



HOKKAIDO UNIVERSITY

Title	Intra- and infra-specific morphological variation in selected coccolithophore species in the equatorial and subequatorial Pacific Ocean
Author(s)	Hagino, Kyoko; 萩野, 恭子; Okada, Hisatake
Citation	Marine Micropaleontology, 58(3), 184-206 https://doi.org/10.1016/j.marmicro.2005.11.001
Issue Date	2006-01-30
Doc URL	https://hdl.handle.net/2115/5820
Type	journal article
File Information	MB58-3.pdf



**Intra- and Infra-specific morphological variation in selected coccolithophore
species in the equatorial and subequatorial Pacific Ocean**

Kyoko Hagino^{1*} and Hisatake Okada¹

¹ Division of Earth and Planetary Sciences, Graduate School of Science, Hokkaido
University, N10 W8, Sapporo, 060-0810 Japan

Phone: +81-11-706-4498, Fax: +81-11-746-0394,

E-mail: hagino@nature.sci.hokudai.ac.jp

* Corresponding author: Kyoko Hagino

Abstract

The ecological preferences of morphological groups within major coccolithophore taxa were studied in surface water samples from the equatorial and subequatorial Pacific Ocean. *Emiliana huxleyi* was subdivided into three morphological groups: Type A, Type C, and variety *corona*. The most probable factors limiting the occurrence of *E. huxleyi* Types A and C were high temperatures and low nutrient concentrations, respectively. *Emiliana huxleyi* var. *corona* had an affinity for oligotrophic conditions. *Calcidiscus leptoporus* ssp. small was adapted to fertile waters. *Umblicosphaera foliosa* and *Umblicosphaera sibogae* preferred mesotrophic upwelling waters and stratified marginal waters surrounding the upwelling front, respectively. Among the three *Umbellosphaera tenuis* morphotypes observed in this study (Types I, III, and IV), only Type I was found in very warm tropical surface. Both Types III and IV were found in subtropical waters, and Type III differed from Type IV in that its distribution was constrained to hemi-pelagic waters. Habitat segregation among the morphotypes of major taxa indicates that the observed global distributions of

these major taxa are, in fact, combinations of discrete morphological groups.

Key words; coccolithophore, extant, morphological variation, biogeography, *Emiliana huxleyi*, *Calcidiscus leptoporus*, *Umbilicosphaera*, *Umbellosphaera*.

1. Introduction

In a typical coccolithophore population, only 1-5 abundant taxa represent over 60% of the assemblage, while the remainder is composed of 10-40 rare taxa, each of which contribute to less than 5% (e.g., Hagino and Okada, 2004; Thierstein et al., 2004). Most studies of living coccolithophores refer to the abundant taxa as major taxa, and focus on their ecology. The composition of the major taxa differs among water masses. Subpolar assemblages are dominated by *Emiliana huxleyi*, and often contain *Coccolithus pelagicus*. The surface assemblages in oligotrophic warm waters consist of abundant *Umbellosphaera irregularis* or *Umbellosphaera tenuis*. In warm eutrophic

waters, *Calcidiscus leptoporus*, *E. huxleyi*, or *Gephyrocapsa* spp. comprise the greater part of the flora (e.g., Jordan and Chamberlain, 1997; Hagino and Okada, 2004). Most of these major taxa display intraspecific morphological variation in their coccoliths, and are subdivided into morphological groups (e.g., Young et al., 2003).

Extensive distributions of morphological groups of *Gephyrocapsa* spp. and *Calcidiscus leptoporus* have been documented in the Atlantic Ocean. For example, Bollmann (1997) classified medium-sized *Gephyrocapsa* from marine surface sediments into five morphotypes with discrete environmental preferences. Extant *Calcidiscus leptoporus* can be subdivided into at least three size groups: large, intermediate, and small forms. The intermediate form is dominant while the large form tends to be more abundant in warm waters (e.g., Knappertsbusch et al., 1997; Ziveri et al., 2004). In the Pacific Ocean, the detailed distribution of morphological subgroups has not been studied, even though the morphotype assignments of selected species have been examined in several studies. McIntyre et al. (1970) showed horizontal distributions of morphotypes of *Calcidiscus leptoporus* over the entire Pacific. Okada and Honjo (1973b) determined the morphotype assignments of *E. huxleyi* and *Calcidiscus*

leptoporus in the central North Pacific Ocean. Hagino and Okada (2004) separated *E. huxleyi*, *Calcidiscus leptoporus*, *Umbellosphaera irregularis* and *Umbellosphaera tenuis* into several morphological groups during their original floral observations, but combined the groups at the species level in their floral analysis.

Recent studies have revealed that morphological variation observed in the major species often reflects genetic differences. *Emiliana huxleyi* Types A and B are distinguishable not only by coccolith morphology, but also by immunological responses (Young and Westbroek, 1991; Medlin et al., 1996). Also Schroeder et al. (2005) discovered a genetic marker to separate *E. huxleyi* Type A from Type B. The largest form of *Calcidiscus* ($> 8 \mu\text{m}$) is distinguishable from smaller forms in the life-cycle association with holococcoliths, and by molecular genetics; therefore, it has been raised to species rank as *Calcidiscus quadriperforatus* (Geisen et al. 2002; Sáez et al. 2003). Extant Coccolithus consist of two morphological groups that can be differentiated by size, and ecology, and which produce different holococcoliths during the haploid phase of the life-cycle (e.g., Cachao and Moita, 2000; Geisen et al., 2002). Molecular phylogenetic studies have shown a sufficient number of substitutions in the base

sequences of these morphological groups to raise them to species rank, with the large temperate groups as *Coccolithus braarudii* and the small sub-polar groups as *Coccolithus pelagicus* (Sáez et al., 2003; Geisen et al., 2004). Sáez et al. (2003) have concluded that *Umbilicosphaera foliosa*, which has often been classified as a variety of *Umbilicosphaera sibogae*, is a discrete species, based on differences in morphology and molecular phylogenetics. These results suggest that the traditional species-level classification is too coarse to recognize true coccolithophore biodiversity, and a more refined taxonomy is required to discuss the ecology of coccolithophores.

Here we present information on the horizontal distributions of morphological groups observed in *Emiliana huxleyi*, *Calcidiscus leptoporus*, *Umbilicosphaera foliosa*, *Umbilicosphaera sibogae*, *Umbellosphaera irregularis*, and *Umbellosphaera tenuis* in the equatorial-subequatorial Pacific Ocean by combining the published quantitative data by Okada and Honjo (1973b), and unpublished data obtained during the studies for Hagino and Okada (2004). The aim of this study is to reveal the habitat preferences of the morphological groups for each major species in order to understand true coccolithophore ecology.

2. Oceanographic setting

The equatorial and subequatorial Pacific (20° N-20° S) is characterized by seven major surface currents: westward-flowing North and South Equatorial Currents (NEC and SEC), eastward-flowing North and South Equatorial Counter Currents (NECC and SECC), western boundary flows of the Philippines and East Australian Currents, and the eastern boundary Peru/Chile Current (Fig. 1). The NEC is the south boundary current of the North Pacific subtropical Gyre. The strong westward flows of the NEC and SEC arrive at the Philippines and Indonesian archipelago, and amasses warm, less saline surface waters in the western Pacific, forming the extremely warm and oligotrophic Western Pacific Warm Pool (WPWP; Fig. 2; Tomczak and Godfrey, 1994). In contrast, in the central and eastern equatorial Pacific, the surface water is relatively cool and remarkably eutrophic because of the Equatorial Divergence (upwelling). Moreover, a strong coastal upwelling off the coast of Ecuador and Peru supplies

nutrient-rich, cold, deep water to the surface of the Eastern Pacific (Figs. 2c-d). Consequently, the equatorial Pacific varies longitudinally in sea level, sea surface temperature, surface nutrient concentration, and intensity of water stratification (Brown et al., 1989).

3. Overview of Surface Flora in the equatorial and subequatorial Pacific

Hagino and Okada (2004) have documented the distribution of living coccolithophores in the surface waters of the equatorial and subequatorial Pacific by combining data from Okada and Honjo (1973b) with their new floral data (Fig. 3a). Based on Q-mode Cluster analysis and floral composition, they identified three main assemblages that included seven sub-assemblages: *Umbellosphaera irregularis* common assemblages (UCA-a, UCA-b, UCA-c), *Gephyrocapsa oceanica* common assemblages (GCA-a, GCA-b), and *Emiliana huxleyi* common assemblages (ECA-a, ECA-b) (Table 2). All the sub-assemblages proposed by Hagino and Okada (2004) are pelagic, except

for ECA-b. The floral composition is controlled by water temperature and nutrient concentration, and the six pelagic sub-assemblages display zonal distributions, with ECA-a as a center (Fig. 3b). ECA occurs in moderately warm waters ($< 28^{\circ}\text{C}$), regardless of nutrient level. In warm conditions ($> 28^{\circ}\text{C}$), GCA appears to prefer eutrophic-mesotrophic waters, while UCA occurs in oligotrophic waters. Floral seasonality is observed in the north subequatorial Pacific ($10\text{-}20^{\circ}\text{ N}$), with a floral change from UCA-c in winter to UCA-b summer, but no such seasonality is obvious in the equatorial waters ($10^{\circ}\text{ N-}10^{\circ}\text{ S}$; Hagino and Okada, 2004).

4. Materials and Methods

All surface water samples studied by Hagino and Okada (2004) were collected using a bucket (Fig. 3a). After pre-filtration through $63\mu\text{m}$ metal sieve, the water samples were filtered on-board through a Millipore filter with a pore size of 0.45 or $0.8\ \mu\text{m}$. The in situ sea surface temperature (SST), salinity, and nutrient concentration

were measured for selected samples during Cruise KH69-4 (Marumo, 1970), and for all samples on Cruises KH90-3 and KH92-4 (Nozaki et al., 1990, 1992). However, only SST was measured at the sampling time for samples on Cruises Conrad 9-12 and Vema 24. For light microscopy an elongate strip running from the center to the rim of the filter was cut out, and was rendered transparent with a drop of immersion oil. The total coccolithophore cell density was estimated by counting the number of coccospheres on 0.6-3.6 mm² of the filter corresponding to > 5ml of water sample in a cross-polarized microscope with a calculation expressed as follows:

$$\text{Coccolithophore concentration (no./litre)} = F \cdot C / V \cdot A$$

where F= effective filtration area (mm²), C = number of coccosphere encountered, V= filtered water volume, and A= investigated filter area (mm²).

Another portion of the filter (ca. 7 x 7 mm) was mounted on a brass stub and sputter coated with gold for observation under a scanning electron microscope (SEM). The morphotype composition of the major species was recorded during species identification by Okada and Honjo (1973b) and Hagino and Okada (2004). The definition of each morphotype is discussed in the following sections. Cell density of

each morphotype in sample was calculated based on the cell density of total coccolithophores and relative abundance of each morphotype in the total coccolithophore assemblages.

5. Results and Ecological Interpretation

The morphotype classification of the major species has been greatly revised in the last decade; thus, some morphotypes identified by Okada and Honjo (1973b) are no longer valid. Here, we use the quantitative data on morphotypes presented in Okada and Honjo (1973b) only when the morphotypes are comparable to those from more recent studies. Therefore, different numbers of samples were studied for each morphological group (Table 2). The ecology of each morphotype was analysed based on the relationship between the cell density of each morphotype, the in situ SST, and mean annual salinity, phosphate, and nitrate concentrations. However, preliminary plotting of the data revealed no significant trend between morphotype cell density and salinity, so

salinity was not considered further.

5-1. *Emiliana huxleyi*

Emiliana huxleyi consists of at least four well-established morphological groups: Type A (i.e., the “warm water” type of McIntyre and Bé, 1967), Type B (i.e., the “subarctic” type of Okada and Honjo, 1973a), and Type C (i.e., the “cold water” type of McIntyre and Bé, 1967), and *E. huxleyi* variety *corona* (*Emiliana* species ‘a’ of Okada and Honjo, 1973b) (Okada and McIntyre, 1977; Young et al., 2003; Hagino et al., 2005).

Emiliana huxleyi Type A and var. *corona* can easily be identified based on morphological observations under an SEM. However, identification of Types B and C are rather difficult. Both Types B and C possess a fragile distal shield, and Type B differs from Type C in that it possesses a relatively large distal shield consisting of elevated shield elements (Young and Westbroek, 1991; Hagino et al., 2005). In addition, transitional forms between Types B and C have been reported (Young et al., 2003; Hagino et al., 2005). Medlin et al. (1996) confirmed the observation of Young and Westbroek (1991) that cultured strains of types A and B maintained their morphology

and concluded that they must be distinct genotypes. They emended Types A, B, and C as varieties *huxleyi*, *pujosiae*, and *kleijneae*, respectively. However, the identification of the transitional forms between Types B and C causes problems and the transitional forms lack formal names. The samples examined here contained various transitional forms between Types B and C. Therefore, to avoid further classification confusion, we followed Young et al. (2003) for the classification of *E. huxleyi* morphotypes.

We subdivided *E. huxleyi* into three morphological groups: Type A, Type C, and var. *corona* (Plate I). We identified all *E. huxleyi* specimens with fragile distal shield elements and a solid/open central area as Type C without using morphometric measurements; thus, our Type C included variants of Types B/C. We note that the Type R and over calcified forms of Type A (Young et al., 2003) were not found in this study. Okada and Honjo (1973a, b) found Type A (their warm type), Type B (their subarctic type), Type C (their cold type), and var. *corona* (their species 'a') in samples collected during Cruise KH69-4. However, in quantitative analyses, they distinguished only 'var. *corona*' from the others, and combined Types A-C at the species level as *E. huxleyi*. Therefore, quantitative data for *E. huxleyi* morphotypes was only available for var.

corona for the samples from Cruise KH64-2. As a result, the number of samples included here for *E. huxleyi* Type A, Type C, and var. *corona* was 124, 124, and 227, respectively (Table 2).

Emiliana huxleyi Type A was distributed over the entire equatorial and subequatorial Pacific surface, except in the surface waters of the western equatorial Pacific (Fig. 4a). The highest concentration of Type A (4.9×10^4 cells/L) was observed at station 125 of Conrad 11. Type A cell density was usually high ($\geq 1.0 \times 10^4$ cells/L) in the eastern equatorial Pacific, moderate ($\geq 1.0 \times 10^3$ cells/L) in the hemi-pelagic waters off Peru and Australia, and relatively low ($< 1.0 \times 10^3$ cells/L) in the north subtropical Pacific (10-20° N). Its relative abundance exceeded 60% at the hemi-pelagic stations, but was usually lower than 50% in the open ocean (Fig. 4a). It was absent from extremely warm waters ($\geq 29^\circ\text{C}$), although it occurred rarely (340-630 cells/L) in two samples collected from extremely warm waters (30.3°C; Fig. 5a). In warm oligotrophic-mesotrophic waters (27-29°C, $\text{PO}_4 < 0.4 \mu\text{mol/L}$; $\text{NO}_3 < 2 \mu\text{mol/L}$), Type A was mostly common, with a low cell density of $< 1.0 \times 10^3$ cells/L. In moderately warm waters ($< 27^\circ\text{C}$), it occurred consistently, regardless of nutrient concentration, and it

increased in abundance in response to both decreasing temperature and increasing nutrient concentrations. These results suggest that high temperature, rather than nutrient depletion, constitutes a limiting factor for the occurrence of *E. huxleyi* Type A in the study area, although both temperature and nutrient concentration affect its cell density.

Emiliana huxleyi Type C was common in the eastern equatorial Pacific, whereas it was absent or rare in the western equatorial to subequatorial Pacific, and the central north subequatorial Pacific (Fig. 4b). The highest cell density of this morphotype (2.1×10^5 cells/L) occurred at station 134 of Conrad 11, off Ecuador. At this station, Type C comprised 86.8% of the total coccolithophore flora. It occurred almost consistently in mesotrophic and eutrophic waters ($\text{PO}_4 \geq 0.4 \mu\text{mol/L}$; $\text{NO}_3 \geq 0.1 \mu\text{mol/L}$), and was absent or rare ($< 10^3$ cells/L) in oligotrophic conditions ($\text{PO}_4 < 0.2 \mu\text{mol/L}$; $\text{NO}_3 < 0.05 \mu\text{mol/L}$), regardless of temperature. These results suggest that the depletion of nutrients not high temperature affect the occurrence of *E. huxleyi* Type C in the study area, although both temperature and nutrient concentration may affect its cell density.

Emiliana huxleyi var. *corona* exhibits transitional morphological characters between *E. huxleyi* Types A and B/C, although it is different from Types A and B/C in

the possession of an elevated central collar (Plate I). Its central area is composed of curved elements, similar to Type A, while the size-range of its distal shield (3.5-4.5 μm) overlaps that of Type B/C. *E. huxleyi* var. *corona* differed from the other types in its distribution; it was restricted to the western and central north subequatorial waters (10-20° N), and to the Coral Sea off Australia (Fig. 4c). Its cell density was consistently < 350 cells/L, and its maximum relative abundance was only 6.0%. It was absent from very warm (> 30°C) and temperate (< 24°C) waters, and from mesotrophic-eutrophic waters regardless of water temperature ($\text{PO}_4 > 0.4 \mu\text{mol/L}$; $\text{NO}_3 > 1 \mu\text{mol/L}$; Fig. 5c). Thus, *E. huxleyi* var. *corona* has an affinity for oligotrophic conditions, as noted by Cortes et al. (2001).

