Haptophyta

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Abstract

Haptophyta are predominantly planktonic and phototrophic organisms that have their main distribution in marine environments worldwide. They are a major component of the microbial ecosystem, some form massive blooms and some are toxic. Haptophytes are significant players in the global carbonate cycle through photosynthesis and calcification. They are characterized by the haptonema, a third appendage used for attachment and food handling, two similar flagella, two golden-brown chloroplasts, and organic body scales that serve in species identification. Coccolithophores have calcified scales termed coccoliths. Phylogenetically Haptophyta form a well-defined group and are divided into two classes Pavlovophyceae and Coccolithophyceae (Prymnesiophyceae). Currently, about 330 species are described. Environmental DNA sequencing shows high haptophyte diversity in the marine pico- and nanoplankton, of which many likely represent novel species and lineages. Haptophyte diversity is believed to have peaked in the past and their presence is documented in the fossil record back to the Triassic, approximately 225 million years ago. Some biomolecules of haptophyte origin are extraordinarily resistant to decay and are thus used by geologists as sedimentary proxies of past climatic conditions.

Keywords

Biogeochemical cycles • Coccoliths • Ecology • Evolution • Fossil record • Haptophyta • Morphology • Ocean acidification • Phylogeny

Summary Classification

- **Haptophyta**
  - **Pavlovophyceae**
    - **Pavlovales** (e.g. *Pavlova*, *Rebecca*)
  - **Coccolithophyceae** (=*Prymnesiophyceae*)
    - **Phaeocystales** (*Phaeocystis*)
    - **Isochrysidales** (e.g. *Isochrysis*, *Emiliania*)
    - **Coccolithales** (e.g. *Coccolithus*, *Syracosphaera*)
    - **Prymnesiales** (e.g. *Prymnesium*, *Chrysochromulina*)

Introduction

The division Haptophyta is a group of unicellular algae that are predominately marine, although there are a few freshwater and terrestrial records. Haptophyte nutrition is mainly phototrophic, but many exhibit phagotrophy and some are exclusively heterotrophic. Haptophytes usually appear yellow-brown at high population densities (in culture or blooms) because of accessory carotenoid pigments. Most haptophytes occur as solitary motile or nonmotile forms, but a few form colonies or short filaments. The cells are usually covered with scales of varying degrees of complexity ranging from elaborate calcified structures termed “coccoliths” that are usually visible in light microscopy to ornamented unmineralized organic scales, many of which can only be observed in electron microscopy. Scales and coccoliths are used in species identification (e.g., Bendif et al. 2011, Chrétiennot-Dinet et al. 2014, and http://ina.tmsoc.org/Nannotax3). Motile forms usually possess two equal (isokont) or subequal flagella, which can beat similarly (homodynamic) or differently (heterodynamic). The two flagella are similar in microanatomy and do not have superficial structures except in the class Pavlovophyceae, where they are markedly different in length and are covered by knob scales and fine hairs. Haptophytes possess a unique structure called a haptonema, a filiform organelle associated with the flagella, but different in structure. The name of the division is based on the name of this organelle (from the Greek, hapsis meaning touch).

The Haptophyta includes some 80 extant genera with approximately 330 species in two classes, the Coccolithophyceae (Prymnesiophyceae) with around 76 genera and 318 species (Jordan et al. 2004) and the Pavlovophyceae with 4 genera and 13 species (Bendif et al. 2011). Its members often form an important component of oceanic and coastal plankton and several species are known to form blooms; some of them toxic. Coccolithophores have been recognized in sedimentary rocks and ocean sediments since the early nineteenth century (Ehrenberg 1836) from their calcified scales called “coccoliths” (Huxley 1858). Haptophyte diversity probably peaked in the past, as documented in the coccolith fossil record that extends back to the late Triassic, ca. 225 million years ago.

Habitats and Ecology

Habitats

Haptophytes are found worldwide as common components of coastal and oceanic habitats, and most genera have representatives in the marine plankton. They constitute a considerable percentage of the numbers and biomass of nanoplankton (2–20 μm) (Marchant and Thomsen 1994; Thomsen et al. 1994; Masquelier et al. 2011) as well as in the picoplankton (<3 μm) (Thomsen 1986; Liu et al. 2009; Jardillier et al. 2010; Kirkham et al. 2011). Few estimates have been made, however, of the fraction of production attributable to haptophytes, partly because of the difficulty in identifying and quantifying them in preserved samples. Molecular methods have been instrumental in revealing haptophyte diversity and distribution. Environmental sequencing of clone libraries have repeatedly shown a large diversity including haptophyte clades with no cultured representatives (e.g., Moon-van der Staay et al. 2000; Liu et al. 2009; Shi et al. 2009; Cuvelier et al. 2010), an indication of a large hidden biodiversity in the group. The use of high-throughput sequencing (HTS) has revealed unprecedented haptophyte diversity in marine waters with most of the OTUs (operational taxonomic units) with best match to an environmental sequence, of which some may represent novel taxa from class to species levels (Bittner et al. 2013; Egge et al. 2015a).

Many species are euryhaline, such as the pavlovophyte *Diacronema vlkianum* and the toxic and economically important *Prymnesium* species, *P. parvum* (Green 1975; Green and Hibberd 1977; Green et al. 1982; Larsen 1999). It also grows epipsammically in sand and may thrive in ponds, lakes, and river basins with slightly elevated salinity (Edvardsen and Imai 2006; Johnsen et al. 2010; Southard et al. 2010). Filamentous and palmelloid forms of taxa such as *Ruttnera* and *Chrysotila* (see Andersen et al. 2014, 2015 for update on taxonomy) occur in the splash zone of marine cliffs and are found on damp, usually basic substrates, such as chalk cliffs, and limestone walls (Green and Parke 1975b). Coccolithophores in the related genera *Jomonlithus*, *Ochrosphaera*, and *Hymnenomonas* are typically found in nearshore coastal zones and estuaries.

Only a few genera and about 15 species have been reported from freshwater environments (Preisig 2002; Nicholls 2014). Freshwater records include reports of *Hymenomonas roseola* (Manton and Peterfi 1969), several *Prymnesium*, and *Chrysochromulina.* Freshwater phylotypes belonging to both classes have been revealed by 18 S rDNA environmental sequences (Shalchian-Tabrizi et al. 2011). Some Prymnesiales thrive in and under ice (Hällfors and Niemi 1974; Takahashi 1981).

Several species of *Phaeocystis* occur as symbionts of the widespread and abundant zooplanktonic acantharians (Decelle et al. 2012), and haptophytes have also been found in symbiosis with polycystine radiolarians (Febvre and Febvre-Chevalier 1979; Anderson et al. 1983) and foraminifers (Gast et al. 2000). A haptophyte with affinities to *Braarudosphaera* harbors a nitrogen-fixing cyanobacterium (Thompson et al. 2012). Several members of Prymnesiales and the motile stage of *Emiliania* *huxleyi* and *Coccolithus pelagicus* phagocytize bacteria, artificial particles, or other algae (Parke et al. 1955; Jones et al. 1994; Tillmann 1998; Rokitta et al. 2011). Many coccolithophores, for example, species of *Balaniger*, *Ericiolus*, *Pappomonas*, and *Trigonaspis*, have been reported to be heterotrophic (Marchant and Thomsen 1994; Thomsen et al. 1994, 1995). Growth of some haptophytes is stimulated by the uptake of dissolved organic compounds (Pintner and Provasoli 1968; Antia 1980).

Biogeography

Investigations of the distribution and community structure of coccolithophores have been conducted since Lohmann (1912), who was probably the first to examine the oceanic distribution of extant coccolithophores. In Winter et al. (1994), rough biogeographic coccolithophore zones were established largely based on two pioneering oceanic transect studies in the Atlantic (McIntyre and Bé 1967) and the Pacific (Okada and Honjo 1973). Hagino and Young (2015) reviewed recent literature and defined eight coccolithophore floras based on the dominance of one (*E. huxleyi*, *G. oceanica*, or *U. irregularis*) and common occurrence of accompanying taxa. Their compilation supports the latitudinal zonal distributions of the Atlantic (McIntyre and Bé 1967; Winter et al. 1994), but highlights that floras of the equatorial Pacific are also subdivided along east–west gradients, likely corresponding to nutrient content (Hagino and Young 2015).

Distinct floral assemblages are found in the subarctic, temperate, subtropical, tropical, and subantarctic biogeographic zones, largely associated with major ocean current systems. Species diversity generally increases from polar to subtropical–tropical regions, where distinct vertical zonations are often related to (permanent) stratification and deep light penetration of oligotrophic surface waters. The majority of species are restricted to either an upper photic (0–80 m) or a lower photic (120–220 m) zone. The intermediate zone (80–120 m) may contain species from the assemblages above and below. Holococcolithophores are often found in relation with (seasonal) surface water stratification (Cros and Estrada 2013).

The lower photic zones are dominated by *Florisphaera profunda* (with occurrences of *Algirosphaera* spp. and *Gladiolithus* spp.) in temperate to tropical waters, whereas these deeper-dwelling taxa are absent in subpolar–polar waters (Hagino and Young 2015). Placolith-bearing taxa occur in the upper and intermediate photic zones. *Emiliania huxleyi* dominates the upper photic zone in all temperate to polar waters, as well as the equatorial Atlantic and eastern equatorial Pacific. Assemblages with *Umbellosphaera irregularis* are found in the oligotrophic Atlantic subtropical gyres and western equatorial Pacific. Notably, *Gephyrocapsa*-dominated assemblages have been, to date, only reported from the equatorial Indo-Pacific regions and western Pacific coastal waters (Hagino and Young 2015).

The open waters of the Arctic host *Coccolithus pelagicus*, *Calciopappus caudatus*, *Algirosphaera robusta*, and *Emiliania huxleyi* (Winter et al. 1994), and several representatives from the partially calcified genera, such as *Pappomonas*, *Papposphaera*, and *Wigwamma*, are also present (Thomsen et al. 1991; Thomsen et al. 1994). The latter group is most abundant in coastal Arctic waters and is sometimes even observed in ice (Marchant and Thomsen 1994; Winter et al. 1994).

In Antarctic waters, common species of the Papposphaeraceae include *Papposphaera*, *Pappomonas*, *Trigonaspis*, and *Wigwamma* (Marchant and Thomsen 1994; Winter et al. 1994), and more than 20 species have been identified including *E. huxleyi* and several species of *Gephyrocapsa*. The abundance and diversity of Southern Ocean coccolithophores decreases poleward (Findlay et al. 2005). The presence of considerable concentrations (>1000 cells/L) of *E. huxleyi* in all sectors of the Southern Ocean and far south of the Antarctic Polar Front (Winter et al. 2014) affirms the eurythermal character of this species.

Less is known about the distribution of members of the Prymnesiales, such as *Chrysochromulina, Chrysocampanula*, *Haptolina*, *Dicrateria*, and *Prymnesium* species, because species identification usually requires electron microscopy examination or genetic methods, and in many instances species delimitation is difficult. Investigations of the Prymnesiales have been carried out in most regions of the world (Leadbeater 1972; Leadbeater 1974; Estep et al. 1984; Hoepffner and Haas 1990; Gao et al. 1991; Marchant and Thomsen 1994; Jensen 1998; LeRoi and Hallegraeff 2004; LeRoi and Hallegraeff 2006), but with present knowledge of species delimitation and their geographical distribution, a reasonable biogeography of the different Prymnesiales species cannot be outlined. Many species such as *Chrysocampanula spinifera*, *Chrysochromulina acantha*, *C. leadbeateri*, *C. simplex*, *C. mantoniae*, *Dicrateria rotunda*, *Haptolina ericina*, and *H. hirta* are reported from areas stretching from the sub-Arctic to the Antarctic and would in this sense be considered cosmopolitan. It has been claimed by De Vargas et al. (2007) that species we recognize as cosmopolitan and display a high genetic and/or ecological plasticity are in fact sibling species within morphological superspecies. The morphological variation in many species as for instance *C. leadbeateri* and *C. simplex* is considerable, and the different morphotypes may in the future prove to constitute sibling species or even different species. Very few of the species of this order have been reported to have their distribution restricted to certain geographical areas of the world. Of those that have, *Chrysochromulina tenuisquama* has only been reported from the southern part of the Atlantic Ocean (Estep et al. 1984), *C. elegans* has been reported only from the southern part of the North Atlantic and Scandinavian waters (Estep et al. 1984; Jensen 1998), and *C. throndsenii* has so far only been recorded from Scandinavian waters (Eikrem 1996; Jensen 1998).

In the Phaeocystales *Phaeocystis globosa* is recognized in the North Sea and English Channel and in temperate waters worldwide; *P. pouchetii* is common along the Norwegian coast, in the Barents Sea, and in Northern Pacific; *P. antarctica* is usual in the Antarctic; and *P. jahnii* is known from the Mediterranean Sea. All of these species have a colonial phase in their life cycle. *Phaeocystis cordata* described from the Mediterranean and *P. scrobiculata*, which has been reported from temperate and subtropic regions, are only known as flagellates (Lange et al. 1996; Medlin and Zingone 2007).

Species of the Pavlovales genera *Diacronema*, *Rebecca*, *Pavlova*, and *Exanthemachrysis* are commonly found in nearshore coastal environments (Carter 1937; Butcher 1952; Droop 1953; Van der Veer 1979), and reports from oceanic environments are scarce. *Diacronema vlkianum* has been recorded a few times from ponds and lakes in Europe, including the British Isles (Preisig 2002).

In a seasonal study of haptophyte diversity (assessed by OTU composition) in Skagerrak (Egge et al. 2015b), a strong seasonal pattern was revealed. Pavlovales representatives were only present in the summer and early spring communities and were dominated by Phaeocystales and Prymnesiales reads, together with environmental sequences that suggest a new haptophyte class. In summer and fall, reads representing coccolithophores and *E. huxleyi* in particular were frequent. Some taxa, e.g., *Phaeocystis cordata* and *Chrysochromulina simplex*, were detected all year.

Ecological Impacts of Bloom-Forming Haptophytes

Haptophytes thrive in both oceanic low-chlorophyll recycling systems and in high-biomass new-production systems, but only few species form intense blooms over large areas of the ocean. *Emiliania huxleyi* and *Gephyrocapsa oceanica* are by far the most prominent bloom formers. They can create blooms in temperate and boreal regions that cover very large areas, for instance, >200,000 km^2 in the Bering Sea (Sukhanova and Flint 1998) or about 250,000 km^2 in the North Atlantic (Holligan et al. 1993; Malin et al. 1993). Whereas cell densities of blooms in the North Sea, Western English Channel, North Atlantic, or the Bering Sea are usually below 6×10^6 cells/L (Holligan et al. 1983; Malin et al. 1993; Sukhanova and Flint 1998), higher densities have been recorded in the Black Sea (up to 30×10^6 cells/L; Mihnea 1997) and in Norwegian fjords (up to 115×10^6 cells/L, Berge 1962).

The conditions for such blooms are created in the course of seasonal succession cycles, typically after diatom blooms when waters have low amounts of macronutrients, especially silicate, prohibiting further growth of diatoms (Townsend et al. 1994; Nanninga and Tyrrell 1996). In these low-nutrient regimes that become more stratified as the season progresses, *E. huxleyi* can typically thrive and outgrow other phytoplankton. This ecological success may be attributed to key physiological traits identified in most or all *E. huxleyi* strains. Photosynthesis, for example, seems not to be inhibited by high irradiances. Cells can grow with near-maximal rates at intensities equal to or exceeding surface sunlight at 1000–1700 μmol photons m^{-2} s^{-1} (Nielsen 1995; Nanninga and Tyrrell 1996). An efficient phosphate acquisition system enables high affinity uptake of inorganic phosphate but also allows scavenging of organically bound phosphates (Riegman et al. 2000; Xu et al. 2010). *Emiliania huxleyi* tolerates nitrogen deprivation much better than diatoms (Löbl et al. 2010). The efficient metabolic recycling of organic N and the direct malate oxidation by malate-quinone oxidoreductase seem to be additional features that increase *E. huxleyi*'s competitiveness in oligotrophic environments (Rokitta et al. 2014). To overcome trace metal limitation and especially that of iron, *E. huxleyi* is able to substitute Fe-containing enzymes by functionally equivalent forms that bind other metal cofactors (Read et al. 2013). Although *E. huxleyi* usually does not form blooms in Fe-limited regions (the Southern Ocean), these adaptations certainly broaden its ecological scope and explain its abundance and persistence in contemporary oceans.

