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Bente Edvardsen^a, Wenche Eikrem^{a,b}, Jahn Throndsen^a, Alberto G. Sáez^c, Ian Probert^d & Linda K. Medlin^e

^a University of Oslo, Department of Biology, Marine Biology, NO-0316 Oslo, Norway

^b Norwegian Institute for Water Research, Gaustadalléen 21, NO-0349 Oslo, Norway

^c Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, 28006 Madrid, Spain

^d CNRS/UPMC, FR2424, Station Biologique de Roscoff, BP74 29682 Roscoff cedex, France

^e University of Pierre and Marie Curie/CNRS, Laboratoire Arago, 66651 Banyuls-sur-mer, France

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Ribosomal DNA phylogenies and a morphological revision provide the basis for a revised taxonomy of the Prymnesiales (Haptophyta)

BENTE EDVARDSEN¹, WENCHE EIKREM^{1,2}, JAHN THRONDSSEN¹, ALBERTO G. SÁEZ³, IAN PROBERT⁴ AND LINDA K. MEDLIN⁵

¹University of Oslo, Department of Biology, Marine Biology, NO-0316 Oslo, Norway

²Norwegian Institute for Water Research, Gaustadalléen 21, NO-0349 Oslo, Norway

³Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, 28006 Madrid, Spain

⁴CNRS/UPMC, FR2424, Station Biologique de Roscoff, BP74 29682 Roscoff cedex, France

⁵University of Pierre and Marie Curie/CNRS, Laboratoire Arago, 66651 Banyuls-sur-mer, France

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Nucleotide sequences of the nuclear-encoded small subunit (18S rDNA) and partial large subunit (28S rDNA) ribosomal DNA were determined in 30 different species of the haptophyte genera *Prymnesium*, *Chrysocampanula*, *Chrysochromulina*, *Imantonia* and *Platyochrysis*, all belonging to the order Prymnesiales. Phylogenies based on these and other available haptophyte 18S, 28S and plastid 16S rDNA sequences were reconstructed, and compared with available morphological and ultrastructural data. The rDNA phylogenies indicate that the genus *Chrysochromulina* is paraphyletic and is divided into two major clades. This is supported by ultrastructural and morphological data. There is a major split between *Chrysochromulina* species with a saddle-shaped cell form (clade B2) and the remaining species in the genus (clade B1). Clade B2 includes the type species *C. parva* and taxa belonging to this clade thus retain the name *Chrysochromulina*. The non-saddle-shaped *Chrysochromulina* species analysed are closely related to *Hyalolithus*, *Prymnesium* and *Platyochrysis* species. *Imantonia* species are sister taxa to these species within clade B1. An amendment to the classification of the order Prymnesiales and the genera *Prymnesium*, *Platyochrysis* and *Chrysochromulina* is proposed with one new and one emended family (Chrysochromulinaceae and Prymnesiaceae, respectively), two new genera (*Haptolina* and *Pseudohaptolina*), and one new species (*Pseudohaptolina arctica*). We suggest a revision of the taxonomy of the Prymnesiales that is in accordance with available molecular evidence and supported by morphological data.

Key words: Chrysochromulinaceae, Haptophyta, phylogeny, phytoplankton, Prymnesiales, *Pseudohaptolina arctica*, ribosomal DNA, taxonomy

Introduction

The haptophyte order Prymnesiales Papenfuss *sensu* Edvardsen & Eikrem currently contains one family, the Prymnesiaceae Conrad *ex* O.C. Schmidt, which at present comprises the genera *Chrysocampanula* R.O. Fournier, *Chrysochromulina* Lackey, *Corymbellus* J.C. Green, *Imantonia* N. Reynolds, *Hyalolithus* M. Yoshida, T. Nakayama & I. Inouye, *Platyochrysis* Geitler *ex* Gayral & Fresnel and *Prymnesium* Massart (Jordan *et al.*, 2004). Most members of this order are unicellular, planktonic and scaly biflagellates, although colonial flagellates (e.g. *Corymbellus* species) occur, and some have benthic filaments or amoeboid stages in their life cycle

(see reviews by Hibberd, 1980; Green *et al.*, 1990). Most inhabit marine or brackish waters, but a few *Chrysochromulina* and *Prymnesium* species occur in fresh water (Preisig, 2003). Some species (e.g. of *Chrysochromulina* and *Prymnesium*) may form blooms, occasionally harmful to fish and other biota (reviewed by Moestrup, 1994; Edvardsen & Paasche, 1998; Edvardsen & Imai, 2006). All known species are photosynthetic, but mixotrophy appears to be common in some genera (e.g. *Chrysochromulina* and *Prymnesium*: Kawachi *et al.*, 1991; Nygaard & Tobiesen, 1993; Jones *et al.*, 1994; Tillmann, 1998), and uptake of dissolved organic carbon may also occur (Pintner & Provasoli, 1968).

The genus *Chrysochromulina* was erected by Lackey (1939) with the description of *C. parva*, one of only four freshwater species in the genus

Correspondence to: Bente Edvardsen. E-mail: bente.edvardsen@bio.uio.no

(Preisig, 2003). In the 1950s, Parke and colleagues (Parke *et al.*, 1955, 1956, 1958, 1959) described a series of marine *Chrysochromulina* species, and since then many others have been added (see e.g. Leadbeater, 1972; Hällfors & Niemi, 1974; Estep *et al.*, 1984; Moestrup & Thomsen, 1986; Kawachi & Inouye, 1993; Eikrem & Moestrup, 1998). At present, 57 *Chrysochromulina* species have been formally described. Fifty-four were listed by Jordan and colleagues (Jordan *et al.*, 2004), to which can be added *C. papillata* Y. Gao, C.K. Tseng & Y. Guo (Gao *et al.*, 1993), *C. planisquama* X.Y. Hu, M.Y. Yin & C.K. Tseng (Hu *et al.*, 2005), and *C. palpebralis* (Seoane *et al.*, 2009). Several others await formal description (Jensen, 1998; LeRoi & Hallegraeff, 2004; Marchant *et al.*, 2005), and the true number of species may exceed 100 (Thomsen *et al.*, 1994). Recent environmental clone library studies revealed the likely existence of hundreds of taxa within the current confines of the genus *Chrysochromulina* (Moon-van der Staay *et al.*, 2000; Liu *et al.*, 2009).

Ten species of *Prymnesium* were listed by Jordan *et al.* (2004). Of these, six have been described in detail and can be delineated by scale morphology and DNA sequences (*P. annuliferum*, *P. calathiferum*, *P. faveolatum*, *P. nemamethecum*, *P. parvum* and *P. zebrinum*), to which can be added *P. lepalleurii* Fresnel (Probert & Fresnel, 2007). Five additional species have been reported in the literature (*P. czosnowskii* Starmach, *P. gladiociliatum* (Büttner) R.W. Jordan & J.C. Green, *P. minutum* N. Carter, *P. papillatum* Jiao-Fen Chen & C.K. Tseng and the type species *P. saltans* Massart), but these have not been well characterized by electron microscopy (EM) or molecular genetics. Type material of *P. saltans* no longer exists and was never studied using EM. Reports of this species (e.g. Wang & Wang, 1992) have been scarce since its description by Massart (1920) and it has been suggested that *P. parvum* is identical to *P. saltans* (e.g. Moestrup, 1994; Guo *et al.*, 1996).

Although the number of species in both of these genera is likely to increase as new taxa are described, it may also be reduced as organisms formerly believed to be different species are found to be life-cycle stages. The two forms of *P. parvum*, f. *parvum* and f. *patelliferum*, were previously recognized as separate species, but are now considered to be stages in the life cycle of the same species (Larsen & Medlin, 1997; Larsen & Edvardsen, 1998; Larsen, 1999). *Chrysochromulina polylepis* also has an alternate stage with distinct scales (Paasche *et al.*, 1990; Edvardsen & Paasche, 1992), which is similar in overall cell morphology and ultrastructure as well as having identical gene

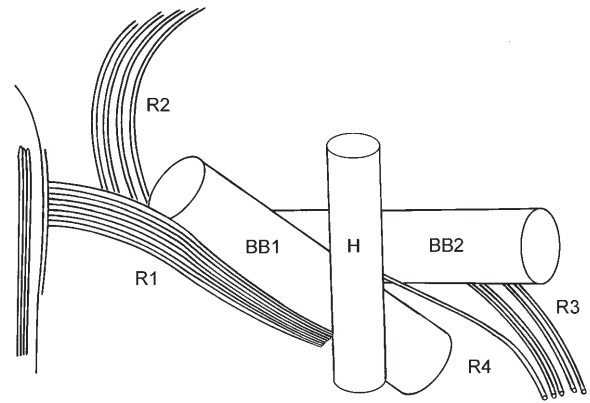


Fig. 1. Drawing of the simple flagellar apparatus in *Chrysochromulina scutellum* showing the positions of the microtubular roots R1–R4, haptonemal base (H) and the basal bodies 1 and 2 (BB1 and BB2). Modified from Eikrem & Moestrup (1998).

sequences (Edvardsen *et al.*, 1996; Edvardsen & Medlin, 1998) to the authentic cell type described by Manton & Parke (1962). Flow cytometric analysis has documented the presence of two ploidy levels in *C. polylepis* (Edvardsen & Vaultot, 1996) and in four other *Chrysochromulina* species (Edvardsen, 2006), suggesting that heteromorphic life cycles may be common in the Prymnesiales.

Members of the order Prymnesiales possess two smooth flagella that are equal or unequal in length, but similar in form (Green & Hori, 1994). An organelle unique to haptophytes, termed the haptonema (Parke *et al.*, 1955; Inouye & Kawachi, 1994), is located between the flagella. The haptonema differs structurally from the flagella, usually containing six or seven microtubules in the Prymnesiales, but sometimes eight as in *Chrysochromulina kappa* (Manton & Leedale, 1961a). The length of the haptonema varies greatly, from being many times the cell diameter in some species of *Chrysochromulina* (e.g. 160 μm long in *C. camella*: Leadbeater & Manton, 1969a) and coiling, to short and non-coiling in *Prymnesium* species (Green *et al.*, 1982), and even absent in *Imantonia* (Green & Pienaar, 1977). The flagellar apparatus typically consists of two basal bodies, the base of the haptonema, microtubular (simple or compound) roots (R1–R4), fibrous roots ('cytoplasmic tongue'), and accessory and connecting fibres (Green & Hori, 1994; Jordan *et al.*, 1995; Inouye, 1997; Billard & Inouye, 2004). The microtubular root R1, which is connected to the basal body of the mature or left flagellum (BB1), is present in all prymnesial species hitherto examined. R1 may be either simple (a sheet of microtubules; Fig. 1), or compound (a sheet and a crystalline bundle of microtubules; Fig. 2), and its structure varies within

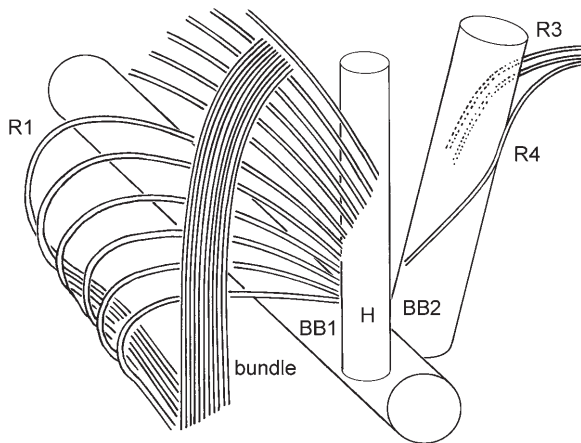


Fig. 2. Drawing of the flagellar apparatus in *Prymnesium palpebrale*, *comb. nov.* (syn. *Chrysochromulina palpebralis*) including a compound R1 flagellar root with the crystalline bundle of microtubules. R2 not shown. Abbreviations as in Fig. 1. Modified from Birkhead & Pienaar (1995a).

genera as they are presently defined. The microtubular root R2, with its origin between the two basal bodies, is simple and comprises a few microtubules in all species examined so far (Billard & Inouye, 2004). The microtubular roots R3 and R4, associated with the right or immature flagellar base (BB2), contain only a few microtubules, which also appears to be the rule in other haptophytes (Green & Hori, 1994).

The two yellow-brown chloroplasts are enveloped by four chloroplast membranes and have lamellae composed of three thylakoids, as in most members of the Haptophyta. The prymnesialean chloroplasts have immersed or bulging pyrenoids traversed by thylakoids or tubes. The nucleus is posterior or central and the outer nuclear membrane is continuous with the outer chloroplast membrane. The large Golgi body, in which scales are produced, lies anterior to the nucleus and just beneath the basal bodies, and is composed of a single fan-shaped dictyosome with many cisternae. The cisternae can be dilated, as in *Chrysochromulina chiton* (Manton, 1967a; Hibberd, 1976, 1980; Pienaar & Birkhead, 1994).

There are no clear-cut morphological or ultrastructural features that distinguish existing genera within the Prymnesiales. The main character used for species identification within genera is the morphology and ornamentation of body scales, features that can usually be seen only by using the electron microscope. The cell surface of all prymnesialean species is covered by one to several layers of these scales. The basic scale is a round or oval plate composed of microfibrils. In many species, the proximal side consists of microfibrils arranged in a radial pattern, whereas the pattern of the distal side is more variable. The scales consist of organic

substances, mainly proteins and carbohydrates (Leadbeater, 1994 and references therein). In *Hyalolithus neolepis*, one scale type of the non-motile form undergoes intracellular silicification (Yoshida *et al.*, 2006). *Chrysochromulina parkeae* J.C. Green & B. Leadbeater has scales that may be slightly calcified (R. Andersen, pers. comm., in Sáez *et al.*, 2004), and may be allied with coccolith-bearing species (Sáez *et al.*, 2004, Edvardsen & Medlin, 2007). The length of the haptonema relative to either the flagellar length or cell body diameter, the cell size and form, and the swimming behaviour are additional features used in species identification that can be seen under the light microscope (LM). In addition, some species have large conspicuous scales that are visible by LM. Early on, Parke and co-workers recognized the great morphological variation within the genus *Chrysochromulina*, but were reluctant to erect new genera on characters that could only be seen in the electron microscope (Parke *et al.*, 1955). Suggestions that the genus *Chrysochromulina* is an artificial grouping have also been based on ultrastructural studies (Birkhead & Pienaar, 1995a), 18S rDNA (Medlin *et al.*, 1997; Edvardsen *et al.*, 2000; Edvardsen & Medlin, 2007) and *rbcL* DNA sequence analysis (Inouye, 1997; Fujiwara *et al.*, 2001).

