass divergence	420			177	
Plastid origin		263		322	293

the secondary endosymbiotic event leading to the transfer of photosynthetic capacity to heterokont organisms. If the haptophytes obtained their plastids at the origin of their lineage, then the timing of their symbiosis should be widely disparate from that of the pigmented heterokonts given no plastid loss.

The average date for the origin of the pigmented heterokonts calculated from the SSU rRNA gene (170 Ma) is close to the mean of the average date for a secondary endosymbiotic origin of the heterokont plastid estimated from three plastid genes (274 Ma) (Figs. 6–8C, Table 1). This provides support for hypotheses 1c and 1d over the remaining two hypotheses. The consistent separation of the haptophytes from the heterokonts in all of the plastid phylogenies provides evidence to support hypothesis 1d over 1c.

The estimated average date for the origin of the haptophyte plastid is considerably younger than the origin of the host lineage. This would suggest that early members of the haptophytes were not photosynthetic and that the endosymbiosis occurred somewhere along the internode leading to the diversification of the haptophytes. Interestingly, the divergence of the two subclasses of the haptophytes, as estimated from the SSU rRNA and RUBISCO genes, is very close to the origin of the haptophyte plastid (Table 1). We hypothesise that the endosymbiotic event in the haptophyte lineage occurred just prior to the divergence of the two subclasses. Therefore, it follows that all early ancestors in the haptophyte lineage were heterotrophic and are extinct, or are undersampled.

Interestingly, the estimated times for the two secondary endosymbiotic events in haptophytes and heterokonts, respectively, are remarkably close. The mean divergence time of the two groups estimated from three genes is 281 Ma ($n = 5$, Table 1). Thus, the transfer of the photosynthetic capacity to these lineages occurred approximately the same time at or before the Permian-Triassic boundary (250 Ma). MEDLIN & al. (1997) have presented evidence for the correlation of the Permian-Triassic mass extinction with the re-radiation of the modern phytoplankton following this event. Both the pigmented heterokonts and the haptophytes comprise the bulk of today's eukaryotic phytoplankton in the oceans. Our molecular clock calculations would support a theory that the Permian-Triassic extinction opened many niches in the world's oceans and those organisms capable of engulfing and maintaining a photo-autotroph had an adaptive advantage. This suggests that multiple secondary endosymbioses could have occurred at a similar time.

Summary

Although remarkable progress has been made during the past 40 years, a satisfactory understanding of the phylogenetic relationships among the "golden algae" remains elusive. From the data presented in Figs. 1–3 and from other published phylogenies, no clear sister relationship has been demonstrated for the haptophyte and heterokont host cells. This is the minimum that must be presented to invoke a monophyletic origin for the two groups. Instead, a relationship between the alveolates and the heterokonts consistently reoccurs with up to 100% bootstrap support in some phylogenies. The *Haptophyceae*, once part of the *Chrysophyceae*, have been shown to be a distinct taxonomic group, however, their closest living relative remains unresolved. Further work should be undertaken from other genes to resolve conclusively the relationship between heterokonts and haptophytes: nevertheless existing date do not support a monophyletic origin for the two groups.

Both ultrastructural (two extra membranes around the plastid) and molecular data suggest the plastids of the haptophytes and heterokont chromophytes are the result of secondary endosymbiosis (probably a red algae or red alga-like organism). The plastid-encoded SSU rRNA (bootstrap = 67%) and *rbc*L data (bootstrap = 75%) support separate endosymbioses for the plastids of haptophytes and heterokont chromophytes because other groups occupy branches in phylogenetic trees between them (cryptophytes and red algae in SSU rRNA – Fig. 7; cryptophytes and red algae in *rbc*L – Fig. 8). Although bootstrap support of $\geqslant 70\%$ can indicate a probability $\geqslant 95\%$ that the recovered clade is real (Hillis & Bull 1993), we cannot state conclusively that the haptophyte and heterokont plastids are a monophyletic group (hypothesis 1a or 1c) or are two distinctly different lineages (hypotheses 1b and d). However, the consistent separation of the haptophyte and heterokont plastids in the phylogenetic analyses, taken in combination with our molecular clock calculations, favour hypothesis 1d that the two lineages are not monophyletic.