Blooms of *E. huxleyi* are unique in their optical properties. As a bloom progresses, coccoliths or entire coccospheres are shed (Balch et al. 1991; Paasche 2002) and scatter the incoming light, turning surface waters turbid and milky, a phenomenon visible from ships and remote sensing satellites (Holligan et al. 1983; Balch et al. 1991). At high concentrations in surface water, the average light intensity is increased due to the scattering of light by the $CaCO_3$ crystals. With depth, however, the irradiance is strongly attenuated (Balch et al. 1991; Holligan et al. 1993; Tyrrell et al. 1999). Increased light and heat trapping in the surface layers can cause a further decrease in the depth of the euphotic zone and increase the stratification (Balch et al. 1991; Tyrrell et al. 1999).

Blooms of *E. huxleyi* can sustain considerable populations of zooplanktic herbivores and are, because of high growth rates, usually not "top-down" regulated by grazing (Nejstgaard et al. 1997). Instead, mature blooms grow into nutrient starvation and are typically lysed by specific viruses (Castberg et al. 2002). Phycodnaviruses, like animal viruses (Mackinder et al. 2009), take control of cellular machinery, replicating and inducing cell lysis and viral burst within 2–3 days (Kegel et al. 2010). This top-down regulation of E. *huxleyi* blooms enables the transition to the next stage of ecological succession (Martínez et al. 2007). *Emiliania huxleyi* exhibits a haplo-diplontic life cycle and the haploid stage is a scale-bearing, non-calcified cell that possesses flagella. The distinct morphology and striking resistance to viral attacks (Frada et al. 2008) point toward an important ecological function of life cycling: growing diploid populations sporadically produce haploid cell stages, and this "background population" of haploid individuals can function as a new founding population in the case of a virally mediated termination of the parental diploid bloom, a phenomenon termed the "Cheshire cat escape strategy" (Frada et al. 2008; Frada et al. 2012). The distinct genetics of the haploid phase and consequentially also the distinct morphology and metabolism of the haploid phase are also thought to extend the species' ability to occupy new ecological niches (Rokitta et al. 2011).

The biogeochemical impacts of such coccolithophore blooms on the global carbon cycle are well studied. As phytoplankton, coccolithophores contribute to primary production and to the export of organic matter to the deep sea. Whereas this process, termed the *organic carbon pump*, causes a net drawdown of CO_2 from the atmosphere into the ocean, the production and export of calcium carbonate has the opposite effect on air/sea CO_2 exchange, causing a net release of CO_2 to the atmosphere (Rost and Riebesell 2004). This counteracting effect on the ocean–atmosphere CO_2 flux is referred to as the *carbonate counter pump*. As coccolithophores contribute to both carbon pumps, the drawdown of CO_2 caused by organic carbon production is partly compensated by the release of CO_2 via calcification. Primary production by coccolithophores is therefore a smaller sink for CO_2 when compared to a non-calcifying production system, such as a diatom bloom (Robertson et al. 1994). In cases of calcite overproduction, a phenomenon typical for *E. huxleyi* blooms growing into nutrient limitation can become a net source of CO_2 and effectively release CO_2 into the atmosphere (Purdie and Finch 1994). $CaCO_3$ also acts as a "ballast" mineral that increases the transfer efficiency of organic matter from surface waters to depth (Klaas and Archer 2002). It has been shown that coccoliths in fecal pellets and marine snow enhance their sedimentation rate (Buitenhuis et al. 1996).

Estimates of global calcium carbonate production ranges from 0.64 to 2 Gt C per year (Morse and Mackenzie 1990; Milliman 1993; Westbroek et al. 1993; Wollast 1994; Milliman and Droxler 1996). Global annual marine primary production are ~45–50 Gt C (Antoine and Morel 1996; Field et al. 1998; Carr et al. 2006), of which 5 ~ 10 Gt C are exported to the deep sea (Laws et al. 2000; Palmer and Totterdell 2001; Henson et al. 2011). A significant part is driven by the ballast effects derived from $CaCO_3$ created by *E. huxleyi* and *G. oceanica*, but also other heavily calcified, non-blooming coccolithophore species, such as *Calcidiscus leptoporus* or *Coccolithus pelagicus*, may contribute substantially to $CaCO_3$ vertical fluxes (Baumann et al. 2004; Ziveri et al. 2007).

Phaeocystis is a cosmopolitan mucilage-producing genus whose species may occur in high concentrations in both northern and southern hemispheres (Veldhuis et al. 1986; Moestrup and Thomsen 2003; Schoemann et al. 2005), and the species *Phaeocystis globosa*, *P. pouchetii*, and *P. antarctica* (Edvardsen and Imai 2006; Medlin and Zingone 2007) are important bloom formers. Off the British coast and along the coasts of Netherlands and Germany, *P. globosa* regularly blooms in late spring or early summer, producing mucilage in sufficient quantity to clog fishing nets. High winds cause the decomposing mucilage to foam and accumulate on beaches and shores where it is considered a nuisance for recreational beach use (Moestrup 1994). Blooms of *Phaeocystis* may cause harmful effects on animals and *P. pouchetii* may be toxic to cod larvae (Aanesen et al. 1998; Hansen et al. 2004). *Phaeocystis* blooms have influenced herring migration through the North Sea (Savage 1930).

Phaeocystis and other haptophytes are known to be prodigious producers of dimethylsulfoniopropionate (DMSP) (Keller et al. 1989), the precursor compound to dimethyl sulfoxide (DMS), a climate-active gas that induces atmospheric cloud formation and thus may affect planetary albedo. *Phaeocystis* species produce DMSP that is cleaved into acrylic acid and DMSP, but is not considered toxic to animals (Schoemann et al. 2005). It is unclear whether the observed harmful effects derive from toxin production, the toxic nature of breakdown products as blooms decays, or deoxygenation of the water during bloom decays.

The controversial CLAW hypothesis (Charlson et al. 1987; Quinn and Bates 2011) proposed that this DMS-cloud albedo system would represent a negative feedback acting to stabilize climate. Several studies on *Phaeocystis* and *E. huxleyi* in culture and in natural blooms have contributed significantly to our current understanding of DMSP production and cycling (Keller et al. 1989; Malin and Steinke 2004). Many haptophytes, both calcifying and non-calcifying species, produce high amounts of DMSP (Holligan et al. 1983; Holligan et al. 1993; Malin et al. 1993; Malin and Steinke 2004), and this compound has typical properties of a compatible solute/osmolyte (Kirst 1996) but may also have additional physiological roles (Stefels 2000), e.g., acting as a mitigator of oxidative stress (Sunda et al. 2002). Several environmental factors (nutrients, irradiance, and temperature) alter intracellular DMSP content; while little DMSP is produced in exponential growing cultures, nutrient limitations or stress causes unbalanced growth and increased DMSP cell quotas (Stefels and van Boekel 1993; Sunda et al. 2002). Grazing or viral lysis releases DMSP to the water column (Malin et al. 1993). Despite a large marine DMSP pool, only a very small proportion is ever emitted as DMS to the atmosphere, the rest being recycled within the marine food web or transported to deeper waters (Malin and Steinke 2004). *Phaeocystis* is one of few microalgal genera found to be able to enzymatically convert DMSP into the antibacterial compound acrylic acid and the volatile compound DMS (Stefels 2000). Already Sieburth emphasized the antibiotic properties of acrylic acid produced by *Phaeocystis* to the intestinal bacterial flora of animals (Sieburth 1961).

Widely reported from coastal and inland saline waters in Britain, Denmark, the Netherlands, Israel, Norway, China, and North Africa (Holdway et al. 1978; Moestrup 1994; Edvardsen and Paasche 1998; Edvardsen and Imai 2006; Johnsen et al. 2010) as well as lakes and river basins in southern United States (Baker et al. 2007; Southard et al. 2010), *Prymnesium parvum* causes serious economic losses because of its association with extensive fish kills. It produces toxins with ichthyotoxic, cytotoxic, neurotoxic, antibacterial, and allelopathic activity (Shilo 1981; Meldahl et al. 1994; Edvardsen and Imai 2006; Graneli et al. 2012), which act on biological membranes. The ichthyotoxic effect is assumed to be caused by increased permeability in fish gills resulting in disturbed ion balance and possibly in higher susceptibility to any toxic agents in the water, including other components of the *P. parvum* toxins (Yariv and Hestrin 1961; Ulitzur and Shilo 1966). Several different compounds have been ascribed the toxic effects of *P. parvum* such as proteolipids (Ulitzur and Shilo 1970), glycolipids (Kozakai et al. 1982), hemolysins (Kozakai et al. 1982), polyethers (Igarashi et al. 1996), prymnesins −1 and −2 (e.g., Igarashi et al. 1995; Igarashi et al. 1996), and recently fatty acid amides (Bertin et al. 2012a; Bertin et al. 2012b). Toxins produced by *Prymnesium polylepis* show the same type of toxicity as *P. parvum* including toxicity to the brine shrimp *Artemia* (Yasumoto et al. 1990; Meldahl et al. 1994). In 1988 an extensive bloom of this species occurred in the Skagerrak, northern Kattegat, west coast of Sweden, and coastal waters of Southern Norway (Dahl et al. 1988; Granéli et al. 1993). Both natural fauna and flora were affected severely, and 900 metric tons of caged fish were killed (Gjøsæter et al. 2000). A number of additional haptophytes, such as members of *Chrysochromulina*, *Haptolina*, *Phaeocystis*, and *Chrysotila* (*Pleurochrysis*), have been associated with fish kills or with other toxic or allelopathic activities (Edvardsen and Paasche 1998; Edvardsen and Imai 2006). Several species of *Chrysotila* and *Jomonlithus littoralis* have been shown to be toxic to the brine shrimp *Artemia salina.* Local blooms of *Chrysotila* species have been reported with some harmful effects (Houdan et al. 2004b), and also *Chrysochromulina* *parva* has been associated with fish kills (Hansen et al. 1994). A bloom (5–9 million cells L^{-1}) of *C. breviturrita* was reported to have produced lake-wide obnoxious odors in Ontario and New Hampshire, North America (Nicholls et al. 1982).

Coccolith Function and Calcification

Despite intensive research on coccolithophores, the ecophysiological function of calcification is not yet understood (Young 1994; Paasche 2002; Brownlee and Taylor 2004). It has been hypothesized that the coccosphere serves to protect against grazing and/or virus attack, but as yet no experimental proof of these hypotheses has been reported (Harris 1994; Young 1994). Coccoliths could optimize the light interception by the algal cell (Young 1994), or calcification could provide a way of dissipating absorbed light energy to avoid photodamage under nutrient limitation (Paasche 2002), but these hypotheses also remain unproven. It was suggested that cells use the coccosphere to regulate their buoyancy, because controlled sinking might provide means of escaping high irradiances or reaching depths with higher nutrient concentrations (Young 1994; Balch et al. 1996). However, calculated rates of sinking vary significantly among authors and are numerically negligible compared to the wind-driven mixing and wave turbulence. It therefore seems unlikely that a coordinated sinking in response to environmental situations is a reasonable evolutionary purpose of calcification.

There is some support from a theoretical basis for a function of calcification as a carbon-concentrating mechanism (CCM). $CaCO_3$ precipitation releases CO_2 or protons in the course of calcification according to the following reactions:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + CO_2 + H^+$$

Consequently, CO_2 derived from calcification can in principle be used in photosynthesis, or protons could be used in the conversion of HCO_3^- to CO_2 for the same purpose. In either case, this may provide the calcifying cell with a mechanism to access HCO_3^-, which represents the largest pool of inorganic carbon in seawater. Such functional coupling between calcification and photosynthesis was first proposed by Sikes et al. (1980) and has since then been discussed by various authors (Nimer and Merrett 1993; Anning et al. 1996; Buitenhuis et al. 1999).

Experimental data, however, suggest that calcification in coccolithophores is neither a prerequisite for efficient photosynthesis nor is it particularly effective in mitigating CO_2 limitation. The latter is indicated, for example, by the fact that photosynthesis continues unaffected when calcification ceases (Paasche 1964; Herfort et al. 2002; Trimborn et al. 2007), and non-calcifying haploid cells of *E. huxleyi* can photosynthesize as efficiently as or even more efficiently than the diploid calcifying ones (Rost and Riebesell 2004). Moreover, non-calcifying cells of *E. huxleyi* are equally capable of direct HCO_3^- utilization for photosynthesis, which implies that HCO_3^- utilization is not tied to calcification (Trimborn et al. 2007; Rokitta and Rost 2012). Ultimately, calcification appears unsuited to prevent CO_2 limitation in *E. huxleyi*, which is indicated by observations that the rate of photosynthesis often decreases with decreasing CO_2 concentration despite a concomitant increase in calcification rate (Riebesell et al. 2000; Berry et al. 2002; Hoppe et al. 2011).

The removal of Ca^{2+} ions from the cytoplasm is a prime necessity of literally all organisms to prevent cytotoxic effects of uncontrolled Ca^{2+} signaling. Calcification in coccolithophores may thus represent a form of "irreversible removal" from the cell (Young 1994). Recent data indeed show that, in contrast to non-calcifying phytoplankton, coccolithophores can tolerate massively increased $[Ca^{2+}]$ and maintain fitness even at concentrations 4–5fold higher than in today's oceans. Most strikingly, strains that were earlier described as "non-calcifying" started to re-calcify after ~2 weeks in high Ca^{2+} medium. Hence, the key evolutionary advantage of intracellular calcification may lie in the removal of potentially toxic Ca^{2+} ions, thus enabling cells to have a better control on the transient regulation of cytoplasmic enzyme activity (Müller et al. 2015).

Calcification and Ocean Acidification

Several future emission scenarios predict an increase from currently 400 to more than 750 µatm CO_2 for the year 2100 (IPCC scenario IS92a). As the ocean takes up CO_2, concentrations of dissolved inorganic carbon increase and the pH of seawater decreases. This ocean acidification (OA) is expected to intensify, so that pH levels will have dropped by 0.4 units relative to preindustrial values by the end of this century (Wolf-Gladrow et al. 1999). Ongoing OA will decrease saturation states for $CaCO_3$ minerals. Whereas only "true" undersaturation will cause dissolution, a lowered saturation state can already to some extent affect the biological mineralization processes, simply because $CaCO_3$ precipitation under low pH is thermodynamically less favored (Ridgwell and Zeebe 2005).

Specific differences in the sensitivity to OA do exist (Langer et al. (2006)). In *Calcidiscus leptoporus*, an optimum curve was observed with maximum calcification rates at present-day CO_2 levels, but calcification rates did not vary significantly with pH in *Coccolithus pelagicus*. In both species, photosynthetic carbon fixation rates remained constant at CO_2 levels ranging between 150 and 920 µatm. Most of our current understanding of the processes and sensitivities of calcification and photosynthesis derives from studies on *E. huxleyi* and the closely related species *G. oceanica*. Both belong to an evolutionary very young lineage of rather atypical coccolithophores in terms of structure, physiology, and ecology (Sáez et al. 2004; De Vargas et al. 2007). Different OA response patterns have also been observed between different strains of *E. huxleyi*, with strains largely showing negative or no effects on calcification at pCO_2 values expected for the end of the century (Langer et al. 2009). Overall, most studies have observed negative responses in growth, positive or no responses in biomass production, and negative or no responses in calcification under high CO_2, leading in most of the studies to a lowered PIC:POC ratio under OA (Zondervan 2007; Hoppe et al. 2011). When assessing possible responses of this group to global change, the intraspecific variability and the diversity within coccolithophores have to be acknowledged, including the different life cycle stages (haploid and diploid) because they display different morphologies and modes of calcification or no calcification and thus may differ in their sensitivity to ocean acidification.

