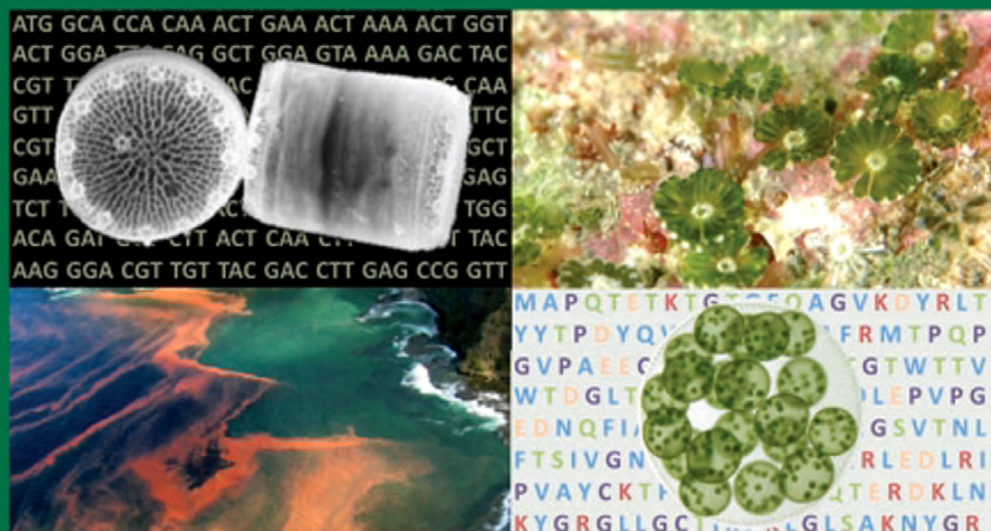


Advances in
**BOTANICAL
RESEARCH**

GENOMIC INSIGHTS INTO THE
BIOLOGY OF ALGAE



Volume 64

Edited by
GWENAËL PIGANEAU

Series Editors
**JEAN-PIERRE JACQUOT &
PIERRE GADAL**





VOLUME SIXTY FOUR

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VOLUME SIXTY FOUR

GENOMIC INSIGHTS INTO THE BIOLOGY OF ALGAE

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Diversity and Ecology of Eukaryotic Marine Phytoplankton

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Abstract

Marine phytoplankton, the photosynthetic microorganisms drifting in the illuminated waters of our planet, are extremely diverse, being distributed across major eukaryotic lineages. About 5000 eukaryotic species have been described with traditional morphological methods, but recent environmental molecular surveys are unveiling an ever-increasing diversity, including entirely new lineages with no described representatives. Eukaryotic marine phytoplankton are significant contributors to major global processes (such as oxygen production, carbon fixation and CO₂ sequestration, nutrient recycling), thereby sustaining the life of most other aquatic organisms. In modern oceans, the most diverse and ecologically significant eukaryotic phytoplankton taxa are the diatoms, the dinoflagellates, the haptophytes and the small prasinophytes, some of which periodically form massive blooms visible in satellite images. Evidence is now accumulating that many phytoplankton taxa are actually mixotrophs, exhibiting alternate feeding strategies depending on environmental conditions (e.g. grazing on prey or containing symbiotic organisms), thus blurring the boundary between autotrophs and heterotrophs in the ocean.



1. PHYTOPLANKTON FEATURES

1.1. Diversity of Phytoplankton

This chapter provides an overview of current knowledge on the diversity and ecology of the phytoplankton that drift in the illuminated waters of seas and oceans. The term phytoplankton here corresponds to the functional grouping of single-celled organisms (prokaryotes and eukaryotes) that have the capacity to perform oxygenic photosynthesis. Marine phytoplanktonic prokaryotes all belong to the phylum Cyanobacteria within the domain Bacteria. In contrast, eukaryotic phytoplankton, the focus of the present chapter, is taxonomically very diverse, having representatives in all but one lineage of the eukaryotic tree of life (Fig. 1.1). The early evolutionary history of eukaryotic phytoplankton (and more generally of all plastid bearing eukaryotes) was shaped by series of endosymbiotic events, involving the engulfment of a cyanobacterium by a eukaryote (Chapter II of this volume, De Clerck, Bogaret, & Leliaert, 2012) or the engulfment of a photosynthetic eukaryote by another eukaryote (Chapter III of this volume, Archibald

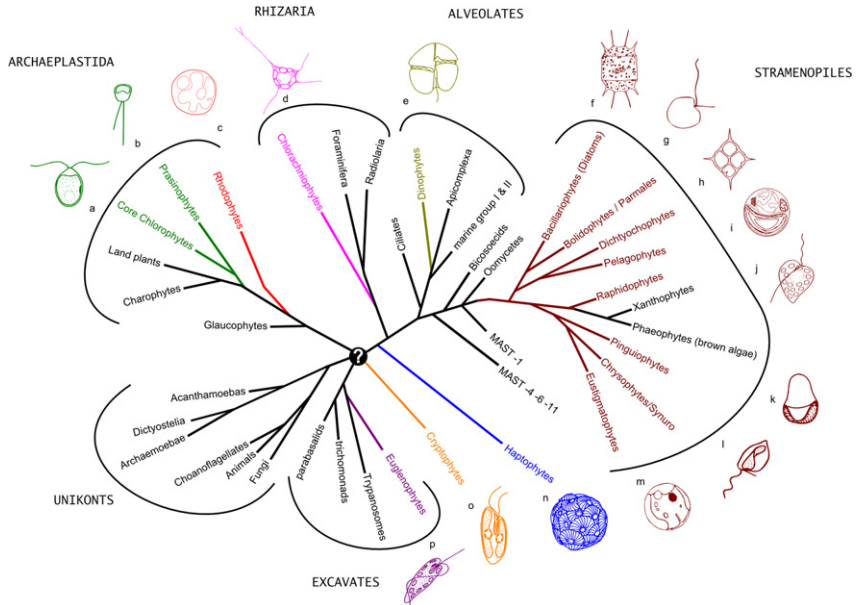


Figure 1.1 Schematic phylogenetic tree representing the distribution of phytoplanktonic taxa across eukaryote lineages (in color). Illustrations of (a) Chlorophyceae, (b) *Pseudocourfieldia* sp., (c) *Porphyridium cruentum*, (d) *Gymnochloa dimorpha*, (e) Dinoflagellates, (f) *Odontella* sp. (g) *Bolidomonas pacifica*, (h) *Dictyocha* sp., (i) *Aureococcus anophagefferens*, (j) *Heterosigma akashiwa*, (k) *Pinguiochrysis pyriformis*, (l) *Ochromonas* sp., (m) *Nannochloropsis salina*, (n) *Calcidiscus* sp., (o) *Cryptomonas* sp., (p) Euglenids; 'a, b, e, f, h, j, l, n, o and p' are adapted from Tomas (1997), 'c' adapted from Lee (1999), 'd' from Ota, Kudo, and Ishida (2011), 'g' adapted from <http://tolweb.org/Bolidomonas/142186>, 'i' from Andersen and Preisig (2000), 'k' from Kawachi *et al.* (2002) and 'm' from Van Den Hoek, Mann, and Jahns (1995). For colour version of this figure, the reader is referred to the online version of this book.

2012). These endosymbiotic events were accompanied by massive gene transfers from the genomes of the endosymbionts to the genome of the host, traces of which can be detected in modern eukaryotic primary producers.

Historically, the diversity of eukaryotic phytoplankton has been assessed by microscope-based comparison of morphological features. Based on these observations, less than 5000 species have been described to date (Simon, Cras, Foulon, & Lemée, 2009; Sournia, Chretiennot-Dinet, & Ricard, 1991; Tett & Barton, 1995), but there is a general agreement that this number largely underestimates the real extent of phytoplankton diversity. In the last decade, evaluation of environmental diversity using molecular approaches has highlighted massive undescribed diversity, including whole lineages without any

cultured representatives and for which only environmental sequences are available (Massana & Pedros-Alio, 2008; Vaulot, Eikrem, Viprey, & Moreau, 2008). Some of these environmental lineages are so distantly related to all other groups that they may represent new phyla, one example being the picobiliphyte (Not *et al.*, 2007). Combination of molecular phylogenetic and morphological analyses has repeatedly demonstrated the existence of cryptic (or pseudocryptic) species, even in supposedly well-known groups such as diatoms and coccolithophores, fuelling the debate concerning species delineation in protists (Amato *et al.*, 2007; Saez *et al.*, 2003). In addition, detailed studies comparing well-defined species complexes demonstrate that commonly used molecular markers (e.g. the 18S rRNA gene) often underestimate diversity, particularly for organisms like phytoplankton that have huge population sizes and high turnover rates (Piganeau, Eyre-walker, Grimsley, & Moreau, 2011a). Information on the genetic diversity of phytoplankton is likely to significantly increase in the future with the advent of environmental meta-barcoding surveys (Bik *et al.*, 2012; Toulza, Blanc-Mathieu, Gourbiere, & Piganeau, 2012). This is particularly true for small-sized phytoplankton for which very few distinguishing morphological characters are available.

1.2. Size Matters

Eukaryotic phytoplankton cells are not only taxonomically very diverse but also span an exceptionally wide size range both between and within taxonomic groups. Size spectra can even vary temporally and/or spatially in response to varying environmental conditions or succession of life cycle stages. Phytoplankton cells span more than three orders of magnitude in size, ranging from picoplankton (0.2–2 μm) up to mesoplankton (0.2–2 mm). Individual cells of most species are solitary, but many species (e.g. most species within the diatom genera *Chaetoceros* and *Thalassiosira*, the dinoflagellate *Alexandrium catenella*, or the haptophyte *Phaeocystis*) also have the ability to form chains or colonies. Although exceptions exist, the largest size classes of marine phytoplankton are generally dominated by ‘golden brown’ groups, notably diatoms and dinoflagellates, whereas smallest size classes essentially consist of green algae from the prasinophyte lineage (e.g. *Ostreococcus tauri*, which has a cell diameter less than 1 μm ; Chrétiennot-Dinet *et al.*, 1995). In practice, this wide range of cell sizes requires the deployment of various collecting devices (plankton nets and filtration on various mesh sizes) and observation methodologies (optical and electronic microscopy) to characterize phytoplankton diversity. Cell

size also affects numerous functional characteristics of phytoplankton. For instance, because of their large surface to volume ratio which facilitates passive nutrient uptake, small cells are particularly well adapted to stable and oligotrophic (nutrient poor) waters, whereas larger cells typically perform better in mixed and eutrophic (nutrient rich) settings (Finkel *et al.*, 2010; Marañón *et al.*, 2001). Because the marine environment exhibits heterogeneous physicochemical structures across space and time, cell size is an important feature to consider from an ecological point of view.

1.3. Global Ecological Patterns

Phytoplankton plays a significant role in global ecology and ecosystem functioning. First and foremost, phytoplankton species are primary producers and contribute to about half of the primary production on the planet, of which one forth is estimated to occur in oligotrophic waters (essentially performed by the cyanobacteria *Prochlorococcus*), one forth in eutrophic waters and half in mesotrophic regions (Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. 1998). Phytoplankton participates to the global carbon cycle through the so-called biological pump, by fixing carbon, a portion of which is subsequently sequestered at depth. Carbon is ultimately buried at the sea floor for centuries or longer (Falkowski, 2012). Phytoplankton is also at the base of virtually all marine food webs. Under specific light and nutrient conditions, some phytoplankton taxa can form large blooms, particularly in coastal waters of temperate seas. Some bloom-forming phytoplankton produce toxins that affect higher trophic levels (Harmful Algal Blooms or HABs), thus having significant ecological and economic impacts (Hinder *et al.*, 2011; Imai & Yamaguchi, 2012). Classically, study of the ecology of phytoplankton communities involves one or a combination of microscope-based morphological studies, flow cytometric cell counting, molecular surveys and/or measurement of the presence of specific photosynthetic pigments (Jeffrey, 1997). Although each approach has its inherent limitations, general ecological patterns can be drawn from the literature. Eutrophic coastal and continental shelf waters are classically dominated by diatoms, dinoflagellates and calcifying haptophytes (coccolithophores), groups that contain species that have the capacity to form large blooms, while other groups such as the euglenophytes, cryptophytes and raphidophytes produce more localized blooms (Assmy & Smetacek, 2009). Open oceans tend to be dominated by groups such as green algal

prasinophytes, *Chrysochromulina*-like haptophytes and small stramenopiles like pelagophytes and chrysophytes (Not *et al.*, 2008; Reynolds, 2006).