Data presented above (nuclear-encoded SSU rRNA, actin) suggest that the heterokont chromophytes are a monophyletic assemblage, which supports hypothesis 2a. Bootstrap support for the molecular data is too weak to determine the phylogenetic branching pattern among this assemblage. Thus, we cannot support either hypothesis regarding the monophyly of taxa with reduced flagellar apparatuses (hypotheses 3a, 3b). The SSU rRNA data (Fig. 5) suggest that the two carotenoid types arose independently more than once, which lends some support to hypothesis 4a.

Molecular clock-calculated dates suggest that the haptophyte host cell lineage is relatively ancient (Proterozoic-Paleozoic) but that the haptophyte plastid was acquired more recently (Mesozoic). The molecular clock-calculated dates for origin of the heterokont chromophytes are more recent (Mesozoic), both with respect to the host cell and the plastid. Perhaps coincidentally, the estimated dates for the origin of plastids in both haptophytes and heterokont chromophytes are nearly identical. Data suggest both groups first became photosynthetic at, or shortly before the Permian-Triassic boundary. This would support a hypothesis that secondary endosymbioses, which represent a major evolutionary step in the

advancement of the algae, may be associated with the major climatic changes at the end Permian and the mass extinctions that followed (ERWIN 1994).

We gratefully acknowledge the technical support of U. WELLBROCK, S. WRIEDEN, and NATHALIE SIMON. Dr M. RAPPÉ kindly provided access to unpublished 16S rRNA sequence data from the diatoms, haptophytes and cryptophytes and Dr D. BHATTACHARYA provided Fig. 3. This research was supported in part by grants from the BMBF (03F0161B) and DFG (ME1480/1-2) of Germany to LKM, from the NSERC of Canada to GWS, and from the NSF (BRS94-19498) and ONR (N001492J1717) of the USA to RAA. This is AWI contribution no. 1290.

References

AGARDH, C. A., 1820: Species Algarum. **1**. – Lund.

ANDERSEN, R. A., 1987: *Synurophyceae* classis nov., a new class of algae. – Amer. J. Bot. **74**: 337–353.

– 1991: The cytoskeleton of chromophyte alga. – Protoplasma **164**: 143–159.

– SAUNDERS, G. W., PASKIND, M. P., SEXTON, J. P., 1993: The ultrastructure and 18S rRNA gene sequence for *Pelagomonas calceolata* gen. & sp. nov., and the description of a new algal class, the *Pelagophyceae* classis nov. – J. Phycol. **29**: 701–715.

ARIZTIA, E. V., ANDERSEN, R. A., SOGIN, M. L., 1991: A new phylogeny for chromophyte algae using 16S-like rRNA sequences from *Mallomonas papillosa* (*Synurophyceae*) and *Tribonema aequale* (*Xanthophyceae*). – J. Phycol. **27**: 428–436.

BHATTACHARYA, D., MEDLIN, L., 1995: The phylogeny of plastids: A review based on comparison of small subunit ribosomal RNA coding regions. – J. Phycol **31**: 489–498.

– EHLTING, J., 1995: Actin coding regions: gene family evolution and use as a phylogenetic marker. – Arch. Protistenk. **145**: 155–164.

– MEDLIN, L., WAINRIGHT, P. O., ARIZTIA, E. V., BIBEAU, C., STICKEL, S. K., SOGIN, M. L., 1992: Algae containing chlorophylls *a* and *c* are paraphyletic: molecular evolutionary analysis of the *Chromophyta*. – Evolution **46**: 1801–1817.

– HELMCHEN, T., MELKONIAN, M., 1995: Molecular evolutionary analyses of nuclear-encoded small subunit ribosomal RNA identify an independent rhizopod lineage containing the *Euglyphidae* and the *Chlorarachniophyta*. – J. Eukaryote Microbiol. **42**: 65–69.

