

Morphological variation in the deep ocean-dwelling coccolithophore *Florisphaera profunda* (Haptophyta)

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Detailed analysis of the morphology of *Florisphaera profunda* from plankton samples collected at three sites in the Atlantic and Pacific Oceans reveals wide variation in this deep ocean-dwelling coccolithophore. In addition to the two varieties described previously, we found a third distinctive form, *Florisphaera profunda* var. *rhinocera* var. nov. All three varieties occur at each of the sampling sites. The analysis of monthly samples from different levels in the lower photic zone (LPZ) (100–200 m) at the Hawaii Ocean Time series station suggests that the varieties have similar distributions, which are correlated to primary productivity and the availability of light. The analysis of coccolith and coccosphere size in *F. profunda* reveals the existence of several size modes in *Florisphaera profunda* var. *profunda* and *F. profunda* var. *elongata*. The biological significance of these modes, or morphotypes is not known. However, their co-occurrence in single samples from different oceanic areas suggests that they are not ecophenotypes. In the light of recent molecular genetic analyses of intraspecific groups within commonly occurring coccolithophores, the varieties and size morphotypes of *F. profunda* are of significant interest for the study of marine phytoplankton biodiversity. Coccolithophores inhabiting the LPZ may be adapted to the low light, high nutrient conditions of this layer and hold great potential as a means to reconstruct past oceanographic conditions such as the position of the nutricline. However, coccolithophore biodiversity in the LPZ is poorly documented and the number of species may be much higher than previously thought.

Key words: coccolithophores, lower photic zone, morphotypes, biodiversity

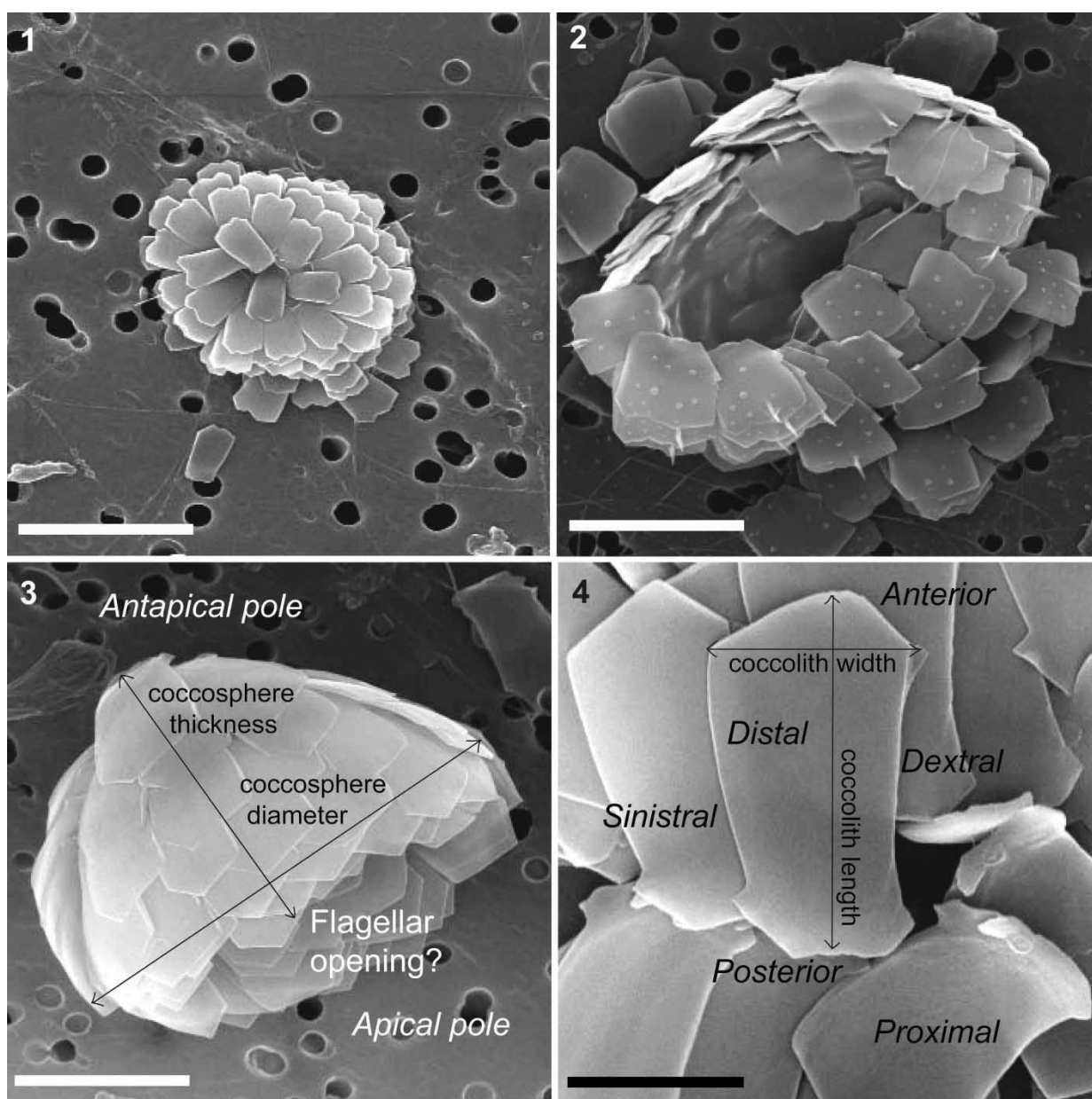
Introduction

Recent analyses of selected coccolithophore species have indicated that the biodiversity in this single-celled marine phytoplankton group may be severely underestimated, as fine scale ‘intraspecific’ morphological groups appear to display ecological and genetic differences (Bollmann, 1997; Knappertsbusch *et al.*, 1997; Young & Westbroek, 1991; Sáez *et al.*, 2003). Such morphological groups may in fact represent different species which diverged several million years ago, as already demonstrated for other microscopic planktonic organisms e.g., planktonic foraminifera (Darling *et al.*, 1996; Norris & de Vargas, 2000; de Vargas *et al.*, 2004). These findings are crucial to understanding the biodiversity, ecology and evolution of coccolithophores as well as for their application as proxies in palaeoenvironmental reconstructions (Bollmann *et al.*, 2002). On the basis of these facts,

detailed morphological as well as genetic analyses of other coccolithophore taxa, of which some 180 species occur in the ocean at the present day (Jordan & Kleijne, 1994), are necessary.