Since phytoplankton are primary producers living in a dispersive environment, abiotic physico-chemical factors exert a strong control on the composition and dynamics of phytoplankton communities. Several bloom-forming phytoplankton taxa have the ability to bio-mineralize silica or calcium, which, among other biogeochemical impacts, drives long-term carbon sequestration by accentuating sinking to the sea floor after bloom events. Valuable fossil records exist for these bio-mineralizing taxa and these are extensively used for paleo-stratigraphy and paleo-climatology. Bio-mineralization is also probably involved in mechanical defense and probably explains, at least in part, why diatoms and coccolithophores are ubiquitous in spring blooms in temperate and boreal systems (Smetacek, 2001).

Besides the control exerted by the zooplankton, which feed upon phytoplankton, the impact of biotic parameters on the global ecology of phytoplankton has generally not been studied in great detail. There is now a growing awareness of the impact of viruses and of parasitic and mutualistic symbiotic interactions on phytoplankton community structure (Brussaard *et al.*, 2008; Siano *et al.*, 2011) and ultimately on global biogeochemical cycles (Strom, 2008). We refer to Chapter IX of this volume for a review on genomic insights into the diversity of algal viruses (Grimsley *et al.* 2012).

Each phytoplankton lineage employs diverse trophic strategies. Indeed, although phytoplanktonic organisms are primarily photosynthetic, many exhibit mixotrophic behavior, feeding on prokaryotes or other small phytoplankton in addition to conducting photosynthesis. This has been well characterized for certain dinoflagellates (e.g. species within the genera *Gymnodinium* or *Amphidinium*; Lee, 1999) and haptophytes (e.g. *Chrysochromulina* sp.; Kawachi, Inouye, Maeda, & Chihara, 1991). Phytoplankton can also live in symbiosis with larger heterotrophic protists such as foraminifers or radiolarians and also with metazoans (e.g. in coral reefs; (Weber & Medina, 2012). Recently, several lines of evidence (e.g. stable isotope labelling) promote the conclusion that such mixotrophic strategies are more frequent than previously thought in the marine environment (Frias-Lopez, Thompson, Waldbauer, & Chisholm, 2009; Liu *et al.*, 2009; Stoecker, Johnson, de Vargas, & Not, 2009). While exogenous abiotic and biotic factors exert key controls on phytoplankton growth and mortality, internal factors such as life cycle traits (D'alelio *et al.*, 2010) or control of cell death (Biddle & Falkowski, 2004) fine-tune the regulation of population dynamics.

1.4. Current Conceptual Challenges

Studies of phytoplankton diversity and ecology, and more generally of microbial ecology and evolution, are driven by a number of major unresolved conceptual challenges, perhaps the foremost of which is the definition of what is a species. The species stands as a key concept, a basic unit and a common currency for studies of diversity and ecology in any environment; yet, there is no consensus on how to define a species. Phytoplankton species are traditionally defined according to morphological features, but (1) comparisons of morphological and molecular data often provide evidence for cryptic diversity within ‘morphospecies’ (Amato *et al.*, 2007), (2) morphological traits are prone to change under varying environmental conditions (Pizay *et al.*, 2009), and (3) the smallest phytoplanktonic cells usually lack distinctive features (Potter, Lajeunesse, Saunders, & Anderson, 1997). As for prokaryotes, the classical biological species concept defined by E. Mayr in 1969 (i.e. members of an interbreeding population reproductively isolated from other such groups and capable of producing fertile descendants; Mayr, 1969) cannot be applied to most microbial eukaryotes due to the lack of knowledge on sexual reproduction (Silva, 2008). Other species concepts have been proposed (e.g. ecological, phylogenetic, morphological) (De Queiroz, 2007). While progress has been made towards the proposition of a unified species concept (De Queiroz, 2007; Samadi & Barberousse, 2006), operationally applicable non-subjective criteria are lacking to circumscribe phytoplankton, and more generally microbial, species.

Another major scientific puzzle that has its historical roots in the nineteenth century (O’malley, 2007) and is currently at the centre of an intense debate in the field of microbial ecology concerns the conceptual principle of ‘everything is everywhere, but the environment selects’, postulating that the abundance of individuals in microbial species is so large that dispersal is never restricted by geographical barriers (Finlay, 2002). Intuitively, this might be thought to be particularly true for oceanic phytoplankton, unicellular eukaryotes drifting in a dispersive environment. This statement still structures the ecological and evolutionary understanding of microbial distribution (De Wit & Bouvier, 2006). However, microbial ecology no longer relies on culture-based studies. With the advent of molecular tools, evidence is accumulating that tends to show that physico-chemical barriers do exist for marine plankton and that species are not globally distributed (Casteleyn *et al.*, 2010) and can occupy distinct niches (Foulon *et al.*, 2008). In the near

future, the use of relevant molecular markers coupled to massive sequencing depth provided by high throughput technologies will probably allow this question to be fully addressed.

Finally, another unresolved question is that of the paradox of the plankton formulated by G.E. Hutchinson in 1961, who asked ‘why do so many planktonic species co-exist in a supposedly homogeneous habitat? (i.e. under the competitive exclusion principle of Gause, given the limited range of resources required for their growth)’. For specific ecosystems, proposed mechanisms to explain the extreme diversity of phytoplankton include spatial and temporal heterogeneity in physical and biological environments at different scales, oscillation and chaos generated by internal and external causes and self limitation by toxin-producing phytoplankton. A general and well-accepted theory to explain environmental plankton diversity is still, however, lacking (Roy & Chattopadhyay, 2007). This question is extremely challenging in the context of the uncertainties mentioned above concerning species delineation and enumeration in natural phytoplankton assemblages.



2. THE GREEN PHYTOPLANKTON: THE CHLOROPHYTES

2.1. General Considerations

The Chlorophyta together with the land plants form the green lineage (Viridiplantae). This group arose after an endosymbiotic event between a cyanobacterium-related organism and a heterotrophic eukaryote that was at the origin of the Plantae, also named Archaeplastida, a super group of eukaryotes that also includes the red algae and glaucophytes (Leliaert, Verbruggen, & Zechman, 2011). The extant Streptophyta include the land plants as well as diverse freshwater algal lineages, while the Chlorophyta include some freshwater algae and all marine representatives (De Clerck *et al.*, 2012). The Chlorophyta and Streptophyta possess the following common unique features: a double membrane bound plastid containing chlorophyll *b* as the main accessory pigment and starch as well as a unique stellate structure linking pairs of microtubules in the flagellar base. The Chlorophyta form a strongly supported group in molecular phylogenies and are characterized by unique biochemical and ultrastructural features (Leliaert *et al.*, 2011).

In marine waters, Chlorophyta are especially important within the smallest size classes, in particular the picoplankton and nanoplankton, which are formally defined as cells between 0.2–2 and 2–20 μm , respectively.

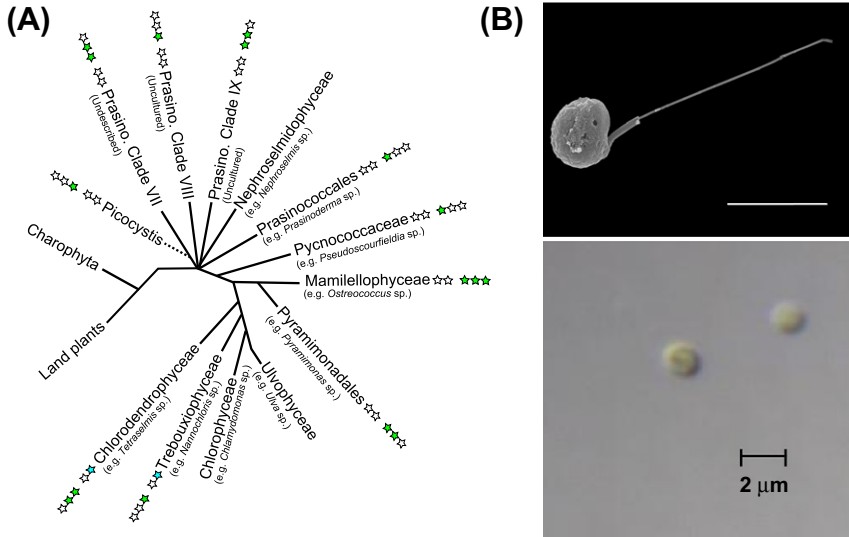


Figure 1.2 A) Schematic phylogenetic tree of the green algae and land plants lineages showing the relationships among major phytoplanktonic taxa and an estimation of their ecological significance. Typical representative of each lineage is indicated in brackets. The overall ecological significance (illustrated by a five-star ranking) is subjective and has been established based on parameters such as abundance, distribution, bloom formation, trophic strategies, toxicity, etc. (color code is 1 blue star = having freshwater members, 1 red star = important toxic or harmful species, 1–3 green stars range = other relevant ecological parameters, no stars means multi-cellular or no marine species). (B) Illustration of two important prasinophytes belonging to the Mamiellophyceae. Top: scanning electron microscopy of the common and abundant *Micromonas* sp. (E. Foulon). Bottom: the smallest photosynthetic eukaryote *Ostreococcus* sp. (D. Vault). See the colour plate.

A rather small proportion of the lineage belongs to the Trebouxiophyceae (that are mostly freshwater or terrestrial species), but most of the species described from marine isolates (Vaulot *et al.*, 2008) and most 18S rRNA gene sequences recovered from the oceanic environment correspond to prasinophytes. Prasinophytes form a polyphyletic assemblage (Fig. 1.2) with very few common characters and taxonomists are slowly re-organizing this group by creating new classes for each of the existing clades (Guillou *et al.*, 2004; Marin & Melkonian, 2010).

2.2. The Mamiellophyceae

The recently defined class Mamiellophyceae (Marin & Melkonian, 2010) encompasses three orders (Mamiellales, Dolichomastigales, and Monomastigales).

From an ecological point of view, the Mamiellales is the most important order (Fig. 1.2), containing three key genera: *Micromonas* (Butcher, 1952), with the first described picoplanktonic species *Micromonas pusilla*, *Ostreococcus* (Chrétiennot-Dinet *et al.*, 1995), containing the smallest known photosynthetic algal species (0.8- μm cell diameter), *Ostreococcus tauri*, and *Bathycoccus* (Eikrem & Throndsen, 1990), with a single scale-bearing coccoid species, *Bathycoccus prasinos*. These three related genera, which share few morphological features, are typical of coastal waters (Cheung *et al.* 2010; Collado-Fabri, Ulloa, & Vaultot 2011; Medlin, Metfies, Wiltshire, Mehl, & Valentin, 2006; Not *et al.* 2004) but can also bloom under specific conditions in oceanic waters (Treusch *et al.*, 2011) or be dominant in Arctic ecosystems (Lovejoy *et al.*, 2007). Members of these three genera can relatively easily be isolated into pure laboratory culture, facilitating their adoption as biological and ecological models. Full genome sequences for the three representative genera cited above are now available (Derelle *et al.*, 2006; Moreau *et al.*, 2012; Worden *et al.*, 2009), and their analysis has started to reveal genes that are relevant to studies of ecology and speciation (Piganeau, Grimsley, & Moreau, 2011b).

The single described species of the genus *Micromonas*, *M. pusilla*, is characterized by naked cells with a short flagellum with a characteristic hair-point and is genetically differentiated into at least three (but probably more) clades (Foulon *et al.*, 2008; Guillou *et al.*, 2004; Slapeta, Lopez-Garcia, & Moreira, 2006). Two of the major clades (A and B; sensu Guillou *et al.* 2004) are found in coastal waters, while clade C is typically oceanic (Foulon *et al.*, 2008). Within clade B, a specific lineage seems to be restricted to Arctic waters (Lovejoy *et al.*, 2007), where it can completely dominate the picophytoplankton size fraction (Balzano, Marie, Gourvil, & Vaultot, 2012).