The aim of this study was to reconstruct the phylogeny of the Prymnesiales based on nuclear and plastid-encoded ribosomal DNA (18S, partial 28S, 16S and concatenated) sequences and available morphological and ultrastructural data, and to revise the taxonomy in accordance with these data, thereby attempting to identify synapomorphic and systematically useful morphological character states within this ecologically and economically important group of photosynthetic protists.

Materials and methods

Cultures

Twenty-one strains representing 14 prymnesialean species, presently maintained in the University of Oslo Culture Collection of Algae (UIO), were isolated from Norwegian coastal waters by the serial dilution method or by single cell capillary isolation (Table 1). Of these 11 were used in this study. Twenty-six additional strains of Prymnesiales were obtained from the Alcobank Caen Collection de Cultures de Microalgues – Université de Caen Basse-Normandie (ALGOBANK-CAEN, previously ALGO), Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP) and the Plymouth Culture Collection of Marine Algae (PCC) (Table 1). These originated mainly from North Atlantic coastal waters, but also from the Arctic, the Pacific, New Zealand and from freshwater lakes.

Table 1. Summary of strains and nucleotide sequences used in the present study. Accession numbers for the sequences from this study are in bold. n/a = not available. Old strain codes are in brackets.

| Species | Strain code | Culture collection | Collection site | Isolator, year | Accession no 18S rDNA | Accession no 28S rDNA | Accession no 16S rDNA |
|--|--------------|--------------------|---------------------|--------------------|--------------------------|----------------------------|--------------------------|
| Prymnesiales | | | | | | | |
| <i>Chrysocampanula spinifera</i> R.O. Fournier | PCC 328 | PCC | English Channel, UK | R. Jowett, 1964 | AM491022 | AM850679 | n/a |
| <i>Chrysochromulina acantha</i> B. Leadbeater & Manton | UIO024 (T20) | UIO | Skagerrak, Norway | B. Edvardsen, 1994 | AJ246278 | n/a | EF051758 |
| <i>Chrysochromulina acantha</i> B. Leadbeater & Manton | HAP 78 | ALGO | Atlantic France | J. Fresnel, 1994 | FN599059 | n/a | n/a |
| <i>Chrysochromulina acantha</i> B. Leadbeater & Manton | RCC339 | RCC | Atlantic Morocco | L. Garczarek | n/a | n/a | AY702152 |
| <i>Chrysochromulina acantha</i> B. Leadbeater & Manton | S-6 | RCC | Atlantic Spain | n/a | n/a | EU729437 | n/a |
| <i>Chrysochromulina brevifilum</i> Parke & Manton | PCC 143 | PCC | n/a | n/a | AM491012 | AM850680 | n/a |
| <i>Chrysochromulina brevifilum</i> Parke & Manton | S-3 | RCC | N Atlantic Spain | n/a | n/a | EU729456 | n/a |
| <i>Chrysochromulina camella</i> B. Leadbeater & Manton | CCMP289 | CCMP | N Atlantic Ocean | n/a | n/a | EU729440 | n/a |
| <i>Chrysochromulina camella</i> B. Leadbeater & Manton | RCC1185 | RCC | Atlantic France | J. Fresnel | n/a | EU729439 | n/a |
| <i>Chrysochromulina campanulifera</i> Manton & B. Leadbeater | J10 | UIO | Skagerrak, Norway | J. Throndsen, 1984 | AJ246273 | AJ876800 | EF051761 |
| <i>Chrysochromulina chiton</i> Parke & Manton | PCC 146A | PCC | England | M. Parke | AM491029 | n/a | n/a |
| <i>Chrysochromulina cymbium</i> B. Leadbeater & Manton | R18 | UIO | Skagerrak, Norway | W. Eikrem, 1992 | AM491018 | n/a | EF051760 |
| <i>Chrysochromulina cymbium</i> B. Leadbeater & Manton | UIO137 | EHU/UIO | Atlantic Spain | S. Seoane, 2003 | n/a | AM779761 | n/a |
| <i>Chrysochromulina ericina</i> Parke & Manton | UIO026 (Q17) | UIO | W Norway | W. Eikrem, 1989 | AM491030 | AM850681 | n/a |
| <i>Chrysochromulina ericina</i> Parke & Manton | CCMP 281 | CCMP | North Pacific | R. Waters, 1976 | =AM491030 | AM850682 | n/a |
| <i>Chrysochromulina fragaria</i> Eikrem & Edvardsen ¹ | UIO029 (S19) | UIO | Skagerrak, Norway | B. Edvardsen, 1994 | AM491013 | AM850683 | n/a |
| <i>Chrysochromulina</i> cf. <i>herdlensis</i> B. Leadbeater | CCMP 284 | CCMP | North Pacific | R. Waters, 1976 | AM491011 | AM850684 + EU729453 | EF051749 |
| <i>Chrysochromulina hirta</i> Manton | UIO030 (1Y) | UIO | W Norway | B. Edvardsen, 1989 | AJ246272 | n/a | EF051751 |
| <i>Chrysochromulina hirta</i> Manton | UIO134 | EHU/UIO | Atlantic Spain | S. Seoane, 2003 | n/a | AM779758 | n/a |
| <i>Chrysochromulina hirta</i> Manton | S-17 | RCC | Atlantic Spain | n/a | n/a | EU729454 | n/a |
| <i>Chrysochromulina kappa</i> Parke & Manton | UIO033 (EN3) | UIO | Oslofjord, Norway | W. Eikrem, 1989 | AJ246271 | AM850685 | n/a |

(continued)

Table 1. Continued.

| Species | Strain code | Culture collection | Collection site | Isolator, year | Accession no 18S rDNA | Accession no 28S rDNA | Accession no 16S rDNA |
|---|----------------|--------------------|---------------------|--------------------|-----------------------|-----------------------|-----------------------|
| <i>Chrysochromulina kappa</i> Parke & Manton | CCMP 288 | CCMP | Main, USA | M. Keller, 1986 | n/a | AM850686 | n/a |
| <i>Chrysochromulina leadbeateri</i> Estep, P.G. Davis, Hargraves & Sieburth | UIO035 (ERIK) | UIO | Lofoten, N. Norway | W. Eikrem, 1991 | AM491017 | AM850687 | n/a |
| <i>Chrysochromulina leadbeateri</i> Estep, P.G. Davis, Hargraves & Sieburth | TJE | UIO | Lofoten, N. Norway | W. Eikrem, 1991 | =AM491017 | n/a | EF051759 |
| <i>Chrysochromulina minor</i> Parke & Manton | PCC 304 | PCC | English Channel, UK | n/a | AM491010 | n/a | n/a |
| <i>Chrysochromulina palpebralis</i> Seoane, Eikrem, Edvardsen & Pienaar | UIO133 | EHU/UIO | Atlantic Spain | S. Seoane, 2003 | AM779755 | AM779756 | n/a |
| <i>Chrysochromulina parva</i> Lackey | CCMP 291 | CCMP | Lake Colorado, USA | P. Kugrens | AM491019 | n/a | |
| <i>Chrysochromulina polylepis</i> Manton & Parke | B152j | UIO | Oslofjord, Norway | B. Edvardsen, 1988 | n/a | n/a | AF172719 |
| <i>Chrysochromulina polylepis</i> Manton & Parke | A | UIO | Oslofjord, Norway | E. Paasche, 1988 | n/a | n/a | ≈AF172719 |
| <i>Chrysochromulina polylepis</i> Manton & Parke | UIO036 (B11) | UIO | Oslofjord, Norway | B. Edvardsen, 1988 | AJ004866 | n/a | n/a |
| <i>Chrysochromulina polylepis</i> Manton & Parke | UIO037 (B1511) | UIO | Oslofjord, S Norway | B. Edvardsen, 1988 | n/a | AM850688 | n/a |
| <i>Chrysochromulina</i> aff. <i>polylepis</i> Edvardsen & Eikrem | PCC 200 | PCC | English Channel, UK | I. Adams, 1958 | AJ004868 | AM850689 | EF051752 |
| <i>Chrysochromulina rotalis</i> Eikrem & Thronsen ² | UIO045 (P16) | UIO | Skagerrak, Norway | W. Eikrem, 1990 | AM491025 | n/a | n/a |
| <i>Chrysochromulina rotalis</i> Eikrem & Thronsen ³ | UIO044 (TH2) | UIO | Skagerrak, Norway | W. Eikrem, 1990 | =AM491025 | FN599061 | n/a |
| <i>Chrysochromulina scutellum</i> Eikrem & Moestrup | UIO046 (G7) | UIO | Skagerrak, Norway | W. Eikrem, 1990 | AJ246274 | n/a | n/a |
| <i>Chrysochromulina scutellum</i> Eikrem & Moestrup | HAP72 | ALGO | Atlantic France | C. Billard, 1993 | =AJ246274 | n/a | n/a |
| <i>Chrysochromulina simplex</i> Estep, P.G. Davis, Hargraves & Sieburth | UIO047 (JomfB) | UIO | Oslofjord, Norway | W. Eikrem, 1989 | AM491021 | AM850690 | EF051754 |
| <i>Chrysochromulina simplex</i> Estep, P.G. Davis, Hargraves & Sieburth | UIO136 | EHU/UIO | Atlantic Spain | S. Seoane | n/a | AM779760 | n/a |
| <i>Chrysochromulina strobilus</i> Parke & Manton | PCC 43 | PCC | N. Atlantic | n/a | FN599060 | n/a | n/a |
| <i>Chrysochromulina thronsenii</i> Eikrem | UIO049 (K11) | UIO | Skagerrak, Norway | W. Eikrem, 1989 | AJ246279 = AJ246277 | AJ876799 | EF051755 |

| | | | | | | | |
|---|--------------|---------|------------------------|------------------------------|------------------|---------------------------|----------|
| <i>Chrysochromulina thronsenii</i> Eikrem | UIO048 (L12) | UIO | Skagerrak, Norway | W. Eikrem, 1989 | AJ246277 | n/a | n/a |
| <i>Chrysochromulina thronsenii</i> Eikrem | UIO135 | EHU/UIO | Atlantic Spain | S. Seoane, 2003 | n/a | AM779759 | n/a |
| <i>Chrysochromulina</i> sp. | CCMP 1204 | CCMP | Arctic | R. Selvin, 1989 | AM491016 | AM850692 | n/a |
| <i>Chrysochromulina</i> sp. | MBIC10513 | MBIC | Japan | n/a | AB199882 | n/a | n/a |
| <i>Chrysochromulina</i> sp. | NIES 1333 | NIES | Japan | n/a | n/a | DQ980468 | n/a |
| <i>Chrysochromulina</i> sp. | MBIC10518 | MBIC | Japan | n/a | AB058358 | n/a | AB196967 |
| <i>Chrysochromulina</i> sp. | MBIC10513 | MBIC | Japan | n/a | n/a | n/a | AB200263 |
| <i>Chrysochromulina</i> sp. | MBIC10516 | MBIC | Japan | n/a | n/a | n/a | AB196966 |
| <i>Chrysochromulina</i> sp. | clone 124 | n/a | Taiwan | n/a | n/a | n/a | DQ187908 |
| <i>Imantonia rotunda</i> N. Reynolds emend. J.C. Green & Pienaar | UIO101 | UIO | Skagerrak, Norway | B. Edvardsen, 1994 | AJ246267 | AM850693 | EF051762 |
| <i>Imantonia rotunda</i> N. Reynolds emend. J.C. Green & Pienaar | UIO138 | EHU/UIO | Atlantic Spain | S. Seoane, 2003 | n/a | AM779757 | n/a |
| <i>Imantonia rotunda</i> | RCC305 | RCC | Mediterranean Sea | S. Boulben | n/a | n/a | AY702150 |
| <i>Imantonia rotunda</i> | RCC406 | RCC | English Channel | F. Le Gall | n/a | n/a | AY702162 |
| <i>Imantonia</i> sp. | MBIC10497 | MBIC | Japan | n/a | n/a | n/a | AB196965 |
| <i>Imantonia</i> sp. | CCMP 1404 | CCMP | Maine, USA | M. Keller | AM491015 | AM850694 | n/a |
| <i>Hyalolithus neolepis</i> M. Yoshida, M.-H. Noël, T. Nakayama, Naganuma & I. Inouye | NIES 1393 | NIES | Japan | n/a | AB183265 | n/a | n/a |
| <i>Hyalolithus neolepis</i> M. Yoshida, M.-H. Noël, T. Nakayama, Naganuma & I. Inouye | RCC1453 | RCC | Mediterranean Sea | I. Probert | n/a | FJ696922 | n/a |
| OLI 16029 | n/a | n/a | Pacific | n/a | AF107080 | n/a | n/a |
| OLI 16108 | n/a | n/a | Pacific | n/a | AF107082 | n/a | n/a |
| OLI 26017 | n/a | n/a | Pacific | n/a | AF107083 | n/a | n/a |
| OLI 51033 | n/a | n/a | Pacific | n/a | AF107087 | n/a | n/a |
| OLI 51056 | n/a | n/a | Pacific | n/a | AF107087 | n/a | n/a |
| OLI 51059 | n/a | n/a | Pacific | n/a | AF107089 | n/a | n/a |
| OLI 51102 | n/a | n/a | Pacific | n/a | AF107092 | n/a | n/a |
| <i>Platyochrysis pienzaarii</i> Gayral & Fresnel | HAP50bis | ALGO | France | J. Fresnel, 1978 | AM491027 | EU729451 | n/a |
| <i>Platyochrysis pigra</i> Geitler | HAP83 | ALGO | n/a | M.J. Chrétiennot-Dinet, 1980 | AM491003 | AM850695 +EU729458 | n/a |
| <i>Platyochrysis simplex</i> Gayral & Fresnel | HAP51bis | ALGO | Thailand | J. Fresnel, 1981 | AM491028 | n/a | n/a |
| <i>Platyochrysis</i> sp. | RCC1385 | RCC | Mediterranean Sea | J. Fresnel | n/a | EU729452 | n/a |
| <i>Prymnesium annuliferum</i> Billard | HAP47 | ALGO | Atlantic France | C. Billard, 1980 | AM491007 | FN599062 | n/a |
| <i>Prymnesium calathiferum</i> F.H. Chang & K.G. Ryan | CCMP 707 | CCMP | Northland, New Zealand | F. Chang, 1983 | AM491008 | EU729444 | n/a |
| <i>Prymnesium calathiferum</i> F.H. Chang & K.G. Ryan | HAP53bis | ALGO | Atlantic France | J. Fresnel, 1990 | =AM491008 | AM850696 | n/a |
| <i>Prymnesium faveolatum</i> Fresnel | HAP79 | ALGO | Atlantic France | J. Fresnel, 1996 | AM491005 | AM850701 | n/a |
| <i>Prymnesium nemamethecum</i> Pienaar & Birkhead | South Africa | S.A. | S. Africa | R. Pienaar | AJ246268 | AJ876798 | n/a |