The intracellular mechanism of calcification by coccolithophores was for a long time not well understood (Brownlee and Taylor 2004), despite the wealth of information on the effects of environmental conditions on coccolith production (see review in Paasche 2002). Recently molecular techniques have yielded insights into the functions of calcification-related genes and their responses to environmental change (Mackinder et al. 2010; Rokitta et al. 2012; Rokitta and Rost 2012). Unlike other calcifying organisms, where calcification occurs in extracellular fluids, calcite precipitation in coccolithophores takes place in intracellular vesicles (at least in the case of heterococcoliths) and hence is under control of the cell. It is therefore surprising that calcification in *E. huxleyi* shows such a strong dependency on seawater carbonate chemistry, similar to that observed in Foraminifera and corals (Gattuso et al. 1998; Wolf-Gladrow et al. 1999; Riebesell et al. 2000).

Coccolithophores have been at the center of debate about the consequences of ocean acidification. Numerous findings from laboratory and mesocosm studies (Riebesell et al. 2000; Zondervan et al. 2002; Delille et al. 2005; Hoppe et al. 2011), suggest that calcification by *E. huxleyi* will be reduced in response to OA. In most studies, the decrease in calcification was accompanied by an increase in biomass production (Hoppe et al. 2011), which has been attributed to an intracellular reallocation of acquired carbon away from the impacted calcification toward photosynthesis (Rokitta and Rost 2012). Under OA, multiple parameters of the carbonate system change, which affect the different physiological processes in various ways. The cell-integrated changes in calcification and photosynthesis under elevated pCO_2 have typically been ascribed to the decrease in carbonate ion concentration and thus calcite saturation levels (Cyronak et al. 2015), but more recent and systematic investigations could show how exactly the particular processes are affected by the isolated parameters of the carbonate system. To elucidate the effects of the different parameters of the carbonate system, Bach and coworkers have uncoupled the carbonate chemistry in their experiments (Bach et al. 2011; Bach et al. 2013). They were able to show that growth, biomass production, and carbon acquisition are primarily controlled by CO_2 supply at low (subsaturating) levels, whereas at higher concentrations, they are adversely affected by the concomitant decrease in pH. Further (also transcriptomic) evidence suggests that calcification of scales varies with HCO_3^- supply and is impaired by low pH (Bach et al. 2013). Despite some differences between coccolithophore species and strains, these authors derived a unifying mathematical concept that robustly describes the dependence of calcification rates on carbonate chemistry speciation (Bach et al. 2015).

The precipitation of $CaCO_3$ and the consequential shift in carbonate chemistry inevitably cause acidosis in the cytoplasm of the cells. This is prevented by rapid H^+

effluxes via voltage-gated H^+ channels that are situated in the plasma membranes of many eukaryotes. The increased acidity under OA causes a less steep proton gradient, therefore impairing channel gating and leading to overall higher $[H^+]$ in the cytoplasm of cells (Suffrian et al. 2011; Taylor et al. 2011). The impairment of calcification leads to an intracellular reallocation of inorganic carbon toward the competing reaction, biomass production. This happens in such way that OA often does not significantly affect production of total carbon, i.e., biomass and calcite (Zondervan et al. 2002; Feng et al. 2008; Langer et al. 2009). This effect of carbon reallocation from calcification toward photosynthesis seems to be dependent on light intensity (Rokitta and Rost 2012). Under high light conditions, cells can mitigate and prevent the effects of OA, whereas under limiting light intensities, the reallocation effects are large. This modulation of OA effects by light intensity is accompanied by metabolic reconstellations, in which reductive, anabolic pathways, for example, may synthesize glucans and fatty acids to be upregulated over oxidative, catabolic pathways such as glycolysis and respiration (Rokitta et al. 2012). Cellular redox levels appear to be the informational “hub” that mediates the modulation of OA responses by integrating light intensity into cellular organic carbon metabolism.

In general, a reduction in the degree of calcification is assumed to put coccolithophores at an ecological disadvantage, suggesting a rather “grim future” for this phytoplankton group. This view is derived mostly from short-term acclimation studies over several generations. However, there are also new lines of evidence regarding the midterm and long-term adaptability to OA. Lohbeck et al. (2011) have grown monoclonal cultures of *E. huxleyi* under OA scenarios for ~500 generations and observed that these strains were still affected by OA, but exhibited higher calcification rates than unadapted strains when confronted with OA. This implies that evolution occurring at timescales of months may help to maintain calcification as a cellular function under global change. Data from present-day phytoplankton assemblages and sediment cores covering the last 40,000 years indicate, however, that changes in carbonate chemistry impose a massive selective pressure on the degree of calcification and induce floristic shifts from heavily calcified to less calcified species and strains with increasing CO_2 levels (Beaufort et al. 2011). In the Chilean upwelling system, a rare but distinct over calcified morphotype of *E. huxleyi* thrives under permanently low pH conditions, suggesting that this species is in general able to cope with and adapt to changing ocean acidity.

Characterization and Recognition

Cell Shape and Organization

Unicellular haptophytes exhibit a variety of cell shapes (Fig. 1). In the Coccolithophyceae, cells may be more or less spherical as in *Dicrateria* (Fig. 1e) and many coccolithophores, e.g., *E. huxleyi* and *C. pelagicus* (Figs. 1j and 7a). Saddle-shaped species are confined to the *Chrysochromulina* species (*C. campanulifer*a and

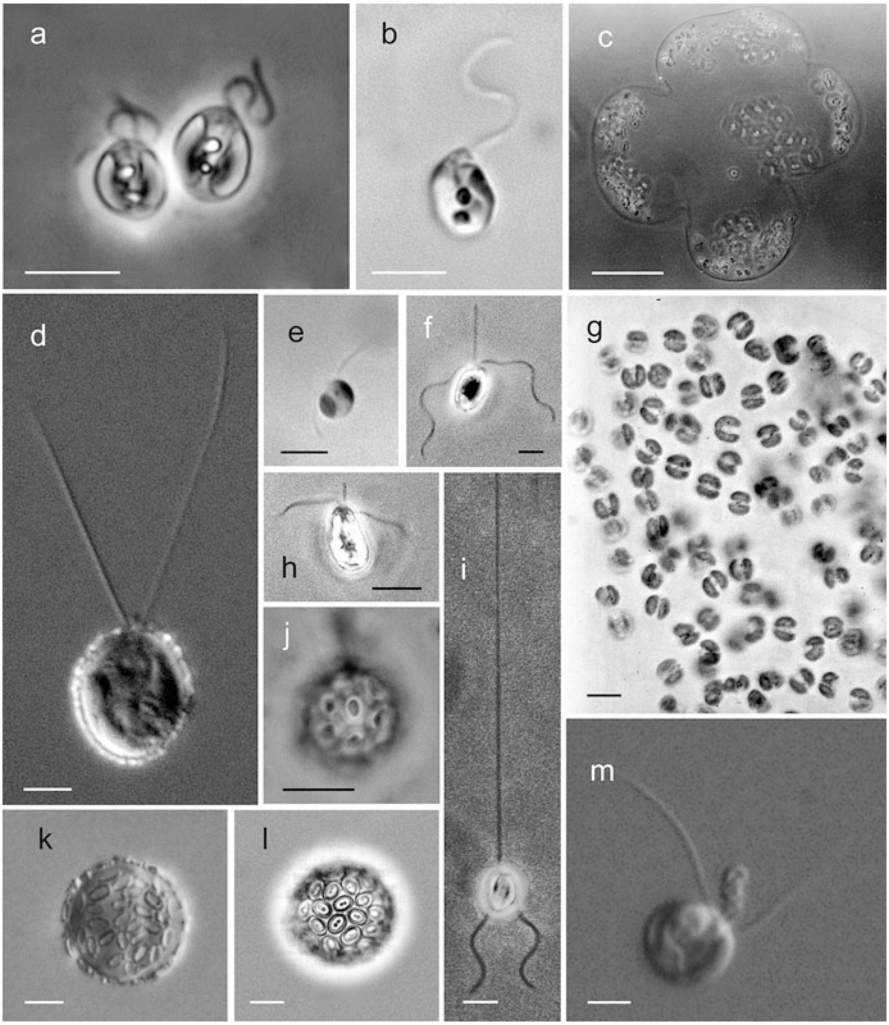


Fig. 1 Light micrographs of representatives of the Haptophyta. (**a**, **b**) Pavlovophyceae, (**a**) *Pavlova*, (**b**) *Rebecca*; (**c**–**m**) Coccolithophyceae, (**c** and **g**) *Phaeocystis pouchetii* colonies; (**d** and **k**) *Chrysotila carterae*; (**e**) *Dicrateria rotunda*; (**f**) *Prymnesium polylepis*; (**h**) *Prymnesium parvum*; (**i**) *Chrysochromulina simplex* with extended haptonema; (**j**) *Emiliania huxleyi*; (**k**) *Chrysotila carterae*; (**l**) *Coronosphaera binodata*; (**m**) *Chrysochromulina campanulifera* with coiled haptonema. Scale bars 5 μm Figs. **a**–**b**, **d**–**m**; Fig. **c**, 100 μm

C. rotalis, Fig. 1l) in which the flagella and haptonema (the appendages) are inserted ventrally. Elongate forms are found in *Prymnesium* (Fig. 1f, h), *Haptolina*, and *Chrysocampanula* and some coccolithophores such as *Calciopappus caudatus* and *Syracosphaera pirus*. Many species such as *Haptolina brevifila*, *Dicrateria rotunda* (Fig. 1e), the motile stage of *E. huxleyi*, and *Calyptrosphaera sphaeroidea* have their appendages inserted in a polar position, sometimes in association with clusters of spine scales (coccoliths) as can be seen in *Michaelsarsia elegans*. Clusters of spine scales may be located both at the apex and antapex as in *Calciosolenia murrayi* and *Acanthoica quatrospina. Ophiaster hydroideus* has spines only at the antapex. Spines may also cover the entire cell body as in *Haptolina ericina* and *Rhabdosphaera clavigera*. In *Prymnesium*, *Isochrysis*, *Chrysoculter*, and *Chrysotila* species, the appendages are usually inserted sub-anteriorly. The appendages may arise from a depression as in *Corymbellus aureus* and the large, amoeboid cells of *Prymnesium pigrum.*

In the class Pavlovophyceae, cells may be variously shaped, but are almost always flattened with the appendages arising sub-anteriorly on the ventral side. In *Diacronema lutheri*, the insertion of the appendages is almost in the center of the ventral face (Green and Hibberd 1977; Bendif et al. 2011). Species of *Isochrysis*, *Ruttnera*, and the Pavlovophyceae have an asymmetric appearance that is accentuated by the possession of only one plastid, whereas most members of the Haptophyta appear to have two (rarely four, *Dicrateria inornata*). There are a few instances of colonial, stalked, and filamentous stages within the Coccolithophyceae. In *Phaeocystis* (Fig. 1c, g), several species form palmelloid colonial stages. *Phaeocystis* are commonly observed as gelatinous bladders that, in their younger condition, are roughly spherical and up to a few millimeters in diameter, but which later become irregular in form and may be several centimeters in their longest dimension. The cells themselves are small (approximately 3–8 μm diameter) and are arranged in a layer at the periphery. They are separated from each other and with no apparent connection between them. The exact nature of the colorless bladder is not known, but from its physical properties, it seems the surface has some of the elastic properties of a membrane and the matrix, while holding the cells in position, is apparently semiliquid, dispersing readily once the bladder is punctured (Kornmann 1955; Zingone et al. 2011). Benthic palmelloid forms are also known from cultures (Gaebler-Schwarz et al. 2010).

Motile colonies are seen in *Corymbellus aureus* in which the flagellated cells are attached to each other laterally and the colony has an annular shape (like a doughnut ring). In the motile colonies of *Prymnesium radiatus*, the cells are attached to each other at the posterior end and the colony has a ball shape with the flagella extending outward (Sym et al. 2011).

Ruttnera lamellosa and *Chrysotila stipitata* form benthic colonies made by concentric layers (*Ruttnera*) or stalks (*Chrysotila*) of gelatinous material. The motile cells of *R. lamellosa* are asymmetrical and *Isochrysis*-like, but after they have settled, they become spherical with a thick, lamellate mucilage sheath (Andersen et al. 2014). The benthic “Apistonema” stage of *Chrysotila* has cell walls composed of organic scales in a cementing substance and forms branched filaments. The motile cells bear coccoliths (Pienaar 1994). Nonmotile palmelloid cells have been reported in a number of species. Parke et al. (1955) described palmelloid cells in cultures of *Chrysochromulina* species, and nonmotile mucilage-embedded stages are known in *Ochrosphaera* (Fresnel and Probert 2005) and several species of *Pavlova* (Bendif et al. 2011).

Flagella, Transition Zone, and Flagellar Roots

In the Pavlovophyceae, the two flagella are markedly unequal, the more posteriorly inserted one is much shorter than the anterior flagellum. In *Rebecca salina*, the short flagellum is reduced to a short stump, detectable only with electron microscopy. The longer flagellum in the Pavlovophyceae usually carries a covering of fine hairs and small, dense, knob-like scales (Fig. 6a). The fine hairs do not resemble the tubular hairs (mastigonemes) of the heterokont algal groups in their structure and consist only of a fine non-tubular thread (Green 1980; Green and Hori 1994; Bendif et al. 2011). The knob scales (Fig. 6a) are often regularly arranged on the longer flagellum as in *Pavlova gyrans*, but in *Diacronema lutheri*, they are irregularly arranged and they are totally absent in *Exanthemachrysis* and *Diacronema vlkianum* (Bendif et al. 2011).

Coccolithophyceae flagella when present are more or less equal and naked. In flagellated species, two flagella are common, but *Chrysochromulina quadrikonta* has four (Kawachi and Inouye 1993).

The flagellar action may be homo- or heterodynamic. In Pavlovophyceae, the longer flagellum is directed forward with respect to the direction of swimming and beats with a sinuous S-like beat. The shorter flagellum is directed posteriorly, away from the cell body, and beats with a stiff, inflexible action. In swimming Coccolithophyceae cells, the flagellar pole may be in front with the flagella moving along the cell surface, or posterior with the flagella directed away from the cell because the smooth flagella always push the cell. The action of the flagella may be homodynamic and display an undulating motion as in *Chrysochromulina*, *Haptolina*, and many Coccolithales or be heterodynamic as in *Chrysocampanula* and many *Prymnesium*.

