

What is new in coccolithophore biology?

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Summary

Knowledge of the biology of coccolithophores has progressed considerably in recent years thanks to culture studies and meticulous observations of coccospheres in wild samples. It has been confirmed that holococcolithophores and other "anomalous" coccolithophores are not autonomous but stages in the life cycle of oceanic heterococcolithophores. The existence of such heteromorphic life cycles linking former "species" has far reaching consequences on the taxonomy and nomenclature of coccolithophores and should foster research on the environmental factors triggering phase changes. The cytological characteristics of coccolithophores are reviewed in detail with special attention to the cell covering, coccolithogenesis and the specificity of appendages in this group. There have been comparatively few recent studies concerning the cytology of oceanic representatives. Important issues such as status of aplastidic groups, mode of synthesis of holococcoliths/nannoliths and details of the flagellar apparatus need to be addressed. Such morphological data will enable a more natural classification of modern coccolithophores in a phylogenetic perspective.

Introduction

Coccolithophores include all haptophyte algae possessing calcified scales (coccoliths) at some stage in their life cycle. Following the taxonomic revision of the division Haptophyta recently proposed by Edvardsen et al. (2000), coccolithophores belong to the class Prymnesiophyceae which also features non-calcifying organisms.

The biology of extant coccolithophores has been the subject of excellent previous reviews: Pienaar (1994), and relevant chapters in Green and Leadbeater (1994) where coccolithophores were often taken as examples of the haptophytes (see also Inouye 1997). A recent monograph focuses on the model coccolitho

phore *Emiliana huxleyi* (Paasche 2001). Of the approximately 300 haptophytes in modern oceans, about 200 are in fact coccolithophores and these contribute significantly to the biodiversity of the group (Jordan and Chamberlain 1997). However, as a consequence of recent multidisciplinary research projects such as CODENET (1998–2001), with better sampling/preservation methods and focus on culturing coccolithophores, the number of biological species recognized as authentic is currently in a state of flux. Indeed, one of the more challenging aspects concerning the biology of these organisms is the fact that holococcolithophores and other "anomalous" coccolithophores are not autonomous but stages in the life cycle of heterococcolith-covered oceanic species. In this presentation, current knowledge relative to the different types of heteromorphic life cycles present in coccolithophores and their implications for the taxonomy of the group as a whole will be emphasized. Whereas such heteromorphic life cycles linking two (or more!) former "species" consequently reduce the number of valid species, conversely recent studies demonstrate the existence of fine scale speciation in certain well established taxa (see Sáez et al. 2003; Geisen et al. this volume). The complexity of the existing taxonomy has not hampered the description of new species, whether heterococcolithophores (e.g. Kleijne et al. 2001) or holococcolithophores (e.g. Sym and Kawachi 2000) and a clade of prymnesiophytes of unknown morphology (based on clone library samples only) may represent a new group of coccolithophores (see Sáez et al. this volume). Phylogenetic reconstructions of the Haptophyta are also an area in progress; this should result in a better understanding of the systematics of coccolithophores as well as their status relative to the non-calcifying members of the division (Sáez et al. this volume). The major taxonomic groups within the Prymnesiophyceae featuring extant coccolithophores are listed in Table 1.

In contrast, since the earlier works mentioned in the reviews above, there have been very few studies devoted to the fine structure of coccolithophores. Such studies usually reflect the availability of cultured material and, notably, most recent works are concerned with the description of coastal species, and among these *Pleurochrysis* is still a notorious model organism. Blooms of *P. roscoffensis*, shown to be moderately toxic to brine shrimp, have recently been recorded in saline inland waters (Reifel et al. 2001), which goes to show that such coastal species are more important in the environment than generally thought. However, a number of oceanic species have now been brought into culture (see Probert and Houdan this volume) and this available resource will foster new investigations in coccolithophore research as a whole. For general haptophyte terminology relevant to extant coccolithophores, the reader is referred to the glossary of Jordan et al. (1995).

Table 1. Major taxonomic groups within the Haptophyta featuring heterococcolithophores and place of extant genera mentioned in the text. Based on Young and Bown (1997), Edwardsen et al. (2000) and Kleijne et al. (2001).

DIVISION: Haptophyta
CLASS: Prymnesiophyceae
Order: Isochrysidales

- Family Noelaerhabdaceae (*Emiliania*[°], *Gephyrocapsa*[°])
- Zygodiscales
 - Family Helicosphaeraceae (*Helicosphaera*^{*})
 - Family Pontosphaeraceae
- Syracosphaerales
 - Family Calciosoleniaceae
 - Family Syracosphaeraceae (*Coronosphaera*^{*}, *Syracosphaera*^{*})
 - Family Rhabdosphaeraceae (*Acanthoica*^{*}, *Algirosphaera*^{*})
 - Family incertae sedis (*Alisphaera*^{**}, *Canistrolithus*^{**})
- Coccolithales
 - Family Coccolithaceae (*Coccolithus*^{*}, *Cruciplacolithus*)
 - Family Calcidiscaceae (*Calcidiscus*^{*}, *Umbilicosphaera*)
 - Family Pleurochrysidaceae (*Pleurochrysis*[°])
 - Family Hymenomonadaceae (*Hymenomonas*[°], *Ochrosphaera*[°], *Jomolithus*)
 - Family Papposphaeraceae (*Pappomonas*^{*}, *Papposphaera*^{*}, *Wiggwamma*^{*})
 - Family Ceratolithaceae (*Ceratolithus*^{***})
- Order incertae sedis
 - Family Braarudosphaeraceae (*Braarudosphaera*)

[°] alternate stage non-calcifying

^{*} alternate stage with holococcoliths

^{**} alternate stage with aragonitic coccoliths

^{***} alternate stage with nannoliths

Cytological aspects

Coccolithophores generally occur as single cells and their typical features have been previously compiled in earlier reviews (Pienaar 1994; Inouye 1997) and are summarized in Fig. 1. Most of the available information is provided from investigations relative to heterococcolithophores since only two holococcolithophores have been sectioned for detailed ultrastructural studies (Klaveness 1973; Sym and Kawachi 2000). The relevant cytological characteristics of coccolithophores are described in the following sections, with emphasis on new findings.

