Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data

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Most haptophytes are unicellular, photosynthetic flagellates, although some have coccolithic, colonial, amoeboid, or filamentous stages. Nearly all have a characteristic filamentous appendage, the haptonema, arising between the two flagella. The small subunit ribosomal RNA gene (18S ribosomal DNA) from 18 haptophyte species has been sequenced, and the sequences aligned with those of more than 300 published and unpublished chlorophyll $a+c$ algae. Phylogenies were constructed using maximum likelihood, neighbor-joining, and weighted maximum parsimony analyses. The high divergence (6%) between members of Pavlova and the remaining haptophytes supports the division of Haptophyta into two classes: Prymnesiophyceae and Pavlovophyceae. Three major clades that correspond to known taxa within the Prymnesiophyceae were identified: one clade embraces Phaeocystis spp.; the second includes members of the genera Chrysochromulina, Prymnesium, and Hanaolia; and the third includes coccolithophorid genera and the genus Isochrysis. Two other clades contain taxa whose sequences were derived from a gene clone library. These taxa are not strongly related to any of the cultured taxa included in this study. Based on 18S ribosomal DNA sequence data and available information on morphological structure and ultrastructure, we propose that the class Prymnesiophyceae be divided into four orders: Phaeocystales ord. nov., Prymnesiales, Isochrysidales, and Coccolithales. A total of 1–2% divergence at this level in the 18S ribosomal RNA gene analysis warrants a separation above the level of family. Within the Pavlovophyceae, a new genus is established, Rebecca J.C. Green gen. nov., into which Pavlova salina and Pavlova helicata are moved.

INTRODUCTION

The Haptophyta represent a major lineage of chlorophyll $a + c$ algae. Although a few species thrive in freshwater, most known haptophytes occur as planktonic forms in coastal and oceanic environments (Hibberd 1980; Green & Jordan 1994; Thomsen et al. 1994). Several can form extensive blooms (Birkenes & Braarud 1952; Berge 1962; Dahl et al. 1989; Blackburn & Cresswell 1993; Brown & Yoder 1994; Wal et al. 1995; Lancelot et al. 1998), occasionally harmful both to the natural biota and to fish farming industries (Moestrup 1994; Edvardsen & Pasche 1998).

These algae exhibit a variety of life forms, ranging in size from the nanoplanктон (Thomsen 1986) to the macroscopic colonies of Phaeocystis. They may occur as nonmotile single cells (many coccolithophorids), nonmotile colonies of single cells embedded in mucilage (i.e., species of Phaeocystis), or motile single cells (e.g., species of Chrysochromulina), or colonial flagellates (species of Cymbellales). Some have benthic filaments, whereas others may have amoeboid stages in their life cycle (Hibberd 1980). Several have morphologically distinct alternate forms, e.g., Isochrysis galbana Parke (Parke 1949), Phaeocystis globosa Scherffel [as Phaeocystis pachetti (Hariot) Lagerheim in Parke et al. 1971], Chrysochromulina polylepis Manton et Parke (Edvardsen & Pasche 1992), and many coccolithophorids (see Gayral & Fresnel 1983; Thomsen et al. 1991; Billard 1994).

The characteristic structural feature of nearly all haptophytes is the haptonema, a filiform appendage situated between the flagella. The haptonema may be very long, up to 160 μm in Chrysochromulina camella Leadbeater et Manton, with the capacity to coil and uncoil (Leadbeater & Manton 1969), or it may be short and flexible, reduced to a few microtubules (MTs) inside the cell, or (rarely) absent. The haptonema may be used for attachment and in food capture (Inouye & Kawachi 1994).

The haptophytes contain one or two chloroplasts, each with a pyrenoid that may be immersed or bulging. The nucleus is usually situated toward the antapical end of the cell, and the outer membrane of the nuclear envelope is continuous with the chloroplast endoplasmic reticulum (ER). Haptophyte cells have ER, the peripheral ER, that lies just beneath the plasmalemma (Jordan et al. 1995). The peripheral ER is absent only in the area immediately around the flagella, but it extends up into the haptonema. The Golgi body of haptophytes lies
close to the flagellar basal bodies and haptonemal base. The stack of cisternae is often in a fanlike arrangement perpendicular to the long axis of the cell. In addition to other functions, the Golgi body produces body scales in some species (Manton & Parke 1962; Jordan et al. 1995). For further discussion on fine-structural features of the Haptophyta, see Hibberd (1980), Green et al. (1990), Jordan et al. (1995), and Green & Jordan (in press).

The haptophyte algae can be considered as a single class, the Prymnesiophyceae Hibberd (= Haptophyceae Christensen ex Silva) (Christensen 1962; Hibberd 1976; Christensen 1980; Chrétiennot-Dinet et al. 1993; Jordan & Green 1994) separated into two groups at the subclass level (Prymnesiophycidae and Pavlovophyceae; Cavalier-Smith 1986; 1989; Jordan & Green 1994). However, Cavalier-Smith (1993) raised both subclasses to the class level, which he named Patelliferae and Pavlovec, although the latter name was not validly published (see below). In 1996, Cavalier-Smith emended Pavlovec to Pavlovophycidae and changed the concept of the Prymnesiophyceae to include two subclasses: the Prymnesiophycidae with Patelliferae as a synonym and the Flavoretophycidae, which included only the enigmatic genus Reticulosphaera (Cavalier-Smith 1996). Information on ultrastructure, including details of the flagellar apparatus (Green and Hori 1994) and the process of mitotic division (Hori & Green 1994), and sequence data from the nuclear small subunit ribosomal RNA (18S rRNA) (Medlin et al. 1997; Simon et al. 1997) and the ribulose 1,5-bisphosphate carboxylase/oxygenase (rbcL) gene (Fujiswa et al. 1994; Inouye 1997) support this separation, and we shall, therefore, follow Cavalier-Smith's (Cavalier-Smith 1993) proposal in this article and refer to the two classes as Prymnesiophyceae Hibberd (Cavalier-Smith 1996) and Pavlovophyceae Cavalier-Smith (Cavalier-Smith 1993; for validation, see below). For further comment on the status of the Haptophyta and a new Latin diagnosis, see below.

In the class Prymnesiophyceae, the flagella are usually equal to subequal and homodynamic or heterodynamic. The mature flagellum of several species has been shown to contain an autofluorescent substance (Kawai & Inouye 1989). Members of the Prymnesiophyceae usually have organic, fundamentally plastid body scales that may become elaborate and have complex forms (Leedhafter 1994 and references therein). In the coccolithophorids, the body scales are calcified and termed coccoliths. The identification of prymnesiophycean algae to species level relies heavily on scale and coccolith ornamentation. In the unmineralized genera, similarity in scale morphological structure has not been used as a taxonomic character above the species level, but in the coccolithophorids, both species determination and classification at higher levels are dependent on coccolith morphological structure (Deflandre 1952; Braarud et al. 1955; Heimdal 1993; Jordan & Kleijne 1994 and references therein).

Complex life cycles that involve haploid and diploid generations have been hypothesized for the group, and several alternating morphological forms living in different habitats have been demonstrated in Pleurochloris pseudoschoenensis Gayral et Frenzel (Gayral & Frenzel 1983) and other coccolithophorids (Billard 1994). The presence of scales with dimorphic or monomorphic scale faces has also been suggested to be diagnostic of haploid and diploid generations in the coccolithophorids (Billard 1994).

In contrast to members of the Prymnesiophyceae, the Pavlovophyceae have strongly anisokont flagella that are markedly heterodynamic, and autofluorescent flagella are unknown. The haptonema is always short. The longer, anterior flagellum is often adorned with a covering of fine hairs and knoblike bodies considered to be either modified scales (Green 1980) or modified hairs (Cavalier-Smith 1994). Species identification in the Pavlovophyceae is primarily based on the morphological structure of these knoblike bodies because plastid body scales are absent. Stigmata (eyespots) are found within the chloroplast in several species, but they are often not associated with an overlying flagellum, as in some heterokont algae. For further discussion on fine structural features of the Haptophyta, see Jordan et al. (1995) and Green & Jordan (in press).

As new information concerning life cycles and ultrastructural details have emerged from both classes, the need for a comprehensive revision of the group has become increasingly evident. The purpose of the present study is to compare genetic and ultrastructural information where available and to relate these data to the current classification system of the haptophyte algae.