The axoneme consists of the usual “9 + 2” arrangement of microtubules except where reduction has taken place as in the short posterior flagellum of *Rebecca salina* in which the axoneme consists only of a ring of nine single microtubules (Green 1976). At the proximal end of the normal flagellum, there is a short transition region in which there are two transverse partitions, the more distal often appearing rather diffuse and thickened in the center. The central pair of axoneme microtubules stops at this partition. The double partition structure appears to extend throughout the phylum (Green and Hori 1994), having been recorded in *Chrysochromulina* (Eikrem and Moestrup 1998), *Haptolina* (Eikrem and Edvardsen 1999), *Prymnesium* (Manton 1964b; Green and Hori 1990), *Phaeocystis* (Parke et al. 1971), and *Dicrateria* (Green and Pienaar 1977), although in species of *Isochrysis* (Hori and Green 1991), *Hymenomonas*, and *Chrysotila* (*Pleurochrysis*) only the proximal partition is present. A helical band is located distally to it in the flagella in *Chrysotila carterae* (Beech and Wetherbee 1988) and *Hymenomonas coronata* (Roberts and Mills 1992). Tiers of tubular rings may also be present proximal to the axosome (Green and Hori 1994; Billard and Inouye 2004). In *Prymnesium*, tubular rings are present in addition to the double partition structure (Birkhead and Pienaar 1994, 1995). Some Pavlovales appear to have one partition only in the posterior flagellum (Green and Hibberd 1977). *Chrysoculter rhomboides* and *Diacronema vlkianum* are

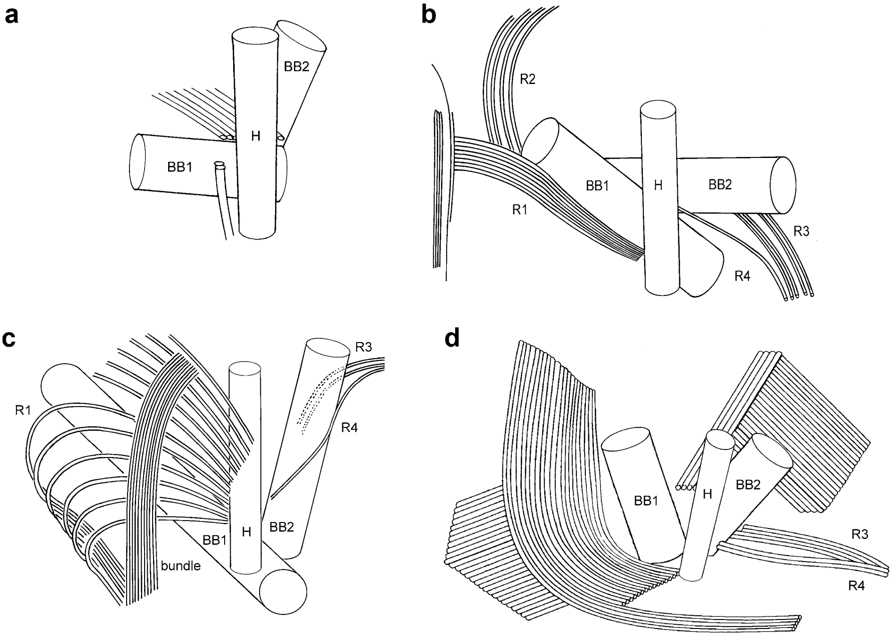


Fig. 2 Schematic three-dimensional reconstruction of the flagellar apparatus with microtubular roots of the Haptophyta: (**a**) *Diacronema*; (**b**) *Chrysochromulina scutellum*; (**c**) *Prymnesium palpebrale*; (**d**) *Chrysotila*

unusual in that the shorter posterior flagellum has a swelling on the side adjacent to the cell body (Green and Hibberd 1977).

The flagella bases and flagellar roots anchor the flagella within the cell and are components of the cell's cytoskeleton as well as being involved in cellular functions, such as mitosis. Both microtubular (Fig. 2) and fibrous flagellar roots are recorded in the Haptophyta and the microanatomy of the flagellar apparatus is believed to offer phylogenetic information because its morphology appears to evolve very slowly. Coccolithophyceae flagellar roots R1 and R2 are associated with the left flagellum and R3 and R4 with the right flagellum (see Eikrem and Moestrup 1998). R1 originates close to the base of the haptonema. The roots are numbered in a clockwise direction starting at the left flagellum with R1 (Green and Hori 1994). Over generations, the right flagellum and its basal body develop into the left flagellum. The left flagellum is termed the mature flagellum and it corresponds to the longer flagellum; the right flagellum is shorter and will transform into a mature flagellum in the next generation (Beech et al. 1988).

Inouye and Pienaar showed that the coccolithophores, *Umbilicosphaera foliosa*, and a species of *Chrysotila* (*Pleurochrysis*) have a complex root system with some components consisting of as many as two hundred microtubules (Inouye and Pienaar 1984; Inouye and Pienaar 1985). Such roots have been termed compound roots and consist of a sheet of microtubules and a closely packed bundle of numerous microtubules. The bundles are termed crystalline roots (CR1, CR2) and are associated with the roots (R1, R2) of the basal body of the left flagellum (Fig. 2d). Some species have crystalline roots in both R1 (termed CR1) and R2 (termed CR2), whereas others have only one of them (Billard and Inouye 2004). Crystalline roots (Fig. 2c) are also found in *Isochrysis* (Hori and Green 1991) and *Prymnesium* (Birkhead and Pienaar 1994; Birkhead and Pienaar 1995).

Syracosphaera pulchra (Inouye and Pienaar 1988), *Algirosphaera robusta* (Probert et al. 2007), and the saddle-shaped species of *Chrysochromulina* seem to lack crystalline roots, and the R1 of saddle-shaped *Chrysochromulina* species (Fig. 2b) often consist of less than ten microtubules (Moestrup and Thomsen 1986; Eikrem and Moestrup 1998; Jensen and Moestrup 1999). In non-saddle-shaped Prymnesiaceae species, they may number more than 20 and form a broad sheet of microtubules.

The cytoplasmic tongue (Beech and Wetherbee 1988) is a complex formed by some microtubules of the R1 sheet and the fibrous root originating on the left basal body. It is delineated by an extension of the peripheral endoplasmic reticulum and extends deep into the cell in a narrow space of cytoplasm (Billard and Inouye 2004) in several *Chrysotila* species (Gayral and Fresnel 1983; Beech et al. 1988; Fresnel and Billard 1991) and *Prymnesium palpebrale* (Birkhead and Pienaar 1995). In *Prymnesium nemamethecum*, it is present, but reduced (Birkhead and Pienaar 1994).

R2 may be compound, but only a few microtubules constitute the sheet of R2, and it originates between the basal bodies close to the left basal body. Possible homologies between the complex and simple systems have been suggested (Inouye and Pienaar 1984; Moestrup and Thomsen 1986; Green and Hori 1994). Roots R3 and R4 are never compound and consist of only a few microtubules. R3 originates from the right side of the right basal body and R4 from the left. Accessory and connecting fibers link the flagellar bases and the haptonema base. They may appear striated or nonstriated in electron micrographs of thin sections (Green and Hori 1994; Pienaar 1994). The fibrous root associated with the cytoplasmic tongue is the most prominent.

The flagellar roots found in the Pavlovophyceae are different. Two microtubular roots, R1 and R2, with only a few microtubules are accompanying the basal body of the short flagellum. A conspicuous fibrous root is associated with the basal body of the long flagellum extending deep into the cell close to the nucleus. Accessory and connecting fibers are present (Green and Hori 1994).

Haptonema

In its most extreme form, the haptonema is very long, often many times the diameter of the cell body in length as in *Chrysochromulina rotalis* (Fig. 1i). In *C. strobilus*, the extended haptonema may be up to 100 μm in length, the cell diameter being only approximately 8 μm. The reduced haptonema of *Isochrysis* and *Chrysotila* is very short (1–2 μm) and barely detectable with light microscopy. In *Dicrateria rotundata*, reduction is more extreme such that the haptonema is represented by at most a small

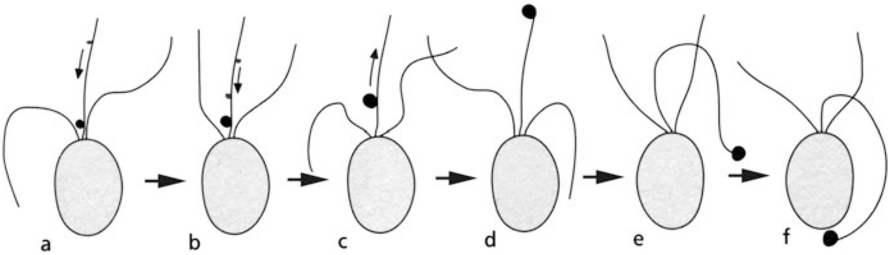


Fig. 3 Schematic interpretation of nutrient particle uptake studies in *Haptolina hirta* by Inouye and Kawachi (1994) from Graham and Wilcox (2000): (**a**–**b**), food particles captured by the distal part of the haptonema and transported along the haptonema surface to aggregate in a larger particle on the proximal part; (**c**–**d**) food particle aggregates move to the haptonema tip; (**e**–**f**) haptonema manipulating the food aggregate to be deposited on the posterior cell surface where it will be engulfed

elevation between the flagella. Between these extremes may be seen a variety of haptonema lengths. In some taxa, the haptonema coils (*Haptolina ericina*, *Chrysochromulina campanulifera*), whereas in others (*Prymnesium parvum*, *Phaeocystis pouchetii*), the haptonema flexes, but without any organized pattern of movement.

The haptonema may function as a feeding organelle (Inouye and Kawachi 1994; Kawachi and Inouye 1995), and the process is demonstrated in Fig. 3. In swimming cells of *Haptolina hirta* with the haptonema directed forwardly and the flagella alongside the cell, prey particles adhere to the haptonema. They are transported to a point ca 2 μm from the base where an aggregate of prey is formed. The aggregate is moved to the tip of the haptonema and the haptonema bends to deliver it to the surface of the rear end of the cell where it is phagocytized into a food vacuole.

In both long and short haptonema-bearing species, the cell may attach to the substrate (slide, cover glass) either at the tip or, in the case of a long haptonema, by any point along its length. Attached haptonema may show gliding or sliding movements in a sinuous manner, whereas unattached, extended haptonema may demonstrate bending or flicking movements. Coiling often occurs at contact with an obstacle. The most spectacular movements, however, are seen in the coiling and uncoiling of long haptonema of *Chrysochromulina* species. Coiling is a very rapid process and may take only 1/60–1/100th of a second, whereas the uncoiling process is much slower and may take between 2 and 10 s. Coiling is usually initiated from the haptonema tip, and the direction and orientation of coiling and number and diameter of gyres is predetermined (Parke et al. 1971; Leadbeater 1971a; Inouye and Kawachi 1994). Coiling is believed to be the result of an influx of Ca + from the medium in which the cells live and into the lumen of the haptonema (Gregson et al. 1993; Inouye and Kawachi 1994).

The fine structure of the haptonema (Fig. 4) is quite unlike that of the flagella. The plasmalemma of the free part typically encloses a fenestrated cylinder of endoplasmic reticulum (ER) within which there is a ring of 6 or 7 single microtubules. The

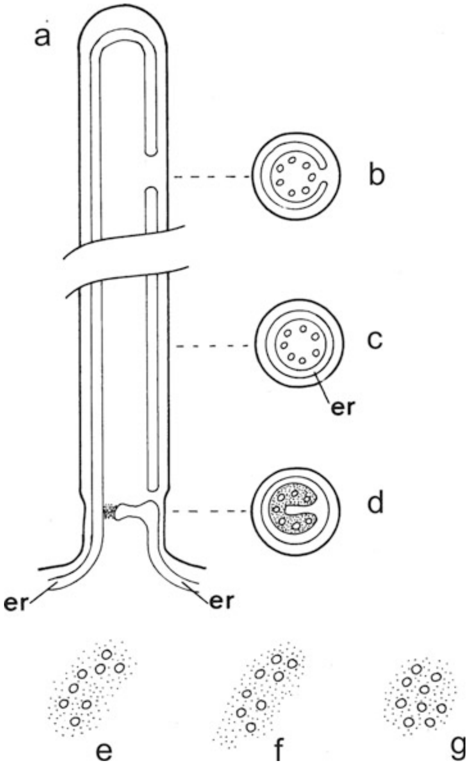


Fig. 4 Schematic view of a haptonema: (**a**) longitudinal section with endoplasmic reticulum, microtubuli not shown; (**b**–**d**) transverse section at indicated positions showing endoplasmatic reticulum and microtubuli; (**e**–**g**) microtubuli within the cell. Abbreviation: *er*, endoplasmic reticulum

plasmalemma of the haptonema is confluent with that of the cell body, and the ER is continuous with the peripheral ER of the cell. Toward the proximal end of the haptonema, the ring of microtubules becomes reoriented into an arc facing one of the flagella, with the ER cisterna lying on the outer curved side of the arc. Immediately beneath this level, the ER once again forms a complete cylinder, but with a flat-ended, fingerlike component projecting inward; in transverse section, the projection appears to be capped by the arc of microtubules. On entering the cell, additional microtubules appear and further rearrangements of the microtubules take place to form two superposed arcs of 4 microtubules each, followed in some cases by the addition of a ninth microtubule (Manton 1964b; Moestrup and Thomsen 1986; Billard and Inouye 2004).

Reduced haptonema are known in many Coccolithophyceae species, such as the short bulbous haptonema of many *Chrysotila* species (Manton and Peterfi 1969; Billard and Inouye 2004). In *Isochrysis* and *Ruttnera*, the haptonema is reduced to a small protrusion containing ER profiles and only three microtubules, increasing to four within the cell. In *Emiliania huxleyi* and *Dicrateria inornata*, there is no trace of a haptonema (Klaveness 1972; Green and Pienaar 1977).

In the Pavlovophyceae, the free part of the haptonema is short and the number of microtubules reduced (only one to four microtubules), although the number increases to seven or eight inside the cell (Green and Hibberd 1977; Gayral and Fresnel 1979; Inouye and Kawachi 1994). The ER is strongly fenestrated and may be reduced to a small vesicle on one side of the organelle (Green 1980).

Haptonemal roots have been described in a few species of the Pavlovophyceae where they appear as fibrous structures originating at the proximal end of the haptonema microtubules (Green and Hori 1994). Haptophyte haptonema microtubules may also be attached to one of the kinetids by a fibrous connection (Manton 1964a; Moestrup and Thomsen 1986; Green and Hori 1994).

Plastid, Nucleus, Golgi Apparatus, Pyrenoids, and Stigma

Haptophyta cells normally possess 1–2 plastids containing three thylakoid lamellae and there is no girdle lamella (Fig. 5). Pyrenoids may be immersed within the plastid and penetrated by one or a few pairs of thylakoids (Fig. 5c), but in some genera, they may bulge from the inner face of the plastid. The plastid and pyrenoid are surrounded by endoplasmic reticulum confluent with the nuclear envelope (Fig. 5b, c), the nucleus itself usually lying close to the plastid (Pienaar 1994). Coccolithophyceae cells usually lack a stigma, but an autofluorescent substance has been associated with the flagella in several species (Kawai and Inouye 1989).

A stigma or “eyespot” consisting of a single layer of lipid globules occurs in many Pavlovophyceae (Fig. 5d), although its position is variable. It is absent in *Diacronema ennorea* and *Rebecca helicata*. In some, it lies on the inner face of the

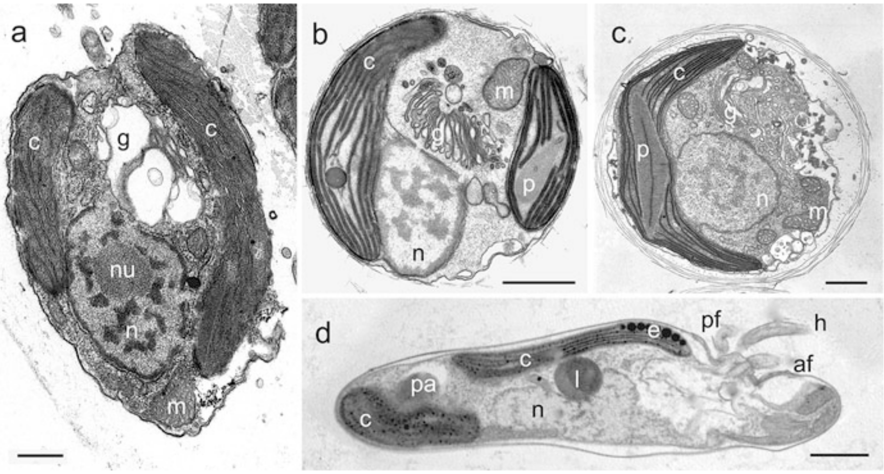


Fig. 5 Ultrastructure, TEM sections. (**a**) *Phaeocystis antarctica*, (**b**) *Chrysochromulina throndsenii*, (**c**) *Calyptrosphaera sphaeroidea*, (**d**) *Diacronema noctivaga*. Abbreviations: c, chloroplast; n, nucleus; nu, nucleolus; pf, posterior flagellum; af, anterior flagellum; h, haptonema; p, pyrenoid; l, lipid droplet; m, mitochondrion. Scale bars 5 µm (Image of *Phaeocystis* by courtesy of Adriana Zingone, *Calyptrosphaera* by Dag Klaveness and *Diacronema* by Madhi Bendif)

plastid close to the anterior flagellum, but in *Diacronema lutheri* and *D. vlkianum*, it is found on the outer face of the plastid lying beneath the shorter flagellum (Green 1980; Van Lenning et al. 2003; Bendif et al. 2011).

