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On the ultrastructure of *Gephyrocapsa oceanica* (Haptophyta) life stages

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Résumé – *Gephyrocapsa oceanica* est une espèce cosmopolite de coccolithophores appartenant à la famille des Noëlaerhabdaceae dans l'ordre des Isochrysidales. Exclusivement pélagique, *G. oceanica* est fréquemment retrouvée dans les océans modernes ainsi que dans les sédiments fossiles. Aussi, elle est apparentée à *Emiliania huxleyi* chez qui un cycle haplodiplobiontique a été décrit, avec un stade diploïde ou les cellules sont immobiles et ornées de coccolithes, qui alterne avec un stade haploïde ou les cellules sont mobiles et ornées d'écailles organiques. Alors que la cytologie et l'ultrastructure des autres membres des Noëlaerhabdaceae n'ont jamais été étudié, ces caractères peuvent être uniques au genre *Emiliania* ou communs à la famille. Dans cette étude, nous présentons pour la première fois l'ultrastructure des stades non-calcifiants mobiles et calcifiants immobile de *G. oceanica.* Nous n'avons pas retrouvé de différences ultrastructurales significatives entre ces deux morpho-espèces apparentées au niveau des stades diploïdes et haploïdes. Les similarités entre ces deux morpho-espèces démontrent une importante conservation des caractères cytologiques. Par ailleurs, nous avons discuté de la vraisemblance de ces résultats en rapport au contexte évolutif des Noelaerhabdaceae.

Calcification / coccolithophores / *Gephyrocapsa oceanica* / cycle de vie / microscopie électronique à transmission / ultrastructure

Abstract – *Gephyrocapsa oceanica* is a cosmopolitan bloom-forming coccolithophore species belonging to the haptophyte order Isochrysidales and family Noëlaerhabdaceae. Exclusively pelagic, *G. oceanica* is commonly found in modern oceans and in fossil assemblages. Its sister species *Emiliania huxleyi* is known to possess a haplo-diplontic life cycle, the non-motile diploid coccolith-bearing cells alternating with haploid cells that are motile and covered by non-mineralized organic scales. Since the cytology and ultrastructure of other members of the Noëlaerhabdaceae has never been reported, it is not clear whether these features are common to the family. Here, we report on the ultrastructure of both the non-motile calcifying stage and the non-calcifying motile stage of *G. oceanica*. We found no significant ultrastructural differences between *E. huxleyi* and *G. oceanica* either in the calcifying diploid stage or the haploid phase. The similarities between these two morpho-species demonstrated a high degree of conservation of cytological features. We discuss the significance of these results in the light of the evolution of the Noelaerhabdaceae.

Calcification / coccolithophores / *Gephyrocapsa oceanica* / life cycle / transmission electron microscopy / ultrastructure

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INTRODUCTION

Coccolithophores (Prymnesiophyceae, Haptophyta) produce composite cell coverings of minute calcite platelets (coccoliths) and consequently have been key contributors to both the oceanic carbon pump and counter-pump, and thus to the flux of CO_2 between the atmosphere and oceans (Rost and Riebesell, 2004), since their origin in the Triassic. They can be grouped within the sub-class Calcihaptophycideae, a clade in which all prymnesiophytes that use organic plate scales as a substrate for calcification are found (de Vargas *et al.*, 2007). A haplo-diplontic life cycle appears to be a synapomorphic feature of the Calcihaptophycideae and possibly of the whole haptophyte phylum. Typically calcihaptophytes calcify in both phases producing different coccolith types, heterococcoliths and holococcoliths respectively in the diploid and haploid phase. In some families, however, calcification only occurs in the diploid phase (Noelaerhabdaceae, Pleurochrysidaceae, Hymenomonadaceae) or in neither phase (Isochrysidaceae) (Young *et al.*, 2005).