5-2. *Calcidiscus leptoporus*

Lohmann (1920) first reported morphotypes of *Calcidiscus leptoporus*. McIntyre et al. (1970) subdivided the extant *Calcidiscus leptoporus* into Types B and C based on the number of distal shield elements; however, they only gave the average number of elements and did not display the range of variation in the number of shield

elements for each morphotype. Kleijne (1993) subdivided living *Calcidiscus leptoporus* into three groups: Type A (3-4.9 μm), Type B (7.5-9.6 μm), and Type C (4.9-7.2 μm). She also stated that the largest form (her Type B) is characterized by obscure central-area elements. Knappertsbusch et al. (1997) identified three morphoclines: small, intermediate, and large, separated at coccolith diameters of 5 and 8 μm . The intermediate morphocline (5-8 μm) dominates *Calcidiscus* populations in all oceans, except the eastern equatorial Pacific, which is characterized by the dominance of the small morphocline ($< 5 \mu\text{m}$; note that the legends of $\leq 5 \mu\text{m}$ and 5-8 μm in Fig. 10 of Knappertsbusch et al., 1997 should be inverted; Knappertsbusch, pers. comm.).

The large and intermediate forms of *Calcidiscus leptoporus* have different life-cycle associations, with distinctly different holococcoliths during the haploid phase. The large forms produce holococcoliths with high walls and internal septa formerly regarded as a separate species *Syracolithus quadriperforatus*. By contrast the intermediate forms produce flat holococcoliths formerly classified as *Crystallolithus rigidus* (e.g., Kleijne, 1991; Cortes, 2000; Geisen et al., 2002). Molecular phylogenetic studies show a large number of base sequence substitutions between the large and

intermediate forms (Sáez et al., 2003). Consequently, the large morphotype of *Calcidiscus leptoporus* ($> 8 \mu\text{m}$) was emended as *Calcidiscus quadriperforatus* (Sáez et al., 2003). The size range of *Calcidiscus quadriperforatus* slightly overlaps that of *Calcidiscus leptoporus* ssp. *leptoporus*. Therefore, morphological observation of the central-area elements is essential to distinguish these forms (Quinn et al., 2004). *Calcidiscus leptoporus* ssp. *small* ($< 5 \mu\text{m}$) is probably a discrete species from *Calcidiscus leptoporus* ssp. *leptoporus* (5-8 μm). At present, it is classified as *Calcidiscus leptoporus* because the particular evidence by which it may be differentiated from *Calcidiscus leptoporus* ssp. *leptoporus* (5-8 μm) has not yet been found, owing to the lack of a culture strain (Quinn et al., 2004).

Okada and Honjo (1973b) identified Types B and C of *Calcidiscus leptoporus* following the classification scheme of McIntyre et al. (1970). However, these *Calcidiscus leptoporus* morphotypes are no longer valid because McIntyre et al. (1970) only determined the average size of the distal shield elements for each morphotype, and not the range in sizes. Therefore, the data on *Calcidiscus leptoporus* morphotypes in Okada and Honjo (1973b) were excluded from this study (Table 2).

Instead, we subdivided specimens of *Calcidiscus* into three size-groups, small ($< 5 \mu\text{m}$), intermediate (5-8 μm), and large ($> 8 \mu\text{m}$) forms, but without morphological observation of the central area (Plate II). Therefore, our intermediate form may included both *Calcidiscus leptoporus* ssp. *leptoporus* and relatively small specimens of *Calcidiscus quadriperforatus*.

In the equatorial-subequatorial Pacific, the small form was the most common form *Calcidiscus*. It was abundant (up to 1×10^5 cells/L) in the eastern equatorial to south subequatorial Pacific, rare (< 500 cells/L) in the central equatorial Pacific, and absent from the entire north subequatorial Pacific (10-20° N) and western equatorial-subequatorial Pacific (Fig. 6a). The abundance of the small form in the eastern equatorial Pacific was consistent with the results of previous studies (Knappertsbusch et al., 1997; Broerse, 2000). Comparisons between the cell density of the small form and hydrographic parameters showed that this form had an affinity for eutrophic conditions (Fig. 7b). It was mostly absent from oligotrophic waters ($\text{PO}_4 < 0.3 \mu\text{mol/L}$; $\text{NO}_3 < 1 \mu\text{mol/L}$), regardless of water temperature. Its cell density was consistently $< 5.0 \times 10^2$ cells/L in warm waters ($> 27^\circ\text{C}$), although it could persist at

high temperatures ($> 30.0^{\circ}\text{C}$) if nutrients were available.

The occurrence of intermediate specimens was rather sporadic. The cell density of this form was consistently < 610 cells/L in the study area (Fig. 6b). The highest in situ SST of the samples in which the intermediate form was found was 27.8°C (Fig. 7b). It seems that the depletion of nutrients ($\text{PO}_4 < 0.2 \mu\text{mol/L}$; $\text{NO}_3 < 1.0 \mu\text{mol/L}$) constitutes the limiting factor of occurrence of the intermediate specimens although the presence of nutrient not always induces the occurrence of them. The large form was found at a detectable relative abundance (i.e., $> 0.3\%$) at only one station (AQ11 of Cruise KH90-3; Fig. 6c). At this station, the absolute and relative abundances of the large form were 166 and 1.7%, respectively.

5-3. *Umblicosphaera foliosa* and *Umblicosphaera sibogae*

Umblicosphaera foliosa was originally described as *Cycloplacolithus foliosus* by Kamptner (1963). After Okada and McIntyre (1977) combined it into *Umblicosphaera sibogae*, it was usually identified as *Umbellosphaera sibogae* var. *foliosa* until Sáez et al (2003) re-raised it to species rank, based on morphological

differences, morphological stability in culture and molecular phylogenetic differences. However, this species has been consistently distinguished from *Umbilicosphaera sibogae* in numerous studies of plankton conducted over the past quarter century, despite the confusion in taxonomy, since despite the similarity of the coccoliths the coccospheres are very different. The extent of the distribution of *Umbilicosphaera foliosa* and *Umbilicosphaera sibogae* in the Pacific is not well known because these two species are mainly found in warm open ocean, and are not easily collected over a sufficient geographic extent.

Okada and Honjo (1973b) differentiated *Umbilicosphaera foliosa* (their *Umbilicosphaera sibogae* var. 'a') from *Umbilicosphaera sibogae* (their *Umbilicosphaera sibogae* var. 'b'); therefore, the abundance of these two species was available in all 229 samples collected during the eight cruises (Table 2, Fig. 3a, Plate III). The distributions of these species overlapped, but were concentrated in different areas. *Umbilicosphaera foliosa* was common in the central equatorial Pacific, and occurred sporadically in the hemi-pelagic waters off Australia and the American continents (Fig. 8a). The absolute and relative abundances of this species were

consistently $< 1.0 \times 10^3$ cells/L and 6.0%, respectively. There was no clear relationship between cell density and either temperature, or nutrient concentration (Fig. 9a).

Umblicosphaera sibogae displayed different patterns of occurrence on the western and eastern sides of the Date Line. On the western side, it occurred near the Equator, but was absent from the subequatorial zones, except in the Coral Sea off Australia. On the eastern side, however, it was absent or rare near the equator, and was abundant from 5-13° N and from 8-12° S (Fig. 8b). The highest concentration of this species (4.0×10^3 cells/L) occurred at station 129 of Conrad 11, where its relative abundance was also highest (47.7%; Fig. 8b). Its absolute abundance was high ($> 1.0 \times 10^3$ cells/L) in the subequatorial samples where the mean annual phosphate and nitrate concentrations ranged from 0.3-0.7 and 0.5-6.1 $\mu\text{mol/L}$, respectively. However, it was rare or absent in the most eutrophic waters of the central-eastern equatorial Pacific (Figs, 8b and 9b).

The habitat preferences of *Umblicosphaera foliosa* and *Umblicosphaera sibogae* have been examined in several studies. Kleijne (1993) recorded a decreasing abundance of *Umblicosphaera sibogae* in surface waters of the western Arabian Sea

approaching upwelling areas. Broerse (2000) compared the coccolith flux of *Umbilicosphaera foliosa* and *Umbilicosphaera sibogae* in the eastern equatorial and subequatorial Pacific, and associated *Umbilicosphaera foliosa* with eutrophic, upwelling water, and *Umbilicosphaera sibogae* with oligotrophic, stable water. Hagino and Okada (2004) reported the common occurrence of *Umbilicosphaera sibogae* in *Umbellosphaera irregularis* Common Assemblage-a, which is distributed in the infra-marginal waters of upwelling zones. In this study, the abundance of *Umbilicosphaera foliosa* was higher than that of *Umbilicosphaera sibogae* in the moderately eutrophic central equatorial Pacific waters, but was lower than that of *Umbilicosphaera sibogae* in oligotrophic western equatorial Pacific and mesotrophic subequatorial central Pacific waters (Fig. 8). Therefore, *Umbilicosphaera foliosa* is more opportunistic than *Umbilicosphaera sibogae*, although the factors controlling their abundance are unknown.

5-4. Family UMBELLOSPHAERACEAE

Kleijne (1993) studied the morphological variation in living

Umbellosphaeraceae in surface water samples collected from the Mediterranean Sea, Red Sea, Northern Indian Ocean, and western equatorial Pacific Ocean. She subdivided specimens of the family Umbellosphaeraceae into *Umbellosphaera irregularis* and *Umbellosphaera tenuis* Types 0-IV based on the morphology of the distal surface. However, Young et al. (2003) combined *Umbellosphaera tenuis* Type 0 of Kleijne (1993) into *Umbellosphaera irregularis*. In addition, they subdivided Type III of Kleijne (1993) into Types IIIa and IIIb on the basis of the intensity of calcification of the sutural and secondary ridges.

Here, we classified specimens of Umbellosphaeraceae into five groups:

Umbellosphaera irregularis sensu strictu (s.s.), *Umbellosphaera irregularis* Type 0, *Umbellosphaera tenuis* Type I, *Umbellosphaera tenuis* Type III, and *Umbellosphaera tenuis* Type IV (Plates IV-V). Our *Umbellosphaera irregularis s.s.*, *Umbellosphaera irregularis* Type 0, and *Umbellosphaera tenuis* Type IV correspond to *Umbellosphaera irregularis*, *Umbellosphaera tenuis* Type 0, and *Umbellosphaera tenuis* Type IV of Kleijne (1993), respectively. Several poorly preserved specimens with Type II-like morphological characters were hardly distinguishable from Type IV of Kleijne (1993);

therefore, they were included in Type IV. Some Type III-like specimens, which are characterized by both heavily calcified sutural ridges and partly papillate secondary ridges, were observed (Plate 5-3). These were included in Type III because they had peripherally intense calcification of the sutural ridges, similar to Type III. We did not differentiate Types IIIa and IIIb of Young et al. (2003) because we did not find the possible division between them before our studies was carried out. Okada and Honjo (1973a, b) only identified *Umbellosphaera irregularis* and *Umbellosphaera tenuis* at the species level; therefore, we did not include their data for Umbellosphaeraceae here. We note that *Umbellosphaera irregularis* Type 0 of this study (i.e., *Umbellosphaera tenuis* Type 0 of Kleijne, 1993) was incorporated into *Umbellosphaera irregularis* in all our previous studies. In addition, *Umbellosphaera irregularis* of Hagino et al. (2005) solely consists of *Umbellosphaera irregularis s.s.*

Umbellosphaera irregularis s.s. was distributed in all open-ocean waters studied in this study, except for some samples collected from the western equatorial Pacific (Fig. 10a). This species was not found in the hemi-pelagic waters off North and South America. The highest concentration of this species (2.0×10^4 cells/L) occurred at

station 35 of Vema 25 from the eastern equatorial Pacific Ocean. Its cell density was generally high ($\geq 1.0 \times 10^4$ cells/L) in the eastern equatorial Pacific, moderate ($\geq 5.0 \times 10^3$ cells/L) in the western equatorial and southern subequatorial Pacific, and relatively low ($< 5.0 \times 10^3$ cells/L) in the central equatorial and north subequatorial Pacific. The relative abundance of this species appeared unrelated to its absolute abundance. In the western equatorial Pacific, *Umbellosphaera irregularis s.s.* comprised more than 80% of the flora, with relatively low abundance (ca. 3.0×10^3 cells/L). Its cell density was not correlated with water temperature, but was correlated with nutrient concentrations (Fig. 11a). In the moderately warm waters (23-27°C), its cell density exceeded 5.0×10^3 cells/L when the nutrient concentration was sufficiently high ($\text{PO}_4 > 0.5 \mu\text{mol/L}$, $\text{NO}_3 > 4.0 \mu\text{mol/L}$). *Umbellosphaera irregularis s.l.* (*Umbellosphaera irregularis s.s.* and *Umbellosphaera irregularis* Type 0) has been considered an oligotrophic dweller because it is one of the most common species in the nutrient-depleted Subtropical Gyres (e.g., Brand, 1994; Young, 1994a). The concentration of this species, however, had no relation to nutrient concentrations except that it is absent when phosphate concentration is higher ($> 0.7 \mu\text{mol/L}$). It is clear that the dominance of this species in the oligotrophic

flora is the result of the varying abundance of other species, and does not ensue from the high abundance of *Umbellosphaera irregularis s.s.* itself.

Umbellosphaera irregularis Type 0 was most common in the western and central Pacific, and occurred sporadically in the eastern equatorial Pacific (Fig. 10b). Its highest cell concentration (4.7×10^3 cells/L) occurred at station AQ16 of KH90-3, where its relative abundance was also highest (55.1%). Its cell density was nearly consistent between very warm oligotrophic and moderately warm eutrophic waters; therefore, it is clear that this type is an oligotrophic dweller (Fig. 11b). Kleijne (1993) reported *Umbellosphaera irregularis* Type 0 (her *Umbellosphaera tenuis* Type 0) from the Arabian Sea and equatorial Indian Ocean, which are characterized by high temperatures ($> 28.2^\circ\text{C}$). This type is absent or rare in temperate waters of the Mediterranean Sea (Kleijne, 1993) and northwestern Pacific off Japan (Hagino, pers. obs.). Therefore, it is evident that Type 0 has a preference for even higher temperatures than *Umbellosphaera irregularis s.s.*

Umbellosphaera tenuis Type I was rare or absent in most of the study area, but is common in some oceanographic settings: the hemi-pelagic waters off North

America, the southeastern subequatorial Pacific, and the western equatorial Pacific area around 160° S. Its highest concentration occurred at station 26 of Vema 24, where the highest relative abundance was also recorded (40.7%; Fig. 12a). Among the four morphotypes of *Umbellosphaera tenuis*, only Type I had a tolerance for high temperatures (> 28°C; Fig. 13a). Kleijne (1993) has noted that this type increases in abundance with increasing temperature, and that it shows the highest concentration at 30-32°C. Here, however, there was no clear relationship between cell density, in situ SST, and mean annual nutrient concentrations, although there is no doubt that Type 0 is the only morphotype of *Umbellosphaera tenuis* that has an affinity for very warm equatorial waters.

Umbellosphaera tenuis Type III showed a constrained distribution in the hemi-pelagic subtropical waters off South America, although it was also found in subsurface waters of the northwestern subequatorial Pacific covered by WPWP (Hagino, pers. obs.). The highest concentration of this type (1.6×10^5 cells/L) occurred at station 62A of Conrad 9, where its relative abundance was also highest (64.8%; Fig.12b). The in situ SST of common Type III samples ranged from 21.9-25.3°C. The cell density of

this type was very low ($< 10^2$ cells/L) in oligotrophic waters ($\text{PO}_4 < 0.3 \mu\text{mol/L}$; $\text{NO}_3 < 0.3 \mu\text{mol/L}$), but reached 1×10^3 cells/L in temperate eutrophic waters ($\text{PO}_4 > 0.5 \mu\text{mol/L}$; $\text{NO}_3 > 1.6 \mu\text{mol/L}$; Fig. 13b). These results suggest that Type III prefers moderately warm eutrophic waters.

The distribution of our *Umbellosphaera tenuis* Type IV, which probably includes some Type II specimens, overlapped with that of Type III in the neritic waters off South America and Australia, but differed from Type III in its common occurrence in the open ocean of the Northwest Subtropical Gyre (Fig. 12c). Its highest concentration (1.3×10^4 cells/L), occurred at station 60A of Conrad 9, where its relative abundance was also highest (54.2%). It was absent from extremely warm waters ($> 29^\circ\text{C}$), and was rare in warm waters ($27\text{-}29^\circ\text{C}$). In moderately warm conditions ($< 27^\circ\text{C}$) its cell density increased with nutrient concentrations (Fig. 12c). Kleijne (1993) reported that *Umbellosphaera tenuis* Type IV is common over the entire Mediterranean Sea and Northeast Atlantic Ocean. Hagino et al. (2005) reported Type IV from the temperate northwestern Pacific off Japan. Thus, it is clear that *Umbellosphaera tenuis* Type IV has an affinity for subequatorial to temperate temperatures.

6. Discussion

Subspecies-level classification showed differences with respect to the distribution and habitat preferences of morphological subgroups of so-called major taxa. The results of this study indicate that the global distributions observed for major taxa are, in fact, mixtures of several discrete subspecies/varieties, as suggested by Ziveri et al. (2004). Traditional species-level studies can detect general floral variations in response to environmental changes; however, this type of study inhibits our understanding of the ecology of coccolithophores. For example, *Emiliana huxleyi* has often been referred to as “cosmopolitan” and “eurythermal”. The taxonomies of several well-known morphological groups have already been ascertained using molecular phylogenetic studies and observations of life-cycles in population cultures and from combination coccospheres (e.g., Geisen et al., 2004), but the status of most of the morphological groups that have not yet been cultured is still in question. In the section that follows, we

discuss unsolved questions regarding the taxonomy and ecology of these, as-yet uncultured, morphological groups.