**MATERIAL AND METHODS**

**Cultures**

The cultured taxa used in this study are listed in Table 1. Species of *Chrysochromulina*, *Inaniontis*, and *Isochrysis* were grown as batch cultures (0.5-2 l) in Erlienmeyer flasks with filtered, autoclaved seawater diluted to 30 psu. Nutrients, vitamins, and trace metals were added as in IMR 1/2 medium (Epplley et al. 1967) supplemented with 10 nM selenite. Other cultures were grown in f/2 (Guillard & Ryther 1962). Typically, cultures were grown at 15°C under white fluorescent light, with a quantum flux of 50-100 μmol photons m² s⁻¹ and a 12 : 12-h light : dark cycle. Cultures were harvested by filtration or centrifugation.

**DNA extraction and polymerase chain reaction amplification**

Total nucleic acids were extracted using a modified CTAB extraction (Doyle & Doyle 1987) and served as a template for amplification of the 18S rRNA gene following Medlin et al. (1988) or Chenick et al. (1997). Most of the polymerase chain reaction products were directly sequenced using a solid-phase sequencing method with radioisotopes (Chenick et al. 1997) or cycle sequenced (Sequitherm, BtOZYM) using infrared-labeled primers and analyzed with the LiCor automated sequencer (MWG Ebersberg, Germany), whereas others were cloned (LigAgor, R&D Systems) before automated sequencing or were gel purified before solid-phase sequencing (Potter et al. 1996).

**Phylogenetic analyses**

Sequences were manually aligned in an algal database that contained more than 300 published and unpublished chloro-
<table>
<thead>
<tr>
<th>Species</th>
<th>Strain code</th>
<th>Collection site</th>
<th>Culture collection</th>
<th>Isolator, year</th>
<th>Accession no.</th>
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<td>Chrysotrichium compactumifera Manton et Leadbeater</td>
<td>J10</td>
<td>Skagerrak</td>
<td>UIO</td>
<td>J. Thronsen, 1984</td>
<td>AJ246273</td>
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<tr>
<td>Chrysotrichium hirta Manton</td>
<td>JY</td>
<td>Off Austevoll, W Norway</td>
<td>UIO</td>
<td>B. Edwardsen, 1989</td>
<td>AJ246272</td>
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<td>Chrysotrichium kappae Park et Manton</td>
<td>EN3</td>
<td>Oslofjord, S Norway</td>
<td>UIO</td>
<td>W. Eikrem, 1989</td>
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<td>Chrysotrichium polylepis Manton et Park</td>
<td>B11</td>
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<td>UIO</td>
<td>B. Edwardsen, 1988</td>
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<td>Skagerrak, S Norway</td>
<td>UIO</td>
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<td>W. Eikrem, 1989</td>
<td>AJ246279</td>
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<td>Off Arenal, S Norway</td>
<td>UIO</td>
<td>W. Eikrem, 1989</td>
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<td>Coccolithus pelagicus (Wöllich) Schillier</td>
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<td>English Channel</td>
<td>PML</td>
<td>J. C. Green, 1990</td>
<td>AJ246261</td>
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<td>Cricriocystis novellis (McIntyre et Bé) Reinhardt</td>
<td>CCMP 298</td>
<td>La Jolla, CA</td>
<td>CCMP</td>
<td>K. Lee, 1984</td>
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<td>Emiliana huxleyi (Lohmann) Hay et Mohler</td>
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<td>English Channel</td>
<td>PML</td>
<td>J. C. Green 1975</td>
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<td>Gephyrocapsa oceanica Kromas</td>
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<td>Mutsu Bay, Aomori Prefecture, Japan</td>
<td>CCMP</td>
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<td>See University of Washing</td>
<td>CCMP</td>
<td>NA</td>
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<tr>
<td>Pavlova gyrans Butcher emend. Green et Manton</td>
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<td>See University of Washing</td>
<td>CCMP</td>
<td>NA</td>
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<td>Gulf of Maine</td>
<td>CCMP</td>
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<td>Weddeli Sea</td>
<td>AWI</td>
<td>M. Baumann</td>
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<td>North Sea</td>
<td>AWI</td>
<td>M. Baumann</td>
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<td>Phaeocystis poucheri (Harriott) Lagerheim</td>
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<td>Greeneland Sea</td>
<td>AWI</td>
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<td>Phaeocystis sp. 1</td>
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<td>Gulf of Naples, Italy</td>
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<td>Phaeocystis sp. 2</td>
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<td>Gulf of Naples, Italy</td>
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<td>A163148</td>
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<td>Pleurochrysis carterae (Braarud et Fagerland) Christensen</td>
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<td>BAH</td>
<td>H. A. von Storch</td>
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<td>Pleurochrysis sp.</td>
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<td>See PLY 181</td>
<td>CCMP</td>
<td>M. Parke</td>
<td>AJ246275</td>
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<td>Pleurochrysis elongata (Droop) Jordan</td>
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<td>NE Atlantic, coastal</td>
<td>CCMP</td>
<td>M. Droop</td>
<td>AJ246264</td>
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<td>Pleurochrysis sp.</td>
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<td>Plymouth 181</td>
<td>CCMP</td>
<td>M. Parke</td>
<td>AJ246265</td>
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<td>Prymnesium nannochloris Pienaar et Birkhead</td>
<td>K-0081</td>
<td>Flade S, Denmark</td>
<td>SCCAP</td>
<td>T. Christensen</td>
<td>AJ246269</td>
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<td>Prymnesium pateliferum Green, Hibberd et Pienaar</td>
<td>PLY 527</td>
<td>S coast of England</td>
<td>PML</td>
<td>D. J. H. Herder 1976</td>
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<td>Grell</td>
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<td>Fucus distichus Linnaeus</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>M34847</td>
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*NA, not applicable.*
phyll a + c algae using maximum primary and secondary structural similarity with the Olsen sequence editor (Larsen et al. 1993). This data set also includes 14 sequences from a clone library obtained from amplified 18S rRNA genes from water samples taken in oligotrophic Pacific waters (Moon et al. in press). A final data set of 46 species was used for phylogenetic analyses with the brown alga Fucus and the dinoflagellate Cryptophyceae as outgroups. A total of 1764 nucleotides were used for the data analysis, of which 351 were informative for the maximum parsimony analysis. Maximum likelihood analyses were performed using the fastDNAML program (version 1.0) (Larsen et al. 1993). Maximum parsimony analyses were implemented with the PAUP computer program (Swoford 1993). Introductions gaps were treated as missing data; informative characters were treated as multistate unrooted. Unweighted maximum parsimony trees were obtained using the tree-bisection-reconnection branch swapping option in a heuristic search with random taxon addition. A weighted maximum parsimony analysis of the data was also performed (Kostrita & Medlin 1996). Distance analyses were performed using the PHYLIIP computer program (Felsenstein 1993). Dis-similarity values (Fitch & Margoliash 1967), based on pairwise comparisons of sequences, were transformed into distances using the Kimura two-parameter models (Kimura 1980). Distance matrices were converted into trees using the neighbor-joining method (Felsenstein 1993). Stability of monophyletic groups in weighted maximum parsimony and distance trees was estimated with a bootstrap analysis (500 replicates) (Felsenstein 1985).

RESULTS

A phylogenetic reconstruction of the haptophyte algae based on nucleotide sequences of the 18S rRNA gene is presented in Fig. 1. All analyses recovered a major split in the haptophyte algae corresponding to its two classes: Prymnesiophyceae and Pavlovophyceae. All available ultrastructural characters for described prymnesiophycean species in our tree are presented in Table 2.

In the class Pavlovophyceae, Pavlova aff. salina is distinctly separate from other pavlovophycean species, with strong bootstrap support. This finding is supported by earlier morphological data that suggest that Pavlova salina (Carter) Green and its close relative Pavlova helicata van der Veer are distinct (Green 1976). With regard to clones CCMP 1394 (a coccoid unciell) and CCMP 1416, little can be said other than further study is required to establish their identity. From their positions in the tree, both probably are related to Pavlova.