Ostreococcus is characterized by small naked coccoid cells with no specific morphological features except a very salient starch grain in the pyrenoid (Ral *et al.*, 2004). As in the case of *Micromonas*, it can be subdivided into at least four clades based on phenotypic, genetic and genomic traits (Rodriguez *et al.*, 2005). While clade C is mostly restricted to environments where it was initially discovered (coastal lagoons), clade A is typical of surface coastal waters and clade B appears to be associated with deeper layers of the euphotic zone, displaying specific photoacclimation strategies (Six *et al.*, 2008). However, analyses of the distribution of the 18S rRNA gene of *Ostreococcus* clades in the Pacific Ocean as well as in the subtropical and tropical North Atlantic indicate that the ecophysiological parameters influencing clade distribution are more complex than irradiance alone, with factors such as temperature and nutrients also being involved in the control

of the distribution of ecotypes (Demir-Hilton *et al.*, 2011). *Ostreococcus* can form localized blooms not only in coastal waters (O’Kelly, Sieracki, Thier, & Hobson, 2003) but also in open ocean regions (Treusch *et al.*, 2011). In certain ecosystems, such as the coastal upwelling off Chile, it is the most abundant picophytoplankton species (Collado-Fabri *et al.*, 2011).

The third member of the Mamiellales, *B. prasinos*, is characterized by spider-like scales covering the cell surface (Eikrem & Throndsen, 1990). In contrast to the two other genera, there is little evidence as yet for the existence of distinct clades with the genus *Bathycoccus* (Guillou *et al.*, 2004). Although initially described from the bottom of the euphotic zone (hence the prefix ‘Bathy’; Eikrem & Throndsen 1990), *Bathycoccus* is typical of surface coastal waters (Collado-Fabri *et al.*, 2011; Not *et al.* 2004). The analysis of metagenomes obtained from sorted cells from coastal and pelagic deep chlorophyll maximum waters suggests that there may indeed be distinct *Bathycoccus* ecotypes or species adapted to these different environments (Monier *et al.*, 2012; Vaultot *et al.*, 2012). Two other nanoplanktonic genera, *Mantionella* and *Mamiella*, also belong to Mamiellales. The order Dolichomastigales contains two genera *Dolichomastix* and *Crustomastix* with nanosized cells possessing two very long flagella. The 18S rRNA gene sequences related to these four genera have been found in the Mediterranean Sea (Viprey, Guillou, F  r  ol, & Vaultot, 2008), in the Atlantic, and even associated to deep sediment samples (Marin & Melkonian, 2010), but very little information is available on their global distribution and ecology.

2.3. Other Prasinophytes

The Pyramimonadales (prasinophyte clade I) encompasses more than 35 species (Guiry & Guiry, 2012) within the main genus *Pyramimonas*, characterized by nanosized cells typically possessing four flagella. This order can be ecologically important in coastal areas (Bergesch, Odebrecht, & Moestrup, 2008) as well as in polar waters (Balzano *et al.*, 2012; Rodriguez, Varela, & Zapata, 2002).

The Chlorodendrophyceae (prasinophyte clade IV) is a recently established class (Massjuk, 2006), which contains one major genus, *Tetraselmis*, with around 30 species (Guiry & Guiry, 2012). Cells possess four equal flagella arranged in two opposite pairs and a theca composed of aggregated scales. This group does not appear to be ecologically important in marine waters, although related sequences have been found in the Mediterranean

Sea (Viprey *et al.*, 2008). Cultured strains are widely used for applications such as aquaculture (Mohammady, 2004).

The Pycnococcaceae (prasinophyte clade V) contains only two major species: *Pseudoscurfieldia marina*, a flagellate, and *Pycnococcus provasolii*, a coccoid cell. The two species share 100% 18S rRNA gene identity and could actually be the two forms of a single life cycle (Fawley, Yun, & Qin, 1999; Guillou *et al.*, 2004). *Pycnococcus* has been found to be abundant in specific ecosystems such as the Magellan Straits (Zingone, Sarno, Siano, & Marino, 2011). These species are easily isolated from oceanic waters and similar sequences have been found, for example, in the Mediterranean Sea (Viprey *et al.*, 2008), suggesting that this group may be widespread.

As in the case of the Pycnococcaceae, the order Prasinococcales (prasinophyte clade VI) contains only two genera, *Prasinoderma* and *Prasinococcus*, both falling in the picoplankton size range and containing in total three species (Guiry & Guiry, 2012). All three species produce some kind of gelatinous matrix, which, in the case of *Prasinococcus capsulatus*, has been identified as consisting of a sulfated and carboxylated polyanionic polysaccharide named capsulan (Sieburth, Keller, Johnson, & Mykkestad, 1999). They are easily isolated from marine waters (Le Gall *et al.*, 2008), but few 18S rRNA gene sequences are recovered from planktonic environmental clone libraries (Viprey *et al.*, 2008), suggesting that they may be associated to specific marine habitats such as marine particles.

Prasinophyte clade VII has not yet been formerly described, despite the fact that it contains cultured strains, all of which are picosized and coccoid (Vaulot *et al.*, 2008). It is divided into two well-supported subclades (A and B) and, depending on phylogenetic analyses, can include *Picocystis salinarum*, a small species found in inland saline lakes (Lewin, Krienitz, Goericke, Takeda, & Hepperle, 2000). The 18S rRNA gene sequences from this clade have been recovered from moderately oligotrophic areas from the Pacific Ocean and Mediterranean Sea (Shi, Marie, Jardillier, Scanlan, & Vaulot, 2009; Viprey *et al.*, 2008) as well as from coastal waters (Romari & Vaulot, 2004).

In contrast to clade VII, no cultures have yet been isolated from prasinophyte clades VIII and IX that were first discovered from 18S rRNA gene sequences in the Mediterranean Sea (Viprey *et al.*, 2008). Sequences from clade IX (but not VIII) have also been found in the very oligotrophic waters of the South East Pacific gyre (Shi, Lepère, Scanlan, & Vaulot, 2009). These clades appear to be extremely diversified and are probably an important component of the photosynthetic picoplankton in the central oceanic gyres.

2.4. Trebouxiophyceae

Trebouxiophyceae are mostly terrestrial algae, in particular associated with lichens. However, several genera, including *Picochlorum* (erected to regroup salt-tolerant *Nannochloris* species; Henley *et al.*, 2004), *Stichococcus* and *Chlorella*, can be isolated from marine waters and have been found in environmental 18S rRNA gene clone analyses from coastal waters (Medlin *et al.*, 2006).



3. THE PHYTOPLANKTON WITH CALCAREOUS REPRESENTATIVES: THE HAPTOPHYTES

3.1. Origins of the Haptophytes

The haptophytes are a distinct and almost exclusively photosynthetic protistan lineage that is widespread and often very abundant in diverse marine settings. Haptophytes are characterized by the presence of a unique organelle called a haptonema (from the Greek *hapsis*, touch, and *nema*, thread), which is superficially similar to a flagellum but differs in the arrangement of microtubules and in function, being implicated in attachment or capture of prey. The group includes some well-known taxa, such as *Phaeocystis*, *Prymnesium* and *Chrysochromulina*, that form periodic harmful or nuisance blooms in coastal environments, the calcifying species (coccolithophore) *Emiliania huxleyi* that produces massive ‘white-water’ blooms in high latitude coastal and shelf ecosystems (Fig. 1.3), and *Pavlova lutheri* and *Isochrysis galbana* that are species extensively used as feedstock in aquaculture. *E. huxleyi* has become a model species, notably for studies of the effects of ocean acidification on coccolithophore calcification (Beaufort *et al.*, 2011; Iglesias-Rodriguez *et al.*, 2008; Riebesell *et al.*, 2000), and is the only haptophyte for which extensive genomic data (including full genome sequences) are currently available.

The origin and evolutionary affiliations of the Haptophyta remain contentious. Haptophytes were tentatively grouped within stramenopiles (Cavalier-Smith, 1981) since in both lineages plastids contain chlorophylls *a* and *c* as well as various carotenoids, typically giving them a golden or brown colour, and the photosynthetic carbohydrate storage product is a β -1,3-linked glucan. Haptophytes possess a network of endoplasmic reticulum immediately below the cell membrane that was suggested to be homologous to alveoli of ciliates, amphiesmal vesicles of dinoflagellates, the inner membrane complex of apicomplexans, the periplast of

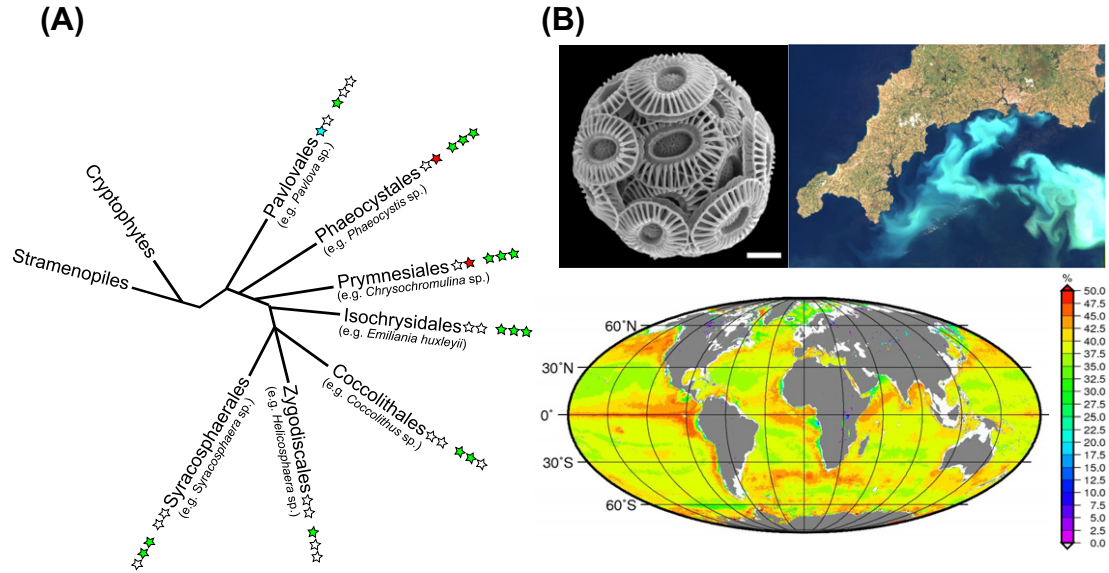


Figure 1.3 A) Legend as Figure 1.2 A but for Haptophyta. (B) Scanning electronic microscopy illustration of the coccolithophore *Emiliana huxleyi*, scale bar 1 μm (top left), satellite image showing a coccolithophore bloom off south-western England (image source: http://ina.tmsoc.org/galleries/photodujour/source/cornwall-bloom_ehux.htm) (top right) and Haptophyta pigment concentration estimates across the world oceans. (adapted from Liu et al., 2009). See the colour plate.

cryptophytes and possibly mucosal structures of heterokont algae (Andersen, 2004; Cavalier-Smith, 2002; Daugbjerg & Andersen, 1997), supporting the hypothesis that these lineages form a supergroup termed the chromalveolates, with plastids originating from a single secondary endosymbiosis event (Cavalier-Smith, 1999). Data from plastid genes have generally supported the monophyly of chromalveolate lineages (e.g. Fast, Kissinger, Roos, & Keeling, 2001; Harper & Keeling, 2003), including evidence from a lateral gene transfer common to the plastids of haptophytes and cryptophytes (Rice & Palmer, 2006). However, the chromalveolate hypothesis also implies that host nuclear lineages are monophyletic, which has not been confirmed despite the use of substantial genetic data sets. Nuclear-based phylogenomics have consistently shown that the heterokonts (Stramenopiles) and Alveolates are closely related, forming a strongly supported group with Rhizaria, together constituting the so-called SAR (Stramenopiles, Alveolates, Rhizaria) group (Burki *et al.*, 2007). Haptophytes generally branch together with cryptophytes, picobiliphytes and several heterotrophic groups (telonemids, centrohelids and katablepharids) in these analyses (e.g. Burki *et al.*, 2009). Based on congruent plastid and nuclear data, haptophytes and cryptophytes were proposed to be a distinct chromalveolate lineage, the Hacrobia (Okamoto, Chantangsi, Horak, Leander, & Keeling, 2009). A recent phylogenomic study based on alignment of 258 genes provided strong support for the hypothesis that haptophytes are sister to the SAR group, possibly together with telonemids and centrohelids, but that cryptophytes and katablepharids have a common origin and are not related to other hacrobian rather than branching with plants (Burki, Okamoto, & Keeling, 2011).