6-1. *Emiliana huxleyi*

Emiliana huxleyi Type C, whose culture strain has not been established, has often been regarded as a cold-water dweller, despite its occurrence in relatively low concentrations in warm waters. Surprisingly, Type C exceeded Type A in both absolute and relative abundance in the eastern equatorial Pacific, and was associated with high nutrient concentrations, but not with low temperatures. It may be tempting to interpret Type C as a true eurythermal and eutrophic taxon because the cool subpolar waters dominated by Type C are usually eutrophic (e.g., Findlay and Giraudeau et al., 2000; Hagino et al., 2005). However, it would be ill-advised to make this conclusion solely on the basis of observations in this study, because *E. huxleyi* Type A not Type C usually makes huge bloom in the eutrophic North Atlantic ocean (e.g., Holligan et al., 1983; Young 1994b), and our Type C may include several variants of Type B/C, and there is no evidence available to demonstrate a genetic relationship between morphotypes from

previous studies and our Type C.

If our Type C population is closely related to the typical subpolar Type B/C or C, the possession of a fragile distal shield and solid/open central area are common morphological features of the eutrophic morphotypes of *E. huxleyi*. However, this characteristic conflicts with that observed in intra- and infra-specific morphological variation in the other members of the family Noëlaerhabdaceae, whose populations, found in eutrophic waters, often possess an over-calcified central area. In the western tropical Pacific, the relative size of the central area of *Gephyrocapsa oceanica* is large (av. 50%) in specimens from the oligotrophic upper photic zone of the WPWP, but is small (av. 37%) in those from the mesotrophic lower photic zone of the WPWP, and in the eutrophic surface water of the upwelling front (Hagino et al., 2000). The distributions of *Gephyrocapsa crassipons* and *Reticulofenestra punctata*, which also possess over-calcified closed central areas, are constrained to the upwelling area of the Equatorial Divergence (Okada and Honjo, 1973b; Hagino and Okada, 2001). To determine the true ecology of *E. huxleyi* Type B/C in tropical and subtropical waters, an elaborated classification, based on morphometric measurements and molecular

phylogenetics, is needed.

Emiliana huxleyi var. *corona* is the only variety of *E. huxleyi* that has large ($\geq 4 \mu\text{m}$) coccoliths in tropical and subtropical waters. Among all morphotypes of *E. huxleyi*, only *E. huxleyi* var. *corona* has a consistent affinity for oligotrophic conditions. This variety coexists with medium-sized ($< 4 \mu\text{m}$) Type A in the western subequatorial Pacific; therefore, it is not likely to be an ecophenotype of Type A. Its size range overlaps that of Type B/C, but it is not likely to be an ecophenotype of Type B/C because its central area consists of curved elements, similar to those of Type A. There currently exists no evidence with which to discuss the genetic relationships between variety *corona* and other varieties of *E. huxleyi*.

6-2. *Calcidiscus leptoporus* ssp. small

Among the extant morphological groups of *Calcidiscus*, only *Calcidiscus leptoporus* ssp. small has not been cultured or studied genetically. This taxon dominates the *Calcidiscus* populations in the eastern equatorial and subequatorial Pacific, but is usually rare in the tropical waters of Atlantic Ocean (e.g., Knappertsbusch et al., 1997;

Broerse, 2000; Ziveri et al., 2004). We did not find any specimens of hetelococolith-holococolith combinations in this taxon, and elucidation of the taxonomy of *Calcidiscus leptoporus* ssp. small is still pending.

6-3. Family Umbellosphaeraceae

Umbellosphaera irregularis s.s. has the widest distribution among the morphotypes of family Umbellosphaeraceae in the Pacific surface waters, and is distributed in the entire studied area. In contrast, other morphotypes of the family Umbellosphaeraceae display habitat segregation, between equatorial and subequatorial waters, and can be classified into two groups: equatorial, lightly calcified taxa including *Umbellosphaera irregularis* Type 0 and *Umbellosphaera tenuis* Type I; and subequatorial heavily calcified taxa including *Umbellosphaera tenuis* Types III and IV. Since *Umbellosphaera irregularis* s.s. coexists with all other morphotypes of the family Umbellosphaeraceae, it is evident that *Umbellosphaera irregularis* s.s. is a discrete species, and not an ecophenotype of the other morphotypes. Taxa co-existing within each of the equatorial/subequatorial waters are likely to be genetically discrete, but the

genetic relationship between the equatorial and subequatorial-temperate groups is not clear at this time.

7. Summary

- (1) The morphotype assignments of selected coccolithophore taxa were studied over the entire equatorial and subequatorial Pacific by compiling data from Okada and Honjo (1973b) and the original unpublished data used in Hagino and Okada (2004).
- (2) The *Emiliana huxleyi* population consisted of at least three morphological groups: Type A, Type C, and variety *corona*. The most probable factors limiting the occurrence of Types A and C are high temperatures and low nutrient concentrations, respectively. *Emiliana huxleyi* var. *corona* is adapted to oligotrophic waters.
- (3) *Calcidiscus leptoporus* ssp. small was abundant in the eastern equatorial and

subequatorial Pacific, and so has an affinity for eutrophic conditions.

(4) *Umbilicosphaera foliosa* is adapted to mesotrophic conditions, whereas, *Umbilicosphaera sibogae* prefers stratified marginal waters surrounding the eutrophic upwelling zone.

(5) The morphotypes of *Umbellosphaera tenuis* displayed habitat segregation in the study area. Only Type I was found in very warm tropical waters. Both Types III and IV occurred in subtropical latitudes, but Type III differed from Type IV in that its distribution was constrained to hemi-pelagic waters.

8. Acknowledgements

We are grateful to A. McIntyre, formerly at Lamont-Doherty Earth Observatory, and H. Matsuoka at Kochi University for kindly providing samples and for their helpful suggestions. We acknowledge H. Andrleit at Bundesanstalt für Geowissenschaften und Rohstoffe, R. Jordan at Yamagata University and J.R. Young at

the Natural Science Museum for their critical reviews and constructive comments. This research is supported by a 21st Century COE Program on "Neo-Science of Natural History" at Hokkaido University and by a Grant-in Aid for Scientific Research (No. 17740337; awarded to K. Hagino) financed by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

9. References

- Bollmann, J., 1997. Morphology and biogeography of *Gephyrocapsa* coccoliths in Holocene sediments. *Mar. Micropaleontol.*, 29, 319-350.
- Brand, L.E., 1994. Physiological ecology of marine coccolithophores. In: Winter A. and Siesser, W.G. (Eds.), *Coccolithophores*. Cambridge University Press, Cambridge, pp. 39-49.
- Broerse, A.T.C., 2000. *Coccolithophore export production in selected ocean environments*. Ph.D. Thesis, Free University Amsterdam, the Netherlands, pp.

185.

Brown, J., Colling, A., Park, D., Phillips, J., Rothery, D., Wright, J., 1989. *Ocean Circulation*. The Open University / Pergamon. Oxford, pp. 238.

Cachão, M., Moita, M.T., 2000. *Coccolithus pelagicus*, a productivity proxy related to moderate fronts off Iberia. *Mar. Micropaleontol.*, 39, 131-155.

Conkright, M.E., Levitus, S., Boyer, T.P., 1994. *World Ocean Atlas 1994: Nutrients*, vol. 1. National Oceanographic Data Center, Ocean Climate Laboratory.

Cortés, M.Y., 2000. Further evidence for the heterococcolith-holococcolith combination *Calcidiscus leptoporus-Crystallolithus rigidus*. *Mar. Micropaleontol.*, 39, 35-37.

Cortés, M.Y., Bollmann, J., Thierstein, H.R., 2001. Coccolithophore ecology at the HOT station ALOHA, HAWAII. *Deep-Sea Research II*, 48, 1957-1981.

Findlay, C.S., Giraudeau, J., 2000. Extant calcareous nannoplankton in the Australian Sector of the Southern Ocean (austral summers 1994 and 1995). *Mar. Micropaleontol.*, 40, 417-439.

Geisen, M., Billard, C., Broerse, A.T.C., Cros, L., Probert, I., Young, J.R. 2002.

Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? *Eur. J. Phycol.*, 37, 531-550.

Geisen, M., Young, J.R., Probert, I., Sáez, A.G., Baumann, K.-H., Sprengel, C., Bollmann, J., Cros, L., de Vargas, C., Medlin, L.K., 2004. Species level variation in coccolithophores. In: Thierstein, H.R. and Young, J.R., (Eds.), *Coccolithophores, From Molecular Processes to Global Impact*. Springer, pp. 327-366.

Hagino, K., Okada, H., Matsuoka, H., 2000. Spatial dynamics of coccolithophore assemblage in the Equatorial Western-Central Pacific Ocean, *Mar. Micropaleontol.*, 39, 53-57.

Hagino, K., Okada, H., 2001. Morphological observations of living *Gephyrocapsa crassipons*. *J. Nannoplankton Res.* 23, 3-7.

Hagino, K., Okada, H., 2004. Floral response of coccolithophores to progressive oligotrophication in the South Equatorial Current, Pacific Ocean, *In* Shiyomi, M., Kawahata, H., Koizumi, H., Tsuda, A., Awaya, Y. (Eds.) *Global environmental change in the ocean and on land*, TERRAPUB, pp. 121-132.

- Hagino, K., Okada, H., Matsuoka, H., 2005. Coccolithophore assemblages and morphotypes of *Emiliana huxleyi* in the boundary zone between the cold Oyashio and warm Kuroshio currents off the coast of Japan. *Mar. Micropaleontol.*, 55, 19-47.
- Holligan, P.M., Viollier, M., Harbour, D.S., Camus, P., Champagne-Philippe, M., 1983. Satellite and ship production along a continental shelf edge. *Nature*, 304, 339-342.
- Jordan, R.W., Chamberlain, A.H.L., 1997. Biodiversity among haptophyte algae. *Biodiversity and Conservation*, 6, 131-152.
- Kamptner, E., 1963. Coccolithineen-Skelettreste aus Tiefseeablagerungen des Pazifischen Ozeans. *Annalen des Naturhistorischen Museums in Wien*, 66, 139-204.
- Kleijne, A., 1991. Holococcolithophorids from the Indian Ocean, Red Sea, Mediterranean Sea and North Atlantic Ocean. *Mar. Micropaleontol.*, 17, 1-76.
- Kleijne, 1993. Morphology, taxonomy and distribution of extant coccolithophorids (calcareous nannoplankton). Drukkerij FEBO, Enschede, The Netherlands, pp.

1-315.

Knappertsbusch, M.W., Cortes, M.Y., Thierstein, H.R., 1997. Morphologic variability of the coccolithophorid *Calcidiscus leptoporus* in the plankton, surface sediments and from the Early Pleistocene. *Mar. Micropaleontol.*, 30, 293-317.

Levitus, S., Boyer, T.P., 1994. World Ocean Atlas 1994: Temperature, vol. 4, National Oceanographic Data Center, Silver Spring, MD.

Levitus, S., Burgett, R., Boyer, T.P., 1994. World Ocean Atlas 1994: Salinity, vol. 3, National Oceanographic Data Center, Silver Spring, MD.

Lohmann, H. 1920. Die Coccolithophoridae, eine Monographie der Coccolithen bildenden Flagellaten, zugleich ein Beitrag zur Kenntnis des Mittelmeerauftriebs. *Archiv. für Protistenkunde*, 1, 89-165.

Marumo R., editor, 1970. Preliminary report of the Hakuho Maru Cruise KH69-4 (IBP Cruise). R. Marumo, editor, *Ocean Res. Inst., Univ. Tokyo*, pp. 68.

McIntyre, A., Bé, A.W.H., 1967. Modern Coccolithophoridae of the Atlantic Ocean – 1. Placoliths and cyrtoliths. *Deep-Sea Res.*, 14, 561-597.

McIntyre, A., Bé, A.W.H., Roche, M.B., 1970. Modern Pacific Coccolithophorida: A

- paleontological thermometer. New York Acad Sci series II (32), 720-731.
- Medlin, L.K., Barker, G.L.A., Campbell, L., Green, J.C., Hayes, P.K., Marie, D.,
Wrieden, S., and Vaultot, D., 1996. Genetic characterization of *Emiliana
huxleyi* (Haptophyta). J. Mar. Syst. 9, 13-31.
- Nozaki, Y., editor, 1990. Preliminary report of the Hakuho Maru Cruise KH90-3. Ocean
Res. Inst., Univ. Tokyo, pp. 47.
- Nozaki, Y., editor, 1992. Preliminary report of the Hakuho Maru Cruise KH92-4. Ocean
Res. Inst., Univ. Tokyo, pp. 59.
- Okada, H., Honjo, S., 1973a. The distribution of oceanic coccolithophorids in the
Pacific. Deep-Sea Res., 20, 355-374.
- Okada, H., Honjo, S., 1973b. Distribution of coccolithophorid in the North and
Equatorial Pacific Ocean: Quantitative data on samples collected during Leg
30, Oshoro-Marui, 1968 and Leg HK69-4, Hakuho-Marui, 1969. WHOI Tech.
Rep., WHOI-73-81, Woods Hole Oceanogr. Inst., pp. 59.
- Okada, H., McIntyre, A., 1977. Modern coccolithophores of the Pacific and North
Atlantic Oceans. Micropaleontology, 23(1), 1-55.

Quinn, P.S., Sáez, A.G., Baumann, K.-H., Steel, B.A., Sprengel, C., Medlin, L.K. 2004.

Coccolithophorid biodiversity: evidence from the cosmopolitan species
Calcidiscus leptoporus. In: Thierstein, H.R. and Young, J.R., (Eds.),
Coccolithophores, From Molecular Processes to Global Impact. Springer, pp.
299-326.

Sáez, A.G., Probert, I., Geisen, M., Quinn, P., Young, J.R., Medlin, L.K. 2003.

Pseudo-cryptic speciation in coccolithophores. Proc. Natn. Acad. Sci. USA,
100 (12), 7163-7168.

Schroeder, D.C., Biggi, G.F., Hall, M., Davy J., Martínez, J.M., Richardson, A.J., Malin,

G., Wilson, W.H., 2005. A genetic marker to separate *Emiliana huxleyi*
(Prymnesiophyceae) morphotypes. J. Phycol., 41, 874-879.

Thierstein H.R., Cortés, M.Y., Haidar, A., 2004. Plankton community behavior on

ecological and evolutionary time-scales; when models confront evidence. In:
Thierstein, H.R. and Young, J.R., (Eds.), Coccolithophores, From Molecular
Processes to Global Impact, Springer, pp. 455-497.

Tomczak M., Godfrey J.S., 1994. Regional Oceanography: An Introduction. Pergamon,

London. pp. 422.

Young, J.R., 1994a. Function of coccoliths. In: Winter A., Siesser, W.G. (Eds.),
Coccolithophores. Cambridge University Press, pp. 63-82.

Young, J.R., 1994b. Variation in *Emiliana huxleyi* coccolith experiment in samples
from the Norwegian EHUX experiment, 1992. *Sarsia*, 79, 417-425.

Young, J.R., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I., Ostergaard, J.,
2003. A guide to extant coccolithophore taxonomy. *J. Nannoplankton Res.*,
Special Issue 1, pp. 125.

Young, J.R., Westbroek, P., 1991. Genotypic variation in the coccolithophorid species
Emiliana huxleyi. *Mar. Micropaleontol.*, 18, 5-23.

Ziveri, P., Baumann, K.-H., Böckel, B., Bollmann, J., Young, J.R., 2004. Biogeography
of selected Holocene coccoliths in the Atlantic Ocean. In: Thierstein, H.R. and
Young, J.R., (Eds.), *Coccolithophores, From Molecular Processes to Global
Impact*. Springer, 327-366.

Appendix I

Sampling location, date, in situ and annual mean hydrographic information, and cell concentration of studied morphotypes in studied samples. Annual mean temperature and salinity, were referred from Levitus and Boyer (1994) and Levitus et al. (1994), respectively. Annual mean phosphate and nitrate concentrations were quoted from Conkright et al. (1994).

Plate Captions

Plate 1. Scanning electron micrographs of *Emiliana huxleyi*. Scale bars 2 μ m. 1. *E. huxleyi* Type A from st.45 of Vema 24 (19°18'N, 161°19'W). 2. *E. huxleyi* Type A from st.92 of Conrad 12 (13°39'S, 161°37'W). 3. *E. huxleyi* Type C from st.26 of Vema 24 (6°15'N, 90°40'W). 4. *E. huxleyi* Type C from st.134 of Conrad 11 (1°32'S, 85°49'W). 5. *E. huxleyi* variety *corona* from st. AQ01 of

KH90-3 (14°30'N, 167°42'E). 6. *E. huxleyi* variety *corona* from st.45 of Vema 24 (19°18'N, 161°19'W).

Plate 2. Scanning electron micrographs of *Calcidiscus leptoporus* s.l. Scale bars 2µm.

1-2. *C. leptoporus* small form (< 5µm) from st.126 of Conrad 11 (5°59'S, 115°37'W). 3-4. *C. leptoporus* intermediate (5-8µm) from st.131 of Conrad 11 (11°36'S, 95°38'W). 5-6. *C. leptoporus* large (≥ 8µm) from st.AQ11 of KH90-3 (4°00'S, 178°99'E).

Plate 3. Scanning electron micrographs of *Umbilicosphaera foliosa* and

Umbilicosphaera sibogae. Scale bars 2µm. 1-2. *U. foliosa* from st.82 of KH69-4 (2°00'N, 155°2'W). 3. *U. sibogae* from st.129 of Conrad 10 (13°46'N, 116°38'W). 4. *U. sibogae* from st.131 of Conrad 11 (11°36'S, 95°38'W).

Plate 4. Scanning electron micrographs of *Umbellosphaera irregularis*. Scale bars 2µm.

