Analysis of crystallographic directions in *Florisphaera profunda, Braarudosphaera bigelowii* and Neogene discoasters: preliminary report on nannolith crystallography

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Abstract Selected-area electron diffraction (SAED) was combined with high-resolution transmission and scanning electron microscopic analyses to determine the crystallography of three nannoliths, *Florisphaera profunda, Braarudosphaera bigelowii* and *Discoaster*. *F. profunda* produces pentagonal-shaped plates whose [0001] direction (*c*-axis) is oriented parallel to its surface and in the elongation axis of the plate-like element. *B. bigelowii* produces thick, pentagonal plates, composed of five trapezoidal crystals with the *c*-axis oblique to the crystal plane. This species is characterised, crystallographically, by the development of cleavage faces, called 'laminae'. Conversely, *Discoaster* specimens are star-shaped calcite crystals that are composed of several bent, bar-shaped calcite elements, called rays. Based on SAED observations and calcite decoration experiments, *Discoaster* rays are single calcite crystals. The [0001] direction is likely perpendicular to the tangential plane of the central area, and each ray joins in the plane that is parallel to [0001].

   The difference in the crystallographic nature between nannoliths and heterococcoliths suggests that the phylogenetic origin, and the evolutionary history, of nannolith-producing phytoplankton are completely different from those of heterococcolith-bearing coccolithophores.

Keywords Crystallography, nannolith, *Florisphaera profunda*, *Braarudosphaera bigelowii*, *Discoaster brouweri*, *Discoaster variabilis*

1. Introduction

The crystallographic nature of calcareous nannofossils has been investigated by various authors, in order to understand the ultrastructure of nannofossils, their growth, biomineralisation and phylogenetic relationships (*e.g*. Mann & Sparks, 1988; Young *et al*., 1992, 1999, 2005; Didymus *et al*., 1994; Davis *et al*., 1995; Young & Henriksen, 2003; Henriksen *et al*., 2004). There are some different crystallographic types among nannofossils, based on variations in crystal shape with various optical orientations, and combinations of crystals (*e.g*. Braarud *et al*., 1955). Calcareous nannofossils that were produced by ancestral organisms of cocccolithophores are called coccoliths (Huxley, 1858). They are divided into heterococcoliths and holococcoliths (see Young *et al*., 1999, fig.1), depending on morphology of the shape and size of the calcite crystals (Braarud *et al*., 1955; Young *et al*., 1997, 1999, 2003). Heterococcoliths are formed of crystal-units with complex shapes and sizes, whereas holococcoliths are composed of an assembly of numerous, minute calcite crystals, each with similar shape and size (Braarud *et al*., 1955). According to Young *et al*. (1999), all holococcolith crystallites are simple rhombohedra. Most crystallographic and ultrastructural studies have focused on specimens obtained from wild (oceanic) or cultured coccolithophores, while nannoliths have been much less often studied.

   Nannoliths are distinguished from hetero- and holococcoliths on the basis of crystallography and morphology (*e.g*. Young *et al*., 1999). Phytoplankton that bear nannoliths may be considered similar organisms to coccolithophores, and they may also have appeared in the Late Triassic (*e.g*. Perch-Nielsen, 1985a; Bown & Young, 1997; Bown, 1998). The crystallography of nannoliths has not been investigated in any detail, although nannolith-type taxa are one of the major groups of calcareous nannofossils. Black (1972) estimated crystallographic orientations of some nannoliths (*Discoaster* and *Braarudosphaera*), mainly based on shape and optical orientations, estimated by light-microscopic observations. No clear data, however, have been provided, although crystal faces have been examined on some species of *Discoaster* and *Braarudosphaera*.