The mitochondrion has tubular cristae and is probably single and highly reticulated (Beech and Wetherbee 1984). The Golgi apparatus consists of a single dictyosome (Fig. 5b) and lies between the nucleus and the kinetids. In sections, the cisternae are arranged in a fan formation with the edges of the cisternae closer and less dilated on the side of the stack nearer the kinetids. The central parts of several adjacent cisternae may be inflated in a manner that seems to be unique to members of the Coccolithophyceae (Manton 1967; Hibberd 1980; Pienaar 1994) and may be concerned with carbohydrate polymerization during scale formation (Romanovicz 1981).

Storage Metabolites, Lipids, and Sterols

The primary storage metabolite of most members of the Haptophyta is assumed to be the water-soluble 1–3 glucan chrysolaminarin, as demonstrated for *Phaeocystis globosa* motile cells (Janse et al. 1996) and *Emiliania huxleyi* (Vårum et al. 1986). In the Pavlovales, another 1–3 glucan, the solid paramylon, commonly found in the euglenoids, has been identified by X-ray analysis (Kreger and Van der Veer 1970). Lipid bodies are also commonly present in the Haptophyta. Lipids and sterols found in the Haptophyta have been reviewed by Conte et al. (1994) and Marlowe et al. (1984). Many Isochrysidales species are unusual as they produce long-chain polyunsaturated C_{36} fatty acids and C_{37}–C_{39} sterols and diverse other neutral lipid compounds that can be collectively referred to as “polyunsaturated long-chained (C_{37}–C_{39}) alkenones, alkenoates, and alkenes” (PULCAs) (Eltgroth et al. 2005). The relative composition of the latter compounds, their degree of unsaturation, and their ^{13}C and ^{2}H signatures are often well correlated with environmental parameters such as temperature, salinity, and also CO_2 concentration (Marlowe et al. 1984; Pagani 2002; Van der Meer et al. 2007). Additionally, they are comparably resistant to digenesis in sediments (see Rontani et al. (2013) for a recent review), which makes PULCAs a tool widely used by geologists for paleo-reconstructions of temperature, salinity, and CO_2 (Conte et al. 1998; Pagani 2002; Beltran et al. 2007; Liu et al. 2008). These genera also have particularly high concentrations of the sterol 24-methylcholesta-5,22E-dien-3-ol, which occurs as well in some other coccolithophores but has not been recorded in the Prymnesiales taxa examined and only occurs in low concentrations in the Pavlovophyceae. Other sterols occurring in significant concentrations, though not universally distributed throughout the class, include cholest-5en-3-ol (cholesterol), 24-methylcholest-5-en-3-ol, 24-ethycholesta-5,22E-dien-3-ol, and 24-ethylcholest-5-en-3-ol. Well-known PULCA producers include the Isochrysidales species *Emiliania huxleyi*, *Gephyrocapsa oceanica*, and *Isochrysis galbana* (Conte et al. 1998); PULCA production has also been observed in the benthic lacustrine *Ruttnera* (Sun et al. 2007). Biochemically, these compounds derive from specialized cellular lipid synthetic pathways, possibly similar to polyketide synthesis, as can be interpreted on the basis of genomic evidence (Read et al. 2013) and gene expression patterns (Rokitta et al. 2011). PULCAs are typically deposited in cytoplasmic liposomes and may function as sinks of cellular reductive energy and carbon; this latter function may especially be important in situations when growth is arrested, under nutrient starvation (Eltgroth et al. 2005). In experimental mesocosm blooms, cells of *E. huxleyi* accumulated up to 6 pg. PULCA cell^{-1} during exponential phase growth, a significant portion of the cellular biomass (Benthien et al. 2007). In addition, PULCAs appear also to serve as energy stores as they can be degraded in the dark (Eltgroth et al. 2005).

Pigments

Haptophytes have high pigment diversity. Chl *a* and the accessory pigments divinyl protochlorophyllide (MgDVP), Chl c_2, diadinoxanthin, diatoxanthin, and β,β-carotene comprise the common haptophyte pigments (Van Lenning et al. 2004). The haptophytes can then be divided into eight pigment types based on the content of nine chl *c*-type pigments (DV-Chl c_{PAV}, MV-Chl c_{PAV}, DV-Chl c_3, MV-Chl c_3, MgDVP, Chl c_2, Chl c_1, Chl c_2-MGDG$_{Ehux}$, Chl c_2-MGDG$_{Cpoly}$) and five fucoxanthin derivatives (Unk-Fx, 4kFx, Bfx, HFx, 4kHFx) (Van Lenning et al. 2003; Van Lenning et al. 2004; Zapata et al. 2004). Among the coccolithophores, the pigment profiles follow large phylogenetic groups and/or ecological preferences (Van Lenning et al. 2004). The pigment profiles of the class Pavlovophyceae agrees with SSU rDNA phylogenies and some ultrastructural features (Van Lenning et al. 2003). The pigment types within the non-calcifying order Prymnesiales do, however, not appear to correlate with phylogenetic groups (Zapata et al. 2001; Edvardsen et al. 2011).

The fucoxanthin derivative 19-hexanoyloxyfucoxanthin (HFx) was suggested as a pigment marker to determine the contribution of haptophytes to phytoplankton assemblages (Everitt et al. 1990), but was later found to be absent in the coastal families Isochrysidaceae, Pleurochrysidaceae, and Hymenomonadaceae and to be present in some dinoflagellates with plastids of haptophyte origin such as species of *Karenia* and *Karlodinium* (Berger et al. 1977), as well as in the ochrophytes *Dictyocha speculum* (Daugbjerg and Henriksen 2001) and *Pseudochattonella farcimen* (Edvardsen et al. 2007). The chemotaxonomic approach should be based on the combined use of chlorophylls and carotenoids to define pigment types (Seoane et al. 2009) and validated by microscopy or molecular species identification.

Scales

The unmineralized scales of the Coccolithophyceae (Fig. 6d–q) are composed of microfibrils (see Leadbeater 1994 and references therein) usually arranged in two layers where the proximal face (facing the cell membrane) has a radial pattern of

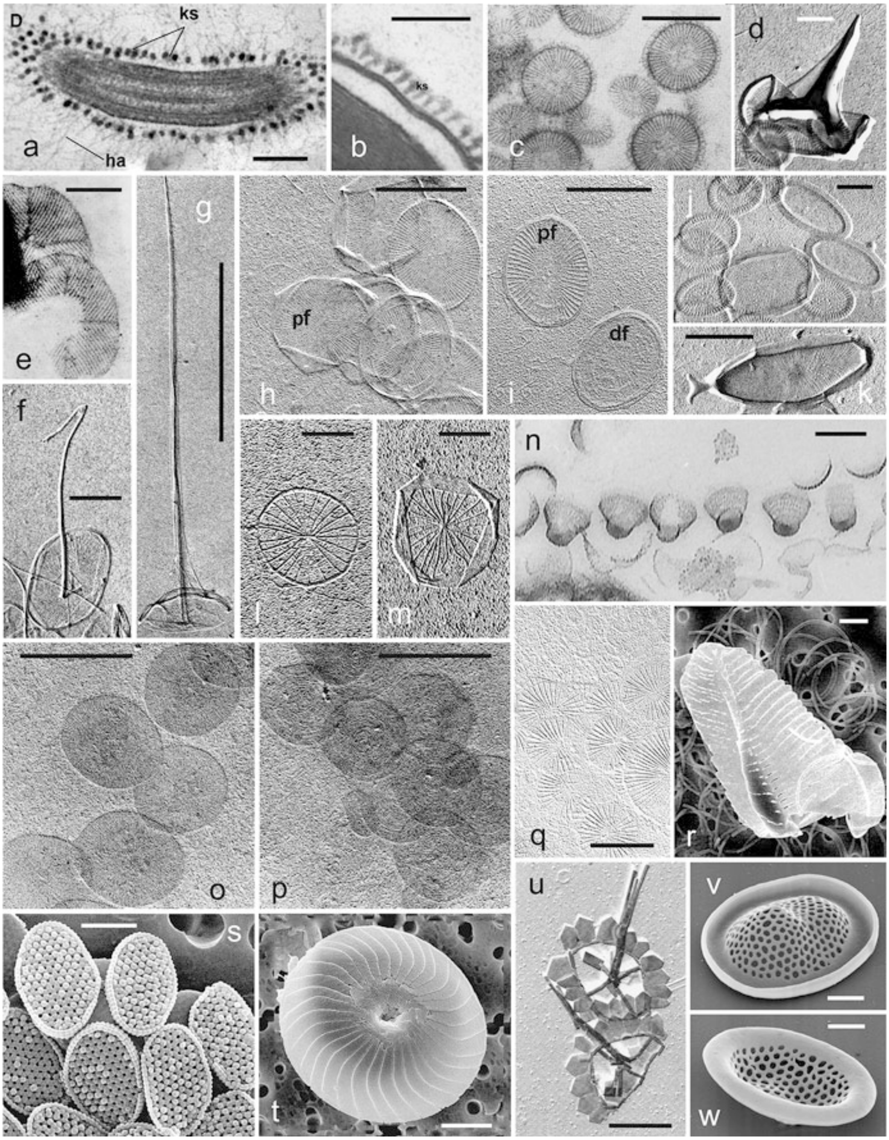


Fig. 6 Scales and coccoliths in Haptophyta. (**a**–**b**) Pavlovophyceae: (**a**) *Pavlova pinguis*, knob scales on flagella; (**b**) cell surface with knob scales in *Rebecca salina*. (**c**–**w**) Coccolithophyceae: (**c**–**d**) *Phaeocystis*, (**c**) small and large body scales; (**d**) *Prymnesium polylepis*, spine scale aberrant type. (**e**) *Emiliania*, body scale covering flagellate stage; (**f**–**g**) *Haptolina hirta*, (**g**, **h**) body scales; *H. fragaria*, (**i**) body scales; *Chrysochromulina scutellum*, body scales; (**j**, **k**) *Prymnesium polylepis*, (**j**) flat body scales, (**k**) scale with fishtail like extension. (**l**, **m**) *Dicrateria rotunda*: (**l**) flat body scale, (**m**) body scale with raised rim; (**n**) *Chrysochromulina campanulifera* cup scales. (**o**, **p**) *Calyptrosphaera sphaeroidea*: (**o**) body scale proximal side, (**p**) body scale distal side; (**q**) *Chrysochromulina simplex* body scales; (**c**) *Ceratolithus cristatus* nannolith-ceratolith. (**s**, **t**) *Calcidiscus leptoporus*: (**s**) holococcoliths, crystallolith (*Crystallolithus rigidus* stage), (**t**)

microfibrils, often arranged into quadrants (Fig. 6e, h), whereas the distal face is more variable with spiral and interwoven patterns that are common and modifications often form spines, cylinders, or cup-shaped structures (Fig. 6d, g, n). The distal face may have marginal thickenings as in the plate scale of *Chrysochromulina campanulifera* or more substantial rims that can be inflexed as in *Prymnesium polylepis* (Fig. 6j) or upright as in outer layer scales of *Chrysochromulina throndsenii* and *Dicrateria rotunda (*Fig. 6m). The scales may be arranged in one or more layers and with scales bearing spines (*Haptolina hirta*) (Fig. 6f, g), cups (*C. campanulifera*) (Fig. 6o), or cylinders (*C. microcylindra*) constituting the outer layer when present. Scale form and ornamentation are important taxonomic characters at the species level. In *Prymnesium neolepis*, both organic scales and silicified scales (Fig. 6v, w) are present (Yoshida et al. 2006).

Scales occur in a variety of structures from apparently simple plates, such as those found in *Isochrysis* (Green and Pienaar 1977), *Chrysotila*, and the motile stage of *Emiliania huxleyi* (Fig. 6e), to scales formed into spines that can be elaborated as in the alternate stage of *Prymnesium polylepis* (Fig. 6d) and sometimes of considerable length as in *Haptolina ericina* (9–15 μm long). Spines are often formed by hypertrophy of the distal face of the scale, either wholly or in part, such as the long spines of *H. ericina* (Manton and Leedale 1961) or *Chrysochromulina mantoniae* (Leadbeater 1972). Some spines are closed as in *Haptolina* (Fig. 6f, g) whereas others such as those covering *H. ericina* and *Chrysocampanula spinifera* are open-ended tubes (Manton and Leedale 1961; Pienaar and Norris 1979). Lateral development of the scales is shown in the authentic stage of *Prymnesium polylepis* in which there are a variety of oval plate scales, some of which have fishtail extensions at one end (Fig. 6j, k).

The scales are usually distributed over the entire cell surface, and where there is more than one type of scale, these may be in discrete layers. In *C. campanulifera*, the cuplike scales form a distal layer overlying the plate scales (Manton and Leadbeater 1974), and in *Phaeocystis globosa* (Fig. 6c) and *Dicrateria rotunda* (Fig. 6l, m), the scales with erect rims overlie the scales without such rims. However, the scales are not always evenly distributed and examples of this may be seen in the spine scales of *Chrysochromulina mantoniae*, which occur in clusters at the poles of the ovoid cells, or the very small scales found only on the haptonema in *Isochrysis* species (Green and Pienaar 1977) and in *Prymnesium nemamethecum* (Pienaar and Birkhead 1994).

Fig. 6 (continued) heterococcolith, placolith; (**u**) *Papposphaera sarion*, heterococcolith, pappolith; (**v**, **w**) *Hyalolithus neolepis*, silicified body scales. Abbreviations: pf, proximal face; df, distal face. Scale bars Figs. **a**–**c**, 0.2 μm; Fig. **d**, 1 μm; Fig. **e**, 0.2 μm; Fig. **e**, 1 μm; Fig. **f**, 0.5 μm; Fig. **g**, 10 μm; **h**–**i**, Fig. 0.5 μm; Fig. **j**–**k**, 1 μm; Fig. **l**–**n**, 0.2 μm; Figs. **o**–**p**, 1 μm; Fig. **q**, 0.5 μm; Figs. **r**–**s**, 1 μm; Fig. **t**, 2 μm; Figs. **u**–**w**, 1 μm. TEM images of knob scales in *Pavlova* and *Rebecca,* by courtesy of Madhi Bendif, *Emiliania* and *Calyptrosphaera* scales by Dag Klaveness and pappolith by Helge A. Thomsen. SEM images of holococcolith, placolith, and ceratolith, by LLuïsa Cros. Silicified scales, by courtesy of Masaki Yoshida

The cells in the filamentous "Apistonema" stage of Chrysotila have several layers of tightly packed scales (Leadbeater 1971b; Pienaar 1994).

Pavlovophycean scales are small spherical or clavate dense bodies (6 a, b) known as "knob scales," produced in the Golgi body and found particularly on the longer flagellum, although in some species they may also be found also on the cell body (Fig. 6b) as in *Pavlova gyrans* and occasionally on the haptonema, for example, in *Diacronema lutheri*. Such scales are usually smaller and sparser than those of the flagellum. The latter are often arranged in regular rows longitudinally, each row being slightly displaced relative to those adjacent. In a few species, however, the flagellar knob scales are disposed randomly on the flagellum. Most members of the Pavlovophyceae lack a distinct cell covering, but nonmotile cells may be invested with layers of mucilage (Green 1980; Leadbeater 1994; Bendif et al. 2011).

Scale Composition and Formation

Unmineralized Coccolithophyceae scales consist largely of complexed carbohydrates and some protein (Allen and Northcote 1975; Klaveness and Paasche 1979; Romanovicz 1981; Leadbeater 1994). The structural aspects of scale formation were first reviewed by Hibberd (1980) and Romanovicz (1981). The Golgi body is the site of scale formation. Scales are released to the cell surface by fusion of the plasmalemma with the cisternal membrane. Scales are often composed of four distinct components where the proximal radial microfibrils are to a large extent composed of sulfated polysaccharides, whereas the distal spiral microfibrils contain cellulose and protein. A glycoprotein covers the spiral microfibrils and there is also an amorphous layer composed of acidic polysaccharides. The radial fibrils are laid down before the spiral fibrils and then the amorphous matrix is added. In the Pavlovophyceae microfibrillar, scales are unknown (Leadbeater 1994).