BJØRNLAND, T., LIAAEN-JENSEN, S., 1989: Distribution patterns of carotenoids in relation to chromophyte phylogeny and systematics. – In GREEN, J. C., LEADBEATER, B. S. C., DIVER, W. L., (Eds): The chromophyte algae: problems and prospectives, pp. 37–60. – Syst. Ass. Spec. Vol. **38**. – Oxford: Clarendon Press.

BLACKMAN, F. F., 1900: The primitive algae and the flagellata. An account of modern work bearing on the evolution of algae. – Ann. Bot. **14**: 647–688.

BOURRELLY, P., 1957: Recherches sur les Chrysophycées: morphologie, phylogénie, systématique. – Rev. Algol. Mém. Hors-Séri. **1**: 1–412.

– 1968: Les Algues d'eau douce. 2. Algues jaunes & brunes. – Paris: Boubée.

CARON, L., DOUADY, D., QUINET-SZELY, DE GOËR, S., BERKALOFF, C., 1996: Gene structure of a chlorophyll a/c binding protein from a brown alga: Presence of an intron and phylogenetic implications. – J. Molec. Evol. **43**: 270–280.

CAVALIER-SMITH, T., 1975: The origin of nuclei and of eukaryotic cells. – Nature **256**: 463–468.

– 1982: The origins of plastids. – Biol. J. Linn. Soc. **17**: 289–306.

– 1986: The kingdom *Chromista*: Origin and systematics. – In ROUND F. E., CHAPMAN D. J., (Eds): Progress in phycological research, **4**, pp. 319–358. – Bristol: Biopress.

– 1989: The kingdom *Chromista*. – In GREEN, J. C., LEADBEATER, B. S. C., DIVER, W. L., (Eds): The chromophytic algae: problems and perspectives, pp. 381–407. – Oxford: Clarendon Press.
– 1993: Kingdom *Protozoa* and its 18 phyla. – Microbiol. Rev. **57**: 953–994.
– CHAO, E. E., 1996: 18S rRNA sequence of *Heterosigma carterae* (*Raphidophyceae*), and the phylogeny of the heterokont algae (*Ochrophyta*). – Phycologia **35**: 500–510.
– ALLSOPP, M. T. E. P., CHAO, E. E., 1994: Chimeric conundra: Are nucleomorphs and chromists monophyletic or polyphyletic? – Proc. Natl. Acad. Sci. USA **91**: 11368–11372.
– CHAO, E. E., ALLSOPP, M. T. E. P., 1995: Ribosomal RNA evidence for chloroplast loss within *Heterokonta*: Pedinellid relationships and a revised classification of Ochristan algae. – Arch. Protistenk. **145**: 209–220.
– ALLSOPP, M. T. E. P., HAUEBER, M. M., GOTHE, G., CHAO, E. E., COUCH, J. A., MAIER, U.-G., 1996: Chromobiote phylogeny: the enigmatic alga *Reticulosphaera japonensis* is an aberrant haptophyte, not a heterokont. – Eur. J. Phycol. **31**: 255–264.
CHADEFAUD, M., 1950: Les cellules nageuses des algues dans l'embranchment des Chromophycées. – Compt. Rend. Hebd. Séances Sci. **231**: 788–790.
CHESNICK, J. M., MORDEN, C. W., SCHMIEG, A. M., 1996: Identity of the endosymbiont of *Peridinium foliaceum* (*Pyrrhophyta*): Analysis of the *rbc*LS operon. – J. Phycol. **32**: 850–857.
CHRISTENSEN, T., 1962: Alger. – In BÖCHER, T. W., LANGE, M., SØRENSEN, T., (Ed.): Botanik, **2**/2, pp. 1–178. – Copenhagen: Munksgaard.
– 1989: The *Chromophyta*, past and present. – In GREEN, J. C., LEADBEATER, B. S. C., DIVER, W. L., (Eds): The chromophyte algae: problems and perspectives, pp. 1–12. – Oxford: Clarendon Press.
CRAIGIE, J. S., 1974: Storage Products. – In STEWART, W. D. P., (Ed.): Algal physiology and biochemistry, pp. 206–235. – Berkeley, CA: University of California Press.
DELWICHE, C. F., PALMER, J. D., 1996: Rampant horizontal transfer and duplication of Rubisco genes in *Eubacteria* and plastids. – Molec. Biol. Evol. **13**: 873–882.
– KUHSEL, M., PALMER, J. D., 1995: Phylogenetic analysis of *tuf*A sequences indicates a cyanobacterial origin of all plastids. – Molec. Phylogenet. Evol. **4**: 110–128.
DODGE, J. D., 1975: The fine structure of algal cells. – New York: Academic Press.
EHRENBERG, C. G., 1838: Die Infusionsthierchen als vollkommene Organismen. – Leipzig: Voss.
ERWIN, D. H., 1994: The Permo-Triassic extinction. – Nature **367**: 231–236.
FELSENSTEIN, J., 1985: Confidence limits on phylogenies: an approach using the bootstrap. – Evolution **39**: 783–791.
FRITSCH, F. E., 1945: The structure and reproduction of the algae. **2**. – Cambridge: Cambridge University Press.
FUJIWARA, S., SAWADA, M. H., SOMEYA, J., MINAKA, N., NISHIKAWA, S., 1995: Molecular phylogenetic analysis of the *rbc*L in *Prymnesiophyta*. – J. Phycol. **30**: 863–871.
GERSONDE, R., HARWOOD, D. M., 1990: Lower Cretaceous diatoms from ODP Leg 113 site 693 (Weddell Sea). I: Vegetative cells. – In BARKER, P. F., KENNETT, J. P., et al. (Eds): Proceedings of the Ocean Drilling Program, scientific results, **113**, pp. 365–402. – College Station, TX: Ocean Drilling Program.
GIBBS, S. P., 1978: The chloroplasts of *Euglena* may have evolved from symbiotic green algae. – Canad. J. Bot. **56**: 2882–2889.
– 1981: The chloroplasts of some algal groups may have evolved from endosymbiotic eukaryotic algae. – Ann. New York. Acad. Sci. **361**: 193–208.
– 1993: The origin of algal chloroplasts. – In LEWIN, R. A., (Ed.): Origins of plastids, pp 107–121. – New York: Chapman and Hall.