(continued)

Table 1. Continued.

| Species | Strain code | Culture collection | Collection site | Isolator, year | Accession no 18S rDNA | Accession no 28S rDNA | Accession no 16S rDNA |
|--|--------------------|--------------------|-------------------|-------------------------|-----------------------|----------------------------|-----------------------|
| <i>Prymnesium parvum</i> f. <i>patelliferum</i> (J.C. Green, D.J. Hibberd & Pienaar) A. Larsen | PCC 527 | PCC | S. England | D.J. D.J. Hibberd, 1976 | L34670/71 | n/a | n/a |
| <i>Prymnesium parvum</i> f. <i>patelliferum</i> (J.C. Green, D.J. Hibberd & Pienaar) A. Larsen | CCMP 1926 | CCMP | Maine, USA | R. Guillard, 1997 | n/a | AM850697 | n/a |
| <i>Prymnesium parvum</i> f. <i>patelliferum</i> (J.C. Green, D.J. Hibberd & Pienaar) A. Larsen | R1pat93 | UIB | W. Norway | A. Larsen, 1993 | n/a | AJ876795 | n/a |
| <i>Prymnesium parvum</i> f. <i>parvum</i> N. Carter | K-0081 | SCCAP | Flade Sø, Denmark | T. Christensen | AJ246269 | AJ876802 | n/a |
| <i>Prymnesium parvum</i> f. <i>parvum</i> N. Carter | CCMP 1927 | CCMP | Maine, USA | R. Guillard, 1999 | n/a | AM850698 | n/a |
| <i>Prymnesium parvum</i> f. <i>parvum</i> N. Carter | CCMP 2941 | CCMP | Lake, Russia | R. Andersen, 2005 | n/a | AM850699 | n/a |
| <i>Prymnesium zebrinum</i> Billard | HAP29 | ALGO | Atlantic France | C. Billard, 1977 | AM491001 | AM850700 + EU729448 | n/a |
| <i>Prymnesium zebrinum</i> Billard | RCC1432 (HAP29bis) | RCC | Atlantic France | C. Billard, 1977 | n/a | EU729447 | n/a |
| <i>Prymnesium</i> sp. | HAP_Pt | ALGO | n/a | n/a | AM491000 | n/a | n/a |
| <i>Prymnesium</i> sp. | RCC1440 (HAP27bis) | RCC | Mediterranean Sea | J. Fresnel | n/a | EU729449 | n/a |
| Other haptophytes: | | | | | | | |
| <i>Coccolithus pelagicus</i> ssp. <i>pelagicus</i> (G.C. Wallich) J. Schiller | IBV 73 | ALGO | | | AJ544117 | n/a | n/a |
| <i>Cruciplacolithus neohelis</i> (McIntyre & Bé) P. Reinhardt | CCMP 298 | CCMP | | | AJ246262 | n/a | n/a |
| <i>Cruciplacolithus neohelis</i> (McIntyre & Bé) P. Reinhardt | RCC1206 | RCC | | | n/a | EU729467 | n/a |
| <i>Dicrateria inornata</i> Parke | CCMP 355 | CCMP | | | n/a | n/a | EF051750 |
| <i>Emiliana huxleyi</i> (Lohmann) W.W. Hay & H. Mohler B morphotype | PCC 92D | PCC | | | M87327 | n/a | n/a |
| <i>Emiliana huxleyi</i> (Lohmann) W.W. Hay & H. Mohler | BOF | UIO | | | n/a | n/a | EF051753 |
| <i>Emiliana huxleyi</i> (Lohmann) W.W. Hay & H. Mohler | RCC1251 | RCC | | | n/a | EU502880 | n/a |
| <i>Gephyrocapsa oceanica</i> Kamptner | PCC 574 = GO1 | PCC | | | AJ246276 | n/a | n/a |
| <i>Isochrysis galbana</i> Parke emend. J.C. Green & Pienaar | UIO102 | UIO | | | AJ246266 | n/a | n/a |
| OLI 16010 | n/a | n/a | | | AF107081 | n/a | n/a |
| OLI 26041 | n/a | n/a | | | AF107084 | n/a | n/a |
| OLI 26047 | n/a | n/a | | | AF107085 | n/a | n/a |
| OLI 51004 | n/a | n/a | | | AF107086 | n/a | n/a |

| | | | | | |
|---|----------|------|----------|----------|-----|
| OLI 51050 | n/a | n/a | AF107088 | n/a | n/a |
| OLI 51076 | n/a | n/a | AF107090 | n/a | n/a |
| OLI 51080 | n/a | n/a | AF107091 | n/a | n/a |
| <i>Pavlova gyrans</i> Butcher <i>emend.</i> J.C. Green & Manton | CCMP 607 | CCMP | U40922 | n/a | n/a |
| <i>Phaeocystis antarctica</i> G. Karsten | SK 23 | AWI | X77481 | n/a | n/a |
| <i>Phaeocystis cordata</i> Zingone & Chrétiennot-Dinet | c2a1 | ZSN | AF163147 | n/a | n/a |
| <i>Phaeocystis globosa</i> Scherffel | SK 35 | AWI | X77476 | n/a | n/a |
| <i>Phaeocystis jahnii</i> Zingone | B5 | ZSN | AF163148 | n/a | n/a |
| <i>Phaeocystis pouchetii</i> (Hariot) Lagerheim | SK 34 | AWI | X77475 | n/a | n/a |
| <i>Pleurochrysis carterae</i> (Braarud & Fagerland) T.A. Christensen | HAP1 | ALGO | AJ544120 | n/a | n/a |
| <i>Pleurochrysis carterae</i> (Braarud & Fagerland) T.A. Christensen | RCC1418 | RCC | n/a | EU819084 | n/a |
| <i>Pleurochrysis dentata</i> (J.R. Johansen & Doucette) Probert | HAP6 | ALGO | AJ544121 | n/a | n/a |
| <i>Pleurochrysis elongata</i> (Droop) R.W. Jordan | CCMP 874 | CCMP | AJ246264 | n/a | n/a |
| <i>Rebecca salina</i> (N. Carter) J.C. Green | PCC 486 | PCC | L34669 | n/a | n/a |

¹*Chrysochromulina fragaria* is *C. sp. 2* in Simon *et al.* (1997).

²*Chrysochromulina rotalis* strain P16 is *C. sp. 3* in Simon *et al.* (1997).

³*Chrysochromulina rotalis* strain TH2 is *C. sp. 1* in Simon *et al.* (1997).

Cultures of these strains were generally grown in IMR 1/2 medium (Eppley *et al.*, 1967), supplemented with 10 nM selenium, in a 12:12 h light:dark cycle at a photon flux rate of 50–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Cultures from the ALGOBANK culture collection were grown as in Probert & Houdan (2004). Cultures were harvested by filtration or centrifugation (20 min, 7000 rpm, 4°C, RC-5B Sorvall Centrifuge).

PCR amplification and DNA sequencing

Total nucleic acids were extracted using a modified CTAB extraction (Doyle & Doyle, 1990) or by the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), and these served as the template for amplification of the 18S rDNA following Medlin *et al.* (1988), Chesnick *et al.* (1997), and Edvardsen & Medlin (1998). 28S rDNA was amplified as in Edvardsen *et al.* (2003). 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 analysed with a LICOR automatic sequencer (MWG, Everbat, Germany). Alternatively, purified PCR products were sequenced directly using the DYEnamic ET terminator Cycle sequencing kit (Amersham Biosciences, USA) according to the manufacturer's recommendations. The PCR fragments were then bidirectionally sequenced using the primers as described in Edvardsen *et al.* (2003) on a MEGABACE (Amersham Biosciences, Germany) automatic sequencing device at the Department of Biology, University of Oslo, Norway. Some templates were cloned (LigAator, R&D Systems, the Netherlands) prior to automatic sequencing. Amplification and sequencing of the partial 16S rDNA region of some of our strains were undertaken by S. McDonald (McDonald *et al.*, 2007).

Phylogenetic analyses

The rDNA sequences generated in this study and all publicly available nearly full-length 18S, and partial 28S and 16S rDNA sequences of cultured prymnesialean taxa were included, and then duplicates removed (Table 1). A set of 18S rDNA environmental sequences of oceanic picoplankton (Moon-Van der Staay *et al.*, 2000, Table 1) were also included to increase the number of taxa. Sequences of haptophyte taxa basal to Prymnesiales in previous phylogenetic studies (e.g. Edvardsen *et al.*, 2000; Medlin *et al.*, 2008) were selected as outgroups to root the trees. Four datasets were generated: 18S (63 taxa, 1838 characters), 28S (37 taxa, 1086 characters), 16S (22 taxa, 701 characters) and a concatenated dataset of the three genes, including taxa where both 18S and 28S rDNA sequences were available (30 taxa, 3625 characters), with the 16S rDNA for 13 taxa (Table 1). The single gene datasets were aligned using MAFFT v6 Q-INS-I model (Katoh & Toh, 2008), considering secondary RNA structure (default parameters) followed by editing by eye in BioEdit v7.0.9 (Hall, 1999). The concatenated dataset was generated by combining the three alignments. The 28S

rDNA and concatenated alignments included hypervariable regions and were treated with Gblocks v0.91b (Castresana, 2000), under default parameters, to exclude poorly aligned positions from the phylogenetic inference, reducing the number of characters to 2758 (1669 in 18S, 389 in 28S, 700 in 16S) in the concatenated and 384 in the 28S rDNA datasets. All four datasets were then analysed with MODELTEST (Posada & Crandall, 1998) to establish the optimal model of nucleotide evolution; for all alignments the general time reversible model (GTR) was preferred for both the Akaike and Bayesian information Criterion (AiC and BiC). Maximum-likelihood (ML) analyses were performed using RAxML v.7.0.3 (Stamatakis, 2006). The general time reversible model with parameters accounting for invariable sites (I) and gamma-distributed (G) rate variation across sites with four discrete rate categories was used for all four rDNA datasets. The bootstrap analyses had 100 replicates for the ML analysis. Bayesian inference (BI) under the same evolutionary model was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). Two Markov Chain Monte Carlo (MCMC) runs each with four chains were performed for 5 000 000 generations, where the average standard deviation of split frequencies were <0.01. Trees were sampled every 100 generations. Bayesian posterior probabilities (PP) were calculated from the majority-rule consensus of the tree sampled after the initial burn-in phase. The MAFFT alignments were performed online at CBRC (www.mafft.cbrc.jp), the Gblock at Phylogeny.fr (www.phylogeny.lirmm.fr), and the Modeltest, ML and BI at BioPortal (www.bioportal.uio.no). The alignments are available as supplementary files (available via the Supplementary Content tab on the article's online page at <http://dx.doi.org/10.1080/09670262.2011.594095>).

Results and discussion

Molecular phylogenetics

This study produced 53 new sequences from 37 different strains representing 30 species in the haptophyte order Prymnesiales: 29 nearly complete 18S and 24 partial 28S rDNA sequences (Table 1). Bayesian and ML analyses recovered trees of almost identical topology. The four consensus Bayesian rDNA trees generated are shown in Fig. 3 (18S), Fig. 4 (28S), Fig. 5 (16S) and Fig. 6 (combined 18S, 28S and 16S = concatenated) using the new names proposed in this study. These four phylogenies are largely congruent with each other and subsequently we refer mainly to the 18S rDNA tree because it contains the most characters and taxa. The single gene partial 28S rDNA dataset, including only 384 characters, generated a weakly resolved phylogeny.