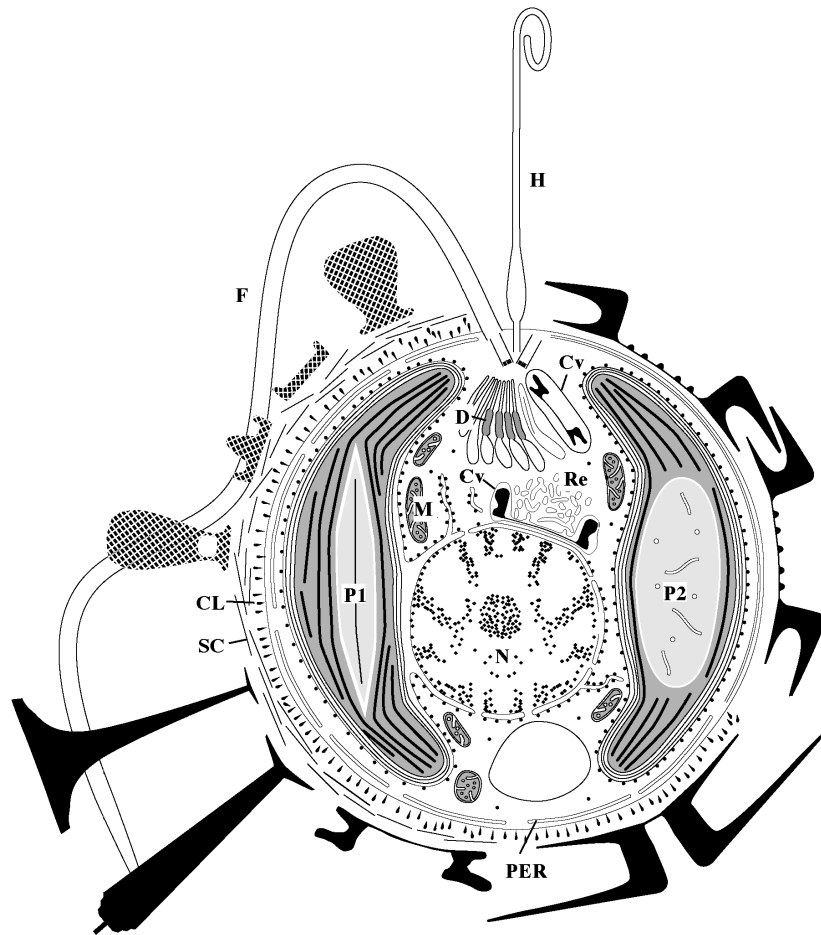


Fig. 1. Diagrammatic representation of cell structures of coccolithophores. Morphological features seen in various coccolithophores are combined in a single figure. Various types of coccoliths are drawn as silhouettes. Two types of coccolith-forming vesicles found in *Pleurochrysis* (top) and *Emiliana* (bottom) are illustrated. Pyrenoid (P1) is typical in the coccolithophores and pyrenoid (P2) is seen in *Emiliana* and *Gephyrocapsa*. Heterococcoliths are blotted black and holococcoliths are blotted by a lattice pattern. Abbreviations CL: columnar deposit, Cv: coccolith forming vesicle, D: peculiar dilation of Golgi body, F: flagellum, H: haptonema, M: mitochondrial profiles, N: nucleus, P1: pyrenoid traversed by thylakoids, P2: pyrenoid traversed by tubular structures, PER: peripheral endoplasmic reticulum, Re: reticular body, SC: unmineralized organic scales.

Cell covering

As in other members of the Prymnesiophyceae, the cell wall (periplast) in coccolithophores basically consists of various layers of organic scales held in place by fibrillar or columnar material with presumably adhesive properties (Fig. 1). The unit of cell covering in prymnesiophytes is considered to be a two-layered microfibrillar scale (Leadbeater 1994). Coccolithophores are distinctive in that the distal scales of the periplast are generally calcified, termed coccoliths, and visible with the light microscope. Cell coverings (including chemical composition) in prymnesiophytes or in coccolithophores have previously been extensively reviewed by Leadbeater (1994) and Pienaar (1994) respectively. Here we present aspects of cell coverings which are relevant to life cycles and systematics.

Organic body scales

Body scales in coccolithophores designate the layers of non-calcified scales located closest to the plasmalemma and produced by the Golgi apparatus. The majority of coccolithophores (including holococcolithophores) examined possess such scales and these represent the only type of cell wall in certain families where the haploid stage of the life cycle is non-calcifying: Noelaerhabdaceae (*Emiliana*; *Gephyrocapsa*, Probert unpublished results), Pleurochrysidaceae (*Pleurochrysis*), Hymenomonadaceae (*Hymenomonas* (Fresnel 1994); *Ochrosphaera* (Fresnel and Probert in press)). Notable exceptions are *Emiliana huxleyi* and *Umbilicosphaera foliosa* (= *U. sibogae* var. *foliosa*) where the heterococcolith-covered stages lack an underlayer of body scales (see references in Pienaar 1994).

Relative sizes, shape and ornamentations of organic body scales are variable and such aspects must be carefully observed in shadowcast whole mounts or thin sections, and viewed with the transmission electron microscope (TEM). Reduced body scales, whatever their shape or patterning, are sometimes observed at the flagellar pole of certain motile stages of coccolithophores and have been termed haptonematal scales. While they may be related to the presence of a haptonema (e.g. *Crystallolithus*-stage of *Coccolithus*), in some instances they have been reported despite the absence of an emergent haptonema. Such is the case in the Hymenomonadaceae where the haptonema is vestigial: haptonematal scales are remnant in members of the genera *Hymenomonas* and *Jomonolithus*, while they are altogether absent in *Ochrosphaera* (Fresnel and Probert in press). Remnant haptonematal scales could therefore indicate recent secondary loss of the haptonema.

Two main types of body scales may be distinguished differing in their general shape and ornamentations: (1) circular body scales, generally rimmed, with apparently identical ornamentations on both sides, i.e. patterned with concentric plus radial fibrils; in favorable cases however, when thick scales are present, the two-layered organization typical of prymnesiophyte scales is recognizable, the concentric pattern showing on the distal face, the radiating fibrils on the proximal face, as in *Coccolithus* (see recent observations by Houdan et al. in press) and also in *Cruciplacolithus* (see Fresnel 1986, although interpretation of the two sides of

the body scales was different); (2) elliptical, rimless body scales with distinctly different ornamentations on each face (distal pattern of concentric fibrils; proximal pattern of radiating fibrils arranged in four quadrants). Billard (1994) argued that this heteromorphism in body scales might be indicative of ploidy levels in the biphasic life cycle, type (1) scales being found in heterococcolithophores (presumably the diploid generation) and type (2) scales in holococcolithophores or non-calcifying stages (presumably alternate haploid generations).

The haploid, unmineralized motile stage of *Emiliana* features a third type of body scale (Klaveness 1972; Green et al. 1996) which is also present in motile stages of *Gephyrocapsa* (Probert unpublished results): type (3) scales are variable in shape, circular to elliptical, and monomorphic (with a simple pattern of radiating fibrils arranged in four segments which do not meet at a common point at the center of the scale). These thin scales, which are smaller than types (1) or (2), are considered single-layered (Green et al. 1996). Identical body scales are present in the non-mineralized genus *Isochrysis* (Billard and Gayral 1972; Green and Pienaar 1977), and are so far distinctive of the order Isochrysidales to which the Noelaerhabdaceae belong (Edvardsen et al. 2000).

Another type of body scale has been recently observed in the heterococcolithophore *Algirosphaera robusta*, a member of the Rhabdosphaeraceae: these minute scales (half the size of the scales of *Emiliana*) are strongly elliptical, rimmed, and bear a pattern of radiating fibrils arranged in four quadrants with a well defined elongated central ridge (Probert et al. in press). They may represent a novel type of apparently monomorphic body scales, which so far has not been reported in any other heterococcolithophore.

Coccoliths

Coccolithophores produce two main types of coccoliths which differ in their morphology: heterococcoliths, formed of crystal-units of variable sizes and shapes and holococcoliths, made of a single type of minute crystallites (see Young et al. this volume). Within these basic categories, various terms are used by workers on living coccolithophores and based on morphological characteristics of certain taxa: cricoliths, helicoliths, pappoliths, etc. for heterococcoliths; calyptroliths, crystalloliths, laminoliths, etc. for holococcoliths (see Young et al. 1997 for terminology). The abundance of such terms reflects the high diversity of coccolith morphology. The possible biological functions of coccoliths have been comprehensively reviewed by Bown and Young (1998).

Two families of extant coccolithophores produce so-called nannoliths (Ceratolithaceae, Braarudosphaeraceae) which are anomalous calcareous structures lacking the typical features of hetero- or holococcoliths. Whereas the Ceratolithaceae have heterococcolithophore stages in their life cycle, the affinities of the Braarudosphaeraceae are still uncertain, although living cells have golden brown plastids, and motile cells (with two equal flagella and no haptonema) have been reported once in *Braarudosphaera magnei* (Lefort 1972). *Polycrater galapagensis* produces unusual aragonitic coccoliths (often considered as nannoliths) and its assignment to the haptophytes was tentative until it was shown to possess a hap-

tonema (Thomsen and Buck 1998). Furthermore, various "species" of *Polycrater* have recently been shown by Cros et al. (2000a) to be in fact stages in the life cycle of heterococcolithophores. It is therefore becoming increasingly clear that coccolithophores produce different types of coccoliths/nannoliths during their life cycle, each generation being characterized by the presence of a distinct type of calcareous structure or by the absence of calcified structures altogether (see Table 1).