GREEN, B. R., DURNFORD, D. G., 1996: The chlorophyll-carotenoid proteins of oxygenic photosynthesis. – Annual. Rev. Pl. Physiol. Pl. Mol. Biol. **47**: 685–714.

GREEN, J. C., 1989: Relationships between the chromophyte algae: the evidence from studies of mitosis. – In GREEN, J. C., LEADBEATER, B. S. C., DIVER, W. L., (Eds): The chromophyte algae: problems and perspectives, pp. 189–206. – Oxford: Clarendon Press.

– LEADBEATER, B. S. C., (Eds), 1994: The haptophyte algae. – Oxford: Clarendon Press.

– – DIVER, W. L., (Eds), 1989: The chromophyte algae: problems and perspectives. – Oxford: Clarendon Press.

GU, X., LI, W.-H., 1996: Bias-corrected paralinear and LogDet distances and tests of molecular clocks and phylogenies under nonstationary nucleotide frequencies. – Molec. Biol. Evol. **13**: 1375–1383.

HARVEY, W. H., 1836: *Algae*. – In MACKAY, J. T., (Ed.): Flora Hibernica, pp. 157–254. – Dublin.

HIBBERD, D. J., 1976: The ultrastructure and taxonomy of the *Chrysophyceae* and *Prymnesiophyceae* (*Haptophyceae*): a survey with some new observations on the ultrastructure of the *Chrysophyceae*. – Bot. J. Linn. Soc. **72**: 55–80.

– LEEDALE, G. F., 1971: A new algal class – the *Eustigmatophyceae*. – Taxon **20**: 523–525.

HILLIS, D. M., BULL, J. J., 1993: An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. – Syst. Biol. **42**: 182–192.