3.2. Haptophytes Diversity

The known diversity of extant haptophytes is relatively low compared to other ecologically predominant microalgal groups, with only ca. 400 extant species having been described (Jordan, Cros, & Young, 2004). Most haptophytes occur as solitary planktonic cells possessing two smooth flagella (i.e. completely lacking mastigonemes) in addition to the haptonema, but solitary non-motile planktonic or benthic cells, pseudofilamentous forms and colonies also exist. Most described haptophytes fall into the nanoplankton size class (cells 2–20 μm in diameter), but results of environmental molecular surveys indicate the existence of numerous taxa of very small (<2–3 μm) undescribed pico-haptophytes (Liu *et al.*, 2009; Moon-van der Staay *et al.*,

2000). Common ultrastructural characters of the group include the presence of plastids surrounded by four membranes, chloroplast lamellae consisting of three thylakoids without girdle (interconnecting) lamellae, tubular mitochondria and characteristic distension of Golgi vesicles in which organic scales are produced prior to being exported onto the cell surface via exocytosis. A number of haptophytes are known to undergo a haplodiplontic life cycle, with alternation between haploid and diploid phases both capable of independent asexual division, each phase characterized by distinct scale morphology.

Both morphological and molecular evidence support the division of the Haptophyta into two classes, the Pavlovophyceae and the Prymnesiophyceae (Edvardsen *et al.*, 2000). The likely existence of one or more haptophyte lineages that occupy an intermediate phylogenetic position between the two described classes has been revealed by analysis of molecular data from environmental surveys in marine (Shi *et al.*, 2009) and freshwater (Shalchian-Tabrizi, Reier-Roberg, Ree, Klaveness, & Brate, 2011; Slapeta, Moreira, & Lopez-Garcia, 2005).

The Pavlovophyceae contains only 13 described species classified in a single order, the Pavloales (Fig. 1.3). Structural features common to all or most members of the Pavlovophyceae that distinguish them from the Prymnesiophyceae include the markedly anisokont (i.e. unequal in length) nature of the flagella and the relatively simple arrangement of microtubular and fibrous roots of the pavlovophycean flagellar-haptonematal basal complex (Hori & Green, 1994). The Pavlovophyceae are also known to synthesise certain specific sterols and conjugates, the pavlovols (Véron, Dauguet, & Billard, 1996; Volkman, Farmer, Barrett, & Sikes, 1997) and a unique photosynthetic pigment signature with unknown xanthophyll and two polar chlorophyll *c* forms (Van Lenning *et al.*, 2003). Intriguingly, some pavlovophytes possess an eyespot (Lee, 1999). The organic scales of Pavlovophyceae, when present, consist of small dense bodies ('knob scales') in contrast to the plate scales of the Prymnesiophyceae. The phylogenetic relationships between known Pavloales were recently elucidated, leading to a taxonomic revision of the group (Bendif *et al.*, 2011).

The vast majority of the known diversity of haptophytes occurs in the Prymnesiophyceae, which comprises two orders of non-calcifying taxa, the Phaeocystales and the Prymnesiales, together with the coccolithophores making up a monophyletic sublineage (the subclass Calcihaptophycidae) containing four orders (Isochrysidales, Coccolithales, Syracosphaerales and Zygodiscales; Fig. 1.3).

The Phaeocystales contains a single genus, *Phaeocystis*, with less than 10 non-calcifying species, several of which have complex life histories involving solitary cells and colonies. The Prymnesiales contains two families of non-calcifying taxa, the Prymnesiaceae and the Chrysochromulinaceae (Edvardsen *et al.*, 2011), the organic plate scales of which are often relatively highly elaborated. The two families contain roughly equivalent numbers of species (30–40), but the Chrysochromulinaceae, which are known to be able to catch prey using their characteristically long haptonema (Kawachi *et al.*, 1991), appear to contain a massive undescribed diversity (possibly hundreds of genotypes) of extremely small taxa (Liu *et al.*, 2009). One recently described Prymnesiacean species produces siliceous scales (Yoshida, Noel, Nakayama, Naganuma, & Inouye, 2006).

The coccolithophores possess an exoskeleton of calcareous plates called coccoliths. Calcification occurs intracellularly in Golgi-derived vesicles, using organic base-plate scales as the substrate for crystal nucleation. Two main types of coccoliths exist: heterococcoliths, formed of a radial array of complex-shaped interlocking crystals units, and holococcoliths, constructed of numerous small, similar sized and simple-shaped calcite elements. Heterococcolith- and holococcolith-bearing taxa were originally thought to be morphologically and phylogenetically distinct species, but they are now known to be different phenotypes exhibited within the life cycle of coccolithophore species (typically, diploid life cycle stages bear heterococcoliths and haploid stages bear holococcoliths). Although the underlying structures of coccoliths are universal (Young, Didymus, Bown, Prins, & Mann, 1992), coccolith morphology is extremely diverse, with several morphological categories recognized (Young *et al.*, 2003). Coccolithophore taxonomy is almost exclusively based on comparison of coccolith morphology; however, molecular studies have demonstrated significant cryptic genetic diversity within several coccolithophore species (Saez *et al.*, 2003). Despite the existence of numerous theories (such as involvement in intracellular supply of CO₂ for photosynthesis, protection from predators or concentration of light towards plastids; see Young, 1994), the function of coccoliths remains unknown.

3.3. Haptophytes Evolution

Coccoliths are extremely abundant as microfossils, providing an outstanding tool for biostratigraphic dating and studies of evolution. The earliest appearance of coccoliths in the fossil record (corresponding to the origin of

the calcareous haptophytes) is dated at ca. 220 Mya and several clear evolutionary transitions through the subsequent evolutionary history of coccolithophores have been accurately dated (Bown, 1998). This allows calibration of multiple nodes on phylogenetic trees and hence molecular clock analysis of the evolution of the group as a whole. A recent molecular clock study based on multigene analysis (nuclear 18S rRNA gene or SSU, 28S rRNA gene or LSU and plastid *tufA* and *rbcL* genes) estimated that the haptophytes diverged from other chromists in the Neoproterozoic Era ca. 824 Mya (1031–637 Mya) around the time of the onset of the Cryogenian ‘snowball Earth’ (Liu, Aris-Brossou, Probert, & de Vargas, 2010). In the same study, the divergence of the two extant haptophyte classes was estimated to have occurred 543 Mya, early in the Cambrian period that witnessed the most rapid and widespread diversification of life in Earth’s history. The primary radiation within the Prymnesiophyceae (the divergence of Phaeocystales from other prymnesiophytes) was estimated to have occurred 329 Mya in the Carboniferous period, which was characterized by the presence of widespread shallow epicontinental seas. The timing of the next two divergences within the Prymnesiophyceae, that of the Prymnesiales and the primary radiation of the Calcihaptophycidae, both apparently followed important Earth system transitions early in the Permian and the Triassic, respectively (Liu *et al.*, 2010). Within the Calcihaptophycidae, extant coccolithophores appear to have diversified from a few lineages that survived the major extinction at the Cretaceous/Tertiary (K/T) boundary ca. 65 Mya, whereas non-calcifying haptophytes were not affected by the K/T extinction (Medlin, Saez, & Young, 2008). The adaptation of non-calcifying haptophytes to eutrophic coastal environments and their ability to switch nutrition modes from autotrophy to mixotrophy were posited as possible explanations for their survival during this abrupt global change event. Members of the Isochrysidalean family Noelaerhabdaceae have numerically dominated coccolithophore communities for the last 20 million years and continue to do so in modern oceans. The noelaerhadacean *E. huxleyi* dominates modern coccolithophore assemblages despite the fact that it is a very young species in geological terms, having originated only 220 Kya (Thierstein, Geitzenauer, Molino, 1977).

3.4. Distribution and Ecology of Haptophytes

Haptophytes adopt diverse ecological strategies, being present in open-ocean, shelf, upwelling, coastal, littoral, brackish and freshwater environments. In

addition to undertaking photosynthesis, many haptophytes are known to be capable of using particulate and/or dissolved organic food sources, the group probably therefore being predominantly mixotrophic (de Vargas, Aubry, Probert, & Young, 2007). Life cycle transitions, with each phase adapted to distinct ecological niches (Noel, Kawachi, & Inouye, 2004), may also be an integral part of the ecological strategy for different haptophytes.

Pavlovophytes have been described mostly from cultures isolated from littoral, brackish water and in some cases freshwater environments, and they are common components of near-shore planktonic and benthic microalgal communities in widespread locations. Due to the difficulty in identifying pavlovophytes in the light microscope, it is not clear whether they commonly occur in open-ocean environments. *Phaeocystis* is ubiquitous from poles to tropics and from coastal to open ocean waters and certain species regularly produce extensive blooms, notably in Arctic, Antarctic and North Sea waters. *Phaeocystis* blooms can be detrimental to the growth and reproduction of shellfish and zooplankton, and hemolytic and toxic effects have been reported on fish (Schoemann, Becquevort, Stefels, Rousseau, & Lancelot, 2005). Some Prymnesiales species also form periodic blooms in coastal waters, occasionally harmful to fish and other biota. The Prymnesiaceae tend to be restricted to coastal waters, whereas the Chrysochromulinae also thrive in oligotrophic open ocean regions (Liu *et al.*, 2009) where mixotrophic nutrition is likely an important strategy. Through the process of calcification and export of calcite to the deep ocean following cell death, coccolithophores play a key role in the 'biological pump' that contributes significantly to global carbon cycling (Rost & Riebesell, 2004). Massive annual blooms of *E. huxleyi* in temperate and subpolar coastal and shelf environments are visible in satellite images (Fig. 1.3). Among the factors that may contribute to the ecological success of *E. huxleyi* are high affinities for inorganic nutrient uptake (Paasche, 2002) and physiological mechanisms for maintaining growth under high irradiance (Loebl, Cockshutt, Campbell, & Finkel, 2010; Ragni, Aris, Leonardos, & Geider, 2008). Specific viruses play an important role in regulation of *E. huxleyi* blooms (Wilson *et al.*, 2002). Although it does not form bloom populations in subtropical and tropical environments, *E. huxleyi* (and the closely related species *Geophyrocapsa oceanica*) is widespread and relatively abundant in these ecosystems. The Coccolithales genus *Coccolithus* includes relatively large cells (ca. 20 μm) that, together with *E. huxleyi*, dominate coccolithophore communities in subpolar and temperate regions of the North Atlantic as well as occurring in western-margin upwelling zones

around the globe. The Coccolithales, Syracosphaerales and Zygodiscales include species that contribute significantly to communities in warm water mesotrophic settings, including *Calcidiscus*, *Umbilicosphaera*, *Syracosphaera*, *Helicosphaera*, *Discosphaera*, *Rhabdosphaera* and *Scyphosphaera*. The genus *Syracosphaera* is notably very diverse in mesotrophic to oligotrophic coccolithophore communities. *Umbellosphaera*, a genus of uncertain phylogenetic affinities, is abundant in oligotrophic surface layer communities. The coccolithophores *Florisphaera* and *Gladiolithus* are important members of deep-photic zone communities, but the adaptations that allow them to thrive in conditions of extremely low light are unknown.



4. THE MULTIFACETED PHYTOPLANKTON: THE DINOFLAGELLATES

4.1. Dinoflagellates as Members of the Alveolate Lineage

The Alveolata constitutes a diverse group of single-celled eukaryotes present in both marine and terrestrial ecosystems, the principal shared morphological feature of which is the presence of flattened vesicles (cortical alveoli) packed into a continuous layer supporting the cell membrane (Cavalier-Smith & Chao, 2004). These structures have been associated by immunolocalization to a family of proteins, named alveolins, common to all alveolates (Gould, Tham, Cowman, Mcfadden, & Waller, 2008). Alveolates exhibit extremely diverse trophic strategies, including predation, photo-autotrophy and intra-cellular parasitism. Most alveolates fall into one of three main subgroups (or phyla): ciliates, dinoflagellates and Apicomplexa, all sharing a common ancestor (Leander & Keeling, 2003). Apicomplexans are obligate parasites of animal cells, including humans (e.g. *Plasmodinium* which causes malaria). Ciliates are mainly aquatic predators that perform essential roles as consumers in microbial food webs, although some taxa can be parasitic or may contain sequestered plastids (e.g. *Myrionecta rubra*). Dinoflagellates are either photo-autotrophs, free-living predators (heterotrophs) or both, either simultaneously or alternatively (mixotrophs), while some also live as parasites or symbionts (Taylor, Hoppenrath, & Saldarriaga, 2008). Other lineages of marine alveolates (MALV) have been identified in culture independent surveys and associated to the class Syndinea (order Syndiniales). These are divided into two main groups, MALV-I and MALV-II, the phylogenetic placement of which is still uncertain (Massana & Pedros-Alio, 2008). Both groups have

been proposed to correspond to heterotrophic parasites of marine organisms (Guillou *et al.*, 2008; Gunderson, John, Boman, & Coats, 2002).