   Some nannolith-bearing taxa have characteristic ecologic occurrences, such as being major components of nannofloras in certain geological intervals (*e.g*. Perch-Nielsen, 1985a), or being identified as deeper-dwellers in the photic zone of oceanic surface-waters (*e.g*. Okada & Honjo, 1973). Thus, it is important to study the crystallographic and morphologic structure of nannoliths, because research on their growth-patterns and development may help to clarify the phylogenetic history of nannolith-bearing taxa and their response to climate change. This paper describes the crystallographic orientation of the nannoliths, *Florisphaera profunda*, *Braarudosphaera bigelowii* and two *Discoaster* species, and presents the basic crystallographic structure of these nannoliths.

2. Material and methods

We investigated three types of nannolith, *Florisphaera profunda*, *Braarudosphaera bigelowii* and two Neogene *Discoaster* species, *D. brouweri* and *D. variabilis* (Pl.1,

fig.1). *F. profunda* is well-known as a lower-photic-zone species (Okada & Honjo, 1973), and it has been used to monitor environmental changes in thermocline waters during the latest Quaternary (*e.g.* Molfino & McIntyre, 1990). *B. bigelowii* plates are pentagonal and composed of five conjugate crystals. *Braarudosphaera* was especially abundant shortly after the Cretaceous/Tertiary boundary event and during the Middle Eocene (*e.g.* Perch-Nielsen, 1985b). *Discoaster* species are found in sediments from the Late Paleocene to the latest Pliocene, and generally are considered to be warm-water, oligotrophic taxa, because of their comparatively limited distribution in lower-latitude regions (*e.g.* Haq, 1980).

In this study, we used four deep-sea samples that were obtained off the Philippines (DSDP Leg 31, Samples 292-

Plate 1

SEM micrographs of studied nannoliths

Florisphaera profunda

1μm

Braarudosphaera bigelowii

5μm

central part

ray

Discoaster brouweri

5μm

Discoaster variabilis

5μm

7-CC and 292-8-CC), off Florida (ODP Leg 171B, Sample 1051A-17X-1, 125-127cm), and from the Caribbean Sea (ODP Leg 165, Sample 999A-1H-1, 0-1cm). These samples are predominantly nannofossil/foraminiferal oozes. The preservation of nannofossil specimens is good in the samples from Site 292 and Hole 999A, but moderate to poor in the samples from Hole 1051A.

Samples for scanning electron microscope (SEM) analysis were prepared using a suspension method, with methanol as the liquid: a few drops of this suspension were dispersed on a carbon transmission electron microscope (TEM) grid using a micropipetter. Two microgrids were prepared for each sample, one of which was platinum-coated for SEM observation. Morphological obser-

vations and crystallographic measurements of the nannoliths were carried out on a Hitachi H-7100FA electron microscope. Selected-area electron diffraction (SAED) during TEM analysis was applied to clarify the crystallographic characteristics.

Specimens of *B. bigelowii* and the two *Discoaster* species were too thick to obtain electron diffraction patterns from and, thus, we also used the calcite decoration method, based on the epitaxial growth of crystals, of Okazaki & Inoué (1976). For decoration with calcite crystals, a few milligrams of samples were immersed for a few minutes in 1ml of $NaHCO_3$ (0.1M), and then ultrasonicated for a few seconds. After that, 1ml of $CaCl_2$ (0.1M) was added. In this case, a specimen becomes fatter and acquires jagged edges within several minutes. The crystallographic orientation and optical axes of these epitaxic overgrowths on a specimen are the same as the underlying nannofossil crystal.

