X. A Review of the Phylogeny of the Haptophyta

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X.1 **ABSTRACT**

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. We have amassed small subunit rRNA gene sequences (18S rDNA) from 125 haptophytes and aligned the sequences with those of over 300 published and unpublished chlorophyll a+c algae. Phylogenies were constructed using Bayesian, maximum likelihood, minimum evolution and weighted maximum parsimony analyses. The high divergence (6%) between members of *Pavlova* and the remaining haptophytes supports the division of the Haptophyta into two classes: the Prymnesiophyceae and the Pavlovophyceae (Edvardsen et al. 2000). Four major clades within the Prymnesiophyceae were identified that correspond to known taxa: one clade embraces Phaeocystales; the second includes members of the Prymnesiales; the third represents the Isochysidales; and the fourth the Coccolithales. Two other clades contain taxa whose sequences were derived from a gene clone library. In the absence of information on cell morphology associated with these sequences we are unable to determine whether they belong to existing orders or if new orders should be erected. These taxa are not strongly related to any of the known cultured taxa. One to two per cent divergence in the 18S rRNA gene analysis warrants a separation above the level of family.

X.2 INTRODUCTION

The Haptophyta are a major lineage of chlorophyll a+c algae. The majority of known haptophytes occur as marine coastal or open oceanic planktonic forms (Hibberd 1980; Green & Jordan 1994; Thomsen et al. 1994), although a few species thrive in freshwater. Many can form massive blooms (Birkenes & Braarud 1952; Berge 1962; Dahl et al. 1989; Blackburn & Cresswell 1993; Brown & Yoder 1994; Wal et al. 1995; Lancelot et al. 1998), in some cases harming natural biota and commercial fisheries (Moestrup 1994; Edvardsen & Paasche 1998).

The haptophytes range in size from nanoplankton (Thomsen 1986) to macroscopic colonies (*Phaeocystis)*, and may occur as non-motile single cells (many coccolithophores), non-motile colonies of single cells embedded in mucilage (*Phaeocystis*), as motile single cells (most non-coccolithophores, e.g. *Chrysochromulina*, many coccolithophores e.g. *Syracosphaera*), or colonial flagellates (*Corymbellus*). Several haptophyte species form benthic filaments, and some may have amoeboid stages in their life cycle (Hibberd 1980). Many have alternate morphologically distinct forms, e.g., *Isochrysis galbana* (Parke 1949), *Phaeocystis globosa* (as *P. pouchetii* in Parke et al. 1971), *Chrysochromulina polylepis* (Edvardsen & Paasche 1992), and many coccolithophores (see Gayral & Fresnel 1983; Thomsen et al. 1991; Billard 1994, this volume). Most of these alternate morphotypes have been shown to be alternate stages in a haplo-diploid life cycle (Billard 1994, this volume).

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

The haptophytes contain one or two chloroplasts, each with an immersed or bulging pyrenoid. The nucleus is usually situated towards the anterior end of the cell, and the outer nuclear membrane is continuous with the CER, i.e., chloroplast endoplasmic reticulum. Haptophytes also have endoplasmic reticulum, the peripheral ER, lying just beneath

immediately around the flagella. In scale-bearing species scales are produced in the Golgi body (Manton & Parke 1962; Jordan et al. 1995), which is often arranged in a fan-like structure perpendicular to the long axis of the cell. Heterococcolith calcification occurs in Golgi-derived vesicles, holococcolith calcification occurs on Golgi derived scales but appears to occur extracellularly (Young et al. 1999).

The standard classification of modern haptophytes was established by Parke & Green (*in* Parke & Dixon, 1976), who recognised four orders: Coccosphaerales (coccolith-bearing), Prymnesiales (non-coccolith-bearing haptophytes, haptonema well developed), Isochrysidales (haptonema diminutive, including some coccolith-bearing genera), and Pavlovales (with flagella of unequal lengths, the longer flagellum with hairs and scales, haptonema diminutive). Green & Jordan (1994), however, argued that only the Pavlovales were an unambiguously well-differentiated group and recommended subsuming the other three orders into the single subclass Prymnesiophycidae, order Prymnesiales.