One important coccolithophore that displays intraspecific morphological variability is *Florisphaera profunda* Okada & Honjo. This unique, deep dwelling species is not clearly related to other coccolithophores and is therefore classified as *incertae sedis* (Young *et al.*, 2003). Two varieties of *F. profunda* have so far been described: *Florisphaera profunda* var. *profunda* and *Florisphaera profunda* var. *elongata* (Okada & Honjo, 1973; Okada & McIntyre, 1977, 1979, 1980) (Figs 1–4) and several additional morphologies have also been figured (Okada, 1983; Cros & Fortuño, 2002). However, the two varieties of *F. profunda* appear to be difficult to distinguish (Young, 1998) and are therefore not always separately recorded in plankton or sediment studies. Furthermore, Kleijne (1991) has suggested that *Florisphaera profunda* var. *elongata* and an unidentified holococcolithophorid ‘type B’ may be different stages of a single life cycle. Therefore,

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Figs 1–4. SEMs showing coccosphere and coccolith morphology of *Florisphaera profunda* var. *profunda* (1, 2) and *Florisphaera profunda* var. *elongata* (3, 4). Fig. 1. Complete coccosphere of *F. profunda* var. *profunda* viewed from antapical pole, composed of small, simple, straight-sided small coccoliths. Fig. 2. Partially incomplete coccosphere viewed from antapical pole, composed of unusual, short-fat coccoliths with distal spine. Fig. 3. Complete coccosphere of *F. profunda* var. *elongata* viewed from side with coccosphere terminology used in this study, composed of elongate coccoliths with anterior peak. Fig. 4. Close-up of *F. profunda* var. *elongata* coccoliths with coccolith terminology used in this study. Scale bars represent 5 μ m (Figs 1–3) and 2.5 μ m (Fig. 4).

the goal of the present study is to re-assess the ecological and biological significance of morphological variation within *F. profunda* from plankton samples.

The ecology of *F. profunda* is poorly understood. However, it appears to be adapted to low light, high nutrient conditions (Jordan *et al.*, 1992; Brand, 1994; Cortés *et al.*, 2001) and may even be heterotrophic (Brand, 1994). The species dominates coccolithophore assemblages in the LPZ (>100 m) throughout the year in the subtropical oligotrophic central gyres (Cortés *et al.*, 2001;

Haidar & Thierstein, 2001) and is a common component of fossil coccolithophore assemblages in marine sediments dating back to four million years (Okada, 1983; Young, 1998). *Florisphaera profunda* therefore holds great potential as a tool for the reconstruction of past oceanographic conditions, such as changes in the position of the nutricline (Molfin & McIntyre, 1990; McIntyre & Molfin, 1996; Henriksson, 2000; Kinkel *et al.*, 2000), surface water turbidity (Ahagon *et al.*, 1993) and primary productivity (Beaufort *et al.*, 1997; 2001).

Material and methods

The morphology of *F. profunda* coccospheres and coccoliths was studied in filtered plankton samples from the LPZ at three locations in the Atlantic and Pacific Oceans; the Hawaii Ocean Time-series (HOT), 22°45'N, 158°0'W, the Bermuda Atlantic Time-series (BATS), 32°10'N, 64°30'W and European Station for Time-series in the Ocean, Canary Island Eastern Boundary Current (ESTOC EBC), Canary Islands, 28°65'N, 13°65'W. A small segment of each filter sample, housed at the Geological Institute of the Swiss Federal Institute of Technology (ETH), Zürich, was mounted on an aluminium stub and coated with 15 nm of gold. Using a Philips XL-30 scanning electron microscope SEM, a total of 1000 consecutive fields of view were scanned along a radial transect of each filter sample at a magnification of 2000 $\times$ (for details see Bollmann *et al.*, 2002). This is equivalent to an area of ca. 2.66 mm² or 17.5 ml of filtered seawater in the HOT samples and 8.75 ml in the Bermuda and Canary Islands samples. The overview images were then viewed on a PC and all complete, partially disintegrated, and totally collapsed *F. profunda* specimens were classified and measured. Coccosphere diameter and coccolith length and width (Figs 3, 4) were recorded using *analysIS 3.0 for Windows*[®] to an accuracy of 0.2 μ m, as determined by micrometric calibration. From the HOT station, Hawaii, monthly plankton samples at the depth of maximum *F. profunda* abundance (100, 150 or 200 m), were analysed for the years 1994 and 1995. Additional LPZ samples with >ca. 8000 *F. profunda* cells l⁻¹ were analysed, as well as samples from a depth transect from 0–200 m, passing through a shallow peak of *F. profunda* at 100 m during March 1995. Selected samples from BATS, Bermuda and Eastern Boundary Current (EBC), Canary Islands were studied for comparison to the HOT samples.

Terminology

In their original description of *F. profunda*, Okada & Honjo (1973) use several informal morphological terms. However, these terms have not been adopted by all subsequent authors, and confusion has arisen with regard to the orientation of coccospheres and isolated coccoliths, and the differentiation between the two varieties.

The description of this artichoke-shaped coccosphere in terms of an apical and an antapical end was previously not possible because no flagellar opening had been observed. Okada & Honjo (1973) therefore used the term *shallow dome top* in their original description of *F. profunda*. However, in many specimens we have observed an opening at the opposite end of the cell to the shallow dome top, which may represent the flagella opening (Fig. 3). We therefore suggest that the terms *apical pole* (the end with the flagellar opening) and *antapical pole* (shallow dome top of Okada & Honjo, 1973) should be used to orient coccospheres of *F. profunda* (Fig. 3). This is in keeping with the description of coccosphere morphology in other coccolithophores (Young *et al.*, 1997).

The polygonal plate-shaped coccoliths that form *F. profunda* cannot be conveniently described by any of the *lith-terms* given by Young *et al.* (1997). We therefore assign the term *polygolith* to this type of coccolith morphology, consisting of a single crystal of flat, polygonal-shaped calcite. Polygoliths of *F. profunda* have a weakly birefringent appearance in the polarizing light microscope (Perch-Nielsen, 1985) and are therefore easily overlooked. In the absence of any terminology for the two flat coccolith surfaces, we name these *proximal* and *distal* (Fig. 4). The proximal surface of *F. profunda* coccoliths faces inwards towards the centre of the coccosphere and the distal surface faces outwards. In keeping with the coccosphere terminology adopted above, the end of the coccolith oriented toward the antapical pole is termed the 'anterior' (Fig. 4). The anterior end of *F. profunda* coccoliths is always wider than the opposite, *posterior end*. This difference can be used to orientate the coccoliths and, in the absence of a visible flagellar opening, the coccosphere upon which it sits. By applying the system proposed for other flat, elongate coccoliths such as *Ceratolithus* (Young *et al.*, 1997), the terms *dextral* and *sinistral* can be used to describe the right and left edges of *F. profunda* coccoliths when viewed from the distal surface, with the anterior end up (Fig. 4).

