

## 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 coccoid, 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DNA) from 18 haptophyte species has been sequenced, and the sequences aligned with those of more than 300 published and unpublished chlorophyll *a* + *c* algae.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 *Imantonia*; 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 & Paasche 1998).

These algae exhibit a variety of life forms, ranging in size from the nanoplankton (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*), as motile single cells (e.g., species of *Chrysochromulina*), or colonial flagellates (species of *Corymbellus*). Some have benthic filaments, whereas others may have amoeboid stages in their life cycle (Hibberd 1980). Several have morphologically dis-

tinct alternate forms, e.g., *Isochrysis galbana* Parke (Parke 1949), *Phaeocystis globosa* Scherffel [as *Phaeocystis pouchetii* (Hariot) Lagerheim in Parke *et al.* 1971], *Chrysochromulina polylepis* Manton et Parke (Edvardsen & Paasche 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  $\mu$ m 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

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close to the flagellar basal bodies and haptonematal 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 Pavlovophycidae; Cavalier-Smith 1986; 1989; Jordan & Green 1994). However, Cavalier-Smith (1993) raised both subclasses to the class level, which he named Patellifera and Pavlovea, although the latter name was not validly published (see below). In 1996, Cavalier-Smith emended Pavlovea to Pavlovophyceae and changed the concept of the Prymnesiophyceae to include two subclasses: the Prymnesiophycidae with Patellifera 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-biphosphate carboxylase/oxygenase (*rbcL*) gene (Fujiwara *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 platelike body scales that may become elaborate and have complex forms (Leadbeater 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 *Pleurochrysis pseudoroscoffensis* Gayral et Fresnel (Gayral & Fresnel 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 platelike body scales are absent. Stigmata (eyespot) 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*, *Imantonia*, and *Isochrysis* were grown as batch cultures (0.5–2 l) in Erlenmeyer flasks with filtered, autoclaved seawater diluted to 30 psu. Nutrients, vitamins, and trace metals were added as in IMR 1/2 medium (Eppley *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  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  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 Chesnick *et al.* (1997). Most of the polymerase chain reaction products were directly sequenced using a solid-phase sequencing method with radioisotopes (Chesnick *et al.* 1997) or cycle sequenced (Sequi-therm, BIOZYM) using infrared-labeled primers and analyzed with the LiCor automated sequencer (MWG Ebersberg, Germany), whereas others were cloned (LigAator, 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 1.** Summary of strains and nucleotide sequences used in the present study. Genbank accession numbers for the sequences from this study are in bold.