Young & Bown (1997a,b) and Bown & Young (1997) provided a comprehensive classification of extant and fossil coccolithophores including 46 families (2 new). They considered that following Green & Jordan (1994) and including all these in the single order Prymnesiales would inhibit description of affinities and so used 12 orders (3 new), including 6 with extant members, but with a clear understanding that the number of orders could be reduced as new information on relationships became available. A slightly revised version of this classification was used by Cros & Fortuno (2002) in their monograph of extant Mediterranean coccolithophores.

Edvardsen et al. (2000) published the most recent phylogeny of the Haptophyta, based on new molecular data and a review of cytological characters. They raised Pavlovales and Prymnesiales to class level (classes Pavlovophyceae and Prymnesiophyceae) because the 6% divergence in the 18S rRNA gene between the groups was consistent with the amount of divergence found in other algal groups at the class level. At the ordinal level, roughly 1-2% differences are noted for the dinoflagellates (Saunders et al. 1997) and for the green algae (Friedel 1996). We find similar levels among the haptophyte orders.

The molecular data of Edvardsen et al. (2000) also allowed identification of four clades within the Prymnesiophyceae at this level of differentiation, three rather closely corresponding to the traditional orders, Prymnesiales, Coccosphaerales, and Isochrysidales and a fourth including the numerous species of the distinctive genus *Phaeocyctis*. Consequently they reinstated the traditional orders and introduced a new order, Phaeocystales. Their sample set did not include any members of the additional four orders of coccolithophores with extant members recognised by Young & Bown (1997b); the Syracosphaerales, Rhabdosphaerales, Zygodiscales and Stephanolithales.

In the class Prymnesiophyceae, the homodynamic or heterodynamic flagella are usually equal to sub-equal. The mature flagellum of several species autofluoresces (Kawai & Inouye 1989). Members usually have organic, fundamentally plate-like scales, which may become complex (Leadbeater 1994 and references therein). In the coccolithophores, the outer layer of scales are calcified and called coccoliths. The identification of haptophyte algae to species level relies heavily on scale and coccolith morphology. In the unmineralised genera, scale morphology has not been used as a taxonomic character above the species level, but in the coccolithophores, all taxon descriptors are dependent on coccolith morphology and structure (e.g., Deflandre 1952; Braarud et al. 1955; Heimdal 1993; Jordan & Kleijne 1994, Bown & Young 1998). A key reason why this is possible and successful is that calcite, unlike cellulose or silica, is an anisotropic crystalline substance, the biomineralisation and shape regulation of which is a significant biochemical problem. This both means that coccolith ultrastructure is more conservative and characteristic than is obvious from the superficial appearance of the coccoliths and that crystallographic orientation provides further key characters (Romein 1979, Young et al. 1992, 1999). One consequence of this is that the primarily palaeontological

might be expected, indeed in some cases more successful than biological classifications based on examination of whole coccospheres; separation of the Noelaerhabdaceae from the Coccolithaceae was for instance made by palaeontologists 20 years before it was accepted by biologists. Complex life cycles involving haploid and diploid generations have been hypothesised and documented for many of the coccolithophores as well as many *Chrysochromulina* spp.

In contrast to members of the Prymnesiophyceae, the Pavlovophyceae have strongly anisokont, heterodynamic flagella with a short haptonema. Autofluorescent flagella are unknown. The longer, anterior flagellum is often adorned with a covering of fine hairs and knob-like bodies, which may be either modified scales (Green 1980) or modified hairs (Cavalier-Smith 1994). Because plate-like body-scales are absent, species identification in the Pavlovophyceae is primarily based on the morphology of the knob-like bodies. Stigmata (eyespots) are found within the chloroplast in several species, but are often not associated with an overlying flagellum, as in some heterokont algae.

X.3 DESCRIPTION OF METHODS

X.3.1 Cultures

A list of the cultured taxa used in this study is presented in Table 1; the majority of these cultures were established as part of the EU Codenet Project. 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 17 ºC under white fluorescent light with a quantum flux of 50-100 µmol photons m-2 s-1 and a 12:12 h light : dark cycle. Cultures were harvested by filtration or centrifugation.

