Evolution / Évolution

Diversity and evolution of marine phytoplankton

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Abstract

Marine phytoplankton organisms account for more than 45% of the photosynthetic net primary production on Earth. They are distributed across many of the major clades of the tree of life and include prokaryotes, and eukaryotes that acquired photosynthesis through the process of endosymbiosis. If the number of extant described species is relatively low compared to the diversity of the terrestrial plants, recent insights into the genetic diversity of natural assemblages have revealed a large unsuspected diversity at different taxonomic levels. Wide infra-specific diversity is also being discovered in many widespread and well known morphological species. This review summarizes data obtained in the fields of ecology, evolutionary biology, physiology and genomics that have improved our understanding of the biodiversity and evolution of marine phytoplankton. *To cite this article: N. Simon et al., C. R. Biologies 332 (2009).*
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Résumé

Diversité et évolution du phytoplancton marin. Le phytoplancton marin est à l'origine de plus de 45% de la production primaire nette sur notre planète. Les organismes de ce compartiment fonctionnel ont des représentants dans la plupart des super-groupes de l'arbre du vivant, au sein des domaines des bactéries et des eucaryotes, ces derniers ayant acquis la photosynthèse au travers d'une, ou de plusieurs endosymbiose(s). Si le nombre d'espèces décrites du phytoplancton marin apparaît faible au regard de la diversité des plantes terrestres, des analyses récentes de la diversité génétique d'assemblages naturels ont révélé une diversité insoupçonnée à différents niveaux taxinomiques. Des variations génétiques infra-spécifiques ont été mises en évidence au sein de taxons cosmopolites. Cet article de synthèse résume les données obtenues dans les domaines de l'écologie, de la biologie évolutive, de la physiologie et de la génomique, et montre comment ces données ont permis de réelles avancées dans notre compréhension de la biodiversité et de l'évolution du phytoplancton marin. *Pour citer cet article : N. Simon et al., C. R. Biologies 332 (2009).*
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Keywords: Marine phytoplankton; Diatoms; Dinoflagellates; Haptophytes; Prasinophytes; Cyanobacteria; Evolution

Mots-clés : Phytoplancton marin ; Diatomées ; Dinoflagellés ; Haptophytes ; Prasinophytes ; Cyanobactéries ; Évolution

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Introduction

Marine phytoplankton, i.e. the autotrophic component of the plankton (from the Greek terms "phyton" or plant and "planktos" or wanderer) obtain energy through photosynthesis and therefore live within the well-lit surface layers of the ocean, down to 200 m in the clearest waters. Most phytoplankton species are microscopic unicellular organisms with a size ranging between 0.4 and 200 μm. Marine phytoplankton represent less that 1% of the Earth's photosynthetic biomass. Yet, this compartment is responsible for more than 45% of our planet's annual net primary production [1]. Continuous grazing and recycling keeps the biomass of this extremely active compartment low, compared to the biomass of terrestrial photosynthetic organisms. The evolution of marine photoautotrophs began in the Archaean period with the origin of photosynthesis [2]. These primitive organisms are at the origin of the diverse photosynthetic biota from which all complex life is dependent. They are also at the origin of the oxygenation of the atmosphere and have profoundly modified the biochemistry of the oceans and the atmosphere. In the past decades, our understanding of the evolution of phytoplankton has increased significantly and our perception of the diversity of this group has been enhanced with the integration of data from the fields of biology (ecology, evolution, physiology, genomics), ocean biogeochemistry and ocean/atmosphere exchanges.