In the class Prymnesiophyceae, several major clades may be recognized (Fig. 1). The branching order for these clades that is recovered in the maximum likelihood analysis is not supported in either the weighted maximum parsimony or neighbor-joining analysis. However, the monophyly of the individual clades, except for the position of the gene clones OLIS1050 and OLIS26041, is strongly supported in all bootstrap analyses. The strongly supported clades correspond to known groups of haptophyte algae: (1) clade A = Phaeocystis spp.; (2) clade B = the nonmineralized taxa containing species of Chrysochromulina, Prymnesium, and Imantonia; and (3) clade C = the mineralized genera belonging to the coccolithophorids (plus Isochrysis). In addition, a series of sequences from a gene clone library from oligotrophic Pacific waters (clade D) represent a group of taxa with some affiliation to Phaeocystis spp. This relationship is recovered only in the weighted maximum parsimony bootstrap analysis (75) and nowhere else (tree not shown). The position of gene clones OLIS1050 and OLIS26041 (clade E) is not stable, and all bootstrap analyses place them with an equal status to all other major clades instead of sister group to the mineralized taxa (clade C). Based on 18S rDNA sequences, the taxa in clades D and E do not appear to be strongly related to any other known haptophyte taxa and may represent novel haptophyte taxa.

Phaeocystis group

The genus Phaeocystis contains, according to our molecular data, at least six distinct species (clade A). Phaeocystis scrobiculata Moestrup was not included in our molecular study but, on morphological grounds, is believed to represent a seventh species. On the basis of the molecular data, at least three colonial species are recovered (Medlin et al. 1994). These include P. globosa, Phaeocystis antarctica Karsten, and P. pachyderma. Gene clone OLIS1004 is most closely related to P. globosa but is not identical (Moon et al. in press). Two undescribed species of Phaeocystis have been isolated from the Mediterranean (Zingone et al. in press) and are distinct taxa based on 18S rDNA sequence analysis.

Nonmineralized genera (excluding Phaeocystis)

The genera that belong to a second major group of the Prymnesiophyceae (clade B) can be divided into two subgroups, clade B1 and clade B2 in the rRNA tree (Fig. 1), and they correspond to clade 1 and clade 2 in the analysis of Chrysochromulina spp. by Simon et al. (1997). Both groups are well supported in all analyses. Species of Chrysochromulina fall into both clades, and thus this genus must be considered para phyletic (Inouye 1996; Simon et al. 1997). It has previously been recognized on morphological grounds that Chrysochromulina is not a natural group (Birkhead & Pienaar 1995). Clade B1 (Simon et al. 1997) contains Imantonia rotunda Reynolds as sister to a clade, including certain Chrysochromulina spp. and all Prymnesium spp. Clade B2 contains only Chrysochromulina spp. Within subclade B1, the position of the clone library taxa (OLIS1059 and OLIS1033 + OLIS1056) is not stable, and their inclusion in the analysis weakens support for clade B1 to only 66 in the neighbor-joining analysis. Support is below 50 in the maximum parsimony analysis, and the branching order between this clade plus clade B2 and I. rotunda collapses to an unresolved polytomy. Omitting these taxa from the analysis raises the bootstrap support for clade B1 to 53/85 (maximum parsimony/neighbor joining) (tree not shown). In the maximum likelihood analysis, Imantonia forms the most deeply divergent branch; in the neighbor-joining analysis, the clone library taxa are most deeply divergent, and the maximum parsimony analysis was unable to resolve the branching order at the base of clades B1 and B2.

Mineralized genera (including Isochrysis and Reticulospaera)

Clade C contains the coccolithophorids and has strong bootstrap support in the maximum parsimony analysis (Fig. 1).
Fig. 1. Phylogenetic tree based upon a maximum likelihood analysis showing the relationships of haptophyte taxa. The tree is rooted on the branch leading to *Fucus* and *Cryptophyceae*. For discussion of clades A through E, see text. Maximum parsimony and neighbor-joining analyses produced similar trees (not shown). Bootstrap values (500 replications) are presented at internal nodes for values more than 50% for neighbor-joining and maximum parsimony analyses, respectively.

Two subgroups are supported in the bootstrap analysis, corresponding to the orders Coccolithales and Isochrysidales. On the basis of the 18S rDNA sequence (Cavalier-Smith et al. 1996), *Reticulosisphaera japonensis* Grell appears as a sister taxon to *Pleurochrysis*.