Coccoliths may be of similar size or morphology on a single cell, or there may be several distinct coccolith forms and sizes (e.g. circum-flagellar coccoliths). In most cases coccoliths (which may be interlocking or not) form a single layer external to the body scales, but species of the large genus *Syracosphaera* consistently exhibit dithecatism, i.e. two discrete layers of heterococcoliths of different types (e.g. Cros 2000). A few coccolithophores over-produce heterococcoliths of a single type, and the coccosphere may thus become multilayered (*Emiliana*, see Paasche 2001; *Cruciplacolithus*, Fresnel 1986). A specific process has recently been described in cultures of the coastal species *Ochrosphaera neapolitana*: coccolith formation does not continue once the coccosphere is complete and the heterococcoliths (tremaliths) progressively undergo extracellular over-calcification which is thought to occur by continued growth of existing coccolith crystals; ultimately individual coccoliths are no longer distinguishable and the resulting over-calcified cell (pseudo-cyst) is thought to represent a resistant stage (Fresnel and Probert in press).

The covering of holococcoliths may be enclosed within a continuous investment termed "skin" or envelope, as in the *Crystallolithus*-stage of *Coccolithus* or in *Calyptrosphaera sphaeroidea*. This envelope has a fibrillar microarchitecture in the former case (Rowson and Leadbeater 1986) and is interpreted by Sym and Kawachi (2000) as being composed of cohesively packed organic scales. This external envelope is lacking in *Calyptrosphaera radiata* (Sym and Kawachi 2000).

Coccoliths are typically based on an organic baseplate scale with microfibrillar components of prymnesiophyte scales. Size, thickness and patterning of the baseplate scale is highly variable among species; in *Syracosphaera pulchra*, the proximal coccoliths have baseplates whereas distal coccoliths do not (see Pienaar 1994). Mature coccoliths of *Emiliana* and *Gephyrocapsa* lack microfibrillar baseplates but a thin layer of polysaccharide material (analogous to an organic baseplate) is present in the coccolith making vesicle of *Emiliana* (see references in Paasche 2001).

Haptonema and flagellar apparatus

In several articles published in the 1990's, the haptonema and flagellar apparatus of the Haptophyta were reviewed in detail (Green and Hori 1994; Inouye and Kawachi 1994; Pienaar 1994). Since then, most studies have been conducted on non-coccolith-bearing members of the Prymnesiophyceae, especially the genus *Chrysochromulina* (Birkhead and Pienaar 1994a, 1995; Eikrem and Throndsen 1998; Eikrem and Moestrup 1998; Eikrem and Edvardsen 1999; Jensen and

Moestrup 1999), and very few investigations have been undertaken on the coccolithophores (Sym and Kawachi 2000). However, these works, in combination with previously published data, have demonstrated both great consistency in certain respects and variations of the haptonema and the flagellar apparatus within the Prymnesiophyceae and the coccolithophores.

Haptonema

The haptonema is a multi-functional organelle unique to the haptophytes (Fig. 1). It adheres to substrata, coils and uncoils. It is responsible for prey capture in some members of *Chrysochromulina* (Kawachi et al. 1991; Jones et al. 1993; Kawachi and Inouye 1995). It is typically comprised of six or seven microtubules surrounded in part or entirely by the haptonematal endoplasmic reticulum (ER) which is an extension of the peripheral endoplasmic reticulum (Fig. 2A). The haptonematal microtubules increase in number up to eight or nine toward the base, changing configuration from a ring in the free part, an arc around the insertion region, to two rows of four microtubules or a diamond pattern at the most proximal end. The transition region from the haptonema base to the emergent part is a complex structure comprised of a tongue-like extension toward the arc of microtubules and electron dense material in which microtubules are embedded (Fig. 2A). All these features seem to be common in the Prymnesiophyceae, although the number of microtubules and their configuration is different in reduced forms.

Although the emergent haptonema is known in various orders and families of coccolithophores, ultrastructural studies have been conducted for few genera. The number of microtubules in the emergent part is six in the holococcolith-bearing motile phase of *Coccolithus* (Manton and Leedale 1963) and *Calyptosphaera sphaeroidea* (Klaveness 1973). It is seven in the emergent part and eight at the base in *Syracosphaera pulchra* (Inouye and Pienaar 1988) while *Algirosphaera robusta* has the same number of microtubules in the base, but one less (six) in the emergent part of the haptonema (Probert et al. in press). *Helicosphaera carteri* (Helicosphaeraceae) also has a haptonema comprised of six microtubules in the free part and eight at the base (unpublished observation). The haptonema of these coccolithophores usually does not coil under normal conditions, but only when cells are exposed to stress such as desiccation or when cells are dead. Rapid coiling as seen in *Chrysochromulina* has never been reported in the coccolithophores. The utilization of the haptonema to adhere to substrata is also not so conspicuous in these coccolithophores. The emergent haptonema of coccolithophores, when present, seems to be morphologically similar to that of other members of the Prymnesiophyceae (e.g. *Chrysochromulina*), but less functional and appears to act mainly as an obstacle-sensing device. When cells sense obstacles with the tip of the haptonema, rapid backward swimming occurs as an avoidance response.

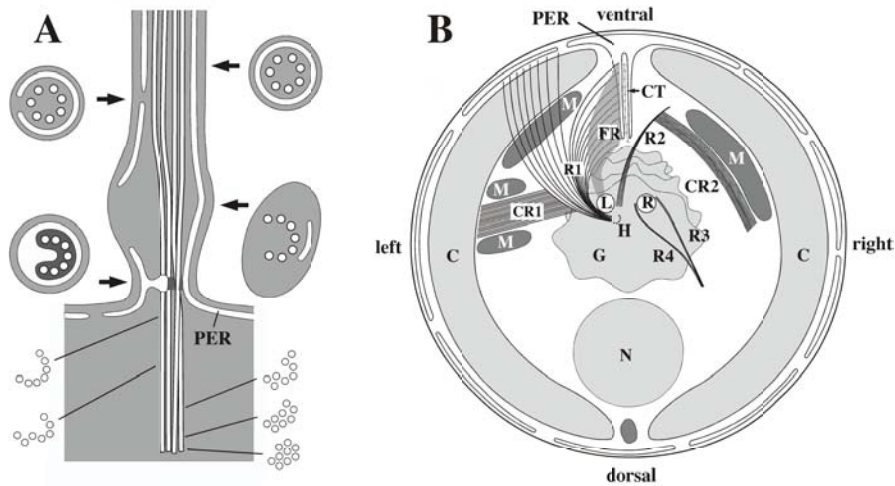


Fig. 2. **A.** Schematic representation of a haptonema showing configuration of microtubules and endoplasmic reticulum. **B.** Diagrammatic representation of a cell viewed from the apex, showing typical configuration of flagellar apparatus components and other organelles.

Abbreviations C: chloroplast, CR1: crystalline root arising from R1, CR2: crystalline root arising from R2, CT: cytoplasmic tongue, FR: fibrous root, G: Golgi body, H: haptonema, M: mitochondrial profiles, L: left basal body, N: nucleus, PER: peripheral endoplasmic reticulum, R: right basal body, R1: root 1, R2: root 2, R3: root 3, R4: root 4.

The haptonema is often vestigial in various groups of coccolithophores. A reduced bulbous haptonema is known in most species of *Pleurochrysis* (Pleurochrysidaceae) and *Hymenomonas roseola* (Hymenomonadaceae) (Manton and Peterfi 1969). In an unnamed species of *Pleurochrysis*, six microtubules are arranged more or less in an arc in the bulbous region and haptonematal endoplasmic reticulum is also arranged in an arc (Inouye and Pienaar 1985). In *H. roseola*, small vesicles occupy the bulbous region. The haptonema base of *Pleurochrysis* seems to be constant (two rows of four microtubules) (Gayral and Fresnel 1983; Inouye and Pienaar 1985; Fresnel and Billard 1991). In many taxa of the Hymenomonadaceae, the haptonema is reduced to the haptonematal base, and the number of microtubules are variable depending on taxa (three in *H. lacuna*, five in *H. coronata* arranged in a U-shape throughout its length, at least five in *H. globosa*, five in *Ochrosphaera neapolitana* and five in *Jomonlithus littoralis*) (Gayral and Fresnel-Morange 1971; Pienaar 1976; Gayral and Fresnel 1976; Inouye and Chihara 1988; Roberts and Mills 1992). The flagellate cells of *Emiliania* and *Gephyrocapsa* (Noelaerhabdaceae) lack a haptonema, but their non-coccolith bearing relative, *Isochrysis*, possesses a very short rudimentary haptonema that is comprised of five microtubules in the free part and at the base (Hori and Green 1991). *Cruciplacolithus neohelis* is the only taxon in the Coccolithaceae of which the haptonematal

base has been described in detail (Fresnel 1986; Kawachi and Inouye 1994). The flagellate cells of *C. neohelis* have five microtubules at the base. In the Calcidiaceae, a haptonematal base consisting of eight microtubules (two rows of four microtubules) has been described in non-motile cells of *Umbilicosphaera foliosa* (Inouye and Pienaar 1984). These tendencies to reduction and less effective functions of the haptonema suggest that the haptonema seems to be a less important organelle in the biology of certain coccolithophores if compared with other prymnesiophytes.

