CHAPTER

12

[AU1]

Origin and Evolution of Coccolithophores: From Coastal Hunters to Oceanic Farmers

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I. COCCOLITHOPHORES AND THE BIOSPHERE

The coccolithophores are calcifying protists that have formed a significant part of the oceanic phytoplankton since the Jurassic. Their role in regulating the Earth system is considerable. Through their secretion of a tiny composite exoskeleton (the *coccosphere* made of multiple *coccoliths*), the coccolithophores are estimated to be responsible for about half of *all* modern precipitation of $CaCO_3$ in the

oceans (Milliman 1993). Coccolithophores thus play a primary role in the global carbon cycle (Figure 1). The ecological and biogeochemical impacts of their skeletons are multiple and act on a wide range of ecological to geological time scales. On ecological time scales, coccolithophore biomineralization plays a major role in controlling the alkalinity and carbonate chemistry of the photic zone of the world ocean. Counter-intuitively, the precipitation of carbonate is a source of CO_2 for the upper-ocean and atmosphere (Figure 1B). On the other hand, the biogenic carbonate produced by coccolithophores constitutes an ideal material for aggregating with the huge reservoir of particulate organic carbon

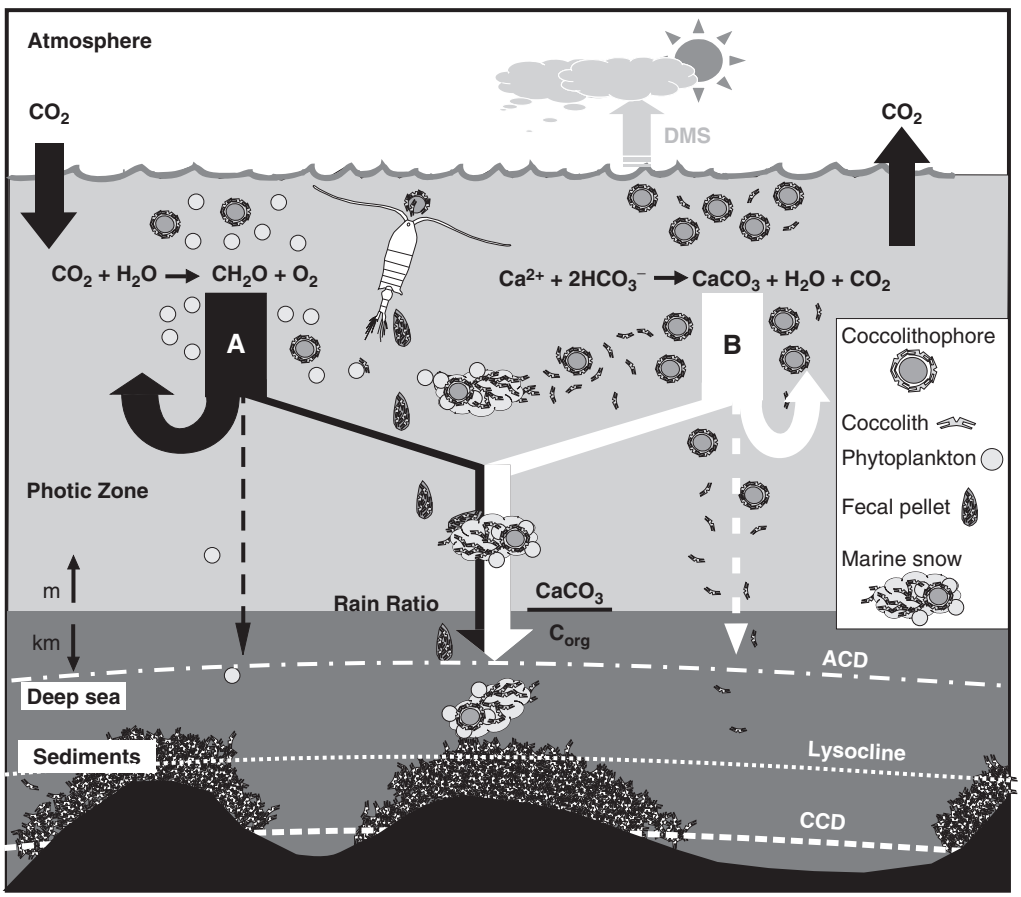

FIGURE 1. Role of coccolithophores in biogeochemical cycles.
Through the production of their $CaCO_3$ coccoliths, coccolithophores are playing a key role in the global carbon cycling. Although they thrive in the photic layer of the world ocean, the coccolithophores actively participate in gas exchange (CO_2, DMS) between seawater and the atmosphere and to the export of organic matter and carbonate to deep oceanic layers and deep-sea sediments. They are the main actors of the *carbonate counter-pump* (**B**), which, through the *calcification* reaction, is a short-term source of atmospheric CO_2. Via the ballasting effect of their coccoliths on marine snow, coccolithophores are also the main driver of the *organic carbon pump* (**A**), which removes CO_2 from the atmosphere. Thus, organic and carbonate pumps are tightly coupled through coccolithophore biomineralization. Ultimately, certain types of coccoliths particularly resistant to dissolution are deposited at the seafloor, where they have built a remarkable fossil archives for the last 220 My. The three main carbonate dissolution horizons are depicted: ACD, aragonite compensation depth; Lysocline (complete dissolution of planktic foraminifera); and CCD, calcite compensation depth. See text for more details.
(Inspired from Rost and Riebesell 2004).

created by photosynthesis in the upper oceanic layers. The accumulation of coccoliths into marine snow ballasts organic matter that otherwise would not sink to deep oceanic layers and, potentially, to the deep seafloor. [AU2] According to Honjo *et al*. (in press), coccoliths are the main driver of the open ocean organic carbon pump (Figure 1A), which removes CO_2 from the atmosphere. In fact, the effect of coccolith ballasting on atmospheric CO_2 concentration could outweigh CO_2 output from biomineralization. Overall, on geological time scales, certain types of coccoliths particularly resistant to dissolution (~30% of the modern diversity, according to Young *et al*. [2005]) slowly accumulate in deep oceanic [AU3] sediments, at rates of less than 10 mm/ka to more than 100 mm/ka (Baumann *et al*. 2004). In the Cretaceous, when they started proliferating in the open oceans, the coccolithophores were responsible for switching the major site of global carbonate deposition from shallow seas to the deep ocean for the first time in the history of the Earth (Hay 2004), thus revolutionizing the regulation of ocean carbon chemistry (Ridgwell and Zeebe 2005). Since this time, coccoliths have been the prime contributors to the kilometers-thick accumulation of calcareous ooze covering ~35% of the ocean floor. This carbonate deposit is one of the main stabilizing components of the Earth system via the mechanism of *carbonate compensation* (Broecker and Peng 1987); its fate is eventually to be subducted into the mantle of the Earth, thus depleting carbon from its surface for millions of years.

Overall, the evolutionary and ecological success of coccolithophores for the last 220 Ma have literally transformed the fate of inorganic and organic carbon in the Earth system, leading to a global decrease in the saturation state of seawater with respect to carbonate minerals (Ridgwell 2005) and participating in the long-term increase of atmospheric O_2 (Falkowski *et al*. 2005). The biological revolution underlying these long-term biogeochemical changes occurred when certain *haptophyte* protists evolved the ability to genetically control the intracellular nucleation and growth of $CaCO_3$ crystals on preexisting organic scales, forming tiny, exquisitely sculptured skeletal plates: the *coccoliths*. Since this invention, the coccolithophores have diversified into more than 4000 morphological species, most of which are now extinct. Although apparently complex, coccolithophore biomineralization (or *coccolithogenesis*) appears to be a rather versatile process that can be quantitatively and qualitatively regulated depending on, for instance, environmental conditions or the stage in the life cycle of the cells. Coccolithogenesis has been modulated multiple times in the course of coccolithophore evolution, either through the continuing innovation of remarkable morphostructures or, in some lineages, even via the complete shut down and possible reinvention of the process itself (see later). Here, we offer an up-to-date summary of coccolithophore evolution, integrating recent stratophenetic, molecular phylogenetic, biogeochemical, and biological data. We discuss the origin and nature of the haptophyte ancestors of coccolithophores, the origin of coccolithophores, and the onset(s) of calcification and illustrate different evolutionary trajectories that succeeding lineages have followed. This evolutionary scheme is then correlated to abiotic and biotic records of historical change in the Earth system, allowing us to evaluate the various extrinsic and possibly intrinsic genomic forces that have driven coccolithophore evolution and the resulting feedbacks of their evolution on the ecosystem. Finally, based on our interpretations of coccolithophore evolutionary history, we envision an uncertain future for this clade in the high CO_2 and high Mg/low Ca world of the Anthropocene.

II. WHAT IS A COCCOLITHOPHORE?

The term *coccolith* was coined by Huxley (1857) for mineral bodies resembling coccoid cells, which he observed in deep-sea

sediments with a relatively low magnification microscope. This word, meaning literally "spherical stone," is a rather unsubtle description for remarkably diverse and exquisitely sculptured calcite platelets (Plate 1). Wallich (1861, 1877) first described the association of coccoliths on single spherical structures, which he termed *coccospheres*, and Lohmann (1902) introduced the word *coccolithophore* for the organisms producing these structures, which appeared to be part of a larger entity of eukaryotic life, the haptophyte microalgae (see later). Biological observations later revealed that many species within the coccolithophores do not calcify during part of their life cycle (Billard and Inouye 2004), and certain taxa such as *Isochrysis galbana* or *Dicrateria inornata* have

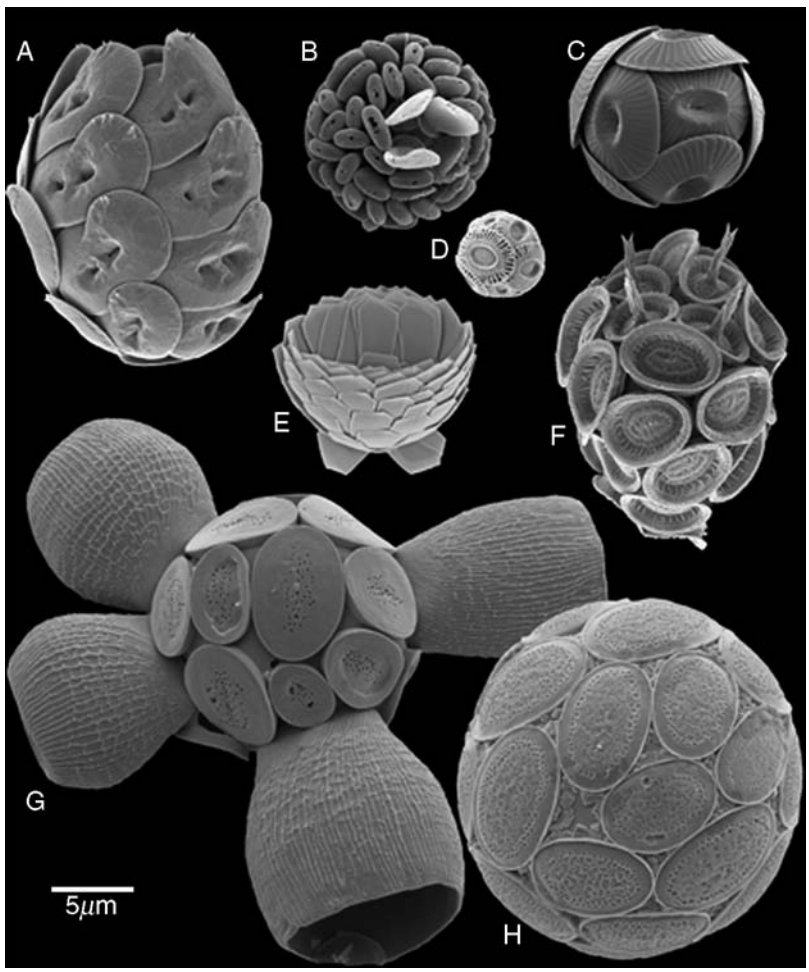

PLATE 1. Morphostructural diversity in extant coccospheres and their heterococcoliths (diploid stage of the life cycle, see Plate 2).
This plate illustrates the astounding calcareous morphostructures observed in modern Calcihaptophycidae (new subclass introduced in this chapter). **(A)** *Helicosphaera carteri*, **(B)** *Algirosphaera robusta*, **(C)** *Coccolithus pelagicus*, **(D)** *Emiliania huxleyi*, **(E)** *Florisphaera profunda*, **(F)** *Syracosphaera pulchra*, **(G)** *Scyphosphaera apsteinii*, and **(H)** *Pontosphaera japonica*. Independent of their size (< 1 to 30 μm) and even though their shape has varied mostly between circular to long elliptical, heterococcoliths exhibit a vast array of morphostructures that have helped reconstructing the phylogenetic history of the coccolithophores (Aubry 1998; Bown 2005; Bown *et al*. 2004).

no known calcifying stages (De Vargas and Probert 2004b). Recently, molecular phylogenetic surveys of environmental and cultured samples have unveiled a growing number of noncalcifying haptophytes, which may partly join the clade comprising more traditional, skeletonized coccolithophores. From geology to biology, and more recently to genetics, the group containing *calcifying haptophytes* is rapidly changing, embracing an increasing diversity of noncalcifying, partly calcifying, or differentially calcifying species. This emerging monophyletic entity of *potentially* calcifying haptophytes has no formal, scientific name, and we propose here the erection of a new *subclass*, the *Calcihaptophycidae*.[1] Further justification of this new taxonomic designation is provided through this chapter.