Results

Morphology and taxonomy

The main difference between the two varieties of *F. profunda* described by Okada & Honjo (1973) is the shape of the apical end of their coccoliths. In this respect, the apical end of *F. profunda* var. *profunda* is straight or zig-zagged and *F. profunda* var. *elongata* is rounded or possesses an obtuse angled peak (Figs 1–4). However, Okada & Honjo (1973) also stated that the coccospheres of *F. profunda* var. *profunda* and *F. profunda* var. *elongata* differ in terms of their size. This may have prompted subsequent authors to adopt coccolith size as a distinguishing feature instead of the shape of the apical end, even though the size of coccoliths and coccospheres in both varieties overlap significantly (Okada & Honjo, 1973). Using the shape of the apical end of the coccoliths as a distinguishing feature, we were able to identify the two varieties of *F. profunda* described by Okada & Honjo (1973) in 26 plankton samples from the Atlantic and Pacific Oceans. Our size measurements demonstrate that whilst *F. profunda* var. *elongata* reaches a greater maximum size, both varieties overlap considerably in terms of coccosphere diameter and coccolith length and width (Figs 3, 4). Furthermore, additional morphological variation was present within both varieties, including striations on the distal surface of *F. profunda* var. *elongata* and the presence of a small dorsal

spine on rare specimens of *F. profunda* var. *profunda* (Fig. 2). Some of these features have been already reported in plankton samples from other locations (e.g. Cros & Fortuño, 2002, fig. 85D).

In addition to the two existing varieties of *F. profunda*, we discovered a third distinctive form which differs from the other two by having strongly asymmetrical, sail-shaped coccoliths with a prominent horn-like spine on the dextral side of their anterior end (Figs 5–8). Specimens of this form are immediately recognisable and occurred in the majority of samples analysed. A single specimen with this morphology was figured by Okada (1983, plate 1, fig. 8) from 125 m in the central Pacific, but was not reported since. Given its distinctive morphology and occurrence in samples containing the two existing varieties from the Atlantic and Pacific Oceans, we propose this form as a new variety *Florisphaera profunda* var. *rhinocera*.

Florisphaera profunda var.
rhinocera var. nov. (Figs 5–8)

Unnamed variety of *F. profunda* Okada, 1983: 185, plate 1, Fig. 8.

DERIVATION OF NAME: On account of distinctive anterior spine, which resembles the horn of the extant mammal rhinoceros (family Rhinocerotidae). Feminine adjective.

LATIN DESCRIPTION: *Coccosphaera constans* ex 30–100 coccolithis simplicibus. Multum delicata. Diameter est 7.1–12 µm longus (medius 9.1 µm). Coccolithi quadranguli ad falcate aut forma veli sunt visione proximale aut distale. Plani aut varie convexi latere distale praeter axem longum. Recti aut leviter curvati, fronte levigata ad inaequabile levante leviter prope dexterum latus. Spina evidens in dextero margine frontis. Spina plerumque acuta atque varie longa est. Latera recta ad dextra curvata, plerumque levigata, se contrahentia prope tergum. Spina frontis processus ingentis incrementi crassitudinis lateris in dextero margine saepe est. Formae infrequentes parvam spinam in dextero margine, proiectam in partem anteriorem, aut distalem superficiem leviter pectinatam habere possunt. Margo laevus minime saepe spissatus est ac minimam spinam obtusam efficere potest. Tergum plerumque rectum, saepe spissatum, leviter curvatum et minime saepe tortuosum est. Coccolithi marginibus curvatis tenuem, rotundatam dexteram extensionem habere possunt. Spinam frontis atque ingentem dextram inconcinnitatem aut curvaturam usas esse ad disponendum recte coccolithos singulos oportet. Longitudo 1.7–3.8 µm (media 2.7 µm), latitudo 0.9–3.1 µm (media 1.0 µm).

DESCRIPTION: Coccosphere composed of ca. 30–100 simple coccoliths. Very delicate. Diameter ca. 7.1–12 µm (mean ca. 9.1 µm). Coccoliths rhombic to almost crescentic or sail-shaped when viewed from proximal or distal surface. Flat or with variable distal convexity along long axis. Straight or slightly curved, smooth to irregular anterior rising gently towards dextral side. Distinctive spine on dextral edge of anterior. Spine is usually sharp and of variable length. Sides straight to dextrally curved, usually smooth, tapering towards posterior. Anterior spine is often a continuation of strong lateral thickening on dextral edge. Rare specimens may possess a small spine on dextral edge, projecting in an anterior direction, or a slightly scalloped distal surface. Sinistral edge is only very rarely thickened and may give rise to a very small blunt anterior spine. Posterior usually straight, often thickened, slightly curved and more rarely zig-zagged. Coccoliths with curved edges may possess a weak, rounded dextral projection. Anterior spine and strong dextral asymmetry or curvature should be used to orientate isolated coccoliths. Length ca. 1.0–3.8 µm (mean ca. 2.7 µm), width ca. 0.9–3.1 µm (mean ca. 1.9 µm).