1. Diversity of the marine phytoplankton

Within the tree of life, which has been drastically redrawn several times based on data from improved microscopic techniques and molecular phylogenies, the extant marine phytoplankton species are found among the domains Bacteria and Eukarya. They have representatives in 4 of the 6 super-groups of eukaryotes described by Adl and collaborators [3] (Figs. 1, 2). Within the extant described marine phytoplankton taxa, the diatoms (one of the major lineages within the stramenopiles), the dinoflagellates and the haptophytes (Figs. 1, 2) appear to dominate phytoplankton communities on continental shelves and are responsible for seasonal blooms in temperate and polar waters. They are more generally the main marine planktonic primary producers within the nano- and microplanktonic size classes (respectively 2–20 and 20–200 μm). Members of the stramenopiles, dinoflagellates and haptophytes, together with the cryptophytes, have been proposed to have a common ancestor and have thus been grouped into the super-group Chromalveolata,
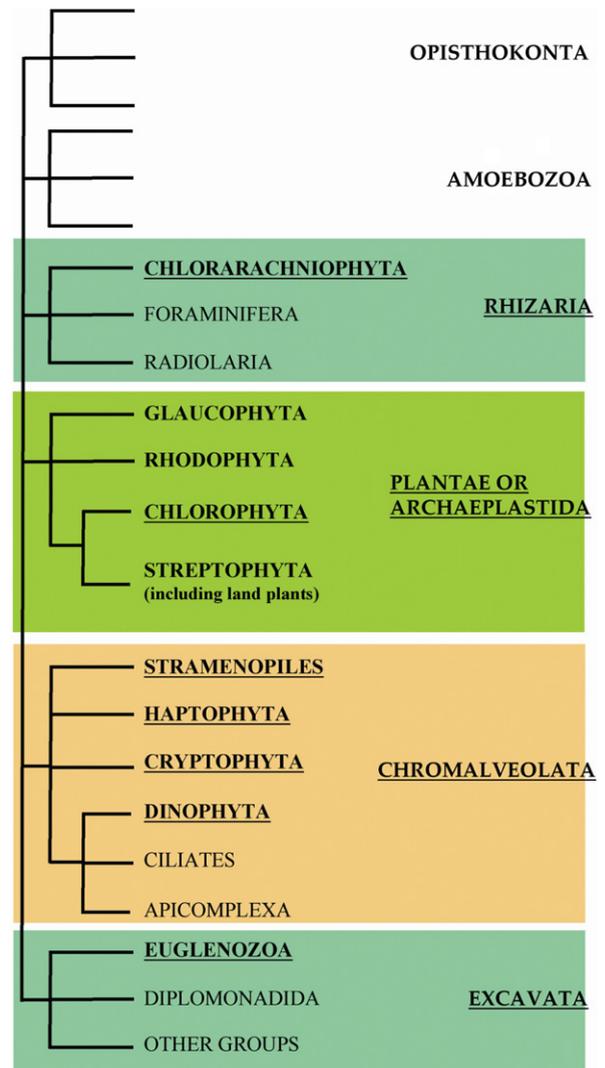

Fig. 1. Schematic tree of the 6 eukaryotic super-groups as described by Adl and collaborators [3] showing the distribution of the major lineages that acquired a plastid through endosymbiosis (bold) and those of these lineages that include marine phytoplanktonic representatives (bold, underlined). Note that the eukaryotic tree is constantly being reshuffled and that the evolutionary links between some groups are yet to be confirmed. Most of the marine phytoplanktonic described species belong to the Chromalveolata and Archaeplastida super-groups while only a handful of species belong to the Chlorarachniophyta. Concerning the Euglenozoa, the existence of truly planktonic species has been questioned by some authors [9]. Note that the taxonomic ranks of each of the groups distinguish in this study (that are indicated by the suffixes) are not always consensual, probably because the nomenclature codes are not adapted to the protists (see [77] and [3]). The suffix -phyta indicates a division (or phylum) in the botanical nomenclature code. Some groups such as the dinoflagellates have been named both according to the botanical and zoological nomenclature codes (Dinophyta, Dinoflagellata).

Fig. 2. Representative species of the major lineages that include marine phytoplanktonic members. A. *Bigelowiella natans* (Chlorarachniophyta, super-group Rhizaria). B, C. *Tetraselmis chuii*, *Nephroselmis pyriformis* (Prasinophyceae within the super-group Archaeplastida). D. *Eutreptiella* sp. (Euglenozoa). E, F, G, H. *Rhodomonas salina*, *Scyphosphaera apsteinii*, *Akashivo sanguinea* and *Coscinodiscus radiatus* (from the super-group Chromalveolata, respectively members of the Cryptophyta, Haptophyta, Stramenopile and more precisely dinophyte, and diatom). Pictures are from Daniel Vaulot (A, E, G) and Fabien Jouenne (B, C, F, H), Station Biologique de Roscoff, France, and Chantal Billard, University of Caen Basse-Normandie, France (D) and were provided by Plankton*Net Data Provider at Station Biologique de Roscoff (hdl: 10013/fr.sb-roscoff.planktonnet).

although the position of the cryptophytes and haptophytes is still poorly resolved [4]. Marine phytoplanktonic green algae belong to the Chlorophyta within the super-group Archaeplastida which also includes the land plants (Figs. 1, 2). Together with other ultra-small eukaryotes and cyanobacteria, green algae play a key role in the open oceans, where the picophytoplankton (0.2–2 μm) dominates both photosynthetic biomass and production [5,6]. Picoplanktonic green algae also seem to dominate picophytoplankton in coastal systems [7, 8]. As for the Chlorarachniophyta, and Euglenophyta (Figs. 1, 2), their contribution to photosynthetic biomass within the micro-, nano- and picophytoplankton appears less significant [6,9].