Coccoliths

The coccolithophores possess external calcified ($CaCO_3$ as calcite) scales termed coccoliths (Figs. 6s-v and 7a -i). There are many living and fossil forms exhibiting an astounding variety in morphology and an extensive literature exists on the subject (see, e.g., Tappan (1980), Kleijne (1993), and Jordan et al. (2004)). The characteristics of these structures are briefly outlined here. Coccoliths have been classified into two main types, heterococcoliths and holococcoliths, based on coccolith ultrastructure and morphology, and there are a number of terms in use to describe both the overall form of the coccolith and its crystal structure (Jordan et al. 1995; Young et al. 1997). Nannoliths are a third group that differs from both holo- and heterococcoliths in structure and architecture (Jordan et al. 1995; Billard and Inouye 2004). Coccospheres are made up of multiple coccoliths and may be multilayered and monomorphic as in *Emiliania huxleyi* (Fig. 7a), have two layers of

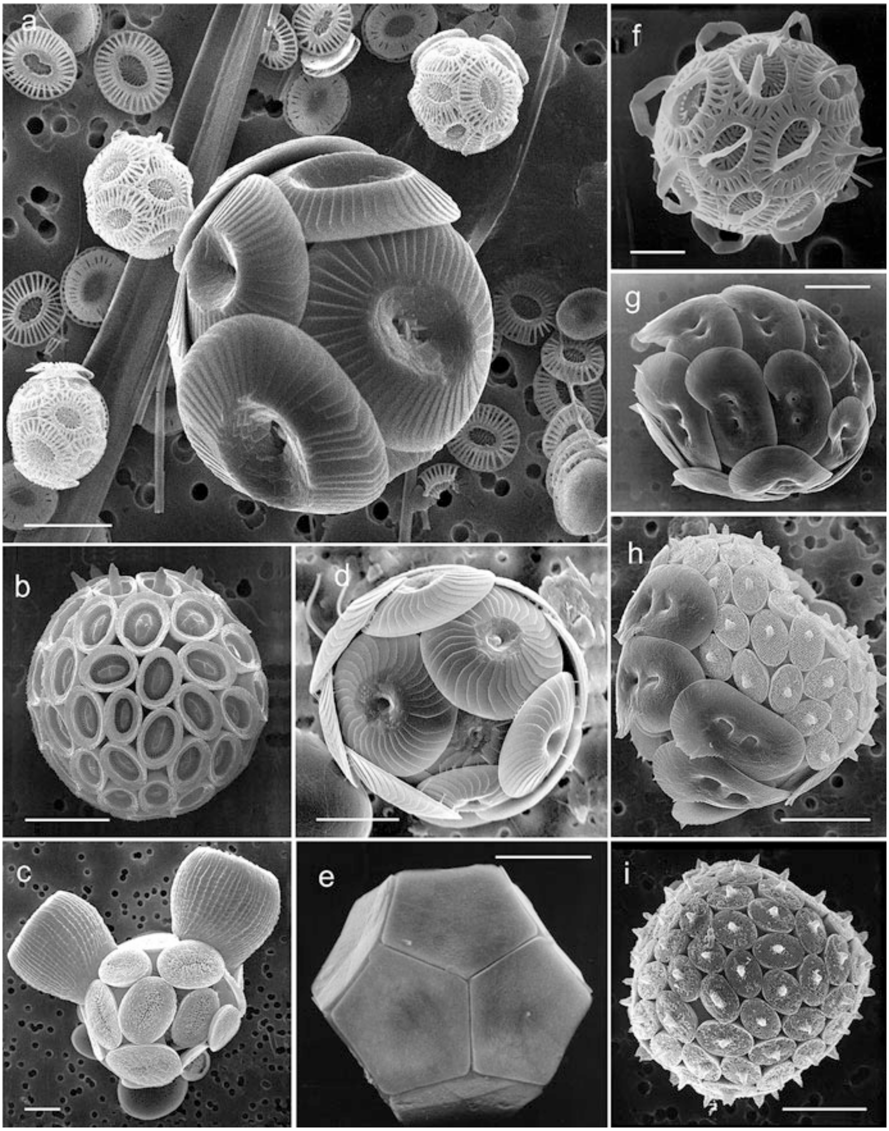


Fig. 7 SEM graphs of Coccolithophores: (**a**) *Coccolithus pelagicus* and *Emiliania huxleyi* (arrows). (**b**) *Coronosphaera mediterranea*: (**c**) *Scyphosphaera apsteinii*. (**d**) *Calcidiscus leptoporus*: (**e**) *Braarudosphaera bigelowi*. (**f**) *Gephyrocapsa ericsonii*: (**g**, **h**, **i**) *Helicosphaera carteri*, (**h**) combination cell, (i) *Syracolithus catilliferus* stage. Scale bars 5 µm. SEM images of *Coccolithus* and *Emiliania* by courtesy of L. Luïsa Cros and J-M. Fortuño. *Coronosphaera*, *Scyphosphaera*, *Gephyrocapsa*, and *Helicosphaera*, by courtesy of LLuïsa Cros. *Braarudosphaera*, by courtesy of Karen R. Gaarder

morphologically different coccoliths (dithecate) as in most *Syracosphaera* species, or have two types of coccoliths within one layer (dimorphic and monothecate) (Fig. 7b).

Heterococcoliths are elaborate interlocking structures composed of multiple strongly modified calcite crystals (Figs. 6u, v and 7a-g). The crystallographic orientation of subvertical “V-units” and sub-radial “R-units” (Young et al. 1992) is key to understanding heterococcolith ultrastructure and phylogenetic relationships (especially when dealing with their fossil record). There are several types of heterococcoliths, such as placoliths (Figs. 6u and 7a, d, f), caneoliths (Fig. 7b), cribriliths, lopadoliths (Fig. 7c), helicoliths (Fig. 7g, h), and pappoliths (Fig. 6v). Pentaliths (Fig. 7e) and ceratoliths (Fig. 6s) are considered nannoliths.

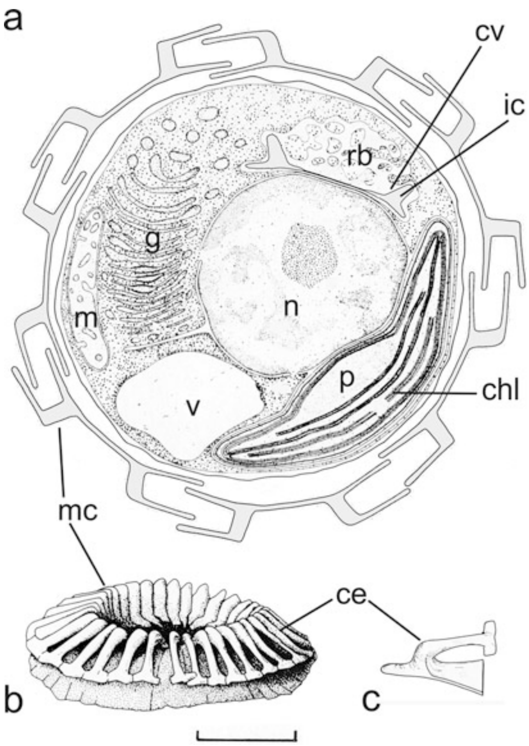
Holococcoliths are non-interlocking structures composed of rhombohedral crystallites of uniform size (ca. 0.1 μm in diameter) that cover the cell surface. Each holococcolith is made up of numerous identical calcite elements in the form of minute rhombohedral or hexagonal prisms (Figs. 6t and 7h, i). A variety of holococcolith types exist including crystalloliths (composed of rhombohedra arranged evenly on an organic baseplate in *Crystallolithus hyalinus* stage of *Coccolithus pelagicus*), calyptroliths (cap-shaped coccoliths of hexagonal crystals in *Calyptrosphaera*), and zygoliths (elliptical rings with crossbars and a distal knob) in *Helladosphaera cornifera*.

Coccolith Formation

Detailed observations of coccolith formation are possible in ultrathin sections of fixed cells in transmission electron microscopy, and complimentary information is provided by biochemical studies. The mechanisms of intracellular formation of heterococcoliths have been most extensively studied in *Emiliania huxleyi* (Wilbur and Watabe 1963; Klaveness 1976; Van Der Wal et al. 1983; Van Emburg et al. 1986; Figs. 9 and 10) and *Chrysotila* (Manton and Leedale 1969; Outka and Williams 1971; Van Der Wal et al. 1983; Marsh et al. 2002), with additional information available from a few taxa such as *Coccolithus braarudii* (Manton and Leedale 1969), *Umbilicosphaera sibogae* (Inouye and Pienaar 1984), *Syracosphaera pulchra* (Inouye and Pienaar 1988), and *Algirosphaera robusta* (Probert et al. 2007). By comparison, little information is available concerning holococcolith formation.

The heterococcoliths (Fig. 8b, c) of *E. huxleyi* are synthesized intracellularly in a system of vacuoles derived from the Golgi body, consisting of a coccolith vesicle (cv) enclosing the growing coccolith and a reticular body (rb) with anastomosing tubes (Fig. 8a). Inside the coccolith vesicle, coccolith production occurs through two discrete processes, controlled nucleation of crystals and their subsequent growth (Young et al. 1999). Crystal nucleation of a “proto-coccolith rim” occurs around the rim of the baseplate scale. These crystals subsequently grow in various directions to form complex crystal units. Crystal growth is regulated by a coccolith-associated

Fig. 8 Schematic drawing *Emiliania huxleyi*: (**a**) transverse section of whole cell showing the chloroplast (chl), coccolith vesicle (cv), Golgi body (g), immature coccolith (ic), mature coccolith (mc), mitochondrion (m), nucleus (n), pyrenoid (p), reticulate body (rb), vacuole (v), (**b**) single mature coccolith showing elements arranged in an oval ring, (**c**) calcite element (ce) of a coccolith. Scalebar 1 μm



polysaccharide (CAP) that has been located in the cv-rb system at four different sites: the outline of the membranes, fine threads inside the lumen, the baseplate, and a thin film surrounding the $CaCO_3$ crystals. The CAP is a highly complex soluble acidic polysaccharide containing at least 13 different monosaccharide residues, including uronic acids, mono- and dimethylated sugars, and sulfate esters (Fichtinger-Schepman et al. 1981). It has been shown that this CAP can inhibit crystal growth (Borman et al. 1982) and influence crystal morphology by site-specific attachment to crystallographic steps (Henriksen et al. 2004). It is therefore thought that this polysaccharide has a regulatory function in the inhibition, termination, and therefore modeling of crystal growth. The regulation of coccolith shape is also thought to be dependent on the morphology of the membrane of the coccolith vacuole. Using various inhibitors, Langer et al. (2010) experimentally demonstrated that cytoskeletal microtubules and actin filaments play a role in coccolith morphogenesis in *E. huxleyi*, presumably by determining the shape of the coccolith vesicle. After it is completed, the coccolith is transported to the cell membrane and exuded to the extracellular coccolith cover. In the motile scale-bearing form of *E. huxleyi*, the scales are formed in the Golgi apparatus, but do not calcify, although a cv-rb system is present (Klaveness 1972).

In contrast to *E. huxleyi*, *Chrysotila carterae* scales and coccoliths are produced in the trans (maturing) part of the Golgi apparatus. Granular elements termed coccolithosomes are formed in cisternae at the cis side of the Golgi. They appear to be transported to vesicles containing calcifying scales where they disintegrate as the formation of a $CaCO_3$-associated matrix proceeds. Coccolithosomes contain high concentrations of calcium and polysaccharide (Van Der Wal et al. 1983). Three types of polysaccharide have been identified in *C. carterae* (PS1, PS2, PS3; (Marsh et al. 2002)). PS1 and PS2 bind calcium and form coccolithosomes, with PS2 probably playing an important role in the nucleation of the proto-coccolith ring, as shown by the fact that mutant *C. carterae* cells deficient in this polysaccharide show very little calcification (Marsh and Dickinson 1997). During coccolith growth, PS3 is located between the crystals and the vesicle, and it is believed to be involved in shape regulation, because *C. carterae* cells not expressing PS3 produce a proto-coccolith ring that does not develop further (Marsh et al. 2002).

Comparison of observations of heterococcolith formation in other taxa reveals a similar overall pattern with calcification occurring in Golgi-derived vesicles and commencing with nucleation of a proto-coccolith ring of simple crystals around the rim of a precursor baseplate scale, followed by growth of these crystals in various directions to form complex crystal units. However, significant diversity is also evident, with, for example, coccolithosomes only being observed in *Chrysotila*, the reticular body being unique to *E. huxleyi* and *Gephyrocapsa*, and the peripheral endoplasmic reticulum playing a role in coccolithogenesis in *Algirosphaera robusta*.

Very few holococcolithophore cultures have been maintained and only three species have been studied in TEM sections; *Coccolithus braarudi* (as *Crystallolithus braarudi)* was studied by Manton and Leedale (1963, 1969) and by Rowson et al. (1986). *Calyptrosphaera sphaeroidea* was studied by Klaveness (1973) and Sym and Kawachi (2000) studied *Calyptrosphaera radiata*. As with heterococcoliths, holococcoliths are underlain by an organic baseplate scale and develop in Golgi vesicles. Individual rhombohedral calcite crystals have been observed within Golgi cisternae in *C. radiata* (Sym and Kawachi 2000), but despite numerous observations, fully formed holococcoliths have not been observed inside cells. It has therefore been inferred that the assemblage of crystals to form holococcoliths occurs outside the cell membrane, after exocytosis of the baseplate scale. This poses obvious problems for understanding how calcification is regulated. A potential explanation is provided by observations that a delicate hyaline "skin" envelopes the coccosphere of holococcolithophores, meaning that even if holococcoliths are assembled outside the cell membrane, this is likely to occur in a regulated environment. Alternatively, it is possible that holococcoliths are assembled just below the cell membrane but that it is a rapid process immediately preceding exocytosis and so has not been captured in TEM preparations.