– MORITZ, C., 1990: An overview of applications of molecular systematics. – In HILLIS, D. M., MORITZ, C., (Eds): Molecular systematics, pp. 502–515. – Sunderland, MA: Sinauer.

– – MABLE, B. K., 1996: Molecular systematics. – Sunderland, MA: Sinauer.

JEFFREY, S. W., 1989: Chlorophyll *c* pigments and their distribution in the chromophyte algae. – In GREEN, J. C., LEADBEATER, B. S. C., DIVER, W. L., (Eds): The chromophyte algae: problems and perspectives, pp. 13–36. – Oxford: Clarendon Press.

JORDAN, R., GREEN, J. C., 1994: A check-list of the extant *Haptophyta* of the world. – J. Mar. Biol. Assoc. U.K. **74**: 149–174.

KEELING, P. J., DOOLITTLE, W. F., 1996: Alpha-tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. – Molec. Biol. Evol. **13**: 1297–1305.

KIMURA, M., 1980: A simple method for estimating evolutionary rates of base substitution through comparative studies of sequence evolution. – J. Molec. Evol. **16**: 111–120.

KLEBS, G., 1893: Flagellatenstudien. II. – Z. Wiss. Zool. **55**: 353–445.

KLEIN, R. M., CRONQUIST, A., 1967: A consideration of the evolutionary and taxonomic significance of some biochemical, micromorphological, and physiological characters in the thallophytes. – Quart. Rev. Biol. **42**: 105–296.

KNOLL, A. H., 1992: The early evolution of eukaryotes: a geological perspective. – Science **256**: 622–627.

KOHLER, S., DELWICHE, C. F., DENNY, P. W., TILNEY, L. G., WEBSTER, P., WILSON, R. J., PALMER, J. D., ROOS, D. S., 1997: A plastid of probable green algal origin in apicomplexan parasites. – Science **275**: 1485–1489.

KOOISTRA, W. H. C. F., MEDLIN, L. K., 1996: Evolution of the diatoms (*Bacillariophyta*): IV. A reconstruction of their age from small subunit rRNA coding regions and the fossil record. – Molec. Phylogenet. Evol. **6**: 391–407.

KOWALLIK, K. V., 1992: Origin and evolution of plastids from chlorophyll-*a* + *c*-containing algae: suggested ancestral relationships to red and green algal plastids. – In LEWIN, R. A., (Ed.): Origins of plastids, pp. 223–263. – New York: Chapman and Hall.

KUMAR, S., TAMURA, K., NEI, M., 1993: MEGA: molecular evolutionary genetics analysis, version 1.0. – University Park, PA: Institute of Molecular Evolutionary Genetics, Pennsylvania State University.

KÜTZING, F. T., 1834: Synopsis diatomearum oder Versuch einer systematischen Zusammenstellung der Diatomeen. – Halle.

LACKEY, J. B., 1939: Notes on plankton flagellates from the Scioto River. – Lloydia **2**: 128–143.

LAMOUROUX, J. V. F., 1813: Essai sur les genres de la famille des thalassiophytes non articulées. – Ann. Mus. Hist. Nat. **20**: 21–47, 115–139, 267–293.

LAROCHE, J., HENRY, D., WYMAN, K., SUKENIK, A., FALKOWSKI, P., 1994: Cloning and nucleotide sequence of a cDNA encoding a major fucoxanthin-chlorophyll *a/c*-containing protein from the chrysophyte *Isochrysis galbana*: implications for the evolution of the *cab* gene family. – Pl. Molec. Biol. **25**: 355–368.

LEIPE, D. D., WAINRIGHT, M. L., GUNDERSON, J. H., PORTER, D., PATTERSON, D. J., VALOIS, F., HIMMERICH, S., SOGIN, M. L., 1994: The stramenopiles from a molecular perspective: 16S-like rRNA sequences from *Labyrintuloides minuta* and *Cafeteria roenbergensis*. – Phycologia **33**: 369–377.