Table 1. . A list of the species used in the rRNA tree. Cultures of these species can be found at CCMP at Bigelow Maine or at http://www.nhm.ac.uk/hosted_sites/ina/CODENET/caencultures.htm

Division Haptophyta Hibberd ex Edvardsen et Eikrem

Class Pavlovophyceae Cavalier-Smith, 1986 Green et Medlin

Order Pavlovales Green

Family Pavlovaceae Green

Species: *Diacronema vlkianum, Exanthemachrysis gayraliae, Rebecca salina, Pavlova gyrans, P. virescens, P.* sp. (‘*pseudogranifera’*), *P. lutheri, Pavlova* sp. CCMP 1416, *Pavlova* sp. CCMP1394, *Pavlova* sp.

Class Prymnesiophyceae (Hibberd) Cavalier-Smith

Order Phaeocystales Medlin **CLADE A**

Family Phaeocystaceae Lagerheim

Genus*: Phaeocystis jahnii, P. cordata, P. globosa, P. antarctica, P. pouchetii, Phaeocystis* sp. PLY 559

New Order **CLADE E**

OLI16010, OLI51080, OLI26047, OLI51076

Order Prymnesiales (Papenfuss) Edvardsen et Eikrem **CLADE B**

Family Prymnesiaceae Conrad ex. O.C. Schmidt, **CLADE B2**

Species: *Imantonia rotunda, Imantonia japonica, Imantonia* sp. CCMP1404, *Chrysochromulina* sp. CCMP 1204; *Prymnesium calathiferum, P. annuliferum, P. faveolatum, Prymnesium* sp. (‘mediteranneum’), *P. nemamethecum, P. parvum, P. patelliferum, P. zebrinum, Prymnesium* sp. (‘tunis’), *Prymnesium* sp., *Platychrysis pigra, P. simplex, P. pienaarii, Chrysochromulina polylepis, C. polylepis* CCMP 200, *C. chiton, C. kappa, C. minor, C. herdelensis, C. hirta, C. ericsonia, C. fraga, C. brevifilum*

New Family

New genus OLI33056, OLI51059

New genus *Chrysochromulina spinifera*

New Family CLADE B1

Species: *Chrysochromulina* cf. *eppiphyra, C. scutellum, C. strobilis, C. campanulifera, C. cymbium, C. simplex,* OLI16029, OLI51102, OLI26017, OLI 16108, *C. leadbeaterii, C. parva, C. acantha, C. thronsendii, C. rotalis, Chrysochromulina* sp. 1

New Family

New genus OLI33056, OLI51059

New genus *Chrysochromulina spinifera*

Family Isochrysidaceae (Bourrelly) Edvardsen et Eikrem

Species: *Isochrysis galbana, I. littoralis,*

Pseudoisochysis paradoxa, Dicrateria sp. 1,

Chrysotila lamellosa

Family Noelaerhabdaceae Jerkovic

Species: *Emiliania huxleyi, Gephyrocapsa oceanica*

Order Coccolithales (E. Schwarz) Edvardsen et Eikrem **CLADE D**

New family CLADE F

OLI 26041, OLI51050

New Family (suggest new family if species position remains stable in the Coccolithales)

Genus: *Chrysochomulina parkeae*

Family Pleurochrysidaceae Fresnel et Billard

Species: *Pleurochrysis scherffelii, P. carterae, P. carterae*

v. *dentata, Pleurochrysis* sp., *P. placolithoides,*

P. elongata, P. roscoffensis, P. pseudoroscoffensis,

P. gayraliae, Pleurochrysis sp. 1, *Pleurochrysis* sp. 2 CCMP 875

Pleurochrysis sp. 3 CCMP 300

Family Coccolithaceae Poche

Species: *Coccolithus pelagicus, Cruciplacolithus neohelis,*

Family *Calcidiscaeae Young & Bown*

Species: *Oolithotus fragilis,*

Calcidiscus leptoporus v. *leptoporus, C. leptoporus* v.

quadriperforatus, Umbilicosphaera sibogae v. *sibogae,*

U. sibogae v. *foliosa, U. hulburtiana*

Family Reticulosphaeraceae Cavalier Smith

Genus: *Reticulosphaera japonensis** NB *Reticulosphaera* Grell is a non-calcifying protist very different from any known haptophyte, DNA sequences obtained by Cavalier Smith 19xx from cultures of *Reticulosphaera* are unquestionably of haptophye origin but we suspect a haptophyte contaminant may have been present in the culture.