Life Cycles

Organisms with heteromorphic phases in their life histories are common in the Haptophyta (Fig. 9a–c). An alternation of a haploid stage with a diploid stage has been documented in all orders and many families within the Coccolithophyceae. So

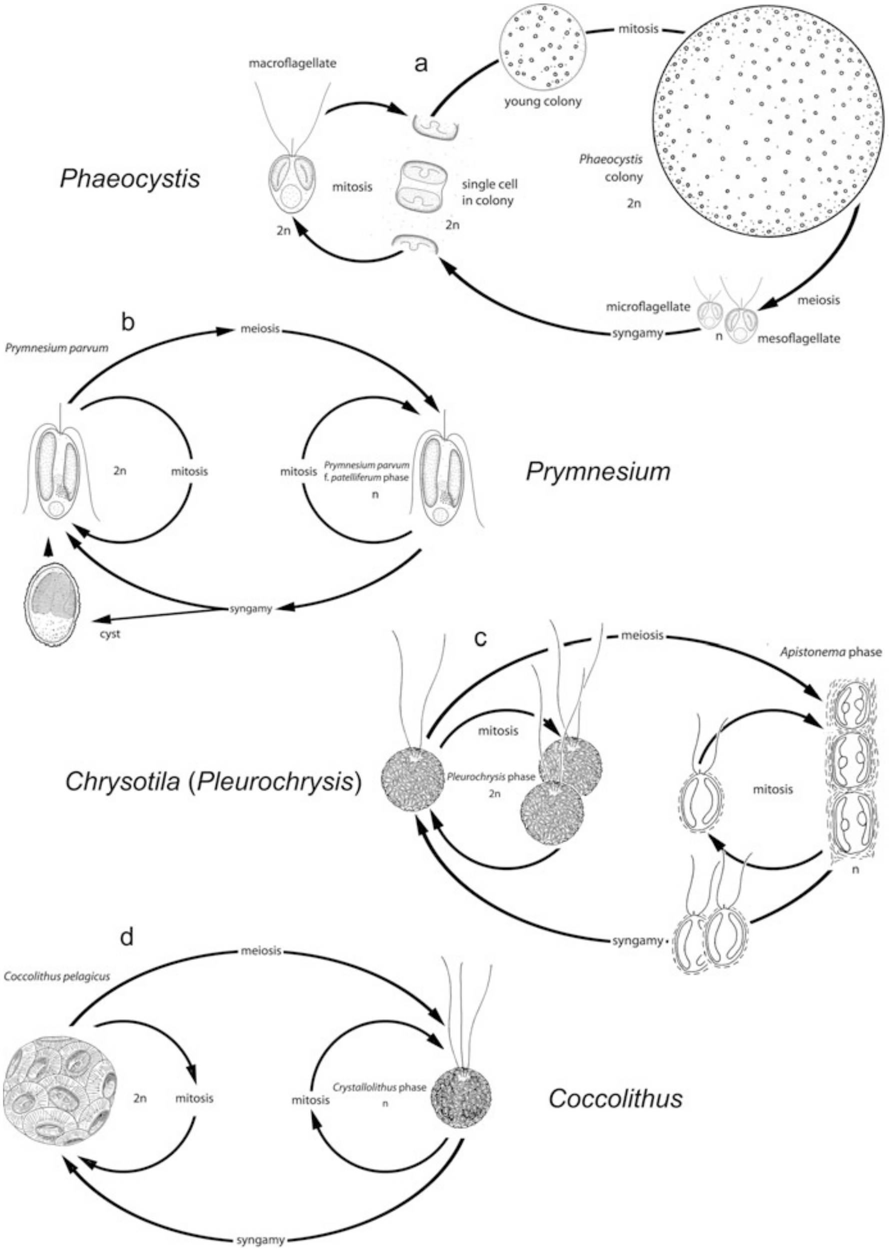


Fig. 9 Schematic life cycles in Coccolithophyceae: (**a**) *Phaeocystis globosa*, (**b**) *Prymnesium parvum*, (**c**) *Chrysotila carterae*, (**d**) *Coccolithus pelagicus*

far alternation of generations has not been demonstrated in members of the Pavlovophyceae, although transitions between motile and nonmotile forms occur in many species (Billard 1994; Bendif et al. 2011).

Alternation between a nonmotile planktonic palmelloid phase (Figs. 1c, g and 9a) and motile swarmers occurs in *Phaeocystis pouchetii*, *P. antarctica*, *P. jahnii*, and *P. globosa.* In *P. globosa*, four different cell types have been distinguished; two of them are haploid: the microflagellate and the slightly larger mesoflagellate. The largest flagellate type, the macroflagellate, and the palmelloid phases are diploid. The function of these cells and their order of appearance in the life cycle are still unknown, but the short-lived macroflagellate is believed to develop into a colony. Micro- and mesoflagellates (meiosis) are produced within the colony and are eventually released and multiply vegetatively. The life cycle is completed by syngamy between a micro- and mesoflagellate that develops into a macroflagellate that forms a new colony (Peperzak et al. (2000) and references therein). A nonmotile zygote linking the haploid unicellular stages and the diploid colonial stages has been documented in *P. antarctica* (Gaebler-Schwarz et al. 2010). The zygote can divide vegetatively as a benthic palmelloid stage and not revert to the colonial stage at least in culture conditions. The characteristic α-chitin containing pentagonal stars (Chrétiennot-Dinet et al. 1997) are produced by the mesoflagellate in *P. globosa* (Peperzak et al. 2000). These star-shaped structures are also produced by *P. antarctica*, *P. pouchetii*, and *P. cordata* but have not been observed in *P. jahnii.* In *P. scrobiculata*, a nine-ray star has been reported. *Phaeocystis scrobiculata* and *P. cordata* are believed to occur as flagellates only. *Phaeocystis* flagellates may be covered by minute scales (Fig. 6d) and the ornamentation of the scales may vary slightly between species (Rousseau et al. 2007). A plausible life cycle for *Phaeocystis* is illustrated in Fig. 9a.

In *Prymnesium*, two distinct cell types with differing scale morphology and cell size may occur within their haplodiploid life cycle as is seen in *Prymnesium polylepis* (Edvardsen and Vaulot 1996; Edvardsen and Medlin 1998) (Fig. 6d, j, k) and *P. parvum* (Larsen and Medlin 1997; Larsen and Edvardsen 1998). Their life cycle may also contain nonmotile cells (Parke et al. 1955) and even a silicified cyst in the case of *P. parvum* (Pienaar 1980) (Fig. 9b).

Life cycles of coccolithophores provide an excellent documentation of the alternation between haploid and diploid generations. Each generation is characterized by a specific cell covering and is capable of vegetative multiplication and dispersal. Diploid generations bear heterococcoliths, whereas haploid generations, depending on the family/genera they represent, are either covered by holococcoliths (Coccolithaceae, Helicosphaeraceae, Figs. 7i and 9d) or, nannoliths (Ceratolithaceae, Figs. 6s and 7e), or are a non-calcifying benthic stage (Pleurochrysidaceae, Hymenomonadaceae, Fig. 9c), or a non-calcifying motile stage (Noëlhaerhabdaceae) (Billard and Inouye 2004).

Parke and Adams (1960) showed that the heterococcolithophore *Coccolithus pelagicus* ssp. *braarudii* (Geisen et al. 2002) phase alternated with a motile holococcolithophore (*Crystallolithus braarudii*) phase. The haploid and diploid state of the holococcolith and heteroccoccolith stage, respectively, has been more recently demonstrated by flow cytometric DNA analysis (Houdan et al. 2004a). Reports of combination cells with the heterococcolithophore placed inside the gametangium (zygote stage) bearing holococcoliths are increasing in occurrence since their first reports in the early 1900s (Kamptner 1941; Thomsen et al. 1991; Kleijne 1993; Cros et al. 2000).

Alternation of a nonmotile stage ("Apistonema stage") with one or more motile forms (Fig. 9c) has been observed in *Chrysotila* (Leadbeater 1970; Gayral and Fresnel 1983) and *Ochrosphaera* Schussnig (Schwarz 1932; Lefort 1975). In *Chrysotila pseudoroscoffensis*, the diploid coccolith-bearing phase produces motile spores without coccoliths after meiosis. These give rise to a haploid benthic filamentous phase that eventually releases isogametes with flagella and a haptonema. Fusion takes place and a zygote is formed that releases diploid, coccolith-bearing motile cells within 24 h. In *Ochrosphaera neapolitana* meiosis, isogamete formation and syngamy were reported already by Schwarz (1932).

In *Ruttnera* species, the benthic, nonmotile, mucilage ensheathed cells form the dominant stage; the nonmotile cell may divide within the mucilage sheath to form a variable number of offspring cells (usually 8 or 16) that are released as swarmers. They settle quickly and secrete a new mucilage sheath after which they divide vegetatively (Green and Parke 1975).

A complex life cycle involving naked, scale-bearing, and coccolith-bearing stages was described in *Emiliania huxleyi* by Klaveness (1972). Flow cytometric analysis has shown that its life cycle includes a diploid and a haploid phase (Green et al. 1996) where the motile scale-covered flagellate is haploid and the coccolithophore may be diploid; however, haploid coccolithophores were reported by Medlin et al. (1996). This is a type of life cycle (Fig. 9d) that is considered typical of the Noëlhaerhabdaceae (Billard and Inouye 2004).

So far alternation of generations has not been demonstrated in members of the Pavlovophyceae, although transitions between motile and nonmotile forms occur in many species (Billard 1994; Bendif et al. 2011).

There are few reports of cysts in the Haptophyta. Cysts of *Prymnesium* were described by Carter (1937) and have been investigated by Pienaar (1980) who has shown that the walls of *Prymnesium parvum* cysts are composed of layers of scales with siliceous material on the distal surfaces. There is a simple sub-anterior pore. Cysts have also been reported in *Isochrysis galbana* (Parke 1949).

Differences in gene expression between the haploid flagellate and the diploid coccolith-bearing stage have been demonstrated (Von Dassow et al. 2009; Rokitta et al. 2011), but the ecological role of the different life cycle stages, their occurrence, and distribution are poorly understood. It has been shown that the colonial diploid stage of *Phaeocystis* may be resistant to viral attacks, whereas single cells are more susceptible to viral infection (Jacobsen et al. 2007). In *Emiliania huxleyi*, the haploid flagellate has been reported to escape viral infection (Frada et al. 2008).

Maintenance and Cultivation

Some haptophytes are easily cultivated, but many are more demanding to isolate and keep in culture. Of those in culture, most are euryhaline with wide nutritional tolerance. *Isochrysis* and *Pavlova* are easily cultivated and extensively used as feed in the aquaculture industry. *Prymnesium parvum* is extremely euryhaline and eurytherm and thrives in eutrophic waters (Edvardsen and Paasche 1998) and is also easily cultivated. About half of the described Prymnesiales species in all genera have at some point been cultured and most *Phaeocystis* species are presently kept in culture. Of the coccolithophore species, approximately half of those currently accepted are in culture, but most culture-based studies on coccolithophore physiology, genetics, and biochemistry use the cosmopolitan *Emiliania huxleyi*. Most cultured coccolithophores are from coastal waters of the families Pleurochrysidaceae, Hymenomonadaceae, Noëlaerhabdaceae, and Coccolithaceae. Few oceanic oligotrophic species have been cultured likely because they are sensitive to high nutrient concentrations (Probert and Houdan (2004) and references therein). The few oceanic picoplanktonic haptophyte cultures partly reflect our lack of information on nutritional requirements and physiology of haptophytes in oligotrophic oceanic waters but also the logistical problems of isolating rare and small species in remote places.

Many isolates were made of single cells by micropipette, either from the original water sample or from an enriched culture (10:1 or 20:1). Motile haptophytes, such as *Chrysochromulina*, tend to swim toward the light and can be concentrated at the surface prior to isolation. It is advisable to have a selection of media and dilutions available. The serial-dilution method can yield small and abundant haptophytes, such as *Dicrateria* and *Chrysochromulina* (Edvardsen et al. 2000). These and other isolation techniques are described in Andersen and Kawachi (2005).

Many marine media (f/2, IMR1/2, ErdSchriber, for a review, see Andersen (2005) are based on natural seawater with added nutrients, vitamins, and trace elements and have proved useful in the maintenance of many haptophytes. IMR1/2 and f/2 have the advantage that no soil extract is used in the enrichment, thus eliminating a variable component. Artificial media, such as ASP- and S-media (Provasoli et al. 1957) and their modifications made by Paasche (1964) have been useful in the culture of some coccolithophores. Paasche's medium is broadly similar to ASP2 but includes a number of minor elements (Br, Sr, Al, Rb, Li, I) and the only vitamins are B_{12}, thiamine, and biotin. A thorough review on culturing coccolithophores was published by Probert and Houdan (2004). Light intensity and quality, day length, temperature, mixing, mode of sterilization of media, and growth containers are other factors that must be taken into consideration for the cultivation of haptophytes, similar to other sensitive microalgae (Probert and Houdan 2004; Andersen 2005).

Axenic cultures of haptophytes have been obtained (Guillard 2005), although not all species readily lend themselves to bacteria-free culturing; presumably the bacteria are providing, or making available, micronutrients or organic compounds not accessible to the algae in the standard media used. Several haptophytes have been shown to be mixotrophic and can feed on bacteria (Jones et al. 1994) or algae (Tillmann 1998). The method generally used to remove bacteria is treatment with a range of concentrations of antibiotics (penicillin, streptomycin, gentamicin, etc.) followed by subculturing into fresh, antibiotic-free medium.

Some haptophytes produce nonmotile cells surrounded by masses of mucilage, and these may be difficult to obtain bacteria-free, seemingly because bacteria are embedded in the mucilage and are protected from the antibiotics. Green and Course (1983) found that *Chrysotila lamellosa* could be obtained apparently bacteria-free by first inducing the formation of motile swarmers, which do not carry a mucilage investment, and exposing these to antibiotics before subculturing them into new medium.

Several haptophyte species are cultivated as feed for both experimental and commercial shellfish farming, particularly for oysters (Jeffrey et al. 1994; Moestrup 1994). *Isochrysis galbana* has proved to be particularly valuable and has been in use since its isolation into culture by Parke (Flagellate "I" in Bruce et al. (1940)) more than 70 years ago. *Tisochrysis lutea* (as *Isochrysis* affinis *galbana* or T-Iso, a culture isolated from the sea off Tahiti) is also widely used as feedstock in bivalve aquaculture (Jeffrey et al. 1994; Brown et al. 1997; Bougaran et al. 2003). The pavlovophytes *Diacronema lutheri* (as *Monochrysis lutheri*, then *Pavlova lutheri*) and *Pavlova gyrans* are also being extensively used as feed in the aquaculture of bivalves, crustaceans, and fish (Green 1975; Meireles et al. 2003; Ponis et al. 2006). Haptophytes are rich in fatty acids, a large proportion of which may be of the valuable, long-chain polyunsaturated omega-3 fatty acids EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3) (Meireles et al. 2003; Guschina and Harwood 2006). Algae are the only producers of EPA and DHA in marine food webs, and cultivation of microalgae, including haptophytes, as supplement in fish feed is rapidly increasing.

Evolutionary History

Fossil Record

Fossil coccoliths and other fossil remains of calcifying nanoplankton (calcareous nannofossils) first appear ca. 225 Ma. Older reports are disputed and represent forms that are not ancestral to the latest Triassic and Early Jurassic coccoliths (Bown 1998).

Pioneering studies of calcareous nannofossils date back to the nineteenth century (Ehrenberg 1836; Huxley 1858; Siesser 1994). Species-level taxonomy is based on nannofossil morphology (Perch-Nielsen 1985a; Perch-Nielsen 1985b; Bown 1998; Jordan et al. 2004). Not all extant coccolithophores are well represented in the fossil record. Selective dissolution of rare and fragile taxa arguably impedes the reconstruction of "true" species richness through time (Young et al. 2005). The fossil record is largely composed of dissolution-resistant heterococcoliths, recording the diploid life stage of coccolithophores and rarely the haploid holococcoliths (Dunkley Jones et al. (2008)). Bown et al. (2004) compiled an overview of calcareous nannofossil morphospecies richness and evolutionary rates over the last 225 Ma.

Mesozoic Era

The earliest known coccoliths, of Late Triassic (Norian and Rhaetian) age, are very small (~2 μm) and possess very simple "murolith" morphologies. The Triassic/Jurassic boundary is characterized by significant extinctions, and only one species (*Crucirhabdus primulus*) survived (Bown 1998). Following this event, calcareous nanoplankton abundance and diversity steadily increased during the Jurassic and Cretaceous periods, with relatively low background extinction and speciation. Maximum morphospecies diversity was reached during the Late Cretaceous (Campanian-Maastrichtian), with large coccoliths and nannofossils with sophisticated architecture (Perch-Nielsen 1985a; Bown et al. 2004). Provincialism in calcareous nannofossil assemblages, between low (tethyan realm) and high (boreal and austral realms) paleolatitudes, is well documented during the Cretaceous (Roth and Bowdler 1981).

The Cretaceous-Paleogene boundary (K-T boundary; 65.5 Ma) is marked by a catastrophic event in which 93 % of all species or 83 % of all genera went extinct leaving only 10 survivors (Perch-Nielsen et al. 1982; Bown et al. 2004; Bown 2005). Survivor species included both common, opportunistic taxa as well as those typically rare in Late Cretaceous assemblages.