In our 18S rDNA tree (Fig. 3), clades are numbered according to our previous work (Edvardsen *et al.*, 2000) and newly recognized clades B1-1 to B1-6. The first divergence within the class

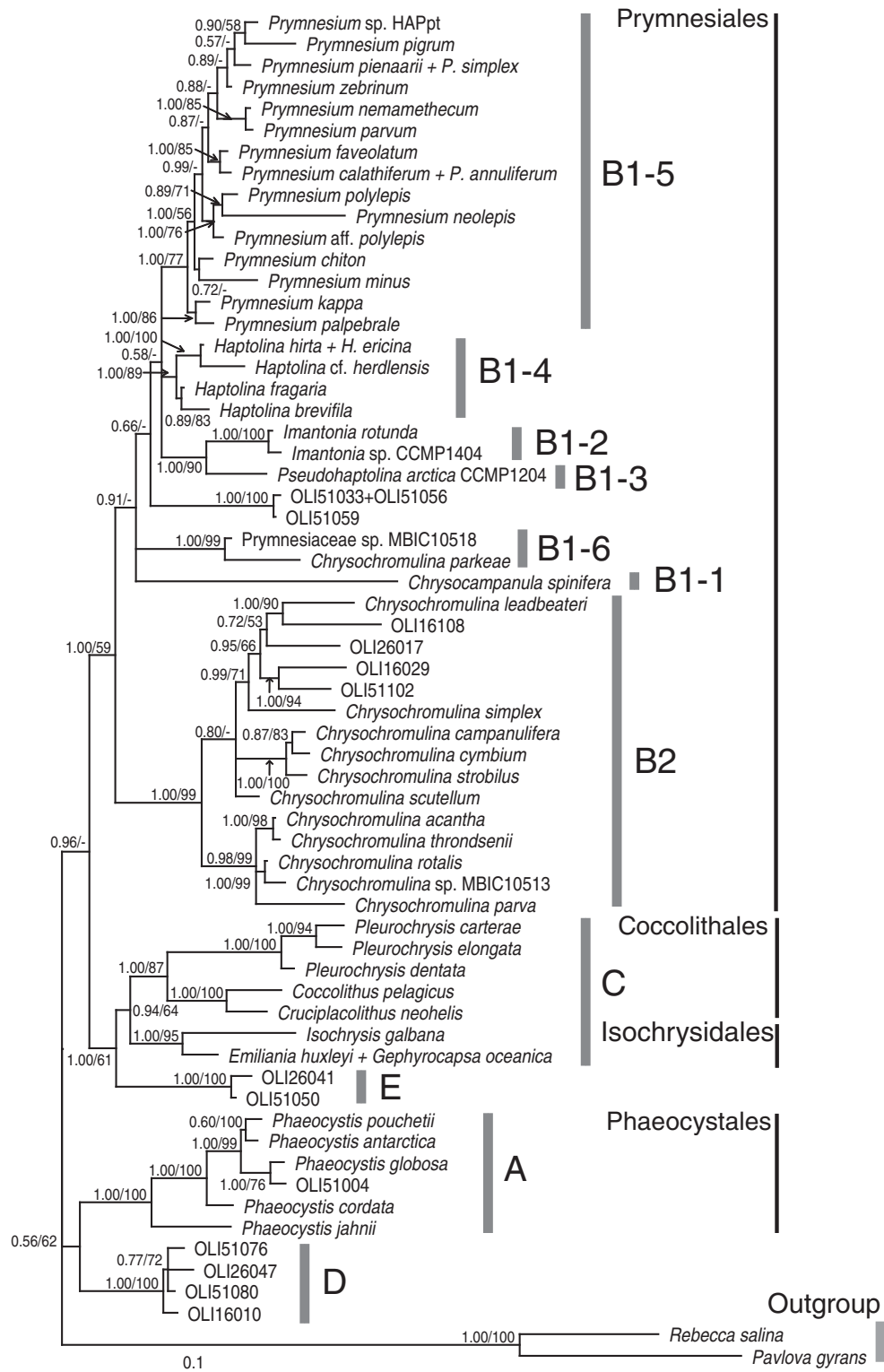


Fig. 3. Consensus Bayesian tree based on nuclear 18S ribosomal encoding DNA sequences of members of the Prymnesiophyceae. Posterior probability (left) and maximum-likelihood bootstrap values (right) $>0.5/50$ are shown above or below the branches. *Pavlova gyrans* and *Rebecca salina* (Pavlovophyceae) were used as outgroups. Scale bar represents number of substitutions/site.

Prymnesiophyceae (the ingroup) corresponds to the order Phaeocystales, clade A (see Lange *et al.*, 2002 for full description) plus clade D, consisting of some environmental sequences known only from a picoplanktonic clone library

originating from oligotrophic waters in the Pacific Ocean, here termed OLI sequences (Moon-Van der Staay *et al.*, 2000). Clade D is sufficiently genetically divergent to be recognized as a separate order of haptophyte algae once the

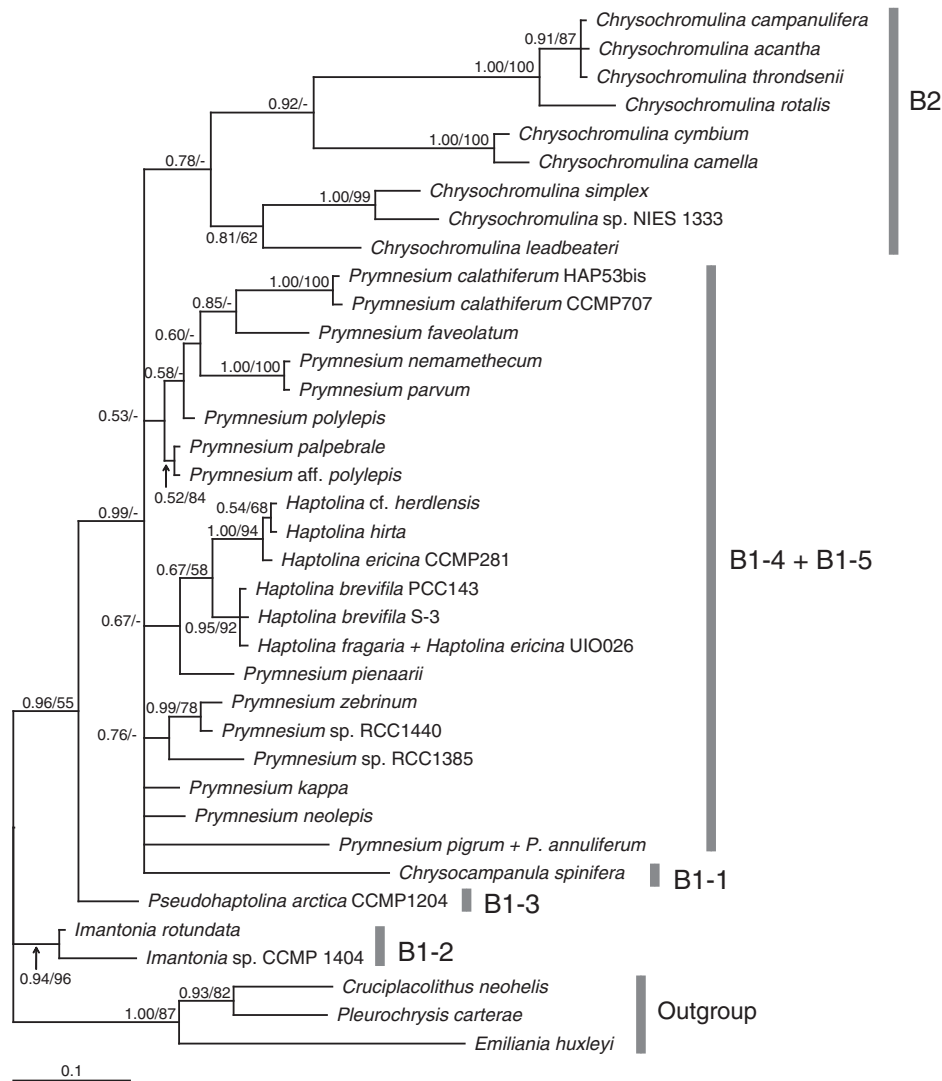


Fig. 4. Consensus Bayesian tree based on partial nuclear 28S ribosomal encoding DNA sequences of members of the Prymniales. Posterior probability (left) and maximum-likelihood bootstrap values (right) > 0.5/50 are shown above or below the branches. *Cruciplacolithus neohelis*, *Emiliana huxleyi* and *Pleurochrysis carterae* were used as outgroups. Scale bar represents number of substitutions/site.

morphology of these cells is known. The next major divergence is that of the order Prymniales, corresponding to clades B1 and B2, from the cluster of taxa in clades C and E. Clade C embraces the orders Coccolithales and Isochrysidales. Clade E here contains two picoplanktonic clone library OLI sequences found to group with a sequence of a newly described non-mineralized species *Chrysoculter rhomboideus* T. Nakayama, M. Yoshida, M.-H. Noël, M. Kawachi & I. Inouye in the phylogeny presented by Nakayama *et al.* (2005).

Prymniales

The divergences within the order Prymniales are the primary focus of this study. In Table 2, available morphological and ultrastructural data for the

prymnesial species analysed here are summarized. In the 18S rDNA Bayesian tree (Fig. 3) we find two distinct clades with high posterior probabilities (PP 0.91 and 1.00, respectively), termed B1 (consisting of several subclades) and B2. These two major clades are also recovered and strongly supported in previous SSU rDNA phylogenies (Medlin *et al.*, 1997; Simon *et al.*, 1997; Edvardsen *et al.*, 2000; Sáez *et al.*, 2004; Edvardsen & Medlin, 2007). This major divergence between clades B1 and B2 is also recovered in the 16S and concatenated rDNA phylogenies (Figs 5, 6, support values PP 0.7–1.0), but with an unresolved placement of *Chrysocampanula spinifera* (Fig. 6, see below). Species of the genus *Chrysochromulina* fall into both of these clades and our findings support the views of Birkhead & Pienaar (1995a), based on ultrastructural data, and

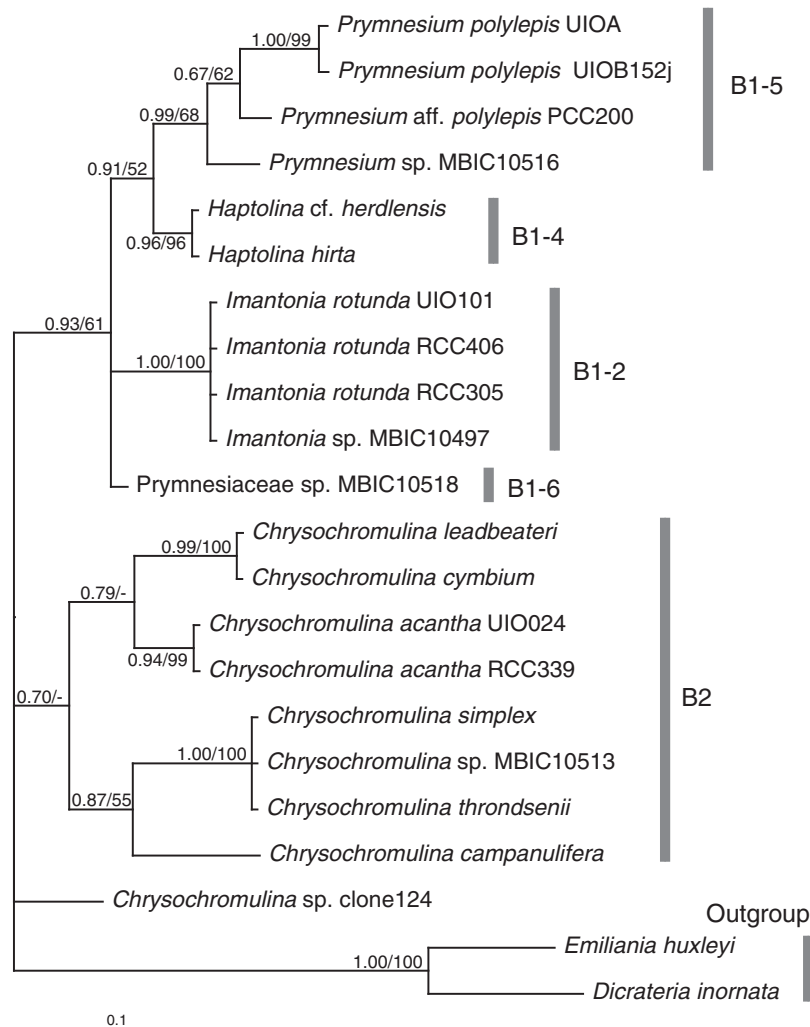


Fig. 5. Consensus Bayesian tree based on partial plastid 16S ribosomal encoding DNA sequences of members of the Prymnesiales. Posterior probability (left) and maximum-likelihood bootstrap values (right) $>0.5/50$ are shown above or below the branches. *Dicrateria inornata* and *Emiliania huxleyi* were used as outgroups. Scale bar represents number of substitutions/site.

of Inouye (1997), based on *rbcL* DNA sequence data, that the genus *Chrysochromulina* is not monophyletic.

Clade B2 – *Chrysochromulina*

Clade B2 contains the type species of the genus *Chrysochromulina*, *C. parva*, and therefore these species retain the name *Chrysochromulina*. All of the taxa in clade B2, except for *C. leadbeateri*, are allied by their basic cell shape and morphology (Fig. 7). This clade includes all of the saddle-shaped *Chrysochromulina* species examined here (*C. acantha*, *C. campanulifera*, *C. cymbium*, *C. leadbeateri*, *C. rotalis*, *C. simplex*, *C. scutellum*, *C. strobilus* and *C. thronsdensei*). Available data on the ultrastructure of the saddle-shaped species have been reviewed by Eikrem & Moestrup (1998). Along with a common shape, these species all have a very long, coiling haptonema with six or

seven microtubules in the free part. Their two flagella have both proximal and distal transitional plates, which is the case in all species of *Chrysochromulina* and *Prymnesium* where this character has been examined. All taxa in clade B2, except for *C. leadbeateri*, have flagella that are inserted subapically and ventrally. The flagellar root R1 is generally simple (only a sheet of microtubules with no crystalline bundle of microtubules) and contains relatively few microtubules (usually <10 , Fig. 1). One exception to this is *C. acantha*, which may have up to 20 microtubules in the R1 in a sheet (Table 2). The cells contain two chloroplasts with immersed pyrenoids traversed by tubes or thylakoids and are covered by many small, delicate scales that may be cup-shaped or have a relatively short spine.