LIAUD, M.-F., BANDT, U., SCHERZINGER, M., CERFF, R., 1997: Evolutionary origin of cryptomonad microalgae: Two novel chloroplast/cytosol-specific GAPDH genes as potential markers of ancestral endosymbiont and host cell components. – J. Molec. Evol. **44**: [Suppl 1]: S28–S37.

LINNEAUS, C., 1753: Species plantarum. – Stockholm.

LOCKHART, P. J., HOWE, C. J., BRYANT, D. A., BEANLAND, M. D., PENNY, D., 1994: Substitutional bias confounds inference of cyanelle origins from sequence data. – J. Molec. Evol. **34**: 153–162.

LOHMANN, H., 1913: Über Coccolithophoriden. – Verh. Deutsch. Zool Ges. **23**: 143–164.

LOISEAUX DE GOËR, S., 1994: Plastid lineages. – Progr. Phycol. Res. **10**: 137–177.

LUTHER, A., 1899: Über *Chlorosaccus* eine neue Gattung der Süsswasseralgen. – Beih. Kongl. Svenska Vetensk. – Akad. Handl. **24** (III, 13): 1–22.

MCFADDEN, G. I., GILSON, P. R., WALLER, R. F., 1995: Molecular phylogeny of chlorarachniophytes based on plastid rRNA and *rbc*L sequences. – Arch. Protistenk. **145**: 231–239.

MARGULIS, L., 1981: Symbiosis in cell evolution. – San Francisco: Freeman.

MEDLIN, L. K., COOPER, A., HILL, C., WRIEDEN-PRIGGE, S., WELLBROCK, U., 1995: Phylogenetic position of the *Chromista* plastids from 16S rDNA coding regions. – Curr. Genet. **28**: 560–565.

– BARKER, G. L. A., CAMPBELL, L., GREEN, J. C., HAYES, P. K., MARIE, D., WRIEDEN, S., VAULOT, D., 1996a: Genetic characterization of *Emiliania huxleyi* (*Haptophyta*). – J. Mar. Syst. **9**: 13–31.

– GERSONDE, R., KOOISTRA, W. H. C. F., WELLBROCK, U., 1996b: Evolution of the diatoms (*Bacillariophyta*): II. Nuclear-encoded small-subunit rRNA sequence comparisons confirm a paraphyletic origin for the centric diatoms. – Molec. Biol. Evol. **13**: 67–75.

– – – SIMS, P. A., WELLBROCK, U., 1996c: Evolution of the diatoms (*Bacillariophyta*): III. The age of the *Thalassiosirales*. – Beih. Nova Hedwigia **11**: 221–234.

– – – – – 1997: Is the origin of diatoms related to the end-Permian mass extinction. – In JAHN, R., MEYER, B., PREISIG, N. R. (Eds): Nova Hedwigia Festschrift für U. GEISSLER pp. 1–13. – Stuttgart: J. Cramer.

MERESCHKOWSKY, C., 1905: Über Natur und Ursprung der Chromotaphoren im Pflanzenreiche. – Biol. Centralbl. **25**: 593–604.

MÜLLER, O. M. F., 1786: Animacula infusoria fluviatilia et marina. – Copenhagen: Moller.

MOESTRUP, Ø., 1992: Taxonomy and phylogeny of the *Heterokontophyta*. – In STABENAU, H., (Ed.): Phylogenetic changes in peroxisomes of algae, phylogeny, of plant peroxisomes, pp. 383–399. – Oldenburg: University of Oldenburg.

PALMER, J. D., 1993: A genetic rainbow of plastids. – Nature **364**: 762–763.

PARKE, M., MANTON, I., CLARKE, B., 1955: Studies on marine flagellates. II. Three new species of *Chrysochromulina*. – J. Mar. Biol. Assoc. U.K. **34**: 579–609.

– – – 1956: Studies on marine flagellates. III. Three further species of *Chrysochromulina*. – J. Mar. Biol. Assoc. U.K. **35**: 387–414.