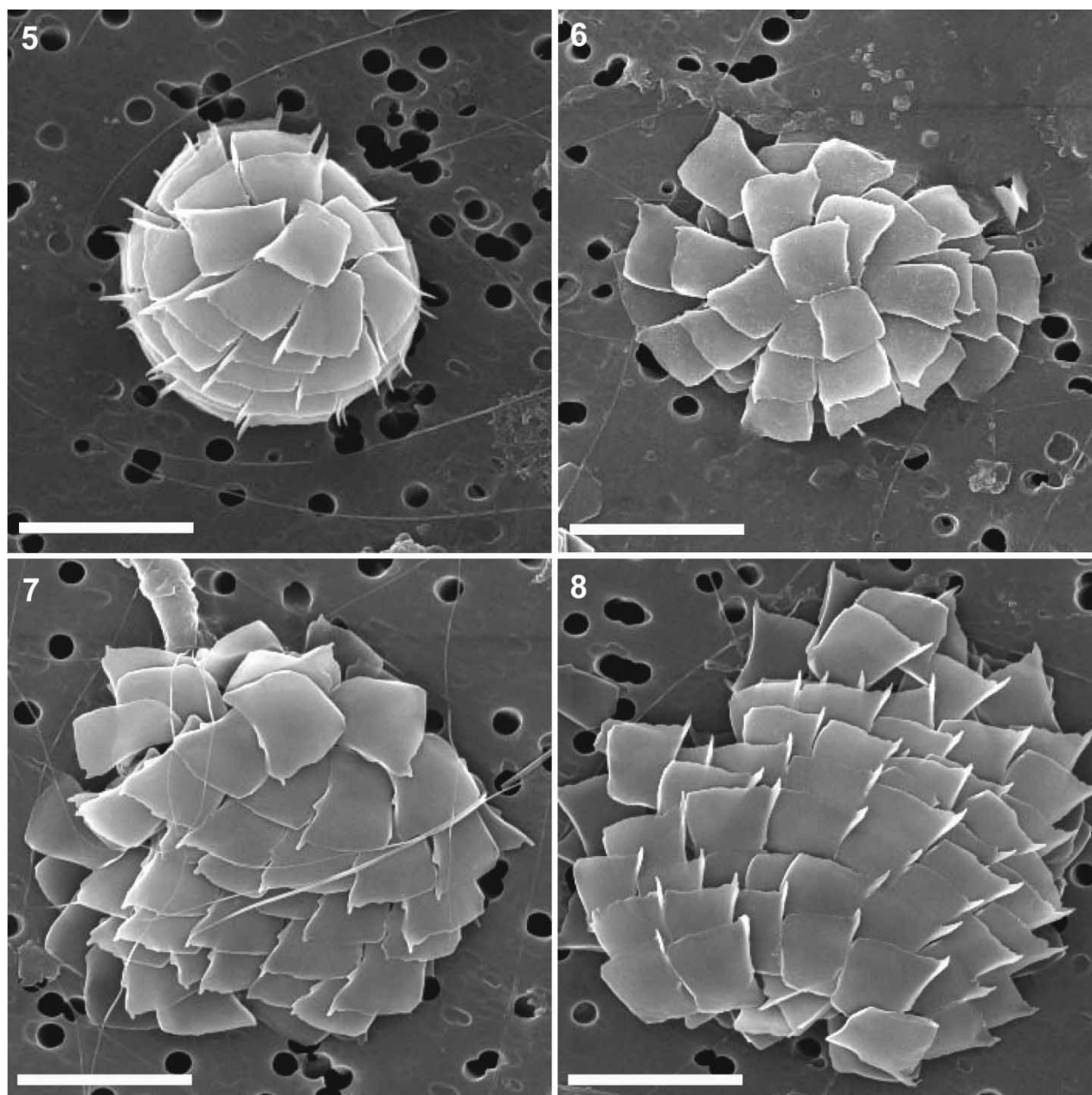
DIFFERENTIAL DIAGNOSIS: Coccospheres of this variety are distinguished from those of *Florisphaera profunda* var. *profunda* and *Florisphaera profunda* var. *elongata* by their strong asymmetry and the presence of a spine on the dextral corner of the anterior end of their coccoliths.

HOLOTYPE: Fig. 5, Plankton sample H61–100, collected from 100 m water depth at 14:04 on 05.03.1995 at HOT time series station, Hawaii, North Pacific (Latitude N° – 22.75, Longitude W° – 158). Sample stored at Geological Institute, ETH, Zürich, Switzerland.

DISTRIBUTION: North Atlantic Ocean, North Pacific, Equatorial Pacific, Caribbean Sea.

Morphometry

Our measurements of coccosphere and coccolith size indicate that wide size variation occurs within *F. profunda* (Fig. 9). Because of the delicate nature of *F. profunda*, coccosphere diameter could only be measured on ca. 200 intact and un-deformed specimens. Nevertheless, this dimension appears to be broadly correlated with the mean coccolith diameter on a coccosphere (Fig. 9). Each of the three varieties of *F. profunda* have different coccosphere size ranges, with *F. profunda* var. *rhinocera* forming medium sized coccospheres (ca. 8–12 µm coccosphere diameter) in relation to the other two (*F. profunda* var. *profunda*: 4–14 µm; *F. profunda* var. *elongata*: 5–15 µm).



Figs 5–8. SEMs showing morphology of *Florisphaera profunda* var. *rhinocera* var. nov. Fig. 5. Complete coccosphere viewed from antapical pole, composed of straight-sided, rhombic coccoliths with prominent anterior spine (Holotype). Fig. 6. Partially flattened coccosphere viewed from antapical pole, composed of curved coccoliths with indistinct anterior spine. Fig. 7. Flattened coccosphere viewed from the side, composed of curved, sail-shaped coccoliths with small anterior and dextral spines. Fig. 8. Collapsed coccosphere viewed from side, composed of straight-sided to slightly curved coccoliths with prominent anterior spine. Scale bars represent 5 μ m.

By measuring the length and width of isolated coccoliths from collapsed *F. profunda* specimens we were able to determine that coccolith size is relatively constant on a single coccosphere (standard deviation ca. $<0.2 \mu$ m). Plotting of the average coccolith size of all specimens in which three or more coccoliths could be measured, reveals that coccolith length and width are linearly correlated in the three varieties of *F. profunda* with a coefficient of 0.59–0.93 (Fig. 10A–C).

The distribution of mean coccolith length in *F. profunda* var. *rhinocera* is unimodal (Fig. 10).

However, *F. profunda* var. *profunda* and *F. profunda* var. *elongata* display distinctly bi- to polymodal mean coccolith length distributions. The coccolith size distribution of *F. profunda* var. *profunda* is composed of a small (mean length 1.9μ m) and a medium sized mode (mean length 3.4μ m), which can be easily separated at 2.5μ m mean coccolith length (Fig. 10A). The mean coccolith length distribution of *F. profunda* var. *elongata* on the other hand is less clear, but may be composed of four modes (Fig. 10B): small (mean length 2.1μ m; $<2.5 \mu$ m), medium (mean length 3.1μ m;