In contrast, the haptonema is remarkable by its length and seemingly coiling abilities in members of the aplastidic Papposphaeraceae (e.g. Thomsen et al. 1998; Thomsen and Buck 1998) and could possibly be involved in prey capture. None of these tiny coccolithophores have been grown in culture, however, and available observations are based exclusively on transmission electron micrographs, except for *Balaniger balticus* (the alternate stage of *Pappomonas virgulosa*, see Østergaard 1993) where the haptonema was seen to coil in living cells (Thomsen pers. com.).

Flagellar apparatus

The flagellar apparatus is a complex structure involved in various cellular functions, such as mitosis and cytoskeleton formation, and its morphological features are believed to be evolutionarily conservative. Analysis of flagellar structure is therefore informative in taxonomy and phylogenetic analysis. In the Haptophyta, the flagellar apparatus is unique and complex, due to the involvement of the haptonema. Two flagellar basal bodies and the haptonematal base are arranged in an absolute configuration. The haptonema is positioned close to the left basal body, and the concave side of the C- or U-shaped array of microtubules is always oriented to the left basal body (Fig. 2B). This basal body is designated as mature in terms of the generation of basal bodies and flagella, and it is seen as the longer flagellum under the light microscope (Beech et al. 1988). The right basal body is situated at a distance from the haptonematal base and corresponds to the shorter flagellum. The latter is the immature basal body (flagellum), and is destined to become the mature basal body in the next generation. This configuration seems to be universal in the Haptophyta. The two classes of the Haptophyta, Pavlovophyceae and Prymnesiophyceae, are however distinct in other aspects of the architecture of the flagellar apparatus (see Green and Hori 1994 for the Pavlovophyceae).

In the coccolithophores, the flagellar apparatus has been studied in detail mainly for members of the Pleurochrysidaceae and Hymenomonadaceae. The flagellar apparatus in these coccolithophores is the most complex among the haptophytes so far investigated, and has been adopted as a standard for comparison within the Prymnesiophyceae. Species in these families possess four microtubular roots, termed R1, R2, R3 and R4 (Fig. 2B). Of these, R1 and R2, both associated with the left basal body, are conspicuous because of the presence of closely packed bundles of microtubules, termed crystalline roots (Beech and Wetherbee 1988). R1 is a large sheet of microtubules, originating in the proximity of the haptonematal base. These microtubules run upward along the flagellar depression,

then, some of these pass over the mitochondrial profile and chloroplast situated at the left side of the cell. Other microtubules extend toward the ventral side of the cell, where they form a complex with the fibrous root originated from the left basal body, and extend into a thin space of cytoplasm delineated by extensions of the peripheral endoplasmic reticulum. This structure is collectively called the cytoplasmic tongue (Beech and Wetherbee 1988). Its functions are not well understood, but it has been suggested that the cytoplasmic tongue is responsible for contraction of the cell (this structure was termed a 'contractile root' by Fresnel and Billard 1991) or related to scale formation due to the proximity to the forming side of the Golgi body (Gayral and Fresnel 1983; Beech and Wetherbee 1988). The cytoplasmic tongue is conspicuous in many flagellate coccolithophores, but it is also found in *Chrysochromulina* sp. (Birkhead and Pienaar 1995). R2 originates from between the basal bodies near the left basal body and beneath the distal connecting fiber, and is comprised of a small number of microtubules (up to seven). Another crystalline root (CR2) is associated with R2 and extends toward the right side of the cell. These two crystalline roots are well developed in most coccolithophores so far investigated, however, some coccolithophores have only one crystalline root or completely lack both. In *Hymenomonas coronata* (Roberts and Mills 1992) and *Calyptosphaera radiata* (Sym and Kawachi 2000), CR1 is absent though CR2 is well developed in both algae. In contrast, *Cruciplacolithus neohelis* possesses a CR1, though it is vestigial and comprised of only about five microtubules, and CR2 is missing. In this species, well developed CR1 and CR2 appear in preprophase, suggesting their conversion to a mitotic spindle (Kawachi and Inouye 1994). The crystalline roots are completely absent in *Syracosphaera pulchra* (Inouye and Pienaar 1988) and *Algirosphaera robusta* (Probert et al. in press). The crystalline roots used to be thought to be unique to the coccolithophores (e.g. Kawachi and Inouye 1994). However, structures comparable to the crystalline roots have been found in several non-coccolithophore prymnesiophytes. *Isochrysis galbana* (a non-coccolith-bearing member of the Isochrysidales) has a very complicated R1 root, i.e. it has CR1 (as r1c) and an additional bundle of microtubules arising from CR1 (as r1b) (Hori and Green 1991). Since *Isochrysis* is believed to be a taxon which has secondarily lost the ability of coccolith formation (it is a close relation of *Emiliana* and *Gephyrocapsa*), it is not surprising that *I. galbana* possesses crystalline roots. However, its complex R1 root, together with other features such as the involvement of a reticular body in coccolith formation, suggests that this group has undergone a unique evolution within the coccolithophore lineage. CR2 is missing in *I. galbana*. The crystalline root is also present in some species of *Prymnesium* (Birkhead and Pienaar 1994b), and *Chrysochromulina* (Birkhead and Pienaar 1995; Edvardsen et al. 1996), which may be phylogenetically distant from the coccolithophores. In the latter, R3 and R4 are simple roots compared with R1 and R2. Root R3 originates from the right side of the right basal body and R4 originates from the left side of the right basal body (Fig. 2B). These two roots merge at a distance and this combined root extends towards the dorsal side of the cell. The function of this combined root is not understood but it is suggested in *Chrysochromulina* that it is involved in determining the site on the

cell membrane where scale release takes place (Jensen and Moestrup 1999), which could also be applicable to the coccolithophores.

Various fibrous bands, some of which appear to be consistently present in most taxa, connect the flagellar basal bodies and the haptonematal base. Most typically, the two basal bodies are interconnected by distal, intermediate and proximal striated connecting fibers. The haptonematal base is connected to the two basal bodies by haptonematal fibers. Variations are present in the presence or absence of the intermediate connecting fibers and the haptonematal fibers and their striated or non-striated nature.

Transition region and flagellar axoneme

Unique structures of the flagellar transitional region are known in *Pleurochrysis carterae* (Beech and Wetherbee 1988). Tiers (up to eight) of electron dense rings are situated at the level of the cell membrane and distal to this is a transitional plate called an axosome. In the proximal part of the flagellar axoneme, a helical band is situated between the doublets and central pair of microtubules. Similar structures seem to be widely distributed in the Pleurochrysidaceae (e.g. Henry et al. 1991) and Hymenomonadaceae (e.g. Manton and Peterfi 1969; Gayral and Fresnel 1983; Roberts and Mills 1992). The axosome also seems to be widely distributed in various coccolithophores, although not so elaborated as that of *P. carterae* (e.g. Sym and Kawachi 2000). In the flagellate cells of *Emiliania* and *Gephyrocapsa*, there is a dense band in the basal region of the axoneme (unpublished observation) that is similar to the fibrous plug known their non-coccolith bearing relative *Isochrysis galbana* (Hori and Green 1991). This structure is probably a characteristic of the Noelaerhabdaceae.