The most abundant and successful extant coccolithophore, *Emiliania huxleyi*, has a haplo-diplontic life cycle, alternating between calcified, non-motile, diploid cells and non-calcified, motile, haploid cells, with both phases being capable of unlimited asexual cell division (Green *et al.*, 1996; Klaveness, 1972a, 1972b). Haploid cells exhibit different growth preferences relative to diploid cells and do not have the exceptional ability to adapt to high light exhibited by diploid cells (Houdan *et al.*, 2004). Transcriptomic and experimental analyses also suggest a potential for mixotrophy in haploid cells (Rokitta *et al.*, 2011). As haploid cells of *E. huxleyi* are not easily recognizable by classical microscope techniques, little is known about their ecological distribution. *E. huxleyi* haploid cells were demonstrated to be resistant to the EhV viruses that are lethal to diploid cells and are involved in terminating massive blooms of diploid cells in nature (Frada *et al.*, 2009). This suggests that haploid cells might have a crucial role in the long-term maintenance of *E. huxleyi* populations by serving as the link for survival between the yearly 'boom and bust' successions of diploid blooms. Moreover, *E. huxleyi* has a number of apparently unique or unusual ultrastructural features. For example, in the diploid phase a distinctive Golgi-derived “reticular body” is involved in coccolithogenesis and the coccoliths lack cellulosic base-plate scales (Klaveness, 1972a; Westbroek *et al.*, 1984). In the haploid phase a refractive body termed the “X-body” is present (Klaveness, 1972b). Since the cytology and ultrastructure of other members of the Noëlaerhabdaceae have never been reported, it is not clear whether these features are unique to *E. huxleyi* or common to the family.

The Noëlaerhabdaceae comprises exclusively pelagic species that inhabit coastal, shelf and open ocean environments, including the extant genera *Emiliania* and *Gephyrocapsa* and various extinct genera. *Gephyrocapsa oceanica* is known to be the closest relative of *E. huxleyi* available in culture collections. Various genetic markers have been used to demonstrate their close relationship. Conservative ribosomal DNA sequences (nuclear and plastidial) demonstrated a high similarity between the two morphospecies, while relatively fast-evolving genes such as plastidial tufA and various mitochondrial genes delineate them respectively partially and completely (Bendif *et al.*, 2014). From paleontological evidence, *Gephyrocapsa* were first unambiguously present in the Pliocene, around 3.5 Mya (Young, 1998), and became abundant from around 1.7 Mya (Raffi *et al.*, 2006) with a succession of different morphospecies occurring (Matsuoka &