Photographs of electron diffraction patterns were processed on a personal computer. Measurements of the lengths and angles of spots were made using the public domain software NIH-IMAGE. Subsequently, the crystallographic indices of each species were determined using the software '*Recipro*'.
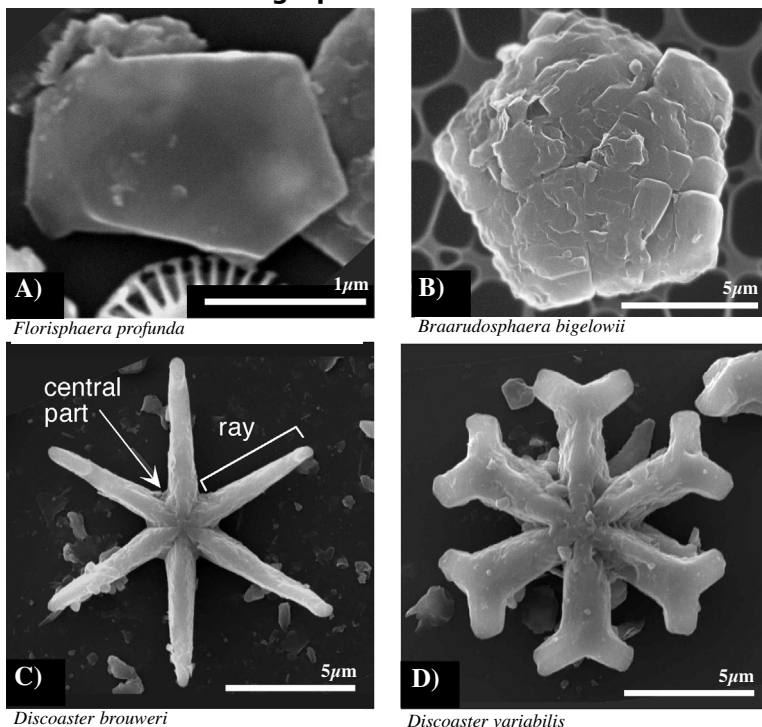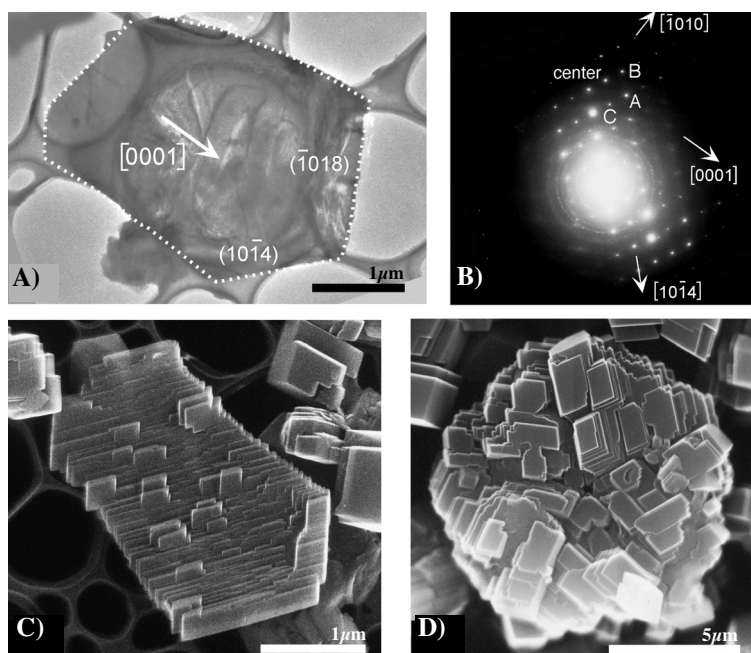
3. Results and discussion

Florisphaera profunda is composed of a slightly bent, elongate five-sided crystal, 1-2μm long and approximately 20nm in thickness (Plate 1). Based on our analysis of the electron diffraction pattern, the *c*-axis is parallel to the longitudinal direction and the plane of a specimen nearly corresponds to $(11\bar{2}0)$ with $(10\bar{1}4)$ and $(\bar{1}018)$ faces at one side of the end (Figure 1). The diffraction pattern is quite clear and there is no streak on spots shown in the pattern. In our crystal decorating experiments, many tiny, sheet-like crystals were grown in a step-like arrangement. The faces on all overgrowth crystals are parallel, so we can infer that there is no change in the *c*-axis direction, even though the specimen is bent. Hence, the apparent 'bending' must be a result of step-wise growth in the calcite crystals, rather than accumulated lattice defects.