A. Coccoliths and Coccolithogenesis

Coccolith is a collective term that designates all of the *biomineralized, calcified scales* produced by extant and extinct haptophytes. Although morphologically highly diverse (Plate 1), the singularity of coccoliths lies in their size (a few μm), rather homogeneous calcitic composition (although aragonitic coccoliths are known [Manton and Oates 1980; Cros and Fortuno 2002]), optical characteristics, and remarkable symmetry. Haptophyte biomineralization is unusual among eukaryotes as it occurs *intracellularly*. Intracellular *coccolithogenesis* requires the maintenance of sustained net fluxes of Ca^{2+} and inorganic carbon from the external medium to the intracellular Golgi-derived vesicle in which calcification occurs (Brownlee and Taylor 2004). Calcium is the main anion used for signal transduction in eukaryotes; consequently, its intracellular concentration and compartmentalization need to be under *extreme control*. Marine organisms must also regulate calcium concentration to prevent the intracellular precipitation of apatite (Constantz 1986).

The complex crystallographic structure of most coccoliths suggests that coccolithogenesis is a *highly organized* process under significant biological control. It is believed to involve proteinic templates to support the nucleation of $CaCO_3$ crystals (Corstjens *et al*. 1998; Schroeder *et al*. 2005), complex

[1]Taxonomic note: within the division Haptophyta and the class Prymnesiophyceae, the new subclass Calcihaptophycidae comprises currently four modern orders (the Isochrysidales, Syracosphaerales, Zygodiscales, and Coccolithales) and XX extinct orders (XXX). It defines the monophyletic group containing the last, probably [AU4] noncalcifying common ancestor of all calcifying haptophytes, commonly named "coccolithophores," and all of its descendants including those that do not calcify or calcify in a single stage of their life cycle. Nakayama et al. (2005) discovered a noncalcifying prymnesiophyte, Chrysoculter rhomboideus, which looks like Chrysochromulina sp. but appears as an ancestral lineage within the Calcihaptophycidae according to genetic analyses of the 18S rDNA and rbcL gene sequences (note, however, that no representatives of the orders Syracosphaerales or Zygodiscales were included in this analysis). A number of ultrastructural characters of the flagellar/haptonematal complex appear to be synapomorphies of the clade including Chrysoculter and the coccolithophores: the fibrous root (F1), the electron-dense plate on the Rl microtubular sheet, the R2 of four microtubules with appendages, and the generally low number of microtubules in the emergent part of the haptonema (when present) (Nakayama et al. 2005). Another feature shared by Chrysoculter and coccolithophores is the presence of a single transitional plate in the transition region of the flagellum (other prymnesiophytes have two). In coccolithophores, this single transitional plate that display or not helical bands (Beech et al. 1988; Kawachi and Inouye 1994). Sym and Kawachi (2000) was interpreted [AU5] as homologous to the proximal transitional plate of other, more primitive prymnesiophytes, based on its position in the flagellum and general morphological feature (a perforated septum with an axosome [Nakayama et al. 2005]). Note that these last authors interpreted the single transitional plate of Chrysoculter as homologous with the distal transitional plate of other prymnesiophytes.

The five ultrastructural features described previously appear to be reliable diagnoses to define the new subclass Calcihaptophycidae, whose formal, Latin description is *Prymnesiophyceae plerumque ferens coccolithos ut minimum ex parte cursus vitae. Species sine crystallis carbonatis calcii descriptae. Apparatus flagellaris plerumque cum radice fibrosa (F1), structura tabulari opaca juxta fasciculum tubularem R1, R2 microtubulis quatuor appendiculatus, et typice microtubulis paucis in parte emergenti haptonemis (si adest). Regio transitiva flagellorum cum tabula transitiva una.*

polysaccharides to control their growth and thus sculpt the coccolith (Marsh 2003), and certainly many other, as yet unknown, proteins, enzymes, and transcription factors needed for the formation, shaping, transport, and cellular addressing of the different vacuoles enclosing the liths and their basic components (Ca^{2+}, CO_3^-). To date, ~100,000 partial messenger RNAs (expressed sequence tags, or ESTs) have been sequenced from a few strains of the coccolithophore species *Emiliania huxleyi* by different research groups (mainly the Joint Genome Institute, California). In total, ~20,000 unique sequences (unigenes) were identified (Betsy Read, personal communication). Quinn *et al*. (2006) used a microarray holding ~2300 unique oligonucleotide sequences to identify a *minimum* number of *46 genes* displaying overexpression associated with coccolithogenesis. These upregulation patterns were confirmed by quantitative polymerase chain reaction (PCR). Even though the function of most of these proteins is currently unknown, these preliminary data support the idea that coccolithogenesis is a rather complex phenomenon, involving multiple structural and regulatory molecules under the control of a significant genetic network.

Coccoliths have long been classified into two broad structural groups, *heterococcoliths* and *holococcoliths* (Braarud *et al*. 1955). Heterococcoliths are complex morphostructures that consist of strongly modified calcite crystals arranged in interlocking cycles. They form within cytoplasmic Golgi-derived vesicles by a process that begins with *nucleation* of a proto-coccolith ring of simple crystals arranged around the rim of an organic base-plate in alternating vertical (V) and radial (R) crystallographic orientations (Young *et al*. 1992). The crystals subsequently grow in various directions to form the final structure (Young *et al*. 2004). In some cases, additional nucleation and growth of calcite crystals occurs in the central area (Young *et al*. 1999, 2004). Holococcoliths, by contrast, are simpler assemblies of noninterlocking rhombohedral crystallites of uniform size (~0.1 µm across) and are thought to be at least partly formed extracellularly (e.g., Rowson *et al*. 1986; Sym and Kawachi 2000; Young *et al*. 2003). Interestingly, heterococcoliths and holococcoliths are produced by *single* species in different stages of their life cycle (Plate 2). Although likely based on a common genetic background, heterococcolithogenesis and holococcolithogenesis should recruit different cellular pathways for at least part of the calcification process.

Other biogenic calcareous structures of similar size, but lacking the characteristic features of either heterococcoliths or holococcoliths, have long been *incertae sedis* classified into a category *nannoliths* (Haq and Boersma 1978). Examples include the pentagonal plates of *Braarudosphaera,* the horse-shoe shaped *ceratoliths,* and numerous fossil groups of uncertain affinities such as *discoasters* and *sphenoliths*. However, recent observations have systematically shown that nannoliths are indeed secreted by species within the Calcihaptophycidae. For instance, the coccolithophore *Ceratolithus cristatus* has been shown to produce both *ceratoliths* and two types of heterococcoliths (Alcober and Jordan 1997; Sprengel and Young 2000). The aragonitic cup-shaped nannoliths of *Polycrater* are formed by the haploid stage of the genus *Alisphaera* (Cros and Fortuno 2002). Recent molecular data have even shown that *Braarudosphaera* with its unique pentagonal nannoliths is a deep-branching Calcihaptophycidae (Takano *et al*. 2006). Thus, since their origin, the Calcihaptophycidae appear to have mastered widely different modes of calcification, resulting in several distinctive biomineralized skeletal structures that can all conveniently be referred to as coccoliths.

III. THE HAPTOPHYTES

Today, ~280 different, morphologically defined coccosphere types (Young *et al*. 2003) inhabit the photic zone of the global

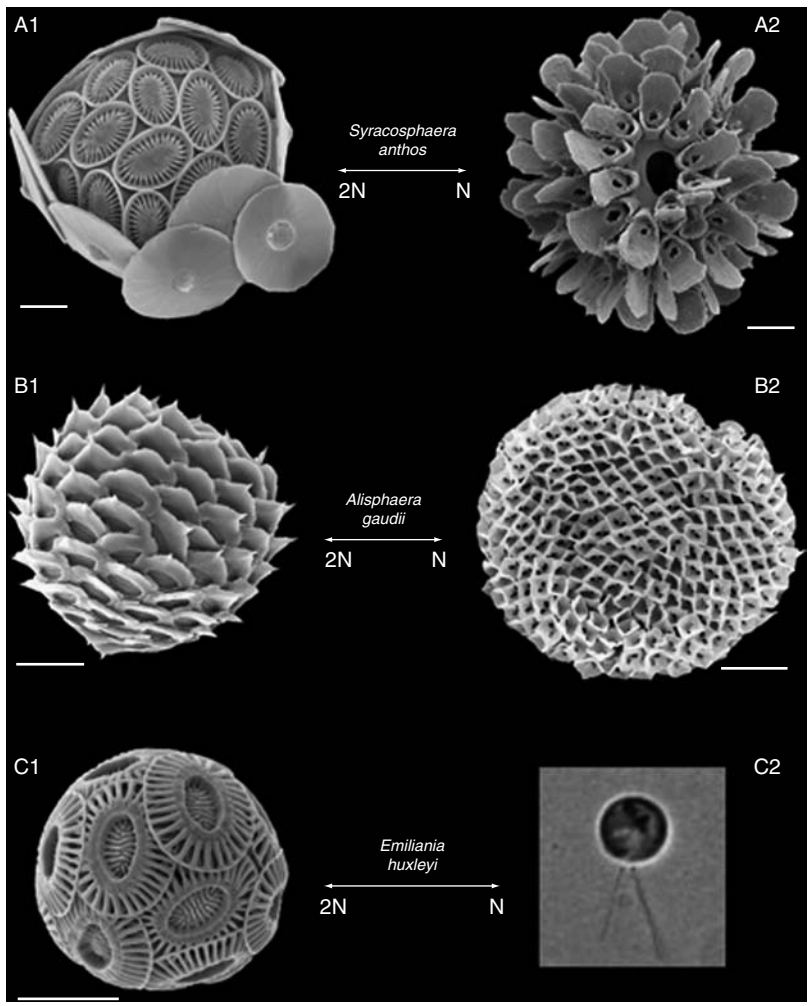

PLATE 2. Haplo-diploid life cycles in the Calcihaptophycidae.
Like most haptophytes, the Calcihaptophycidae are capable of independent asexual reproduction in both the diploid (2N) and haploid (1N) stages of their life cycle. Diploid and haploid stages of a single species express radically different phenotypes and can be either calcifying or noncalcifying. Scale bars are 2 µm. **(A)** *Syracosphaera anthos;* A1: diploid phase producing heterococcoliths; A2: haploid phase with holococcolith, a form previously described as a discrete species, *Periphyllophora mirabilis*. **(B)** *Alisphaera gaudii*. B1 as in A1; B2: Aragonitic-nannolith bearing phase, inferred to be haploid (ex-genus *Polycrater*). This life cycle is based on observations of combination coccospheres (Cros and Fortuno 2002). **(C)** *Emiliania huxleyi*. C1 as in A1; C2: Noncalcifying haploid phase, which occurs repeatedly in culture of initially diploid calcifying cells (e.g., Houdan *et al*. 2005). [AU68]

ocean. Another 6 noncalcifying coastal morphospecies (the family Isochrysidaceae) are included in the Calcihaptophycideae (new subclass, this chapter), which comprises 4 orders and 10 families (Jordan *et al*. 2004). They belong to the class Prymnesiophyceae (6 orders, ~360 species) that, together with the Pavlovophyceae (1 order, 12 species), constitute the division Haptophyta (Jordan *et al*. 2004). Haptophytes are unicellular chlorophyll a + c containing ("red lineage") algae. They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Other forms include

colonies of motile cells, and nonmotile cells that may be solitary and form pseudo-filaments or mucus-bound aggregations. Most haptophytes are marine organisms, inhabiting littoral, coastal, and oceanic waters. In the non-coccolithophore haptophytes, however, brackish and freshwater species are common and a single freshwater coccolithophore, *Hymenomonas roseola,* has been documented (Manton and Peterfi 1969). Haptophytes have also been reported in symbiotic relationships with foraminifers and acantharians (Gast *et al*. 2000), and a coccolithophore was found in skin lesions of dogfishes (Leibovitz and Lebouitz 1985). The production of exotoxins and allelopathic activity has been documented or inferred in various taxa from across the phylogeny of the prymnesiophytes, including the noncalcifying genera *Prymnesium*, *Chrysochromulina,* and *Phaeocystis* and certain members of the coccolithophore genus *Pleurochrysis* (Houdan *et al*. 2004b).

The *Haptophyta* are distinguished by the presence of a unique organelle called a *haptonema* (from the Greek *hapsis*—touch), which is superficially similar to a flagellum but differs in the arrangement of its microtubules and in its use for *prey capture* or *attachment*. The haptonema varies in length among haptophytes and in many coccolithophore species is reduced to a vestigial structure. The use of the haptonema to capture prey has been illustrated for some members of the non-coccolithophore genus *Chrysochromulina* (e.g., Kawachi *et al*. 1991), whereas in the related genus *Prymnesium* ingestion is by means of pseudopodal development at the nonflagellar pole with no involvement of the short haptonema (Tillmann 1998). Particle ingestion has been documented in one species of Calcihaptophycidae (Parke and Adams 1960), and Billard and Inouye (2004) noted that coccolithophores with less rigid coccolith coverings or noncalcifying life cycle stages are likely candidates for *phagotrophy*. The uptake and use of dissolved organic carbon has also been demonstrated for several species of *Chrysochromulina* (Pintner and Pravasoli 1968). *Mixotrophy* thus appears to be widespread in the noncalcifying haptophytes and may also be a significant physiological trait of the coccolithophores. Indeed, although nearly all known haptophytes contain chloroplasts, a number of coccolithophore species found in polar waters (notably from the family Pappsphaeraceae) have been reported to be aplastidial heterotrophic organisms (Marchant and Thomsen 1994). Whether these taxa are genuinely heterotrophic or forms that have secondarily lost the photosynthetic apparatus remains to be verified.