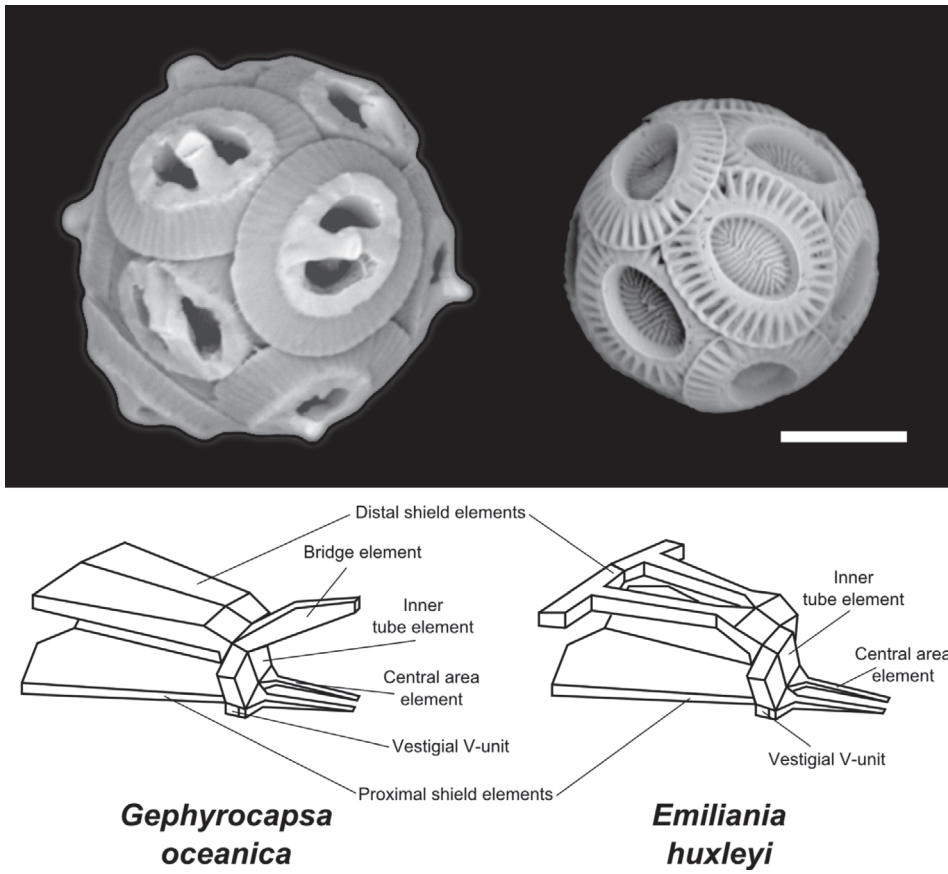


Fig. 1. Coccolith structure of Noëlaerhabdaceae redrawn from Young (1992).

Okada, 1990). The dominance of *Gephyrocapsa* shifted to *E. huxleyi* around 87 Ka (Hine & Weaver, 1998; Thierstein *et al.*, 1977). The coccoliths produced by the Noelaerhabdaceae share a distinctive coccolith structure, with crystals with sub-radial crystallographic c-axes (R-units) forming the grill, both shields and the two-layered tube of the coccoliths, whilst crystals with sub-vertical c-axes are only vestigial (Young *et al.*, 1992; Young *et al.*, 2004; Hoffmann *et al.*, 2014). However, a conjunct bridge over the central area made by the extension of some inner tube crystals on opposite sides of the coccolith is inherent to *Gephyrocapsa* (Fig. 1).

The ultrastructure of both coccolith-bearing and non-mineralized phases in the life cycle of *E. huxleyi* was described several decades ago (Klaveness, 1972a, 1972b) from culture observations. Many cultures of the diploid stage of *G. oceanica* are available in culture collections, and the motile haploid stage of this species occasionally occurs in these cultures and can relatively easily be isolated into pure asexually propagating culture. Nonetheless, the *G. oceanica* life cycle stages remain undescribed at the ultrastructural level. We present here the first ultrastructural records of both life cycle phases of *G. oceanica* and the first cytological comparison of *Gephyrocapsa* and *Emiliania*.

MATERIALS AND METHODS

Origin and morphological characterisation of analysed strains

Gephyrocapsa oceanica strains from the Roscoff Culture Collection (RCC: www.roscoff-culture-collection.org) were maintained in K/2(-Si,-Tris,-Cu) medium (Keller *et al.*, 1997) at 17°C with 50 µmol-photons·m^{-2}·s^{-1} illumination provided by daylight neon tubes with a 14:10h L:D cycle.