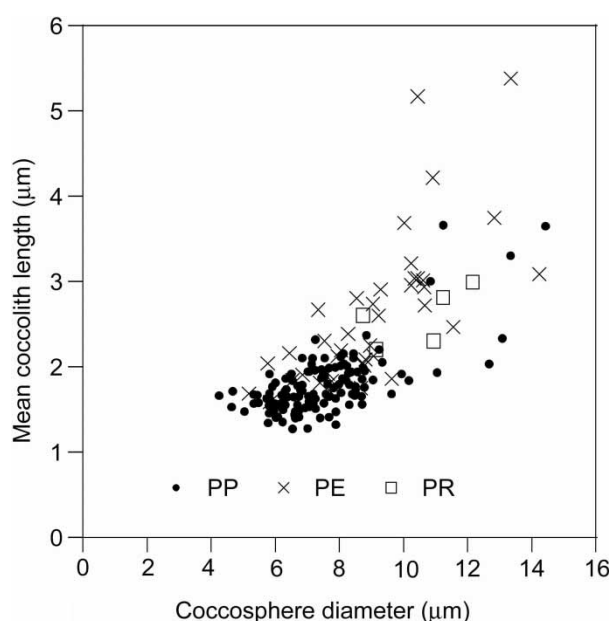


Fig. 9. Coccosphere and coccolith size of *F. profunda* from HOT, BATS and EBC plankton samples. Coccosphere diameter and mean coccolith length of all complete, undeformed cells upon which at least three coccoliths could be measured. The individual distributions of the three varieties of *F. profunda* are shown: PP – *F. profunda* var. *profunda*, PE – *F. profunda* var. *elongata*, PR – *F. profunda* var. *rhinocera*. Coccosphere diameter and mean coccolith length is correlated with a coefficient (r) of 0.46.

2.5–4 μm), large (mean length 4.9 μm; 4–6 μm) and extra large (>7 μm). Many of these size modes can be distinguished in the mean coccolith length distribution of single samples from the three localities (Figs 10D, E). We suspect that they represent different size groups within the two varieties (Figs 11–16). However, the low number of intact, un-deformed specimens in which coccosphere diameter could be reliably measured prevented the reliable documentation of these size groups of coccospheres (Fig. 9).

Distribution in plankton samples

All three varieties of *F. profunda* were present in the majority of samples analysed. In most samples, *F. profunda* var. *profunda* and *F. profunda* var. *elongata* occurred in comparable numbers (average ca. 1500–2000 cells l⁻¹) and accounted for the majority of the *F. profunda* specimens. The new variety *F. profunda* var. *rhinocera* was generally less abundant than the other two (average ca. 500 cells l⁻¹) but was recorded at all three sampling sites and reached densities of >2000 cells l⁻¹ in some samples.

The analysis of monthly samples from HOT at the depth of maximum *F. profunda* abundance in the LPZ during 1994–1995 show a clear seasonal

distribution of each of the varieties with high cell densities in summer and autumn 1994 and spring 1995, and low cell densities in winter 1994 and 1995 and summer 1995 (Fig. 17). All three exhibit similar fluctuations in their cell densities throughout this period, which mirror those of total *F. profunda*, as reported by Cortes *et al.* (2001).

By calculating the average coccolith length on cells of *F. profunda*, we were able to classify specimens of *F. profunda* var. *profunda* and *F. profunda* var. *elongata* into the six size morphotypes described above. The vast majority of *F. profunda* var. *profunda* cells encountered at HOT belong to the small size morphotype. Consequently, cells of this size morphotype exhibit a similar distribution pattern to that of total *F. profunda* var. *profunda* during 1994–1995 (Fig. 17). The medium size morphotype of *F. profunda* var. *profunda* occurred in very low absolute numbers (<500 cells l⁻¹) at the depth of maximum *F. profunda*, though its abundance relative to the small size morphotype was much higher during summer and autumn 1995 when total *F. profunda* was low. The small and medium size morphotypes of *F. profunda* var. *elongata* accounted for the majority of cells of this variety at HOT. They occur in similar absolute numbers throughout 1994–1995 and exhibit distinct fluctuations, which follow that of total *F. profunda* var. *elongata* during this period. The large size morphotype of *F. profunda* var. *elongata* occurred in low numbers (<500 cells l⁻¹) in all HOT samples. During 1994–1995 it showed minor fluctuations, including an increase in absolute abundance during the shallow peak of *F. profunda* at 100 m in March 1995 (Fig. 17). The extra large size morphotype of *F. profunda* var. *elongata* occurred during this month at HOT, but below the depth of maximum *F. profunda* abundance at 150 m. Cells belonging to this biggest morphotype (mean coccolith length >7 μm) were not recorded in any other samples at HOT.

All varieties of *F. profunda* were present in samples collected from 100, 150 and 200 m depth at HOT. For each variety, its highest abundance during any given month always occurred at the depth of maximum *F. profunda* cell density. However, the proportion of each of the varieties at the three sampling depths suggests that *F. profunda* var. *elongata* is more abundant at deeper levels in the LPZ relative to *F. profunda* var. *profunda* and *F. profunda* var. *rhinocera* (Fig. 18). This is the result of an increase in the relative abundance of the medium and large morphotypes of *F. profunda* var. *elongata* and a reduction in *F. profunda* var. *rhinocera* and the small morphotype of *F. profunda* var. *profunda* with depth (Fig. 18).

In order to investigate whether there were any dominant ecological controls on the distribution