Species	Strain code	Collection site	Culture collection	Isolator, year	Accession no.
<i>Chrysochromulina acantha</i> Leadbeater et Manton	T20	Off Arendal, S Norway	UIO	B. Edwardsen, 1994	<b>AJ246278</b>
<i>Chrysochromulina campanulifera</i> Manton et Leadbeater	J10	Skagerrak	UIO	J. Thronsdén, 1984	<b>AJ246273</b>
<i>Chrysochromulina hirta</i> Manton	1Y	Off Austevoll, W Norway	UIO	B. Edwardsen, 1989	<b>AJ246272</b>
<i>Chrysochromulina kappa</i> Parke et Manton	EN3	Oslofjord, S Norway	UIO	W. Eikrem, 1989	<b>AJ246271</b>
<i>Chrysochromulina polylepis</i> Manton et Parke	B11	Oslofjord, S Norway	UIO	B. Edwardsen, 1988	AJ004866
<i>Chrysochromulina scutellum</i> Eikrem et Moestrup	G7	Skagerrak, S Norway	UIO	W. Eikrem, 1990	<b>AJ246274</b>
<i>Chrysochromulina thronsdénii</i> Eikrem	K11	Off Arendal, S Norway	UIO	W. Eikrem, 1989	<b>AJ246279</b>
<i>Chrysochromulina thronsdénii</i> Eikrem	L12	Off Arendal, S Norway	UIO	W. Eikrem, 1989	<b>AJ246277</b>
<i>Coccolithus pelagicus</i> (Wallich) Schiller	PLY 182G	English Channel	PML	J. C. Green, 1990	<b>AJ246261</b>
<i>Cruciplacolithus neohelis</i> (McIntyre et Bé) Reinhardt	CCMP 298	La Jolla, CA	CCMP	K. Lee, 1984	<b>AJ246262</b>
<i>Imantonia rotunda</i> Reynolds emend. Green et Pienaar	UIO 101	Off Arendal, S Norway	UIO	B. Edwardsen, 1994	<b>AJ246267</b>
<i>Isochrysis galbana</i> Parke emend. Green et Pienaar	UIO 102	Ryfylke, W Norway	UIO	W. Eikrem, 1990	<b>AJ246266</b>
<i>Emiliana huxleyi</i> (Lohmann) Hay et Mohler	PLY 92D	English Channel	PML	J. C. Green 1975	M87327
<i>Gephyrocapsa oceanica</i> Kamptner	PLY 574 = GO1	Mutsu Bay, Aomori Prefecture, Japan	PML	M. Kawachi 1991	<b>AJ246276</b>
Coccolith haptophyte	CCMP 625	NA <sup>1</sup>	CCMP	NA	U40924
<i>Pavlova gyrans</i> Butcher emend. Green et Manton	CCMP607	See University of Wash- ington culture 349	CCMP	NA	U40922
<i>Pavlova</i> aff. <i>salina</i>	PLY 486	U.K.	PML	M. Turner	L34669
<i>Pavlova</i> sp.	CCMP 1394	Gulf of Maine	CCMP	L. Provasoli	U40925
<i>Pavlova</i> sp.	CCMP 1416	Gulf of Maine	CCMP	M. Keller	<b>AJ243369</b>
<i>Phaeocystis antarctica</i> Karsten	SK 23	Weddell Sea	AWI	M. Baumann	X77481
<i>Phaeocystis globosa</i> Scherffel	SK 35	North Sea	AWI	M. Baumann	X77476
<i>Phaeocystis pouchetii</i> (Hariot) Lagerheim	SK34	Greenland Sea	AWI	M. Baumann	X77475
<i>Phaeocystis</i> sp. 1	c2a1	Gulf of Naples, Italy		A. Zingone	AF163147
<i>Phaeocystis</i> sp. 2	B5	Gulf of Naples, Italy		A. Zingone	AF163148
<i>Pleurochrysis carteræ</i> (Braarud et Fagerland) Christensen	von Stosch isolate	NA	BAH	H. A. von Stosch	<b>AJ246263</b>
<i>Pleurochrysis</i> sp.	CCMP 300	See PLY 181	CCMP	M. Parke	<b>AJ246275</b>
<i>Pleurochrysis elongata</i> (Droop) Jordan	CCMP 874	NE Atlantic, coastal	CCMP	M. Droop	<b>AJ246264</b>
<i>Pleurochrysis</i> sp.	CCMP 875	See Plymouth 181	CCMP	M. Parke	<b>AJ246265</b>
<i>Prymnesium calathiferum</i> Chang et Ryan	CCMP 707	Northland, New Zealand	CCMP	F. Chang, 1983	U40923
<i>Prymnesium nemamethecum</i> Pienaar et Birkhead	South Africa	St James—False Bay, South Africa	S.A.	R. Pienaar	<b>AJ246268</b>
<i>Prymnesium parvum</i> N. Carter emend. Green, Hibberd et Pienaar	K-0081	Flade Sø, Denmark	SCCAP	T. Christensen	<b>AJ246269</b>
<i>Prymnesium patelliferum</i> Green, Hibberd et Pienaar	PLY 527	S coast of England	PML	D. J. Hibberd 1976	L34670/71
<i>Reticulospaera japonensis</i> Grell	NA	NA	NA	Grell	X90992
<i>Fucus distichus</i> Linnaeus	NA	Straits of Georgia	NA	NA	M97959
<i>Cryptocodinium cohnii</i> (Seligo) Chatton	NA	NA	NA	NA	M34847

<sup>1</sup> 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 *Cryptothecodinium* 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 fastDNAm1 program (version 1.0) (Larsen *et al.* 1993). Maximum parsimony analyses were implemented with the PAUP computer program (Swofford 1993). Introduced gaps were treated as missing data; informative characters were treated as multistate unordered. 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 (Kooistra & Medlin 1996). Distance analyses were performed using the PHYLIP computer program (Felsenstein 1993). Dissimilarity 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 unicell) 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 OLI51050 and OLI26041, 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 coccolith-

ophorids (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 OLI51050 and OLI26041 (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. pouchetii*. Gene clone OLI51004 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 paraphyletic (Inouye 1997; 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 (OLI51059 and OLI51033 + OLI51056) 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 *Reticulosphaera*)

Clade C contains the coccolithophorids and has strong bootstrap support in the maximum parsimony analysis (Fig. 1).