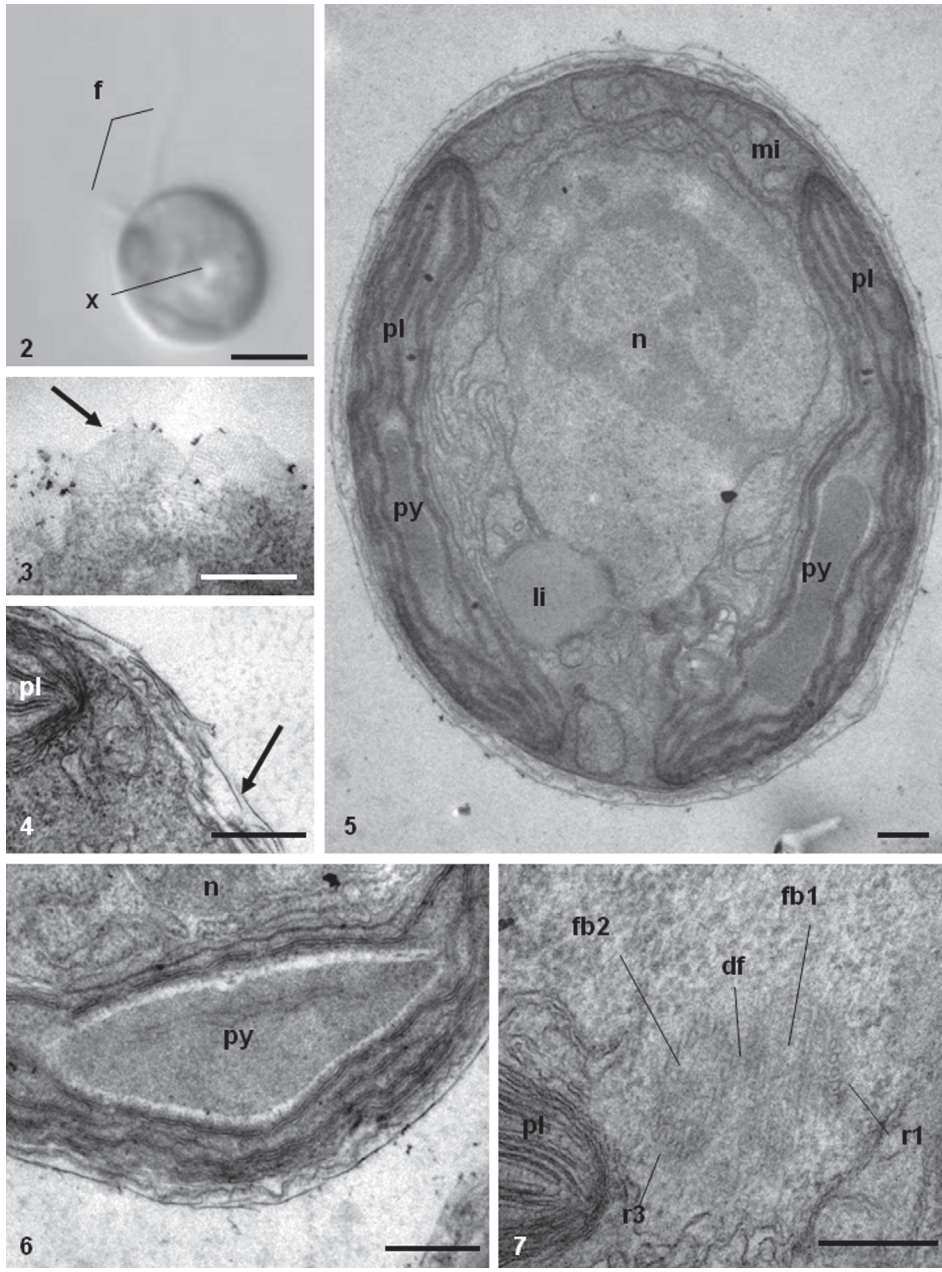
Living cells were observed with an Olympus BX51 light microscope equipped with differential interference contrast (DIC) optics. For transmission electron microscopy (TEM), cells were collected by gentle centrifugation and fixed with a 2.5% glutaraldehyde solution with 0.25M sucrose in 0.1M sodium cacodylate buffer at pH 7.2 for two hours. After three rinses in 0.1M cacodylate buffer with decreasing sucrose concentration (0.25M, 0.1M, 0M), cells were post-fixed for one hour with 1% osmium tetroxide and washed once in 0.1M cacodylate buffer. Dehydration was performed by transfer through a graded ethanol series (30, 50, 70, 85, 90, 95 and 100%) for 10 min each. The dehydrated cells were suspended in a 1:1 (v:v) mixture of Epon's resin and ethanol for one hour and then embedded in 100% Epon's resin. The embedded samples were polymerized at 60°C for twenty-four hours and sectioned using a Leica ultramicrotome with a diamond knife. Thin sections were placed on formvar covered copper grids. Sections were stained with uranyl acetate followed by post staining with lead citrate following the protocol of Reynolds (1963). The sections were examined under a JEOL 1011 and a JEOL JEM 1400 transmission electron microscope at an accelerating voltage of 80 kV. For scanning electron microscopy (SEM), coccolithophore cells were grown until early exponential phase and then filtered onto nitrocellulose filters that were dried in a dessicator before being sputter coated with a thin layer of Au/Pd. Observations were made with a Philips XL 30 FEG.