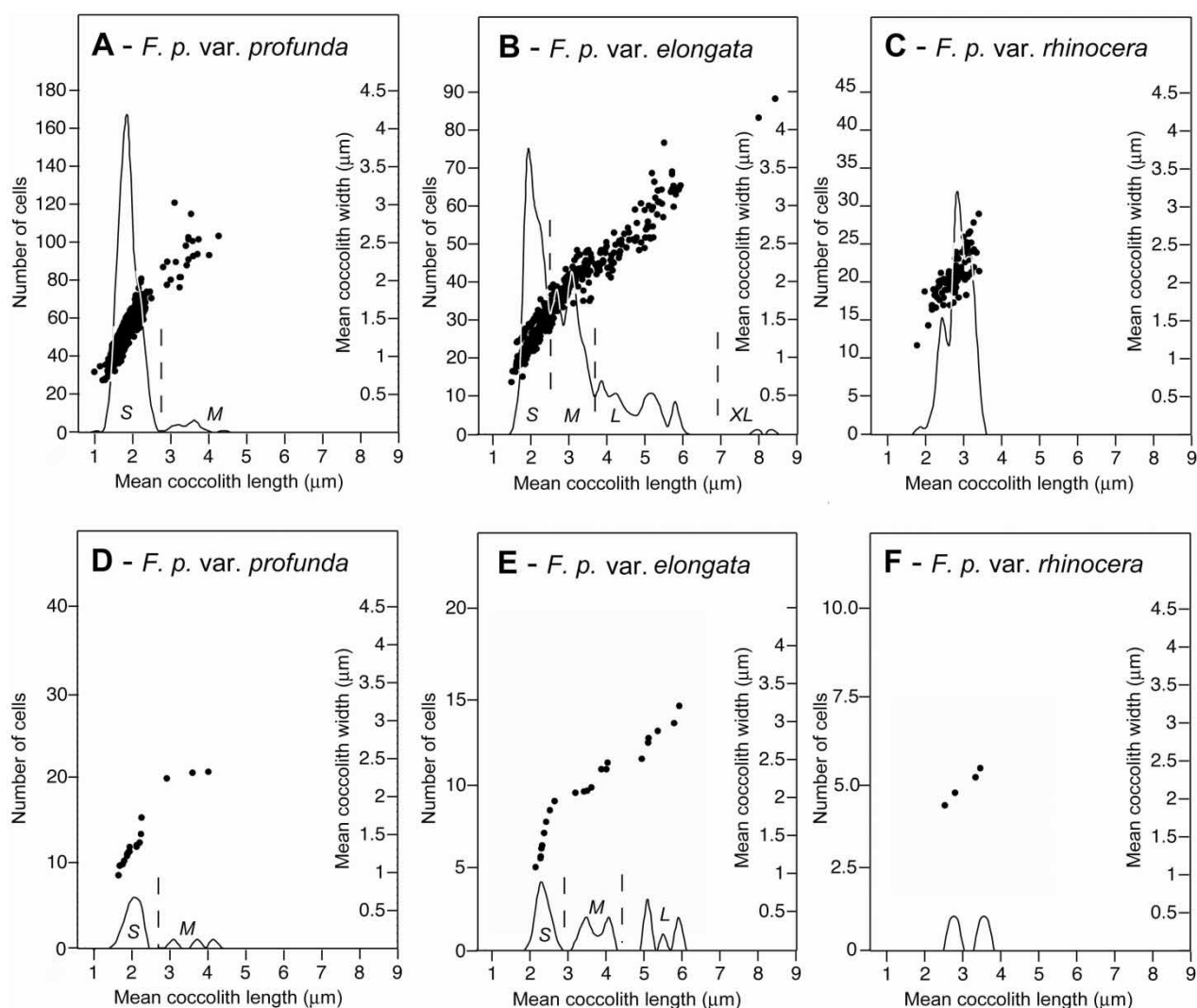


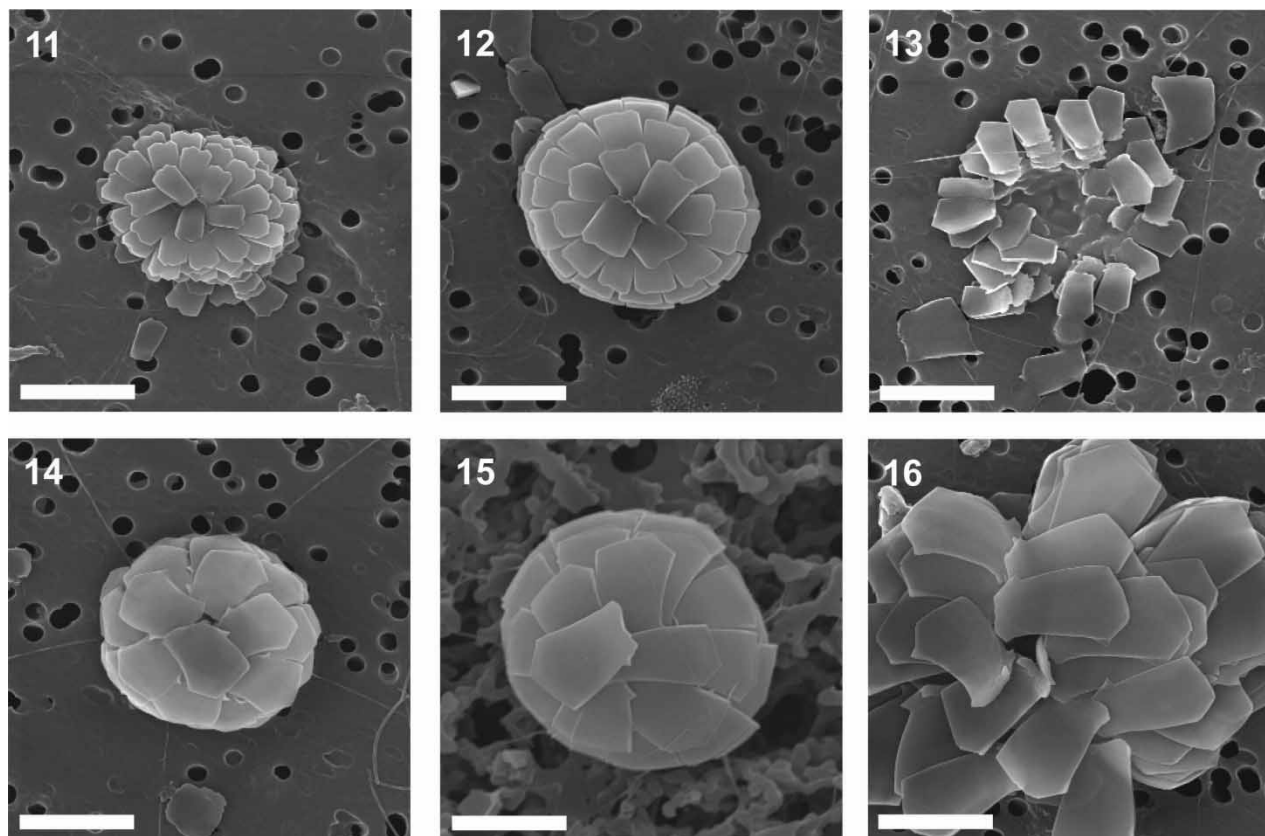
Fig. 10. Graphs showing mean coccolith size distribution and morphotypes of *F. profunda* from HOT, BATS and EBC plankton samples. A–C. Mean coccolith length and width of all complete, deformed or collapsed specimens upon which three or more coccoliths could be measured is plotted as a scatter alongside the smoothed frequency distribution of mean coccolith length, calculated using a bin of 0.2 μm . Mean coccolith length and width is correlated with a coefficient (r) of: (A) – 0.84, (B) – 0.93 and (C) – 0.59. In *F. profunda* var. *profunda* and *F. profunda* var. *elongata* several modes or size morphotypes are visible. These are labelled S, M, L and XL, and their possible subdivision is indicated by dashed lines on the distribution of mean coccolith length. D–F. Mean coccolith size distribution and morphotypes of *F. profunda* in a single plankton sample from October 1995 at 150 m depth at HOT (sample H67–150m). Morphotypes *F. profunda* var. *profunda* S and M and *F. profunda* var. *elongata* S, M and L are visible in this sample. No coccospheres of *F. profunda* var. *elongata* XL were found, however, isolated coccoliths corresponding in size to this morphotype were encountered.

of the varieties of *F. profunda*, their cell densities and relative abundances in all LPZ samples analysed from 1994–1995 at HOT were compared to several *in situ* environmental parameters including light, temperature, nutrients and primary productivity (Cortés *et al.*, 2001). Pearson product-moment correlation analysis revealed no high correlations, suggesting that the distribution of the three varieties cannot be related to a single environmental parameter. Likewise, the cell densities and relative abundances of the size morphotypes of *F. profunda* var. *profunda* and *F. profunda* var. *elongata* were not well correlated to any abiotic parameter in our samples.