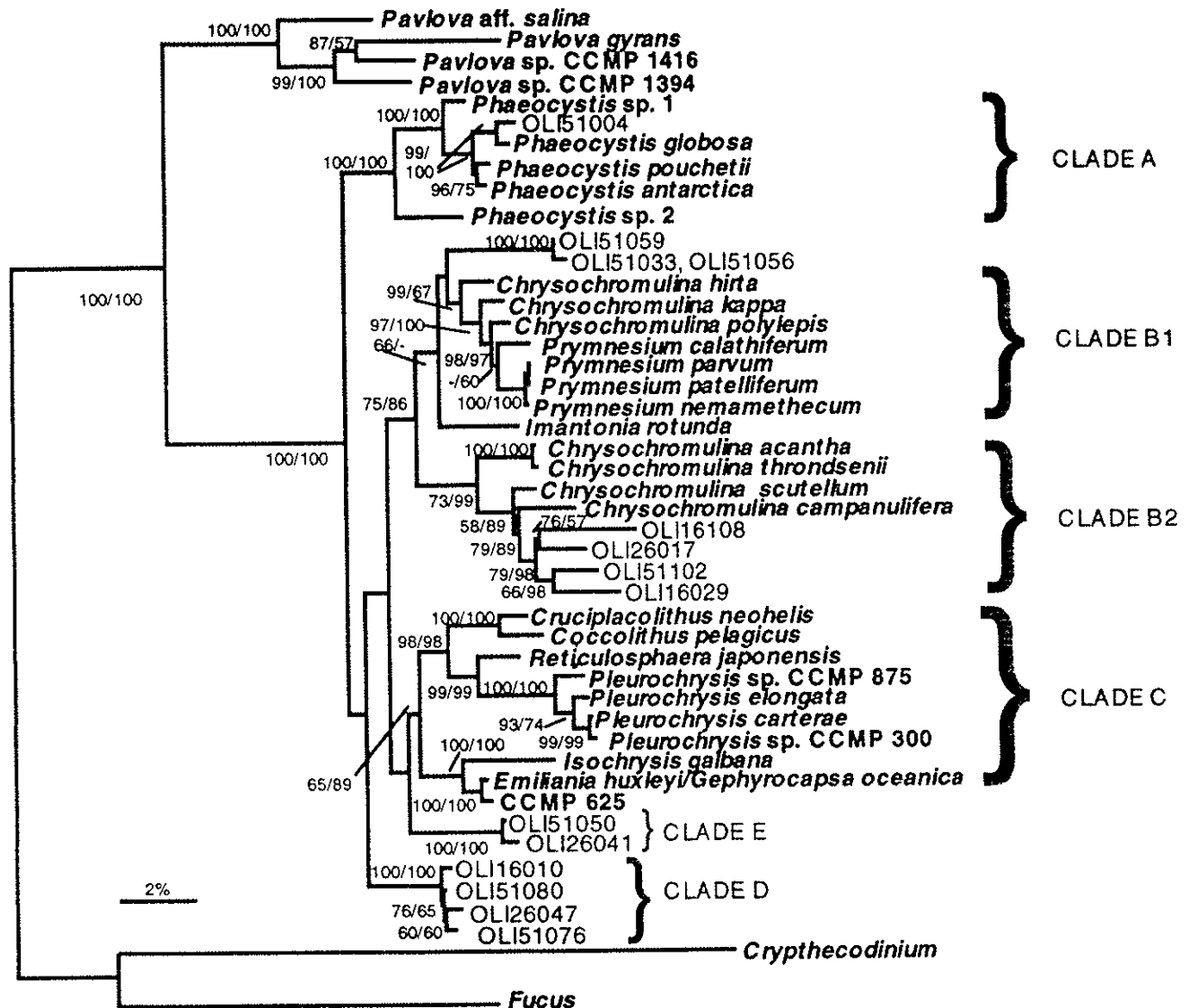


Fig. 1. Phylogenetic tree based upon a maximum likelihood analysis showing the relationships of haptophyte taxa. The tree is rooted on the branch leading to *Fucus* and *Cryptothecodinium*. For discussion of clades A through E, see text. Maximum parsimony and neighbor-joining analyses produced similar 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), *Reticulosphaera 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 (Fujiwara *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 2.** Morphological and ultrastructural information about selected species from the class Prymnesiophyceae. (Abbreviations, p. 26.)