Living cells of *Braarudosphaera bigelowii* are enclosed in a dodecahedral shell formed of 12 pentagonal plates (*e.g.* Young *et al.*, 2003). A fossil usually is a single pentagonal plate, called a pentalith (Gran & Braarud, 1935; Plate 1). A single pentalith consists of five thick, trapezoidal segments. Although it is difficult to obtain electron diffraction patterns from *B. bigelowii* plates, a trapezoidal face of the segment corresponds to a cleavage face, $(10\bar{1}4)$, based on calcite decoration (Figure 1). The *c*-axis of each segment obliquely directs to the trapezoidal

Figure 1: Results of crystallographic analysis of *F. profunda* and *B. bigelowii*. **A**) TEM micrograph of *F. profunda*; **B**) its electron diffraction pattern corresponds to the [1$\bar{2}$10] direction. Reflection A = (0006) (2.86Å); B = ($\bar{1}$012) (3.86Å); C = (10$\bar{1}$4) (3.03Å). Angles: [0001] ^ [$\bar{1}$010] = 90˚; [0001] ^ [10$\bar{1}$4] = 45˚; [10$\bar{1}$4] ^ [$\bar{1}$018] = 68˚. **C**) Calcite decoration on a specimen of *F. profunda*. **D**) Calcite decoration on a specimen of *B. bigelowii*

direction (Figure 2). It indicates that the *c*-axis is closely perpendicular to the ray extension. Our crystal decoration experiments on a *Discoaster* specimen show that the *c*-axis is oriented perpendicularly to the tangential plane of the central area (Figure 2). Thus, the *c*-axis for *Discoaster* specimens corresponds to the direction of the 'handle' of the 'umbrella' (Figure 3), and each ray extends perpendicularly to the direction of [0001] at the central part of the specimen, and bends down toward the ray-tip. Moreover, the crystallographic direction of each decorating calcite crystal on a specimen is completely the same from the central part to the ray-tip, and so a ray is composed of a single calcite crystal, even in *D. brouweri* and *D. variabilis*. The rays of a *Discoaster* specimen show pentagonal and/or hexagonal symmetry, depending on the number of rays, and, in every case, each ray joins together in the plane parallel to [0001]. However, it is not clear whether the crystallographic relationship between rays corresponds to a twin structure.

Black (1972) estimated crystallographic faces of some species of *Discoaster* and *Braarudosphaera*, based on their shape under the SEM, and their optical orientations, using light-microscopic observations. Our study mostly confirms Black's
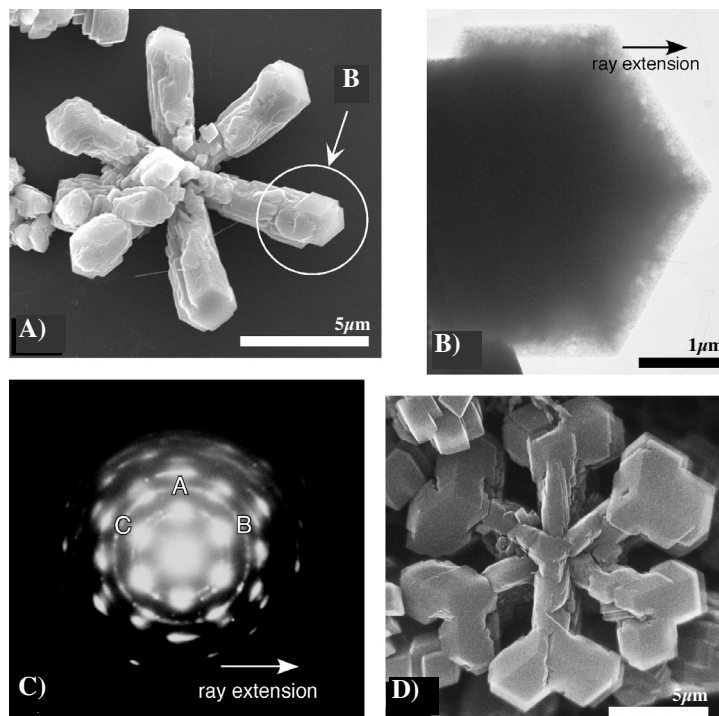
face and its angle is approximately 45˚. The contact face of each segment is perpendicular to the trapezoidal face and, thus, a step-wise growth of calcite crystals is again likely.