In general, most features of the haptonema and flagellar apparatus of the coccolithophores are primitive (plesiomorphic) characters, and consequently, it is difficult to illustrate uniqueness of the coccolithophores by these features.

Chloroplasts

Coccolithophores generally contain two golden brown chloroplasts with chlorophylls *a* + *c*. For further pigment composition, the reader is referred to Van Leeuwen et al. this volume. Presence of a single chloroplast has been documented only in some holococcolithophores (species of *Calyptrosphaera* available in culture) and confirmed by serial sectioning. In the Haptophyta, the existence of only one chloroplast is typical of the members of the Pavlovophyceae (Edwardsen et al. 2000) and a single plastid is also observed in certain non-calcifying prymnesiophytes such as *Isochrysis* and *Chrysothila* (Isochrysidales).

The chloroplast, which features thylakoids in stacks of three, typically lacks a girdle lamella and is surrounded by four membranes, two of which represent the chloroplast endoplasmic reticulum. The presence of these additional membranes is thought to reflect the secondary origin of haptophyte plastids in the endosymbiotic hypothesis of plastid evolution. The outer membrane of the nuclear envelope is

continuous with the chloroplast endoplasmic reticulum. Chloroplast DNA in coccolithophores, as in other haptophytes, is dispersed within the stroma. The plastid genome size of *Ochrosphaera neapolitana* has been recently investigated and it is significantly larger than in other chlorophyll *a* + *c*-containing algae (Sáez et al. 2001).

Each chloroplast always contains a pyrenoid which is immersed in the majority of the coccolithophores examined so far (Fig. 1), except for members of the families Pleurochrysidaceae and Hymenomonadaceae where it is bulging and located on the internal face of each plastid. In *Ochrosphaera* (Hymenomonadaceae) the bulging pyriform pyrenoids are stalked and particularly conspicuous (Fresnel and Probert in press). These bulging pyrenoids may represent a derived character in certain Coccolithales. Immersed pyrenoids of coccolithophores show some diversity at the ultrastructural level: they are usually traversed by a number of thylakoids, except for the Noelaerhabdaceae where the pyrenoid stroma contains tubular profiles (Fig. 1). The product of photosynthesis in coccolithophores is considered to be a β 1-3 glucan (chrysolaminarin) as in other Prymnesiophyceae.

As a rule, there is no stigma in members of the class Prymnesiophyceae, and coccolithophores are no exception. Despite the absence of an eyespot, motile stages of some coccolithophores examined in culture may be positively phototactic such as the *Crystallolithus*-stage of *Coccolithus* (Houdan pers. com.; see also Fresnel 1994; Fresnel and Probert in press).

Although the majority of coccolithophores are photosynthetic (all cultured species at least), polar representatives of the weakly calcified family Papposphaeraceae (including *Pappomonas*, *Papposphaera*, *Wigwamma* and their alternate stages with holococcoliths; Thomsen et al. 1991; Østergaard 1993) and an allied genus of holococcolithophores (*Eriolus*) are heterotrophic organisms featuring long, and apparently coiling haptonemata (Marchant and Thomsen 1994; Thomsen et al. 1995). The lack of chloroplasts (and hence absence of any red chlorophyll autofluorescence) was verified using epifluorescence microscopy (Thomsen et al. 1995). The same genera of Papposphaeraceae have now also been found in temperate areas (Thomsen and Buck 1998; Cros and Fortuño 2002) but it is not known whether these small coccolithophores are also aplastidic outside polar regions characterized by prolonged periods of darkness. As Thomsen and Buck (1998) pointed out, thin-sectioning of embedded material and the search for chloroplasts and/or food vacuoles with the TEM is necessary in the Papposphaeraceae to demonstrate mixotrophy and possibly phagotrophy.

Phagotrophy has frequently been documented in non-mineralized haptophytes with plastids: in *Chrysochromulina*, the long and coiling haptonema may play an active part in prey capture (Kawachi et al. 1991), while in *Prymnesium* ingestion is by means of pseudopodial development at the non-flagellar pole with no involvement of the short haptonema (Tillmann 1998). In coccolithophores, information on phagotrophy is lacking except for the holococcolith-covered *Crystallolithus*-stage of *Coccolithus* where ingestion of graphite particles was once recorded (Parke and Adams 1960). Holococcolithophores being alternate motile stages with presumably a less rigid coccolith covering, are in fact likely candidates for phagotrophy. Non-calcifying motile stages of other coccolithophore

families are also potential candidates, whether a haptonema is present or not (e.g. *Emiliana* motile cells; Paasche 2001). The question of active phagotrophy (or mixotrophy) should therefore be addressed in coccolithophores (and particularly in the Papposphaeraceae), in view of its potential ecological significance in the microbial food web.

Golgi apparatus and coccolithogenesis

The single, large and highly polarized dictyosome, with its peculiar dilated central cisternae, is a distinctive attribute of the Prymnesiophyceae (Fig. 1). In coccolithophores it is involved both in the synthesis of organic scales and coccolithogenesis. Sequential synthesis of the organic body scales is a process well documented (see earlier reviews by Leadbeater 1994; Pienaar 1994) and haploid non-calcifying cells of *Pleurochrysis* are still used as models to study transport mechanisms of secretory products across the Golgi body. A recent study (Hawkins and Lee 2001), using improved fixation and staining methods and quantitative morphology analyses, challenges the cisternal-progression model generally assumed for scale formation in this coccolithophore. Their results in *Pleurochrysis* sp. show that body scales form in the trans-Golgi network and that abstricted cisternal fragments of the basal trans-Golgi network develop into discoid scale-bearing prosecretory vesicles. These mature into secretory vesicles prior to exocytosis of the scales to the cell surface. Secretory vesicles may contain up to five scales following sequential fusions of prosecretory vesicles. They distinguish two morphological types of cisternal dilations which are centers of radial microfibril synthesis. Previous evidence in *P. sherffellii* had shown that radial fibrils are laid down before the spiral/concentric fibrils (see Leadbeater 1994). Hawkins and Lee (2001) also describe novel, bottlebrush-shaped macromolecules which are related to the biogenesis of scales and may also account for the "columnar" deposit outside the cell membrane in various coccolithophores. According to these authors, body scale formation in coccolith-covered stages of *Hymenomonas lacuna* (Pienaar 1994) follows the same vesicle shuttle progression model as in *Pleurochrysis*.

Coccolithogenesis has been investigated mostly in cultured heterococcolith-bearing species and it is now becoming apparent that mechanisms may vary according to the morphological type of coccolith produced. Basically, heterococcolith growth is intracellular and occurs inside Golgi vesicles or Golgi-derived compartments and the coccolith is extruded to the cell surface (generally close to the flagellar pole) when fully calcified. Diversity is observed in the manner in which components for the construction of the coccolith are transported to the coccolith vesicle and in the role of the endomembrane system in shaping the forming coccolith (Fresnel and Probert in press).

In *Pleurochrysis*, once synthesis of the organic baseplate is completed inside a Golgi cisterna, the scale is transferred to distal Golgi-derived vesicles where calcite nucleation and growth occur in the presence of densely stained granules termed coccolithosomes. Recent data on coccolith development and its biochemical aspects in *Pleurochrysis* will be found in Marsh (1999). Presence of discrete

coccolithosomes are typical of *Pleurochrysis* species and no particular cellular component is involved in shaping the small coccoliths (cricoliths). In *Ochrosphaera*, the vesicle containing the baseplate also migrates away from the Golgi, and subsequently dilates when close to the peripheral endoplasmic reticulum (PER). Invaginations of the peripheral endoplasmic reticulum form a tubular matrix, containing densely stained material inside the coccolith vesicle. In *Ochrosphaera* the peripheral endoplasmic reticulum and its tubular invaginations are thought to be involved in modeling the shape of the future coccolith (tremalith) which calcifies at a later stage (Fresnel and Probert in press). In *Emiliana*, the coccolith vesicle seems to result from fusion of smaller Golgi derived vesicles. The coccolith vesicle remains tightly apposed to a flattened section of the nucleus and formation of a thin baseplate, lacking a microfibrillar pattern, is the first stage of the developing coccolith. A reticular body (Fig. 1) of anastomosing tubes is added to the vesicle and calcification proceeds. When it is completed, the coccolith vesicle is detached from the nucleus (see Paasche 2001 for recently acquired information relevant to biochemical aspects of coccolith formation). In both *Emiliana* and *Gephyrocapsa* where the coccoliths are probably formed one by one, the role of the nucleus in shaping the early stages of the growing coccolith (placolith) is morphologically very apparent. *Umbilicosphaera foliosa*, which lacks organic body scales, also produces its large coccoliths (placoliths) one at a time and the endoplasmic reticulum system, together with the nucleus and the Golgi body seem to participate in shaping the coccolith vesicle (Inouye and Pienaar 1984).