Species	Cell	Flagella	Haptonema	Scales	Pattern	Base plate scales	Coccoliths	No. of MT in free part of haptonema
<i>Chrysochromulina campanulifera</i> (Norwegian isolate)	Saddle	Equal	Hapt. $\gg$ flag.	Plate, cup	Rad. patt. both faces	Absent	Absent	6
<i>Chrysochromulina thronsenii</i>	Saddle	Equal	Hapt. $\gg$ flag.	Plate (2 types)	Rad. ribs overlaying spiraling rib(s), central open ring with cross	Absent	Absent	6
<i>Chrysochromulina scutellum</i>	Saddle	Equal	Hapt. $\gg$ flag.	Plate, spine (2 types)	Rad. ribs prox. face, con. fibrils dist. face	Absent	Absent	7
<i>Chrysochromulina acantha</i>	Saddle	Equal	Hapt. $>$ flag.	Plate, spine	Loosely woven rad. ribs overlaying con. ribs	Absent	Absent	7
<i>Chrysochromulina kappa</i>	Spherical	Equal	Hapt. $<$ flag.	Plate (2 types), spine	Rad. ribs prox. face, fibrils dist. face	No obs	No obs.	7-8
<i>Chrysochromulina hirta</i>	Oblong - spherical	Equal	Hapt. $>$ flag.	Plate, spine (2 types)	Rad. ribs. one face, fibrils other face	Absent	Absent	No obs.
<i>Chrysochromulina polytepis</i> (authentic)	Oblong - spherical	Equal	Hapt. $<$ flag.	Plate (4 types)	Rad. ribs both faces	Absent	Absent	7
<i>Chrysochromulina polytepis</i> (alternate)	Oblong - spherical	Equal	Hapt. $<$ flag.	Plate (2 types) spine	Rad. ribs dist. face, con. fibrils prox. face	Absent	Absent	7
<i>Prymnesium nemamethechum</i>	Oblong	Equal-sub-equal	Hapt. $\ll$ flag. with scales	Plate (2 types on body, 1 type on hapt.)	Rad. patt. both scale faces, body scales with superstructures	Absent	Absent	7
<i>Prymnesium parvum</i>	Oblong-spherical	Equal-sub-equal	Hapt. $\ll$ flag.	Plate (2 types)	Rad. ribs dist. face, fibrils prox. face	Absent	Absent	7
<i>Prymnesium patelliferum</i>	Oblong-spherical	Equal-sub-equal	Hapt. $\ll$ flag.	Plate (2 types)	Rad. ribs both faces	Absent	Absent	No obs.
<i>Prymnesium calathiferum</i>	Oblong-spherical	Equal-sub-equal	Hapt. $\ll$ flag.	Plate (2 types)	Rad. ribs both faces	Absent	Absent	No obs.
<i>Imantonia rotunda</i>	Spherical	Equal	Absent	Plate (2 types)	Rad. ribs both faces	Absent	Absent	NA
<i>Cruciplacolithus neohelis</i>	Oblong-spherical	Equal	Vestigial	Plate	Rad. ribs dist. face, con. fibrils prox. face	Rad. ribs both faces	Placoliths	(5 MT in base)
<i>Pleurochrysis carterae</i>	Oblong	Unequal	Bulbous with scales	Plate	Rad. ribs one face, fibrils other face	Rad. ribs prox face, fibrils dist. face	Cricoliths	6 MT
<i>Pleurochrysis elongata</i>	Oblong	Equal-unequal	Bulbous with scales	Plate	Fibrils both faces	Rad. ribs prox face, fibrils dist. face	Cricoliths	No obs
<i>Coccolithus pelagicus</i> (Crystalloolithus stage)	Spherical	Equal	Hapt. $\ll$ flag.	Plate	Rad. ribs one face, con. fibers other face	Present	Crystallooliths	6 (5) MT
<i>Gephyrocapsa oceanica</i>	Spherical	Absent	Absent	Absent		Absent/non-resistant	Placoliths	No obs.
<i>Emiliana huxleyi</i> (S-cells)	Spherical	Equal	Prob. absent	Plate	Rad. ribs both faces	Absent	Absent	NA
<i>Isochrysis galbana</i>	Oblong	Equal	Hapt. $\ll$ flag. with scales	Plate (2 types)	Rad. ribs both faces	Absent	Absent	5 MT
<i>Phaeocystis antarctica</i>	Spherical	Equal	Hapt. $\ll$ flag.	Plate (2 types)	No obs.	Absent	Absent	No obs.
<i>Phaeocystis globosa</i>	Spherical	Equal	Hapt. $\ll$ flat.	Plate (2 types)	Rad. ribs both faces	Absent	Absent	6 MT
<i>Phaeocystis pouchetii</i>	Spherical	Equal	Hapt. $\ll$ flag.	Plate (2 types)	Rad. ribs both faces	Absent	Absent	No obs.