RESULTS

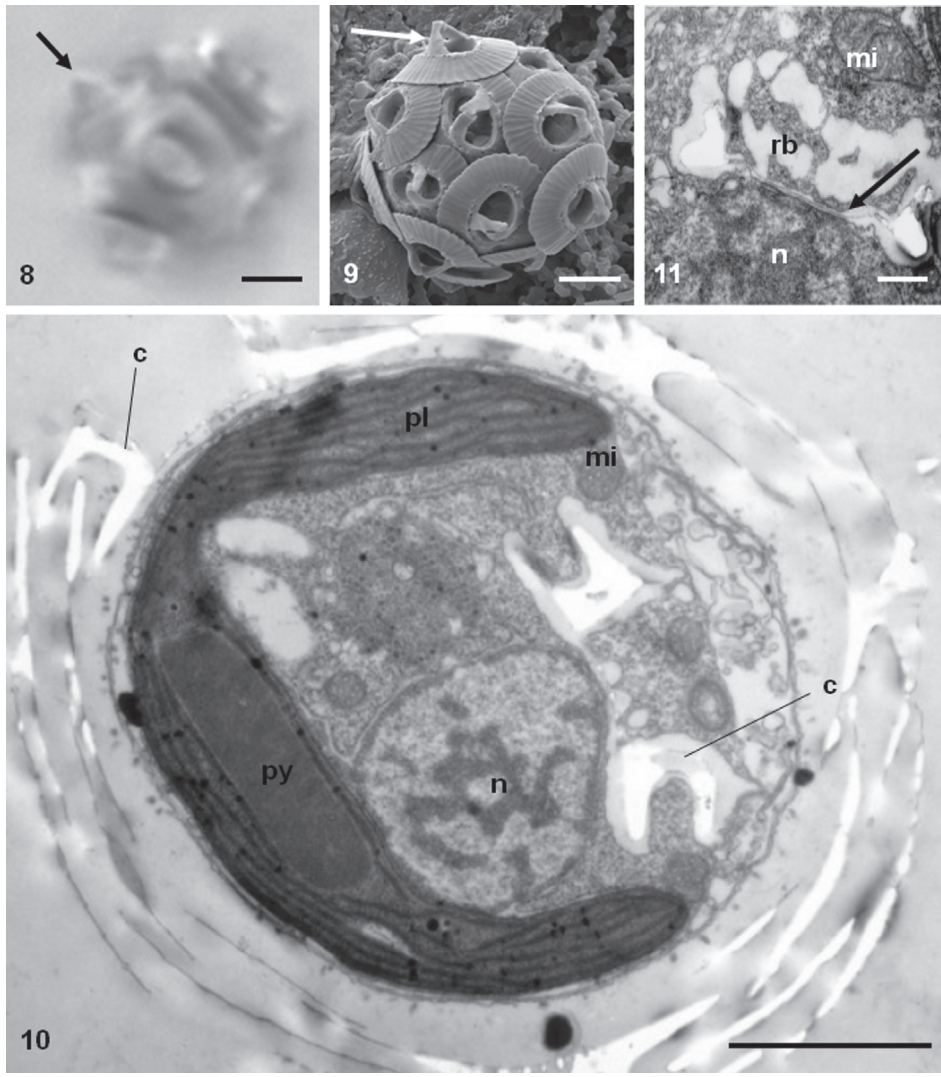
Morphological descriptions of *Gephyrocapsa oceanica* RCC1315/RCC1314 (Figs 2-11)

Gephyrocapsa oceanica exhibits a life cycle identical to that of *Emiliania huxleyi*, a non-motile coccolith-bearing phase alternating with a motile, non-calcifying phase, and both phases are capable of independent asexual reproduction.