Discoaster nannoliths are composed of several radially-spreading, bending, pillar-like calcite crystals, called rays, similar to spreading umbrella-ribs without the handle (Pl.1, fig.2). The morphology of discoasters is more complex than that of *F. profunda* and *B. bigelowii*, even though they are all nannoliths. It is especially difficult to find any clear calcite crystal faces on a specimen, whereas an element of *F. profunda* and a segment of *B. bigelowii* apparently consist of some clear crystal faces. Numerous *Discoaster* species have been defined, based on variation in the number of rays, central-structures, and ray-tip structures (*e.g.* Aubry, 1984). *Discoaster brouweri* is one of the simplest discoasters; it has a slightly bent star-shape with sharp tips (Figure 2). *D. variabilis* is a more complex, six-rayed *Discoaster*, characterised by bifurcated tips at the end of each ray.

Electron diffraction patterns were obtained only around the ray-tip of *Discoaster* specimens (Figure 2), which is the thinnest part of the ray, because the main part of the ray is too thick (200-300nm) to be examined by electron diffraction. Single crystal diffraction patterns from the proximal side clearly exhibit hexagonal symmetry, which corresponds to the [0001]

Figure 2: Results of crystallographic analysis of *D. brouweri* and *D. variabilis*. **A**) Calcite decoration on a specimen of *D. brouweri*. **B**) TEM micrograph of a ray-tip of the *D. brouweri* shown in (A). **C**) Crystal diffraction pattern of a ray-tip of *D. brouweri* corresponds to the [0001] direction at the end of a ray. Reflection A = (1$\bar{2}$10) (2.49Å); B = (2$\bar{1}$10) (2.49Å); C = ($\bar{1}$$\bar{1}$20) (2.49Å). Angles: [1$\bar{2}$10] ^ [2$\bar{1}$10] = 60˚; [1$\bar{2}$10] ^ [1$\bar{1}$20] = 60˚. **D**) Calcite decoration on a specimen of *D. variabilis*
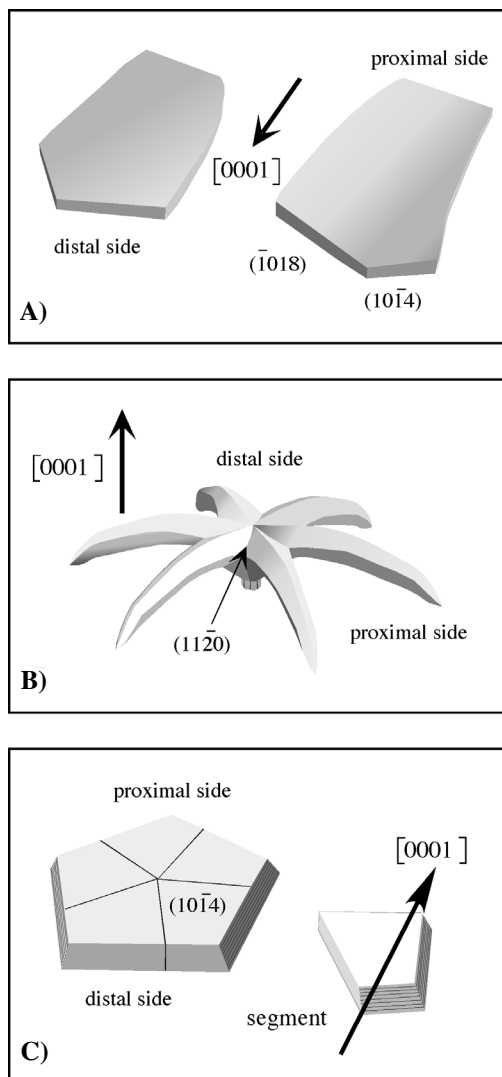
Figure 3: Schematic illustrations of three nannoliths with crystallographic orientations of a single crystal. **A)** *F. profunda*; **B)** *D. brouweri*; **C)** *B. bigelowii*