**DISCUSSION**

**Division Haptophyta**

The division Haptophyta is clearly divided into two clades based on the molecular evidence presented here and elsewhere (Fujwara et al. 1994; Inouye 1997; Medlin et al. 1997). Although it is not strictly valid to equate genetic distance between taxonomic ranks in different phyla and divisions because some groups are more ancient than others and because others are perhaps evolving at a different evolutionary rate, some comparison between groups that emerge at similar points in the rRNA tree may be useful to provide an interpretation of how much distance can be found between similar taxonomic ranks among groups of organisms of similar geological ages. As pointed out by Cavalier-Smith (1996), the amount of divergence in the rRNA gene that separates the two major clades of the haptophyte algae (6%) is in agreement with that found among classes in other algal divisions, which also emerged at the crown group radiation. Using this guide, a rough estimate of 3–5% difference can be found at the class level in the green algae (Friedl 1996; Van de Peer & de Wachter 1997) and the heterokont algae (Medlin et al. 1997), with even more divergence among the red algal clades (Van de Peer & de Wachter 1997). At the ordinal level, roughly 1–2% differences are noted for the dinoflagellates (Saunders et al. ...
<table>
<thead>
<tr>
<th>Species</th>
<th>Cell</th>
<th>Flagella</th>
<th>Hapionema</th>
<th>Scales</th>
<th>Pattern</th>
<th>Base plate scales</th>
<th>Coccolithes</th>
<th>No. of MT in free part of haptonema</th>
</tr>
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<tbody>
<tr>
<td><em>Chrysochromulina causeaeta</em></td>
<td>Saddle</td>
<td>Equal</td>
<td>Hapt. ≫ flag.</td>
<td>Plate, cap</td>
<td>Rad. pat. both faces</td>
<td>absent</td>
<td>Absent</td>
<td>6</td>
</tr>
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<td><em>Chrysochromulina lyra</em></td>
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<td>Equal</td>
<td>Hapt. ≫ flag.</td>
<td>Plate, (2 types)</td>
<td>Rad. ribs overlaying spiraling ribs, central open ring with cross</td>
<td>Absent</td>
<td>Absent</td>
<td>6</td>
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<td>Saddle</td>
<td>Equal</td>
<td>Hapt. ≫ flag.</td>
<td>Plate, spine (2 types)</td>
<td>Rad. ribs prox. face, con. fibrils dist. face</td>
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<td>Absent</td>
<td>7</td>
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<td>Hapt. &gt; flag.</td>
<td>Plate, spine</td>
<td>Loosely woven rad. ribs overlaying con. ribs, fibril dist. face</td>
<td>Absent</td>
<td>Absent</td>
<td>7</td>
</tr>
<tr>
<td><em>Chrysochromulina kampa</em></td>
<td>Spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate (2 types), spine</td>
<td>Rad. ribs prox. face, con. fibrils dist. face</td>
<td>No obs</td>
<td>No obs</td>
<td>7–8</td>
</tr>
<tr>
<td><em>Chrysochromulina hirta</em></td>
<td>Oblong - spherical</td>
<td>Equal</td>
<td>Hapt. &gt; flag.</td>
<td>Plate, spine (2 types)</td>
<td>Rad. ribs one face, fibril other face</td>
<td>Absent</td>
<td>Absent</td>
<td>No obs</td>
</tr>
<tr>
<td><em>Chrysochromulina polyplepis</em></td>
<td>Oblong - spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate (4 types), spine</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>7</td>
</tr>
<tr>
<td><em>Chrysochromulina polyplepis</em></td>
<td>Oblong - spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate (2 types), spine</td>
<td>Rad. ribs dist. face, con. fibril prox. face</td>
<td>Absent</td>
<td>Absent</td>
<td>7</td>
</tr>
<tr>
<td><em>Prymnesium remaneastrevum</em></td>
<td>Oblong</td>
<td>Equal-sub-equal</td>
<td>Hapt. &lt; flag. with scales</td>
<td>Plate (2 types, on body, 1 type on hapt.)</td>
<td>Rad. pat. both scale faces, body scales with superstructures</td>
<td>Absent</td>
<td>Absent</td>
<td>7</td>
</tr>
<tr>
<td><em>Prymnesium parvum</em></td>
<td>Oblong - spherical</td>
<td>Equal-sub-equal</td>
<td>Hapt. &lt; flag. with scales</td>
<td>Plate (2 types)</td>
<td>Rad. ribs dist. face, fibril prox. face</td>
<td>Absent</td>
<td>Absent</td>
<td>7</td>
</tr>
<tr>
<td><em>Prymnesium patelliferum</em></td>
<td>Oblong - spherical</td>
<td>Equal-sub-equal</td>
<td>Hapt. &lt; flag. with scales</td>
<td>Plate (2 types)</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>No obs</td>
</tr>
<tr>
<td><em>Prymnesium coelenteratum</em></td>
<td>Oblong - spherical</td>
<td>Equal-sub-equal</td>
<td>Hapt. &lt; flag. with scales</td>
<td>Plate (2 types)</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>No obs</td>
</tr>
<tr>
<td><em>Imantonia rotunda</em></td>
<td>Spherical</td>
<td>Equal</td>
<td>Absent</td>
<td>Place (2 types)</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>NA</td>
</tr>
<tr>
<td><em>Cruciplocolithus neochelis</em></td>
<td>Oblong - spherical</td>
<td>Equal</td>
<td>Vestigial</td>
<td>Plate</td>
<td>Rad. ribs dist. face, con. fibril prox. face</td>
<td>Rad. ribs both faces</td>
<td>Placoliths</td>
<td>(5 MT in base)</td>
</tr>
<tr>
<td><em>Pleurochrysis carterae</em></td>
<td>Oblong</td>
<td>Unequal</td>
<td>Bulbous with scales</td>
<td>Plate</td>
<td>Rad. ribs one face, fibril dist. other face</td>
<td>Rad. ribs both faces</td>
<td>Cricoliths</td>
<td>6 MT</td>
</tr>
<tr>
<td><em>Pleurochrysis elongata</em></td>
<td>Oblong</td>
<td>Equal-un-equal</td>
<td>Bulbous with scales</td>
<td>Plate</td>
<td>Fibrils both faces</td>
<td>Rad. ribs prox. face, fibril dist. face</td>
<td>Cricoliths</td>
<td>No obs</td>
</tr>
<tr>
<td><em>Coccolithus pelagicus</em> (Cryostephanus stage)*</td>
<td>Spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate</td>
<td>Rad. ribs one face, fibril dist. other face</td>
<td>Present</td>
<td>Crystaloliths</td>
<td>6 (5) MT</td>
</tr>
<tr>
<td><em>Gephyrocapsa oceanica</em></td>
<td>Spherical</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>5 MT</td>
</tr>
<tr>
<td><em>Eucyrella huxleyi</em> (S-cells)*</td>
<td>Spherical</td>
<td>Equal</td>
<td>Prob. absent</td>
<td>Plate</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>No obs</td>
</tr>
<tr>
<td><em>Isochrysis gallina</em></td>
<td>Oblong</td>
<td>Equal</td>
<td>Hapt. &lt; flag. with scales</td>
<td>Plate (2 types)</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>5 MT</td>
</tr>
<tr>
<td><em>Phaeocystis aurantica</em></td>
<td>Spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate (2 types)</td>
<td>No obs.</td>
<td>Absent</td>
<td>Absent</td>
<td>No obs</td>
</tr>
<tr>
<td><em>Phaeocystis globosa</em></td>
<td>Spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate (2 types)</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>6 MT</td>
</tr>
<tr>
<td><em>Phaeocystis paucilis</em></td>
<td>Spherical</td>
<td>Equal</td>
<td>Hapt. &lt; flag.</td>
<td>Plate (2 types)</td>
<td>Rad. ribs both faces</td>
<td>Absent</td>
<td>Absent</td>
<td>No obs</td>
</tr>
</tbody>
</table>
Table 2. Extended.

<table>
<thead>
<tr>
<th>Flagellar microtubular roots</th>
<th>Flagella with proximal and/or distal transition plates</th>
<th>Flagella with helical band or other structures</th>
<th>Cytoplasmic tongue</th>
<th>Pyrenoids</th>
<th>References</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple RI</td>
<td>No obs.</td>
<td>No obs.</td>
<td>No obs.</td>
<td>Immersed, traversed by tubules</td>
<td>Manton &amp; Leadbeater 1974, Eikrem unpublished</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prox. and dist. plates</td>
<td>Absent</td>
<td>Absent</td>
<td>Immersed, traversed by tubules</td>
<td>Eikrem 1996, Eikrem unpublished</td>
<td></td>
</tr>
<tr>
<td>Simple RI</td>
<td>Prox. and dist. plates</td>
<td>Absent</td>
<td>Absent</td>
<td>Immersed, traversed by thylakoids</td>
<td>Eikrem &amp; Moo trop 1998</td>
<td></td>
</tr>
<tr>
<td>Simple RI (many MT)</td>
<td>Prox. and dist. plates</td>
<td>Absent</td>
<td>Absent</td>
<td>Immersed</td>
<td>Leadbeater &amp; Manton 1971, Gregson et al. 1993</td>
<td></td>
</tr>
<tr>
<td>Compound R1</td>
<td>Prox. and dist. plates</td>
<td>Absent</td>
<td>Absent</td>
<td>Bulging, traversed by thylakoids</td>
<td>Parke et al. 1955, Manton &amp; Leedale 1961, Eikrem unpublished</td>
<td></td>
</tr>
<tr>
<td>Compound R1</td>
<td>Prox. and dist. plates</td>
<td>Other structure</td>
<td>Present</td>
<td>Immersed, traversed by thylakoids</td>
<td>Manton &amp; Parke 1952, Eikrem unpublished</td>
<td></td>
</tr>
<tr>
<td>Compound R1</td>
<td>Prox. and dist. plates</td>
<td>Other structure</td>
<td>Present</td>
<td>Immersed, traversed by thylakoids</td>
<td>Piasecki et al. 1990, Edvardsen et al. 1996, Eikrem unpublished</td>
<td></td>
</tr>
<tr>
<td>Compound R1</td>
<td>Prox. and dist. plates</td>
<td>Tubular rings (but reduced)</td>
<td>Present</td>
<td>Immersed, traversed by thylakoids</td>
<td>Birkenhead &amp; Pienaar 1994, Pienaar &amp; Birkenhead 1994</td>
<td></td>
</tr>
<tr>
<td>Simple RI (many MT)</td>
<td>Prox. and dist. plates</td>
<td>No obs.</td>
<td>No obs.</td>
<td>Immersed</td>
<td>N. Carter 1937, Manton &amp; Leedale 1963a, Manton 1964</td>
<td>Nonmobile stage present</td>
</tr>
<tr>
<td>Simple RI (many MT)</td>
<td>Prox. and dist. plates</td>
<td>No obs.</td>
<td>No obs.</td>
<td>Immersed</td>
<td>Green et al. 1982, Green &amp; Hori 1990</td>
<td>Filamentous stage present</td>
</tr>
<tr>
<td>Compound R1 and R2</td>
<td>Prox. plate</td>
<td>Helical band</td>
<td>Present</td>
<td>Bulging</td>
<td>Beech &amp; Wetherbee 1984, Beech &amp; Wetherbee 1988</td>
<td></td>
</tr>
<tr>
<td>Compound R1</td>
<td>No obs.</td>
<td>No obs.</td>
<td>No obs.</td>
<td>Immersed, traversed by thylakoids</td>
<td>Manton &amp; Leedale 1963b, Klaveness 1973</td>
<td>Nonmobile stage placoid covered, with flagella bases</td>
</tr>
<tr>
<td>Compound R1</td>
<td>Prox. plate present</td>
<td>Helical band</td>
<td>No obs.</td>
<td>Immersed, traversed by single tubule</td>
<td>Green &amp; Pienaar 1977, Hori &amp; Green 1991</td>
<td>Scales similar to E. naxi, nonmobile stage present in life cycle</td>
</tr>
<tr>
<td>No obs.</td>
<td>Prox. and dist. plates</td>
<td>No obs.</td>
<td>No obs.</td>
<td>Immersed, traversed by single tubule</td>
<td>Parke et al. 1971, Medlin et al. 1994</td>
<td>Dominating stage nonmobile, spherical gelatinous colonies</td>
</tr>
</tbody>
</table>
1997) and for the green algae (Friedel 1996). Thus, the recognition of the two clades in the Haptophyta at the class level is warranted (6%), and we present a summary of the major morphological features that delineate this division and its two classes and make revisions and emended descriptions of taxonomic levels between class and family based primarily on molecular data supported by morphological data where available.