Compared to the wide diversity at high taxonomic level, species diversity in the marine phytoplankton appears extremely low, especially compared to the almost 300 000 species of terrestrial plants. According to Sournia, less than 5000 species (3444 to 4375) of marine phytoplankton were formerly described at the end of the 1980s [10]. Diatoms, dinoflagellates and to a lesser extend haptophytes and green algae are the most diversified groups (with respectively, approximately, 40, 40, 10 and 6% of the described phytoplanktonic eukaryote species (Fig. 3)). In comparison, the marine planktonic cryptophytes, chlorarachniophytes and euglenophytes appear far less diversified (less than 2% for each group). As for the cyanobacteria, their global
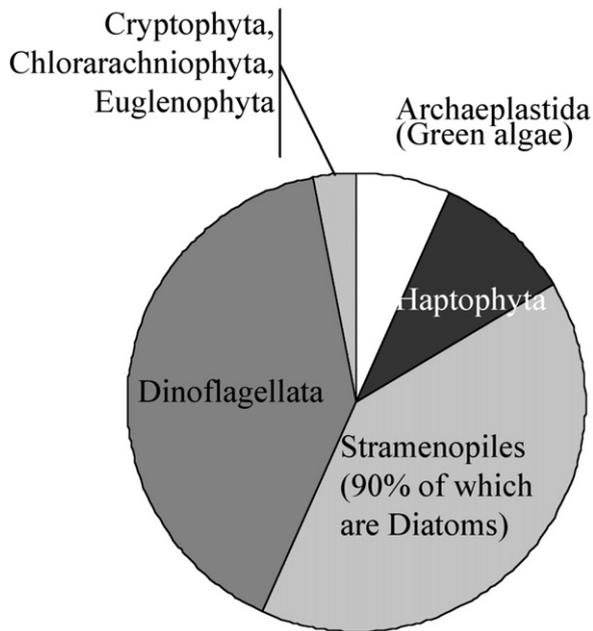
Fig. 3. Contribution of the different phytoplankton groups to the described species inventory of marine phytoplankton (3859 in total). Number of species were compiled from [33] for the dinoflagellates, [10] for the Cyanobacteria, Chlorophyta, Crytophyta and diatoms, [77] and [6] for the Stramenopiles to the exception of the diatoms, and [45] for the haptophytes. For some of the groups, the list of species considered did not distinguish the benthic from the planktonic species (dinoflagellates), or the freshwater from the marine species (Haptophyta). The estimates given are thus overestimated for some taxa, however, in those groups, marine phytoplanktonic species are largely dominant [33,45]. As for the Euglenophyta, as mentioned in the text, the existence of truly phytoplanktonic species is questionable [9].

diversity is difficult to assess in terms of species number. Fourteen genera, some of those containing ecotypes (representing clades restricted to a distinct niche) have been listed but only few genera are known to be strictly marine [11].

However, due to comparatively limited sampling, the relative paucity of morphological distinctive features, and the scarcity of taxonomists, the majority of phytoplankton species is probably still undescribed, while the lists of mammals and higher plants probably reflect in much more detail, the actual diversity. Indeed, new lineages that are quite distantly related from well-known taxa, were discovered within the last decades, especially in the picophytoplanktonic size fraction [12, 13]. Culture independent genetic surveys in a wide range of marine pelagic samples have also revealed the wide undescribed diversity of phytoplankton taxa, again in the picoplanktonic size fraction [6]. In genetic databases established from such approaches, sequences of green algae (and more precisely of prasinophytes) are the most abundant followed by sequences of dinoflagellates and diatoms. The cryptophytes, chlorarachniophytes and stramenopiles (other than diatoms) are also represented in the genetic databases retrieved from the pelagic ocean (Guillou, pers. com.; [6]). Interestingly, while only 2 chlorarachniophyte phytoplanktonic species are described, a significant number of sequences affiliated to this group was retrieved from environmental genetic databases, especially in the Mediterranean Sea (Viprey and Guillou, pers com.), which suggests that this group may be important in some marine systems or niches. In contrast, sequences of euglenophytes are absent from environmental sequences databases (Guillou, pers. com.). Marine euglenophytes occupy mostly near-shore or brackish sands and mudflats environments and their presence in the truly pelagic realm has been questioned [9].