– – – 1958: Studies on the marine flagellates. IV. Morphology and microanatomy of a new species of *Chrysochromulina*. – J. Mar. Biol. Assoc. U.K. **37**: 209–228.

PASCHER, A., 1910: Chrysomonaden aus dem Hirschberger Großteiche. Untersuchungen über die Flora des Hirschberger Großteiches. I. Teil. – Monogr. Abh. Int. Rev. Gesamten Hydrobiol. Hydrogr. **1**: 1–66.

– 1913: *Chrysomonadinae*. – In PASCHER, A., (Ed.): Süsswasser-Flora Deutschlands, Österreichs und der Schweiz, **2**, pp. 7–15.

PATTERSON, D. J., 1989: Stramenopiles: chromophytes from a protistan perspective. – In LEADBEATER, B. S. C., DIVER, W. L., (Eds): The chromophyte algae, pp. 357–379. – Oxford: Clarendon Press.

PÉRASSO, R., BAROIN, A., QU, L. H., BACHELLERIE, J. P., ADOUTTE, A., 1989: Origin of the algae. – Nature **339**: 142–144.

PICKETT-HEAPS, J., SCHMID, A-M. M., EDGAR, L. A., 1990: The cell biology of diatom valve formation. – In ROUND, F. E., CHAPMAN, D. J., (Eds): Progress in phycological research, pp., 1–168. – Bristol: Biopress.

POTTER, D., LAJEUNESSE, T. C., SAUNDERS, G. W., ANDERSEN, R. A., 1996: Convergent evolution masks extensive biodiversity among marine coccoid picoplankton. – Biodiversity conservation. **6**: 99–107.

– SAUNDERS, G. W., ANDERSEN, R. A., 1997: Phylogenetic relationships of the *Raphidophyceae* and *Xanthophyceae* as inferred from nucleotide sequences of the 18S ribosomal RNA gene. – Amer. J. Bot. **84**: 966–972.

RABENHORST, L., 1853: Süsswasser-Diatomeen (Bacillarien), für Freunde der Mikroskopie bearbeitet. – Leipzig.

RAVEN, P. H., 1970: A multiple origin for plastids and mitochondria. – Science **169**: 641–646.

RENSING, S. A., OBRDLIK, P., ROBER-KLEBER, N., MÜLLER, S. B., HOFMANN, C. J. B., MAIER, U.-G., 1996: Molecular phylogeny of the stress-70 protein family with certain emphasis on algal relationships. – In: 1st European Phycological Congress, Abstracts, p. 16.

ROUND, F. E., CRAWFORD, R. M., MANN, D. G., 1990: The diatoms: Biology and morphology of the genera. – Cambridge: Cambridge University Press.

ROWAN, K. S., 1989: Photosynthetic pigments of algae. – Cambridge: Cambridge University Press.

SAITOU, N., NEI, M., 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. – Molec. Biol. Evol. **4**: 406–425.

SAUNDERS, G. W., DRUEHL, D., 1992: Nucleotide sequences of the small-subunit ribosomal RNA genes from selected *Laminariales* (*Phaeophyta*): implications for kelp evolution. – J. Phycol. **28**: 544–549.

– POTTER, D., PASKIND, M. P., ANDERSEN, R. A., 1995: Cladistic analyses of combined traditional and molecular data sets reveal an algal lineage. – Proc. Natl. Acad. Sci. USA **92**: 244–248.

– HILL, D. R. A., TYLER, P. A., 1997a: Phylogenetic affinities of *Chrysonephele palustris* (*Chrysophyceae*) based on inferred nuclear small-subunit ribosomal RNA sequence. – J. Phycol. **33**: 132–134.

– POTTER, D., ANDERSEN, R. A., 1997b: Phylogenetic affinities of the *Sarcinochrysidales* and *Chrysomeridales* (*Heterokonta*) based on analyses of molecular and combined data. – J. Phycol. **33**: 310–318.

SCHIMPER, A. F. W., 1883: Über die Entwicklung der Chlorophyllkörner und Farbkörper. – Bot. Zeitung (Berlin) **41**: 105–112.