1. *U. irregularis* s.s. from st.118 of Vema 24 (17°39'S, 149°39'E). 2. *U. irregularis* s.s. from AQ01 of KH90-3 (14°30'N, 167°42'E). 3. *U. irregularis* Type 0 from st. AQ01 of KH90-3 (14°30'N, 167°42'E). *U. irregularis* Type 0 from st. AQ16 of KH90-3 (2°58'S, 160° 00'E).

Plate 5. Scanning electron micrographs of *Umbellosphaera tenuis*. Scale bars 2 μ m. 1. *U. tenuis* Type I from st.26 of Vema 24 (6°15'N, 90°40'W). 2. *U. tenuis* Type I from st.AQ16 from KH90-3 (2°58'S, 160° 00'E). 3-4. *U. tenuis* Type III from st. 62A of Conrad 9 (6°11'N, 94°95'W). 5. *U. tenuis* Type IV from st.62 of Conrad 10 (6°11'N, 94°95'W). 5. *U. tenuis* Type IV from st.AQ01 of KH90-3 (14°30'N, 167°42'E).

Figure Captions

Fig. 1. Distribution of the surface currents in the equatorial and subequatorial Pacific Ocean.

Fig.2. Hydrography in the equatorial and subequatorial Pacific Ocean. Contour graphs indicate, (a) annual mean values of sea surface temperature (Levitus and Boyer, 1994), (b) salinity (Levitus et al., 1994), (c) nitrate and (d) phosphate (Conkright et al., 1994).

Fig.3. Distribution of (a) samples studied by Hagino and Okada (2004) and this study, and (b) floral assemblages recognized by Hagino and Okada (2004). Note: Abbreviations used in (b) are *Emiliana huxleyi* Common Assemblage (ECA), *Gephyrocapsa oceanica* Common Assemblage (GCA), and *Umbellosphaera irregularis* Common Assemblage (UCA).

Fig.4. Abundance distributions of (a) *Emiliana huxleyi* Type A, (b) *Emiliana huxleyi* Type C, and (c) *Emiliana huxleyi* var. *corona*. The size and filled patterns of the circles indicate the absolute abundance and relative abundance of each species, respectively. Note: Unit of absolute abundance differs between morphotypes.

Fig.5. Correlation between environmental parameters and absolute abundance of (a) *Emiliana huxleyi* Type A, (b) *Emiliana huxleyi* Type C, and (c) *Emiliana huxleyi* var. *corona*. Note: Unit of absolute abundance differs between morphotypes. Annual mean values of nitrate and phosphate of each station were obtained from the World Ocean Atlas (Levitus and Boyer, 1994; Conkright et al., 1994).

Fig.6. Abundance distributions of (a) *Calcidiscus* small ($< 5\mu\text{m}$), (b) *Calcidiscus* intermediate (5-8 μm), and (c) *Calcidiscus* large ($\geq 8\mu\text{m}$). The size and filled patterns of the circles indicate the absolute abundance and relative abundance of each species, respectively.

Fig.7. Correlation between environmental parameters and absolute abundance of (a) *Calcidiscus* small ($< 5\mu\text{m}$), (b) *Calcidiscus* intermediate (5-8 μm), and (c) *Calcidiscus* large ($\geq 8\mu\text{m}$). Note: Annual mean values of nitrate and phosphate of each station were obtained from the World Ocean Atlas (Levitus and Boyer, 1994; Conkright et al., 1994).

Fig.8. Abundance distributions of (a) *Umbilicosphaera foliosa* and (b) *Umbilicosphaera sibogae*. The size and filled patterns of the circles indicate the absolute abundance and relative abundance of each species, respectively.

Fig.9. Correlation between environmental parameters and absolute abundance of (a) *Umbilicosphaera foliosa* and (b) *Umbilicosphaera sibogae*. Note: Annual mean values of nitrate and phosphate of each station were obtained from the World Ocean Atlas (Levitus and Boyer, 1994; Conkright et al., 1994).

Fig.10. Abundance distributions of (a) *Umbellosphaera irregularis* s.s. and (b) *Umbellosphaera irregularis* Type 0. The size and filled patterns of the circles indicate the absolute abundance and relative abundance of each species, respectively.

Fig.11. Correlation between environmental parameters and absolute abundance of (a) *Umbellosphaera irregularis* s.s. and (b) *Umbellosphaera irregularis* Type 0.

Note: Annual mean values of nitrate and phosphate of each station were obtained from the World Ocean Atlas (Levitus and Boyer, 1994; Conkright et al., 1994).

Fig.12. Abundance distributions of *Umbellosphaera tenuis* (a) Type I, (b) Type III, and (c) Types II and IV. The size and filled patterns of the circles indicate the absolute abundance and relative abundance of each species, respectively.

Fig.13. Correlation between environmental parameters and absolute abundances of *Umbellosphaera tenuis* (a) Type I, (b) Type III, and (c) Types II and IV. Note: Annual mean values of nitrate and phosphate of each station were obtained from the World Ocean Atlas (Levitus and Boyer, 1994; Conkright et al., 1994).

annual mean salinity (psu)	annual mean phosphate concentration (µmol/L)	annual mean nitrate concentration (µmol/L)	Emiliania huxleyi Types A and C (cells/liter)	Emiliania huxleyi Type A (cells/liter)	Emiliania huxleyi Type C (cells/liter)	Emiliania huxleyi malformed specimens (cells/liter)	Emiliania huxleyi var. corona (cells/liter)	Caeticiscus leptopus s.l. (cells/liter)	Caeticiscus leptopus large (cells/liter)	Caeticiscus leptopus intermediate (cells/liter)	Caeticiscus leptopus small (cells/liter)	Umbilicosphaera foliosa (cells/liter)	Umbilicosphaera sibogae (cells/liter)	Umbilicosphaera irregularis s.l. (cells/liter)	Umbilicosphaera irregularis s.s. (cells/liter)	Umbilicosphaera irregularis Type 0 (cells/liter)	Umbilicosphaera tenuis s.l. (cells/liter)	Umbilicosphaera tenuis Type I (cells/liter)	Umbilicosphaera tenuis Type III (cells/liter)	Umbilicosphaera tenuis Type IV (cells/liter)
35.22	0.80	2.96	4995	727	0	0	0	0	0	0	91	0	0	0	0	0	0	0	3996	12897
35.23	0.73	2.42	4536	794	0	0	0	0	0	0	38	0	0	0	0	0	0	38	1587	3326
35.24	0.57	1.64	1591	239	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15672	5250
35.26	0.76	2.50	1122	1446	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1761	3081
35.09	0.92	3.08	33186	1281	0	0	0	0	0	0	0	0	0	0	0	0	0	0	233	0
35.09	0.92	3.08	18926	1542	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70	140
35.09	0.86	2.56	845	356	0	0	0	0	0	0	0	0	0	0	0	0	0	0	845	3623
34.19	0.26	0.29	1051	88	0	0	0	0	0	0	0	643	0	1168	1227	0	701	0	0	0
33.72	0.27	2.34	529	1110	0	0	0	0	0	0	496	3969	0	358	193	0	0	0	0	0
34.18	0.26	2.09	107	96	0	0	0	0	0	160	75	0	0	11	0	0	0	0	0	0
34.25	0.31	1.83	198	103	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0
34.62	0.52	2.89	954	163	0	0	0	0	0	191	27	163	0	354	0	0	0	0	0	0
31.57	0.24	0.14	0	262	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33.27	0.36	1.99	348	13	0	0	0	0	0	0	0	27	0	389	27	0	1662	0	0	0
33.74	0.47	3.84	266	832	0	0	0	0	0	0	0	365	0	2030	33	0	0	0	0	0
34.43	0.62	5.54	2701	583	0	0	0	0	0	31	0	0	0	2640	61	0	0	0	0	0
34.41	0.60	5.22	799	2819	0	0	0	0	0	126	0	42	0	3660	0	0	0	0	0	0
34.42	0.62	5.46	8572	2449	0	0	0	0	0	0	0	153	0	14695	0	0	0	0	0	0
34.64	0.62	5.98	7985	853	0	0	0	0	0	233	0	0	0	3799	310	0	0	0	0	0
34.71	0.62	5.98	1322	1862	0	0	0	0	0	0	0	0	0	1189	0	0	0	0	0	0
34.73	0.63	5.52	11538	648	0	0	0	0	0	389	0	0	0	4019	259	0	0	0	0	0
34.78	0.56	4.82	19807	4630	0	0	0	0	0	514	0	0	0	19549	1801	0	0	0	0	0
34.76	0.59	4.30	7400	1139	0	0	0	0	0	569	0	0	0	81	3985	0	0	0	0	0
34.96	0.65	4.19	10120	2344	0	0	0	0	0	2557	0	0	0	8949	1065	0	0	0	0	0
34.95	0.55	3.14	420	78	0	0	0	0	0	67	0	0	0	62	10	0	5	0	0	0
34.95	0.52	3.45	5014	2137	0	0	0	0	0	3041	82	82	0	5753	82	0	0	0	0	0
34.99	0.57	2.49	2056	630	0	0	0	0	0	630	0	0	0	2089	0	0	0	0	0	0
34.92	0.55	1.96	2219	341	0	0	0	0	0	614	68	0	0	410	34	0	0	0	0	0
34.28	0.39	0.78	131	0	33	0	0	0	0	0	0	16	0	2100	1345	0	82	0	0	115
34.28	0.34	0.54	1735	208	208	0	0	0	0	0	0	0	0	3989	486	0	0	0	0	1284
34.52	0.32	0.32	529	0	107	0	0	0	0	0	0	0	0	254	750	0	0	0	0	27
34.78	0.24	0.54	1481	118	326	0	0	0	0	0	0	0	0	1955	1214	0	89	0	0	1303
34.77	0.19	0.77	691	24	262	0	0	0	0	0	0	0	0	1691	1000	0	0	0	0	881
34.62	0.20	0.75	392	87	196	0	0	0	0	0	0	0	0	2725	828	0	0	0	0	828
34.59	0.23	0.59	502	32	65	0	0	0	0	0	0	0	0	1134	1474	0	0	0	0	97
34.53	0.24	0.62	182	21	0	0	0	0	0	0	0	111	0	2033	481	0	0	0	0	11
34.49	0.17	0.18	188	42	0	0	0	0	0	0	0	26	0	564	642	0	0	0	0	58
34.63	0.18	0.11	229	242	64	0	0	0	0	0	0	0	0	572	76	0	0	0	0	127
34.70	0.20	0.07	1309	0	300	0	0	0	0	0	0	0	0	2427	600	0	0	0	0	982
34.90	0.14	0.27	257	71	186	0	0	0	0	0	0	0	0	329	57	0	0	0	0	1328
34.89	0.14	0.54	762	213	142	0	0	0	0	0	0	0	0	1028	142	0	0	0	0	1471
34.36	0.21	0.76	129	43	7	0	0	0	0	0	0	208	0	740	625	0	36	0	0	0
34.45	0.20	0.68	249	32	0	0	0	0	0	0	0	64	0	345	260	0	0	0	0	0
34.59	0.22	0.58	397	0	28	0	0	0	0	0	0	46	0	739	729	0	111	0	0	0
34.58	0.25	0.55	0	0	0	0	0	0	0	0	0	81	0	2486	578	0	591	0	0	0
34.42	0.11	0.42	820	0	52	0	0	0	0	0	0	35	0	1325	1238	0	17	0	0	0
34.85	0.09	0.72	100	0	56	0	0	0	0	0	0	0	0	517	293	0	0	0	0	37
34.51	0.09	0.33	262	31	154	0	0	0	0	0	0	0	0	773	1634	0	0	0	0	0
33.70	0.05	0.74	1461	43	21	0	0	0	0	0	0	21	0	946	1075	0	1096	0	0	0
33.82	0.05	0.52	0	18	0	0	0	0	0	0	0	0	0	4242	0	0	0	0	0	0
34.06	0.12	0.58	194	0	0	0	0	0	0	0	0	0	0	4953	111	0	0	0	0	28
34.42	0.11	0.19	24	0	0	0	0	0	0	0	0	0	0	361	0	0	0	0	0	0
34.39	0.13	0.27	48	0	0	0	0	0	0	0	0	0	0	2373	38	0	0	0	0	0
34.41	0.14	0.24	14	0	0	0	0	0	0	0	0	0	0	3435	42	0	0	0	0	0
34.43	0.15	0.30	13	0	0	0	0	0	0	0	0	127	0	2617	38	0	0	0	0	0
35.04	0.13	0.19	562	48	0	0	0	0	0	0	0	32	0	2072	16	0	16	0	0	209
34.93	0.09	0.00	3723	260	0	0	0	0	0	0	0	58	0	924	0	0	0	0	0	144
35.05	0.15	0.80	4617	380	0	0	0	0	0	0	0	109	0	597	0	0	0	0	0	81
35.04	0.17	0.37	135	292	0	0	0	0	0	0	0	63	0	2117	32	0	21	0	0	42
34.98	0.16	0.33	1197	0	0	0	0	0	0	0	0	0	0	4407	109	0	0	0	0	27
35.11	0.16	0.36	660	262	0	0	0	0	0	25	12	0	0	286	0	0	0	0	0	0
35.18	0.14	0.38	483	15	0	0	0	0	0	0	8	0	0	1124	30	0	8	0	0	0
34.98	0.12	0.00	6345	363	0	0	0	0	0	0	0	0	0	1768	408	0	45	0	0	363
35.00	0.13	0.00	1559	71	0	0	0	0	0	0	0	35	0	543	106	0	35	0	0	71
34.87	0.13	0.35	6904	345	43	0	0	0	0	216	129	0	0	216	43	0	0	0	0	0
34.79	0.14	0.52	1555	0	59	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0
34.89	0.15	0.44	592	0	24	16	0	0	0	0	8	0	0	368	192	0	32	0	0	0
35.17	0.13	0.39	11287	0	485	0	0	0	0	0	0	208	277	277	138	0	0	0	0	0
35.06	0.12	0.00	5052	79	0	0	0	0	0	0	0	79	0	236	26	0	0	0	0	26
34.90	0.12	0.25	219	0	79	0	0	0	0	0	0	39	59	769	99	0	0	0	0	20
34.31	0.17	0.30	3096	0	74	0	0	0	0	0	0	0	0	89	0	0	0	0	0	0
35.86	0.54	1.29	843	422	0	0	0	422	5762	0	843	0	843	4357	2108	0	0	0	0	0
35.66	0.63	2.09	219	73	0	0	0	0	3724	0	365	0	365	1606	876	0	0	0	0	0
35.33	0.60	5.42	1646	1986	0	0	0	0	2100	0	567	0	567	3688	624	0	170	0	0	0
35.04	0.68	6.44	11824	2337	0	0	0	0	778	0	0	0	0	311	0	0	0	0	0	0
35.11	0.69	6.57	4927	20469	0	0	0	0	1365	0	0	0	0	0	0	0	0	0	0	0
35.14	0.65	6.10	3196	1031	0	0	0	309	8247	0	1856	0	1856	3196	0	0	722	0	0	0
35.40	0.56	4.88	2445	2751	0	0	0	611	10085	0	2751	0	2751	11766	306	0	917	0	0	0
35.69	0.50	3.70	1658	0	0	0	0	179	2689	45	1569									