Dinoflagellates and Apicomplexa are the only groups of alveolates that possess plastids. These structures are actively involved in photosynthesis only in dinoflagellates, while they are vestigial in apicomplexans (McFadden, Reith, Munholland, 1996). This common character between these two groups suggests that their common ancestor was photosynthetic (Leander & Keeling, 2003). In contrast to other alveolates, dinoflagellates can develop cellulose-like polysaccharide plates within the cortical alveoli, forming a theca. The arrangement and ornamentation of these plates leads to an astonishing variety of shapes, the description and comparison of which forms the traditional basis of species classification for dinoflagellates.

4.2. Dinoflagellates Diversity

The majority of dinoflagellates (perhaps 80%) are free-living marine planktonic or benthic flagellates, the remainder inhabiting equivalent freshwater habitats (Taylor *et al.*, 2008). Half of the estimated 2000 extant species of dinoflagellates are considered photosynthetic. Dinoflagellates contain a characteristic nucleus with permanently condensed chromosomes (the dinokaryon). The haploid motile stage within dinoflagellate life cycles typically possesses two dissimilar flagella: a ribbon-like flagellum with multiple waves situated in a transverse groove (cingulum) and a more conventional flagellum emerging from a ventral furrow (sulcus) which beats posteriorly to the cell. After sexual recombination, dinoflagellates produce resistant diploid benthic stages (hypnozygotes, also termed resting cysts) to escape predation and adverse environmental conditions as well as to colonize ecosystems. Some dinoflagellates are noxious to humans and other marine organisms due to the production of potent toxins, while others cause hypoxia, anoxia or mechanical damage to marine fauna. Finally, certain dinoflagellates, including some toxin-producing species, can form large blooms ('red tides') that can negatively affect economic activities in coastal areas (Hallegraeff, 2003, 2010) (Fig. 1.4).

Nine major orders (Gonyaulales, Peridiniales, Gymnodiniales, Suesiales, Prorocentrales, Dinophysiales, Blastodinales, Phytodinales and Noctilucales) are recognized within the dinoflagellates (Fig. 1.4), the order Thoracosphaerales being suspected to belong to the Peridiniales (Saldarriaga, Taylor, Cavalier-Smith, Menden-Deuer, & Keeling, 2004). Dinoflagellate orders can be distinguished on the basis of major morphological characters

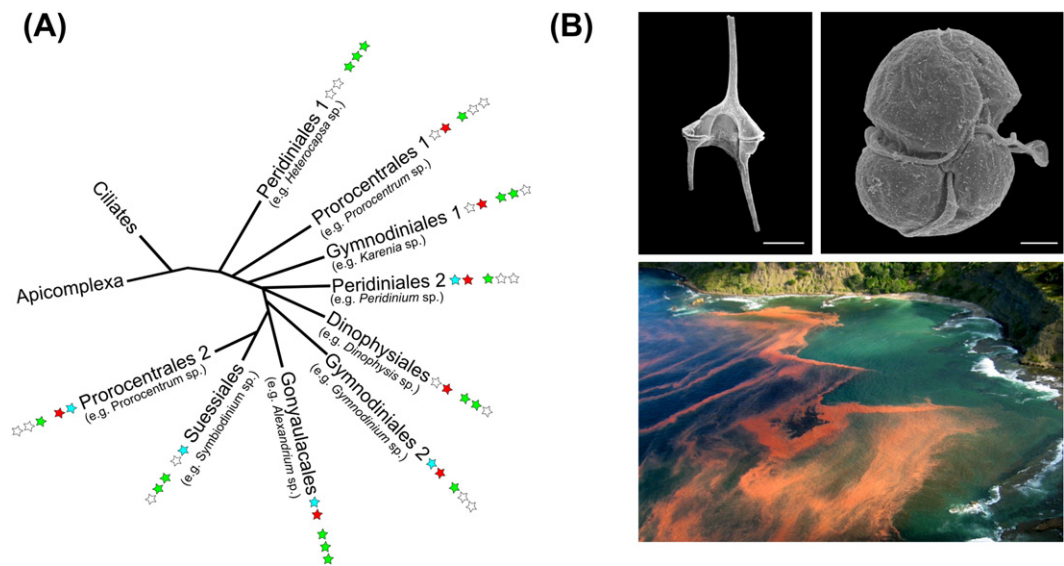


Figure 1.4 A) Legend as Figure 1.2 A, but for dinoflagellates. (B). Scanning Electron Microscopy (SEM) micrographs of *Neoceratium candelabrum*, a non-toxic thecate micro-dinoflagellate (scale bar = 30 μm), a species of the Gonyaulacales lineage (top left) and *Karlodinium veneficum*, belonging to lineage Gymnodiniales 1, an athecate nano-dinoflagellate (scale bar 2 μm) that is toxic for a range of marine invertebrates and fish (top right). Red discoloration caused by the dinoflagellate *Noctiluca scintillans* off Waiheke Island (New Zealand) (<http://www.TeAra.govt.nz/en/plankton/1/4>) (bottom). See the colour plate.

and life cycle features of their members (Table 1.1). The Gonyaulacales and Peridinales are characterized by the presence of cellulose-like thecal plates within the cortical alveoli. The thecae of both groups are constituted of five latitudinal series of plates (apical, anterior intercalary, precingular, post-cingular and antapical) plus the cingular and sulcal series. The two orders were separated by Taylor (1980). Members of the order Blastodinales have

Table 1.1 Main Morphological Features for Major Orders of Dinoflagellates

Order	Main morphological features
Gonyaulacales	Cellulose-like thecal plates within the cortical alveoli Left-handed torsion of the epitheca Small anterior intercalary plates (often absent) Apical pore complex often with a hook-like groove Asymmetric antapical plates
Peridinales	Cellulose-like thecal plates within the cortical alveoli Bilateral symmetry Anterior intercalary plates Small apical pore Two subequal antapical plates
Gymnodinales	Cortical alveoli without thecal plates (athecate, unarmored, naked dinoflagellates) Apical furrow on the cell apex
Suessiales	Cortical alveoli without thecal plates (athecate, unarmored, naked dinoflagellates) Generally 7–10 longitudinal series of cortical alveoli (less than in Gymnodinales)
Prorocentrales	Elongated apical vesicle on the cell apex Division of theca into lateral halves joined by a sagittal suture Lack of the sulcus and the cingulum Two pores, the flagella emerging from the larger one Tiny periflagellar platelets
Dinophysiales	Division of theca into lateral halves joined by a sagittal suture Two pores, the flagella emerging from the larger one
Blastodinales	Temporary dinokaryon Parasitic lifestyle Dinospores with peridinioid plate tabulation
Phytodinales	Shift from a non-calcareous coccoid cell or continuous-walled colonial stage to a vegetative stage
Noctilucales	Highly mobile ventral tentacle Lack (at least in some life stages) of the ribbon-like transverse flagellum or the condensed chromosomes of the dinokaryon

a parasitic lifestyle and a temporary dinokaryon, which fostered the hypothesis that Blastodinales diverged early from the dynokaryotic dinoflagellate lineage (Saldarriaga *et al.*, 2004). However, dinospores of the genus *Blastodinium* have been shown to have peridinoid plate tabulation, suggesting affiliation to the Peridinales. Although the genus *Blastodinium* has been demonstrated to be late-branching, consistent with Peridinales evolution, the affiliation of the genus and of the whole order of Blastodinales to Peridinales is still to be proved (Skovgaard, Massana, & Saiz, 2007). The Prorocentrales and the Dinophysiales share a major synapomorphic feature, unique within dinoflagellates: the division of theca into lateral halves joined by a sagittal suture. The order Phytodinales includes species characterized by a shift from a non-calcareous coccoid cell or continuous-walled colonial stage to a vegetative stage. Similar life shifts have, however, also been observed in genera of other orders (e.g. Suessiales [*Symbiodinium*], Gonyaulacales [*Pyrocystis*]). The Phytodinales presently includes poorly understood genera for which little molecular data are available. The Noctilucales is an early-diverging order that includes aberrant dinoflagellates characterized by a highly mobile ventral tentacle, which is missing in typical dinoflagellates and other alveolates. The tentacle does not play a role in keeping the cell in suspension but seems rather related to food capture. The Noctilucales have the ability to incorporate, replace or lose chloroplasts, a rare phenomenon in other alveolate groups. Whether these chloroplasts are kleptoplastids or derive from ancient endosymbiosis, as in other dinoflagellate families, remain to be demonstrated (Gomez, Moreira, & Lopez-Garcia, 2010).

4.3. Dinoflagellates Evolution

To date, comparison of morphological, cytological and nuclear genetic markers (18S rRNA gene, 28S rRNA gene, ITS rDNA) has not clearly resolved relationships between dinoflagellate orders. This raises the question as to whether all dinoflagellate orders emerged about the same time during a major radiation period (Hoppenrath & Leander, 2010). Phylogenetic studies carried out using plastid genes (*rbcL*, *atpB*) are limited since many dinoflagellates are heterotrophic, and mitochondrial genes (*cob* and *cox 1*) are only useful in combination with other genetic markers and for specific groups of dinoflagellates (Zhang, Bhattacharya, & Lin, 2005). Some major phylogenetic traits can, however, be identified for dinoflagellates (Fig. 1.4). The genus *Oxyrrhis* is often considered a predinoflagellate lineage (Saldarriaga, McEwan, Fast, Taylor, & Keeling, 2003), followed by *Noctiluca*

scintillans (order Noctilucales), which now appears more closely related to Syndinea (Gomez *et al.*, 2010). The Dinophysiales (Gomez, Lopez-Garcia, & Moreira, 2011), Suessiales (Siano, Montresor, Probert, Not, & De Vargas, 2010) and Gonyaulacales (Saldarriaga *et al.*, 2004) are strongly supported holophyletic groups. The Prorocentrales splits into two branches in both 18S rRNA and 28S rRNA genes phylogenies (Saldarriaga *et al.*, 2004), but not in mitochondrial *cox-1* topology (Murray, Ip, Moore, Nagahama, & Fukuyo, 2009). *Cox-1* demonstrates the monophyly of the morphologically very cohesive group of prorocentroid dinoflagellates, but it separates the group into two clades that include species present, respectively, in the two branches of the 28S rRNA gene phylogeny. The Gymnodiniales is a polyphyletic order and together with the Peridiniales are the most evolutionary complex groups of dinoflagellates. The *Karenia*/*Karlodinium* clade separates from other gymnodinioid clades (e.g. *Akashiwo*, *Gymnodinium*, *Amphidinium* clades) in both 18S and 28S rRNA gene phylogenies (Murray, Jorgensen, Ho, Patterson, & Jermilin, 2005; Saldarriaga *et al.*, 2004). The Peridiniales appears to be a complex paraphyletic group of dinoflagellates, that is, its phylogeny comprises non-Peridiniales branches. The *Heterocapsa* clade often has a basal position to other peridinioids and in general to other thecate dinoflagellates in phylogenies inferred from the 18S and 28S rRNA genes, despite having mediocre branching support. Other Peridiniales branches (*Peridinium*, *Scrippsiella* and *Protoperidium*) branch later in phylogenetic trees. General dinoflagellate phylogenies still require molecular data for many dinoflagellate genera (especially for heterotrophic species), taxonomic revision of some species and identification of species likely to correspond to missing branches of phylogenetic trees (Fig. 1.4).