Family Hymenomonadaceae Senn

Species*: Jomonlithus littoralis,*

Ochrosphaera neapolitana, O. verrucosa,

Ochrosphaera sp., *Hymenomonas globosa, H. coronata,*

Family Rhabdosphaeraceae Haeckel

Genus: *Algirosphaera robusta*

Family Helicosphaeraceae Black

Species: *Helicosphaera carteri* v. *carteri*

Family Pontosphaeraceae Lemmermann

Species: *Scyphosphaera apsteinii*

Family Syracosphaeraceae Lemmerman

Species: *Syracosphaera pulchra, Coronosphaera mediterranea*

Holococcoliths *incertae sedis*

Calytrosphaera sphaeroidea, Holococcolithophore sp. 1,
Helladosphaera sp. 1

Here we follow Edvardsen et al. (2000) in classifying all coccolithophores in two orders, the Isochrysidales and Coccolithales, with a diverse set of families in the Coccolithales. This has the advantage of maintaining (rightly or wrongly) the 1-2% molecular divergence between the orders of the haptophytes as in other algal groups where a molecular classification has been presented. In fact, our sample set does not allow us to test robustly the monophyly of the recently proposed additional orders, and a final decision as to whether to subdivide the Coccolithales will depend in part on further molecular analysis and if more species become available in culture.

X.3.2 DNA extraction and PCR-Amplification

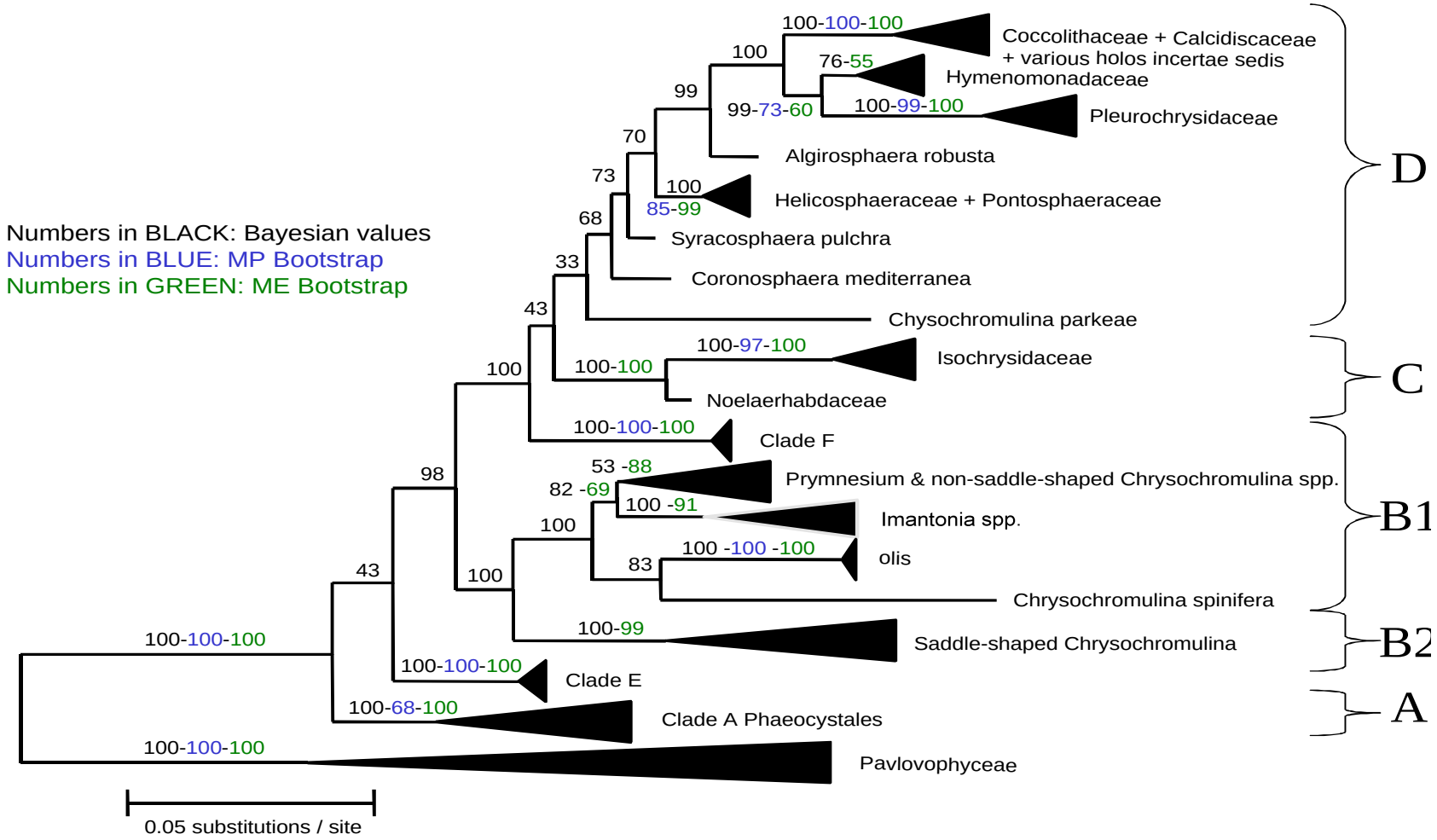
Total nucleic acids were extracted using a modified CTAB extraction (Doyle & Doyle 1987) and served as a template for amplification of the 18S rRNA gene following Medlin et al. (1988) or Chesnick et al. (1997). Most of the coccolithophorid sequences, as well as many others, were obtained with state of the art sequencing technology that resulted in fully resolved sequences (no ambiguities). What follows only refers to this last group of sequences; for the others, further description of methods has been published by Edvarsen et al.(2000) and references therein. The remaining PCR products were directly sequenced using a solid phase sequencing method with radioisotopes (Chesnick et al. 1997) or cycle-sequenced (Sequi-therm, BIOZYM) using infra-red-labelled primers and analyzed with the LiCor automated sequencer (MWG), whereas others were cloned (LigAtor, R&D Systems) prior to automated sequencing or were gel-purified before solid phase sequencing (Potter et al. 1996). The commerically sequenced amplified DNAs were cleaned with a Qiaquick PCR Purification kit (Qiagen). Sequences from the PCR templates (employing both DNA strands) were obtained using an ABI 377 sequencer (Applied Biosystems) and dye terminator cycle sequencing kits (Perkin-Elmer Co.; reactions run by Seqlab). Four oligonucleotides were used as sequencing primers of each template: those that were also used in the amplification reactions, 1F: 5'-aacctggttgatcctgccagt, and 1528R: 5'-tgatccttctgcaggttcacctac from Medlin et al. (1988), plus two internal ones, F743: 5'-tgggataatgaaataggac, and R783: 5'-cccctaactttcgttcttg. The quality of electropherograms were checked when editing sequences of DNA, and revised for confirmation of newly observed substitutions. Xesee 3.2 software (Eric Cabot) was used to align the sequences manually during collection and edition, and electropherograms were viewed with the Chromas 1.45 program (Conor McCarty).