The genetic databases obtained from natural samples also revealed a wide infra-specific genetic diversity, within well known species [14–16]. These findings together with new data obtained in the fields of evolutionary biology, ecology and genomics have allowed formulating new hypotheses concerning both macroevolutionary and microevolutionary patterns and processes for marine phytoplankton.

2. From the primitive to the modern phytoplankton assemblages

2.1. The geological succession of phytoplankton

This subject has been recently reviewed by Knoll and collaborators [17]. The evolutionary history of phytoplankton has been studied through both morphological fossils (well-preserved structures such as cell walls, scales or cysts available for some taxa) and molecular biomarkers such as lipids or nucleic acids. The data suggest that prokaryotes have governed ecosystems during the Proteozoic. Primitive cyanobacteria or other prokaryotic organisms such as anoxygenic photoautotrophs (that might have used H_2 as final electron acceptors) could have dominated the marine flora in the Archean. Biomarkers found in shales (which biosynthesis is known to require oxygen) and observed in extant cyanobacteria suggest that the later had evolved oxygenic photosynthesis by 2.7 Gy, before the initial accumulation of free oxygen in the atmosphere 2.3–2.4 Gy ago. Limited data from proterozoic rocks suggest that cyanobacteria and other photosynthetic bacteria dominated primary production at that time while biochemical evidences suggest that green algae began to play

a role as major primary producers during the late Proterozoic and Cambrian. This primitive phytoplankton assemblage prevailed during the Mesozoic, until the radiation of the chlorophyll $a + c$ algae namely the diatoms, dinoflagellates and haptophytes. Fossils clearly suggest that these groups became dominant during the Mesozoic. The cyanobacteria and green algae, which appeared then to be less important in coastal plankton, were still probably co-dominant or dominant in the oligotrophic central gyres and dominate the modern picophytoplankton size class.

2.2. Endosymbiosis as a major event for the radiation of phytoplankton phyla

The mechanisms by which photosynthesis was acquired, and oxygenic photosynthesis was established in primitive cyanobacteria is still poorly understood [18]. However, the process of photosynthesis acquired by cyanobacteria, spread across the eukaryotic tree of life through the process of endosymbiosis. Early in the evolution of eukaryotes, a heterotrophic eukaryote engulfed and retained a cyanobacterium, converting it into a plastid after intracellular gene transfers between the primitive host and its symbiont. This event, the "primary endosymbiosis" that probably occurred a single time [19,20], gave rise to the extant Archaeplastida which include the Glaucophyta, Rhodophyta (red algae) and Chloroplastida (green algae and land plants). The primary plastid was suggested to be established around 1.25–1.60 Gy ago, during the Proterozoic [4]. Members of these lineages were themselves engulfed by non-photosynthetic protists probably in separate endosymbioses and converted into plastids [20]. A secondary endosymbiosis event (0.8–1.2 Gy, [21,22]) with a primitive red algae gave rise to the cryptophytes, haptophytes, photosynthetic stramenopiles (or heterokontophytes) and peridinin-containing dinoflagellates. Two separate endosymbioses during which the plastids of green algae were integrated are at the origin of euglenophytes and chlorarachniophytes [4]. Some of the extant dinoflagellates arose from a tertiary endosymbiosis in which an alga containing a plastid originating from a secondary endosymbiosis was retained by a heterotrophic protist and reduced to a plastid. In modern oceans, photosynthetic eukaryotes that resulted from a secondary endosymbiosis with a red algae (the so-called "red" lineage), were extremely successful from the Mezozoic era to the present days [23]. According to Falkowski and collaborators [23] this success could be explained by the better transportability of red algal plastids. Indeed, the larger amount of key protein-coding

genes in these plastids compared to the green algal plastids [24], may have increased the transfer probability through secondary endosymbiosis, in new, phylogenetically diverse hosts. Theses hypotheses however, have been questioned [25] and are in direct conflict with the "chromalveolate hypothesis" which proposes that all algae believed to possess secondary red plastids acquired them by a single common endosymbiosis. Overall, endosymbiosis had a major influence on the early evolution of algae. Indeed, this process was not only at the origin of lateral transfer of photosynthetic genes, it also enriched nuclear genomes with cyanobacterial genes (18% of nuclear encoded genes in *Arabidopsis* are estimated to have been acquired from the primitive cyanobacterial endosymbiont [26]).