Table 2. Extended.

Flagellar micro-tubular roots	Flagella with proximal and/or distal transitional plates	Flagella with helical band or other structures	Cytoplasmic tongue	Pyrenoids	References	Comments
Simple R1	No obs.	No obs.	No obs.	Immersed, traversed by tubules	Manton & Leadbeater 1974, Eikrem unpublished	
Simple R1	Prox. and dist. plates	Absent	Absent	Immersed, traversed by tubules	Eikrem 1996, Eikrem unpublished	
Simple R1	Prox. and dist. plates	Absent	Absent	Immersed, traversed by thylakoids	Eikrem & Moestrup 1998	
Simple R1 (many MT)	Prox. and dist. plates	Absent	Absent	Immersed	Leadbeater & Manton 1971, Gregson <i>et al.</i> 1993	
Compound R1	Prox. and dist. plates	Absent	Absent	Bulging, traversed by thylakoids	Parke <i>et al.</i> 1955, Manton & Leedale 1961, Eikrem unpublished	
No obs.	No obs.	No obs.	No obs.	No obs.	Manton 1978	
Compound R1	Prox. and dist. plates	Other structure	Present	Immersed, traversed by thylakoids	Manton & Parke 1962, Eikrem unpublished	
Compound R1	Prox. and dist. plates	Other structure	Present	Immersed, traversed by thylakoids	Paasche <i>et al.</i> 1990, Edwardsen <i>et al.</i> 1996, Eikrem unpublished	
Compound R1	Prox. and dist. plates	Tubular rings	Present (but reduced)	Bulging, traversed by thylakoids	Birkhead & Pienaar 1994, Pienaar & Birkhead 1994	
Simple R1 (many MT)	Prox. and dist. plates	No obs.	No obs.	Immersed	N. Carter 1937, Manton & Leedale 1963a, Manton 1964	
Simple R1 (many MT)	Prox. and dist. plates	No obs.	No obs.	Immersed, traversed by thylakoids	Green <i>et al.</i> 1982, Green & Hori 1990	
No obs.	No obs.	No obs.	No obs.	Immersed	Chang & Ryan 1985	
Vestigial compound R1	Prox. and dist. plates	No obs.	No obs.	Immersed, traversed by thylakoids	Reynolds 1974, Green & Pienaar 1977, Green & Hori 1986	
Compound R1 and R2	Prox. plate	No obs.	Present (but reduced)	Immersed, traversed by thylakoids	Fresnel 1986, Fresnel 1989, Kawachi & Inouye 1994	Nonmobile stage present
Compound R1 and R2	Prox. plate	Helical band	Present	Bulging	Beech & Wetherbee 1984, Beech & Wetherbee 1988	Filamentous stage present
No obs.	No obs.	No obs.	No obs.	No obs.	Droop 1955, Fresnel 1989, Jordan <i>et al.</i> 1993	
Compound R1?	No obs.	No obs.	No obs.	Immersed, traversed by thylakoids	Manton & Leedale 1963b, Klaveness 1973	Nonmotile stage placolith covered, with flagella bases
No obs.	No obs.	No obs.	No obs.	No obs.	Kamptner 1943, Fujiwara <i>et al.</i> 1994, Jordan & Kleijne 1994, Inouye 1997	Motile stage covered by scales similar to those covering S-cells of <i>E. huxleyi</i>
Probably simple	No obs.	No obs.	No obs.	Immersed, traversed by single tubule	Klaveness 1972 a and b, Wal <i>et al.</i> 1983, Green <i>et al.</i> 1996	Dominating nonmobile stage placolith bearing. Lacking resistant base plates "flakelike" structures present. Naked nonmotile stage also present.
Compound R1	Prox. plate present	Helical band	No obs.	1 chloroplast, immersed pyrenoid traversed by tubule	Green & Pienaar 1977, Hori & Green 1991	Scales similar to <i>E. huxleyi</i> , nonmotile stage present in life cycle
No obs.	No obs.	No obs.	No obs.	No obs.	Bauman <i>et al.</i> 1994, Medlin <i>et al.</i> 1994	Dominating stage nonmotile, spherical gelatinous colonies
No obs.	Prox. and dist. plates	No obs.	No obs.	Immersed traversed by single tubule	Parke <i>et al.</i> 1971, Medlin <i>et al.</i> 1994	Dominating stage nonmotile, spherical gelatinous colonies
No obs.	Prox. and dist. plates	No obs.	No obs.	No obs.	Medlin <i>et al.</i> 1994, Eikrem <i>et al.</i> 1998, Eikrem unpublished	Dominating stage nonmotile, lobed gelatinous colonies