Considerable diversity of chloroplast types and pigment composition occurs in photosynthetic dinoflagellates, acquired through secondary and tertiary endosymbioses (Cavalier-Smith, 1999) with in some cases multiple losses and replacement of plastids, as revealed by molecular phylogenetic analysis (Saldarriaga, Taylor, Keeling, & Cavalier-Smith, 2001). Some dinoflagellates harbor foreign plastids that are periodically lost and gained during their life cycle (kleptoplastidy, from the Greek 'klepto' – stealing), or bear photosynthetic endosymbionts that are kept for longer periods but not fully integrated (Moestrup & Daugbjerg, 2007). Beyond chlorophyll *a*, *c*2 and β -carotene, dinoflagellates with permanent plastids can have three other accessory pigments: peridinin (Gonyaulacales, Peridiniales, Prorocentrales, Suessiales and some Gymnodiniales), fucoxanthin or fucoxanthin derivatives (the family Kareniaceae of the order Gymnodiniales) and chlorophyll *b* (the

genus *Lepidodinium* of the order Gymnodiniales). The toxin-producing genus *Dinophysis* (Dinophysiales) is a peculiar case. It is still debated whether *Dinophysis acuminata* has permanent plastids of cryptophyte origin (Garcia-Cuetos, Moestrup, Hansen, & Daugbjerg, 2010) or whether it maintains a temporary plastid (kleptoplast) acquired from prey (Wisecaver & Hackett, 2010). The latter hypothesis is supported by the fact that *Dinophysis* species can only be cultured using the ciliate *Myrionecta rubra* as prey, which itself feeds on cryptophytes and would thus be the source for *Dinophysis* of the cryptophytes and their chloroplasts (Park *et al.*, 2006). The fact that *Dinophysis* needs the ciliate as an intermediary to acquire cryptophyte plastids suggests that it probably lacks critical enzymes to initially process the prey or maintain their plastids (Wisecaver & Hackett, 2010). Future transcriptomic and genomic sequencing would help up to understand if this dinoflagellate possesses a photosynthetic machinery and how it regulates genes that coordinate photosynthetic activity and/or prey capture.

4.4. Ecology of Dinoflagellates

Photosynthetic dinoflagellates are common and abundant in pelagic and benthic habitats of both marine and freshwater ecosystems. Typically, they reach their highest abundances in estuaries and coastal marine waters, in concomitance with high nutrient supply from land sources and/or deep water upwelling. Blooms of noxious species (HABs) are more common under these conditions (Fig. 1.4). Using their two perpendicular flagella, dinoflagellates exhibit directed movement in response to chemical stimuli, physical variations, gravity and light. Due to this motility, dinoflagellates are able to find optimal conditions for growth and survival under high physical disturbance (turbulence and shear forces), intense light stress and nutrient limitation. Because dinoflagellates have various habitat preferences, multiple life strategies and are nutritionally versatile, they are very competitive with other groups of protists for resource acquisition. Although they can sometimes be important in terms of biomass, both micro- and nano-dinoflagellates are rarely reported to dominate in terms of abundance within the phototrophic fraction of plankton communities (Siokou-Frangou *et al.*, 2010). This is probably partly due to the fact that quantitative information is very fragmentary for dinoflagellates because of inadequate identification methodologies, notably for athecate species.

In coastal waters, bloom initiation can be due to germination of vegetative cells from hypnozygotes. The biological and physical factors that trigger bloom initiation are poorly known for most dinoflagellates, including harmful

species (Burkholder, Azanza, & Sako, 2006). During bloom development, many dinoflagellate species are capable of rapid growth, attaining abundances up to 10^9 cells/L (up to 400–500 $\mu\text{g Chla/L}$) (Taylor & Pollinger, 1987). Dinoflagellates can grow at rates of up to 3.5 divisions per day, but only 15% of the larger free-living harmful species have growth rates greater than 1.0 division per day (Smayda, 1997). Photosynthetic dinoflagellates are primarily limited by phosphorous and nitrogen, although they can store these nutrients in a species-specific way that in some cases allows one species to outcompete others (Graham & Wilcox, 2000; Labry *et al.*, 2008). As for other phytoplankton taxa, micro-nutrients, including forms of selenium and iron, have been shown to influence blooms of some harmful phototrophic dinoflagellates (Boyer & Brand, 1998; Doblin, Blackburn, & Hallegraeff, 2000). Apart from nutrient limitation, bloom termination can be caused by water dispersion and dilution, zooplankton grazing and biological endogenous cycles, as well as parasite (Chambouvet, Morin, Marie, & Guillou, 2008) and viral (Nagasaki, Tomaru, Shirai, Takao, & Mizumoto, 2006) infections.

Field observations indicate that bloom-forming dinoflagellate species have neither strict habitat preferences nor uniform responses. Smayda and Reynolds (2001) recognized nine different pelagic habitats where dinoflagellates bloom, arranged along an onshore–offshore gradient of decreasing nutrients, reduced mixing and deepening of the photic water layer. Each of the nine types of habitat is characterized by a specific dinoflagellate life-form, which suggests that dinoflagellates have evolved multiple adaptive strategies, rather than a common ecological strategy. Subsequently, these authors introduced five rules of assembly for marine dinoflagellate communities, which state that specific habitat conditions correspond to specific life forms that are mainly selected on the basis of abiotic factors (turbulence and nutrient availability). Within the species pool of a given habitat, seasonal succession is stochastic and characterized by a high degree of unpredictability (Smayda & Reynolds, 2003). Conversely, for cyst-forming dinoflagellates, the existence of endogenous or exogenous factors determining the presence and succession of species has been hypothesized, suggesting that the apparent random succession of species within a pool of species is understandable and predictable (Anderson & Rengefors, 2006).

The ecological role of dinoflagellates in the functioning of marine ecosystems and in the marine food web can be significant. Some heterotrophic species of the genera *Ornithocercus*, *Histioneis* and *Citharistes* can host photosynthetic endosymbionts. In autumn in oligotrophic subtropical

waters of the Gulf of Aqaba (Israel), peaks of these species coincided with extended nitrogen limitation and high abundances of free-living N-fixing cyanobacteria. It has been proposed that heterotrophic dinoflagellate hosts may provide cyanobacterial symbionts with the anaerobic microenvironment necessary for efficient N fixation, which would in turn determine the ecological success of the hosts (Gordon, Angel, Neori, Kress, & Kimor, 1994). This symbiotic relationship as well as the capacity of N fixation by the cyanobacterial symbionts has been suggested (Foster, Carpenter, & Bergman, 2006). Alternatively, some dinoflagellates are symbionts of benthic organisms (*Symbiodinium*) (Freudenthal, 1962) or of pelagic protists (*Pelagodinium*) (Siano *et al.*, 2010). Many, if not most, photosynthetic dinoflagellates are considered mixotrophic (Smalley & Coats, 2002). It has been suggested that the larger size and lower cell surface-to-volume ratios of dinoflagellates generally result in lower affinities for dissolved nutrients than smaller protists, therefore positively selecting for mixotrophy among photosynthetic species (Smayda, 1997). However, mixotrophy is often difficult to assess clearly because of low feeding rates, intermittent feeding dependent on conditions poorly simulated in cultures, specificity of prey or the fact that organelles can obscure food vacuoles (Stoecker, 1999).



5. THE SILICEOUS PHYTOPLANKTON: THE DIATOMS

Diatoms, also called Bacillariophyceae, are unicellular photoautotrophic stramenopiles, the defining feature of which is the compound silica cell wall, called a frustule. The diatoms constitute one of the most diverse lineages of eukaryotes with possibly over 100,000 extant species (Mann & Droop, 1996). They are ubiquitous in marine and freshwater habitats and in damp terrestrial environments. It is therefore not surprising that diatoms have been studied intensively ever since microscopes became available. We review here the diversity and ecology of diatoms and refer to Chapter VII of this volume for a review on diatom genomics (Mock & Medlin 2012).

5.1. The Hallmark of the Diatom: The Silica Cell Wall

The diatom frustule is composed of two overlapping thecae (the larger called the epitheca and the smaller the hypotheca), each of which consists of a valve and an accompanying series of girdle bands. These frustule elements contain rows of pores, called interstriae, with ribs between them, called striae. The pores in the interstriae form the principal conduits for the uptake of nutrients

and exudation of metabolites. Girdle bands are architecturally relatively simple and always consist of a single layer, whereas valves are more elaborate, with a flat area, called the valve face, and a rim, called the mantle. Valves are either composed of a single layer or two layers. In the latter case, the internal layer has larger pores and the two layers are connected via perpendicular cross walls in a rectangular or honeycomb pattern, giving rise to chambered valves (see [Round, Crawford, & Mann, 1990](#)).

Construction of new silica cell wall elements proceeds in silica deposition vesicles (SDVs) near the plasma membrane. Dissolved silicic acid is actively taken up from water and concentrated in the cytoplasm far above the level at which silica would normally polymerize. Precipitation in the cytoplasm is avoided because silicic acid transporter proteins bind the silica and shepherd it through the cytoplasm into the SDV. Other classes of peptides, including silaffins, silacidins and long-chain polyamines, are produced in the endoplasmic reticulum, transported in vesicles to the Golgi apparatus where they are modified and activated, then transported into the SDV where they form a matrix onto which the super-saturated silica precipitates in an amorphous form ([Hildebrand, 2008](#); [Kroger & Poulsen, 2008](#)).

During vegetative cell division, new thecae are laid down in such a way that the cell is covered completely by silica cell wall elements throughout the division phase. Newly formed thecae must therefore be formed within the confines of existing thecae. The daughter cell inheriting the parental epitheca (the larger half of the frustule) forms a new hypotheca of the same size as that of the parental cell and hence this daughter cell is of the same size as the parent. By contrast, the daughter cell inheriting the parental hypotheca (the smaller half of the frustule) uses it as an epitheca within which a new hypotheca is formed; hence, this daughter cell is slightly smaller than its parent. Consequently, average cell diameter diminishes with ongoing mitotic division. The only escape from this continual miniaturization is sexual reproduction (see [Section 5.3](#)).

5.2. Diatom Diversity

Diatoms are categorized into centrics and pennates based on characteristics of valves, including shape, ultrastructure, ornamentation and types of processes (tubes, slits) ([Fig. 1.5](#)). The principal difference is the way in which the interstriae are organized. Centric diatoms possess radially organized valves with striae radiating from a central region or ring, whereas pennates possess elongated valves with striae oriented perpendicular to a midrib (called

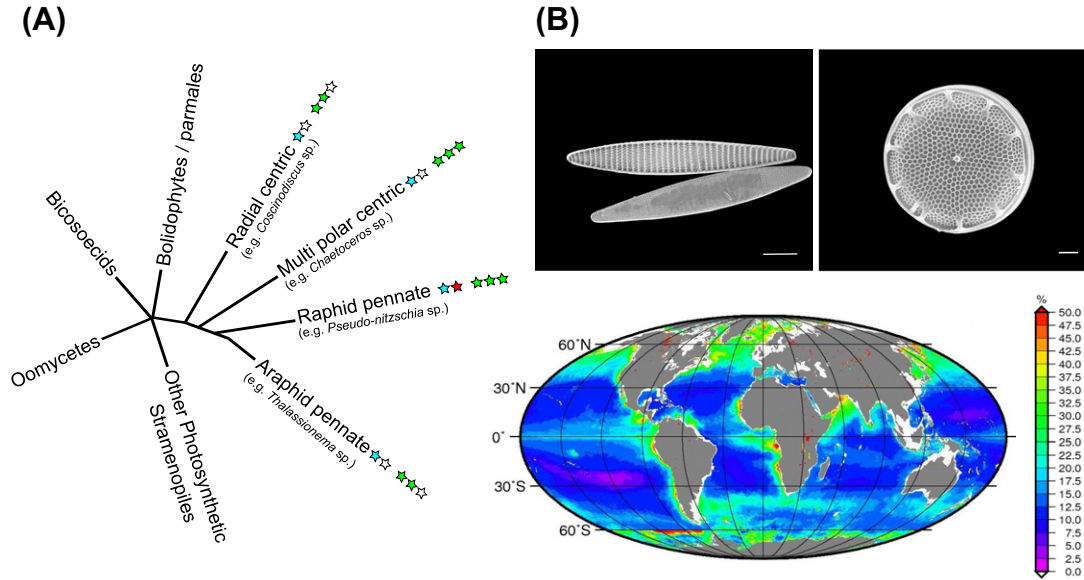


Figure 1.5 A) Legend as Figure 1.2 A, but for diatoms. (B) Pennate diatom *Fragilariopsis kerguelensis*, top valve: interior side, bottom valve: exterior side, scale bar 10 μm (by Marina Montresor) (top left), centric diatom *Thalassiosira tealata* showing valve exterior with 10 peripheral strutted processes, a central strutted process and a peripheral labiate process, scale bar 1 μm (by Diana Sarno) (top right), and diatom pigment concentration estimates across the world oceans (adapted from Liu et al., 2009). See the colour plate.