Chrysochromulina leadbeateri is grouped together with the saddle-shaped species in the 18S rDNA tree (Fig. 3), albeit in a distinct lineage with

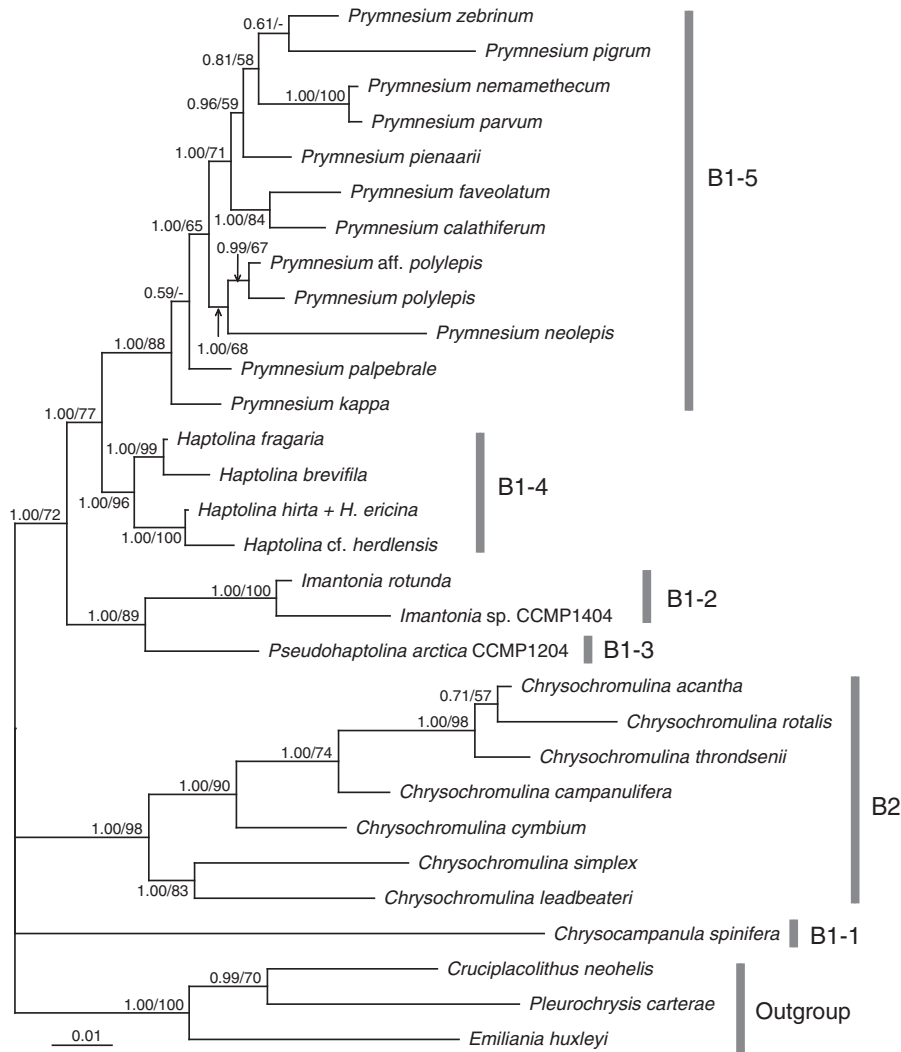


Fig. 6. Consensus Bayesian tree based on concatenated nuclear 18S and partial 28S, and plastid 16S ribosomal encoding DNA sequences of members of the Prymnesiales. Posterior probability (left) and maximum-likelihood bootstrap values (right) > 0.5/50 are shown above or below the branches. *Cruciplacolithus neohelis*, *Emiliania huxleyi* and *Pleurochrysis carterae* were used as outgroups. Scale bar represents number of substitutions/site.

four OLI sequences corresponding to taxa with unknown morphology. *Chrysochromulina simplex* is sister to this lineage in the 18S, 28S and concatenated rDNA trees (Figs 3, 4, 6). Although cells of *C. leadbeateri* may be round and have a coiled haptonema approximately equal in length to the flagella, and flagella that are inserted apically (Eikrem & Thronsen, 1998), it shares other features with the saddle-shaped species, such as immersed pyrenoids, six microtubules in the emergent part of its haptonema and a simple R1 flagellar root. *Chrysochromulina leadbeateri* is believed to constitute a species complex that exhibits some variations in scale and cell morphology, and some cells belonging to this complex possess the saddle-shape characteristic of clade B-2 and a long haptonema (Eikrem & Thronsen, 1998). We therefore suggest that the saddle-shaped cell form is a synapomorphy for clade B2 *Chrysochromulina* species,

but that it has been lost in one or more forms within the *C. leadbeateri* complex. The saddle shape has been reported in the calcified, non-photosynthetic genus *Ericiolus* H.A. Thomsen, which is only known from TEM whole-mounts of natural samples (Thomsen *et al.*, 1995). In our opinion, from the TEM images presented by Thomsen *et al.* (1995), the cells in the two species of *Ericiolus* correspond in shape more to the 'strawberry-shaped' cells of certain coccolithophores (e.g. *Syracosphaera*) than to the typical saddle shapes of clade B2 *Chrysochromulina* species. In addition, unlike the saddle-shaped *Chrysochromulina*, the two species of *Ericiolus* have a haptonema that is shorter than the flagella. *Chrysochromulina campanulifera*, *C. strobilus* and *C. cymbium* are grouped together in our 18S rDNA tree (Fig. 3). Cells of these species are covered by small cup-shaped scales, in addition to an

Table 2. Morphological and ultrastructural information on selected species from the order Prymnesiales. ND = no data; MT = microtubules.

| Species | Cell shape | Flagella | Haptonema (H) length relative to flagella (F) | Scales | Scale patterning | No. of MT in emergent part of haptonema | Flagellar microtubular roots | Flagella with proximal and/or distal plates | Flagella with helical band or other structures | Cytoplasmic tongue | Pyrenoids | 'Peculiar' golgi body | References |
|----------------------------------|-------------|------------------------|---|------------------------|---|---|------------------------------|---|--|--------------------|-----------------------------------|-----------------------|--|
| Clade B2 | | | | | | | | | | | | | |
| <i>Chrysochromulina acantha</i> | saddle | equal | H > F | plate, spine | loosely woven radial ribs overlaying concentric ribs | 7 | simple r1 (many MT) | proximal and distal plates | absent | absent | immersed | ND | Leadbeater & Manton, 1971; Gregson <i>et al.</i> , 1993 |
| <i>C. campanulifera</i> | saddle | equal | H >> F | plate, cup | radial pattern both faces | 6 | simple r1 | ND | absent | absent | immersed, traversed by thylakoids | present | Unpublished results |
| <i>C. campanulifera</i> | saddle | equal | H >> F | plate, cup | radial pattern both faces | 6 | ND | ND | ND | ND | immersed, traversed by thylakoids | ND | Manton & Leadbeater, 1974 |
| <i>C. cymbium</i> | saddle | equal | H >> F | plate, cup | radial pattern both faces | 6 | ND | ND | ND | ND | immersed | present | Parke <i>et al.</i> , 1959; Leadbeater & Manton 1969a |
| <i>C. leadbeateri</i> | spherical | equal–slightly unequal | H = F | plate (2 types) | radial ribs and concentric rings, central open ring with cross | 6 | simple r1 | proximal and distal plates | ND | ND | immersed, traversed by tubules | present | Eikrem & Throndsen, 1998 |
| <i>C. parva</i> | saddle | equal | H >> F | plate | loosely woven radial ribs overlaying concentric ribs | ND | simple r1 | ND | ND | ND | ND | ND | Lackey, 1939; Parke <i>et al.</i> , 1962; Moestrup & Thomsen, 1986 |
| <i>C. rotalis</i> | saddle | equal | H >> F | plate, spine | radial ribs proximal face, concentric fibrils distal face | ND | ND | ND | ND | ND | immersed | ND | Eikrem & Throndsen, 1999 |
| <i>C. scutellum</i> | saddle | equal | H >> F | plate, spine (2 types) | radial ribs proximal face, concentric fibrils distal face | 7 | simple r1 | proximal and distal plates | absent | absent | immersed, traversed by thylakoids | absent? | Eikrem & Moestrup, 1998 |
| <i>C. simplex</i> | saddle | equal | H >> F | plate | radial ribs proximal face, concentric fibrils distal face | 7 | simple r1 | proximal and distal plates | absent | absent | immersed, traversed by tubules | present | Estep <i>et al.</i> , 1984; Birkhead & Pienaar, 1995b |
| <i>C. strobilus</i> | saddle | equal | H >> F | plate, cup | radial pattern both faces | 6 | ND | ND | ND | ND | immersed | present | Parke <i>et al.</i> , 1959; Leadbeater & Manton 1969a, 1969b |
| <i>C. throndsenii</i> | saddle | equal | H >> F | plate (2 types) | radial ribs overlaying a spiralling rib, central open ring with cross | 6 | simple r1 | proximal and distal plates | absent | absent | immersed, traversed by tubules | absent | Eikrem, 1996; Eikrem, unpublished |
| Clade B1 | | | | | | | | | | | | | |
| <i>Chrysocampanula spinifera</i> | bell-shaped | unequal | H < F | plate, spine | radial ribs one face, fibrils other face | ND | ND | ND | ND | ND | ND | ND | Fournier, 1971; Pienaar & Norris, 1979 |
| <i>Haptolina brevifila</i> | spherical | equal | H < F | plate (2 types), spine | radial ribs overlaying concentric fibrils both faces | 7 | simple r1 (many MT) | proximal and distal plates | tubular rings | present | immersed, traversed by thylakoids | ND | Birkhead & Pienaar, 1994a |
| <i>H. ericina</i> | oval–oblong | equal | H > F | plate, spine | radial ribs one face, concentric fibers other face | 7 | ND | ND | ND | ND | immersed | present | Parke <i>et al.</i> , 1956; Manton & Leedale, 1961b |
| <i>H. herdensis</i> | conical | equal | H < F | plate (3 types) | radial ribs | ND | ND | ND | ND | ND | ND | ND | Leadbeater, 1972 |

(continued)

Table 2. Continued.

| Species | Cell shape | Flagella | Haptonema (H) length relative to flagella (F) | Scales | Scale patterning | No. of MT in emergent part of haptonema | Flagellar microtubular roots | Flagella with proximal and/or distal plates | Flagella with helical band or other structures | Cytoplasmic tongue | Pyrenoids | 'Peculiar' golgi body | References |
|--------------------------------------|---------------------------|-------------------------|---|--|--|---|---|---|--|--------------------|-----------------------------------|-----------------------|--|
| <i>H. hirta</i> | spherical-oblong | equal | H > F | plate, spine (2 types) | radial ribs, one face, fibrils other face | ND | ND | ND | ND | ND | ND | ND | Manton, 1978 |
| <i>H. fragaria</i> | spherical | equal | H < F | plate (2 types) | radial ribs overlaying concentric fibrils both faces | 7 | ND | ND | tubular rings | ND | immersed | ND | Eikrem & Edvardsen, 1999 |
| <i>Imantonia rotunda</i> | spherical | equal | Absent | plate (2 types) | radial ribs (few) some concentric ribs | no hapt. | simple r1 (possible vestige of crystal) | proximal and distal plates | ND | ND | immersed, traversed by thylakoids | ND | Reynolds, 1974; Eikrem, unpublished; Green & Pienaar, 1977; Green & Hori, 1986 |
| <i>P. annuliferum</i> | oblong | subequal | H << F | plate (2 types) | radial ribs one face, concentric fibrils other face | ND | ND | ND | ND | ND | ND | ND | Billard, 1983 |
| <i>P. calathiferum</i> | oblong-spherical | equal-subequal | H << F | plate (2 types) | radial ribs, both faces | ND | ND | ND | ND | ND | immersed | ND | Chang & Ryan, 1985 |
| <i>P. chiton</i> (PCC 146) | spherical-oblong | subequal-equal | H > F | plate (2 types) | radial ribs distal face, fibrils proximal face | 7 | ND | ND | ND | ND | bulging, traversed by thylakoids | present | Parke <i>et al.</i> , 1958; Manton, 1967a |
| <i>P. chiton</i> (Leeds isolate) | probably spherical-oblong | probably subequal-equal | Probably H > F | plate (2 types) | radial pattern both faces | 7 | ND | ND | ND | ND | bulging, traversed by thylakoids | present | Manton, 1966, 1967a, 1967b |
| <i>P. faveolatum</i> | oblong-very elongated | subequal | H << F | plate (2 types) | radial ribs, both faces | 7 | simple r1 (many MT) | ND | tubular rings | present | immersed, traversed by thylakoids | ND | Fresnel <i>et al.</i> , 2001 |
| <i>P. kappa</i> | spherical | equal | H < F | plate, spine | radial ribs proximal face, fibrils distal face | 7-8 | ND | ND | ND | ND | bulging | present | Parke <i>et al.</i> , 1955; Manton & Leedale, 1961a |
| <i>P. kappa</i> (Norwegian isolate) | spherical | equal | H < F | plate (2 types), spine | radial ribs proximal face, fibrils distal face | 7 | compound r1 | proximal and distal plates | ND | ND | bulging, traversed by thylakoids | present | Eikrem, unpublished |
| <i>P. minus</i> | spherical-oval | equal | H < F | plate (2 types) | radial pattern both faces | 7 | ND | ND | ND | ND | bulging, traversed by thylakoids | | Parke <i>et al.</i> , 1955; Manton & Leedale, 1961a; Eikrem <i>et al.</i> , 1998 |
| <i>P. nemamethecum</i> | oblong-spherical | equal-subequal | H << F | plate (2 types on body, 1 type on haptonema) | radial ribs both faces | 7 | compound r1 | proximal and distal plates | tubular rings | present (reduced) | bulging, traversed by thylakoids | present | Birkhead & Pienaar, 1994b; Pienaar & Birkhead, 1994 |
| <i>P. neolepis</i> (motile form) | subspherical, pear-shaped | equal | H ≤ F | spine (1 type) | perforated pores in radiating pattern | ND | ND | ND | ND | ND | ND | ND | Yoshida <i>et al.</i> , 2006 |
| <i>P. neolepis</i> (non-motile form) | spherical-subspherical | equal | H > F | plate (1 type) siliceous | radial and concentric ribs | 7 | compound r1 | proximal and distal plates | ND | ND | immersed, traversed by thylakoid | present | Yoshida <i>et al.</i> , 2006 |
| <i>P. neustophilum</i> | rounded or angular shape | subequal | H << F | ND | ND | ND | ND | ND | ND | ND | ND | ND | Norris, 1967 |
| <i>P. palpebrale</i> | oblong-asymmetrical | equal-subequal | H < F | plate (2 types), spine (1 type) | radial ribs both faces | 7 | compound r1 | proximal and distal plates | tubular rings | absent | immersed, traversed by thylakoids | ND | Birkhead & Pienaar, 1995a; Seoane <i>et al.</i> , 2009 |
| <i>P. parvum</i> f. <i>parvum</i> | oblong-spherical | equal-subequal | H << F | plate (2 types) | radial ribs distal face, fibrils proximal face | 7 | simple r1 (many MT) | proximal and distal plates | ND | ND | immersed | ND | Manton & Leedale, 1963; Manton, 1964 |