Formation of holococcoliths is a process not yet fully understood, with only two case studies. In the *Crystallolithus*-stage of *Coccolithus*, the baseplate scales of the crystalloliths are synthesized inside Golgi-associated cisternae, extruded into the periplast, and calcium carbonate deposition is thought to take place extracellularly (Rowson et al. 1986). The envelope or "skin" surrounding the external layers of holococcoliths is suggested to play a role in crystallogenesis, maintaining a favorable environment for precipitation of calcium ions on the distal side of the baseplate. The baseplate scales of *Calyptrosphaera radiata* are produced as in *Crystallolithus*, but Sym and Kawachi (2000) furnish some evidence that the calcite crystals form within scale-containing cisternae of the Golgi. As opposed to *Crystallolithus*, an outer envelope is lacking in *C. radiata*. However, since no mature calyptroliths are found in the dictyosome, crystal assemblage is thought to occur externally. Clearly further investigations are needed to elucidate the system of calcite assembly in holococcoliths.

Life cycles

Coccolithophores reproduce asexually by binary fission and, generally following mitotic division, the coccoliths are redistributed on the daughter cells. In cultures of *Ochrosphaera neapolitana*, the cell divides inside the coccosphere and one of the daughter cells escapes and forms a new covering of coccoliths while the other

conserves the initial coccosphere (Fresnel and Probert in press). When motile and non-motile generations alternate in the life cycle, each is capable of vegetative reproduction as confirmed by culture studies: whether in oceanic forms (e.g. non-motile *Emiliana* and its alternate flagellate scaly stage; non-motile *Coccolithus* and its alternate motile stage *Crystallolithus*) or in littoral forms (e.g. motile *Pleurochrysis* and its alternate benthic scaly stage) (see references in Billard 1994). Benthic non-calcifying stages may also produce flagellate cells, considered as swimmers since they perpetuate the same generation. This is the case in families of coastal coccolithophores (Pleurochrysidaceae, e.g. Fresnel and Billard 1991; Hymenomonadaceae, Fresnel 1994; Fresnel and Probert in press), where such swimmers may be considered as an ecological adaptation for dispersion of the species during the benthic phase.

Concerning sexual reproduction, Billard (1994) postulated that probably all coccolithophores (as most prymnesiophytes) had a heteromorphic life cycle with alternating haploid and diploid generations. Since this earlier synthesis, a wealth of new data has accumulated, confirming this hypothesis. Arguments for the existence of a haplo-diplontic life cycle in coccolithophores are based on the following type of evidence: (a) observation of "combination coccospheres" in field samples; (b) visualization of phase changes in culture; (c) electron microscopic examination of body scale types; (d) nuclear staining and relative chromosome counts; (e) flow cytometric analyses of relative ploidy levels; (f) observations of syngamy and meiosis. Conceptually, observation of syngamy and meiosis, such as described earlier for *Pleurochrysis* (Gayral and Fresnel 1983), provide the ultimate direct evidence of the existence of sexuality in coccolithophores but they are difficult to observe and furthermore restricted to species in culture. A recent study (Houdan et al. in press), using such material, illustrates these stages for the first time in *Coccolithus*, some 40 years after the historical paper by Parke and Adams (1960) reporting presence of two distinct phases in cultures of this coccolithophore. Using flow cytometric DNA analysis, they confirm that *Coccolithus* cells are diploid whereas the alternate holococcolithophorid stage is haploid; other species were analyzed in this study (see below) confirming the earlier hypothesis based on body scale morphologies, that heterococcolithophores are diploid whereas holococcolithophores are haploid. Furthermore, Houdan et al. (in press) report that the 18SrDNA sequence from a pure culture of *Crystallolithus braarudii* (the haploid phase of *Coccolithus* from temperate waters) is identical to a sequence of the same gene from the *Coccolithus* stage, providing genetic evidence that they belong to the same taxon (see Sáez et al. 2003 and Geisen et al. this volume, for new nomenclatural updates on this and other recently discovered pseudocryptic and cryptic species).

The fact that holococcolithophores are not autonomous species was suspected earlier by observations of combination cells (Kamptner 1941; Thomsen et al. 1991; Kleijne 1991). A growing number of illustrations of such cells, bearing both heterococcoliths and holococcoliths, and representing various species are now available (Cros et al. 2000b; Cortés and Bollmann 2002; Geisen et al. 2002; Saugestad and Heimdal 2002). Other combination cells featuring heterococcoliths and nannoliths (Alcober and Jordan 1997; Sprengel and Young 2000), or hetero-

coccoliths and aragonitic coccoliths (Cros et al. 2000a) have now been recently documented.

Heteromorphy is thus expressed in two different ways in each generation of a coccolithophore; (1) the morphology of the organism as a whole (e.g. motile vs. non-motile) and (2) the nature of the cell covering, and from evidence now available, the presence of heterococcoliths is in fact probably indicative of a diploid stage. Digenetic heteromorphic life cycles in coccolithophores show remarkable diversity with, so far, five different types, two of which were unsuspected in the earlier review by Billard (1994). Table 2 is a summary of the various types of life cycles known in heterococcolithophores.

The *Emiliana* life cycle

In *Emiliana*, non-motile heterococcolith-bearing cells (C-cells) alternate with motile scaly cells (S-cells); haplo-diploidy was confirmed by flow cytometric analyses (Green et al. 1996). As mentioned previously, the thin body scales of the S-cells differ from those of other prymnesiophytes. The cycle is the same in the closely related genus *Gephyrocapsa* (unpublished results) and is typical of the Noelaerhabdaceae. Nonmotile naked cells of *Emiliana* (N-cells) which are totally void of body scales are to be considered as mutant diploid stages having lost the ability to produce heterococcoliths (Paasche 2001).

A recent report (Laguna et al. 2001), describing peculiar S-cells, representing "a possible gametic stage" of *Emiliana*, must be considered with caution. These minute, bacteroid-like cells (see their Fig. 1) which grow on agar plates (where they can be maintained for over two years) are much smaller than the usual swimming S-cells of *Emiliana* (see Paasche 2001): although reported to be motile, the flagella were not illustrated, and the presence of scales was not confirmed.

Table 2. Summary of different life cycle types known in heterococcolithophores.

2 N (diploid) generation (HETEROCCOLITHS)	N (haploid) generation
Noelaerhabdaceae	non-calcifying motile stage
Coccolithaceae Calcidiscaceae Helicosphaeraceae Papposphaeraceae Rhabdosphaeraceae Syracosphaeraceae	HOLOCCOLITHS
Family incertae sedis (<i>Alisphaera/Canistrolithus</i>)	aragonitic coccoliths (<i>Polycrater</i>)
Ceratolithaceae	nannoliths (ceratoliths)
Pleurochrysidaceae Hymenomonadaceae	non-calcifying benthic stage

Heterococcolithophore-holococcolithophore life cycles

Well documented experimentally in members of the genera *Coccolithus*, *Calcidiscus* and *Coronosphaera* (Houdan et al. in press), heterococcolithophore-holococcolithophore life cycles may now be safely extended to all holococcolithophore "species" (previously classified in the Calyptosphaeraceae) which are to be considered as haploid stages of certain heterococcolithophores. According to Sym and Kawachi (2000), the presence of a single plastid in holococcolithophores could also be an indication of haploidy. Details of the transformation from the diploid to the haploid phase probably vary between coccolithophores, but observation of combination cells point to its existence in other genera as well: *Pappomonas*, *Papposphaera*, *Wigwamma* (Thomsen et al. 1991; Østergaard 1993; Thomsen and Buck 1998), *Syracosphaera* (Cros et al. 2000b; Geisen et al. 2002; Saugestad and Heimdal 2002), *Acanthoica* and *Helicosphaera* (Cros et al. 2000b). Although they have not been reported since, combination coccospheres featuring *Algirosphaera robusta* and holococcoliths of *Sphaerocalyptra quadridentata* were illustrated by Kamptner (1941).