(1972) results and provides some important evidence for crystal orientation of Neogene *Discoaster*s and *B. bigelowii*.

Young *et al.* (1992) proposed the V/R model as the fundamental crystallographic structure of all heterococcoliths, based on detailed studies of *Emiliania huxleyi* (Mann & Sparks, 1988; Westbroek *et al.*, 1989; Young & Westbroek, 1991), ontogenetic study of Jurassic *Watznaueria* (Young & Bown, 1991), and comparative studies of numerous other coccoliths. In heterococcoliths, growth begins from a chain of small, rhombohedral calcite crystals, called a proto-coccolith ring (Young, 1989), with the *c*-axes oriented subradially (R-units) and subvertically (V-units), relative to the coccolith plane (Young *et al.*, 1992). The R-units or V-units can overgrow the others, and a single shield of a heterococcolith may be composed of R-units or V-units, depending on the family/genus. However, it is impossible to apply the V/R model to the development of nannoliths, because they are typically composed of a single cycle of crystal units (*e.g. Discoaster*, *Braarudosphaera*), or of a single calcite crystal (*e.g. Florisphaera*, *Ceratolithus*). Moreover, a single crystal comprising these nannoliths resembles neither the V- nor R-unit crystals of heterococcoliths.

As a result, there is a significant crystallographic difference between nannoliths and heterococcoliths; generally, nannoliths are composed of single crystals with a unique crystallographic orientation, and the growth-pattern seems to be completely different. Originally, 'nannolith' was a general descriptive term for neither heterococcoliths nor holococcoliths (Young *et al.*, 1997, 2003), and so phytoplankton groups producing nannoliths seemed to be polyphyletic (Young & Henriksen, 2003; Young *et al.*, 2005). Some nannoliths are considered to be produced as an alternate phase in the life-cycle of haptophyta (Young & Henriksen, 2003; Young *et al.*, 2005), specifically *Ceratolithus-Neosphaera* and *Alisphaera-Polycrater*. Significant differences in the crystallographic nature of *Florisphaera*, *Discoaster* and *Braarudosphaera* compared to heterococcoliths, as demonstrated herein, indicate that their phylogenetic origin is quite different from that of coccolith-producing organisms. Obviously, a new model for nannolith development is needed.

4. Conclusions

Crystallographic analyses of nannoliths were carried out to determine the optical directions of the crystals of three nannoliths, *Florisphaera profunda*, *Braarudosphaera bigelowii* and discoasters. A pentagonal-shaped *F. profunda* lith is a plate-like nannolith with [0001] direction (*c*-axis) parallel to its surface and along the long axis. A pentagonal crystal of *B. bigelowii* (pentalith) is composed of five trapezoidal crystals, with the *c*-axis oblique to the pentalith plane and characteristically developing cleavage faces. On the other hand, a star-shaped *Discoaster* is composed of several bent, bar-shaped calcite elements, called rays. Results of SEM observation and calcite decoration on specimens indicate that each ray is composed of a single calcite crystal. The [0001] direction is likely perpendicular to the tangential plane of the central area.

These results indicate that the phylogenetic origin and evolutionary history of nannolith-producing phytoplankton are different from those of heterococcolith-bearing coccolithophores.

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