X.3.3 Phylogenetic analyses

Sequences were manually aligned in an algal database containing over 300 published and unpublished chlorophyll 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. 2000). A final data set of 127 sequences was used for phylogenetic analyses with the brown alga *Fucus* and the dinoflagellate *Crypthecodinium* as outgroups pruned from the tree. A total of 1712 nucleotides were used for the data analysis of which 465 were considered informative for

and Crandall 1998; Sáez and Medlin 2002) to determine the model of evolution that best fit the data set. This program selected the General Time Reversal model (Rodríguez et al. 1990), with a gamma distribution and allowing for invariant positions. The parameters for base variation, gamma, and number of invariant positions were then loaded into PAUP* (Swofford 2000) which was used to run the distance (minimum evolution) analyses, where distances where calculated by maximum likelihood. Maximum parsimony analyses were implemented with PAUP* as well. This procedure was carried out by weighting substitution types. Weight assignations were performed with MacClade 3.04 software (Maddison and Maddison 1992) using default options. The substitution types were weighted inversely to their observed frequency on an unweighted maximum parsimony tree. In all our analyses introduced gaps were treated as missing data. Stability of monophyletic groups in weighted maximum parsimony and distance trees was estimated with a bootstrap analysis (500 replicates) (Felsenstein 1985). We also ran a Bayesian search for the trees with "higher posterior probabilities" (Huelsenbeck and Ronquist 2001). We ran that search using the General Time Reversible model with an undefined gamma distribution, during 2 million generations, and saving every 100th tree. We discarded the first two thousand trees, and repeated this process two times more, collecting 54,000 high-posterior-probability trees in total, which were then used to construct a consensus tree. On this tree (Fig. 1), "credibility values" for each clade are shown, which represent the percentage, out of those 54,000 trees, having the corresponding clades.