The non-calcified motile cells are ellipsoidal in shape (Fig. 2), typically about 5.5 µm wide and 6 µm long. The two flagella are around 7 µm long and emerge from an apical insertion and no emergent haptonema is present. In LM, the parietal plastids are yellow-green in colour and a refractive body can be observed within the cell (Fig. 2). In TEM, a layer of organic scales organised in staggered rows can be seen to cover the entire cell membrane (Figs 3-4). Thin sections show cells with an ellipsoidal shape containing one or two plastids enclosed within the nucleoplastidial membrane. Each plastid contains a pyrenoid with a three cornered or trapezoidal shape traversed by a thylakoid lamella



Figs 2-7. *Gephyrocapsa oceanica* haploid phase (RCC1315) – **2.** LM micrograph of a motile cell; **3.** TEM micrograph of body scales (arrow) in glancing section; **4.** TEM micrograph of body scales (arrow) in transversal section; **5.** TEM micrograph of a cell in transverse section; **6.** TEM micrograph of a pyrenoid crossed by a thylakoid lamellum in transverse section; **7.** TEM micrograph of basal body in transverse section, showing flagellar bases with the distal fibre and flagellar roots 1 and 3; Scale bars 2: 5 µm, 3-4: 1 µm, 5: 500 nm, 6-7: 200 nm. (Abbrev: df: distal fibre; f: flagellum; fb1 and 2: left and right flagellar base; g: Golgi apparatus; li: lipidic droplet; mi: mitochondrion; n: nucleus; pl: plastid; py: pyrenoid; r1-3: flagellar root 1 and 3; x: refractive x-body).



Figs 8-11. *Gephyrocapsa oceanica* diploid phase (RCC1314) – **8.** LM micrograph of a non-motile cell covered by coccoliths with bridge (arrow); **9.** SEM micrograph of a coccosphere displaying coccoliths with bridge over the central area (arrow); **10.** TEM micrograph of cell in transverse section showing detail of coccolith adhesion on cell membrane with columnar material and the intracellular production of a coccolith in close proximity to the nucleus and a parietal plastid; **11.** TEM micrograph of early stage of coccolithogenesis showing a coccolith baseplate scale (arrow) of the coccolith between the nucleus and the reticular body ; Scale bars: 8: 2 µm, 9: 200 nm, 10: 1 µm 11: 500 nm. (Abbrev: c: coccolith; mi: mitochondrion; n: nucleus; pl: plastid; py: pyrenoid; rb: reticular body).

(Figs 5-6). An unusual vacuole sometimes occurs which may be related to the refractive body (x-body). Flagellar roots were found with R1 and R3 adjacent to the left and right flagellar bases, respectively (Fig. 7). No haptonematal root was observed.

Coccolith-bearing cells are non-motile and contain one yellowish-green coloured plastid (Fig. 8). Coccoliths are characterised by the presence of a prominent bridge over the central area of a heavy calcified placolith (Figs 8-9). Thin sections show that a layer of columnar material is present between the coccoliths and the cell membrane and that no non-mineralized body scales are present (Fig. 10). The parietal plastid is enclosed within a nucleopastidial membrane and contains a trapezoidally-shaped pyrenoid (Fig. 10). The coccolith-producing vesicle directly abuts the nucleus and is linked to a reticular body of Golgi origin that is structurally identical to that reported for *E. huxleyi* (Figs 10-11). Organic baseplates are visible below the coccoliths (Fig. 11), as described in *E. huxleyi* by Westbroek *et al.* (1984). Unlike the resistant microfibrillar scales of most coccoliths, these are amorphous structures and extend across the entire proximal surface of the coccolith rather than just the central area.

DISCUSSION

Gephyrocapsa oceanica and *Emiliania huxleyi* are genetically similar but have traditionally been classified in different genera based exclusively on comparison of coccolith morphology, specifically the presence (*Gephyrocapsa*) or absence (*Emiliania*) of a bipartite bridge spanning the central area of the placolith. This is a very visible feature, even in light microscopy, but in structural terms the bridge is simply a conjunct extension of a set of crystals of the inner tube (one to six crystals on either side of the inner tube), i.e. the bridge is formed by variation of the growth pattern of certain existing crystal units, without nucleation of any additional crystals (Young, 1989; Young *et al.*, 2004). Such variation of growth pattern is frequently observed in coccolith evolution, for instance in *E. huxleyi* var. *corona* the inner tube elements extend upwards to form an irregular collar around the central area. Bridge formation in *Gephyrocapsa* is rather unusual in that only a limited set of rim elements are involved and the bridge is invariably oriented at an angle rotated clockwise from the long axis of the coccolith in distal view, i.e. it is a chiral structure that is preserved throughout the geological range of the genus. *Emiliania* coccoliths also show slitting between shield elements which is not seen in *G. oceanica*, however, other species of *Gephyrocapsa* (*G. ericsonii, G. protohuxleyi*) show this feature and it is the presence/absence of a bridge which is traditionally used to separate the genera.