| | | | | | | | | | | | | | |
|--|---|----------------|--------|------------------------|--|----|--------------------------------|----------------------------|-----------------|---------|--|---------|--|
| <i>P. parvum</i> f. <i>patelliferum</i> | oblong-spherical | equal-subequal | H << F | plate (2 types) | radial ribs both faces | ND | simple r1 (many MT) | proximal and distal plates | ND | ND | immersed, traversed by thylakoids | ND | Green <i>et al.</i> , 1982; Green & Hori, 1990 |
| <i>P. pienaarii</i> | rounded or angular | subequal | H << F | plate (2 types) | radial ribs and concentric pattern | ND | compound r1 | ND | ND | ND | immersed, traversed by thylakoids | present | Gayral & Fresnel, 1983 |
| <i>P. pigrum</i> | motile cells spherical with apical depression | equal-subequal | H << F | plate (2 types) | radial ribs and concentric pattern | 7 | broad root >30 MT, compound r1 | ND | ND | ND | immersed, traversed by thylakoids, with stigma | present | Chrétiennot, 1973 |
| <i>P. polylepis</i> (alternate type) | oblong-spherical | equal | H < F | plate (2 types), spine | radial ribs both faces overlaying concentric fibrils | 7 | compound r1 | proximal and distal plates | other structure | present | immersed traversed by thylakoids | present | Manton & Parke, 1962; Eikrem unpublished |
| <i>P. polylepis</i> (authentic type) | oblong-spherical | equal | H < F | plate (4 types) | radial pattern both faces, perforations | 7 | compound r1 | proximal and distal plates | other structure | present | immersed traversed by thylakoids | present | Paasche <i>et al.</i> , 1990; Edvardsen <i>et al.</i> , 1996; Eikrem unpublished |
| <i>P. aff. polylepis</i> (PCC 200) | oblong-spherical | equal | H < F | plate (5 types) | radial ribs both faces | ND | ND | ND | ND | ND | ND | ND | Edvardsen & Medlin, 1998; Edvardsen & Eikrem, unpublished |
| <i>P. simplex</i> | rounded or angular | | H << F | plate (2 types) | radial ribs both faces | ND | compound r1 | ND | ND | ND | immersed, traversed by thylakoids | ND | Gayral & Fresnel, 1983 |
| <i>P. zebrinum</i> | oblong | subequal | H << F | plate (2 types) | radial ribs both faces | ND | ND | ND | ND | ND | ND | ND | Billard, 1983 |
| <i>Pseudohaptolina arctica</i> (CCMP 1204) | spherical | equal | H ≈ F | plate (2 types) | radial ribs both faces | ND | ND | ND | ND | ND | ND | ND | Eikrem & Edvardsen, 1999 |

inner layer of plate scales. *Chrysochromulina camella*, whose 18S rDNA sequence has not been analysed, clusters with *C. cymbium* in the 28S rDNA tree (Fig. 4), as expected from similarities to this group in body shape and scale morphology (Leadbeater & Manton, 1969a, 1969b). Ultrastructural data are missing for *C. cymbium*.

Chrysochromulina ahrengotii M.Ø. Jensen & Moestrup, *C. alifera* Parke & Manton, *C. apheles* Moestrup & H.A. Thomsen, *C. ephippium* Parke & Manton and *C. pontica* Rouchijajnen also have a saddle-shaped cell form, ventrally inserted flagella and a long, coiling haptonema and are expected to belong to clade B2.

Clades B1 and B1-1

Clade B1 is morphologically more heterogeneous than clade B2 and is also less robust in all trees (Figs 3–6). It contains members of the current genera *Chrysocampanula* (in the 18S rDNA tree), *Chrysochromulina*, *Hyalolithus*, *Imantonia*, *Platychrysis* and *Prymnesium*. We propose several taxonomic revisions within this clade and the proposed new names (for which see ‘Taxonomic recommendations’) are henceforth used in figures and tables and introduced in the text below. In the 18S rDNA tree, clade B1 is a polytomy of three lineages: the first (B1-1) is composed of *Chrysocampanula spinifera*, the second (B1-6) of *Chrysochromulina parkeae* and the third consists of three well-supported subclades (B1-2 + B1-3, B1-4, B1-5, all PP=1.0) and a clade of picoplanktonic OLI sequences. The placement of *Chrysocampanula spinifera* (B1-1) within the B1 clade is well supported by the 18S Bayesian analysis (PP>0.9), but the B1 clade is weakly supported in ML analysis of this gene. In the 28S rDNA (Fig. 4) and the concatenated (Fig. 6) trees the position of *C. spinifera* is unresolved. *Chrysocampanula spinifera* was transferred to *Chrysochromulina* by Pienaar & Norris (1979), but was moved back to *Chrysocampanula* by Jordan *et al.* (2004) based on the molecular data presented in Sáez *et al.* (2004). *Chrysocampanula spinifera* has bell-shaped cells covered by plate scales and conspicuous spine scales (Fig. 8). The haptonema is rigid and does not coil and is shorter than the heterodynamic and unequal flagella (Pienaar & Norris, 1979). The flagellar root R1 is probably compound (Kawachi, pers. comm., in Birkhead & Pienaar, 1995a). Morphological characters thus suggest that *C. spinifera* is affiliated to clade B1. The clear divergence of its 18S and 28S rDNA sequences from the rest of the Prymnesiales and its unique combination of characters justify the reinstatement of the genus *Chrysocampanula*. Its unresolved placement within the Prymnesiales

in our rDNA phylogenies and its aberrant cell form could suggest that it represents a separate family but more molecular and morphological data are needed for *C. spinifera* and closely related taxa to clarify their systematic position.

In this study *Chrysochromulina parkeae* was placed within Prymnesiales, in a well-supported clade together with the cultured strain MBIC10518 (B1-6). Previous phylogenetic analyses of haptophytes based on 18S rDNA placed *C. parkeae* within the coccolithophorid clade (Sáez *et al.*, 2004; Edvardsen & Medlin, 2007). As with *C. spinifera*, more molecular and morphological data of *C. parkeae* are needed to clarify its systematic position.

Clades B1-2 and B1-3

Clade B1-2 + B1-3, recovered in the 18S and concatenated rDNA phylogenies, is composed of *Imantonia* and strain CCMP 1204. *Imantonia rotunda* and *Imantonia* sp. (strain CCMP 1404) form a monophyletic lineage (clade B1-2), and together with strain CCMP 1204 (B1-3) form a sister group to the remaining clade B1 species (clades B1-4 and B1-5) in the concatenated rDNA tree (Fig. 6) and a polytomy with these clades in the 18S rDNA phylogeny (Fig. 3). *Imantonia rotunda* has immersed pyrenoids and flagella with distal and proximal transitional plates, which supports a closer relationship to species of *Chrysochromulina* and *Prymnesium* than to *Isochrysis*, with which it was previously grouped (Jordan & Green, 1994). *Imantonia* was also placed near the non-saddle-shaped *Chrysochromulina* species in the *rbcL* gene tree presented by Inouye (1997). Members of *Imantonia* are picoplankters with spherical cells and with no traces of an emergent haptonema except for a proboscis (Fig. 9). The cells possess a deviant flagellar apparatus with many flagellar roots, each composed of only a few microtubules. There is no bundle of microtubules associated with R1 or R2, but the root termed R5 by Green & Hori (1986) may be interpreted as a vestige of a crystalline array (a bundle of many closely packed microtubules branching off the sheet) associated with R1 (R1c, Inouye, 1997; Eikrem & Moestrup, 1998). We suggest that the haptonema was lost in the ancestors of *Imantonia* and its absence is a derived character for this genus. *Imantonia rotunda* is the type species of the genus and is included in all our rDNA phylogenies. Our molecular data and morphological revision support the interpretation that taxa within the *Imantonia* group should all retain their genus name.

In contrast, the sister taxon to *Imantonia*, represented by strain CCMP 1204 isolated from Arctic

waters, has many features in common with the remaining non-saddle-shaped *Chrysochromulina* species in clade B1-4 (rounded cell shape, non-coiling haptonema shorter than flagella, and plate-like body scales; Eikrem & Edvardsen, 1999), suggesting these are shared primitive characters (symplesiomorphies) of clade B1. Based on a combination of morphological and molecular rDNA data, we propose the erection of a new genus for this species, *Pseudohaptolina arctica*, *gen. et sp. nov.*

Clades B1-4 and B1-5

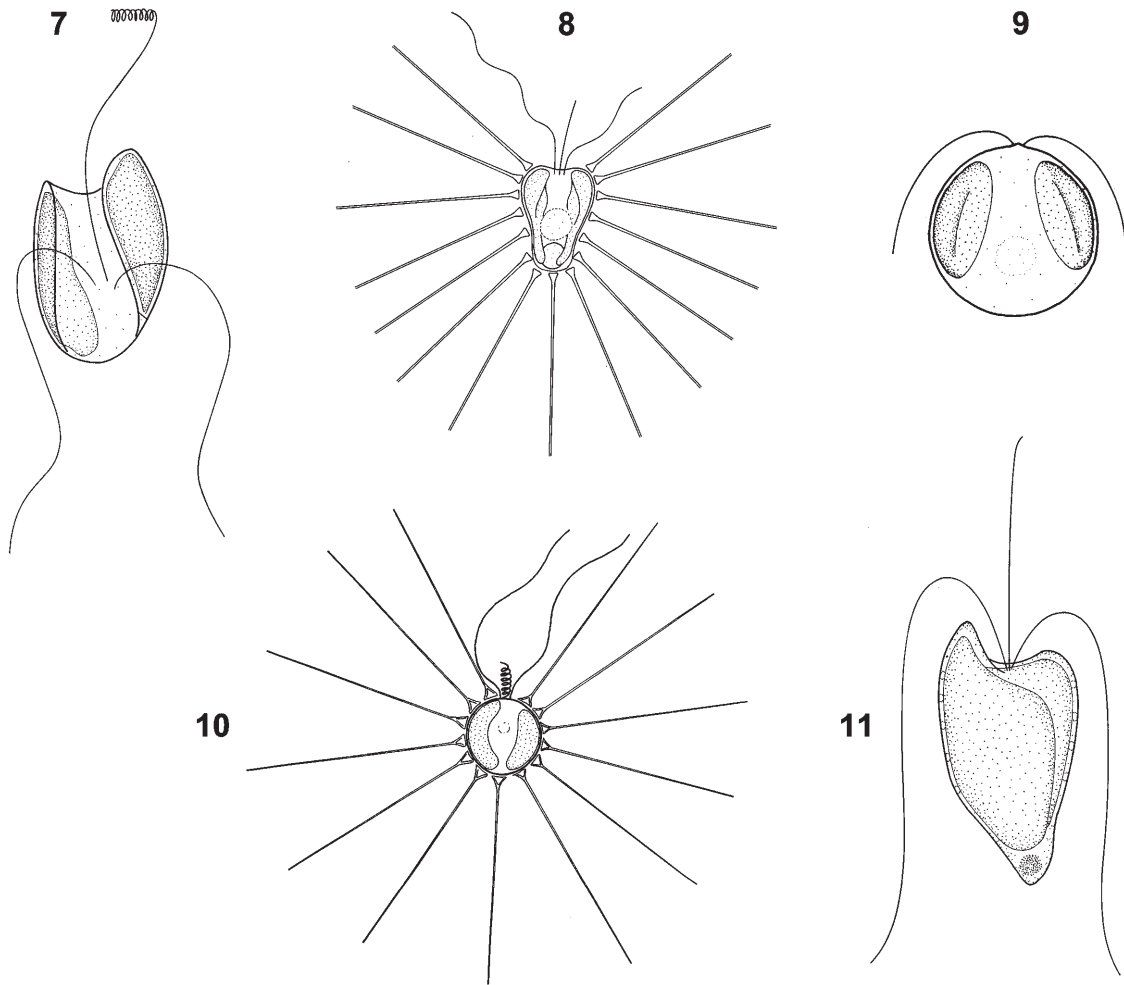
The remaining taxa in clade B1 fall into two well-supported clades, B1-4 and B1-5, in our 18S, 16S and concatenated rDNA Bayesian inferences (Figs 3, 5, 6, PP \geq 0.96). Clade B1-4 contains the current species *Chrysochromulina brevifilum*, *C. ericina*, *C. fragaria*, *C. cf. herdlensis* and *C. hirta*, and we propose the creation of a new genus, *Haptolina*, to embrace species in this clade. Members of the genera *Hyalolithus* (*H. neolepis*), *Platyachrysis* (*Pl. pienaarii*, *Pl. pigra*, *Pl. simplex* and *Pl. sp.* strain RCC1385 in this study) and *Prymnesium*, together with some non-saddle-shaped *Chrysochromulina* species (*C. chiton*, *C. kappa*, *C. minor*, *C. palpebralis*, *C. polylepis* and *C. sp.* strain MBIC 10516 in this study) cluster together in clade B1-5 in the 18S, 16S and concatenated trees, and are here transferred to the genus *Prymnesium* (see below). Members of *Haptolina* (clade B1-4) form a sister group to *Prymnesium* (clade B1-5) with high support (PP > 0.96) in the concatenated rDNA tree.