Fig X.1. . Phylogenetic tree based upon a Bayesian analysis showing the relationships of haptophyte taxa. The tree is rooted on the branch leading to *Fucus* and *Crypthecodinium*, which have been pruned from the tree. For discussion of Clades A - F, see text. Maximum parsimony and minimum evolution analyses produced relatively similar trees (not shown). Bootstrap values (500 replications) are represented at internal nodes for values > 50% for maximum parsimony/minimum evolution analyses, respectively.



All bayesian clade-support values for this tree are shown, but BT values present only when > 50%.

X.4 DESCRIPTIONS OF PHYLOGENETIC CLADES

A phylogenetic reconstruction of the haptophyte algae based on nucleotide sequences of the 18S rRNA gene is presented in Fig. 1. We have collapsed the tree into higher taxonomic levels for ease of viewing the relationships among the families, orders, and classes. A list of all taxa in the tree and their assignment to higher taxa represented in the tree can be found in Table 1. The tree presented is the consensus tree from the Bayesian analysis, and bootstrap values from the MP and ME analysis are also plotted onto the tree.

All analyses recovered a major split in the haptophyte algae corresponding to its two classes: the Prymnesiophyceae and Pavlovophyceae. In the class Pavlovophyceae, van Lenning et al. (2003) have shown that there are three clades within the class, which can be defined by morphological and pigment features. *Exanthemachrysis* is the basal divergence followed by *Rebecca salina*. Then there is a split in the group with true *Pavlova* spp. forming one clade (this clade is defined as 'true' *Pavlova* because it contains the type species *P. gyrans*), sister to a clade containing *Diacronema* and *Pavlova* species with different stigamata and pyrenoids from those in the true *Pavlova* spp. clade. For example, in *P. lutheri* and *Diacronema vlkianum*, 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 specialised swelling on the shorter flagellum (Green & Hibberd 1977; Green 1980).

In contrast to the Pavlovophyceae, motile cells of the Prymnesiophyceae usually have their flagella apically inserted. 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 (*Imantonia rotunda* and *Emiliania huxleyi*). The morphological and ultrastructural features of taxonomic importance for the prymnesiophycean species include cell shape, features of the haptonema (e.g., length, ability to coil, number of microtubules in the emergent part, presence of scales), scale investment (e.g., scale form, calcification, presence or absence of under-layer scales, presence or absence of resistant base plates, and presence of a continuous outer investment or 'skin'), pyrenoid type, flagellar apparatus (e.g., presence or absence of compound roots and a cytoplasmic tongue, the number of microtubules in the sheet of the R1 root of the mature flagellum, and the presence or absence of helical structures in the flagella) (Edvardsen et al. 2000).

In the class Prymnesiophyceae, several major clades may be recognised (Fig. 1). These clades can be recognised at the order level in the taxonomy of the Haptophyta. (1) Clade A = Phaeocystales; (2) Clade B = Prymensiales; (3) Clade C = Isochrysidales; and (4) Clade D = Coccolithales. In addition, a series of sequences from a gene clone library obtained from oligotrophic Pacific waters are present (Clade E and Clade F, see Moon et al. 2000). The morphology of members of these clades is unknown but oligonucleotide probes have been made to these clades in the hope of retrieving the cells in a FISH hybridization format. Clade E represents a group of taxa with some affiliation to *Phaeocystis* spp.. Clade F is at the base of the Coccolithales and Isochrysidales. Because these are clone library sequences we cannot comment on anything other than their size. All of the OLI clones are from the picoplankton fraction of natural samples, so these cells must be smaller than 2 µm. Either these are very small haptophytes or we have picked up gametes or zoospores in the water sample. Clearly these clades represent novel haptophyte taxa.