A recent study (Noël et al. 2002) reports an alternation between *Calyptosphaera sphaeroidea* and a previously undescribed non-motile heterococcolithophore. In contrast to other studies, the life cycle was initiated from the holococcolithophore stage, the first organism isolated, and the change of phase to the coastal (?) heterococcolithophore stage was induced by alteration of environmental conditions in the culture; reversal to the motile *Calyptosphaera*-stage was obtained using "opposite" environmental conditions. Both stages in this life cycle may be flagellate (e.g. species of *Syracosphaera*, *Coronosphaera* or *Helicosphaera*) which suggests that the advantages of such alternations are probably ecologically diverse within genera (or species) and remain to be established. Very few holococcolithophores are predominantly non-motile (Sym and Kawachi 2000) and such rare cases may indicate that their alternate heterococcolithophore stage could be restricted to coastal areas.

Families of typically oceanic coccolithophores concerned with this probably widely distributed life cycle are so far as follows: Helicosphaeraceae, Syracosphaeraceae, Rhabdosphaeraceae, Coccolithaceae, Calcidiscaceae and Papposphaeraceae (see Table 1). Considering that over 70 holococcolithophores have been observed or formally described in the literature, a large number of "missing couples" remain to be found, linking holococcolithophores with established species of heterococcolithophores. However, a number of the latter can be associated with two different holococcolithophore "species". Geisen et al. (2002) investigated this phenomenon and show that while some cases reflect ecophenotypic variations in the haploid phase (e.g. in *Helicosphaera carteri*), others are significant, and discrete variations in holococcoliths indicate either fine scale speciation (in *Coccolithus* and *Calcidiscus*) or cryptic speciation (e.g. in *Syracosphaera pulchra*) (see also Sáez et al. 2003 and Geisen et al. this volume).

Life cycles involving heterococcolithophores and stages with aragonitic coccoliths

Based on the observation of combination cells, this life cycle is so far unique to the genera *Alisphaera* and *Canistrolithus*. The alternate stage of these oceanic heterococcolithophores are various types of *Polycrater* (Cros et al. 2000a), a unique genus featuring small, aragonitic coccoliths (Manton and Oates 1980). *Polycrater galapagensis*, the only "species" formally described, is motile (Thomsen and Buck 1998) and so are certain species of *Alisphaera*.

Recent removal of *Alisphaera* and *Canistrolithus* from the Syracosphaeraceae (Kleijne et al. 2001) is consistent with the distinctiveness of their heterococcoliths (compared to *Syracosphaera*) and the existence of an alternate stage producing aragonitic structures. Cros et al. (2000a) suggest that the latter may replace holococcoliths in this particular life cycle.

Life cycle involving heterococcolithophores and stages with nannoliths

Combination cells of *Ceratolithus cristatus* featuring a single, large, horseshoe-shaped ceratolith (nannolith) inside a coccosphere of delicate, hoop-shaped heterococcoliths are well documented (see references in Cros et al. 2000b). Another association involving the hoop-shaped coccoliths with heterococcoliths (planoliths) of *Neosphaera coccolithophorpha* was first observed by Alcober and Jordan (1997), and Sprengel and Young (2000) provide the final direct evidence that all three calcareous structures are linked in the complex life cycle of *C. cristatus*. According to Young et al. (1998), the simplest hypothesis is that the ceratoliths are equivalent to holococcoliths and represent a haploid stage; the *Neosphaera*-type planoliths are normal heterococcoliths produced on diploid stages, the hoop-shaped coccoliths being alternative morphotypes produced by the same coccolith formation mechanism as planoliths. Living cells of *C. cristatus*, a warm water species of the upper photic zone, have not been observed since the earlier report of Norris (1965) and cultures of this organism are needed to test the above hypothesis based on the observation of combination coccospheres. *Ceratolithus cristatus* is the correct name for this unusual coccolithophore (see Young et al. 1998), which belongs to the monotypic family Ceratolithaceae.

Life cycles involving heterococcolithophores and non-calcifying stages

Historically, this is the best documented life cycle (see references in Billard 1994), and it is typical of coccolithophores inhabiting near-shore marine waters. While the diploid generation is a heterococcolithophore, with relatively small coccoliths (cricoliths or tremaliths), the haploid scaly generation is generally benthic, either pseudofilamentous (so-called *Apistonema*-stage of *Pleurochrysis*), forming pack-

ets (*Hymenomonas*) or palmelloid, i.e. embedded in mucilage (*Ochrosphaera*). Haploid cells of *Pleurochrysis* sp. have recently been observed as symbionts in a benthic foraminifer host, and were subsequently isolated in culture (Hawkins and Lee 2001). This suggests that other coastal coccolithophores could be temporary symbionts within heterotrophic hosts.

Whereas haploid stages of *Pleurochrysis* display a single type of unmineralized, rimless body scale (type 2, see above), both *Hymenomonas* (Fresnel 1994) and *Ochrosphaera* (Fresnel and Probert in press) produce two types of scales: the proximal layer is identical patternwise to type 2 scales of *Pleurochrysis*, while the distal layer is distinctive: these distal scales are rimmed, elevation and localization of the rim being species specific. These distal scales could be homologous to holococcoliths having lost their ability to calcify.

This life cycle, which has been substantiated by observation of syngamy and meiosis (*Pleurochrysis*) or by chromosome counts of stained nuclei (*Hymenomonas*, *Ochrosphaera*), is typical of the Pleurochrysidaceae and the Hymenomonadaceae (Table 1). The freshwater coccolithophore *Hymenomonas roseola* probably has the same type of life cycle as its marine counterparts (Fresnel 1994). *Jomonolithus*, a monotypic genus with distinctive heterococcoliths shows affinities with both the Pleurochrysidaceae and the Hymenomonadaceae with respects to its internal cellular organization (Inouye and Chihara 1983), but because of the absence of coccolithosomes it is tentatively placed in the Hymenomonadaceae (Table 1); its body scales are of the diploid type, but no alternate stage has yet been reported.

Anomalies exist in this life cycle, with one or the other generation potentially missing, and this is documented in both the above families (see Billard 1994). In any case, when a coccolithophore produces "naked" cells in culture, i.e. without apparent coccoliths, the cell covering should be checked with the TEM: if such cells produce type (1) body scales, then the cells have only lost the ability to calcify; if the cell covering consists of differently patterned scales, this may be indicative of a heteromorphic life cycle (Fresnel 1994).

To conclude, it is probably safe now to assume that the life cycle of all coccolithophores involves a haplo-diploid alternation of generations, each characterized by its cell covering, and each capable of asexual reproduction and of ensuring dispersal (Table 2). The advantages of the haplo-diploid life cycle, which are likely to broaden the ecological range of the species, are further discussed in Houdan et al. (in press). This contrasts with the situation in diatoms and dinoflagellates, other major groups in marine phytoplankton, where the life cycle is monogenetic (diploid and haploid, respectively). A distinctive feature in the biology of coccolithophores is that resting stages, i.e. hypnospores or cysts (whether sexual or vegetative) are seemingly absent. The pseudo-cysts described in the coastal genus *Ochrosphaera* could be an exception but they have not been observed in natural samples and may be opportunistic structures (as their mode of formation suggests), related to a favorable microenvironment in culture. It has also been suggested that the coccospheres of *Braarudosphaera bigelowii*, because they lack test perforations, are resting stages or cysts, but this remains to be confirmed.