3. Diversity and evolution of the phytoplankton groups that dominate the modern oceans

3.1. Diatoms, dinoflagellates and haptophytes

Diatoms can be planktonic, benthic, epiphytic, epizoic, endozoic, endophytic and can also live in air [27]. Approximately 40% of all marine phytoplankton described species are diatoms and they are of crucial importance from an ecological and biogeochemical point of view, especially in nutrient-rich systems [23]. Diatom cells are encased within a special cell wall made of silica, the frustule, which is well preserved in the fossil record. They often form chains and colonies. They are traditionally divided into 2 groups. The centric diatoms have valve striae (rows of pores) arranged basically with central symmetry. In this case, the symmetry can be unipolar (radial centrics) or multipolar. Pennate diatoms have valve striae arranged basically in relation to a line. Within the latter, the frustule of raphid pennates has a slit called a raphe. Different phylogenetic studies showed that "radial centrics" represent the deepest branch while "bi- and multi-polar centrics" appeared later. Both groups form clades or sometimes ill-supported basal ramifications, but many well-supported terminal lineages [28]. All pennates, emerged from bipolar centrics, and form a well-supported clade. This group differentiated into the "araphids" and the subsequent "raphids", rendering the araphid pennates paraphyletic. Molecular phylogenies of diatoms are in general agreement with evolutionary hypotheses proposed based on fossil records [29,30].

Species belonging to the Dinophyta can be planktonic, benthic, symbionts or parasites [31], but the majority of photosynthetic species are free-living and planktonic. The main characteristic of dinoflagellates

is the presence of a large nucleus, with permanently condensed chromosomes. A typical cell displays 2 flagella, one in an equatorial groove, the cingulum, and the other projecting toward the posterior of the cell, often in a groove, the sulcus (dinokont species). Some species (desmokonts) have both flagella at the cell apex. Dinoflagellates can harbour thick cellulose plates located in alveoli under the plasmalema and forming a rigid theca (armoured dinoflagellates). These plates can also be inconspicuous or absent in the unarmoured species. Less than half of all dinoflagellates are photosynthetic [32,33], the majority of them harbor peridinin as the main accessory pigment [34]. All the photosynthetic dinophytes belong to the class Dinophyceae *sensu stricto* [35]. Dinoflagellates phylogeny is not consensual [36]. Phylogenies inferred from the ribosomal genes often present problems of long branch attractions and are sensitive to analytical method [37,38]. Moreover, dinoflagellates rDNAs present regions with different rates of evolution, containing stem regions with sites that do not evolve independently [39]. Phylogenies based on sequences of plastid genes are often inappropriate, due to the complex endosymbiotic history of these organisms [40], and because the plastid genome is highly reduced, organized in minicircles, and exhibits a strikingly high rate of evolution and a tendency to unique rearrangements [41,42]. However, the Dinophyceae *sensu stricto* often form a separate clade within the Dinophyta. This clade includes the Dinophyceae with a peridinin-containing plastid, that is thought to be the ancestral plastid of Dinophyceae, but also some non-photosynthetic species that presumably lost their plastids, as well as photosynthetic species without peridinin that acquired other plastids through a secondary or tertiary endosymbiosis [36]. Dinokont/desmokont or armoured/unarmored species do not form separate clades. Orders and genera within the phytoplanktonic dinoflagellates are not always supported by molecular phylogenies. For example, the order Gymnodiniales (dinokont, unarmoured cells) is polyphyletic according to Murray and collaborators [39] while the analysis of LSU rDNA sequences resulted in the splitting of the genus *Gymnodinium* into four genera [37]. Evolutionary hypotheses that emerge from molecular phylogenies are not easily compared to fossil records. This is mainly due to the poor fossilization of vegetative cell walls. Resistant cysts (resting stage) are found in fossils records, but only 13% of modern dinoflagellates are known to form them [43]. Despite many phylogenetic studies, the evolution of the major dinoflagellate lineages remains uncertain and morphogenetic studies are necessary, especially concerning the small unarmoured species.