The name Haptophyta was introduced by Hibberd (1972), who proposed to raise the various classes previously assigned to the Chrysophyta or Chromophyta to a divisional rank that would more clearly express their basic diversity and remote ancestry. He proposed the divisional name Haptophyta but without validating descriptions. It appears that the name Haptophyta never has been validly published under the terms of the International Code of Botanical Nomenclature (Silva, personal communication) in that there has never been a Latin diagnosis or full reference to a previously published Latin diagnosis, although a description in English was provided by Loeblich & Loeblich (1979) and thereafter by Cavalier-Smith (1986). Therefore, the name Haptophyta is validated with a Latin diagnosis.

Division Haptophyta D.J. Hibberd et Edvardsen et Eikrem, nom. desc.

Diagnosis: Cellula in statu erratico duobus plurumque flagellis aequabilibus vel inequicubulis instructa; si flagella aequi interdum pleuquem duo; si flagella notabilier inaequalia, breviora eorum interdum vestigiale. Flagella plurumque glabra pilis tubularibus nullis, si inequalia longius interdum pilis gneilibus non-tubularibus et corporibus minusit sphäreïcis vel claviformibus armata; regio transitoria eorum umum vel duo diseripitens transversa, nullam helicem praebens. Haptenoma, orpaillem biula divisionis proprium, prope flagella situm, longum interdum abbreviatum, rarum deficiens. Chloroplastus unus vel duo, lamellae et terms thylacoïdites formatis, lamella circulari nulla. Stigma plurumque nullo, si praecens rarissimo flagello associatum. Cellula plurumque uno vel pluribus statis squamari in corpore Gröggumi formatum et, ut videatur, sine substantia minerali interdum, ut videtur, carentes.

Swimming cells with usually two equal or unequal flagella; if equal, occasionally more than two flagella; if markedly unequal, the shorter sometimes vestigial. Flagella usually smooth, never with tubular hairs (mastigonemes); if unequal, sometimes the longer one with fine nontubular hairs and minute spherical or elavate bodies. Transitional region with one or two transverse partitions, never with a transitional helix. A haptonema, an organelle characteristic of this division, situated close to the flagella, long, abbreviated, or, rarely, absent. Chloroplasts one or two, lamellae formed from three thylakoïds, girdle lamella absent. Stigma usually lacking; if present, rarely associated with flagella. Cells usually with one or more layers of apparently unmineralized scales, the scales formed in the Golgi body; distal face with spiral fibrils, the proximal face with radial fibrils. Additional calcified scales (coccoliths) present in some members of the division. Cells with coccoliths sometimes apparently lacking unmineralized scales.

Class Pavlovophyceae (Cavalier-Smith) J.C. Green et Medlin


Cavalier-Smith erected the class Pavlovophyceae in 1993 (Cavalier-Smith 1993), basing it on the order Pavlovales Green. However, he did not provide a full citation in accordance with the International Code of Botanical Nomenclature (Greuter et al. 1988, 1994), and the name was, therefore, not validly published. The class name is validated herein, the name being based on that of the validly published subclass Pavlovophycidae ("Pavlovidae"), Cavalier-Smith 1986.

Members of the Pavlovophyceae are almost exclusively flagellates, with flagella inserted subapically or almost in a median position on the concave face of the cell (Green 1980). There are several distinct features that separate them from members of the Prymnesiophyceae. The most obvious feature is the markedly anisokont nature of the flagella. There is a long, anteriorly directed flagellum that beats with a strongly S-shaped wave form in contrast to the much shorter second flagellum that is directed laterally or posteriorly and beats with a stiff, inflexible action. Between the two flagella is a short haptonema that is difficult to detect with the light microscope. The basal apparatus of the flagellar-haptonematal complex has an arrangement of MTs and fibrous roots so far not found elsewhere (Green 1980; Green & Hori 1994).

Typical cells are often elongate and concave compressed (Green 1980). However, cell shape is often irregular, and in some species (e.g., Pavlova granifera (Mack) Green, Pavlova gynans Butcher, Pavlova pinguis Green, Pavlova viridescens Billard, and Exanthemachrysis gayraliae Lepailleur) cells are somewhat metabolically with respect to morphological structure (Green 1980). Except for P. gynans, the cell body is not covered by scales, and when scales occur, they are not of the plate-scale type found in the Prymnesiophyceae. Instead, small dense bodies often form a dense investment on the longer flagellum together with fine nontubular hairs. The dense bodies are considered to be modified scales (Green 1980) or modified hairs (Cavalier-Smith 1994). Dense bodies have also been occasionally observed on the haptonema of Pavlova lutheri (Droop) Green (1980).

Cells have a single chloroplast, which is often strongly bilobed, sometimes with a bulging basal pyrenoid (e.g., P. gynans, P. pinguis). Stigmata (eyespots) have been observed in some species. In P. gynans, P. pinguis, and P. granifera, the stigma consists of a concave layer of osmiophilic droplets at the periphery of the chloroplast near the flagellar bases. In P. lutheri and Diacronema vikatuni Prausnitz, the stigma is composed of a layer of osmiophilic droplets close to or beneath

> , longer than or equal to; ≥ , much longer than; < , shorter than; ≤, much shorter than; ext., extending; flag., flagella; Hapt., haptophyta; MT, microtubules; NA, not applicable; con., concentric; dist., distal; obs., observations; pat., pattern; prox., proximal; rad., radiating.
Rebecca J.C. Green gen. nov.

**DIAGNOSIS:** Cellulare solitariae, libere natantes, elongatae, interdum compressae, flagellis duobus et haptonemone breve. Flagellum anticum longius, pilis gratulibus non-tubularibus et corporebus minitus sphæricis vel claviformibus armato; flagellum posticum vestigialis. Fovea vel canalis cellulare ante flagellum longum penetrans. Chloroplastus unus, laeviter flavovirens; stigma nullum.

Cells solitary, free-swimming, elongate, slightly compressed, with two unequal flagella and a short haptonema. The longer anterior flagellum with fine non-tubular hairs and rows of minute spherical or clavate bodies; posterior flagellum vestigial. A pit or canal penetrating the cell near the long anterior flagellum. Chloroplast single, pale yellow-green, stigma absent.

**TYPE SPECIES:** Rebecca salina (N. Carter) Green, comb. nov.


**ETYMOLOGY:** Named by J. C. Green after his daughter, Rebecca Jane Victoria Green.

**Rebecca helicata** (van der Veer) J. C. Green, comb. nov.


**Class Prymnesiophyceae** D.J. Hibberd, 1976

This class name was introduced by Casper (1972), who provided a German description. A Latin diagnosis was first provided by Hibberd (1976). In contrast to the Pavlovoyphycaceae, motile cells of the Prymnesiophyceae usually have their flagella inserted apically. The two flagella are smooth, and the haptonema may vary from a few micrometers in species of Phaeocystis and Prymnesium to more than 100 μm in some species of Chrysochromulina or may be lacking entirely (e.g., *I. rotunda* and *Emiliania huxleyi* (Lohmann) Hay et Mohler).

The part of the haptonema emerging from the cell may contain five to seven MTs (in rare cases, eight, as occasionally in *Chrysochromulina kapp Parke* et Manton). The flagellar apparatus typically consists of two basal bodies, the haptonematial base, fibrous and microtubular roots (sometimes associated with a cytoplasmic tongue, surrounded by ER, which is confluent with the peripheral ER), and accessory and connecting fibers (Green & Hori 1994). The microtubular roots associated with the mature (i.e., left, no. 1) flagellum may be complex in their arrangement, which varies between genera and species. The roots R1 and R2 may be simple (a sheet of MTs) or compound (a sheet of MTs connecting with a secondary bundle of closely packed MTs, the latter often termed a crystaline bundle). The microtubular roots that are connected with the immature (i.e., right, no. 2) flagellum contain only a few MTs and are less conspicuous. They have been given less emphasis as systematic indicators but are valuable at the level of class in the haptophytes. The proximal parts of the flagella contain a proximal transitional plate that varies in shape between species. Distal to it tubular rings, a helical structure or a distal transitional plate may be found depending on species.