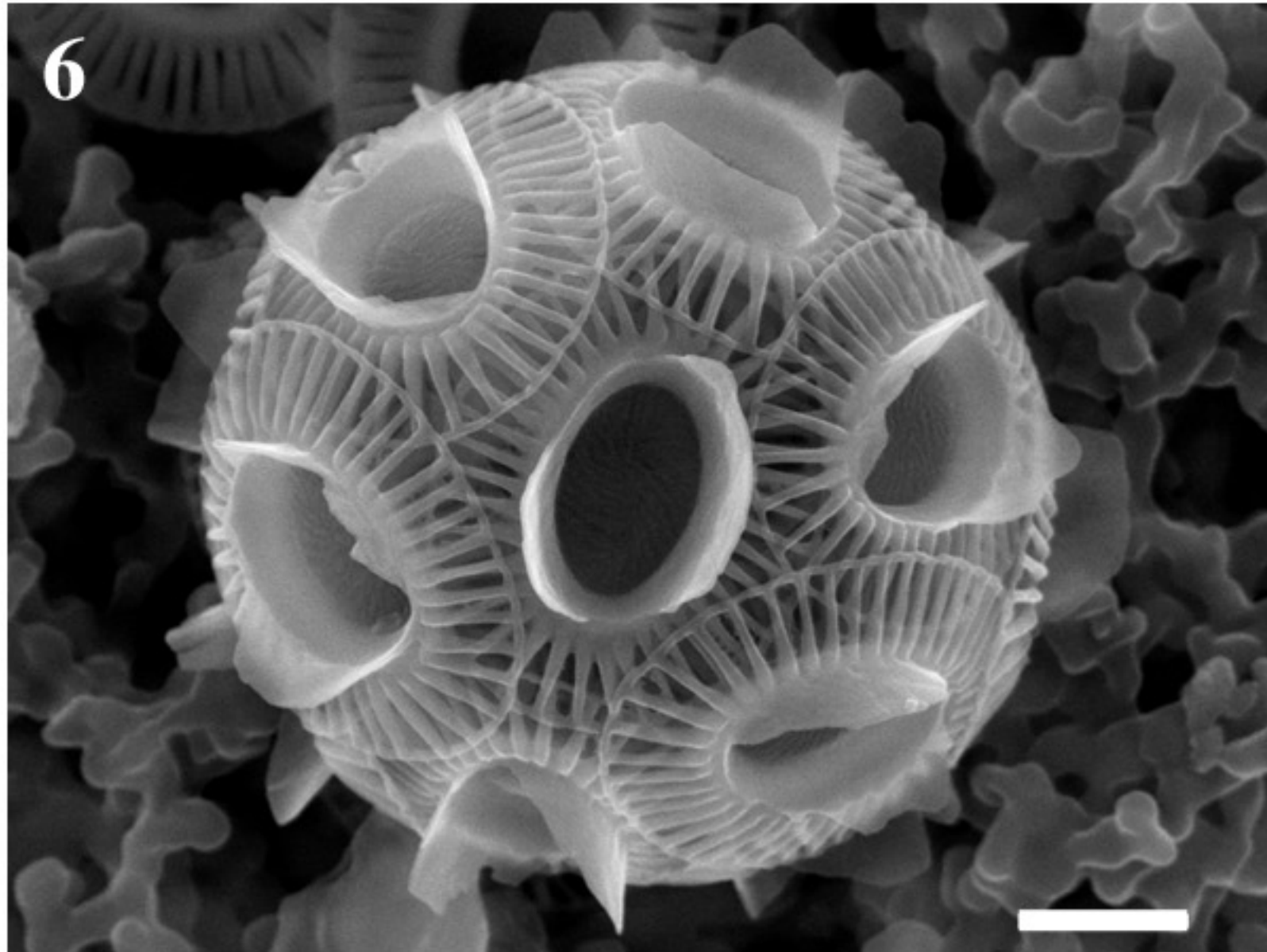
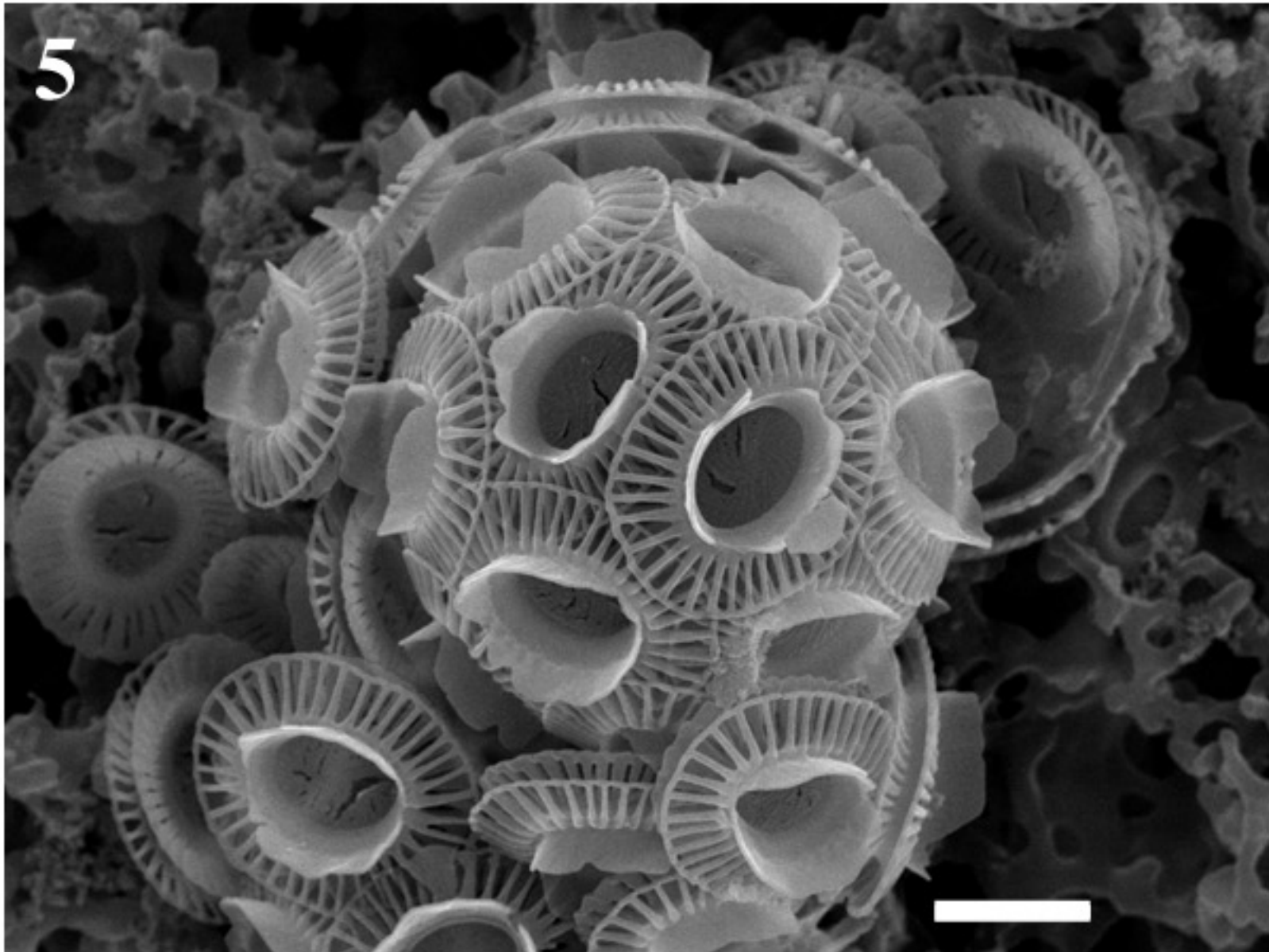
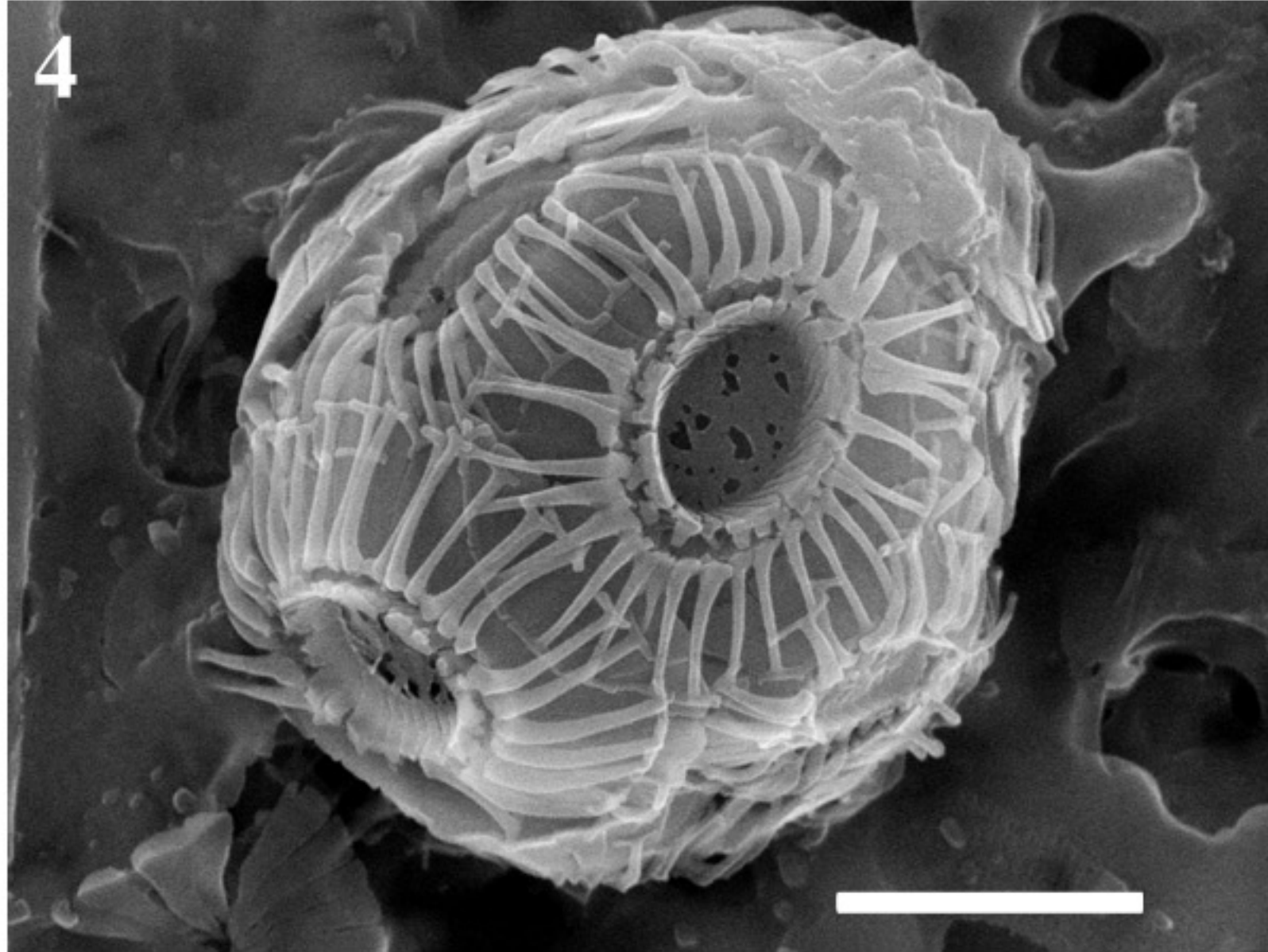
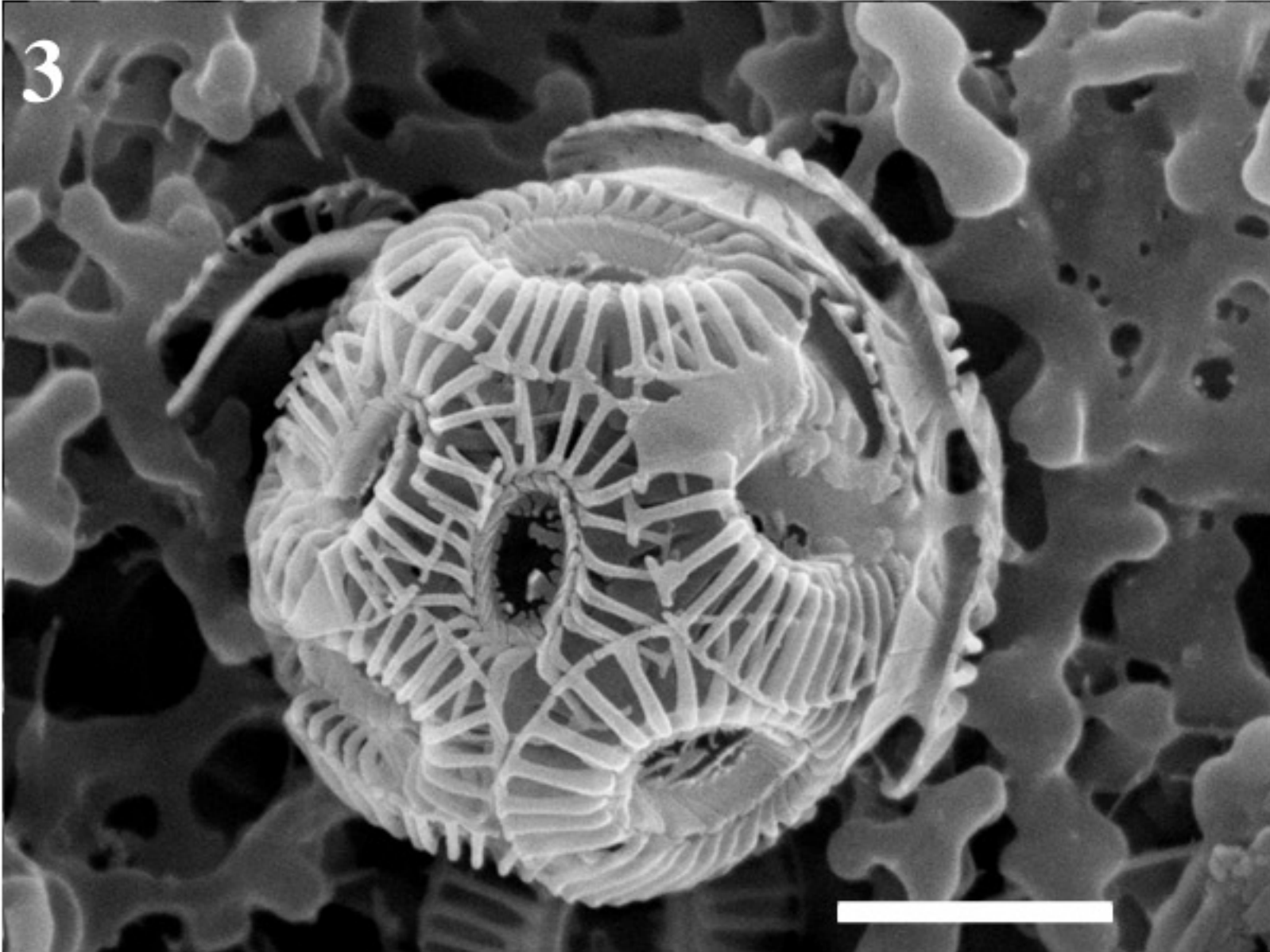
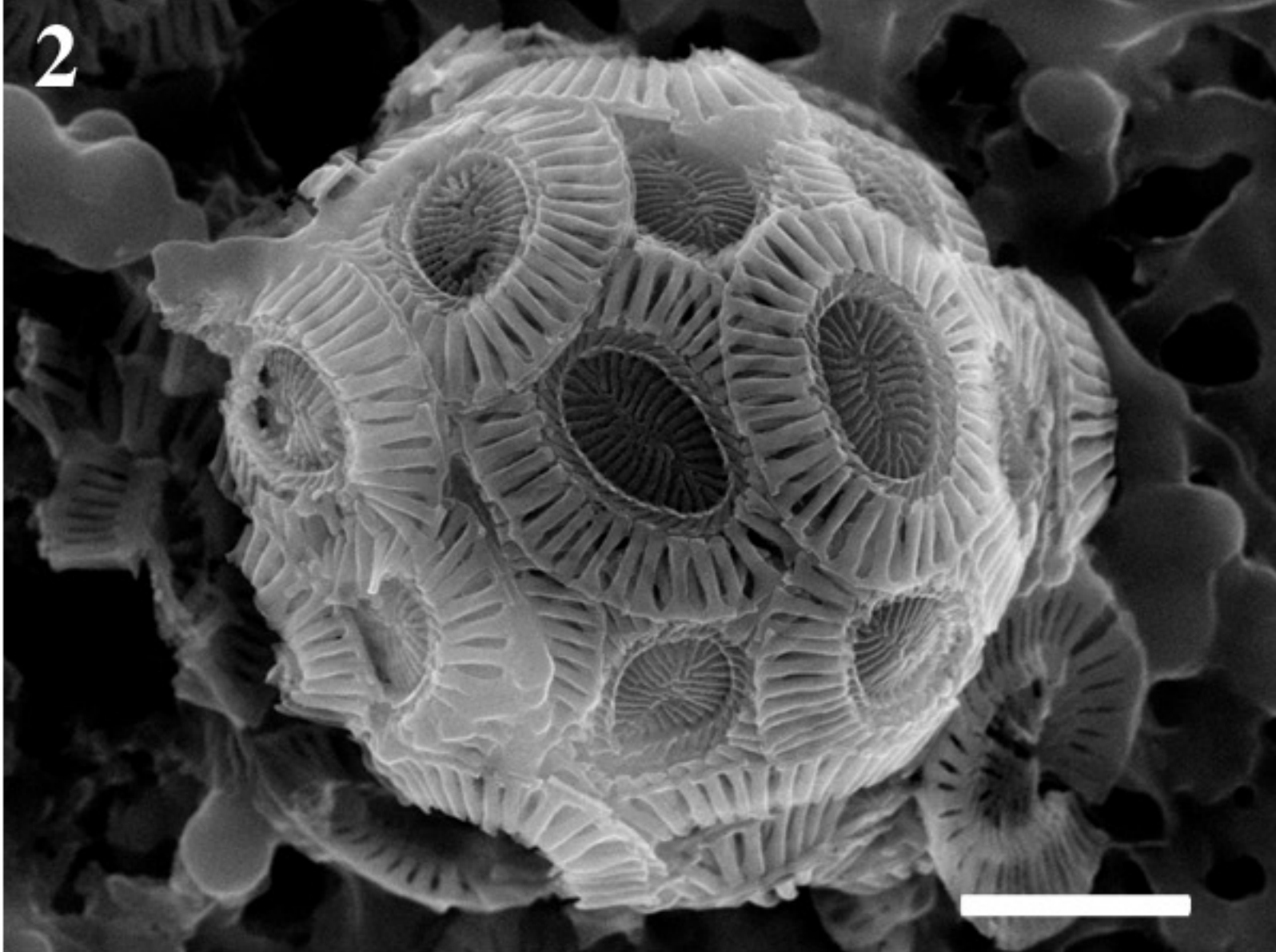
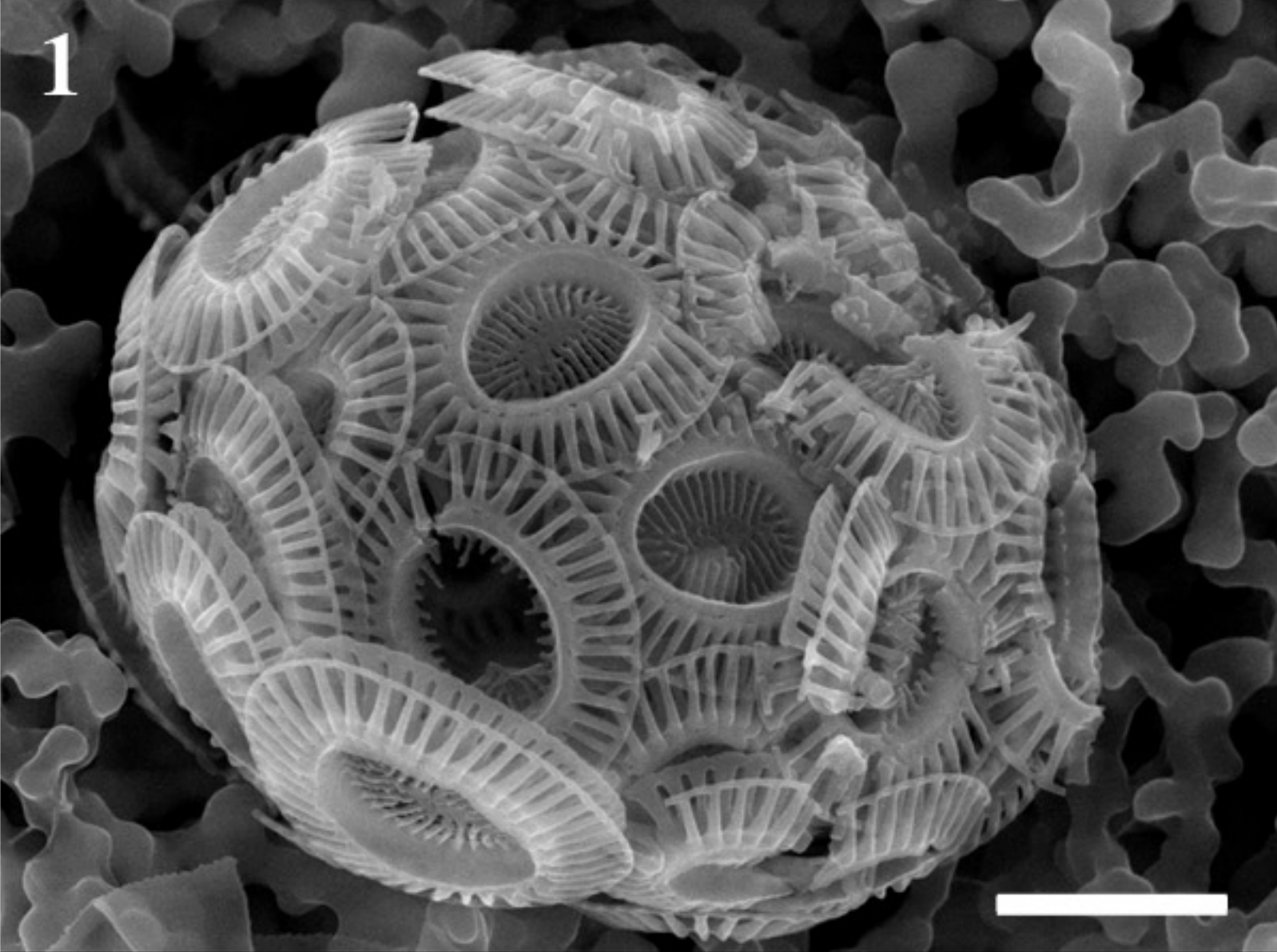
Table 1. Average floral composition of each assemblage observed by Hagino and Okada