Haptophytes are mainly composed of small planktonic unicellular species, occurring single or in colonies. Heterotrophy is not common. Cells usually have 2 flagella, with an associated structure, the haptonema sometimes used for the cell anchoring or prey capture [44]. Organic scales often cover the cell body surface. The division Haptophyta is composed of two classes: the Pavlovophyceae (with 2 unequal flagella) and the Prymnesiophyceae (with 2 more or less equal flagella). Within the Prymnesiophyceae, the Calcihaptophycidae are potentially calcifying haptophytes [45]. This group includes the coccolithophores, which are covered by small regular calcareous plates (coccoliths) which are important in biogeochemical cycles since they are responsible for about half of all modern precipitation of $CaCO_3$ in the ocean [46]. Haptophytes belong to one of the deepest branching groups in the phylogeny of the eukaryotes [47]. Comparative analyses of rDNA sequences also showed that the Calcihaptophycidae are monophyletic, but whether coccolithogenesis has single or multiple origins is still discussed [45]. The lack of geological records makes reconstruction of the primitive evolutionary history of uncalcified haptophytes very difficult.

3.2. Green phytoplanktonic algae

Within the super-group Archaeplastida, the marine phytoplanktonic green algae have representatives in the classes Trebouxiophyceae and Prasinophyceae (Fig. 1) that are distinguished based on morphological and ultrastructural characters (flagellar types and number, flagellar insertion structures, cell wall, type of mitosis, [48]), as well as molecular phylogenies [49]. The ubiquity and contribution of green algae to marine phytoplanktonic assemblages and more precisely to the picoplanktonic fraction has been established using microscopy [50], pigment analyses, and molecular probing [8,16]. The prasinophytes, which is the most diversified group among marine phytoplankton green algae, occupies a critical position at the base of the green algal tree of life. Members of this group are supposed to have retained primitive characters such as the presence of organic scales on the cell bodies. The diversity in their cell shapes, flagellar number, flagellar apparatus organization, scale morphology and cell division features led some authors to suggest that they were not a monophyletic group (Lewis et al. [48]). Molecular phylogenies have echoed the morphological diversity and identified at least seven phylogenetic groups [49]. Genetic studies on the diversity of picoplanktonic green algae investigated by culture independent approaches in

the Mediterranean Sea [16] confirmed the prevalence of the prasinophytes within the Archaeplastida (99% of the sequences detected), and revealed new clades, as well as an important diversity at the sub-genus level.

3.3. Cyanobacteria

Both filamentous (heterocystous or not) and unicellular forms of this group have representatives in the oceans. Marine cyanobacteria have a global ecological significance, not only for the carbon but also for the nitrogen cycles. Marine phytoplanktonic genera are dispersed within the Cyanobacterial-tree. Two closely related genera, *Prochlorococcus* [51] and *Synechococcus* [52] are particularly abundant and ubiquitous in the ocean but do not fix atmospheric nitrogen. Each of these genera contains distinct phylogenetic clades representing physiologically and ecologically distinct populations (or ecotypes [53,54]). At least two marine, nitrogen-fixing genera are also known: the colony-forming, filamentous *Trichodesmium* and the coccoid *Crocosphaera*, both restricted to tropical waters [55]. The genetic variability within the latter genus was recently be found to be remarkably low [55]. More diversity, at different taxonomic levels, is probably to be discovered in the marine planktonic cyanobacteria, both for non-N_2-fixing and N_2-fixing taxa [56,57].

3.4. Key evolutionary forces in phytoplankton evolution

Dinoflagellates, diatoms and coccolithophores, each with plastids derived from red algae by secondary endosymbiosis rose to ecological prominence during the Mesozoic and continue to dominate the phytoplankton biomass. Hypotheses involving competition for light and nutrients as major driving forces in phytoplankton evolution have been proposed to explain this prominence [23,28]. The presence of specific accessory pigments [34] has been proposed as an important selective character; these would have allowed better light absorption, protecting the cells from light saturation, as well as allowing photoacclimation [40]. Moreover, these algae also possess efficient mechanisms for uptake and internal recycling of nutrients and can sometimes take up particulate or dissolved organic matter or be associated with diazotrophic organisms [28]. The ability to form resting stages has also been considered to provide an important selective edge [28]. Smetacek [58] argues that the enormous diversity of shapes and lineages must do more than improve the photosynthetic efficiency of chloroplasts, and that evolution in the plankton is ruled

by protection against predation and pathogens (viruses and bacteria), and not only competition.

The reduction of the size, as well as the acquisition of efficient nutrient absorption pathways are probably key driving forces for the small phytoplankton size classes. These aspects of evolution are started to be elucidated as data from the fields of ecology and genomics are being integrated [59].