Usually, the nonmotile and flagellate cells of the Prymnesiophyceae are scale and/or coccolith covered (except for *Diceratium*, which lacks both). The ornamentation of both structures may vary from simple to elaborate. Most coccoliths have base plates, which consist of an organic scale, whereas others apparently lack them (Leadbetter 1994). The similarity in form between the coccoliths and many of the organic scales in certain noncoccolithophorids (e.g., several *Chrysochromulina* species) is striking: they are regarded as both homologous (same origin) and analogous (same function) structures (Manton 1986; Young 1994). Manton & Leedale (1969) suggested that the coccoliths are organic scales with a calcified rim.

Most species contain two chloroplasts (*Calyptraphaera sphenoides* Schiller, *Chrysotella lamellosa* (Anand) Green et Parke, and *I. galbana* have only one), with immersed or bulging pyrenoids traversed by thylakoids or tubes. The nucleus is posterior or central, and the Golgi body lies anterior to the nucleus and just beneath the basal bodies. The large Golgi body is composed of a single dictysome with many, often dilated cisternae (Manton 1967; Hibberd 1976; 1980; Pierson 1994). One reticulated mitochondrion has been demonstrated in *Pleurorchysis carterae* (Braund et Fagerland) Christensen (Beech & Wetherbee 1984), and a highly branched and retic-
ulate mitochondrion may be present in most or all pynmsesiophyceans. A layer of ER, the peripheral ER, is located beneath the cell membrane and also extends into the haptonema (Pienaar 1994).

The morphological and ultrastructural features of taxonomic importance for the pynmsesiophycean species include cell shape, the features of the haptonema (e.g., length, ability to coil, number of MTs in emergent part, the presence of scales), scale investment (e.g., scale form, calcification, presence or absence of underlayer scales, presence or absence of resistant base plates, and presence of a continuous outer investment (skin)), pyrenoid type, and flagellar apparatus (e.g., presence or absence of compound roots and a cytoplasmic tongue, the number of MTs in the sheet of the R1 root of the mature flagellum, and the presence or absence of helical structures in the flagella). Although combinations of these features are valuable for defining genera and species, there is some overlap in ultrastructural characters between the various subgroups (genera, families, and orders), making it difficult to divide this class into well-defined higher taxa on morphological features alone. Therefore, we have relied heavily on the molecular data to provide an objective framework within which to comment on the systematics of the Pynmsesiophyceae.

At present, there is strong bootstrap support for three clades (A, B, C) within the Pynmsesiophyceae (Fujinaka et al. 1994; Inouye 1997; this study), which encompass many known families that have been erected on the basis of structural and morphological data (Jordan & Green 1994). These include the following unmineralized families: the Phaeocystaceae (species of Phaeocystis), Pynmsiaceae (species of Chrysocromulina and Pynmsium), Isochrysidaceae (Isochrysis), and Noellacehocladiaceae (Emiliania and Gephyrocapsa). Within the mineralized taxa (i.e., the coccolithophorids), species of Prochlorothys are included in the Pleurochrysidaceae and Coccolithus and Cruciplacolithus in the Coccolithaceae. Strong bootstrap support also exists for two clades that contain unknown taxa, but these clades must await formal descriptions until cells belonging to these clades are brought into culture and studied more thoroughly.

From the molecular data presented in Fig. 1, we have considered various classifications for the clades of the Pynmsesiophyceae that contain known taxa. Given the amount of bootstrap support and the genetic divergence between the clades, we feel that there are sufficient grounds to erect a new order to accommodate Phaeocystis and to resurrect the orders Isochrysidales and Coccolithales as distinct from the Pynmsiaceae. We provide new and emended descriptions where needed.

Order Phaeocystales Medlin ord. nov.

Diagnosis: Cellulara planctonica flagellis binis plusminusve aequilibus et haptonemate non-cincinato breviori instructae; chloroplast 1–4, parietales; corpus cellulara squamous complanatis amplitudinis duobus vestita. Cellulae interdum electosoma ex a-chitino compositis effrentes. Cursus vitae involuto; cellulae in statu sedimento et cellulae flagellaribus descripsae sed cellulae in statu sedentario de speciebus totis non cognitae; si praesentes, plerumque sine appendicibus et squamis vel solitariis vel parietaliter in colonias gelatinarum sphaerarum lobatis irregulares dispositae; appendices si praesentes plerumque brevis vel imperfectae.

Swimming cells with two more or less equal flagella and a short, noncoiling haptonema; chloroplasts 1–4, parietal; cell body covered with flat scales of two sizes. Cells sometimes with ejectile material composed of a-chitin. Life cycle complex with nonmotile and motile stages though nonmotile cells not known in all species; if present, situated parietally in gelatinous spherical or irregularly lobed colonies, usually with no appendages or scales; the former, if present, usually short or incomplete.

Type genus: Phaeocystis Lagerheim, 1893.

Family Phaeocystaceae Lagerheim, 1896

There are nine species of Phaeocystis validly published, although Sournia (1988) recognized only two: P. scrobiculata and P. pouchetti, with P. globosa as its later synonym. Sournia recommended the use of Phaeocystis sp. for organisms that could not be assigned to either of the two species. Our analysis (Medlin et al. 1994) supports the recognition of three colonial species (P. globosa, P. antarctica, and P. pouchetti) plus two new species that will be described elsewhere (Zinke & al. in press). Phaeocystis scrobiculata is recognized on morphological grounds even though molecular data are not available.

In the light microscope, colonies of P. pouchetti can be identified by their lobed colony morphological features, whereas the spherical colonies of P. globosa and P. antarctica cannot be distinguished from one another, except by size as colonies age (Baumann et al. 1994; Medlin et al. 1994). At present, motile stages of these species have not been fully characterized morphologically. Swimming cell ultrastructure is known for only one species (Parke et al. 1971), identified as P. pouchetti, but it was probably P. globosa (Medlin et al. 1994; Vaulot et al. 1994). The flagellate cells have two chloroplasts with immersed pyrenoids traversed by single tubules. They have a short, stiff haptonema, with six MTs in the emergent part, flagella with proximal and distal bands, and ejectile organelles discharging filamentous five-armed starlike structures (Parke et al. 1971) that later were shown to have an α-chitin crystalline structure (Chriéniel-Dinet et al. 1997). Details of the flagellar apparatus were not described.

Order Pynmsiaceae Papenfuss, 1955, emend. Edvardsen et Eikrem

Description: Motile single cells with two more or less equal flagella and usually a well-developed bending or coiling haptonema; haptonema rarely absent. Cells covered by simple to elaborate organic scales; scales rarely absent.

Type genus: Pynmsium Massart, 1920.

Family Pynmsiaceae Conrad ex O.C. Schmidt, 1931

Species of this family fall into two subclasses in our molecular analysis. Clade B1, previously identified by Simon et al. (1997), contains Pynmsium species plus certain species of Chrysocromulina that may be irregular to spheroid in shape, with a haptonema that is equal to or shorter than the two flagella. One exception is Chrysocromulina hirta Manton, where the haptonema is longer than the flagella.