Haptophytes typically produce nonmineralized, *organic scales* in Golgi-derived vesicles. These scales are subsequently extruded onto the cell surface and in rare cases onto the haptonema or one of the flagella. In the Pavlovophyceae, these scales are relatively simple *knoblike* structures, whereas in the Prymnesiophyceae more elaborate and usually ornamented *plate* scales, reminiscent of the coccoliths, are produced. The prymnesiophyte ancestor of the coccolithophores evolved the ability to control the intracellular precipitation of calcite onto such organic plate scales and the assembly of the mature carbonate scales at the cell surface (typically exterior to a layer of nonmineralized plate scales).

An increasing number of species from across the phylogeny of the Prymnesiophyceae have been shown to exhibit *haplodiploid life cycles* (e.g., Vaulot *et al*. 1994; Green *et al*. 1996; Larsen and Edvardsen 1998; Houdan *et al*. 2004a). In a haplodiploid life cycle both stages, haploid and diploid, are capable of *independent asexual reproduction*. This life cycle strategy, possibly a synapomorphy for all haptophytes, is arguably the most significant biological feature differentiating the haptophytes from the diatoms (diploid life cycle) and dinoflagellates (haploid life cycle). In the prymnesiophytes, the morphology of the cell covering (scales and coccoliths) differs between haploid and diploid stages. In the

coccolithophores, in particular, current evidence indicates that diploid stages typically produce heterococcoliths, whereas haploid stages produce holococcoliths or nannoliths or do not calcify at all (Plate 2). The absence of calcification or the production of nannoliths may also occur in as yet undiscovered diploid stages (De Vargas and Probert 2004b).

IV. TOOLS AND BIASES IN THE RECONSTRUCTION OF COCCOLITHOPHORE EVOLUTION

In the pelagic realm, most of the functional and biological diversity is found in unicellular organisms (prokaryotes, viruses, protists) that are rapidly remineralized in the water column after death. Coccolithophore skeletons thus represent a rare opportunity to reconstruct the tempo and mode of evolution in a group of marine planktonic microbes. Despite its superior quality in terms of completeness and continuity, the fossil record of coccolithophores is difficult to interpret for several preservational and biological reasons recently reviewed in Young *et al*. (2005). First, the assemblages of living coccolithophores dwelling in the photic oceanic layers are significantly altered before they reach the ocean floor. Most, if not all, coccoliths sink to the ocean bottom attached to marine snow or packed into the fecal pellets of copepods (Figure 1). In addition to the potential damage caused by their passage through copepod mandibles and digestive tracts, the coccoliths may be dissolved by metabolic CO_2 produced by the degradation of organic matter concentrated in both marine snow and feces. The increased acidity of deep oceanic waters further influences dissolution of coccoliths, which are ultimately subject to diagenesis at the sediment-water interface. A recent detailed study of sinking planktonic assemblages (Andruleit *et al*. 2004) has shown that most of the morphological diversity is entirely dissolved in the upper water column. Among the ~280 types of coccosphere (morphospecies) known from the modern plankton, only 57 are common to rare in Holocene sediments (Young *et al*. 2005). Up to 70% of the diversity is thus erased from the readily accessible recent fossil record.

In modern nannoplankton, the coccoliths lost to dissolution are typically tiny (< 3 µm long) and consist of delicate structures. However, dissolution appears to be more taxon- than size-specific, such that coccoliths are either almost entirely preserved in a few *morphostructural* groups (e.g., the *placoliths*) or largely dissolved or even totally erased in others (e.g., holococcoliths and Syracosphaeraceae, except for a few particularly large species). Overall, censuses of paleodiversity are mostly dependent on the abundance of available strata with ideal preservation conditions, and obviously the time spent by experts on these samples. Another major bias of the sediment record is the relative absence of *coccospheres*. The carbonate ooze consists essentially of detached coccoliths. However, these are merely single building blocks of the coccolithophore skeleton, and their number, type (*polymorphism* or *varimorphism*), and arrangement (imbrication, multiple layers, *dithecatism,* etc.) onto a complete coccosphere is likely ecologically and physiologically more relevant than the morphostructure of the lith itself (Aubry in press-b).

Recent advances in the biological knowledge of the coccolithophores further challenge fossil-based assessments of their paleobiodiversity. The broad phylogenetic distribution of haplo-diploid life cycles in prymnesiophytes (including coccolithophores) suggests that this is the ancestral state for this group. Maintaining the physiological ability to grow vegetatively under both haploid and diploid genomes expressing radically different phenotypes is not frequent among eukaryotes, which have mostly been channeled into either the haploid or the diploid mode of life. This haplo-diploid

"double life" should therefore be of primary evolutionary and/or ecological significance. It is likely a strategy to rapidly escape negative selection pressures exerted on one stage, such as grazing, parasite or virus infection, or abrupt environmental changes. However, the factors triggering shifts from diploid to haploid stages in coccolithophores are virtually unknown, as are the ecology and physiology of the *haploid* stages. In fact, it is not exaggerated to state that research on coccolithophores has almost entirely ignored half of their life cycle up to now. This significant gap is even more pronounced in the fossil record. Although most haploid stages are covered with delicate, tiny holococcoliths with high dissolution potential, others, like the haploid cell of *Emiliania,* are simply naked.

Finally, molecular phylogenetic data have highlighted two additional problems. First, a few studies of bulk ribosomal DNA sequences in natural communities of eukaryotic picoplankton (cells smaller than 3 μm) have revealed a potentially important and ancient diversity of unknown pico-haptophytes (e.g., Moon-Van Der Staay *et al*. 2000; Diez *et al*. 2001). Despite limited sequencing efforts, the new clades are widely divergent and dispersed within the haptophyte phylogeny, which suggests that tiny, noncalcifying haptophytes and, potentially, Calcihaptophycidae form a significant component of the group. Further field investigations using DNA fluorescent probes to identify and *quantify* picoplankton have shown that the pico-haptophytes form a major component of the assemblages of this size fraction in Atlantic waters (up to 35% of the pico-eukaryotes, Not *et al*. 2005) and along a basin-wide Indian Ocean transect from oligotrophic to mesotrophic conditions (F. Not, personal communication). This ghost diversity of naked and tiny haptophytes represent likely a fundamental ecological strategy followed by some lineages, which is clearly inaccessible in the sediment record. Last but not least, molecular data seriously challenge the morphological species concepts classically used in coccolithophore taxonomy. Using both nuclear and chloroplastic genetic markers, five classical morphological species have been revealed to be, in fact, *monophyletic groups of sibling species,* isolated by several million years of evolution according to molecular clock estimations (Sáez *et al*. 2003). This disconnection between slow, morphological differentiation and more rapid genetic evolution has been revealed in all kinds of skeletonized eukaryotic plankton (e.g., dinoflagellates [John *et al*. 2003], diatoms [Orsini *et al*. 2004; Amato *et al*. 2005], foraminifera [De Vargas *et al*. 1999]) and synthesized into a concept of "planktonic super-species" (De Vargas *et al*. 2004). The genetic data indicate that the morphological criteria currently used to define coccolithophore species are too broad and most, if not all, current morphospecies are clusters of a few sibling species, often, but not always, distinguished by subtle structural characters of the coccolith. Characters such as the size of coccoliths, minor morphological details, and their number and arrangement on the coccosphere are likely to prove critical to distinguish species. This means that assessment of true *species level diversity* in the fossil record is currently unfeasible. The morphological interpretations of species paleoecology are equally biased, as tiny and discrete morphological differences may reflect isolated biological species adapted to different spatio-temporal ecological niches, as has been demonstrated in several morphospecies of foraminifers ((De Vargas *et al*. 2002).

To sum up, the classical, morphological view of coccolithophore biodiversity and evolution is largely oversimplified. Naked or poorly calcified cells, coccospheres, haploid stages, biological species, and characters such as motility and phagotrophy are fundamental information that is difficult—sometimes impossible—to retrieve from the sediments. A common practice in coccolithophore research is to merge the concept of coccoliths with the considerably more complex protists

responsible for their production. In our view, coccolithophore studies based on coccoliths primarily reflect the ecology, physiology, or evolution of the function *calcification* in coccolithophores, that is, the genetic network involved in *coccolithogenesis*. Although the fossil record remains the main key to the origin and ancient history of coccolithophore calcification, biological and physiological data are clearly needed to anchor the function *calcification* into other equally fundamental processes of the coccolithophore cells and thus broaden the interpretations based on fossil coccoliths. On the other hand, molecular phylogenetics and comparative genomics give access to a detailed, independent understanding of modern diversity and functions, as well as a coarse evolutionary framework to calibrate and interpret the extinct and fossilized diversities. Unfortunately, both biological and molecular data are still very scarce in coccolithophores and largely focused on the recently evolved and atypical species *Emiliania huxleyi* (see later). However, preliminary exploration of the interfaces among coccolithophore palaeontology, geochemistry, and genomics unveils the forthcoming power of such an approach.

V. THE EVOLUTION OF HAPTOPHYTES UP TO THE INVENTION OF COCCOLITH: FROM COASTAL HUNTERS TO OCEANIC FARMERS?

A. The Origin of the Haptophytes and Their Trophic Status

Although the haptophytes are one of the deepest branching groups in the phylogeny of the eukaryotes (Baldauf 2003), the first reliably identified fossil coccolith appears only ~220 Ma (Bown *et al*. 2004). Given the current absence of any other specific biomarkers for the group (except the alkenones characterizing the Isochrysidales, Figure 2), questions concerning the origins of haptophytes and haptophyte photosynthesis cannot be solved by analysis of the geological record. Comparison of cytology and biochemical homologies, integrated into molecular phylogenetics and molecular clocks, are the only tools available for reconstructing the early evolution of the group.

The origin of haptophytes is the subject of considerable debate. The chlorophyll-c–containing algae comprise four major lineages (the alveolates, cryptophytes, haptophytes, and heterokonts, the three latter being sometimes called the chromists), which, based on similarities among their plastids, were grouped together into a super-cluster, the chromalveolates (Cavalier-Smith 1999). According to this theory, the chromalveolate clade originated through a *single* secondary symbiotic event when a biciliate anterokont host enslaved a red alga. Cavalier-Smith (2002) speculates that this event occurred after the Varangerian snowball Earth melted, ~580 Ma. The resulting eukaryote chimaera evolved chlorophyll c_2 prior to diverging into the alveolates and the chromists. If this scenario is true, the first haptophyte would have originally diverged as a photosynthetic protist in the latest Neoproterozoic.

Recent molecular evidence from plastid-targeted and plastid-encoding protein structures (Fast *et al*. 2001; Harper and Keeling 2003), from phylogenies based on multiple plastid genes (Yoon *et al*. 2004; Bachvaroff *et al*. 2005) and from comparison of complete plastid genome sequences (Sánchez Puerta *et al*. 2005), are consistent with a single origin of chlorophyll-c–containing plastids from red algae. However these data, exclusively based on chloroplastic genes, do not preclude the possibility of multiple transfers of related chlorophyll-c–containing plastids among distantly related heterotrophic hosts (Bachvaroff *et al*. 2005). There is growing support for the monophyly of an alveolate/heterokont clade from individual and multigene phylogenies based on *nucleus*-encoded proteins (e.g., Baldauf *et al*. 2000; Ben Ali *et al*. 2001; Stechmann and Cavalier-Smith 2003; Harper *et al*. 2005). In nuclear gene phylogenies, however, the

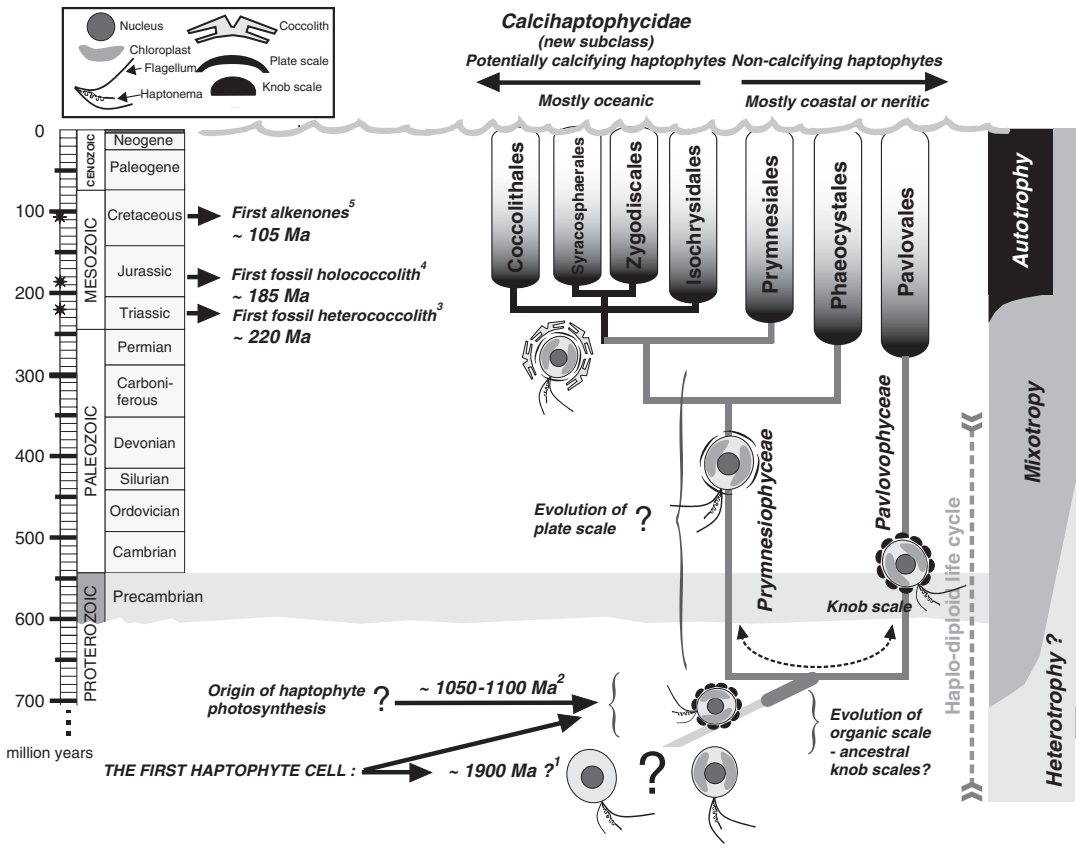

FIGURE 2. Benchmarks in the evolutionary history of the haptophytes and the Calcihaptophycidae. Major innovations are shown along a geological time scale on the left side of the figure, and a synthesis of recent *molecular* phylogenetic data using representative species of the seven extant haptophyte orders (this chapter and [AU61] Saez *et al*. 2004) is depicted on the right side. Biological, phylogenetic, and paleontological data tend to support a scenario according to which the haptophytes have broadly evolved from coastal or neritic heterotrophs/mixotrophs to oceanic autotrophs since their origination in the Proterozoic. Carbonate biomineralization in the Calcihaptophycidae may have been a key evolutionary step for the stepwise invasion of the oligotrophic pelagic realm, starting ~220 Ma. See text for further details. 1, this chapter, Figure 3; 2, Yoon et al. 2004; 3, Bown 1987; 4, Bown [AU62] 1983; 5, Farrimond *et al*. 1986.

haptophytes have an ambiguous, deep and unsolved position, jumping from one sister clade to the other, depending on the analysis. The largest multigene analysis to date places them as the earliest branching group within [AU6] the Chromalveolata (Hackett *et al*. this volume). Nonetheless, even if the chromist and alveolate hosts indeed form a monophyletic group, the hypothesis of multiple secondary endosymbioses via serial transfer is perfectly reasonable, and it is possible that the ancestral haptophyte lineage was originally aplastidial.