4. Microdiversity, microevolution and the species concept in phytoplankton

High growth rates and minute dimensions associated with huge dense populations possessing potentially high dispersal capacities characterize most phytoplankton species, and more broadly most microbes. Consequently these organisms are believed to occur wherever the environment permits and the lack of apparent barriers to gene flow in the open ocean coupled with enormous population sizes and high interoceanic dispersal potential should greatly limit their ability to diversify and speciate through allopatric processes [60]. A related hypothesis states that the local to global species number ratio is high, theoretically approaching 1 [61]. In other words, the diversity of such species found in one drop of seawater in one locality would represent global diversity. Supporting this hypothesis, the described species inventory of marine phytoplankton is relatively poor, while morphological species are often cosmopolitan. However, recent molecular data have shown that genetic diversity within morphospecies can be high [62], and that if some of the infra-specific genetic clades identified are cosmopolitan, others show ecologically and/or geographically restricted distributions. These findings and the discovery of previously unsuspected genetic diversity discussed earlier, have important impacts on our understanding of the processes of phytoplankton evolution in the oceans. They also point to the need of a clearer definition of the species concept for phytoplankton.

4.1. Marine phytoplankton and the species concept

The accurate circumscription of species is an essential requirement for biodiversity assessments, as well as for a proper understanding of their ecology and evolutionary history [62]. Despite long running debates, there is still no consensus and various species concepts have been adopted, often implicitly. While the traditional morphological species concept dominates in the field of phytoplankton taxonomy, several authors have expressed a need for a clearer definition that would include
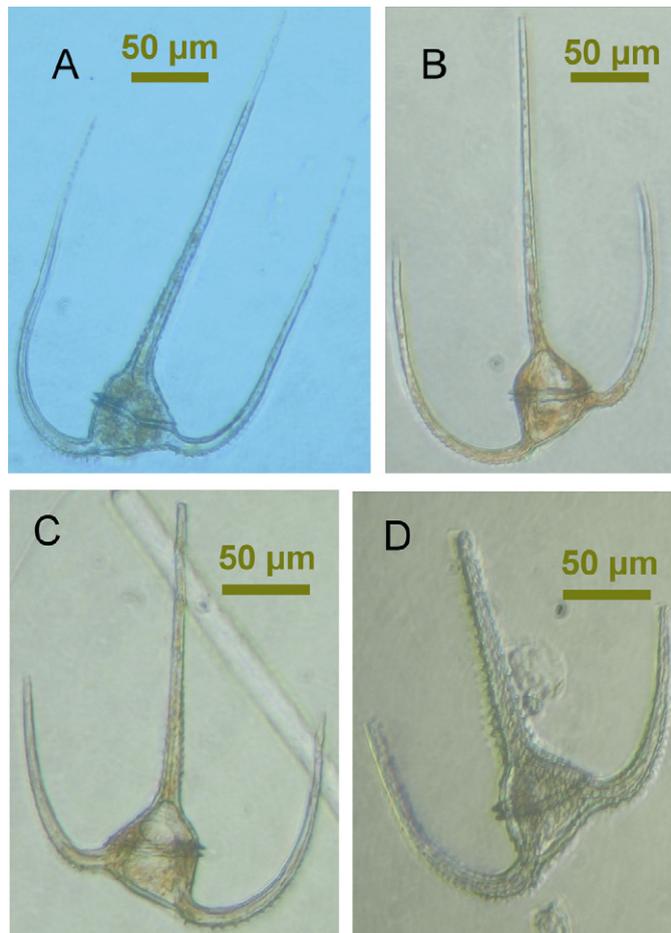
Fig. 4. The difficulties encountered to assess the infra-specific taxonomical status of phytoplankton taxa are exemplified in the genus *Ceratium*. In this genus, infraspecific nomenclature was proposed to take into account the morphological variations and intermediate phenotypes that are commonly encountered, for example, within the species *Ceratium horridum*: A. *Ceratium horridum* var. *horridum*. B. *C. horridum "horridum>buceros"*. C. *C. horridum "horridum-buceros"*. D. *C. horridum* var. *horridum*.

genetic circumscription and mating delineation [62] because morphology is too influenced by external conditions and can change according to life stages. Mating delineation that would allow the definition of biological species as "*groups of interbreeding natural populations that are reproductively isolated from other such groups*" [63] is especially problematic for phytoplankton organisms, as (1) distinctive morphological features are not always easy to identify (Fig. 4) and (2) sexual reproduction is completely unknown in several phytoplankton groups.