Some species in this clade also have features commonly associated with many coccolithophorids (e.g., compound root R1, a cytoplasmic tongue, and bulging pyrenoids) or charac-
ters that are usually not found in the *Chrysochromulina* species in clade B2 (see below, e.g., a simple R1 root with a sheet containing more than 20 MTs). The species of *Chrysochromulina* examined by Birkhead & Pienaar (1995) has several of these features that are associated with coccolithophorids: a compound root associated with its mature flagellum (R1) and a fibrous root resembling a cytoplasmic tongue. In addition to the distal and proximal transitional plates, this species has (as some coccolithophorids) a helical structure in the flagella (Birkhead & Pienaar 1995). The 18S rRNA gene of this species has not been sequenced, but it is expected to fall into clade B1 on the basis of its morphological structure. Of the species included in this study, *C. polylepis* Manton et Parke, *Chrysochromulina kappa* Parke et Manon, and *Prymnesium nemathecum* Pienaar et Birkhead have compound R1 flagellar roots. Both *Prymnesium patelliferum* Green et al. and *Prymnesium parvum* N. Carter have simple R1 flagellar roots, but the sheets of MTs contains many MTs (20), as in *P. nemathecum*. *Prymnesium nemathecum* also has a scale-covered haptonema, a feature that it shares with *I. galbana* and a number of coccolithophorids (Table 2, Fresnel 1989; Pienaar & Birkhead 1994). Except for *C. kappa* and *P. nemathecum*, which have bulging pyrenoids, species of *Chrysochromulina* and *Prymnesium* in clade B1 have immersed pyrenoids.

Our 18S rDNA analysis places *I. rotunda* in clade B1 with *Prymnesium* spp., *C. polylepis*, *C. hirta*, and *C. kappa* (Fig. 1). Unpublished observations (Eikrem et al.) also place *Chrysochromulina ericina* Parke et Manton, *Chrysochromulina brevifillum* Parke et Manton, *Chrysochromulina chiton* Parke et Manton, and *Chrysochromulina minor* Parke et Manton in the same clade. The close relationship of *Imantonia* to *Chrysochromulina* is also supported in an analysis of the rbcL gene (Inouye 1997). In *I. rotunda*, the haptonema is represented by a vestigial proboscis (Green & Pienaar 1977), and it possesses many few-membered roots associated with the flagella. There is no crystalline bundle of MTs associated with R1 or R2, but the root termed R5 by Green & Hori (1986) may be interpreted as a vestige of a crystalline bundle of MTs associated with R1 (R1c) (e.g., Inouye 1997; Eikrem & Moestrup 1998).

In other regards, *I. rotunda* resembles species of *Prymnesium* and *Chrysochromulina* in this clade in having a scaly covering, immersed pyrenoids (except for *C. chiton*), and flagella with distal and proximal transitional plates. The 18S rRNA gene of *Dictateryia inornata* Parke has not been examined, but based on ultrastructural observations (Green & Pienaar 1977), we predict it also belongs in clade B1. Cavalier-Smith erected a separate order for *Dictaterya* based solely on the fact that the cells lack scales (Cavalier-Smith et al. 1996), and this order may not be warranted.

Clade B2 contains the sindle-shaped species of *Chrysochromulina* (including *Chrysochromulina acanthra* Leadbeater et Manton, *Chrysochromulina cymatulifera* Manton et Leadbeater, *Chrysochromulina throni* Eikrem, and *Chrysochromulina scutellum* Eikrem et Moestrup) that have a long, coiling haptonema with six or seven MTs in the emergent part. The two flagella of these species have both proximal and distal transitional plates. They contain two chloroplasts with immersed pyrenoids traversed by tubes or thylakoids. The flagellar root R1, which is associated with the mature flagellum, is simple, and the sheet of MTs usually contains only few MTs (usually c. 10, except *C. acanthra*, which may have approximately 10–20, Table 1).

The large divergence between clades B1 and B2 demonstrates that *Chrysochromulina* is not a natural group and should be divided into two or more genera. Molecular and ultrastructural data show that species of *Prymnesium* are closely related to the *Chrysochromulina* species of clade B1, indicating that nomenclatural changes are needed for species in the family Prymnesiaceae (Eikrem et al. unpublished observations). We classify *Imantonia* in the family Prymnesiaceae.

**Order Isochrysidales Pascher, 1910, emend. Edvardsen et Eikrem**

Isochrysidales was erected as a descriptive name to accommodate taxa with two equal flagella, including *Hymenomonas* (Pascher 1910). The genus *Isochrysis* was first proposed by Parke (1949), and the first valid publication of the family *Isochrysidaceae* is Bourrelly 1957 nom. conserv. (Greuter et al. 1994). Because of the widespread use of the Isochrysidales to classify haptophytes, we are emending Pascher’s (1910) description of this order.

**DESCRIPTION:** Motile cells with two equal to subequal flagella or cells nonmotile; haptonema rudimentary with a few MTs in the emergent part or absent. Motile cells covered by small delicate organic scales; nonmotile cells sometimes with coccoliths.

**TYPE GENUS:** *Isochrysis* Pascher, 1949.

In this clade of the Prymnesiaceae, the unmineralized species *I. galbana* and the coccolithophorids *E. huxleyi* and *Gephyrocapsa* spp. are sister taxa to all other coccolithophorids. The relationship of *Isochrysis* to *Emiliania* and *Gephyrocapsa* has been noted previously, and on the basis of their ultrastructure, the three genera were included in the order *Isochrysidales* by Parke and Dixon (1976, see also comments by Green & Pienaar 1977). In view of the morphological and structural differences between *I. galbana* and the coccolithophorid genera, there is a good case for reinstating the order Isochrysidales (see Parke & Dixon 1976) with *I. galbana* in a separate family from *E. huxleyi* and *Gephyrocapsa*. Indeed, the gene trees based on 18S rRNA analyzed with maximum likelihood analysis (Fig. 1) and based on rbcL gene (Inouye 1997) suggest that both Isochrysidales and Coccolithales could be separate orders. The four genera of the Isochrysidales produce long-chain saturated alkenones (see references in Jordan & Chamberlain 1997), which may be a unifying feature for the order.

**Family Isochrysidaceae Bourrelly, 1957, emend. Edvardsen et Eikrem**

**DESCRIPTION:** With the characters of the order, but coccoliths absent.

**TYPE GENUS:** *Isochrysis* Pascher, 1949.

The unmineralized scales covering the cell body of the motile stage of *I. galbana* are very similar to those covering the motile stage of *E. huxleyi*, supporting the close relationship between the two species indicated by the molecular data. The ultrastructure of *I. galbana* shows several coccolithophorid characters, such as compound roots (R1 has two crystalline bundles of MTs) and a helical structure distal to the proximal
transitional plate of the flagella (Hori & Green 1991). The short haptoma (with five MTs in the emergent part) is covered by minute scales (Green & Pienaar 1977).

Family Noëlaerhabdaceae Jerkovic, 1970

*Emiliania huxleyi* differs morphologically and ultrastructurally from many of the other coccolithophorids. The naked S cells in the life cycle of *E. huxleyi* have chloroplasts with an immersed pyrenoid traversed by single tubules, and they seem to lack a haptoma completely. The coccolith-bearing cells of *E. huxleyi* (C cells), producing placoliths, lack underlayer scales, and the coccoliths do not have base plates. However, a delicate layer of polysaccharide material is present in the coccolith vesicle *E. huxleyi*, but it is quickly obscured once calcification takes place. Also, special staining techniques have shown this alga to have Golgi-produced "flake-like" structures located between the cell membrane and coccolith covering (Wal et al. 1983). These structures may be homologous to the organic scales and the base plates formed by other coccolithophorids.

*Gephyrocapsa oceanica* Kumptner has identical SSU rDNA sequence to that of *E. huxleyi*. The close relationship between the two species is further supported by the findings in recent sediments of coccospheres with both *E. huxleyi* and *G. oceanica* coccoliths (Clocchiatti 1971). The motile phase of *G. oceanica* bears scales reminiscent of those of *E. huxleyi* (S cell) and *I. galbana*. In addition, these three species possess a membranous sheet in the peripheral ER not found elsewhere in the Haptophyta (Inouye 1997).

Order Coccolithales E. Schwarz, 1932 ("Coccolithinales") emend. Edvardsen et Eikrem

**Description:** Cells usually with compound flagellar roots, a helical structure distal to the proximal transitional plate in the proximal part of the flagella, a haptoma shorter than the flagella or a reduced haptoma, and bulging pyrenoids. Coccoliths at some stage in their life cycle; all species with simple organic underlayer scales.

**Type Genus:** Coccolithus Schwarz, 1894.

The coccolithophorids represent another major clade in our rRNA tree. At present, there are too few sequences of the other coccolithophorids to make any strict comparisons between the taxa. However, the following families appear to be confirmed by the rRNA analysis, and ultrastructural data, where available, do not conflict with the molecular data.