In a molecular clock analysis based on multiple chloroplastic genes, Yoon *et al*. (2004) dated the time of divergence between haptophyte and heterokont plastids at ~1050–1100 Ma. Assuming that the plastid phylogeny equals the host phylogeny, the authors proposed this date for the origin of the haptophytes. We have recently sequenced the SSU and LSU rDNA of a wide range of haptophytes, including many species of coccolithophores with an excellent fossil record. The phylogenetic consistency between the two ribosomal

datasets, and between the molecular trees and the morphological taxonomy and stratigraphic ranges of the analyzed taxa, allowed us to infer molecular clocks using multiple maxi-minimum time constraints in the Cenozoic (Figure 3). Our maximum likelihood analyses point toward a significantly earlier origin of the haptophytes, in the Early Proterozoic ~1900 Ma. This time maybe artificially pushed backward by the ML algorithm (Peterson and Butterfield 2005), or if quantum evolution accelerated the transformation of haptophyte rDNA at the origin of the group (the stem branch) [AU7] (Simpson 1944). However, it fits the multigene analyses by (Hedges *et al*. 2004) and the first geological record of putative alveolates ~1100 Ma (Summons and Walter 1990). A Paleoproterozoic origin of the haptophytes matches also the basal position of the group in the eukaryotic tree together with multiple Paleoproterozoic records of eukaryote life as a whole (Knoll *et al*. this volume). Note that the recent attempt by [AU8] Berney and Pawlowski (2006) to date the eukaryotic tree using supposedly accurate microfossil records and relaxed molecular clocks was biased by a miscalibration of the most important node of their tree: the maximum time constraints within the haptophyte. The authors, who estimated a founding date for the haptophyte at ~900 Ma, arbitrarily imposed a post K/T [AU9] divergence time between the branches leading to *Calcidiscus* (Calcidiscaceae) and *Pleurochrysis (*Pleurochrysidaceae, a family without fossil record). According to our data, which include more species and use accurate geological calibrations within entirely fossilized groups, the split between the two families occurred much earlier in the Mesozoic.

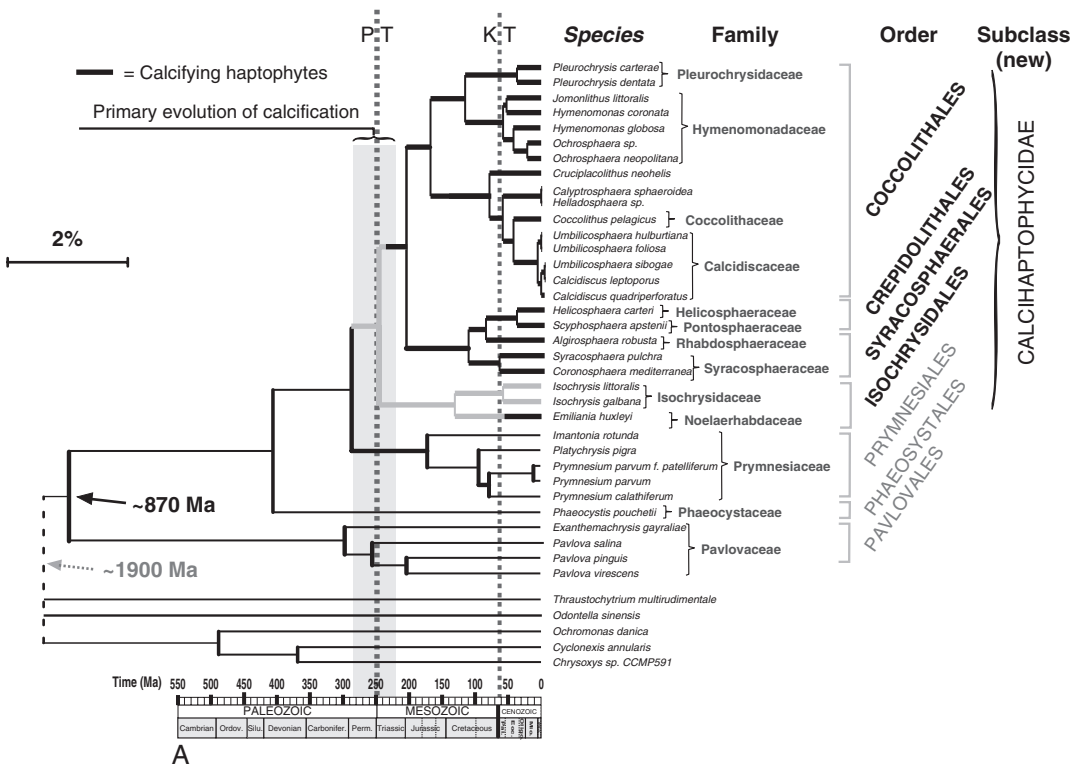

FIGURE 3. The origin and evolution of coccolithophores according to ribosomal DNA. SSU (**A**) and LSU

(Continued)

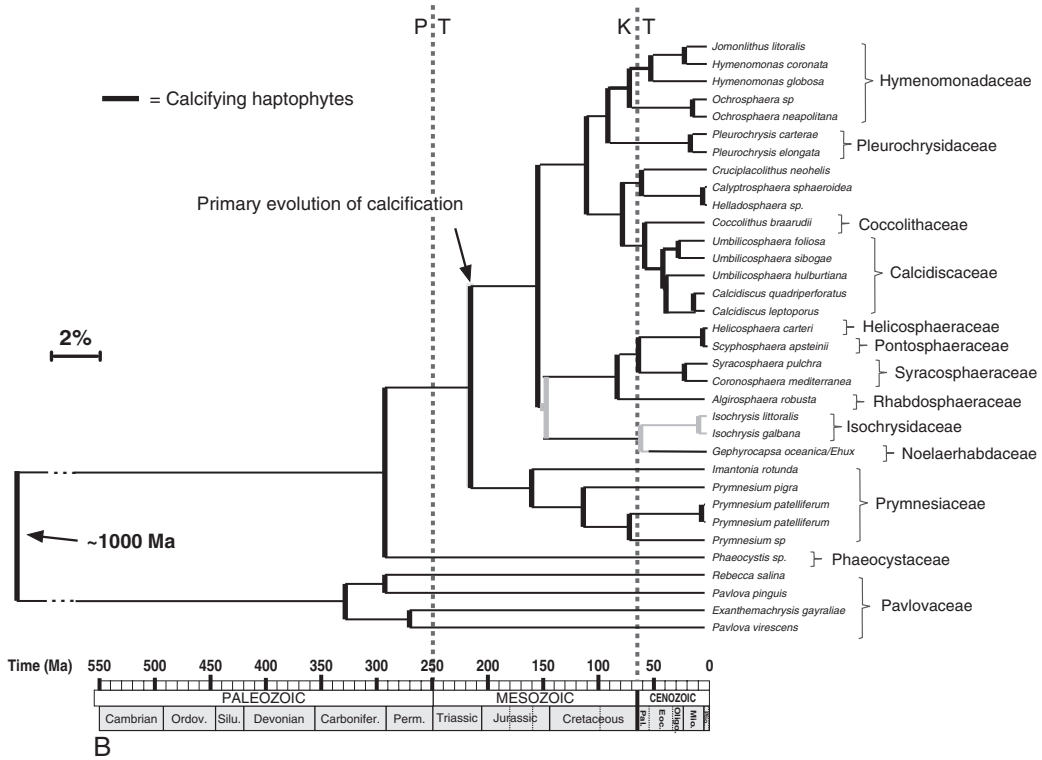

FIGURE 3. Cont'd (**B**) rDNA trees including 34 haptophyte taxa, representatives of the 7 extant orders within the Haptophyta. The SSU rDNA were fully sequenced (alignment of 1750 sites), whereas a 5' fragment containing the D1 and D2 domains was sequenced for the LSU rDNA (950 sites). Unambiguously aligned sites were used for maximum likelihood analyses enforcing a molecular clock, as implemented in *PAUP* (Swofford 2000). Models of DNA substitution that best fit our datasets were selected through a hierarchical likelihood ratio test (Posada and Crandall 1998), and maximum time constraints were based on strato-phenetic events (details presented elsewhere). Despite small topological differences, both trees are in relative good congruence with both classical, morphological taxonomy and interpretation of the fossil record. The monophyly of the haptophytes with potential for calcification (the Calcihaptophycidae, new subclass) is obvious, and the origin of coccolithogenesis is located somewhere between the molecular origin of the group and the first apparent coccolith in the fossil record (~220 Ma).

If the hypothesis of an Early Proterozoic origin of the haptophytes is true, then haptophytes must have been primarily heterotroph and acquired a red plastid via secondary endosymbiosis hundreds of million of years after their origin (Figure 2). The proposed primary function as a *hunting* apparatus of the single most important autapomorphy of the group, the haptonema, fits this heterotrophic scenario, as does fact that many haptophytes from early diverging, noncalcifying lineages (Prymnesiales, Phaeocystales, Figure 2) have conserved heterotrophic behavior (mixotrophy). Strikingly, the early-branching lineages in nuclear molecular phylogenies of extant heterokonts, cryptophytes, and alveolates are systematically represented by heterotrophic (aplastidial) taxa. This observation lends further support to a hypothesis of a wide primordial diversity of heterotrophic predators in *all* ancestral lineages of the modern chlorophyll-c–containing algae, which would have acquired photosynthetic abilities only later via independent serial acquisitions of related plastids (for instance from a red algal prey that was highly efficient at establishing polyphyletic

endocytosymbioses and transferring genes to its hosts nucleus). In fact, the oldest *firm* evidence of photosynthesis in haptophytes can only be dated from the divergence time between the Pavlovophyceae and Prymnesiophyceae, whose modern representatives are almost exclusively mixotrophic or autotrophic (Figure 2). This node was estimated at ~805 Ma using a chloroplastic molecular clock (Yoon *et al*. 2004) and, respectively, at ~780 and 1000 Ma according to our new SSU and LSU rDNA clocks (Figures 3 and 4). For once chloroplastic and nuclear data are in good agreement, so that it seems reasonable to assume that photosynthetic haptophytes were present *at least* from the Neoproterozoic Era (1000–542 Ma). These primordial photosynthetic haptophytes were likely phagoautotrophs, a feature apparently shared by most extant noncalcifying prymnesiophytes.

B. Paleozoic Haptophytes and the Ancestors of the Coccolithophores

Again, without fossil record evidence or specific biogeochemical signatures, it is difficult to infer the morphological, physiological, and ecological features of haptophytes during the Paleozoic, prior to the evolution of the coccolithophores. An intuitively appealing hypothesis, which would account for the fact that coccoliths did not evolve for at least the first half of the evolutionary history of the prymnesiophytes, would be that plate scales, that is, the organic matrices that appear necessary for intracellular coccolith-type biomineralization, originated only shortly after the Permo-Triassic (P/T) boundary event as the group underwent rapid radiation into environmental niches vacated by the massive marine extinction. In fact, proto-prymnesiophytes must have evolved the organic plate scales at least before the Phaeocystales/Prymnesiales split that, according to our molecular clock analyses (Figure 3), occurred in the mid-Paleozoic. It is thus likely that relatively derived and diverse prymnesiophytes were present in the oceans well before the onset of haptophyte calcification. The molecular trees indicate that pavlovophytes were also present in Paleozoic oceans. The deep branches of most analyzed *Pavlova* spp. further suggest that extant lineages survived both the P/T and K/T mass extinctions, probably finding refuge in the coastal ecosytems they typically inhabit nowadays.