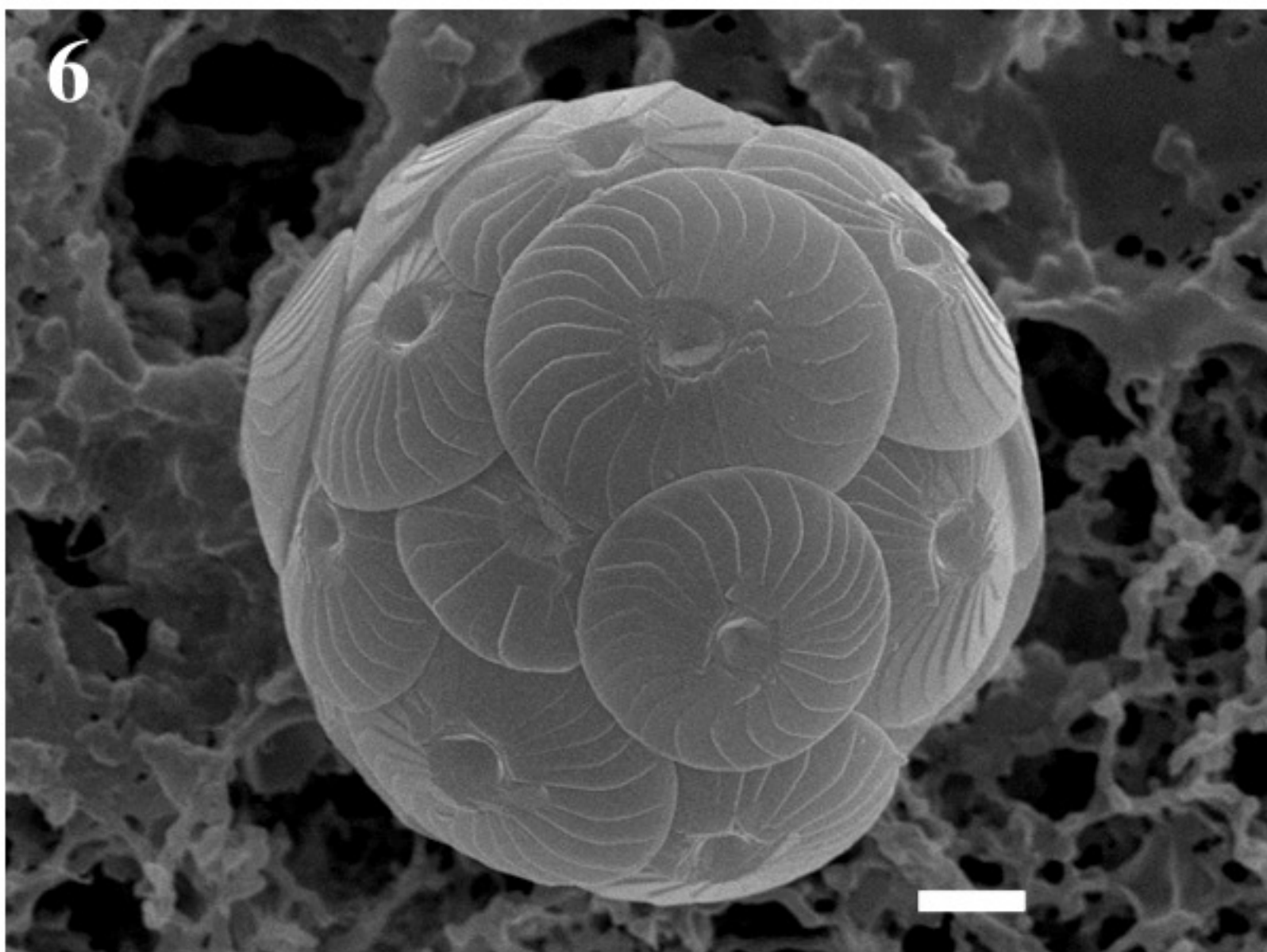
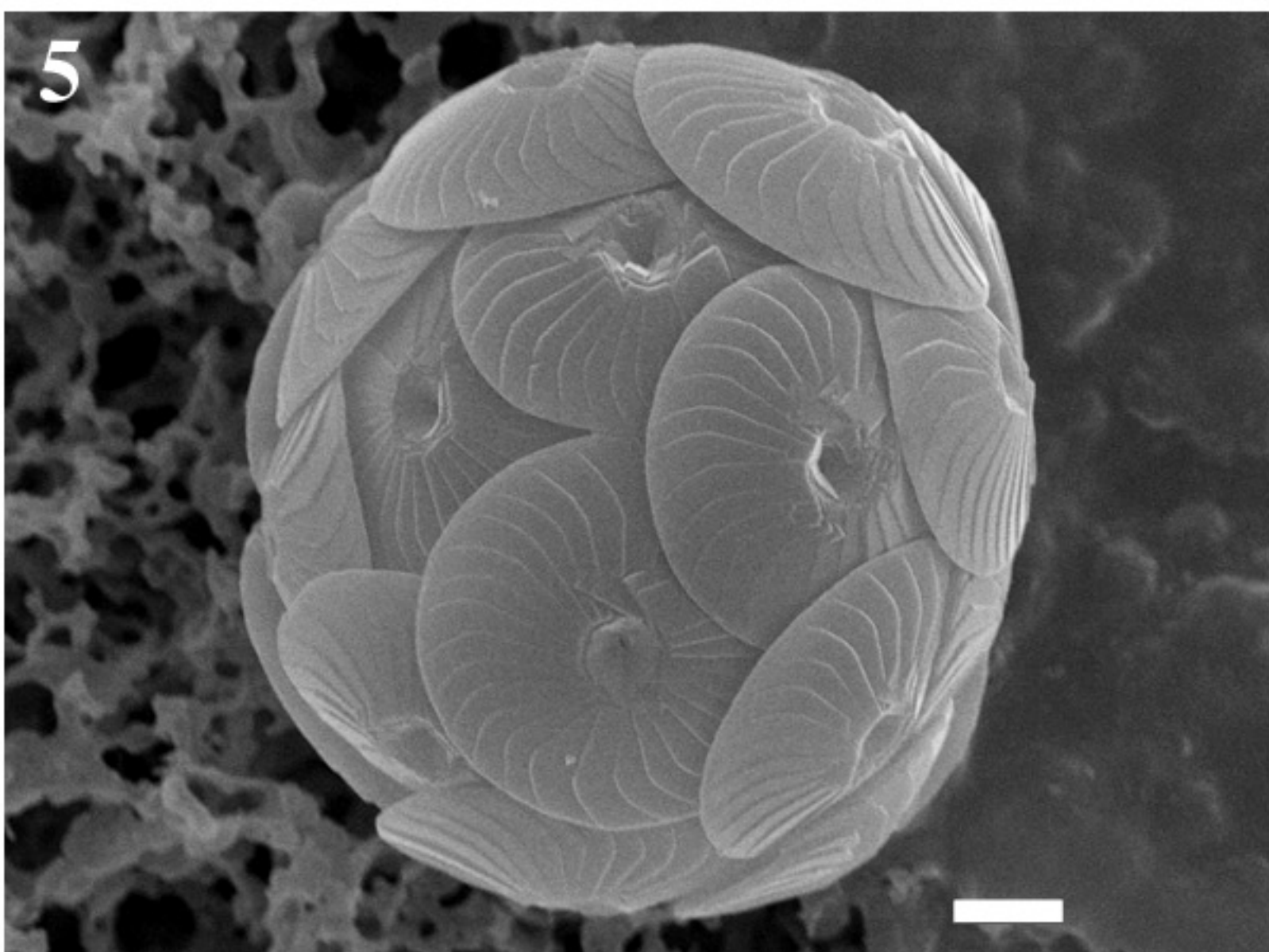
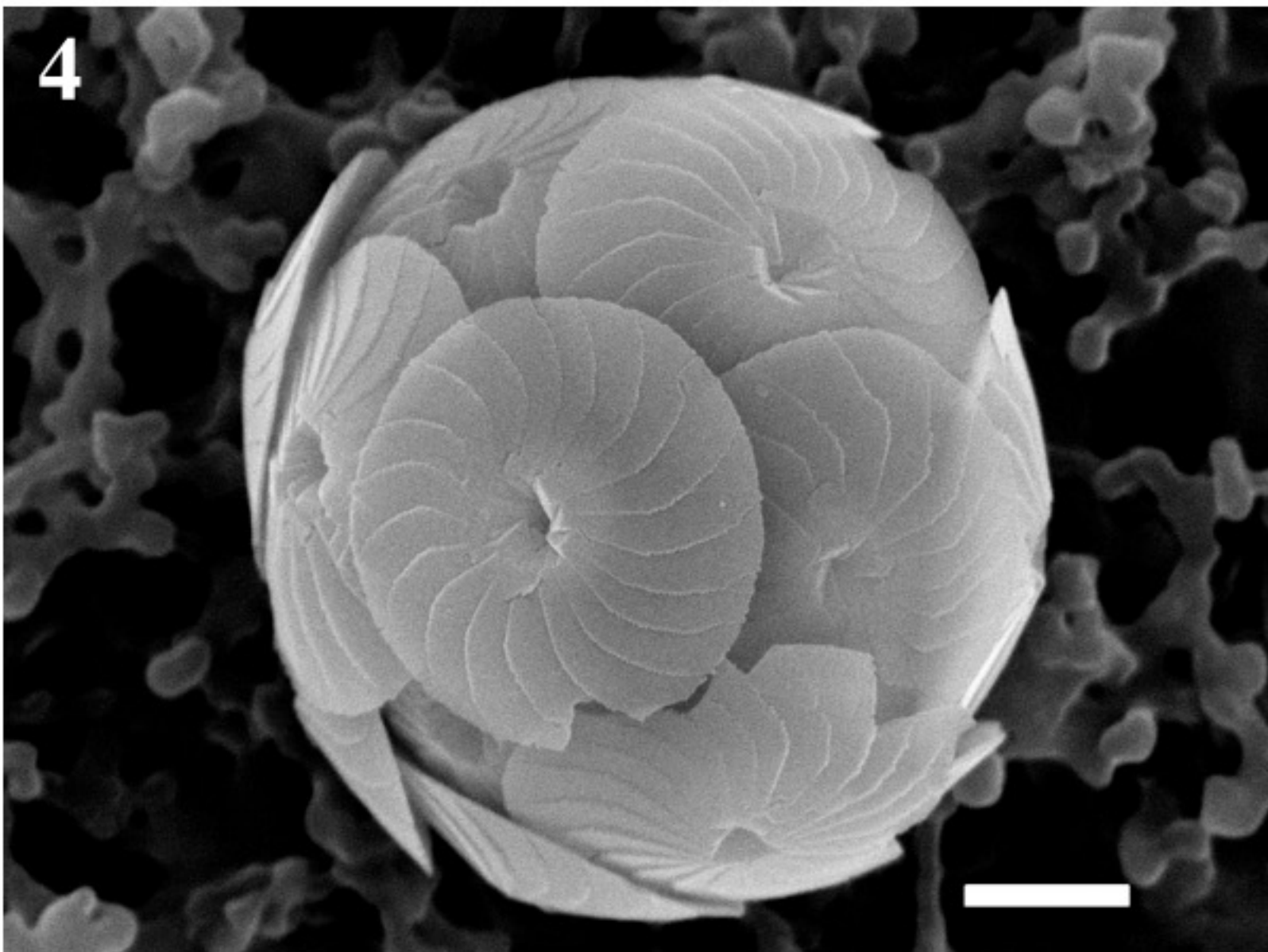
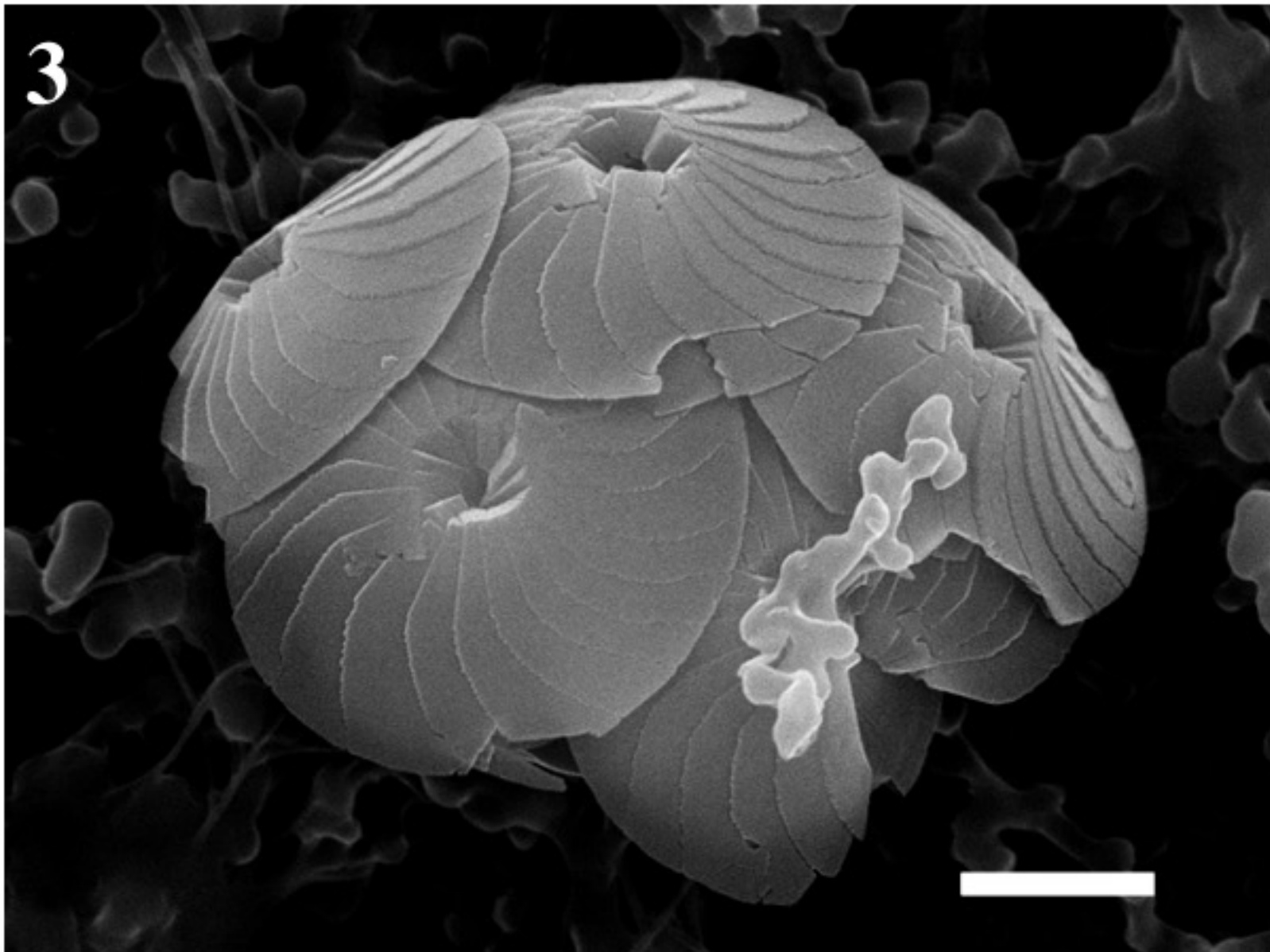
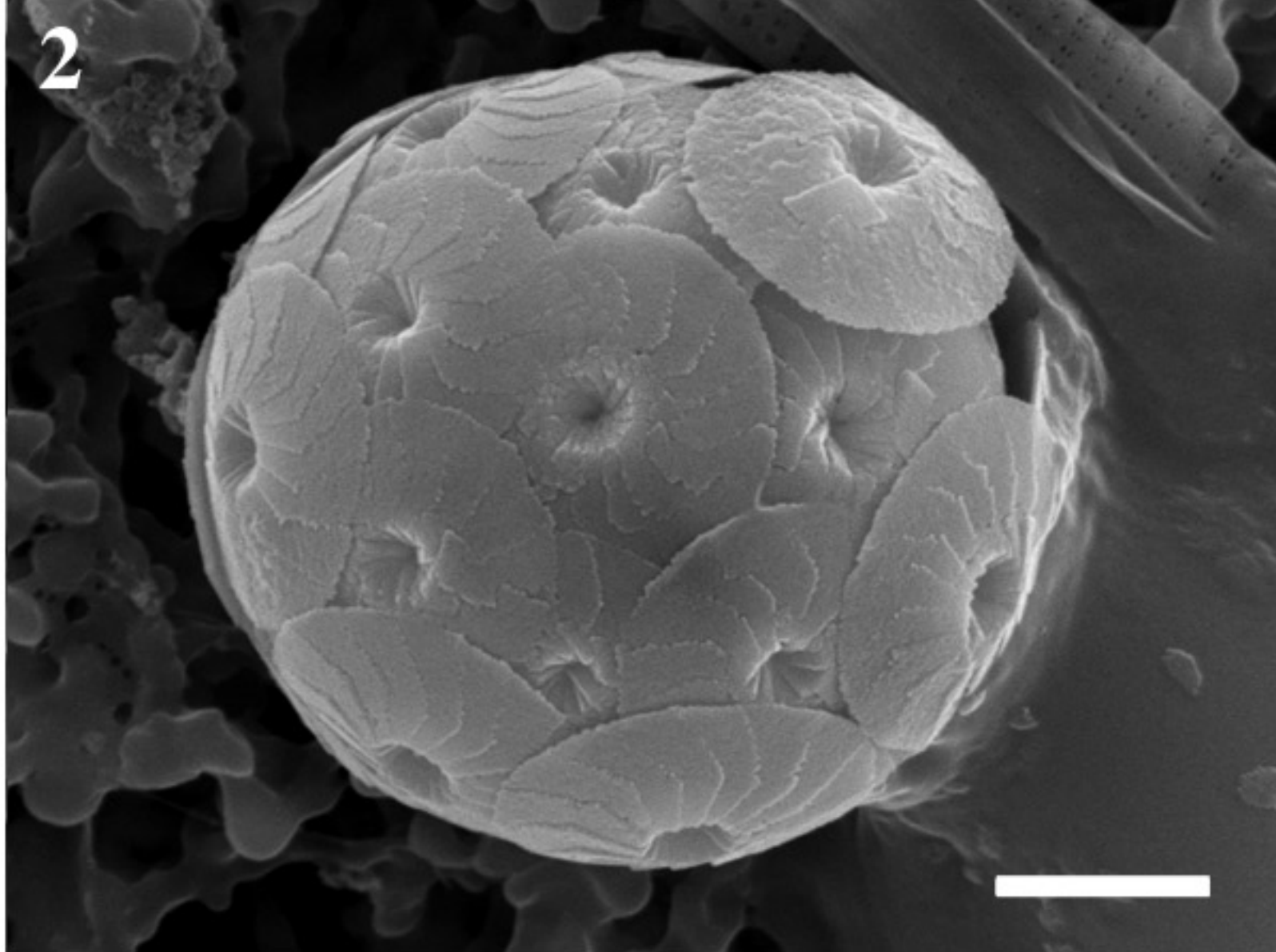
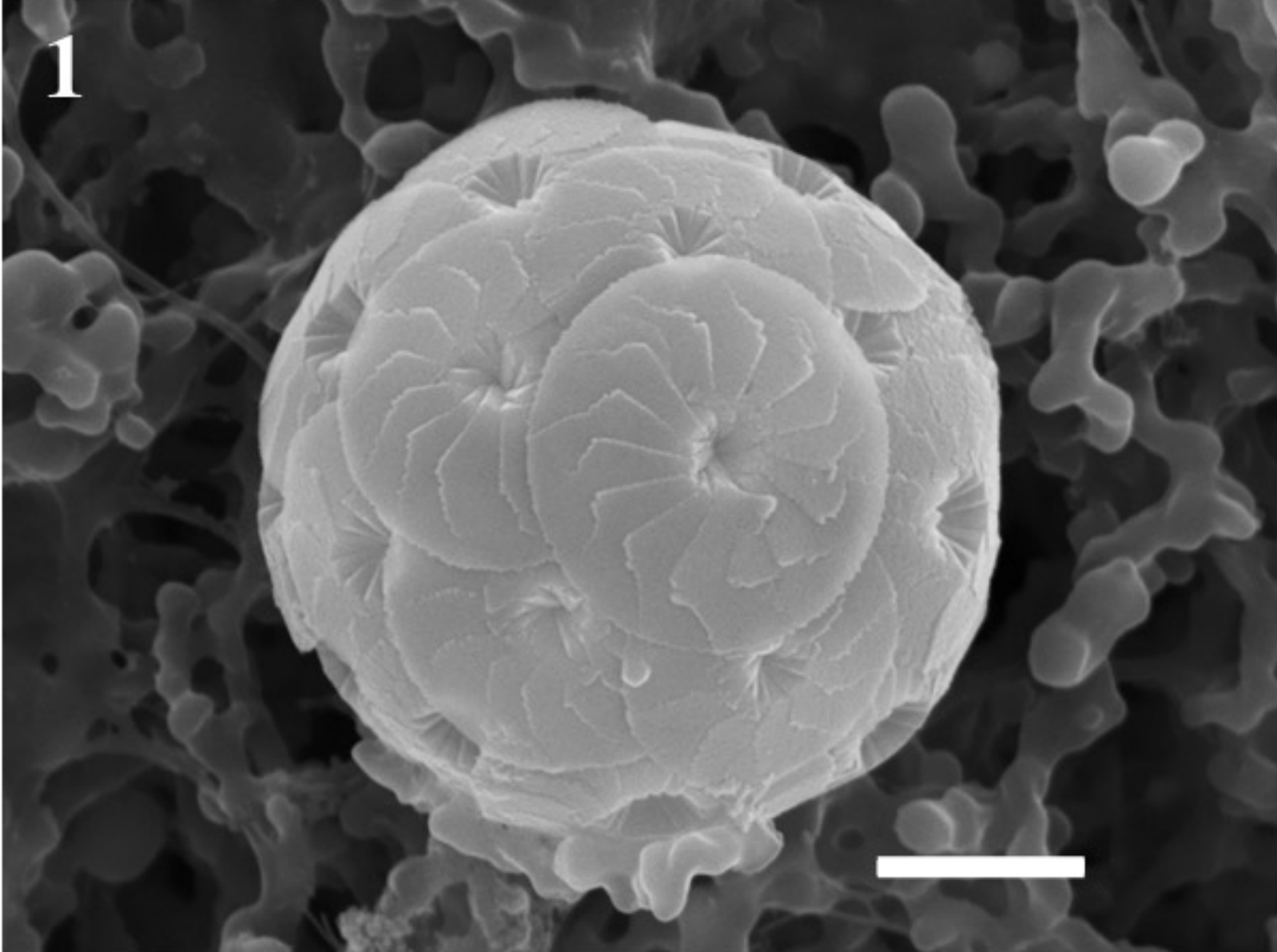
Floral Assemblage Common taxa of each floral assemblage