a sternum), like in a feather (Round *et al.*, 1990). Electron microscope illustrations of ultrastructural details of diatoms can be found in Round *et al.*

One group of centric diatoms, the radial centrics, possesses valves shaped like Petri dishes. Radial centrics usually possess a ring of so-called labiate processes around their valve mantle, forming tubes that may enable intake or secretion of organic material. Important planktonic representatives of radial centrics include *Aulacoseira*, *Corethron*, *Coscinodiscus*, *Leptocylindrus*, *Melosira* and *Rhizosolenia*. A second group of centrics, the so-called multipolar centrics (bi-, tri-, etc., referring to polarity of shape) also exhibit a radial pore organization, but their cell form is usually elongate, triangular or starlike; that is, exhibits polarity. Labiate processes, if present, are located on the central area of valves. Many species possess fields of densely packed pores at their valve apices. Mucilage is exuded through these apical pore fields, enabling benthic or epiphytic species to attach to the substratum or to form chains. Planktonic representatives such as *Eucampia* also form chains this way. Secondarily radial centric genera such as *Lauderia*, *Porosira*, *Skeletonema* and *Thalassiosira* (Order Thalassiosirales) possess specialized tubes in their valves, called strutted processes, through which chitin filaments are exuded. These filaments extend into the surrounding medium and link cells into chains. *Chaetoceros* and *Bacteriastrum* (Order Chaetocerotales) form chains by means of setae; thin hollow silica tubes that are formed following cell division. Setae probably evolved principally as a grazer deterrent, but they have acquired additional functions. In some species, plastids migrate up and down setae. Chaetocerotales and Thalassiosirales are highly diverse and their species form important constituents of phytoplankton blooms.

Pennate diatoms are elongate, their defining feature being the midrib from which striae and interstriae extend perpendicularly. The pennates are subdivided into raphid pennates, which possess a slit-shaped process, called a raphe, and araphid pennates, which lack a raphe, but have apical pore fields and apical labiate processes. The raphe slit enables raphid pennates to move actively by means of a cost-effective system of traction. Long-chain organic compounds protrude partially through one end of the slit and connect to the substratum. Subsequently, the chain is pulled along the slit and then exuded. Consequently, the diatom moves in the opposite direction. Most raphid pennates are benthic, but a few, including *Fragilariopsis* and *Pseudo-nitzschia*, have secondarily acquired a planktonic lifestyle. The araphid pennates are also typically benthic, but there are also several lineages that have acquired a planktonic lifestyle, including *Asterionella*, *Asterionellopsis*, *Lioloma*, *Thalassionema* and *Thalassiothrix*.

Arguably most diatom diversity is not planktonic, but benthic or epiphytic. Many species occur unattached, but their frustules are robust and way too heavy for a planktonic existence, thus abounding drifting over sandy bottoms. Diatom diversity shows several clades and grades containing planktonic species, but these are most often rooted in benthic ancestry. This versatility between planktonic and benthic existence in the evolutionary history of the diatoms means that traits acquired in a benthic setting may later have provided a benefit in the plankton. For example, raphid pennate diatoms are typically found in benthic habitats where they use their raphe to move actively over the substratum. *Pseudo-nitzschia*, a planktonic representative that evolved from benthic ancestry, use their raphe to enable daughter cells to slide along each other adjacent valve faces to assume a position in which they are just attached at their valve apices. In this way, *Pseudo-nitzschia* has acquired a novel way to form chains in the plankton.

5.3. Diatom Life Cycle

The diatoms possess a diplontic life cycle consisting of a long period (up to several years) during which diploid cells divide mitotically and a brief period (a few days) during which sexual reproduction takes place. Gametes fuse to form a zygote, which inflates to generate an auxospore. The new frustule elements of the resultant vegetative cell are formed within the confines of the auxospore wall and once this is completed the cell emerges from the auxospore (Round *et al.*, 1990). Centric diatoms form non-motile macrogametes and flagellated microgametes. In radial centrics and Thalassiosirales, the zygote inflates isometrically and forms an organic wall in which, in some species, small silica elements are embedded. In multipolar centrics, gamete formation and conjugation proceed as in radial centrics, but zygote inflation is anisometric; in this case, a series of silica bands, together called a properizonium, is laid down in sequence to mould the expanding auxospore into a bi- or multipolar shape. Pennate diatoms are isogamous (= produce gametes of equal size, though generally not of equal behaviour) and usually dioecious. Sexual reproduction involves alignment of cells of opposite mating types, gamete formation, migration in an amoeboid fashion to the partner gametes and zygote formation. Zygote inflation is constrained by a double series of perizonial bands into a cigar-like auxospore. The new frustule is formed within the confines of this perizonium. For pennates, it is unclear what provokes sexual reproduction, how potential mates detect each other and how and in what order they initiate the process of gametogenesis.

Several centric diatoms form resting spores (Ishii, Iwataki, Matsuoka, & Imai, 2011; Kooistra *et al.*, 2010). Spores are formed usually in response to deteriorating conditions. Resting spores can remain viable for extended periods (Härnström, Ellegaard, Andersen, & Godhe, 2011) and are often so robust that they exist as fossil markers (Suto, 2006). Auxospores are not resting cysts; their silica elements are flimsy and disintegrate and dissolve soon after the initial cell hatches.

5.4. Diatom Evolution

Results of molecular phylogenetic studies have generally revealed that centrics are the most ancient group of diatoms, forming a grade (= paraphyletic group). Multipolar centrics evolved from radial centric ancestry, but they form a grade because all pennates form a clade within this group. Within the pennates, the raphid pennates form a clade inside a grade of araphid pennates (Fig. 1.5).

This phylogenetic pattern suggests that the properizonial bands of multipolar centric auxospores and the perizonial bands of pennate auxospores are homologous structures and that these bands were acquired in the last common ancestor of the clade containing all multipolar centrics and pennates. The formation of these bands during auxospore formation enabled diatoms to generate shapes diverging from those resembling Petri dishes and tubes. Moreover, the results suggest that isogametogenesis is a derived trait of all pennates and that it evolved from oogenesis of their multipolar ancestor, possibly because it is a more efficient mode of sexual reproduction in benthic environments. The organization of the pennate valve with its midrib evolved from a radial organization mode. The advantage of such a midrib may have been the structural reinforcement it provides in lightweight but elongated diatom valves. Finally, the raphe of raphid pennates evolved from the apical labiate processes of their araphid pennate ancestry (Kooistra, Gersonde, Medlin, & Mann, 2007).

Each of these acquisitions apparently resulted in rapid diversification of the lineage exhibiting the novelty. Radial centrics appear to consist of a restricted number of remnant lineages, as do most multipolar centrics. Within the latter, the Thalassiosirales with their chitin threads and the Chaetocerotales with their setae constitute two highly diverse clades. The pennates as a whole form a huge clade with a poorly resolved basal structure and the same is true for raphid pennates that constitute a relatively novel lineage, but by far the most diverse group. Nonetheless, radial centrics do not

constitute an evolutionary dead-end. Many radial centrics, such as *Lep-
tocyldrus*, are important bloom formers in coastal regions all over the world.

Diatoms possess an extensive fossil record. Frustule elements of vegetative cells and spores are preserved, often in exquisite detail, over millions of years (e.g. Gersonde & Hardwood, 1990; Ishii *et al.*, 2011; Suto, 2006). In addition, biomarkers specific for particular diatom lineages, for instance *Rhizosolenia*, are detectable in petroleum of definable age (Sinninghe-Damsté *et al.*, 2004). These sources of information reveal that radial centrics first appeared in the Jurassic, multipolar centrics in the Early Cretaceous, pennates in the Late Cretaceous and the first raphid pennates at 55 Mya in the Paleogene. This order of appearance corroborates the above-mentioned phylogenetic patterns in extant diversity. The diatoms apparently traversed the K/T boundary relatively unscathed, with diversity subsequently increasing (Harwood, Chang, & Nikolaev, 2004; Stoermer & Smol, 1999).

5.5. Diatom Ecology

Diatoms are ubiquitous in the plankton and benthos of marine and freshwater habitats. Centric diatoms are typically marine, but a few exclusively freshwater genera exist and a few large marine genera have freshwater representatives (e.g. *Thalassiosira*). Araphid pennates are predominantly marine, but there are also many freshwater genera and some marine genera have freshwater representatives. Raphid pennates seem to be well represented in both freshwater and marine environments and many genera have representatives in both. Because of their abundance in shallow coastal seas, it has been estimated that planktonic diatoms account for as much as 20% of global photosynthetic fixation of carbon (~ 20 Pg carbon fixed per year; Mann, 1999), which is more than all the world's tropical rainforests, and that their contribution to nutrient cycling is significant (Boyd *et al.*, 2000). Planktonic diatoms are particularly important bloom-formers in nutrient-rich coastal regions, especially in temperate zones and upwelling zones in warm-temperate and tropical regions. Two characteristics render them particularly well suited to such environments. Firstly, they are well adapted to growth in deeply mixed turbulent water, where cells are only intermittently exposed to high light levels (Falkowski & Raven, 1997). Secondly, they are well adapted to pulsed availability of nutrients because they can use their often large central vacuole for nutrient storage. Diatoms are heavily grazed upon by a plethora of herbivores, in particular copepods. Diatoms seem to have few viral adversaries, but they are subject to attack from a range

of protistan parasites such as oomycetes and thraustochytrids (Hanic, Sekimoto, & Bates, 2009). Defense mechanisms against biological pressures include structural reinforcement of frustule elements with double layers and internal struts and buttresses, such as in the pennate diatom *Fragilariopsis* and in many radial centric diatoms, setae in *Chaetoceros* and *Bacteriastrum*, barbed spines in *Corethron*, or barbed spine-like extensions of the valve as in *Asterionellopsis*. Other strategies include biochemical defense by means of production of metabolites that become bioactive when the diatom cell is disrupted by grazers. Biological attack results in a large part of diatom production being rapidly remineralized in surface waters. Nonetheless, a considerable part of the primary production of coastal diatom blooms sinks. The bulk of this organic material is re-mineralized in deeper water, but given high sedimentation rates in coastal regions, a small part is trapped in sediments. In this way, coastal planktonic diatoms contribute to about half of total long-term organic carbon sequestration in the marine environment. This is why petroleum is found in present and past river plume sediments along so-called passive continental fringes (Brazil, the North Sea, Caspian Sea, Venezuela, Gulf of Mexico and the Middle East).

Diatoms are also important constituents of phytoplankton communities in the Southern ocean. In this region, diatom cell densities are typically low, mainly because of restricted availability of iron, but population sizes are nevertheless considerable given the extent of the region. Southern ocean diatoms possess well-developed physical grazer defenses in the form of spines (e.g. *Corethron*, *Chaetoceros*) or chambered and structurally reinforced valve elements (e.g. *Fragilariopsis*). Diatoms also abound (but do not dominate) in the deep chlorophyll maximum of warm open oceans. In these conditions, species are generally minute and do not form chains, but their diversity is poorly known; hence, it is here that molecular taxonomic surveys might uncover considerable new diversity. Diatoms do not contribute significantly to long-term carbon sequestration in any of these open oceanic communities.



6. LAST, BUT NOT LEAST RELEVANT: OTHER PHYTOPLANKTON TAXA

6.1. Stramenopiles Other Than Diatoms

Within the stramenopile lineage, a number of other phytoplankton groups exist besides diatoms (Fig. 1.1). Although less diverse, some of these groups have important ecological roles in marine ecosystems. They are usually

flagellated cells with heterokont characteristics, that is, two unequal flagella, one being ornamented with hair-like structures called mastigonemes. They possess plastids acquired through secondary endosymbiosis, typically with chlorophylls *a* and *c*. Their main characteristics are summarized in Table 1.2.