Fossils in Paleozoic shales indicate that phytoplankton with morphological features similar to members of extant green algal lineages were abundant and diverse in waters overlying contemporary continental shelves, leading Falkowski *et al*. (2004) and others to suggest that green phytoplankton were taxonomically and ecologically dominant throughout this period. The recurring presence of black shales through much of the Paleozoic is suggestive of frequent anoxia in the deep global ocean (Anbar and Knoll 2002). Basin-wide anoxia would correspond to fundamentally different oceanic chemistry, particularly in terms of the bioavailability of various trace elements, metals, and nutrients (Quigg *et al*. 2003). In particular, the increased availability of Fe, P, and ammonium in reduced oceanic conditions would have given strong ecological advantage to the green microalgae (Falkowski *et al*. 2004) and may have restricted the red lineages, including the haptophytes, to the better oxygenated coastal regions where rivers delivered essential metals (Katz *et al*. 2004). Among modern haptophytes, the morphologically relatively simple pavlovophytes are apparently restricted to near-shore, brackish, or freshwater environments often with semibenthic modes of life, and this may mirror the ancestral ecological strategy of Paleozoic haptophytes, including scale-bearing photophagotrophic proto-prymnesiophytes. In coastal environments, the cells may have complemented their nitrogen and trace metals needs via mixotrophy, a way to offset the presumed competitive advantage of green algae. Modern members of the noncalcifying prymnesiophytes (Phaeocystales and Prymnesiales) are found in both

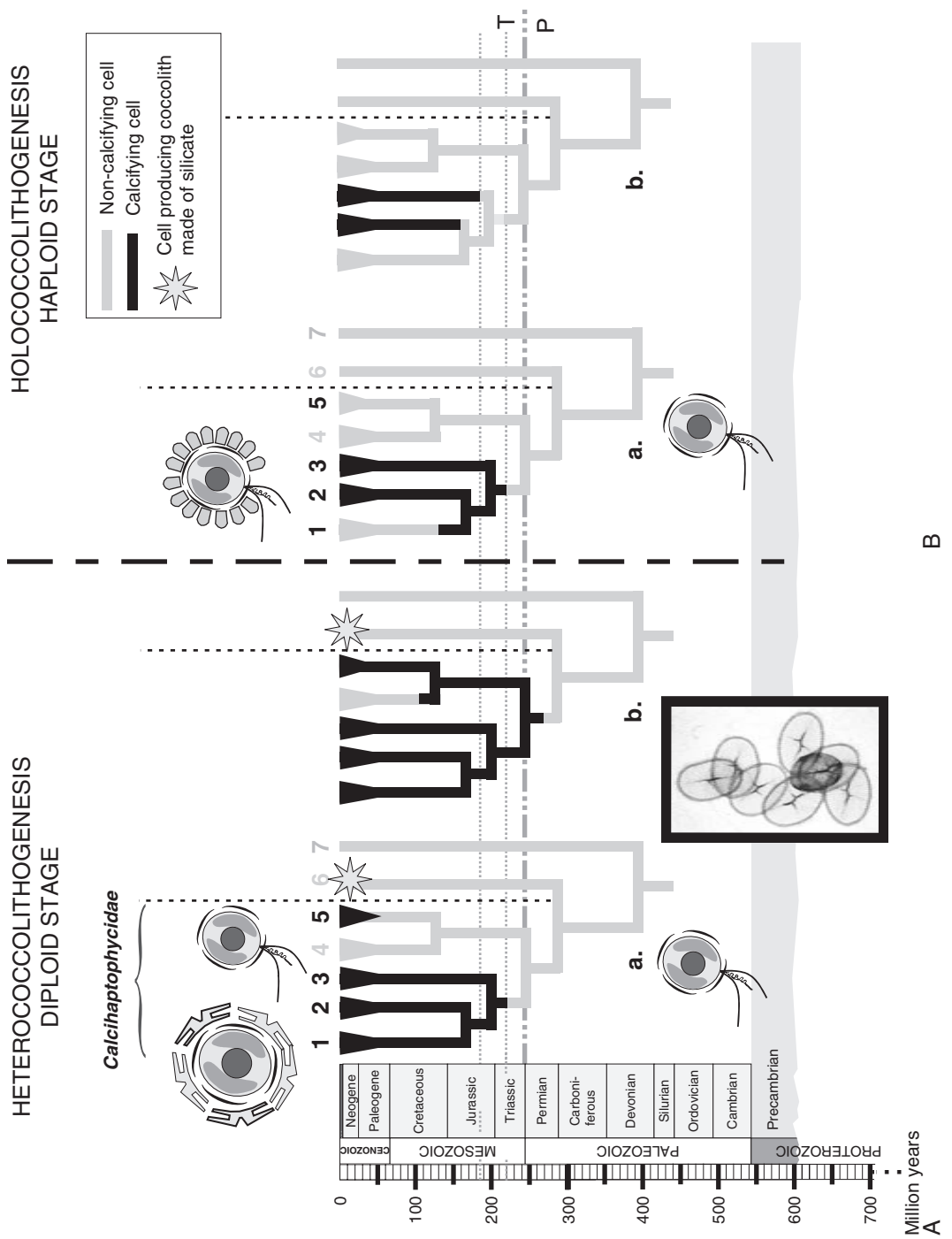

FIGURE 4. For legend see opposite page.

coastal and oceanic environments. This may be indicative of Cenozoic colonization of oceanic realms from coastal pools of diversity across prymnesiophyte phylogeny. This evolutionary pattern is somehow reminiscent of the foraminifers, which became fully planktonic only ~180 Ma. Some time after the P/T catastrophic event, certain prymnesiophytes within the Calcihaptophycidae evolved the ability to biomineralize their organic scales and secrete calcareous coccoliths: they were starting out on the long evolutionary pathway leading to their supremacy in the pelagic realm.

VI. THE ORIGIN OF CALCIFICATION IN HAPTOPHYTES: WHEN, HOW MANY TIMES, AND WHY?

The first *reliable* coccoliths and non-dinoflagellate nannoliths in the fossil record are present from the Late Triassic Norian stage (217–204 Ma) (Bown 1987). The nannofossils present earlier in Carnian sediments (228–217 Ma) are nannoliths of uncertain affinity and calcareous dinoflagellates. Minute (2–6 µm), finely structured, multicrystalline fossils are present in Paleozoic Pennsylvanian and Permian limestones but subject to different interpretations: coccoliths (see review in Tappan (1980) or inorganic calcareous objects or cases of contamination (Bown *et al*. 2004). Triassic coccoliths have simple *murolith* morphologies (i.e., with narrow, wall-like rims) of very small size, 2–3 µm (Bown *et al*. 2004), at the lower limit for preservation in the fossil record, according to Young *et al*. (2005). Modern murolith-producing coccolithophores in the family Hymenomonadaceae are exclusively found in coastal environments and, as a consequence, have not left a fossil record. Should the Norian coccoliths have evolved from even smaller and/or coastal forms, the origin of coccolithogenesis could significantly predate the first fossil appearance of coccoliths. However, coccolithophores are one of the rare cases in which molecular clocks tend to confirm the first appearance of a group of organisms based on fossil data. SSU and LSU rDNA clocks respectively give ~270–240 and ~200 Ma as the earliest possible dates for the origin of the Calcihaptophycidae (Figure 3). As discussed later, these dates correspond to the origin of *potentially* calcifying haptophytes and not necessarily to the onset of biomineralization per se. Thus, molecular data indicate that the ancestral lineage of the Calcihaptophycidae originated around the time of the P/T boundary and evolved coccolithogenesis very soon after their genetic differentiation, as witnessed by the clear heterococcoliths found in ~220-My–old sediments (Bown *et al.*, 2004). As prymnesiophytes with organic plate scales were present in the Paleozoic, why did they start to calcify only between 250 and 220 Ma

FIGURE 4. The multiple origins of coccolithogenesis in the haploid and diploid stages of calcihaptophytes. These schematic trees (based on SSU rDNA data, see Figure 3) illustrate the evolutionary history of biomineralization (within the Prymnesiophyceae **1,** Pleurochrysidaceae and Hymenomonadaceae; **2,** Coccolithaceae and Calcidiscaceae; **3,** Helico-, Ponto-, Rhabdo-, and Syraco-sphaeraceae; **4,** Isochrysidaceae; **5,** Noelaerhabdaceae; **6,** Prymnesiaceae; **7,** Phaeocystaceae). Carbonate coccolithogenesis first evolved in the diploid phase (A) of primitive Calcihaptophycidae after the Permo-Triassic boundary. It was later reinvented, possibly on multiple occasions, in the haploid phase of certain families **(B),** and we suggest that even diploid lineages within the family Noelaerhabdaeae may have reinvented calcification in the early Cenozoic **(Aa.).** Biomineralization based on silicate precipitation independently evolved within the Prymnesiaceae (star symbol). Note that all Prymnesiophyceae, skeletonized or not, secrete and assemble at the cell surface organic plate-scales like the one shown in the black-framed SEM picture in **(A).** The horizontal dotted lines in the middle of the figure indicate, from bottom to top, the P/T boundary, the first fossil heterococcolith, and the first fossil holococcolith.

and not before? Was this triggered by the randomness of rare and complex genomic innovation or rather dominantly driven by extrinsic biotic or abiotic selection pressures?

A. Genetic Novelties?

Unfortunately, too little is known about the structure and functions of genomes of calcifying and noncalcifying haptophytes, and the minimum network of genes required to build a coccolith, to assess which genetic change(s) allowed coccolithogenesis. However, the protein(s) and polysaccharides directly recruited for calcite nucleation and growth are likely *few* (although potentially highly variable) and may not be new but rather be derived from ancestral biochemical pathways. Marin *et al*. (1996) proposed that the polysaccharides involved in the control of skeleton growth first evolved as calcification inhibitors in Proterozoic oceans. In the Precambrian, extreme oceanic supersaturation (Ω) resulted in rapid and sometimes massive abiotic precipitation of $CaCO_3$ (the *Strangelove* ocean mode of Zeebe and Westbroek (2003). Primitive eukaryotes would have thus evolved *anti-calcifying* molecules to avoid spontaneous carbonate encrustation of cells and tissue surfaces. Therefore, the polysaccharides recruited for coccolithogenesis in the Triassic may have existed for hundreds of millions of years before their use as microskeleton architects. Moreover, one of the most typical features of prymnesiophytes, the plate scales made of cellulosic microfibrils and arranged in specific patterns at the cell surface, represent an ideal *preevolved* material for $CaCO_3$ crystal nucleation and growth. In this view, first promoted by Westbroek and Marin (1998), coccoliths, and carbonate skeletons in general, are the "simple" product of *new associations between ancestral biochemical processes* and thus may not be as original as they appear (in other words, they are not dependent on major cellular and biochemical inventions). Supporting this theory, Knoll (2003) estimated that carbonate skeletons evolved independently *at very least* 28 times within the eukaryotes.

B. Multiple Origins for Coccolithogenesis?

The clustering of all calcifying haptophytes into a monophyletic entity (the Calcihaptophycidae, Figure 3) and the early origin of *heterococcolithogenesis,* a highly distinctive biomineralization mode, may argue for a single origin of calcification in haptophytes (Young *et al*. 1992). However, genera branching at the base of the Calcihaptophycidae, such as *Chrysoculter* (Nakayama *et al*. 2005) or *Braarudosphaera* (Nakayama *et al*. 2005; Takano *et al*. 2006), are noncalcifying or secreting relatively simple carbonate scales (nannoliths). Thus, the Calcihaptophycidae ancestor may have been noncalcifying, and its diversification may have given rise to various, non-heterococcolith modes of calcification early in the evolution of the group (such as holococcolithogenesis and diverse nannolithogeneses).

We suggest that coccolithophore biomineralization is a game of *bricolage,* that is, that the assembly of a few necessary, but not necessarily new, materials and cellular processes allows the building of a coccolith. In this game, each individual module of the entire process may be recruited on a different mode, and the potential (re)invention or switch-off of a single or a few module(s) may respectively generate or prevent calcification in a particular taxon. The most striking example supporting this model are *holococcoliths* (Plate 2). Holococcolithogenesis is clearly different from heterococcolith formation in terms of crystal nucleation, growth regulation, and locus of calcification (Young *et al*. 1999; Sym and Kawachi 2000), proving that calcification in haploid stages of the Calcihaptophycidae required some form of *reinvention* of the initial process. Despite the delicate nature and limited preservation potential of holococcoliths in deep-sea sediments, their earliest evidence

in the fossil record dates from ~185 Ma. The ~35-Ma delay between the first records of heterococcoliths and holococcoliths in the fossil archive (Figure 2) does suggest that this reinvention occurred many millions of years after the evolution of heterococcolithogenesis by diploid Calcihaptophycidae. Molecular phylogenies seem to confirm that holococcolithogenesis occurred for the first time after the divergence of the Isochrysidales (whose extant members, such as *Emiliania*, do not calcify in their haploid stage; Figure 4B) and that the Pleurochrysidaceae and Hymenomonadaceae may have secondarily lost the ability to produce holococcoliths (Figure 4Ba). Note that it is also possible that holococcolith biomineralization, a rather flexible and simple process compared to heterococcolithogenesis, evolved independently in at least two and possibly more lineages (Figure 4Bb).