In marine macroalgae, where digenetic life cycles are common, each generation with different ecophysiological traits may occupy a different ecological/seasonal niche. More experiments on coccolithophores in culture such as those initiated by Noël et al. (2002) are needed to determine life cycle strategies in this group and furthermore to determine which environmental factors trigger the change of phases in individual species. Another area of research would be to assess genetic expression in diploid and haploid phases in the life cycle of a coccolithophore, as has recently been done with both generations of the brown alga *Laminaria digitata* (Crépineau et al. 2000). The possibility that such changes are partly controlled by endogenous regulation (biological clock) cannot, however, be ruled out (see discussion in Houdan et al. in press).

Taxonomic concepts

Progress in the phylogeny of coccolithophores

Phylogenetic reconstructions of the Haptophyta inferred from plastid (*rbcL*) (Fujiwara et al. 2001) or nuclear encoded gene sequences (18S ribosomal DNA) (Edwardsen et al. 2000; Sáez et al. this volume), along with supporting morphological data are beginning to provide an objective framework within which to comment on the systematics of extant coccolithophores.

In the monophyletic Prymnesiophyceae, coccolithophores constitute a clade including non-calcifying genera such as *Isochrysis*. Re-instatement of the Isochrysidales (Edwardsen et al. 2000) is supported by significant ultrastructural and biochemical data. Further support is provided by body scale characteristics and by the specific life cycle demonstrated in the calcifying Noelaerhabdaceae. Species in the non-mineralized Isochrysidaceae are now considered as coccolithophores having secondarily lost the ability to produce coccoliths (Fujiwara et al. 2001).

Within the remaining coccolithophores, a number of clades are recognized which support certain current families (see Sáez et al. this volume.): Coccolithaceae, as a sister group to Hymenomonadaceae and Pleurochrysidaceae; Helicosphaeraceae. The Syracosphaeraceae, represented by *Coronosphaera mediterranea* (monothecate) and *Syracosphaera pulchra* (dithecate) may not be a natural group but more representatives need to be sequenced. *Algirosphaera robusta* (Rhabdosphaeraceae) is distinguished by genetic analyses and, as mentioned earlier, its apparently monomorphic body scales are quite distinctive. Nevertheless, the fine structure of *A. robusta* and its coccolith structure show affinities to *S. pulchra* (Probert et al. in press).

The case of *Reticulosphaera*, a genus (two species described) of phototrophic meroplasmoidal protists must be mentioned here. Although originally placed in the Heterokontophyta (Grell et al. 1990), it is considered by some authors as an aberrant haptophyte (Cavalier-Smith et al. 1996), following analysis of the 18SrRNA gene sequence of *R. japonensis*. The latter appears as a sister taxon to *Pleurochrysis* in the tree of Edwardsen et al. (2000). *Reticulosphaera* spe-

cies differ radically from haptophytes in external body form, and the ultrastructural features of the type, *R. socialis*, the only species examined (Grell et al. 1990), are not prymnesiophycean. Pending further studies, it seems unreasonable to include the amoeboid Reticulosphaeraceae in the Coccolithales.

The major taxonomic groups featuring extant heterococcolithophores (Table 1) have so far been based mostly on coccolith architecture. Some currently recognized orders (i.e. Isochrysidales) or families (see above) are supported by genetic data, whereas uncertainties remain for others. A more natural classification system, namely at the ordinal level, must await further analyses of nucleotide sequences of appropriate genes.

Nomenclatural problems

Problems arising from the existence of heteromorphic life cycles in coccolithophores should be addressed. When the alternate phase is non-calcifying, naming these somewhat inconspicuous haploid generations has rarely been an issue except for *Pleurochrysis* where the distinctive pseudofilamentous stages of certain species was sometimes designated as the *Apistonema*-stage; conversely, the genus *Cricosphaera* (a junior synonym of *Pleurochrysis*) was in use for species of *Pleurochrysis* where the benthic stage had not been observed (see Fresnel and Billard 1991). Such practice in the Pleurochrysidaceae has now been generally abandoned.

The situation is different when the alternate (haploid) phase is a distinctive coccolithophore, with coccoliths visible under the light microscope. In the past, both stages were named according to the Linnean system since they were considered as autonomous species. Such is the case with most holococcolithophores (see Jordan and Green 1994) which are presented as distinct taxa in phytoplankton manuals (Heimdal 1993). So far, in all situations where combinations have been reported, the name of the heterococcolithophore (diploid phase) has had priority over the alternate (haploid phase). In agreement with Cros et al. (2000b) we urge that, as far as possible, nomenclature should be based on the heterococcolith phase for the generic name, while the first described epithet should be applied (whether it belongs or not to the heterococcolith phase). As suggested by Young et al. (1998), the original name of the alternate phase could continue to be used in an informal non-Linnean sense. It must be noted that in marine macroalgae with heteromorphic life cycles, there is no standing rule. In the brown algae, for instance, the name of the species is either applied to the sporophytic (diploid) generation (e.g. *Laminaria digitata*) or to the gametophytic (haploid) generation (e.g. *Cutleria multifida*). Similar cases may be found in the red or the green algae. The "trend" in macroalgae is that the binomial with priority corresponds to the dominant, more conspicuous generation, which was formally described first, according to the rules of the ICBN. Nevertheless, for clarity's sake in the complex taxonomy of coccolithophores, it would be wise to apply the informal rule mentioned above.

When an undescribed holococcolithophore is discovered in combination with a previously known heterococcolithophore, a recent practice has been simply to

designate the alternate stage as (HO), e.g. *Acanthoica quattrosolina* (HO) for the holococcolithophore stage of this species (see Cros et al. 2000b). Furthermore, recent diagnoses of new species do not account for or mention the alternate stage and its coccoliths, even when the latter is recorded, e.g. in *Syracosphaera delicata*, a species recently described (Cros et al. 2000b). It would be nevertheless preferable in such diagnoses to furnish some information on the alternate stage of the heterococcolithophore (when available) to allow unambiguous communication, in nannofloral analyses, for instance. For similar purposes, it seems necessary, as a first step, to formally name novel (apparently independent) holococcolithophores (as done by Sym and Kawachi 2000), the ultimate goal being to eventually link them to existing (or new) heterococcolithophore species, via culture studies or observation of combination cells.

The possibility also exists that some life cycles in coccolithophores might be isomorphic, i.e. that both generations could be morphologically identical. Isomorphic life cycles (considered more primitive) are known in different macroalgal groups with alternations of generations, so this possibility cannot be ruled out in coccolithophores (or Haptophyta as a whole). In short, formal knowledge of both phases of a coccolithophore species is important since each phase may have different strategies and these have great implications for understanding the ecology of the group.

Conclusions

In recent years knowledge of the biology of coccolithophores has progressed thanks to culture studies and meticulous observations of coccospores in wild samples. Concerning the cytology of the cells, uncertainties remain, relative, for instance, to the heterotrophic, aplastidic Papposphaeraceae which need to be cultured and examined with the TEM, as well as groups producing nannoliths (e.g. Braarudosphaeraceae) whose status need to be confirmed. Studies of cell coverings in coccolithophores are highly informative, each generation of the life cycle being characterized by a distinctive type of scale or coccolith/nannolith. The mode of synthesis of holococcoliths still remains enigmatic, while diversity is becoming apparent in details of heterococcolith production. Studies of the flagellar apparatus have informative value but few coccolithophores have been thoroughly examined in this sense. Phylogenetic analyses are in progress which, in accordance with morphological or biochemical data, should allow in the future a more natural classification of the coccolithophores. Their haplo-diploid, typically heteromorphic life cycle, shows remarkable diversity and it can now safely be extended to a number of representative families, spanning the diversity of the group. Ecophysiological studies devoted to each generation of a single species and determination of the environmental factors triggering phase changes, should be a promising area of research in order to better understand the distribution and ecology of coastal or oceanic coccolithophores.

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