Haptolina species of clade B1-4 have a symmetrical spherical to oval cell shape, apically inserted appendages and a homodynamic swimming pattern. The haptonema is shorter, equal to or somewhat longer than the flagella and is non-coiling or coiling (see below; Fig. 10). The cells may have large spiny scales (*Haptolina* (*Chrysochromulina*) *ericina* and *Haptolina* (*Chrysochromulina*) *hirta*, Fig. 10). The haptonema of *Haptolina* (*Chrysochromulina*) *fragaria* is shorter than the flagella and has seven microtubules in the emergent part. Like *Haptolina brevifila* (*Chrysochromulina brevifilum*), the proximal part of the flagella of *H. fragaria* has tubular rings (Eikrem, unpublished observations), and the pyrenoids may be bulging (Eikrem & Edvardsen, 1999). The flagellar apparatus in *H. brevifila* includes a R2 root similar to that in *Prymnesium* species, but which was reduced or lacking in two saddle-shaped *Chrysochromulina* species (Birkhead & Pienaar, 1994a). The R1 flagellar root in *H. brevifila*, however, is simple and lacks closely packed bundles of microtubules forming a compound root, as in *Prymnesium* species including *Prymnesium polylepis*

(*Chrysochromulina polylepis*) within clade B1-5 (Birkhead & Pienaar, 1994a, see below). The haptonema in *H. brevifila*, although shorter than the flagella, has the ability to coil and in fact it appears that this is associated with the presence of a simple R1 flagellar root throughout the order Prymnesiales. The cells of *H. ericina* and *H. hirta* have a haptonema that is longer than the flagella and able to coil. Ultrastructural data are limited for these two latter species, but their body shape, scale types (including large spiny scales) and 18S rDNA sequences ally them with clade B1 species.

The species belonging to clade B1-5 are here transferred to *Prymnesium*. They include the species known as *Chrysochromulina polylepis*, *C. chiton*, *C. minor*, *C. kappa* and all members of *Platyachrysis*, as well as species already classified in *Prymnesium*. These species have a non-coiling haptonema slightly or much shorter than their flagella [Fig. 11, Table 2; the haptonema in *Prymnesium* (*Chrysochromulina*) *chiton* is however longer than the flagella], oblong and asymmetrical cell shape, and more or less heterodynamic flagella. They may also have features commonly associated with coccolithophorids, for example a compound R1 root (a sheet plus a bundle of microtubules) and bulging pyrenoids, or they possess features that are usually absent in the *Chrysochromulina* species of clade B2 (see above). Both *P. nemamethecum* and *P. palpebrale* (*Chrysochromulina palpebralis*), which was first described by Birkhead & Pienaar (1994b, 1995a) under the name 'eyelash *Chrysochromulina* sp.' and formally described by Seoane *et al.* (2009), have a compound root associated with their right flagellum (R1) and a fibrous root resembling what is termed a cytoplasmic tongue in the coccolithophorids (Beech & Wetherbee, 1988; Fresnel, 1989; Seoane *et al.*, 2009). In addition to the distal and proximal bands in the flagella, *P. palpebrale* has a helical band in its flagella (Birkhead & Pienaar, 1994b, 1995a; Seoane *et al.*, 2009), as do some coccolithophorids. Of the species we have sequenced, both *P. polylepis* and *P. kappa* (*C. kappa*) have compound R1 flagellar roots (Edvardsen *et al.*, 1996; Eikrem & Moestrup, unpublished observations). Both *P. parvum* f. *patelliferum* and *P. parvum* f. *parvum* have simple R1 flagellar roots, but the sheet contains many microtubules (>20) as in *P. nemamethecum*. Clade B1 species have immersed pyrenoids, except for *P. kappa* and *P. nemamethecum*, which have bulging pyrenoids. Because a compound root associated with R1 is found both in coccolithophorids and in several branches of clade B1, it seems that this may be a primitive feature that has been lost many times in different lineages.

Members of the genus *Platyachrysis* fall within the clade of *Prymnesium* species in our 18S, 28S



Figs 7–11. Drawings of selected species of Prymnesiales. **7.** *Chrysochromulina strobilus*. **8.** *Chrysocampanula spinifera*. **9.** *Imantonia rotunda*. **10.** *Haptolina hirta*, *comb. nov.* (syn. *Chrysochromulina hirta*). **11.** *Prymnesium polylepis*, *comb. nov.* (syn. *Chrysochromulina polylepis*). Modified from Throndsen *et al.* (2007).

and concatenated rDNA trees. *Platychrysis* is known from morphological and ultrastructural studies to have a close affinity to *Prymnesium*, and the two genera have been shown to have closely related *rbcL* DNA sequences (Inouye, 1997). There are at present four described species of *Platychrysis*, *Pl. neustophila* R.E. Norris, *Pl. pianaarii*, *Pl. pigra* and *Pl. simplex*. Their dominant life-cycle phase is non-motile, but they produce flagellate cells that resemble *Prymnesium parvum* in cell microanatomy and the sculpturing of scales. Our rDNA phylogenies support the proposal that all *Platychrysis* species should be transferred to the genus *Prymnesium*. Non-motile cells of *Hyalolithus neolepis* show several of the features characterizing members of clade B1-5: spherical to subspherical cell shape, haptonema shorter than the flagella, a compound flagellar root and cells covered by organic plate scales. The non-motile cells also have siliceous scales. Non-motile cysts of *Prymnesium parvum* similarly have a siliceous cell covering, here composed of layers of organic scales with siliceous material deposited on the

outermost scales (Pienaar, 1980; Green *et al.*, 1982). Electron microscopical X-ray microanalysis of scales of *P. polylepis* indicates that they contain silicate (Jahn Throndsen and Wenche Eikrem, unpublished data), suggesting that silicified scales are not unique to *H. neolepis* within the Prymnesiales. Motile cells covered only by organic scales may also occasionally occur in cultures of *H. neolepis*. This species falls within clade B1-5 in our 18S and 28S rDNA trees and a tree using *rbcL* (Yoshida *et al.*, 2006). In order to avoid having a number of paraphyletic genera in clade B1-5, we propose the transfer of all species within this clade to the genus *Prymnesium*.

Among other *Chrysochromulina* species that have not yet been sequenced, we expect all non-saddle-shaped species (e.g. *C. bergenensis* B. Leadbeater, *C. birgeri* G. Hällfors & Niemi, *C. brachycylindra* G. Hällfors & H.A. Thomsen, *C. fragilis* B. Leadbeater, *C. mantoniae* B. Leadbeater, *C. mactra* Manton and *C. pringsheimii* Parke & Manton) to belong to clade B1. The distinction within this clade between

Table 3. Sites with sequence differences in the 18S ribosomal DNA among the genera of Prymnesiales, where data are available. The site position is related to sequence AJ004866 of *Prymnesium* (*Chrysochromulina*) *polylepis*.

| Genus | Nucleotide site position | | | | | | | | | | | | | |
|-------------------------|--------------------------|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|--|
| | 74 ¹ | 76 ¹ | 181 ³ | 198 ³ | 491 ² | 618 ¹ | 644 ² | 650 ¹ | 678 ² | 704 ² | 1090 ² | 1339 ¹ | 1340 ¹ | |
| <i>Chrysochromulina</i> | – | A | A | T | C | G | G/A | A | C/G | T | A | G | C | |
| <i>Chrysocampanula</i> | T | G | T | A | T | A | G | G | G | T | A | A | T | |
| <i>Imantonia</i> | T | G | G | C | C | A | G | G | G | G | A | A | T | |
| <i>Pseudohaptolina</i> | T | G | G | C | C | A | G | G | C | T | T | A | T | |
| <i>Haptolina</i> | T | G | T | A | C | A | C | G | A | T | A | A | T | |
| <i>Prymnesium</i> | T | G | T/C | A/G | T | A | G | G | G/C | G | T | A | T | |

¹Nucleotide site with sequence difference between Prymnesiaceae (clade B1) and Chrysochromulinaceae (clade B2).

²Nucleotide site with sequence difference between *Haptolina* (clade B1-4) and *Prymnesium* (clade B1-5).

³Nucleotide site with sequence difference between *Imantonia* + *Pseudohaptolina* (clades B1-2 and B1-3) and the rest.

Prymnesium, *Haptolina* and *Pseudohaptolina* is, however, less clear. Thus, we will not transfer additional *Chrysochromulina* species until further ultrastructural information and/or molecular data are available. Similarly, *Corymbellus aureus* has not yet been characterized genetically, but is expected to fall in clade B1 based on its morphology, cells being oblong and irregular in shape and the haptonema being shorter than the flagella. Again, it is unclear whether *Corymbellus* should be transferred into one of *Prymnesium*, *Haptolina* or *Pseudohaptolina*, or whether this genus forms a distinct lineage within clade B1.

Conclusion

Reconstructed phylogenies based on nuclear ribosomal SSU (18S), the plastid ribosomal SSU (16S), and a combination of the 18S, 28S and 16S rDNA were congruent and resulted in very similar major clades. Our data are in close concordance with the trees for the plastid-encoded *rbcL* gene including eight or nine Prymnesiales taxa presented by Fujiwara *et al.* (2001) and Yoshida *et al.* (2006), as well as with previous studies based on 18S rDNA datasets (e.g. Edvardsen *et al.*, 2000; Sáez *et al.*, 2004). *Chrysochromulina* species are clearly distributed in two clades. In clade B1, some *Chrysochromulina* species cluster with members of *Imantonia*, *Hyalolithus*, *Platyochrysis* and *Prymnesium*, whereas clade B2 contains only *Chrysochromulina* species, including the type species *C. parva*.

The ultrastructural relationships between and within genera in the Prymnesiales tend to be rather complicated, as can be seen in Table 2 where we summarize available morphological and ultrastructural data for the species included in this study. Characters showing shared derived states within taxonomic units and thus apparently valuable systematic indicators within the Prymnesiales

are: cell shape, nature of the haptonema, flagellar apparatus including the flagellar roots, and to some extent scale type. Scale morphology appears to be useful for species identification and in some cases may indicate phylogenetic relatedness. However, scale morphology may vary between life-cycle stages within a species, and some features, such as spiny scales, may have evolved more than once (e.g. in *C. parkeae*, *Haptolina ericina* and *Chrysocampanula spinifera*). There is overlap in some of these features between various genetic sub-groups, making it difficult to divide this order based solely on morphology. A number of features appear to have independently been reduced or lost, or have arisen by convergent evolution, obscuring phylogenetic relationships.

Molecular data for different genes are consistent and strengthen inferences from certain morphological and ultrastructural features, making it possible to revise the systematics of this widely distributed group of haptophyte algae. Table 3 lists nucleotide sites with consistent sequence differences in the 18S rDNA among the genera of Prymnesiales, where this information is available, and between clades B1 and B2. Clade B1 (excluding B1-1 and B1-6) is recognized here as Prymnesiaceae, and B2 as Chrysochromulinaceae. *Chrysocampanula spinifera* (B1-1) and *Chrysochromulina parkeae* (B1-6) are considered as *incertae sedis* for the time being. In addition to the specific sites in the 18S rDNA (Table 3), we were able to find two probe regions in this gene that are specific for these families, which can be used as synapomorphic characters. At positions 229–247 (related to sequence AJ004866 of *Prymnesium polylepis*) in 18S rDNA, the sequence TGCCGTTGCGTGCTGAGT is specific for all members of Prymnesiaceae. At positions 168–185 (reference sequence as above), the sequence TACATGCAGGAAGACCCG is specific for all the taxa sequenced so far in the Chrysochromulinaceae.

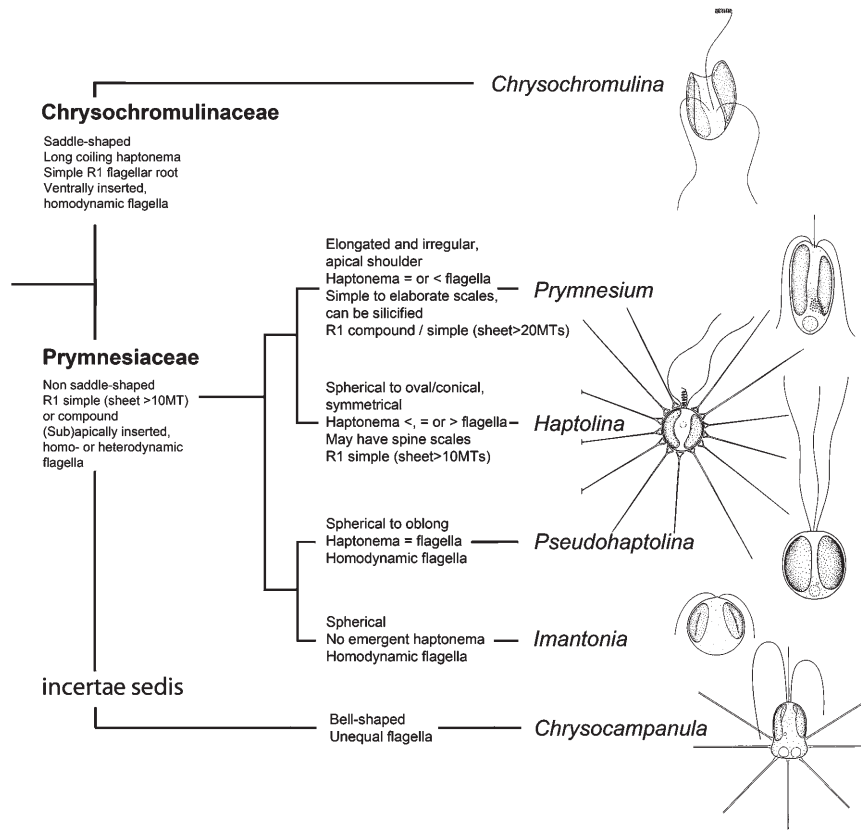


Fig. 12. Typical morphological features in the genera of Prymnesiales included in this study. Illustrations of cells are modified from Thronsen *et al.* (2007).