UCA-a	<i>U. irregularis</i> (38.7), <i>U. sibogae</i> (26.5), <i>G. oceanica</i> (16.3)
UCA-b	<i>U. irregularis</i> (65.7)
UCA-c	<i>U. irregularis</i> (40.0), <i>D. tubifera</i> (8.7), <i>Rhabdosphaera</i> spp. (7.1)
GCA-a	<i>G. oceanica</i> (56.7)
GCA-b	<i>G. oceanica</i> (29.0), <i>E. huxleyi</i> (22.5), <i>C. leptoporus</i> (16.3), <i>O. antillarum</i> (8.6), <i>U. hulburtiana</i> (6.6)
ECA-a	<i>E. huxleyi</i> (27.7), small <i>Gephyrocapsa</i> spp. (13.2), small <i>Reticulofenestra</i> spp. (10.8)
ECA-b	<i>E. huxleyi</i> (53.7)

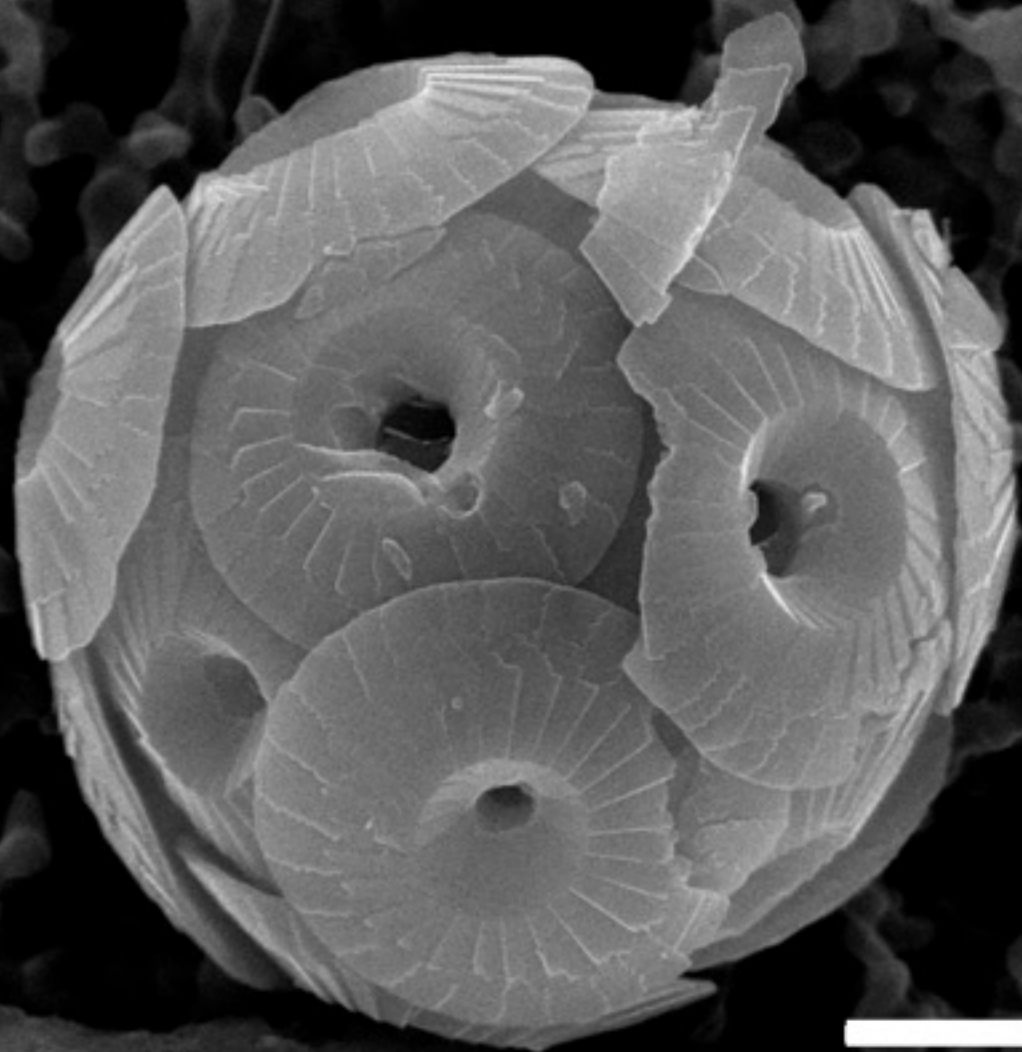
Note: The numbers within the parentheses indicate the mean relative abundance of each taxa.