Cenozoic Era

After the K-T event, calcareous nanoplankton was characterized by the dominance of few survivor species and the appearance of small-sized (~2 μm) Cenozoic newcomers. Paleocene species diversity rapidly increased, with new coccolith morphologies and novel nannolith groups (*Sphenolithus*, *Fasciculithus*, *Heliolithus*, *Discoaster*) that differed significantly from the Mesozoic architectures (Perch-Nielsen 1985a; Bown et al. 2004). The Paleocene-Eocene Thermal Maximum (PETM; ca. 55 Ma) represents a relatively short-lived but rapid climatic perturbation of global warming, elevated atmospheric CO_2, and lowered ocean pH that drove significant evolutionary turnover affecting both fragile and robust taxa across broad ecological preferences (Gibbs et al. 2006).

The transition from the Eocene "greenhouse" into the Oligocene "icehouse" (ca. 34 Ma) was characterized by a rapid decline in diversity and global prominence (Aubry 1992; Hannisdal et al. 2012) and a macroevolutionary decrease in coccolith size (Aubry 1992; Henderiks and Pagani 2008). Nannofossil diversity recovered and increased again in the Middle and Early Late Miocene but decreased sharply during the remainder of the Miocene into the Pliocene and Pleistocene, marked by the loss of discoasters, sphenoliths, and large coccolith morphospecies (*Coccolithus miopelagicus*, *Reticulofenestra pseudoumbilicus*, *Calcidiscus macintyrei*). The latter part of the Neogene is characterized by the dominance of small placoliths and rapid evolution within the *Reticulofenestra*, which gave rise to the modern genera *Gephyrocapsa* and *Emiliania* (Thierstein et al. 1977; Takayama 1993; Bollmann et al. 1998; Okada 2000). Modern coccolithophores are extremely small in comparison to most of their Mesozoic and Cenozoic ancestors (Henderiks et al. 2004; Aubry 2007).

Nannofossil Evolution and Climate Change

In the short term, biocalcification releases CO_2 to the environment. On geological timescales, the burial of calcium carbonate into deep-sea sediments represents a long-term removal (“sink”) of carbon from the surface oceans and the atmosphere. Calcareous nannofossils have been a major component of pelagic carbonates since the Early Jurassic, ca. 180 Ma (Bramlette 1958; Mattioli and Pittet 2002). The widespread Late Cretaceous chalk deposits (the white cliffs of Dover) are prime examples of calcifying nanoplankton as rock-forming organisms. Quantitative estimates of calcareous nannofossils and their corresponding calcite mass, as determined by morphometry (Young and Ziveri 2000) and polarized light microscopy (Beaufort 2005; Beaufort et al. 2014), provide important insights into the process of deep-sea carbonate burial and its climatic feedbacks, from the Jurassic to Recent times (Bornemann et al. 2003; Ziveri et al. 2007). Advances in the geochemical analysis of nannofossils (Stoll and Ziveri 2004; Rickaby et al. 2007), automated microscopy techniques (Beaufort 2005), and the quantification of fossil coccosphere and cell geometries (Henderiks 2008; Gibbs et al. 2013) have expanded investigations of ancient coccolithophore productivity, calcification, and their phenotypic evolution.

It remains a challenge to understand the mechanisms and rates of climatic adaptation by coccolithophores, on both ecological and evolutionary timescales. Scenarios for the future ecological success of coccolithophores are largely informed by short-term experiments on few extant species and field studies. Extrapolation of current physiological knowledge to studies of nanoplankton evolution should be treated with caution because of the very different temporal scales involved. Nevertheless, a range of hypotheses exist linking nanoplankton evolution and ecological prominence to climate variability and ocean chemistry in the past (Aubry 1992; Bown et al. 2004; Erba 2006; Aubry 2007; Henderiks and Rickaby 2007; Henderiks and Pagani 2008).

Not all modern coccolithophore lineages are represented in the fossil record, but the evolution of some morphospecies (*Coccolithus pelagicus*, *Helicosphaera carteri*, and *Braarudosphaera bigelowii*) can be traced back to the Paleocene or even the Cretaceous. Detailed coccolith morphometric analyses can address the evolutionary significance of phenotypic variation in coccolithophores (Knappertsbusch 2000; Reitan et al. 2012).

The first sedimentary evidence of alkenones in Cretaceous black shales, ca. 120 Ma (Farrimond et al. 1986; Brassell and Dumitrescu 2004) post-dates the molecular divergence between the Isochrysidales and other coccolithophore clades (Fig. 10), ca. 195 Ma (Medlin et al. 2008). This supports the notion that the Isochrysidales clade is distinct from all other clades and that modern *Gephyrocapsa* and *Emiliania* may have some unique strategies in adapting to current climatic change (Henderiks and Rickaby 2007).

Molecular Clock Reconstructions

A haptophyte molecular clock with selected extant genera is presented in Fig. 10 and calibrated with the fossil coccolith record. Clocks that average the rate of evolution across all lineages (Takezaki et al. 1995; Medlin and Zingone 2007) and that allowed

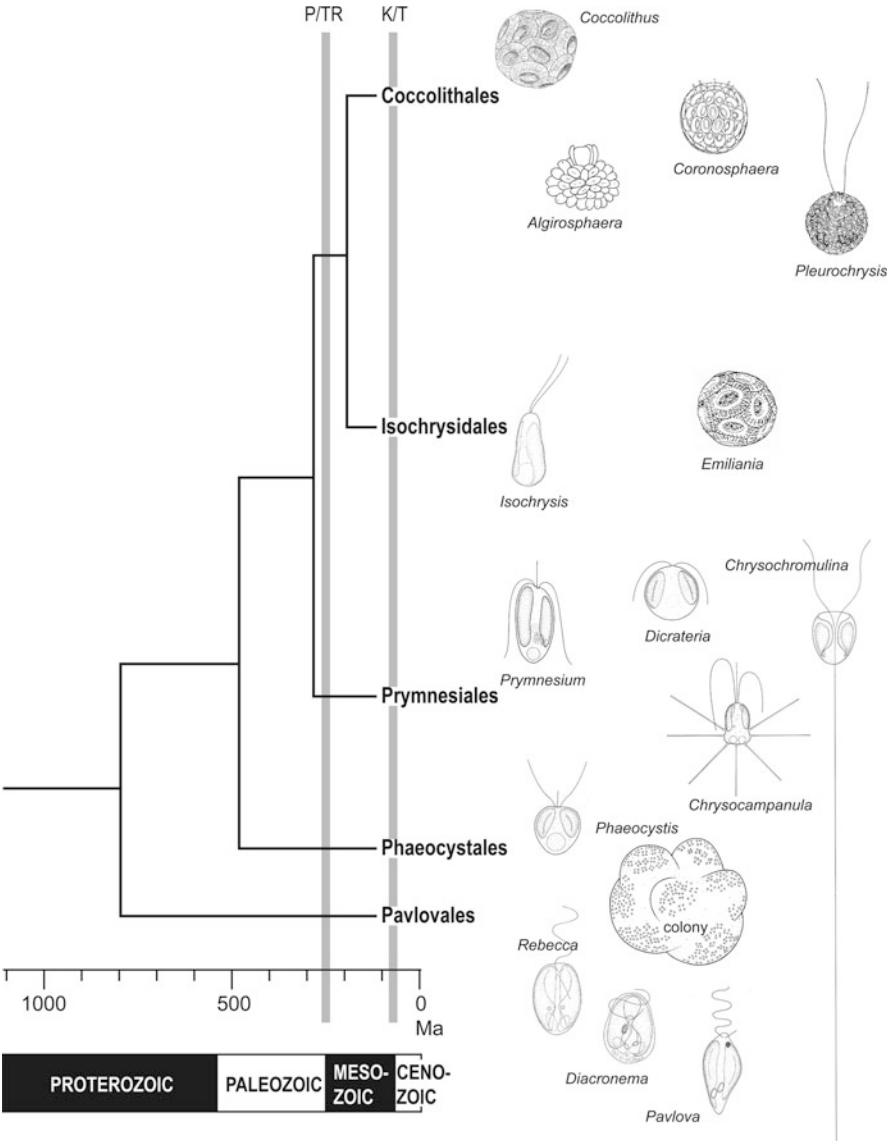


Fig. 10 Haptophyte molecular clock, with selected extant genera, illustrated to represent five orders. Coccolithophyceae, Coccolithales: *Coccolithus*; *Algirosphaera*; *Coronosphaera*; *Chrysotila*. Isochrysidales: *Isochrysis*; *Emiliania*. Prymnesiales: *Prymnesium*; *Dicrateria*; *Chrysochromulina*; *Chrysocampanula*. Phaeocystales: *Phaeocystis*, Pavlovophyceae. Pavlovales: *Rebecca*, *Diacronema*, *Pavlova*. Timescale million years ago (Ma), geologic eras indicated

the evolution to vary across the lineages (Sanderson 2006; Medlin et al. 2007) have been made and calibrated using characters and divergence points, such as the character-based constraint of 195 Ma for the emergence of all coccolithophores and the divergence-based constraints of 64 Ma for the divergence of *Coccolithus* from *Cruciplacolithus* and 50 Ma for the divergence of Helicosphaeraceae from Pontosphaeraceae. The molecular clock extrapolates to dates of origin for some of the undated nodes. Another molecular clock has been made using the SSU and LSU rRNA genes (De Vargas et al. 2007). Divergence dates in that study are slightly older than those found by Medlin and coworkers who used a relaxed molecular clock.

The Haptophyta as a group diverged from other eukaryotes deep in the Proterozoic, >1200 Ma, in the crown group radiation (Medlin et al. 1997). Their true sister group has never been confirmed, and various trees place them in different positions in the crown group radiation. The long time period between the origin of haptophytes and the initial divergence (~800 Ma) of the two classes, Pavlovophyceae and Coccolithophyceae (Fig. 10), indicates that many of the early evolutionary branches in this group are extinct or that they have not yet been sampled (Edvardsen et al. 2000). A new group of picoplankton (Cuvelier et al. 2010) breaks up this long branch substantiating the hypothesis that the latter reason was the cause of this long branch. The order Phaeocystales diverged from all other Coccolithophyceae at ~480 Ma and then the Prymnesiales diverged from the Coccolithales plus Isochrysidales at ~280 Ma, making this a Late Paleozoic-Early Mesozoic event that may be associated with Permian-Triassic boundary (250 Ma). Modern diversifications in these lineages occurred some time after the lineage origin so many taxa were presumably lost during this time.

Within the order Phaeocystales, the divergence of the warm water *Phaeocystis* species from the cold water species occurs at 30 Ma when the Drake Passage opened to isolate the Antarctic Continental waters and dispersal to the Arctic occurred across the equator during a cooling trend at 15 Ma, and then the two polar populations were separated by a warming trend that then isolated the two polar species (Medlin and Zingone 2007).

Molecular diversification occurred earlier within the Prymnesiales than within the Coccolithales plus Isochrysidales where most of these latter divergences occurred fairly late in the haptophyte timetree (Fig. 10). The diversification within the Coccolithales plus Isochrysidales occurred predominantly after the Mesozoic-Cenozoic boundary (66 Ma), as predicted by the fossil record. Mesozoic coccolithophores have been intensively studied, and at the Mesozoic-Cenozoic boundary, an abrupt extinction is documented in the fossil record with ~90 % of end-Cretaceous species disappearing (MacLeod et al. 1997; Bown 2005). After that, there is a major radiation in the Early Cenozoic with new clades rapidly diversifying and forming the origins of the modern coccolithophore biota (Bown et al. 2004).

One significant insight learned from the haptophyte molecular tree is that the Mesozoic-Cenozoic boundary extinction does not seem to have affected the Prymnesiales, Phaeocystales, or Pavlovales to the same degree as the Coccolithales, which is assessed by comparing the depth of clade diversification. In non-calcifying groups, there are 25 clades/lineages that cross the Mesozoic-Cenozoic boundary (Medlin et al. 2007) as compared to 11 coccolithophore clades. This type of branching pattern is suggestive of a major extinction (Medlin et al. 2008). This interpretation of tree branching pattern has been confirmed in other groups

(Vergroeben et al. 2014). One possible explanation for this difference in their survival may be the mode of nutrition in the haptophyte lineages. Those that are mixotrophic (Jones et al. 1994) or who could produce resting stages did not go extinct, whereas those that were obligate phototrophs without resting stages did. There appears to be a selective extinction of the order Coccolithales at the Mesozoic-Cenozoic boundary where calcified organisms were affected by ocean chemistry, and the uncalcified lineages likely switched to mixotrophy to take advantage of the poor light conditions at this extinction event. Modern coccolithophores represent terminal points of a number of evolutionary lines, some extending back to the Triassic and before. These lines have developed to some extent in parallel with each other and with those represented by the Prymnesiales, Phaeocystales, and the Pavlovophyceae.

Taxonomy

A haptophyte taxonomy (Table 1) has been constructed from Silva et al. (2007), de Vargas et al. (2007), and Edvardsen et al. (2011). Two classes are included in the phylum Haptophyta, Pavlovophyceae, and Coccolithophyceae (Prymnesiophyceae) and are separated by features of cell shape, flagellar insertion, and type and location

Table 1 The major taxonomic groups within the Haptophyta, their main characteristics, and examples of the genera of living algae included in them[a]

Phylum Haptophyta	Cells with haptonema
Class: Pavlovophyceae	Cells with knob scales
Order: Pavlovales	Motile cells with two unequal flagella, the longer with an investment of small knob scales and fine hairs and the shorter sometimes vestigial. Haptonema present but reduced (e.g., *Diacronema*, *Pavlova*)
Class: Coccolithophyceae (Prymnesiophyceae)	Cells with organic scales, with or without coccoliths
Order: Isochrysidales	Motile cells with two equal or subequal flagella, haptonema reduced or absent (e.g., *Ruttnera*, *Emiliania*, *Isochrysis*)
Order: Coccolithales	Cells coccolith bearing many genera with a polymorphic life cycle. Haptonemata recorded in several genera (e.g., *Acanthoica*, *Braarudosphaera*, *Calyptrosphaera*, *Chrysotila*, *Coccolithus*, *Corisphaera*, *Crenalithus*, *Cyclolithella*, *Discosphaera*, *Helicosphaera*, *Laminolithus*, *Rhabdosphaera*, *Syracosphaera*, *Umbellosphaera*, *Umbilicosphaera*)
Order: Prymnesiales	Motile cells with two (rarely four) equal or subequal flagella and usually an obvious haptonema which may be very long and coiling (e.g., *Chrysochromulina*, *Haptolina*, *Prymnesium*)
Order: Phaeocystales	Motile cells with two flagella and short haptonema. Flagellate alternating with colonial stage (*Phaeocystis*)

[a]Based on Parke and Adams (1960), Parke and Dixon (1976), Edvardsen et al. (2000), de Vargas et al. (2007), Silva et al. (2007), and Edvardsen et al. (2011) (note that these are not formal taxonomic descriptions)

of scales (Edvardsen et al. 2000). Pavlovophyceae has one order, the Pavlovales, and the class Coccolithophyceae (Prymnesiophyceae) may be divided into four orders: the Phaeocystales, Prymnesiales, Isochrysidales, and Coccolithales. Of these, the Phaeocystales with the genus *Phaeocystis* is the most basal and clearly defined in SSU rDNA phylogenetic trees. The Prymnesiales consists of two well-supported molecular clades, which have been separated at the family level (Prymnesiaceae and Chrysochromulinaceae) based on the shape of cells (saddle shaped or rounded, respectively) and the length and nature of the haptonema (Edvardsen et al. 2011). The coccolithophores have traditionally been classified on the basis of coccolith morphology, and the Coccolithales is the order within the Haptophyta that includes the highest number of described genera and species and is almost certainly a heterogeneous assemblage. De Vargas et al. (2007) erected the subclass Calcihaptophycidae and recognized the orders Isochrysidales, Syracosphaerales, Zygodiscales, and Coccolithales (see also Jordan et al. 2004). Molecular data show Isochrysidales to be the sister of Coccolithales. The Isochrysidales includes two families, the Noëlhaerhabdaceae with the coccolith-bearing genera *Emiliania*, *Gephyrocapsa*, and *Reticulofenestra* and the Isochrysidaeae with the non coccolith-bearing genera *Isochrysis*, *Chrysotila*, and *Tisochrysis* (Bendif et al. 2013).

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