SILVA, P. C., 1980: Names of classes and families of living algae. – Regnum Veg. **103**: 1–156.

SLEIGH, M. A., 1989: Protozoa and other protists. – London: Arnold.

STACKHOUSE, J., 1809: Tentamen marino-cryptogamicum, ordinem novum, in genera et species distributum, in Classe XXIVta Linnaei sistens. – Mém. Soc. Imp. Naturalistes Moscou **2**: 50–97.

STARMACH, K., 1985: *Chrysophyceae* und *Haptophyceae*. – In ETTL, H., GERLOFF, J., HEYNIG, H., MOLLENHAUER, D., (Eds): Süsswasserflora von Mitteleuropa **1**. – Stuttgart: G. Fischer.

STEIN, F. VON, 1878: Der Organismus der Infusionsthiere. **3**. – Leipzig.

STEWART, K. D., MATTOX, K., 1970: Phylogeny of phytoflagellates. – In COX, E. R., (Ed.): Phytoflagellates, pp. 433–462. – New York: Elsevier/North-Holland.

SWOFFORD, D. L., 1993: PAUP, Phylogenetic analysis using parsimony, version 3.1, program and documentation. – Champaign, IL: Illinois Natural History Survey, University of Illinois.

TAGGART, R. E., PARKER, L. R., 1976: A new fossil alga from the Silurian of Michigan. – Amer. J. Bot. **63**: 1390–1392.

TAKEZAKI, N., RZHETSKY, A., NEI, M., 1995: Phylogenetic test of the molecular clock and linearized trees. – Molec. Biol. Evol. **12**: 823–833.

TAYLOR, F. J. R., 1976: Flagellate phylogeny: a study in conflicts. – J. Protozool. **23**: 28–40.

VALENTIN, K., CATTOLICO, R. R., ZETZCHE, K., 1992: Phylogenetic origin of the plastids. – In LEWIN, R. A., (Ed.): Origins of plastids, pp. 193–221. – New York: Chapman and Hall.

VAN DEN HOEK, C., 1978: Algen: Einführung in die Phykologie. – Stuttgart: Thieme.

VAN DER AUWERA, G., DE WACHTER, R., 1996: Large-subunit rRNA sequence of the chytridiomycete *Blastocladiella emersonii*, and implication for the evolution of zoosporic fungi. – J. Molec. Evol. **43**: 476–483.

VESK, M., JEFFREY, S. W., 1987: Ultrastructure and pigments of two strains of the pico-planktonic alga *Pelagococcus subviridis* (*Chrysophyceae*). – J. Phycol. **23**: 322–336.

VLK, W., 1938: Über den Bau der Geissel. – Arch. Protistenk. **90**: 448–488.

VÖGEL, H., FISCHER, S., VALENTIN, K., 1996: A model for the evolution of the plastid sec apparatus inferred from *sec*Y gene phylogeny. – Pl. Molec. Biol. **32**: 685–692.

WAINRIGHT, P. O., HICKLE, G., SOGIN, M. L., STICKEL, S. K., 1993: Monophyletic origins of the *Metazoa*: an evolutionary link with the fungi. – Science **260**: 340–342.

WANG, M. C., BARTNICIA GARCIA, S., 1974: Mycolaminarins: Storage $(1 \rightarrow 3) - \beta$-D-glucans from the cytoplasm of the fungus *Phytophthora palmivora*. – Carbohydrate Res. **37**: 331–338.

WILLIAMS, D. M., 1991: Phylogenetic relationships among the *Chromista*: a review and preliminary analysis. – Cladistics **7**: 141–156.

WRAY, G. A., LEVINGTON, J. S., SHAPIRO, L. H., 1996: Molecular evidence for deep Precambrian divergences among metazoan phyla. – Science **274**: 568–573.

WU, C.-I., LI, W.-H., 1985: Evidence for higher rates of nucleotide substitution in rodents than in man. – Proc. Natl. Acad. Sci. USA **8**: 1741–1745.