Which of the genetic modules involved in heterococcolith biomineralization were recycled in holococcolithogenesis, or, in other words, which molecules and/or cellular processes were newly recruited in holococcolith formation, is a fundamental question to which there is presently no answer. Similarly, the biomineralization of strikingly different nannoliths (Plate 3) or particular structures of certain heterococcoliths (such as the central process in *Algirosphaera*) likely represent partial reinventions or even *independent origins* of the biomineralization process within the Calcihaptophycidae. Here, the case of by far the most famous species of Calcihaptophycidae, *Emiliania huxleyi,* is worth examining. *Emiliania* is the laboratory rat of coccolithophore research, to such a degree that the concept of coccolithophores itself is regularly (and unfortunately!) confounded with this species. However, *Emiliania* and its sister and ancestral species (the Noelaerhabdaceae) are strongly atypical in many ways and may result from an *independent Cenozoic origin* of calcification (heterococcolith-like). In molecular phylogenies based on both rDNA (Figure 3) and chloroplastic genes (Fujiwara *et al*. 2001; Sáez *et al*. 2003), the Noelaerhabdaceae typically branch at the base of the Calcihaptophycidae tree. Moreover, their sister clade, the Isochrysidaceae, contains *noncalcifying* taxa and one species, *Chrysotila lamellosa,* which induces extracellular calcification (see later). Secondary loss of heterococcolith formation in the Isochrysidaceae is a seemingly parsimonious interpretation of this phylogenetic pattern (Figure 4Ab). However, another hypothesis is that the Isochrysidales diverged from the presumably noncalcifying Calcihaptophycidae ancestor prior to the first evolution of heterococcolithogenesis. They remained noncalcifying and possibly coastal (like modern Isochrysidaceae) during the Mesozoic and through the K/T crisis, and independently evolved heterococcolithogenesis ~50 Ma when the first fossil Noelaerhabdaceae appeared in the geological record (Figure 4Aa). Since then, this new mode of calcification dominated Calcihaptophycidae biomineralization. *Emiliania* is the baby of the group; it diverged only ~250,000 years ago from the gephyrocapsids and has played a prominent role in Quaternary oceans (Thierstein *et al*. 1977).

Several ecological, stratophenetic, physiological, and cellular features of *Emiliania* and its sisters favor the hypothesis of an independent invention of calcification in this group. Unlike most other coccolithophore species, which are K-strategists in oligotrophic waters, most of the Noelaerhabdaceae prefer mesotrophic conditions where they can produce atypical massive blooms. The living representatives of the group exhibit unusual life cycles with a *noncalcifying* haploid phase. Most importantly, they possess several ultrastructural features that distinguish them from other coccolithophores, such as the apparent absence of any vestige of a *haptonema*, very thin and unique flakelike scales underlying the coccoliths, the X-body in the haploid phase, and an atypical *reticular body* (a system of membranes separated from the Golgi-body; see Paasche [2002] and references therein) around the coccolith

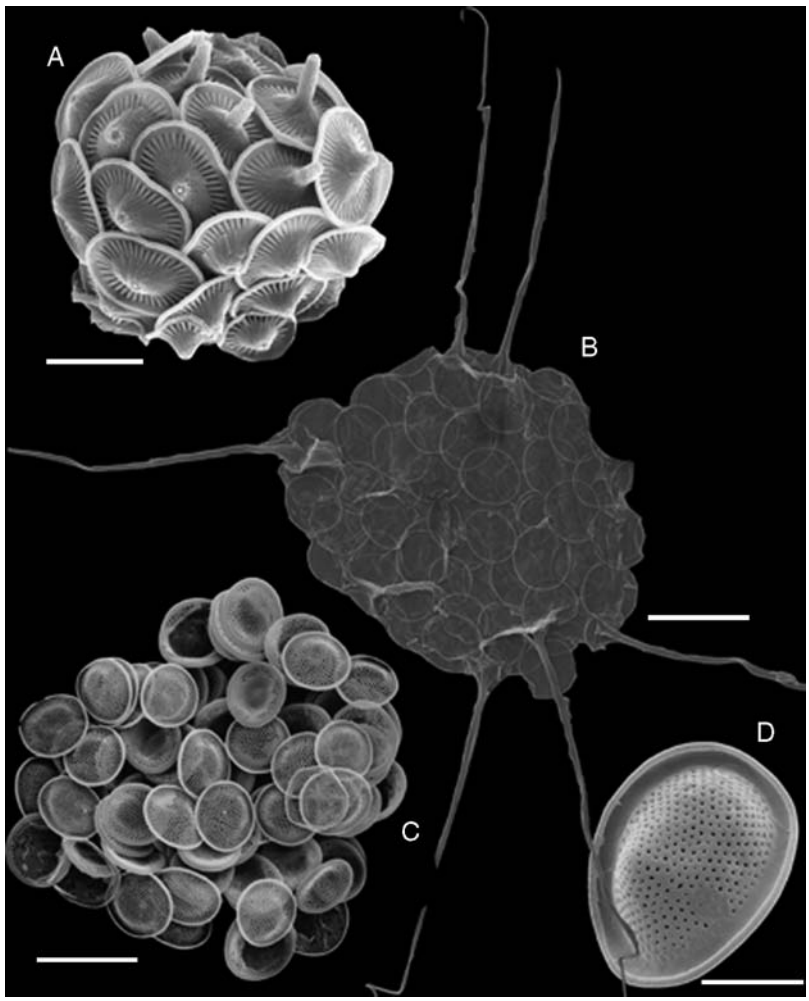

Plate 3. Calcifying, noncalcifying, and silicifying haptophytes.
This plate illustrates different morphostrategies used by the haptophytes to cover the cell membrane: calcite coccoliths in *Cyrtosphaera lecaliae* **(A),** cellulosic scales in a noncalcifying *Chrysochromulina* sp. **(B),** and siliceous scales in *Hyalolithus neolepis* (**C,** specimen courtesy of Rick Jordan; **D,** a single silicoscale). Scale bars are respectively 2, 3, 10, and 2 μm. These remarkable morphological convergences are likely based on common cellular and molecular processes recruiting orthologous and homologous but also convergent and even novel molecules. As in coccolithogenesis, the silicification of *Hyalolithus* occurs within intracellular vacuoles, which are shaped and dispatched around the cell via cytoskeletal forces.

vesicles. Finally, there is no definite fossil link when they first appear in Early Eocene sediments. The proposed derivation from the extinct Prinsiaceae, based on morphological similarities (Romein 1979; Gallagher 1989; Young *et al*. 1992), has not been proven by stratophenetic studies and is challenged in terms of morphostructural evolution by Aubry (in press-a). Although some of these exceptional features are not directly linked to calcification and may be due to derived rather than ancestral evolutionary processes, the combination of them supports the idea that coastal, noncalcifying Isochrysidales, survivors of the K/T extinction, have evolved *de novo*

the necessary conditions for heterococcolithogenesis. (Note that the embryonic V/R pattern of biomineralization observed in the proto-coccolith ring of *Emiliania* [Young *et al*. 1992] imply that this potential independent origin of heterococcolithogenesis was based on homologous [but not necessarily orthologous] proteinic templates).

In this context, it is worth noting that living members of the noncalcifying Isochrysidales may provide clues as to the first evolutionary steps leading toward intracellular biomineralization in the Calcihaptophycideae and may, in fact, be considered to be in the process of reinventing coccolithogenesis in modern oceans. Although *Chrysotila lamellosa* (a species within the Isochrysidaceae, the sister family of *Emiliania* and the Noelaerhabdaceae) does not produce coccoliths, it does produce small organic plate scales and it is typically found as aggregations of nonmotile cells, which are invested with a thick layer of hyaline mucilage. In old cultures, *calcified* deposits are observed to form in interstices within the mucilage mass, presumably partly driven by modifications of the environment (increased pH) due to photosynthetic activity (Green and Course 1983). The chemical nature of the mucilage produced by *C. lamellosa* is not known, but Green and Course (1983) speculate that it is formed of polysaccharides with sulphated and uronic acid residues and perhaps complexed proteins providing a matrix upon which deposition of calcium salts may occur. If chemical analogies between this system and intracellular coccolith formation are confirmed, it can be postulated that extracellular calcification in benthic prymnesiophytes of the type observed in *C. lamellosa* may be a direct evolutionary precursor to coccolithogenesis, the critical step "simply" being the intracellularization of this process. Were this the case, it can further be speculated that the first coccoliths may not have been as complex in crystallographic and structural terms as hetero- and holococcoliths, perhaps being relatively unregulated nannolith-like structures.

Last but not least, biomineralization is actually not restricted to the Calcihaptophycideae and has evolved independently within the Prymnesiales on a remarkably similar mode but using silica rather than carbonate precipitation (Plate 3). Different authors (Pienaar 1980; Green *et al*. 1982) recorded certain species of *Prymnesium* producing layers of scales with electron-dense siliceous material, interpreted as resting cysts. More recently, Yoshida *et al*. (2006) used transmission electron microscopy and molecular phylogenetics to show that *Hyalolithus neolepis,* a haptophyte taxon branching within the Prymnesiales, secretes silica scales by a mode surprisingly similar to the one employed in carbonate coccolithogenesis. The cell controls the intracellular precipitation and growth of silica onto organic templates within vesicles probably derived from the peripheral endoplasmic reticulum, and the silica scales are extruded and arranged into a composite test, strikingly similar to those produced by the organic scales of other Prymnesiales or the coccospheres of the Calcihaptophycidae. A significant part of the genetic network controlling the formation of siliceous scales in haptophytes is likely homologous (and partly orthologous) to the machinery used in haptophyte calcification. This spectacular morphological convergence (Plate 3) provides strong support for a hypothesis of haptophyte biomineralization as a game of *bricolage* and justifies the introduction of the new subclass Calcihaptophycidae for the monophyletic group of potentially *calcifying* haptophytes.

The question of the ease with which Calcihaptophycidae can switch on and off, adapt and modify, and/or independently reevolve intracellular calcification is crucial for understanding the past and present and for predicting the future of these organisms. It highlights the need for further genetic and biochemical investigations on a wider range of taxa. If coccolithogenesis is indeed an interplay between preevolved

modules around a few crucial elements that the Calcihaptophycidae acquired at their origin (most probably specific protein(s) and polysaccharides), then haptophyte calcification may be much more adaptable than previously thought. Coccolithogenesis may have more origins than assumed, and some lineages could stop calcifying and subsequently reactivate the process, even on a different mode, as has been proposed for coral biomineralization (see the "naked coral hypothesis" of Stanley [2003]).

[AU11]

C. Environmental Forcing on the Origin of Haptophyte Calcification

The Late Triassic appears to have been a founding period for the primary diversification of various, unrelated marine calcifiers, including *coccolithophores*, *calcareous dinoflagellates*, and *scleractinian corals* (Figure 5). It thus seems that a global environmental forcing stimulated or at least opened the possibility for the simultaneous development of meters-wide aragonitic coral colonies on the shore and the earliest calcitic microalgae in the plankton. Which factors may have either hindered such biological innovation earlier in the Paleozoic or specifically triggered them in the Triassic?

Atmospheric CO_2 rise has been invoked as a major threat for modern corals (Leclercq *et al*. 2000) and coccolithophores (Riebesell *et al*. 2000b). Because the atmosphere is in equilibrium with the upper ocean, high pCO_2 decreases pH and therefore lowers the carbonate ion concentration of surface waters. This in turns lowers the saturation state (Ω) of the ocean, which can strongly decrease the potential for organisms to secrete calcareous skeletons. However, coccolithophores originated in a high pCO_2 world, where concentrations of atmospheric carbon dioxide appear to have been four to six times higher than today (Figure 5, and Katz *et al*. this volume). Other factors must have buffered the Triassic oceans with high alkalinity to keep Ω high enough for aragonite and calcite deposition. Paleoceanic sea levels and Mg/Ca chemistry have been proposed as driving forces for the colonization of the pelagic realm by calcifying organisms (Ridgwell and Zeebe 2005). The Triassic was characterized by very low sea levels (and therefore reduced area of shallow water carbonate platforms) and seawater with low Ca^{2}+ concentration and a high Mg:Ca ratio (Mg inhibits calcite precipitation) (Figure 5). These conditions of extremely reduced biocalcification on the shelves are thought to have given rise to a highly oversaturated ocean. Weathering of exposed carbonates would have enhanced the effect of decreasing shelf area to further boost the carbonate saturation of the ocean (Walker *et al*. 2002). The very high open ocean Ω deduced in the Late Triassic was certainly a necessary condition for calcite precipitation; however, such conditions (low sea levels, decreased shelf area, high Mg:Ca ratio) seem to have prevailed for around 100 million years prior to the origin of coccolithogenesis (Figure 5). Other factors may have hindered planktonic biocalcification in the late Paleozoic, and these may be related to the reasons suggested to have led to the supremacy of green algae in Paleozoic oceans, while pushing the red algae to near-shore niches. Deep-water anoxia was particularly pronounced near the end of the Permian and persisted through the Early Triassic (Isozaki 1997). Ocean anoxia alter redox chemistry of the oceans and especially increase the concentration of the cations Fe and Mn, which are known to inhibit the precipitation of $CaCO_3$ (Ridgwell and Zeebe 2005). Thus, we propose that the *relaxation* of anticalcifying Paleozoic conditions in the *Aragonite II* open oceans created an environmental matrix favorable for pelagic calcification.

[AU12]

D. Why Were Coccoliths Invented?

The partial reinvention of calcification in the haploid phase of coccolithophore life cycles and the possible multiple origins of coccolithogenesis indicate that biominerali-

zation has been positively selected at least a few times in the evolutionary history of the haptophytes. In fact, widely different groups of protists, in particular the haptophytes, dinoflagellates, and foraminifers, adopted calcification and started to proliferate in this favorable Late Triassic ocean (Figure 5). However, the establishment of oceanic conditions permitting calcification was not the evolutionary force that *directly* drove the invention and maintenance of biomineralization. Strong ecological and/or physiological advantage(s) impacting the survival and fitness of the cells must have selected those individuals that started calcifying and at the same time became clearly planktonic (note that the foraminifers were calcifying since the early Cambrian but invaded the planktonic realm only about 180 Ma). It may be that each group of pelagic microcalcifiers followed these evolutionary steps (calcification and migration to the pelagic realm) for different reasons.