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 ex Edvardsen et Eikrem, nom. descr.

DIAGNOSIS: Cellula in statu erratico duobus plerumque flagellis aequalibus vel inaequalibus instructa; si flagella aequalia interdum plusquam duo; si flagella notabiliter inaequalia, brevius eorum interdum vestigiale. Flagella plerumque glabra pilis tubularibus nullis, si inaequalia longius interdum pilis gracilibus non-tubularibus et corporibus minutis sphaericis vel claviformibus armatum; regio transitoria eorum unum vel duo dissepimenta transversa, nullam heli-ccm praebens. Haptonema, organellum huius divisionis proprium, prope flagella situm, longum interdum abbreviatum, raro deficient. Chloroplastus unus vel duo, lamellis e ternis thylacoidibus formatis, lamella cingulari nulla. Stigma plerumque nullum, si praesens raro flagello associatum. Cellula plerumque uno vel pluribus stratis squamarum in corpore Golgiano formatarum et, ut videtur, sine substantia minerali; facies extrorsa cuiusque squamae fibrillis spiralibus, introrsa radiantibus ornata. In quibusdam membris classis extra squamas (coccolithi) ordinarias saepe stratum continuum squamarum maiorum crystallis carbonatis calcii obsitarum formatum. Cellulae coccolithis instructae squamis 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 clavate 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 thylakoids, 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

BASIONYM: Pavlovophycidae ('Pavlovidae') Cavalier-Smith, *Progress in Phycological Research* (1986), p. 342.

Cavalier-Smith erected the class Pavlovophyceae in 1993 (Cavalier-Smith 1993), basing it on the order Pavloales 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 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 gyrans* Butcher, *Pavlova pinguis* Green, *Pavlova virescens* Billard, and *Exanthemachrysis gayraliae* Lepallieur) cells are somewhat metabolic with respect to morphological structure (Green 1980). Except for *P. gyrans*, 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 (Green 1980).

Cells have a single chloroplast, which is often strongly bilobed, sometimes with a bulging basal pyrenoid (e.g., *P. gyrans*, *P. pinguis*). Stigmata (eyespot) have been observed in some species. In *P. gyrans*, *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 vlkianum* Prauser, the stigma is composed of a layer of osmiophilic droplets close to or beneath



the cell membrane. In *D. vlkianum*, it is associated with a groove in the surface of the cell and a specialized swelling on the shorter flagellum (Green & Hibberd 1977; Green 1980). A further characteristic feature of the cells is an elongate invagination of the plasmalemma that forms a closed canal. The canal opening is near the haptonema, and, for *P. gyrans*, *P. pinguis*, and *P. granifera*, it terminates near the stigma. In some species, the functions of the canal seem to be concerned with the discharge of material from the cell (Green 1980).

The mitotic process in the Pavlovophyceae also differs from that encountered in the Prymnesiophyceae (Green & Hori 1988; Hori & Green 1994). In *P. lutheri* and *P. salina*, the long spindle axis is V-shaped rather than straight, and the fibrous root of the longer flagellum acts as a MT-organizing center. The nuclear envelope is more or less intact at metaphase, and the spindle MTs enter the nucleus via large gaps at the poles. No kinetochores have been observed.