Table 1.2 Main Characteristics of Stramenopiles Containing Phytoplanktonic Representatives Other Than Diatoms

Taxonomy	Main characteristics
Bolidophyceae	Exclusively marine picoplankton Fast swimming cells (Guillou <i>et al.</i> , 1999) Phylogenetically linked to Parmales (i.e. non-motile cells covered with silica plates that are anisometric with a radial organization similar to that of the valves of centric diatoms, (Ichinomiya <i>et al.</i> , 2011))
Dictyochophyceae (also called silicoflagellates by protistologists)	Characterized by tentacles or rhizopodia Presence of a siliceous skeleton in one phase of the life cycle in the order Dictyocales
Pelagophyceae	No real distinct morphological character but supported as a distinct lineage in all phylogenies The order Pelagomonadales contains mainly picoplanktonic representatives (e.g. <i>Pelagomonas</i> , <i>Aureococcus</i>), while the order Sarcinochrysidales contains both planktonic and benthic genera
Raphidophyceae	Characterised by the presence of ejectile bodies (trichocysts) Cells with two flagella, without cell wall Presence of mucilaginous bodies under plasmalemma in some genera Marine raphidophytes form a monophyletic clade and have a pigment composition distinct from their freshwater counterparts (Yamaguchi, Nakayama, Murakami, & Inouye, 2010)
Pinguiophyceae	No real distinct morphological character but supported as a distinct lineage in all phylogenies Characterised by the production of large amounts of omega-3 fatty acids (polyunsaturated fatty acids)

Table 1.2 Main Characteristics of Stramenopiles Containing Phytoplanktonic Representatives Other Than Diatoms—cont'd

Chrysophyceae/Synurophyceae	<p>Taxa in both classes characterised by the ability to form silicified cysts or statospores</p> <p>Most species in these classes inhabit freshwater. Taxa covered by siliceous scales in both classes</p> <p>The Chrysophyceae have been re-defined recently (Andersen, 2004)</p>
Eustigmatophyceae	<p>Characterised by the absence of chlorophyll <i>c</i> (lost through evolution)</p> <p>Zoospores elongated with a basal swelling on the anterior flagellum. Stigma is in cytoplasm (and not in plastid)</p>

Distinctive derived characters (synapomorphies) for each class are indicated when possible. However, it is sometimes difficult to define classes using morphological characters and a combination of ultrastructural and biochemical features is often needed, while some classes are only defined based on phylogenetic analyses.

The stramenopile group that is phylogenetically nearest to diatoms is the Bolidophyceae (Table 1.2). Bolidophyceae are regularly detected in molecular surveys and isolated in to culture from the marine environment, but very few quantitative studies have been performed and their ecological imprint is still unclear (Guillou, 2011). The dictyochophytes are frequently observed in the environment and are regularly detected in molecular surveys of planktonic communities (Massana & Pedros-Alio, 2008). Several species have been recently described (e.g. *Florencia parvula*, Eikrem, Romari, Gall, Latasa, & Vaulot, 2004) or renamed and transferred from the raphidophyte genus *Chattonella* with which confusions were frequent (e.g. *Pseudochattonella* sp., Hosoi-Tanabe *et al.*, 2007). Some dictyochophyte species can produce ichthyotoxins (Skjelbred, Horsberg, Tollefsen, Andersen, & Edvardsen, 2011). Pelagophytes are essentially known because of the ecosystem disruptive algal blooms of the brown tide species *Aureococcus anophagefferens* and *Aureocoumbra lagunensis* (Gobler & Sunda, 2012). Other species such as *Pelagomonas* or *Pelagococcus* are frequently isolated from sea water, and their abundance, estimated through pigment signatures, indicate that they are probably non-negligible phytoplankton contributors in oligotrophic

regions (Not *et al.*, 2008; Shi *et al.*, 2011). The Pinguiphyceae is a class of small-sized phytoplankton erected in 2002 (Kawachi *et al.*, 2002). One of their distinctive features is the production of large amounts of omega-3 fatty acids (polyunsaturated fatty acids), providing them with a yet unexploited potential for biotechnological applications (Kawachi *et al.*, 2002). They are rarely found in environmental surveys and do not seem to be major contributors to phytoplanktonic communities (Fuller *et al.*, 2006).

The raphidophytes, chrysophytes, synurophytes and eustigmatophytes have marine representatives but are most abundant and diverse in freshwater. Yet, some species are of primary importance in marine ecosystems. Raphidophytes are found worldwide, mostly in coastal regions. The genera *Heterosigma* and *Chatonella* can cause important harmful effects in coastal waters and thus have a significant economic impact on aquaculture (Imai & Yamaguchi, 2012). Plastidial and mitochondrial genomes have recently been sequenced and analyzed (Masuda *et al.*, 2011). The most common marine eustigmatophyte genus is *Nannochloropsis*, a small-sized phytoplankton (2–4 μm) for which six species are currently described (Andersen, Brett, Potter, & Sexton, 1998). They have a characteristic pigment signature with violaxanthin and vaucherixanthin like as dominating carotenoids (Karlson, Potter, Kuylenstierna, & Andersen, 1996) and are essentially studied because they produce large quantities of fatty acids and in particular omega 3. Strains of *Nannochloropsis* are commonly used in aquaculture and because these microalgae are good candidates for green energy and blue biotechnologies (Cadoret, Garnier, & Saint-Jean, 2012), *Nannochloropsis* is one of the few phytoplankton taxa for which a full genome sequence is available (Kehou *et al.*, 2011; Oliver, Benemann, Niyogi, & Vick, 2011). Although regularly found in the environment, relatively few studies have been performed on the ecology of eustigmatophytes. Chrysophytes and synurophytes are closely related phylogenetically. Chrysophytes can be strictly phototrophs or heterotrophs or present a dual mixotrophic strategy (Preisig, Vørs, & Hällfors, 1991). A typical genus is *Ochromonas* comprising about 80 known species found in both freshwater and marine environments. Recent environmental molecular surveys of plankton diversity demonstrate the presence of novel clades of marine chrysophytes, in particular in the picoplankton size range, with no cultured representatives (Del Campo & Massana, 2011; Fuller *et al.*, 2006; Shi *et al.*, 2009).

6.2. The Cryptophytes

Cryptophytes are unicellular algae characterized by features including (1) asymmetrical cell shape, (2) presence of an invagination (either a tubular gullet, a furrow, a combination of furrow and gullet or a groove) lined with structures termed ejectosomes that discharge ribbon-like threads upon mechanical or chemical stress (Morrall & Greenwood, 1980), (3) a layered structure surrounding the cells that consists of proteinaceous inner and surface periplast components sandwiching the plasma membrane (Brett, Perasso, & Wetherbee, 1994), (4) a plastid surrounded by four membranes, with the periplastidial space containing a highly reduced remnant nucleus termed the nucleomorph (Gillott & Gibbs, 1980) and (5) a light-harvesting complex consisting of chlorophylls *a* and *c*₂, xanthophylls, and either red or blue phycobiliproteins (Hill & Rowan, 1989). Different combinations and/or concentrations of the pigments give cryptophytes brown, red or blue-green coloration. Cryptophyte cells have two unequal flagella, the longer one with two opposite rows of stiff flagellar hairs that provide reverse thrust, the shorter one with a single row of flagellar hairs (Hibberd, Greenwood, & Griffiths, 1971). The flagella are inserted subapically in the vestibule of an invagination often shifted to the cell's right, whereas the cell apex is shifted to the left.

Most cryptophytes are photosynthetic, but some have lost their photosynthetic pigments and returned to a heterotrophic mode of nutrition with retention of a leucoplast (remnant chloroplast) (Hoef-Emden, 2005). The phagotrophic *Goniomonas* is the only known cryptophyte genus without a plastid (Mcfadden, Gilson, & Hill, 1994) and also differs from plastid-containing cryptophytes in cell shape (flattened in lateral plane), cell invagination(s) and flagellar structure. Ultrastructural characters such as differences in periplast structure, type of invagination, flagellar apparatus and/or position of the nucleomorph have been used to define cryptophyte genera. In addition, each taxon produces only one of the seven known types of biliprotein. Cryptophytes thrive in all kinds of aqueous habitats, including marine, brackish and freshwater, but seem to be more diverse in marine than in freshwater habitats (Clay, Kugrens, & Lee, 1999; Novarino, 2003). Marine as well as freshwater cryptophytes are often abundant components of planktonic communities and may form blooms but are not known to have harmful impacts. Red-coloured genera like *Rhodomonas* and *Rhinomonas* are predominately marine, blue-green genera such as *Chroomonas* are present in both marine and freshwater habitats, while the brown to olive coloured

genus *Cryptomonas* is restricted to freshwater habitats (Hoef-Emden & Melkonian, 2003). The origins and phylogenetic affiliations of cryptophytes are uncertain (see section 3.1).

6.3. The Chlorarachniophytes

Chlorarachniophyta are a small group of algae, the first described members of which were amoeboid and associated with debris (Ishida, Yabuki, & Ota, 2007). They present a strong phylogenetic interest because they possess a four membrane plastid containing green algal pigments (chlorophyll *b* in particular) as well as a remnant of the endosymbiont nucleus called the nucleomorph, the genome of which has been sequenced for at least one species (Gilson *et al.*, 2006). At present, only 13 species and 8 genera are known. In recent years, planktonic chlorarachniophytes have been described, one of picoplanktonic size (Moestrup & Sengco, 2001; Ota, Vaultot, Le Gall, Yabuki, & Ishida, 2009). Their ecological importance remains unknown, although 18S rRNA gene environmental sequences related to chlorarachniophytes have been obtained from the Mediterranean Sea and the upwelling off Chile using specific primers (Ota, personal communication). Moreover, several new species have been isolated into culture from the Mediterranean Sea, hinting that this group could have a narrow biogeographical distribution (Ota & Vaultot, 2012).

6.4. The Euglenids

The Euglenozoa form a monophyletic lineage within the Excavata, a lineage that contains parasites, photo-autotrophs and predators. Typically, excavates have flagella inserted into a reservoir, paramylon (α -1,3 glucan) as the main carbohydrate reserve and a peculiar type of closed mitosis. The photosynthetic euglenids, the class Euglenophyceae, acquired a green plastid through secondary endosymbiosis (Marin, 2004). They mostly occur in freshwater habitats, but a few marine species belonging to the Eutreptiellales are known.



7. CONCLUDING REMARKS

Analysis of the diversity and ecology of phytoplankton has largely benefited from molecular data, and, as for many research fields in biology, phytoplankton research is entering a new era with the advent of

high throughput sequencing technologies. Large-scale environmental meta-barcoding allows quasi-exhaustive analysis of the diversity of communities. Working at the community level allows integration of functional information, even for uncultured taxa (Toulza *et al.*, 2012, in this volume). In the near future, if carefully contextualized with environmental meta-data, the panel of ‘-omics’ tools now available will facilitate eco-systemic approaches (Raes & Bork, 2008) to the analysis of phytoplankton communities and lead to a better understanding of phytoplankton ecology. It will also promote detailed experimental and physiological studies on particular taxa, ultimately fostering improvement of predictive models of phytoplankton distribution at global scales (Barton, Dutkiewicz, Flierl, Bragg, & Follows, 2010).

However, in this environmental genomic context, the improvement of knowledge on the diversity and ecology of phytoplankton strongly relies on high-quality reference databases and is tightly linked to our ability to clearly bridge molecular data and phenotypes. The potential to simultaneously and quantitatively assess the occurrence of phytoplankton taxa at relevant spatial and temporal resolution is also essential. These goals can only be achieved through multidisciplinary research involving strong taxonomic expertise and the development of high throughput microscopy and automatic molecular and imaging tools (Olson & Sosik, 2007; Preston *et al.*, 2011). While some phytoplankton species can be harmful for ecosystems and/or human activities, others provide great potential for providing natural products through blue biotechnology and alternative green energy (Larkum, Ross, Kruse, & Hankamer, 2011). Although little genomic data are currently available for phytoplankton (Rynearson & Palenik, 2011), genomics and postgenomics approaches will undoubtedly significantly contribute to unraveling the nature and implications of biological processes across ecological scales and will help addressing some of the current conceptual challenges in phytoplankton research and more generally in microbial ecology.

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