X.4.1 CLADE A - Phaeocystales

The order Phaeocystales contains, according to our molecular data, at least six distinct species. *Phaeocystis scrobiculata* 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, P. antarctica,* and *P. pouchetii*. Gene clone OLI51004 is included in this clade and is most closely related to *P. globosa*, but is not identical (Moon et al. 2000). The unicellular warm water *Phaeocystis jahnii*, *P. cordata,* and an undescribed *Phaeocystis* sp. PML 559 are basal in the Phaeocystales and give rise to the colonial species. *Phaeocystis* likely arose as a warm water unicellular genus, which later diversified into colonial species that spread into both polar regions (Lange et al. 2002).

X.4.2 CLADE B - Prymnesiales

Usually, the non-motile and flagellate cells of the Prymnesiales are scale- and/or coccolith-covered (except for *Dicrateria*, which lacks both). The ornamentation of both structures may vary from simple to very elaborate.

The genera belonging to the Prymnesiales (Clade B) can be divided into two sub-groups, Clade B1 and Clade B2 in the rRNA tree (Fig. 1, also the same in Edvardsen et al. 2000), and they correspond to Clade 1 & Clade 2 in the analysis of *Chrysochromulina* spp. by Simon et al. (1997). Both groups are well supported in all analyses and can be a defined by the shape of the cell and its pyrenoid and the flagellar root structure. Species of *Chrysochromulina* fall into both clades, and thus this genus must be considered paraphyletic (Simon et al. 1997; Inouye 1997). It has previously been recognised on morphological grounds that *Chrysochromulina* is not a natural group (Birkhead & Pienaar 1995) and a revision of the genus is underway (Eikrem et al. Unpubl.). Clade B1 contains *Imantonia* spp. as sister to a clade including certain non-saddle shaped *Chrysochromulina* spp. and all *Prymnesium* spp. This clade should correspond to the Family Prymnesiaceae. Within a sub-clade B1, the position of the clone library taxa (OLI51059 and OLI51033 + OLI51056) is sister to *Chrysochomulina spinifera*, another flagellate with a distinct morphology (Fournier, 1971). Clade B2 contains only *Chrysochromulina* spp. that are saddle shaped, including the type of the genus, *Chrysochromulina parva*. This clade should be given a new family name, but retain the generic name *Chrysochromulina*.

X.4.3 CLADES C, D, & F

There is strong support in Bayesian analysis (Fig. 1) for the clade containing all taxa bearing calcified scales, providing strong support for the hypothesis that calcification has only arisen once in the evolution of the Haptophyta, an observation also inferred from biomineralisation characteristics (Young et al. 1999). These include Clade F, and the orders Isochrysidales and Coccolithales, respectively. Here we are assuming that Clade F represents another group of coccolithophorid algae because their removal from the tree increases the support for the combination of the Isochrysidales and the Coccolithales. The branching order of these three groups is not stable. We cannot be certain whether or not this is an artifact of taxon sampling, but the removal of *Chrysochormulina parkeae* makes a more stable tree.

CLADE F

This clade originates from the clone library samples and has an unknown morphology.

CLADE C – Isochrysidales

This clade includes members of the families Isochrysidaceae (*Isochrysis*) and Noelaerhabdaceae (*Emiliania* and *Gephyrocapsa*). The relationship of *Isochrysis* to *Emiliania* and *Gephyrocapsa* has been noted previously, and on the basis of possession of a vestigial haptonema, these three genera, plus *Imantonia*, *Dicrateria*, *Pseudoisochrysis* and *Chrysotila* were included in the Isochrysidales by Parke and Dixon (1976, see also comments by Green & Pienaar 1977). The validity of this character was questioned by Green & Jordan (1994) and molecular data has indeed shown that *Imantonia* is a member of the Prymnesiales (Edvardsen et al. 2000). However, the molecular evidence of Edvardsen et al. (2000) strongly supported separation of the other genera so they reinstated the order Isochrysidales. Young and Bown (1997b) independently argued, on grounds of coccolith ultrastructure, that the Noelaerhabdaceae should be placed in a separate order, also including the fossil family Prinsiaceae. Their classification was exclusively based on coccolith morphology they introduced a new order, the Prinsiales, for these families. Because the molecular data has supported the reinstatement the order Isochrysidales, this name has priority over Prinsiales. The four genera of the Isochrysidales produce long-chain saturated alkenones and these have not been identified in any other haptophytes (see references in Jordan & Chamberlain 1997, Stoll et al. this vol.), so this appears to be a unique feature for the order. As noted above, calcification appears to have evolved only once in the haptophytes, in which case it must be inferred that in the Isochrysidaceae calcification has been lost secondarily.