Stepwise multiple regression models (MR) were therefore calculated to explain the variability in the cell densities of the *F. profunda* varieties and size morphotypes described above. The best models for the distribution of the three varieties of *F. profunda* were:

F. profunda var. *profunda* (multiple $R^2 = 0.67$)

$$\begin{aligned} \text{cells l}^{-1} = & 116 + (-1658 \times \text{light}) \\ & + (5247 \times \text{primary productivity}) \\ & + (28526 \times \text{phosphate}) \\ & + (-2505 \times \text{nitrate}) \end{aligned}$$



Figs. 11–16. SEM images of *F. profunda* cells corresponding to the size morphotypes identified in this study. *F. profunda* var. *profunda* small (Fig. 11), medium (Fig. 12), *F. profunda* var. *elongata* small (Fig. 13), medium (Fig. 14), large (Fig. 15) and extra large (Fig. 16). Scale bars represent 5 μm .

F. profunda var. *elongata* (multiple $R^2 = 0.48$)

$$\begin{aligned} \text{cells l}^{-1} &= 25845 \\ &+ (11776 \times \text{primary productivity}) \\ &+ (-1142 \times \text{light}) + (183 \times \text{depth}) \end{aligned}$$

F. profunda var. *rhinocera* (multiple $R^2 = 0.56$)

$$\begin{aligned} \text{cells l}^{-1} &= 564 + (2163 \times \text{primary productivity}) \\ &+ (-58 \times \text{light}) \end{aligned}$$

These models suggest that the abundances of all three varieties of *F. profunda* are influenced by light and primary productivity. In their analysis of total *F. profunda* in the LPZ at HOT, Cortés *et al.* (2001) concluded that the species was most abundant when light was low. In their MR model, the availability of nutrients explained a smaller but significant percentage of the variability in total *F. profunda* cell density. This is also recorded in our model for *F. profunda* var. *profunda* above. Whilst the MR model for *F. profunda* var. *elongata* only explains 48% of the cell density variability in this variety, the importance of depth in this model

confirms our suggestion, that it may prefer deeper levels in the LPZ.

Multiple regression analysis of the size morphotypes of *F. profunda* var. *profunda* and *F. profunda* var. *elongata* was less conclusive. The large and extra large size morphotypes of *F. profunda* var. *elongata* were too rare in most samples to perform stepwise MR. However, we were able to explain 68% of the variance in the cell density of the small morphotype of *F. profunda* var. *profunda* in the LPZ at HOT using the model below. The similarity of this model to that calculated for *F. profunda* var. *profunda* is explained by the fact that cells belonging to this size morphotype account for the vast majority of *F. profunda* var. *profunda* encountered in our samples.

F. profunda var. *profunda* small morphotype (multiple $R^2 = 0.68$)

$$\begin{aligned} \text{cells l}^{-1} &= 93 + (-1672 \times \text{light}) \\ &+ (5378 \times \text{primary productivity}) \\ &+ (27406 \times \text{phosphate}) \\ &+ (-2434 \times \text{nitrate}) \end{aligned}$$