The distinct separation of *P. aff. salina* from *P. gyrans* in our molecular analysis (Fig. 1) is substantiated by clear morphological differences. *Pavlova aff. salina* differs from *P. gyrans* (the type species) in having no obvious pyrenoid, no stigma, and a vestigial posterior (mature or number 1) flagellum that is reduced to approximately 0.2  $\mu\text{m}$ , with an axoneme composed of only a ring of nine single MTs (Green 1976, 1980). *Pavlova helicata* also has a vestigial posterior flagellum and lacks a stigma (Green 1980). Green (1976) suggested that there were fine structural grounds for the separation of *P. salina* into a separate genus but deferred this until more information was available. With the weight of our new molecular data added to these ultrastructural differences, we remove *P. salina* and *P. helicata* from *Pavlova* and establish a new genus, *Rebecca* gen. nov. Within the Pavlovophyceae, we retain one order, the Pavlovales, containing one family, the Pavlovaceae (Green 1976; Jordan & Green 1994), for the genera *Diacronema*, *Exanthemachrysis*, *Pavlova*, and *Rebecca* gen. nov.

### *Rebecca* J.C. Green gen. nov.

DIAGNOSIS: Cellulae solitariae, libere natantes, elongatae, interdum compressae, flagellis duobus et haptonemo breve. Flagellum anticum longius, pilis gracilibus non-tubularibus et corporibus minutis sphaericis vel claviformibus armata; flagellum posticum vestigialis. Fovea vel canalis cellulam 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.

BASIONYM: *Nephrochloris salina* N. Carter (*Arch. f. Protistenk.*, 90: 16–18, pl.2, figs 10–22. 1937).

SYNONYMS: *Pavlova mesolychnon* van der Veer (*Acta Bot. Neerl.* 18: 496–510, figs 1–21. 1969), *P. salina* (N. Carter) Green (*J. Mar. Biol. Assoc. UK*, 56: 595–602, pls 1, 2. 1976).

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

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

BASIONYM: *Pavlova helicata* van der Veer (*Nova Hedwigia* 23: 131–159, figs 1–8. 1972).

### 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 Pavlovophyceae, 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  $\mu\text{m}$  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 kappa* Parke et Manton). The flagellar apparatus typically consists of two basal bodies, the haptonemal 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 *crystalline 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 *Dicrateria*, 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 (Leadbeater 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 (*Calyptrorphaera sphaeroidea* Schiller, *Chrysochrysis 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 dictyosome with many, often dilated cisternae (Manton 1967; Hibberd 1976; 1980; Pienaar 1994). One reticulated mitochondrion has been demonstrated in *Pleurochrysis carterae* (Braarud et Fagerland) Christensen (Beech & Wetherbee 1984), and a highly branched and retic-

ulate mitochondrion may be present in most or all prymnesiophyceans. 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 prymnesiophycean 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 Prymnesiophyceae.

At present, there is strong bootstrap support for three clades (A, B, C) within the Prymnesiophyceae (Fujiwara *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*), Prymnesiaceae (species of *Chrysochromulina* and *Prymnesium*), Isochrysidaceae (*Isochrysis*), and Noëliaceae (*Emiliana* and *Gephyrocapsa*). Within the mineralized taxa (i.e., the coccolithophorids), species of *Pleurochrysis* 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 Prymnesiophyceae 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 Prymnesiales. We provide new and emended descriptions where needed.

#### Order Phaeocystales Medlin ord. nov.

DIAGNOSIS: Cellulae natantes flagellis binis plusminusve aequalibus et haptonemate non-circinato brevioribus instructae; chloroplast 1–4, parietales; corpus cellulae squamis complanatis amplitudinibus duobus vestita. Cellulae interdum eictosomata ex  $\alpha$ -chitino composita efferentes. Cursus vitae involutus; cellulae in statu sedentario et cellulae flagellaribus descriptae sed cellulae in statu sedentario de speciebus totis non cognitae; si praesentes, plerumque sine appendicibus et squamis vel solitariae vel parietaliter in colonias gelatinosas sphaericas, lobatasve irregularibusve 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  $\alpha$ -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. pouchetii*, 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. pouchetii*) plus two new species that will be described elsewhere (Zingone *et al.* in press). *Phaeocystis scrobiculata* is recognized on morphological grounds even though molecular data are not available.

In the light microscope, colonies of *P. pouchetii* 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. pouchetii*, 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  $\alpha$ -chitin crystalline structure (Chrétiennot-Dinet *et al.* 1997). Details of the flagellar apparatus were not described.