During the last decades, the genetic diversity within morphologically-defined phytoplankton taxa has been explored and has revealed a wide cryptic diversity. Coccoid picoplankters are extreme cases in this respect. Their minute size and simple spherical morphology limit the taxonomic level to which these cells can be identified to phyla or classes [64]. Flagellates that do not harbour hard structures are also extremely difficult to identify to the species level. Beyond these extreme cases recent genetic studies have also pointed out to the cryptic diversity within well-characterized species. Indeed, clear genetic clades are commonly found within widespread phytoplankton species. However, the ecological and evolutionary significance of these genetic variations found within well-known morphological taxa and in culture independent molecular survey [16], is often not well understood.

In some cases, the careful examination of the morphology of the clades identified genetically have revealed the existence of pseudo-cryptic species, i.e. species that can be differentiated only on very subtle morphological features. For example, clear genetic clades corresponding to fine scale morphovariants suspected to represent eco-phenotypes or ecotypes, were identified using the 18S rDNA and tufA genes within

the coccolithophore *Calcidiscus leptoporus* [65]. Similarly, detailed analyses of ITS sequences and morphological features allowed to distinguish 3 clear genetic groups within the diatom *Pseudo-Nitzschia delicatissima* [66].

Genetic variability associated with specific biogeographical origin, can also be found in cosmopolitan morphospecies of diatoms such as *Skeletonema costatum* [67], *Skeletonema marinoi* [68] or dinoflagellates such as *Scrippsiella* spp. [69].

In some other cases, if the genetic clades identified are indistinguishable based on detailed morphological examination and appear to be cosmopolitan, fine scale analyses of their distribution shows that they occupy specific niches in the marine ecosystems. This is the case for *Micromonas pusilla*, a widespread green picoplankter. Between 3 and 5 clades were recently identified within this species, with genetic divergence higher than divergences estimated between traditional genera [70]. While those clades are commonly found in sympatry, they seem to occupy specific ecological niches and to compete in some environments [71]. Most interestingly in the context of the delineation of the phytoplankton ideal species, the mating compatibility of genetic variants was recently studied for the diatoms *Pseudo-nitzschia delicatissima* [62] and *Sellaphora pupula* [72]. These studies showed that mating compatibility occurred only for strains presenting highly homologous ITS2 sequences.

More studies combining genetic analyses on several genes and ecological, morphological, physiological and biogeographical analyses have to be conducted in order to clarify the taxonomic status of many phytoplankton species. These studies would also help formulating a new species concept that would in return allow: (1) much more satisfactory biodiversity assessment and monitoring; (2) advances in our knowledge of the ecology, physiology and genetics of phytoplankton; and (3) understanding the evolutionary processes that prevail in the ocean.

4.2. Speciation and phytoplankton

The ecological and evolutionary processes of speciation and the mechanisms by which diversity is maintained in the pelagic microbial realm are poorly understood. Indeed, the coexistence of dozens of phytoplankton species in natural assemblages where only a few resources are potentially limiting (the so-called paradox of the plankton, [73]) has puzzled the biologists for decades. The discoveries of a previously unsuspected diversity in the picoplankton size fraction, and of large genetic variations within widespread taxa amplifies this paradox. In phytoplankton, many populations appear to be so large and so dense that allopatric divergence and speciation seem unlikely, or would be too slow to be able to account for the presently observed diversity. Moreover, it is likely that these organisms do not possess complex behaviours associated with reproduction that would limit reproductive isolation. Recently, analyses of the fossil records for cosmopolitan taxa [74], genomic analyses [59] and theoretical modelling [75] have provided new evidences supporting the potential widespread occurrence of sympatric speciation in the pelagic environment. These findings are important for the understanding of speciation in the pelagic realm. In parallel genomic studies conducted recently on individual picophytoplanktonic strains have shown the occurrence of new gene acquisition through horizontal transfer both in eukaryotes [59] and cyanobacteria [76] and have provided insights into the mechanisms of species divergence and niche partitioning through the gain or loss of genes.

5. Conclusion

The recent studies conducted in the field of phytoplankton systematics, ecology, physiology and genomics unveiled a vast unsuspected diversity, in particular in the picoplanktonic size fraction and at the infra-specific level. These findings have major impacts on our understanding of phytoplankton evolution. The new data and hypotheses obtained contribute more generally to a better understanding of the processes of evolution and diversification in the ocean. Understanding and assessing diversity (that still has to be precisely defined in terms of taxonomical entities) will be essential to understand and predict the impact of environmental forcing on this major compartment.

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