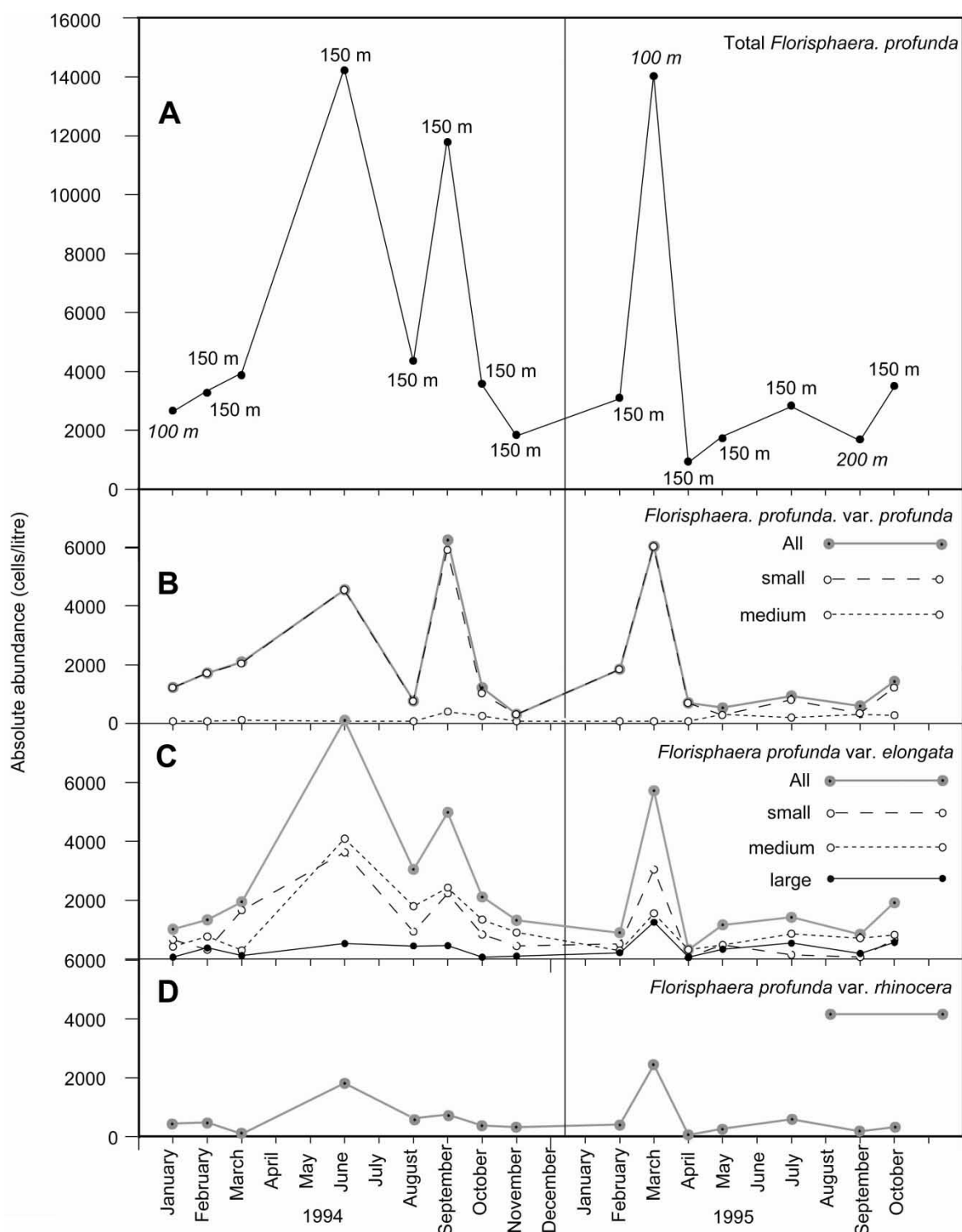


Fig. 17. Graphs showing *Florisphaera profunda* cell density at the HOT time series station. **A** Total *F. profunda* cell density at the depth of maximum abundance during 1994–1995. **B–D.** Cell density of the three varieties and six size morphotypes at the depth of maximum total *F. profunda* abundance: *F. profunda* var. *profunda* (**B**), *F. profunda* var. *elongata* (**C**) and *F. profunda* var. *rhinocera* (**D**). Total *F. profunda* abundance data from Cortés *et al.* (2001).

Discussion and conclusions

Our analysis of the shape and size of the calcareous skeletons of *F. profunda* reveals that several morphological groups exist in this coccolithophore species. The two varieties of *F. profunda* described by Okada & Honjo (1973) could be easily distinguished on the shape of the anterior end of their

calcite coccoliths. At all three sampling sites, a third separate morphological variety of *F. profunda*, characterised by having strongly asymmetrical coccoliths with a horn-like spine was also present. Morphometric analysis of *F. profunda* from our plankton samples reveals the presence of several size groups within *F. profunda* var. *profunda* and

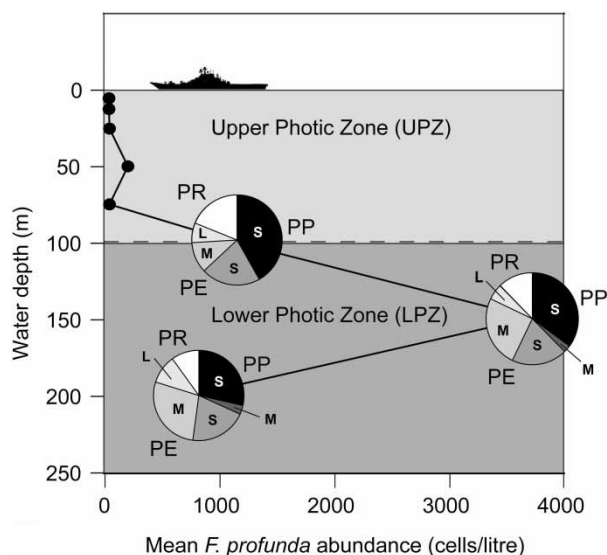


Fig. 18. Diagram showing *Florisphaera profunda* cell density at different depths in the photic zone during 1994–1995. Mean total *F. profunda* cell density for 1994–1995 is shown by black line, with the relative abundance of each of the three varieties of *F. profunda* and their size-morphotypes at 100, 150 and 200 m in the LPZ indicated. PP – *F. profunda* var. *profunda*, PE – *F. profunda* var. *elongata*, PR – *F. profunda* var. *rhinocera*. Total *F. profunda* abundance data from Cortés *et al.* (2001).

F. profunda var. *elongata*. These size groups occur in many samples from the three areas and seem to represent separate size morphotypes of these taxa.

Similar intraspecific morphological variation has been reported within several coccolithophores inhabiting the upper photic layers of the ocean, e.g., *Gephyrocapsa*, (Bollmann, 1997), *Calcidiscus leptoporus* (Kleijne, 1993; Knappertsbusch *et al.*, 1997; Quinn *et al.*, 2004). Analyses on the distribution (Knappertsbusch *et al.*, 1997), ecology (Renaud *et al.*, 2002) and life cycle (Geisen *et al.*, 2002) of the intraspecific groups of *C. leptoporus* have indicated that these may represent separate species. This has subsequently been confirmed by laboratory culture experiments (Quinn *et al.*, 2003) and molecular genetic analyses (Sáez *et al.*, 2003). We now know that this coccolithophore is composed of at least two ‘sibling’ species: *Calcidiscus leptoporus* (Murray & Blackmann) Loeblich & Tappan as emended by Sáez *et al.*, 2003 and *Calcidiscus quadriperforatus* (Kamptner) Quinn & Geisen in Sáez *et al.*, 2003, which may have diverged as long as 11 million years ago.

The discovery of ‘sibling’ species in *Calcidiscus* and other coccolithophores, e.g., *Umbilicosphaera sibogae* and *Coccolithus pelagicus* (Sáez *et al.*, 2003) is changing our view of biodiversity in this phytoplankton group. In the light of these discoveries, the occurrence of intraspecific morphological groups in *F. profunda* is of great interest and requires further investigation. Much less is known about the coccolithophores

inhabiting the LPZ of the ocean. They are morphologically quite different from the coccolithophores in the upper photic zone (Young, 1994) and appear to be adapted to low light, high nutrient conditions (Jordan *et al.*, 1992), or may even be heterotrophic (Brand, 1994).

Our analysis suggests that the three varieties and six size groups of *F. profunda* may have a widespread geographic distribution in the Atlantic and Pacific Oceans. They co-occur in single samples and exhibit similar seasonal distributions and ecologies at the HOT time series station, suggesting that they are not ecophenotypes of a single species. Such a hypothesis may be tested in laboratory culture under varying environmental conditions (Bollmann, 1997; Quinn *et al.*, 2003) or with molecular genetics (Sáez *et al.*, 2003). However, despite our recent attempts *F. profunda* has not yet been successfully isolated and maintained in culture. In fact, only a small proportion (ca. 20%) of all living coccolithophore species have so far been cultured (Probert & Houdan, 2004) and the taxonomy of this phytoplankton group therefore remains mainly based on the morphology of their calcareous skeletons. Nevertheless, the application of new techniques for the combined morphometric and molecular genetic analysis of microscopic plankton (de Vargas *et al.*, 2004) is likely to improve this situation.

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