CLADE D - Coccolithales

The clade including the Coccolithales is less well supported (68%). It contains a diverse set of coccolithophores all of which have well-developed base-plate scales and one non-calcifying flagellate *Chrysochromulina parkae*. *C. parkae* falls at the base of Clade D and has a flagellar root structure most similar to the Coccolithales (W. Eikrem pers. comm.). Thus, this species is closely related to the ancestral state from which all coccolithophores evolved. As mentioned above, if this taxon is removed from the tree, the support for the Coccolithales increasing significantly. Also, in some analyses *C. parkeae* falls into clade B1 of the Prymnesiales, so its correct position in the tree is not stable.

At the apex of the clade is a very well supported sub-clade consisting of the Hymenomonadaceae, Pleurochrysidaceae, Coccolithaceae, Calcidiscaceae and several poorly characterised holococcoliths The other members of clade D are divided into several sub-clades, which correspond more or less into groups at the family level. The Hymenomonadaceae and Pleurochrysidaceae form discrete sister clades, which agrees well with predictions from cytology and ecology; they are both coastal groups, with non-calcifying haploid phases in the life cycle. Coccolith structure is somewhat different: the Hymenomonadaceae have rather simple coccolith structures relative to the Pleurochrysidaceae. A more in-depth discussion of the relationships of the taxa within this clade will appear in a subsequent publication.

The other members of clade D are all coccolithophores for which there was no previous molecular data and which were of very uncertain relationships. They belong to five genera and four families, and in the classification of Young & Bown (1997b), to three orders. In general, the very low sampling makes it difficult to draw strong conclusions and we note that many bootstrap values are rather low and that different types of phylogenetic analyses (not shown) produced different relationships between these taxa. A revision of the classification would therefore be premature, nonetheless several valuable observations can be made.

1. Clearly all of these taxa occupy an intermediate position between the Isochrysidales and Coccolithales (*sensu* Young & Bown 1997b). This was a robust result from all of our analyses. In some analyses they formed a separate clade, in others, as here, a paraphyletic grouping in the stem of the clade including the Coccolithales. A likely interpretation of

the variable results is that the divergence between these groups dates back to the Early Jurassic radiation of the coccolithophores described by Bown (1987).

2. *Helicosphaera carteri* and *Scyphosphaera apsteinii* are sister taxa in all analyses. This is an interesting result; their coccoliths have very different shapes and they are classified in separate families, the Helicosphaeraceae and Pontosphaeraceae. However, the coccoliths do share various structural characteristics and on stratophenetic grounds these two families have both been inferred to have evolved from the extinct family Zygodiscaceae in the Palaeogene (Romein 1977, Aubry 1989). Young & Bown (1997b) placed these families in the order Zygodiscales.

3. The two species of the Syracosphaeraceae, *Syracosphaera pulchra* and *Coronosphaera mediterranea* cluster together, although in different clades. This is a species-rich family so further analyses are likely to resolve this, however, it is worth noting that *C. mediterranea* is an atypical member of the Syracosphaeraceae in terms of coccolith structure so it would not be entirely surprising if it proved to be phylogenetically distinct from the true Syracosphaeraceae.

4. *Algirosphaera robusta*, the only representative of the Rhabdosphaeraceae cultured to date (Probert et al. in prep.) appears to be closely related to the crown group of the clade. However, in terms of coccolith structure and cytology it shows more affinities to the Syracosphaeraceae.

X.5 CONCLUSIONS

It is encouraging to note that in general the molecular data available for the Haptophyta supports the systematic schemes based on traditional morphological information. The uniqueness of some of the clades from the gene clone library (Clades E & F) 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, even coccolithophores 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 in Table 1. More complete checklists of genera in each family can be found in Jordan & Green (1994) and Young & Bown (1997b). Their inclusion in each family is based on morphological and ultrastructural evidence. The detailed, revised and formal descriptions of the orders and families, other than the Coccolithales, confirmed by molecular analysis can be found in Edvardsen et al. (2000) and in Eikrem et al. unpubl. For coccolithophores, a formal original publication with the new reported DNA sequences is under preparation (Sáez et al. in prep.).

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