The ultrastructure of a number of previously cultured species within the Prymnesiales has not been thoroughly investigated and many more described species have not yet been brought into culture. In addition, prymnesialean diversity newly discovered in environmental DNA samples (Moon-Van der Staay *et al.*, 2000; Liu *et al.*, 2009) awaits morphological study and description. Future ultrastructural and genetic studies may well lead to further revisions of the taxonomy proposed here. Nevertheless, in light of the clear deficiencies in previous taxonomic schemes, there is an immediate need for a revised taxonomy that more accurately reflects phylogenetic relationships and provides a more informative framework for interpreting the ever-increasing amount of metagenomic data (e.g. Cuvelier *et al.*, 2010). Here we propose a conservative revision of the taxonomy of the Prymnesiales that is in accordance with available molecular evidence and supported by morphological data. Figure 12 shows typical morphological character states for the genera of Prymnesiales included in this study (all genera described to present except for *Corymbellus*).

Taxonomic recommendations

We propose to make the following taxonomic changes to the taxa falling in clades B1 and

B2: The order Prymnesiales is to be divided into two families. The family Prymnesiaceae is emended and the Chrysochromulinaceae *fam. nov.* is erected. The latter family should be distinguished from a family Chrysochromulinidae proposed by Lackey in 1939 to accommodate his new species *Chrysochromulina parva* and other species with three appendages, for which no description or diagnosis was provided. The previously described family Prymnesiaceae Conrad (1926) with the same distinguishing feature made Lackey’s new family superfluous. The genera *Haptolina* and *Pseudohaptolina* are erected, *Pseudohaptolina arctica* is described, and selected members of *Chrysochromulina*, and all members of *Platyachrysis* and *Hyalolithus* are transferred to *Prymnesium*. The previous reinstatement of the genus *Chrysocampanula* is confirmed. Taxonomic reassignment of *Chrysochromulina parkeae* will be published by others elsewhere.

Family Prymnesiaceae W. Conrad ex O.C. Schmidt emend. Edvardsen, Eikrem & Medlin

DESCRIPTION: Cells oblong and asymmetrical to spherical and symmetrical, not saddle-shaped, with two smooth, equal to unequal, homodynamic or heterodynamic flagella. Haptonema predominantly shorter than or equal in length to flagella,

and predominantly non-coiling. Flagella and haptonema inserted apically or slightly subapically. Cells covered by simple to elaborate scales. Flagellar apparatus with a compound R1 flagellar root with a bundle of microtubules, or simple with a sheet of > 10 microtubules. Cells with two yellow-brown chloroplasts with immersed or bulging pyrenoids. Nucleotide sequences for the SSU and LSU rDNA distinct.

TYPE GENUS: *Prymnesium* Massart, 1920. Genera included in the family: *Chrysocampanula*, *Corymbellus*, *Haptolina*, *Imantonia*, *Prymnesium*, *Pseudohaptolina*.

Genus *Prymnesium* Massart emend. Edvardsen, Eikrem & Probert

DESCRIPTION: Cells predominantly elongated and irregular, often with an apical shoulder with two equal or subequal, homodynamic or heterodynamic flagella. Haptonema predominantly shorter or equal in length to flagella, and usually unable to coil. Flagella and haptonema inserted apically or slightly subapically. The flagellar apparatus has a compound R1 flagellar root with a bundle of microtubules or a sheet of many (>20) microtubules. The organic body scales are simple to elaborate plate scales and may have spines or other protuberances. Scales and cysts may be silicified. Life cycles with alternating haploid and diploid phases occur. Several species are toxic.

SYNONYMS: *Chrysochromulina pro parte excl. typus*, *Platyachrysis*, *Hyalolithus*.

TYPE SPECIES: *Prymnesium saltans* Massart, 1920.

NEW COMBINATIONS:

Prymnesium neolepis (M. Yoshida, M.-H. Noël, T. Nakayama, T. Naganuma & I. Inouye) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Hyalolithus neolepis* M. Yoshida, M.-H. Noël, T. Nakayama, T. Naganuma & I. Inouye. Yoshida *et al.* (2006). *Protist*, **157**: 214, fig. 2A.

Prymnesium palpebrale (Seoane, Eikrem, Edvardsen & Pienaar) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Chrysochromulina palpebralis* Seoane, Eikrem, Edvardsen & Pienaar. Seoane *et al.* (2009). *Phycologia*, **48**: 168, figs 5–8.

Prymnesium polylepis (Manton & Parke) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Chrysochromulina polylepis* Manton & Parke. Manton & Parke (1962). *J. Mar. Biol. Assoc. UK*, **42**: 575, figs 1–28, ‘Type’ culture PCC 136.

Prymnesium kappa (Parke & Manton) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Chrysochromulina kappa* Parke & Manton. Parke *et al.* (1955). *J. Mar. Biol. Assoc. UK*, **34**: 583, figs 1–19, ‘Type’ culture PCC K.

Prymnesium chiton (Parke & Manton) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Chrysochromulina chiton* Parke & Manton. Parke *et al.* (1958). *J. Mar. Biol. Assoc. UK*, **37**: 225, figs 1–37, ‘Type’ culture PCC 146.

Prymnesium minus (Parke & Manton) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Chrysochromulina minor* Parke & Manton. Parke *et al.* (1955). *J. Mar. Biol. Assoc. UK*, **34**: 594, figs 36–64, ‘Type’ culture PCC 52.

Prymnesium neustophilum (R.E. Norris) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Platyachrysis neustophila* R.E. Norris. Norris (1967). *Bull. S. Cal. Acad. Sci.*, **66**: 241, figs 1 h, i; 6.

Prymnesium pienaarii (Gayral & Fresnel) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Platyachrysis pienaarii* Gayral & Fresnel. Gayral & Fresnel (1983). *Phycologia*, **22**: 43, figs 1–10.

Prymnesium pigrum (Geitler) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Platyachrysis pigra* Geitler. Geitler (1930). *Arch. Protistenk.*, **69**: 631, pl. 25b, c, text fig. 14a–e.

Prymnesium simplex (Gayral & Fresnel) Edvardsen, Eikrem & Probert, *comb. nov.*

BASIONYM: *Platyachrysis simplex* Gayral & Fresnel. Gayral & Fresnel (1983). *Phycologia* **22**: 43, figs 19–28.

***Haptolina* Edvardsen & Eikrem, gen. nov.**

DESCRIPTIO: Cellulae symmetricae, sphaericae seu ovaes vel conicae. Haptonema paululo brevius, aequale aut longius quam flagella, tortum aut non tortum. Haptonema cum flagellis duobus apicaliter inserta. Cellulae squamis spineis magnis interdum ornatae. Cellulae chloroplastis praeditae. Apparatus flagellaris radice flagellari simplici RI interdum instructus. Sequentiae nucleotidis genorum SSU et LSU rRNA distinctae.

DESCRIPTION: Cells symmetrical, spherical to oval or conical, with two equal, homodynamic flagella. Haptonema slightly shorter, equal or longer than the flagella, and can be coiling or non-coiling. Haptonema and flagella inserted apically. Cells may have large organic spine scales. The flagellar apparatus may have a simple R1 flagellar root. Nucleotide sequences for the SSU and LSU rDNA distinct.

SYNONYM: *Chrysochromulina pro parte excl. typus*.

TYPE SPECIES: *Haptolina brevifila* (Parke & Manton) Edvardsen & Eikrem, *comb. nov.*

NEW COMBINATIONS:

Haptolina brevifila (Parke & Manton) Edvardsen & Eikrem, *comb. nov.*

BASIONYM: *Chrysochromulina brevifilum* Parke & Manton. Parke *et al.* (1955). *J. Mar. Biol. Assoc. UK*, **34**: 601, figs 65–81.

Haptolina ericina (Parke & Manton) Edvardsen & Eikrem, *comb. nov.*

BASIONYM: *Chrysochromulina ericina* Parke & Manton. Parke *et al.* (1956). *J. Mar. Biol. Assoc. UK*, **35**: 389, figs 1–19.

Haptolina fragaria (Eikrem & Edvardsen) Edvardsen & Eikrem, *comb. nov.*

BASIONYM: *Chrysochromulina fragaria* Eikrem & Edvardsen. Eikrem & Edvardsen (1999). *Phycologia*, **38**: 150, figs 1–9.

Haptolina herdlensis (B. Leadbeater) Edvardsen & Eikrem, *comb. nov.*

BASIONYM: *Chrysochromulina herdlensis* B. Leadbeater. Leadbeater (1972). *Sarsia* **49**: 73, fig. 1E.

Haptolina hirta (Manton) Edvardsen & Eikrem, *comb. nov.*

BASIONYM: *Chrysochromulina hirta* Manton. Manton (1978). *Br. Phycol. J.*, **13**: 13, figs 5 and 7.

Pseudohaptolina Edvardsen & Eikrem, *gen. nov.*

DESCRIPTIO: Cellulae rotundae vel oblongae flagellis duobus haptonemateque instructae. Haptonema cum flagellis duobus apicaliter insertum. Haptonema similis quam flagella et haptonema non tortum. Periplastus squamis organicis planis costis radiantibus in faciebus et distali et proximali tectus. Cellulae chloroplastis praeditae. Sequentiae nucleotidis genorum SSU et LSU rRNA distinctae. DESCRIPTION: Cells round to oblong with two equal, homodynamic flagella. Haptonema similar in length to flagella and non-coiling. Haptonema and flagella inserted apically. Periplast covered by organic plate scales with radiating ribs on both distal and proximal faces. Cells with two chloroplasts. Nucleotide sequences for the SSU and LSU rDNA distinct.

TYPE SPECIES: *Pseudohaptolina arctica* Eikrem & Edvardsen

Pseudohaptolina arctica Edvardsen & Eikrem, *sp. nov.*

DESCRIPTIO: Cellulae rotundae vel oblongae (8–14 × 6–10 μm), saepe dorsoventraliter compressae chloroplastis aureo-brunneis duobus praeditae.

Haptonema (12–20 μm) cum flagellis duobus (14–24 μm longis) apicaliter inserta. Tegumentum squameum generibus squamarum rotundarum vel ovalium duobus compositum. Stratum squamarum interius (0.7–0.9 × 0.8–1.0 μm) margine imbricato, exterius (0.5–0.8 × 0.6–0.9 μm) margine erecto. Squamae omnes costis radiantibus (c. 75–85) in faciebus et distali et proximali.

DESCRIPTION: Cells round to oblong (8–14 × 6–10 μm), often dorso-ventrally compressed with two golden brown chloroplasts. Haptonema (12–20 μm) and two flagella (14–24 μm long) inserted apically. Scaly covering composed of two round to oval scale types. Inner layer scales (0.7–0.9 × 0.8–1.0 μm) with imbricated rim. Outer layer scales (0.5–0.8 × 0.6–0.9 μm) with upright rim. All scales with radiating ribs (c. 75–85) on both distal and proximal faces.

HOLOTYPE: Eikrem & Edvardsen (1999). *Phycologia* **38**: 153, figs 17, 18 (as *Chrysochromulina* sp. 4), CCMP 1204.

TYPE LOCALITY: Arctic waters, 76°25'N 82°55'W

HABITAT: Marine

ETYMOLOGY: It was isolated by R. Selvin in 1989 from Arctic waters.

Family Chrysochromulinaceae Edvardsen, Eikrem & Medlin, fam. nov.

DESCRIPTIO: Cellulae maxime ephippoideae flagellis duobus levibus, aequalibus, homodynamicis instructae. Haptonema longum ad longissimum atque tortum. Flagella et haptonema ventraliter et subapicaliter inserta. Cellulae squamis organicis parvis tectae, structuris costarum vel fibrillarum radiantium et concentricarum. Apparatus flagellaris radice flagellari R1 simplici microtubulos (<10) saepissime paucos continenti fasciculo microtubulorum carenti. Cellulae chloroplastis flavo-brunneis duobus utroque pyrenoide immersa praeditae. Sequentiae nucleotidis genorum SSU et LSU rDNA distinctae.

DESCRIPTION: Cells predominantly saddle-shaped, with two smooth, equal, homodynamic flagella. Haptonema long to very long and coiling. Flagella and haptonema inserted ventrally and subapically. Cells covered by small organic scales with patterns of radiating and concentric ribs or fibrils. Flagellar apparatus with a simple R1 flagellar root that predominantly contains few microtubules (<10) and lacks a bundle of microtubules. Cells with two yellow-brown chloroplasts each with an immersed pyrenoid. Nucleotide sequences for the SSU and LSU rDNA distinct.

TYPE GENUS: *Chrysochromulina* Lackey 1939.

The Chrysochromulinaceae contains one genus, *Chrysochromulina*.

***Chrysochromulina* Lackey emend. Eikrem & Edvardsen**

DESCRIPTION: Cells predominantly saddle-shaped, with two equal, homodynamic flagella. Haptonema long to very long and coiling. Flagella and haptonema inserted ventrally and subapically. Cells covered by small organic scales with patterns of radiating and concentric ribs or fibrils. Some of the scale types bear short spines or are cup-shaped. Cells with two chloroplasts with immersed pyrenoids traversed by tubes or thylakoids. Flagellar apparatus with a simple R1 flagellar root that predominantly contains few microtubules. Nucleotide sequences for the SSU and LSU rDNA distinct.

TYPE SPECIES: *Chrysochromulina parva* Lackey, 1939. Lackey (1939), *Lloydia* **2**: 128–143.

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Supplementary material

The following supplementary material is available for this article, accessible via the Supplementary content tab on the article's online page at <http://dx.doi.org/10.1080/09670262.2011.594095>.

SSU FIN.txt
LSU FIN.txt
16SFIN.txt
3genesFIN.txt

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