In terms of ultrastructure and life cycle, the two species are strikingly similar. Their life cycle consists of a non-motile placolith-bearing phase, a motile phase that bears non-mineralized organic scales, and non-calcified coccoid or amoeboid cells, the latter being relatively infrequently observed in *G. oceanica* cultures. All of the ultrastructural features that distinguish *E. huxleyi* from other coccolithophores (reticular body involved in coccolithogenesis, lack of non-mineralized body scales underlying coccoliths in the coccolith-bearing phase, "X-body" in the flagellate phase) were also observed in *G. oceanica* (Table 1). As reported by Klaveness (1972b) for *E. huxleyi*, the flagellar basal body of *G. oceanica* was very rarely observed in thin sections. Both species appear to have relatively simple flagellar roots with no trace of a haptonematal base, but in the absence of detailed reconstruction of the basal body of either species it is not

Table 1. Comparison of morphological and ultrastructural characters between *Gephyrocapsa oceanica and Emiliania huxleyi*

Life stage	*Morpho-species*	*Description*	*Cell size (µm)*	*Body scales size (µm)*	*Coccoliths size (µm)*	*Columnar material*	*Insertion of appendages*	*Flagella length (µm)*	*Flagellar action*	*Haptonema*	*Plastids*	*X body*
Motile stage	*G. oceanica*	Spherical, ovate or ellipsoid	5.5 × 6	1.3 × 1	–	–	apical	7	homodynamic	?	1 yellow green	+
	E. huxleyi	Spherical, ovate or ellipsoid	3.5-5.5 × 5	1.3 × 1	–	–	apical	6.5-7	homodynamic	?	1 golden yellow	+
Non-motile stage	*G. oceanica*	Dominant stage, spherical and coccolith bearing; sometimes naked spherical	4.0-6.5	–	3-4 × 3.5-5	+	–	–	–	–	1 yellow green	–
	E. huxleyi	Dominant stage, spherical and coccolith bearing; sometimes naked spherical	3.5-5	–	2.5-3 × 3.5-4	+	–	–	–	–	1 golden yellow	–

possible to determine whether there are any minor distinguishing features. These results provide further support for the conclusion of Bendif *et al.* (2014) that *Emiliania* and *Gephyrocapsa s*hould be classified in the same genus, with *Gephyrocapsa* having taxonomic priority. The combination of *E. huxleyi* into *Gephyrocapsa* was initially proposed by Reinhardt (1972).

According to these data and previous information on life-cycle evolution within the Calcihaptophycideae (Liu *et al.*, 2010), it appears probable that the earliest Noelaerhabdaceae were pelagic organisms that exhibited a haplo-diploid life cycle, calcifying in at least one life cycle stage (diploid), with relatively well-developed flagella/haptonema. The Noëlaerhabdaceae have formed large pelagic populations throughout their geological range (Eocene to recent), as did their putative ancestors the Prinsiaceae (Paleocene-Eocene) and Biscutaceae (Early Jurassic to Paleocene), as outlined in Bown (1998) and Young *et al.* (1999). The suggested role of the life cycle in conferring adaptive advantages indicate that this might have been a key factor in a fundamental ecological change towards the fast growing, opportunistic strategy that distinguishes most noëlaerhabdaceans (both extant and extinct) from other coccolithophores. Noëlaerhabdaceans are structurally relatively simple in comparison to other calcihaptophytes and this simplification might be considered a logical consequence of adoption of such a strategy. In this context, the suspected evolutionary interaction with viruses would have played a major role in maintaining and regulating this exceptional biological system.

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