Family Pleurochrysidaceae Fresnel et Billard, 1991

The morphological and fine structure of *P. carterae* has been widely studied, and it has many of the features commonly found in coccolithophorids. Many coccolithophorids have heteromorphic life histories (Billard 1994). The diploid coccolith-bearing cells of *P. carterae* are covered by underlayer scales with monomorphic scale faces and outer cricoliths, whereas the haploid cells are covered only by organic scales with dimorphic scale faces. The cells have two unequal, apically inserted flagella and a short, bulbous, scale-covered haptoma, with six MTs in the emergent part. The flagella have a proximal transitional plate and, distal to it, a helical band. The microtubular roots are remarkable, with both R1 and R2 being compound roots. A cytoplasmic tongue is also present.

The culture of *Pleurochrysis elongata* (Droop) Jordan et al. that we examined was originally identified as *Syracosphaera (Hymenomonas) elongata* (Droop 1955; Jordan et al. 1993). There are no published micrographs of the coccoliths. In the light microscope, the cells fit the original description, except they no longer produce coccoliths.

According to Cavalier-Smith (1996), the enigmatic *R. japonensis* (class Prymnesiophyceae, subclass Flavoretophydae) is, from SSU rDNA sequence data, a close relative of the coccolithophorids and not a heterokont organism as suggested by Grell et al. (1990). Nevertheless, morphologically, it shows little resemblance to the coccolithophorids. Its life cycle includes loricate meroplanktonia, heliozoanlike cells, and heterotrophic and photosynthetic stages (Grell 1990). The related *Reticulosphaera socialis* Grell has been examined ultrastructurally (Grell et al. 1990), and its possible relationship with the haptophytes has been discussed by Cavalier-Smith et al. (1996). In our analysis, *Reticulosphaera* falls near *Pleurochrysis*, and its relegation to a separate subclass in the Prymnesiophyceae (see Cavalier-Smith 1996) is not warranted on the basis of the current molecular analysis, although its separation at least at the level of subclass is acceptable on morphological grounds.

Family Coccolithaceae Poche, 1913 ("Coccolithidea")

*Cruципlacolithus neohelis* (McIntyre et Bé) Reinhardt shares some features with *P. carterae*, e.g., compound roots and a proximal band in the flagella. A helical band, however, has not been observed, and the cytoplasmic tongue is reduced. The haptoma is vestigial, with five MTs in the base. The chloroplast possesses an immersed pyrenoid. The placolith-bearing cells have underlayer scales with monomorphic scale faces.

The nonmotile, placolith-producing *Coccolithus pelagicus* (Wallich) Schiller is linked in a life cycle with the flagellate formerly known as *Crystalloolithus hyalinus* Gaarder et Markali, which produces crystalloliths (holococcoliths) and bears a haptoma shorter than the flagella with six or sometimes five MTs in the free part (Parke & Adams 1960). Both stages have organic underlayer scales and chloroplasts that contain an immersed pyrenoid traversed by thylakoids.

Ultrastructural data on the *C. pelagicus* stage are limited (Manton & Leedale 1969; Manton & Petersi 1969; Pienaar 1969; Leadbeater 1970). In some aspects of cell morphology and ultrastructure (e.g., appendages and pyrenoids), *C. hyalinus* and another coccolithophorid *C. sphaeroides* (Klavness 1973) resemble *Chrysochromulina* species. Nevertheless, both R1 and R2 are compound roots in *C. sphaeroides*. Following the classification of Jordan & Green (1994), *C. pelagicus* is placed with *C. neohelis* in the Coccolithaceae. According to an rbcL analysis (Inouye 1997), species of *Calyprorosphaera* Lohmann, *Chalcococcis* Kamptner, *Cruципlacolithus* Hay et Mohler, and *Umbilicosphaera* Lohmann are also closely related, suggesting that the Coccolithaceae is a natural group.

*Chrysochromulina* species, such as *Chrysochromulina bergensis* Leadbeater and perhaps *Chrysochromulina herdensis* Leadbeater, have structures on their scales reminiscent of the weakly calcified coccolithophorids (Leadbeater 1994; Eikrem et al. 1998). These species have spherical cells and a haptoma shorter than the flagella. The relationship between
the weakly calcified coccolithophorids and C. herdilensis and C. bergenergus remains obscure, since morphological and ultrastructural data are meager, and genetic information is entirely lacking. The saddle shape, which is typical of many Chrysoschreuma species, has also been found in the weakly calcified genus Eriochilus (Thomasen et al. 1995), but ultrastructural data and genetic information on phylogenetic relationships are lacking.

Of the coccolith-lacking species, only L. gaihama has proved to be closely related to the coccolithophorids. Hitherto, no other unmineralized species has proven to be closely related to a coccolithophorid or vice versa. Unfortunately, species like Syracosphaera pulchra Lohmann, which has many ultrastructural details in common with the saddle-shaped Chrysoschreuma species, has not yet been sequenced. It also lacks persistent base plates on the coccoliths forming the distal layer (Inouye & Pienaar 1987) as does E. luizii.

The great taxonomic challenge, therefore, lies within the main body of the coccolithophorids. Certainly, the present major division between heterococcolithophorids and holococcolithophorids (the latter included in the Calyptrosphaeraceae in Jordan & Green 1994) is artificial. Parke and Adams (1960) were the first to report the relationship between a heterococcolithophorid and a holococcolithophorid as alternating stages in a single life cycle, and recently there have been many reported cases of cells from the plankton covered with a coccolith investment of both holococcoliths and heterococcoliths; representative examples now cover six families of the coccolithophorids (Thomasen et al. 1991; Cros et al. in press). Life cycles have been reviewed by Billard (1994), but so far only a few have been fully elucidated. Thus, until such information is available and more molecular data have been obtained, it is premature to revise the taxonomic arrangement of this major group of organisms.

CONCLUSIONS

It is encouraging to note that in general the molecular data available for Haptophyta supports the systematic schemes based on traditional morphological information. We have resurrected some higher taxa because the amount of genetic divergence between major clades is commensurate with that at the order and class level in other algal divisions. How most unmineralized and coccolith-covered species are related to each other will have to await further molecular studies. The uniqueness of some of the clades from the gene clone library (clades D and E) in terms of their molecular relatedness to other known cultured haptophyte species suggests that there may be many novel as yet undescribed or unseen haptophyte taxa in the world's open oceans.

A summary of the taxonomic ranks above the genus level supported by molecular, morphological, and ultrastructural analysis is listed below. A more complete checklist of other genera in each family can be found in Jordan & Green (1994). Their inclusion in each family is based on morphological and ultrastructural evidence.

Order Pavlovales Green, 1976
Family Pavlovaceae Green, 1976
Genera: Diacronema Prauser emend. Green & Hibblett, 1977; Exanthemachrysis Lepailleur, 1970; Pavlova Butcher, 1952; Rebecca Green gen. nov.
Class Prymnesiophyceae Hibberd, 1976 emend. Cavalier-Smith, 1996
Order Phaeocystales Medlin ord. nov.
Family Phaeocystaceae Lagerheim, 1896
Genus: Phaeocystis Lagerheim, 1893
Order Prymnesiales Papenfuss, 1955 emend. Edvardsen et Eikrem
Family Prymnesiaceae Conrad ex. O.C. Schmidt, 1931
Genera: Imantonia Reynolds, 1974; Prymnesium Massart, 1920; Chrysoschreuma Lackey, 1939
Order Isochrysidales Pascher, 1910 emend. Edvardsen et Eikrem
Family Isochrysidaceae Bourrelly, 1957 emend. Edvardsen et Eikrem
Genus: Isochrysis Parke, 1949
Family Noelaerhabdaceae Jerkovic, 1970
Order Coccolithales E. Schwarz, 1932, ('Coccolithinales') emend. Edvardsen et Eikrem
Family Pleurochrysidaceae Fresnel et Billard, 1991
Genus: Pleurochrysis Pringsheim, 1955
Family Coccolithaceae Poche, 1913 ('Coccolithidae')
Genera: Coccolithus H.E.L. Schwarz, 1894; Cruciplacolithus Hay & Mohler, 1967
Family Reticulosphaeraceae Cavalier Smith, 1996
Genus: Reticulosphaera Grell, 1990

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