Multiple hypotheses to explain the primary function of covering the cell with a carbonate crust have been proposed for each group. In coccolithophores, the debate centers mainly on whether the function of coccoliths is principally *ecological biotic* (protection against grazing and/or viruses, see Hamm and Smetacek, this volume), *ecological abiotic* (light concentration, protection against ultraviolet [UV], dissipation of light energy under high irradiances, control of sinking rate), or *cellular biochemical* (carbon concentration mechanism for photosynthesis, phosphorus metabolism, maintenance of a balance between high external and low intracellular Ca concentration) (see reviews by Young 1994; Paasche 2002). Despite a plethora of hypotheses, relatively few experiments on living coccolithophores have been conducted to actually test these ideas, and the large majority of experiments have been performed on one of the most atypical calcihaptophytes, *Emiliania huxleyi*. The lack of knowledge of the physiology and ecology of divergent coccolithophore lineages, having sometimes drastically different ways of building their coccoliths and coccospheres, is arguably the main barrier to interpretation of their evolution. Key selection pressures, such as predation, light intensity, or infection-parasitism, that may act very differently on naked versus calcified cells have not yet been rigorously tested. Even the generally accepted *trash-can* hypothesis, which claims that the transformation of HCO_3 to CO_2 during calcification (Figure 1) acts as a carbon concentration mechanism (CCM) providing carbon dioxide to photosynthesis, has been seriously challenged in *E. huxleyi* (Herfort *et al*. 2004; Rost and Riebesell 2004), Scarlett Trimborn, personal communication). The fact that most planktonic and benthic foraminifera are nonphotosynthetic (i.e., have no symbionts) further confirms that calcification and photosynthesis can be fully uncoupled processes. We are left with a simple fact: It is obviously less nutritional and harder for zooplankton to eat armored protists rather than naked cells, and this "grazing" hypothesis has the advantage of applying to all sorts of skeletonized microplankton that radiated in the early Mesozoic (Hamm and Smetacek, this volume). Note, however, that the kilometer-thick carbonate ooze at the bottom of the ocean is to some extent the product of the export of coccoliths via copepod fecal pellets, which argues in fact for intense grazing!

Whereas most of the hypothetical functions of coccoliths are testable using living species, it is much harder to know which ones prevailed in the Late Triassic and were primarily selected for at the onset of coccolithogenesis. First, all potentially involved selection pressures, chemical or biological, are *inferred* from paleoproxies and have thus a significant degree of inaccuracy. But mostly, the modern coccolithophores are the products of more than 200 My of evolution, which is equivalent to ~75 billion generations of daily-dividing phytoplankton! Their genomes have had a tremendous amount of time to randomly drift in multiple isolated lineages and evolve under strong

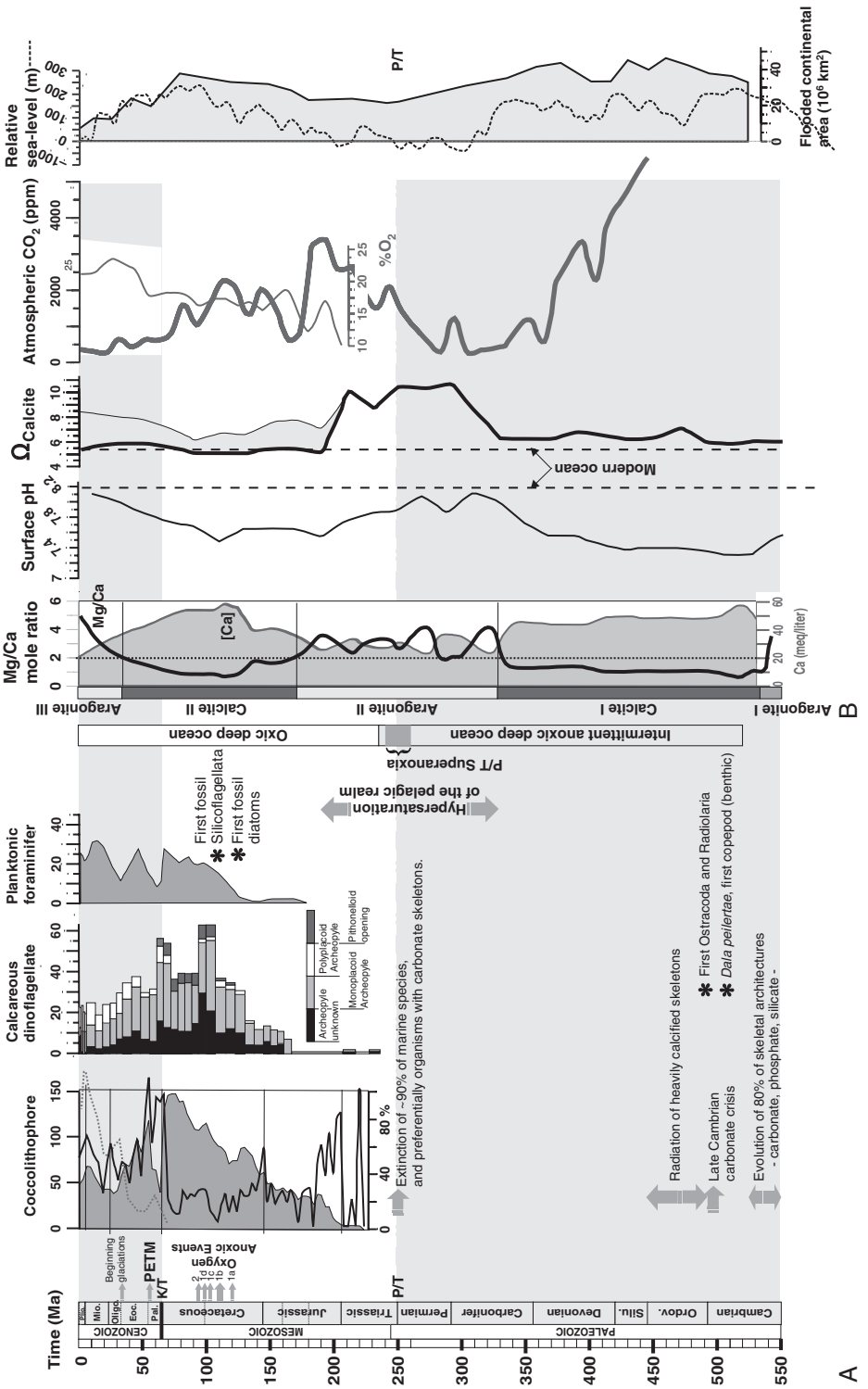

FIGURE 5. For legend see opposite page.

and changing climatic and biotic pressures. The coccolithophores have survived one major extinction event, underwent four important biological turnovers, and have adapted to different oceans since their origin. The relative importance of the forces that originally selected coccolithogenesis in the founding population of naked haptophytes must have changed over time and among their evolving diversity. In the following sections we explore how the morphological diversity of coccolithophores has changed over time and examine which forces may have shaped the different trajectories and ecological strategies that the main lineages have adopted.

VII. MACROEVOLUTION OVER THE LAST 220 MILLION YEARS

A. Forces Shaping the Evolution of Coccolithophores and Coccolithogenesis

The forces driving the ecological and evolutionary success of coccolithophores are multiple, act on different time scales, and affect various taxonomic ranges, from a single population to the entire group. We can classify them into three categories: *extrinsic biotic, intrinsic biotic, and extrinsic abiotic*. Initially, the species endure constant negative selection pressures due to predation, parasitism, viral infection, and competition with other photoautotrophs for nutrients. The biotic arms race is particularly acute in the pelagic realm (Hamm and Smetacek, this volume), where organisms are in perpetual [AU15] motion and have generally very short generation times. In fact, these extrinsic biotic forces largely control population dynamics on ecological time scales and certainly drive a significant part of genomes evolution through natural selection. On longer time scales, new genetic—and in particular *morphogenetic*—inventions can drive novel adaptations, adaptive speciations, and even radiations of a particular taxon into a new ecological niche (De Vargas and Probert 2004a). Finally, many abiotic forces, such as the bioavailability of essential elements or the physical stability of ecological niches, may shape the evolution of the group over even larger taxonomic and time scales. These abiotic factors are typically controlled primarily by the evolution of the Earth system (oceanic currents, tectonics, climate changes) and may ultimately control the fate of planktonic life, particularly sensitive to physico-chemical changes of their environment.

In coccolithophores, the main difficulty is to untie the multiple forces shaping the evolution of the group as a whole, including its entire biological and functional complexity, from those acting on the function

FIGURE 5. Abiotic global forcing on the oceanic carbonate system and the evolution of morphological species richness in pelagic microcalcifiers.
Concerning the carbonate system, the Phanerozoic can be divided into two periods of equal length: a first during the Paleozoic, when the biotic part of the system was essentially coastal benthic and the abiotic components were relatively unstable; a second in the Meso-Cenozoic, when the new planktonic calcifiers originated, diversified, shifted a significant part of carbonate precipitation into the pelagic realm, and at the same time stabilized the concentration of atmospheric CO_2. On this chart, along a geological time scale highlighting a few major paleoceanographic events, several essential biotic **(A)** and abiotic **(B)** components of the carbonate system are represented with, from left to right: coccolithophore morphospecies diversity and their rate of turnover (= rate of extinction + rate of "speciation") (Bown *et al*. 2004, 2005); morpho-species diversity of calcareous dinoflagellates (Streng [AU64] *et al*. 2004); morpho-species diversity in planktonic foraminifers (Tappan and Loeblich 1988); secular variations in absolute concentration of Ca^{2+} and Mg:Ca ratio of seawater (Stanley and Hardie 1999); mean surface saturation (?) with respect to calcite according to Ridgwell (2005); atmospheric CO_2 concentration (Royer *et al*. 2004) and [AU65] atmospheric O_2 concentration (Falkowski *et al*. 2005); and relative sea level (Haq *et al*. 1987) and flooded continental [AU66] areas (Ronov 1994). In addition, a few major ecological events in Paleozoic carbonate biota as described in Knoll [AU67] (2003) are indicated in **(A).** P/T, Permian/Triassic; K/T, Cretaceous/Tertiary; PETM, Paleocene-Eocene Thermal Maximum.

calcification. In oceanography and palaeontology, the coccolithophores are often considered as a single *functional group* and their success is scaled on their ability to produce thick, abundant, and diversified calcareous liths. The coccolithophores actually encompass wide morphological, functional, and ecological diversities and have adopted various strategies at different epochs, most probably in reaction to different forcings.

B. Broad Patterns of Morphological Diversity

Bown *et al*. (2004) recently reviewed current understanding of calcareous nannoplankton evolution through their ~220-Ma history, using a synthesis of *morphospecies* diversity data over time and various inferred rates of evolutionary change. They show that rates of speciation, extinction, and turnover were markedly more variable in the Cenozoic than in the Mesozoic and promote the idea that the main force driving increases in coccolithophore diversity (in fact, strictly speaking, coccolith morphological diversity) over geological time is the *long-term stability* of *oligotrophic* to *mesotrophic* water masses (see also Aubry 1992, 1998). The presence of such environments in the greenhouse world during the Mesozoic and Paleogene promoted long-term diversification of the K-strategist phytoplankton, and in particular the coccolithophores. The shift into an icehouse world in the Oligocene, with the establishment of new, cold and vertically mixed water masses at high latitudes, led to long-term diversity decline and higher rates of both speciation and extinction of morphospecies. Furthermore, these latter environmental conditions, where nutrient delivery is pulsed, became largely advantageous for the diatoms, which may have excluded many coccolithophore species through competition (Bown 2005).

As discussed previously, it is currently not possible to define the species-level based solely on coccolith morphology, and so Bown's diversity curve (Figure 5) should be interpreted with caution. In fact, its general pattern roughly fits previously published curves of morphological diversity at the genus level, and thus it mostly depicts trends of *morphostructural invention* (and the subsequent evolutionary success of these morphostructures) within the 26 coccolithophore families that have built *dissolution-resistant coccoliths* over the last ~220 Ma. Because most of the diversity within the Calcihaptophycidae (species with null or poor preservation potential, noncalcifying species) has been erased from the sediment record, the "real" coccolithophore biodiversity over geological time may actually be radically different. For example, the calcifying species within the families *Pleurochrysidaceae* and *Hymenomonadaceae* are significant components of the modern coastal oceans, and molecular clocks suggest that they have been present for most of the Mesozoic, surviving the K/T mass extinction (Figure 3). Despite this long evolutionary history, no traces of their delicate coccoliths are observed in the fossil record. Generally speaking, the coastal or neritic coccolithophores are not the best calcifiers. They often produce small and poorly calcified coccoliths and their haploid stages are frequently noncalcifying (e.g., the *Pleurochrysidaceae* and *Hymenomonadaceae,* Figure 3). Thus, the Bown's curve of fossil coccolith diversity may be largely skewed toward *oceanic heavy calcifiers* and therefore *intrinsically* reflects long-term stability of oligotrophic water masses. Evolution of the coastal biodiversity may even be driven by forces opposite to those acting on oceanic diversity. Should this be the case, the counts of total Calcihaptophycidae diversity over geological time would tend to be flattened.