#### Order Prymnesiales 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: *Prymnesium* Massart, 1920.

#### Family Prymnesiaceae Conrad ex O.C. Schmidt, 1931

Species of this family fall into two subclades in our molecular analysis. Clade B1, previously identified by Simon *et al.* (1997), contains *Prymnesium* species plus certain species of *Chrysochromulina* that may be irregular to spheroid in shape, with a haptonema that is equal to or shorter than the two flagella. One exception is *Chrysochromulina 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 Manton, and *Prymnesium nemamethecum* 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 sheet of MTs contains many MTs (> 20), as in *P. nemamethecum*. *Prymnesium nemamethecum* 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. nemamethecum*, 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 brevifilum* 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 *Dicrateria 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 *Dicrateria* 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 saddle-shaped species of *Chrysochromulina* (including *Chrysochromulina acantha* Leadbeater et Manton, *Chrysochromulina campanulifera* Manton et Leadbeater, *Chrysochromulina thronsdensii* 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. acantha*, 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 ultrastructure 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. Edwardsen 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* Parke, 1949.

In this clade of the Prymnesiophyceae, 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 species of *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. Edwardsen et Eikrem

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

TYPE GENUS: *Isochrysis* Parke, 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 haptonema (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 haptonema 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* Kamptner 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 haptonema shorter than the flagella or a reduced haptonema, 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 haptonema, 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 Flavoretrophyceae) is, from 18S 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 meroplasmodia, 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 ('Coccolithidae')

*Cruciplacolithus 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 haptonema is vestigial, with five MTs in the base. The chloroplast possess 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 *Crystallolithus hyalinus* Gaarder et Markali, which produces crystalloliths (holococcoliths) and bears a haptonema 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 & Peterfi 1969; Pienaar 1969; Leadbeater 1970). In some aspects of cell morphology and ultrastructure (e.g., appendages and pyrenoids), *C. hyalinus* and another coccolithophorid *C. sphaeroidea* (Klaveness 1973) resemble *Chrysochromulina* species. Nevertheless, both R1 and R2 are compound roots in *C. sphaeroidea*. 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 *Calyptrosphaera* Lohmann, *Calcidiscus* Kamptner, *Cruciplacolithus* 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 haptonema shorter than the flagella. The relationship between

the weakly calcified coccolithophorids and *C. herdlensis* and *C. bergenensis* remains obscure, since morphological and ultrastructural data are meager, and genetic information is entirely lacking. The saddle shape, which is typical of many *Chrysochromulina* species, has also been found in the weakly calcified genus *Ericolus* (Thomsen *et al.* 1995), but ultrastructural data and genetic information on phylogenetic relationships are lacking.

Of the coccolith-lacking species, only *I. galbana* 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 *Chrysochromulina* 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. huxleyi*.

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 Calyptosphaeraceae 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 (Thomsen *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.

Division Haptophyta Hibberd ex Edwardsen et Eikrem  
Class Pavlovophyceae Cavalier-Smith (Green et Medlin ('Pavlovidae'))

Order Pavlovales Green, 1976

Family Pavlovaceae Green, 1976

Genera: *Diacronema* Prauser emend. Green et Hibberd, 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. Edwardsen et Eikrem

Family Prymnesiaceae Conrad ex O.C. Schmidt, 1931

Genera: *Imantonia* Reynolds, 1974; *Prymnesium* Massart, 1920;

*Chrysochromulina* Lackey, 1939

Order Isochrysidales Pascher, 1910 emend. Edwardsen et Eikrem

Family Isochrysidaceae Bourrelly, 1957 emend. Edwardsen et Eikrem

Genus: *Isochrysis* Parke, 1949

Family Noëlaerhabdaceae Jerkovic, 1970

Genera: *Emiliana* Hay et Mohler, 1967; *Gephyrocapsa* Kamptner, 1943.

Order Coccolithales E. Schwarz, 1932, ('Coccolithinales') emend. Edwardsen et Eikrem

Family Pleurochrysidaceae Fresnel et Billard, 1991

Genus: *Pleurochrysis* Pringsheim, 1955

Family Coccolithaceae Poche, 1913 ('Coccolithidae')

Genera: *Coccolithus* E.H.L. Schwarz, 1894; *Cruciplacolithus* Hay & Mohler, 1967

Family Reticulosphaeraceae Cavalier Smith, 1996

Genus: *Reticulosphaera* Grell, 1990

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