C. Oligotrophy and Water Chemistry

Whatever the past diversity of poorly calcified or naked coccolithophores was, a few overarching abiotic forcings are likely to have influenced the *intensity of coccolithogenesis,* reflected in the broad diversity

pattern of coccoliths resistant to dissolution (Bown's curve in Figure 5). Note that these global forcings likely acted on all pelagic microcalcifiers, independent of their taxonomic or trophic status. Indeed, the morphodiversity curves of *calcareous dinoflagellates* and *planktonic foraminifera* show trends of fluctuation through time very similar to the coccolithophore curve (Figure 5).

In coccolithophores in general, there does appear to be a broad connection between *biomineralization* and *pelagic oligotrophy*. The first calcified haptophytes appeared in eutrophic shelf and epeiric environments, where they were confined throughout most of the Jurassic. However, their size, diversity, and impact on carbon cycling was relatively limited until they invaded the *oligotrophic open oceans* in the late Jurassic, where they steadily diversified into an astonishing morphological diversity of larger forms up to the end of the Cretaceous, producing massive amounts of deep-sea chalk. Still today, the large majority of coccolithophore biodiversity displaying strong calcification features is found in oligotrophic stratified water masses, while the shelf or coastal species have a general tendency to produce smaller and more fragile coccoliths or sometimes not to calcify at all. Patterns in haptophyte life cycles may provide additional clues. The rDNA trees indicate that haploid and diploid phases have undergone increased differentiation through the evolutionary history of the haptophytes (Figure 3). Being essentially isomorphic in the ancestral Pavlovales (if haplo-diploidy indeed exists in this lineage), the haploid phases began their morphological (and most probably ecological) differentiation in the Prymnesiales and Phaeocystales, until they reinvented coccolithogenesis under a different mode in the Calcihaptophycidae. However, early branching and predominantly coastal to neritic coccolithophores (Isochrysidaceae, Noelaerhabdaceae, Hymenomonadaceae, Pleurochrysidaceae) are either noncalcifying or do not calcify in their haploid stage (Figure 4). In contrast, all open ocean calcihaptophytes seem to secrete *holococcoliths* or *nannoliths* during this stage (Figures 3 and 4). It seems, thus, that calcification of both diploid and haploid stages has been a *key adaptation for the successful invasion of the oligotrophic pelagic realm by the calcihaptophytes*, and that the success of coccolithophore *biomineralization* (i.e., high diversity of large and robust coccoliths) is indeed roughly positively correlated to the extent and stability of such environmental conditions (Figure 5).

Secular changes in seawater chemistry may also broadly constrain coccolithogenesis. In particular, a seminal study by Sandberg (1983) showed that changes in oceanic conditions promoted abiotic precipitation of alternatively calcite and aragonite over periods of hundreds of millions of years (see *Aragonite* and *Calcite* oceans in Figure 5). Stanley and Hardie (1999) built upon these data and proposed that hydrothermal ridge activity related to rates of seafloor spreading controls the relative seawater concentration of Mg^{2+} and Ca^{2+}. At times of low spreading rates, the release of Ca^{2+} to seawater and the capture of Mg^{2+} that occurs during the transformation of rocks at active ridges are significantly reduced, so that the Mg:Ca ratio of open ocean waters increases and favors the precipitation of high-Mg calcite and *aragonite* (Stanley and Hardie 1999). Conversely, the Mg:Ca ratio decreases when seafloor spreading rates are high, which would promote the precipitation of low-Mg calcite, such as that found in coccoliths. The transition between *calcite* and *aragonite* oceans (Figure 5) appears to have been a strong, overarching evolutionary pressure on organisms building carbonate skeletons (especially those with limited physiological control on biomineralization), as shown by the extinction of calcitic lineages and their replacement by aragonitic taxa in different groups such as corals, sponges, and green and red algae.

The status of the Calcihaptophycidae within this theoretical framework is still ambiguous. Their relatively complex

mechanism of *intracellular* crystal nucleation and growth suggests that these protists exert a significant control on biomineralization, which would thus *escape,* at least to some degree, environmental influence. In particular, coccolithophores should have a strong capacity to buffer the fluids from which they precipitate their calcite skeletons. However, the curve of coccolithophore morpho-species diversity (which roughly represents the ecological and evolutionary success of heavy, dissolution-resistant coccoliths) broadly *correlates* (inversely) with the seawater Mg:Ca ratio, with maxima and minima respectively occurring in the late Cretaceous (Figure 5). The increasingly calcitic oceans in the Cretaceous would have thus selected for the diversification of heavy calcifiers, leading to the formation of massive chalk deposits known from this time. In fact, recent experiments mimicking the ionic composition of Cretaceous seawater seem to confirm that lower Mg:Ca ratio (and high Ca^{2+}) sustains significantly higher rates of calcification and growth in three species within the Calcihaptophycidae (Stanley *et al*. 2005). The authors suggest that increased calcification in low Mg:Ca waters promotes, via the production of CO_2, photosynthesis and thus growth rate. However, several studies have demonstrated that calcification in fact does not promote photosynthesis in *E. huxleyi* (e.g., Herfort *et al*. 2004), and further data using a wider range of taxa are clearly needed. *Emiliania* could, once again, prove to be the exception. It should be noted that, contrary to the generally accepted view that coccoliths are low-Mg calcitic structures, Stanley and collaborators reported that the two coastal species used in their experiments (*Pleurochrysis* and *Ochrosphaera*) secreted liths of high-Mg calcite under modern, high Mg:Ca conditions. This would mean that at least these coastal taxa, which secrete abundant, tiny and relatively simple coccoliths, do not strictly control cation incorporation during intracellular biomineralization. However, our analyses of calcite from a variety of coccolithophore species cultured in present day seawater (Probert, Stoll, and Young unpublished results) have to date never revealed a Mg content as high as those reported by Stanley *et al*. (2005).

D. Changes in Morphostructural Strategies

In their ~220-Ma evolutionary history, the Calcihaptophycidae have produced a large array of morphologies and structures. Much of the history of coccolithophore calcification is locked in the *heterococcoliths,* whose *morphostructures* (i.e., distinctive organization of individual crystals into *cycles*) make them both resistant to dissolution and remarkably useful for macroevolutionary studies. In heterococcolith evolution, the temporal distribution of morphostructures exhibits a pattern that correlates with the main geological events punctuating the history of life. Morphostructural losses and innovations occur at chronostratigraphic boundaries associated with mass extinctions (e.g., the K/T boundary, ~65 Ma) and major biological turnovers (e.g., the Paleocene/Eocene [P/E] boundary, ~55 Ma) (Aubry 1998). This correlation suggests that certain types of calcification/crystallographies may no longer be possible or beneficial under the new ecological circumstances created by the event, thereby causing the extinction or rarefaction of the lineages that produced them, unless survival without calcifying has occurred. It must be appreciated that the loss of numerous morphostructures at the K/T boundary (extinction of 62% of the families [Bown *et al*. 2004]) is evolutionarily more significant than the associated 93% reduction in species richness. However, because coccolith-species are mere morphotypes, a marked decrease in species richness per family is essentially a measure of the evolutionary success of a particular morphostructure.

Using a morphostructural approach at both the coccolith and coccosphere levels, the macroevolution of coccolithophores can

be divided into two modes, separated by the P/E boundary ~55 Ma. The first, the Mesozoic-Paleocene (*MP*) mode, is one of great morphologic diversification. Simply stated, it seems that *MP* coccolithophores expressed their full morphogenetic potential, with little apparent selection pressure on morphostructures. The *MP* mode was abruptly, yet only momentarily, interrupted by the K/T event, which eliminated all taxa that were common through the Late Cretaceous (Perch-Nielsen 1981). Taxa that were uncommon in the Cretaceous, but which can be traced back to an Early Jurassic ancestry, survived the K/T boundary and rapidly evolved into new Cenozoic morphostructures. The rate of Paleocene diversification that produced 11 families (or 60% of the Cenozoic families) in 10 My compares well with the Early Jurassic radiation of the group, when 56% of the Mesozoic families evolved in 17 Ma (Bown *et al*. 2004).

The second mode, Eocene-Oligocene-Neogene (*EON*), is fundamentally different. In the *EON*, coccolith and coccosphere morphologies seem to have been under significantly stronger selection pressure, as independent, polyphyletic lineages have been channeled into similar morphostructural trajectories, or *strategies* (Aubry in press-b). In other words, the paths of morphogenetic diversification became apparently restricted after the P/E event, and this restriction seems to have only grown stronger with time. Different morphological strategies characterize the *EON* mode, such as the increase in coccolith size during the Eocene, but these are still poorly studied. Only the youngest strategy is currently described (Aubry in press-b). It became established 2.8 Ma around the Middle/Late Pliocene boundary. At this time, most large coccoliths became extinct in various *unrelated* lineages (even within the successful family Noelaerhabdaceae), and a polyphyletic shift occurred from morphostructures with overlapping elements to morphostructures characterized by jointive or even disjunct elements. The extant coccolithophores are part of this new strategy. It must be noted that the change from the *MP* to the *EON* mode and the establishment of the youngest strategy did not occur abruptly and thus cannot be compared to massive extinctions or any other biotic changes related to catastrophic environmental change. It appears rather that global extrinsic abiotic or biotic pressures have been driving the selection of morphostructures over long time periods in coccolithophores.

Finally, clear taxonomic shifts have been occurring over time beneath these global morphogenetic trajectories. The evolutionary and/or ecological success of a particular morpho-taxonomic unit may reflect new genomic inventions providing a higher fitness to the group. As an example, the Coccolithales dominated early Paleogene communities until ~40 Ma, both in terms of diversity and amount of biomineralization. They remained an important component of the coccolithophores through the early Oligocene, but the Isochrysidales became increasingly diverse and abundant after the early Eocene, except at high latitudes where *Coccolithus pelagicus* continued to intermittently dominate (Beaufort and Aubry 1992). [AU16] This important taxonomic transition may be related, as we have seen previously, to a reinvention of biomineralization in the Isochysidales.

VIII. THE FUTURE OF COCCOLITHOPHORES

Over the last years, several studies have demonstrated the rapid impact of rising anthropogenic CO_2 on the carbonate system in the oceans. The surface ocean is acting as a sink for CO_2 (Sabine *et al*. 2004), where the water pH and concentration of CO_3^{2-} ions are predicted to drop by respectively 0.4 units and 50% by the end of this century. Carbonate is thermodynamically less stable under such conditions. In addition, Orr and collaborators have recently predicted, using a range of models of the ocean-carbon cycle,

an imminent and dramatic shoaling of the carbonate compensation depth (Figure 1) by several hundreds, if not thousands, of meters (Orr *et al*. 2005). Surface waters at high latitudes will become undersaturated with respect to aragonite within decades, and calcite undersaturation will only lag that of aragonite by 50 to 100 years, which may lead to massive extinction of pelagic calcifiers, including the Calcihaptophycidae. In 2000, Riebesell and collaborators tuned the pH (using HCl and NaOH) of monospecific cultures of *Emiliania* and *Gephyrocapsa* to preindustrial and future high CO_2 world values and reported reduced calcite production and coccolith malformations at increased CO_2 concentrations (Riebesell *et al*. 2000a). Furthermore, the seawater Mg:Ca ratio has shown a significant increase since the K/T, with the onset of a new, Neogene, Aragonite ocean (Figure 5). The modern Mg:Ca ratio of ~5 is higher than ever before in the Phanerozoic and may significantly increase the metabolic cost to the Calcihaptophycidae of producing calcite coccoliths.

Obviously, extant coccolithophores are facing a fast-changing ocean imposing strong pressures on their calcification. However, recent culture and mesocosm studies mimicking predicted pCO_2 conditions (e.g., Delille *et al*. 2005; Riebesell *et al*. 2000a, 2000b) have major limitations. In these studies, the carbonate chemistry was modified *abruptly* in *short-term* experiments involving a *single clone* of a *single species*. This is basically testing the physiological response (or acclimation potential) of an individual to abnormal change, ignoring the ongoing evolutionary adaptation of species and communities. In fact, natural populations of pelagic species are immense, occupying circum-global biogeographic ranges, and thus genetically highly polymorphic (Medlin *et al*. 1996). Pelagic genomes are dividing on daily time scales, thus adapting (i.e., slightly modifying their fitness and ecological range) at exceptionally high pace through the constant and rapid reset of the worldwide population. The intense genetic turnover characterizing pelagic biodiversity may be a key evolutionary strategy for survival in this unstable and climatically responsive environment, which is obviously difficult to test in laboratory conditions.

To sum up, we have shown in this chapter that there is not a singular coccolithophore (and certainly not *Emiliania*!) but several, widely divergent groups of potentially calcifying haptophytes, the Calcihaptophycidae. Our journey through their fossil record and molecular evolution has shown that their biomineralization was originally selected in a high CO_2, low pH, aragonite ocean (Figure 5), whose conditions may actually resemble the future Anthropogenic world after 2100. They radiated into an astounding morphological diversity of highly productive species in the Cretaceous Calcite II Ocean, which was relatively acidic under a high CO_2 atmosphere (Figure 5). And they were bigger than ever, producing thicker and large coccoliths across the Paleocene-Eocene Thermal Maximum (probably the best geologic analogue for future global change), when a massive increase in atmospheric CO_2 over a 10,000-year period caused rapid $CaCO_3$ dissolution at the seafloor and shoaling of the CCD by at least 2 km (Zachos *et al*. 2005). Thus, representing the ultimate haptophyte adaptation to the pelagic realm, the Calcihaptophycidae may in fact be strongly equipped against extinction, capable of multiplying in both haploid and diploid, calcifying or noncalcifying phases of their life cycle and having the potential to reinvent biomineralization at any time from coastal pools of noncalcifying taxa. Future palaeontogenomic approaches (De Vargas and Probert 2004b) will certainly help unveil the biological and functional complexity of the calcareous flowers of the oceans.

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