Hagino and Okada, Table 1

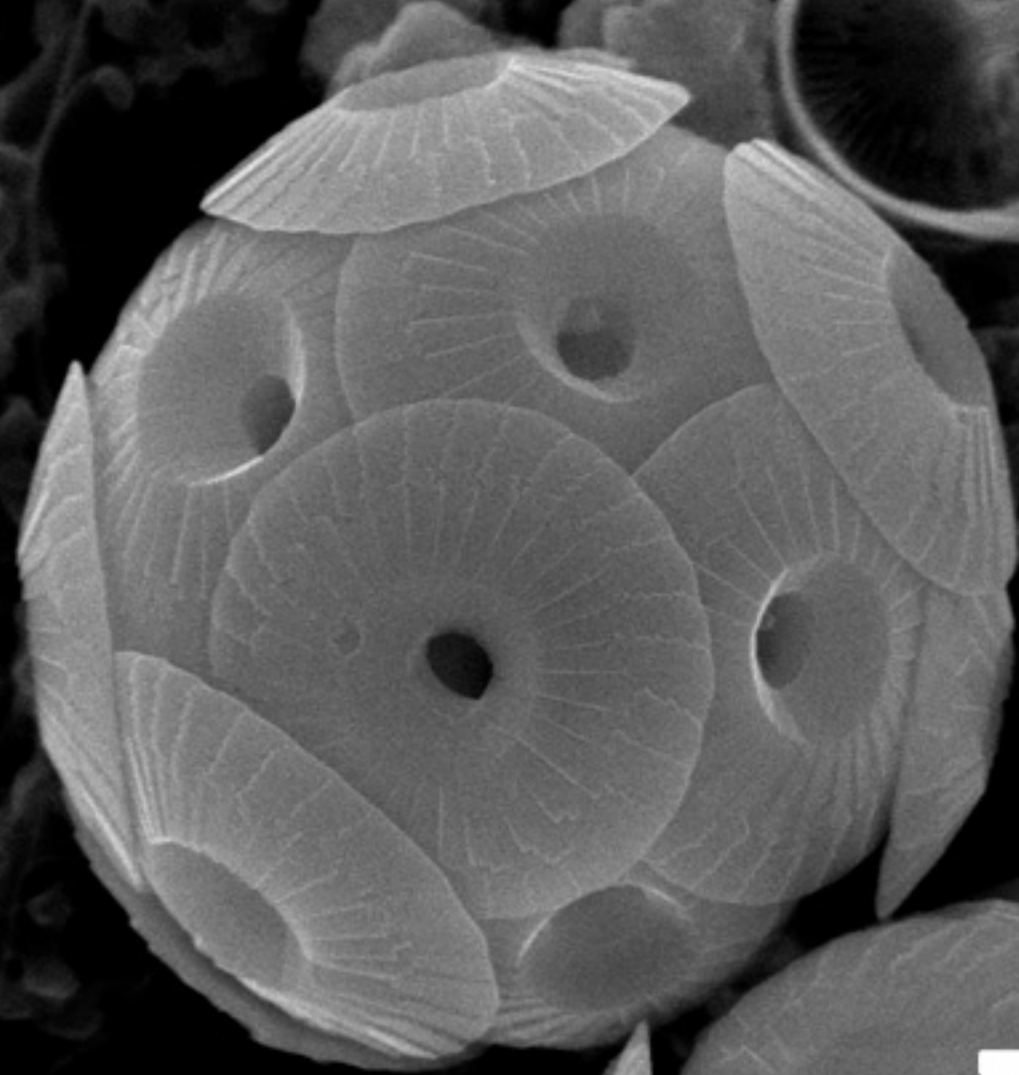




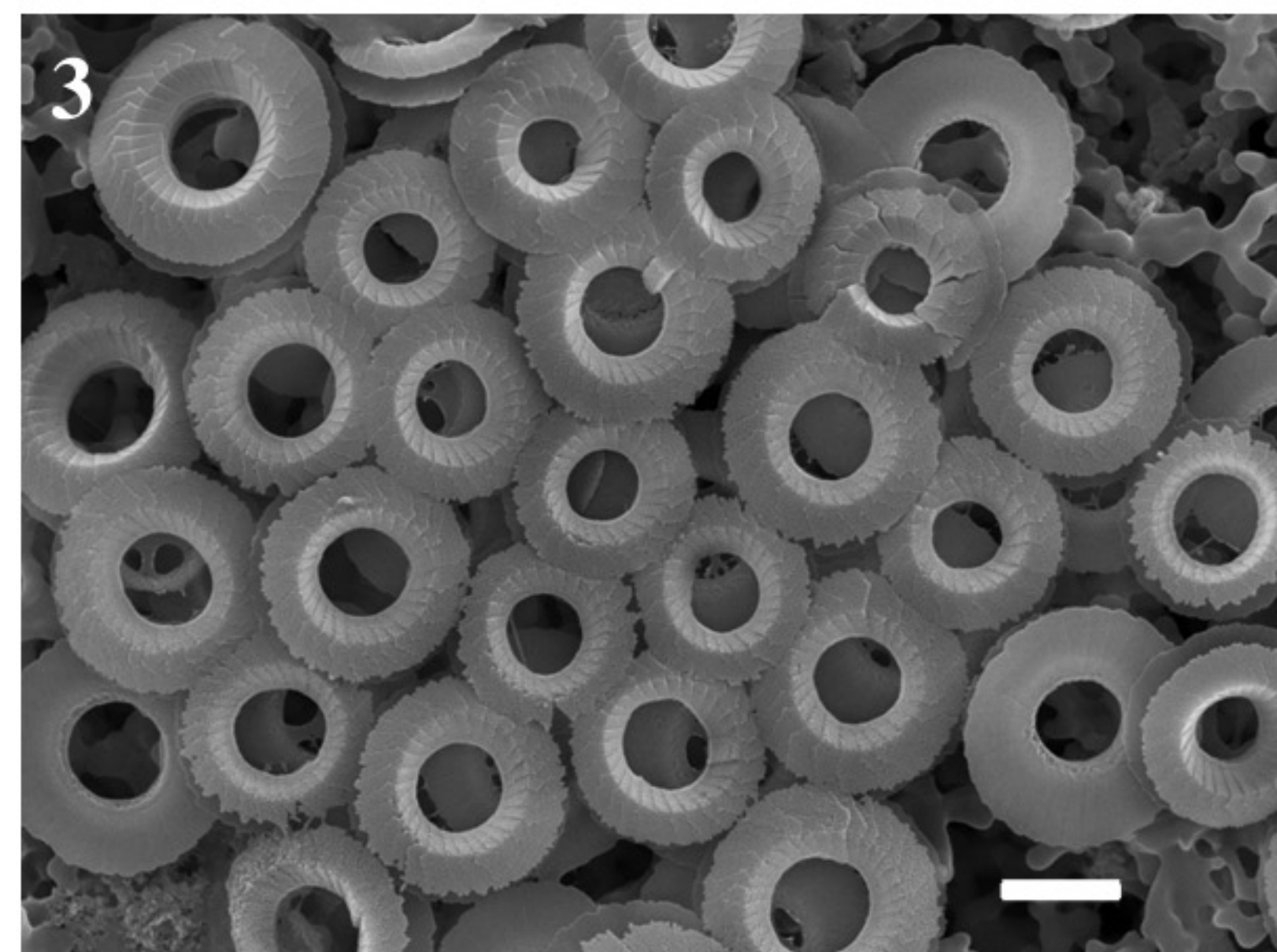
1



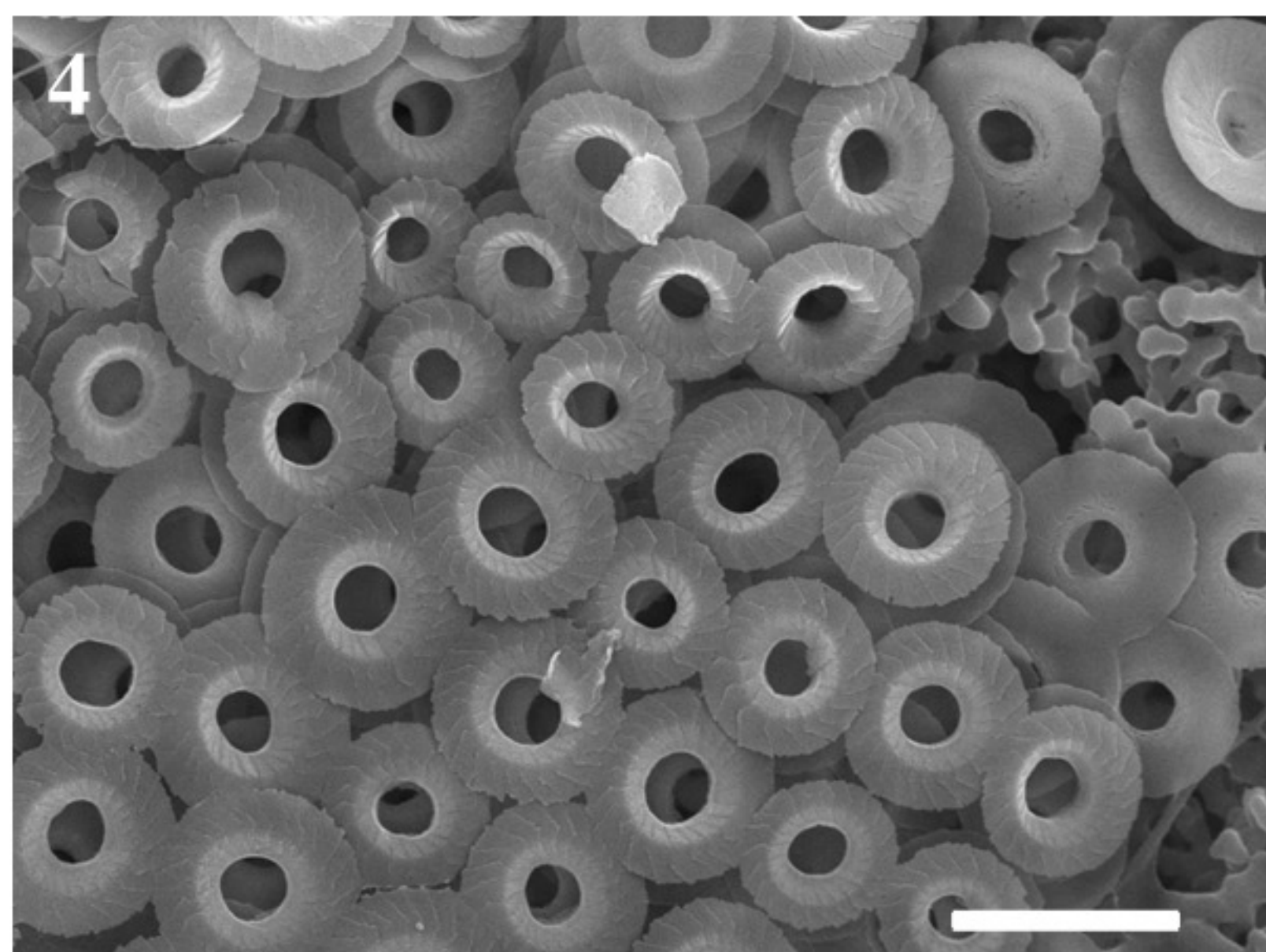
2

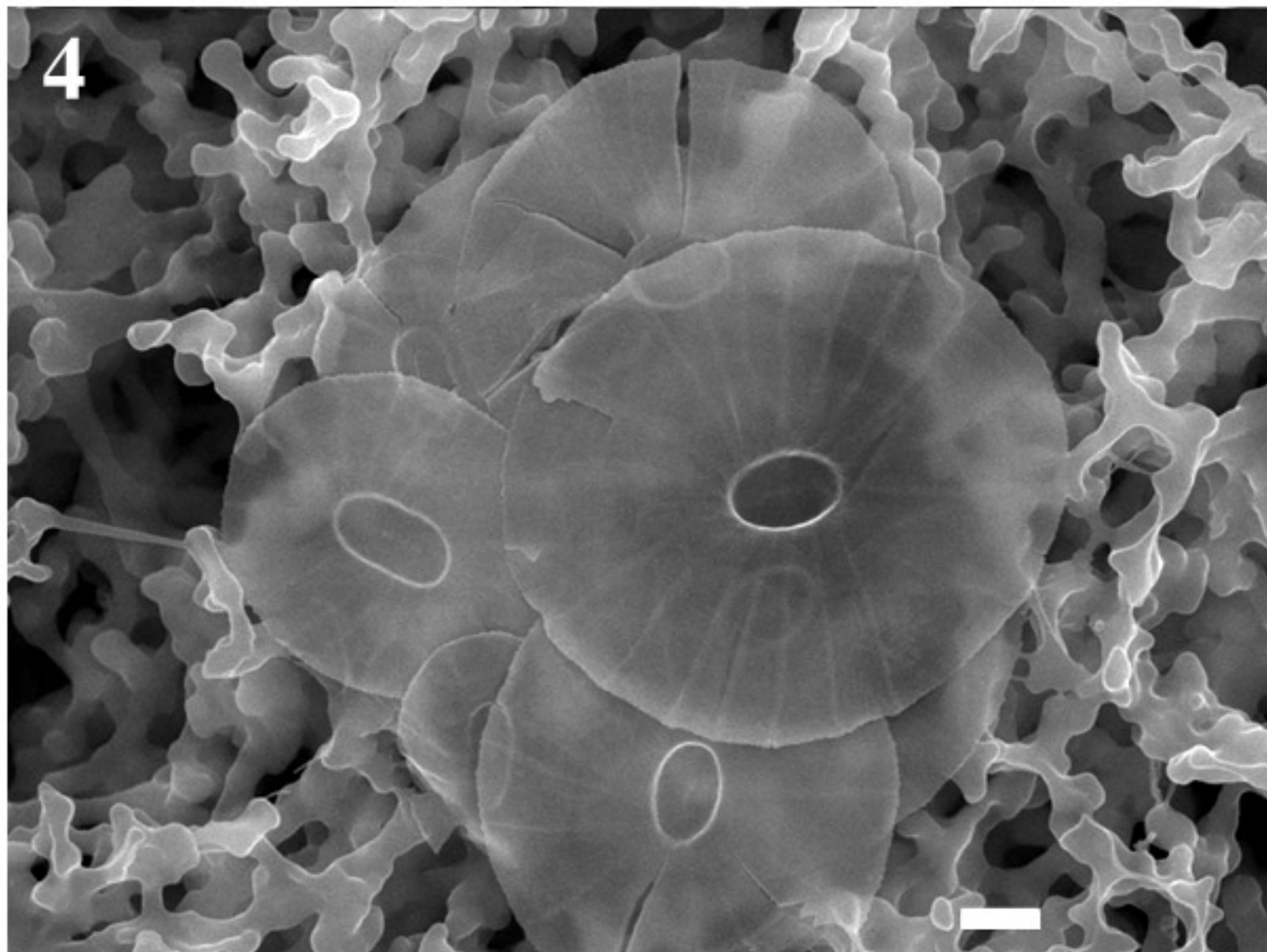
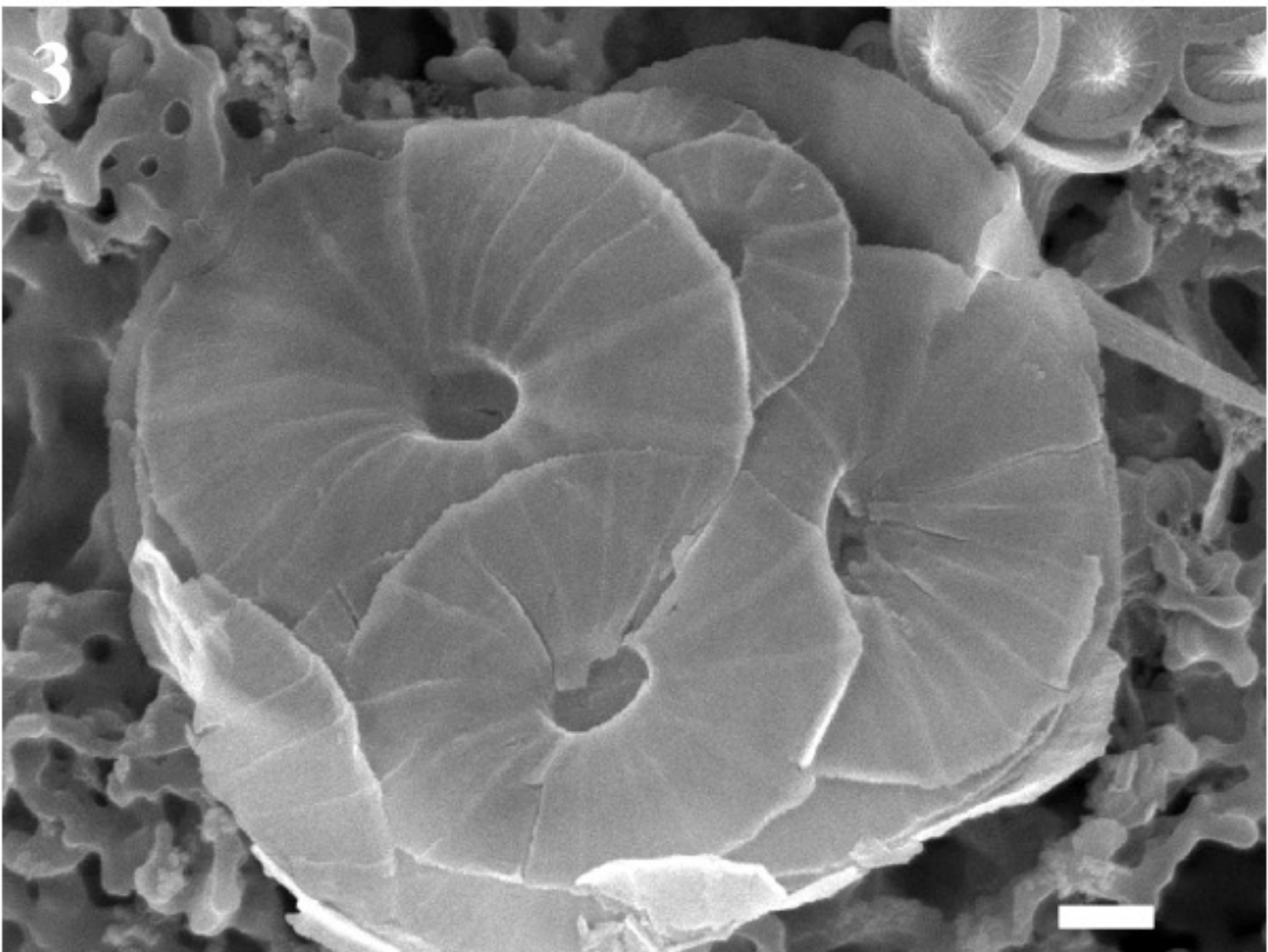
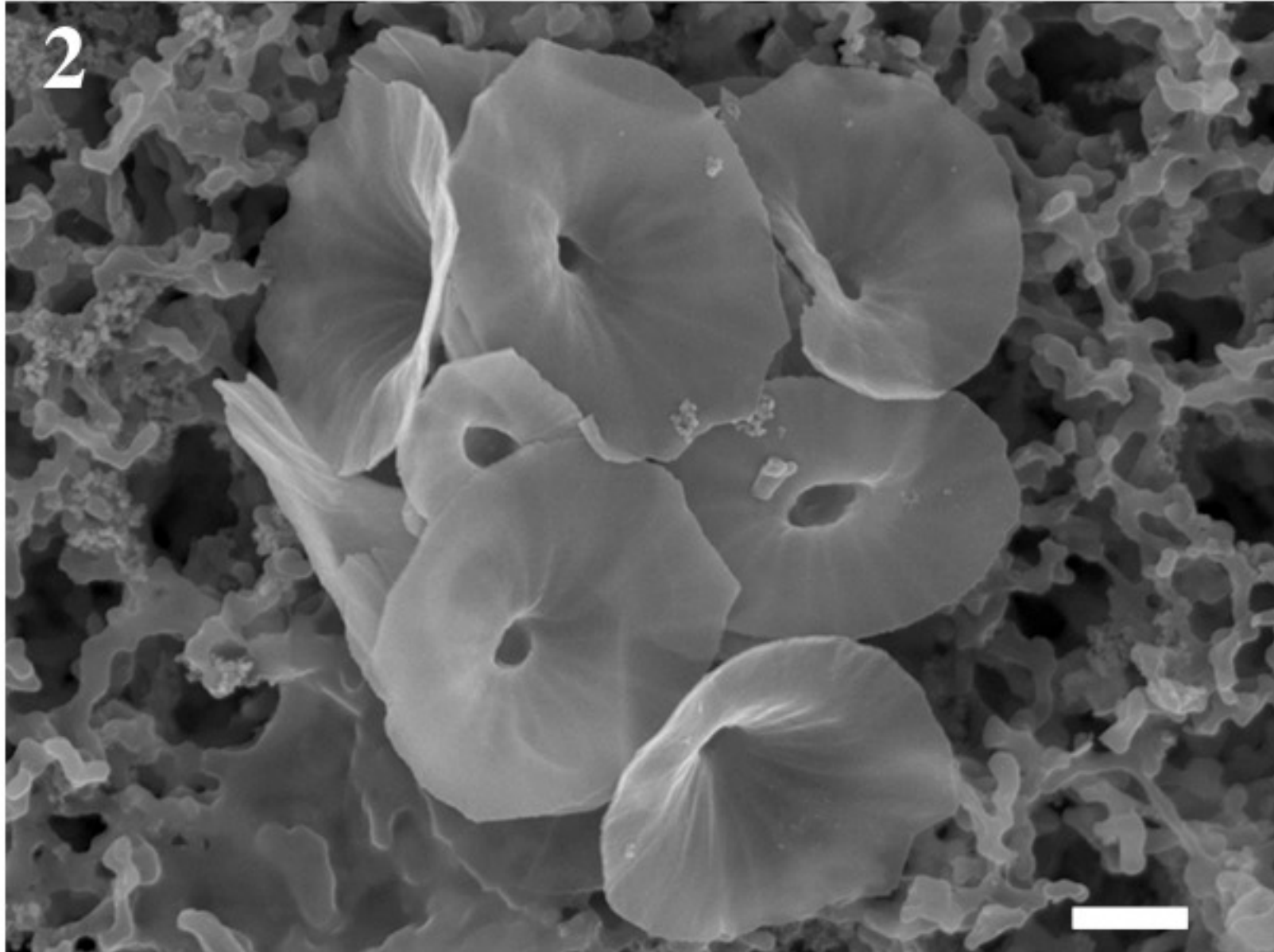
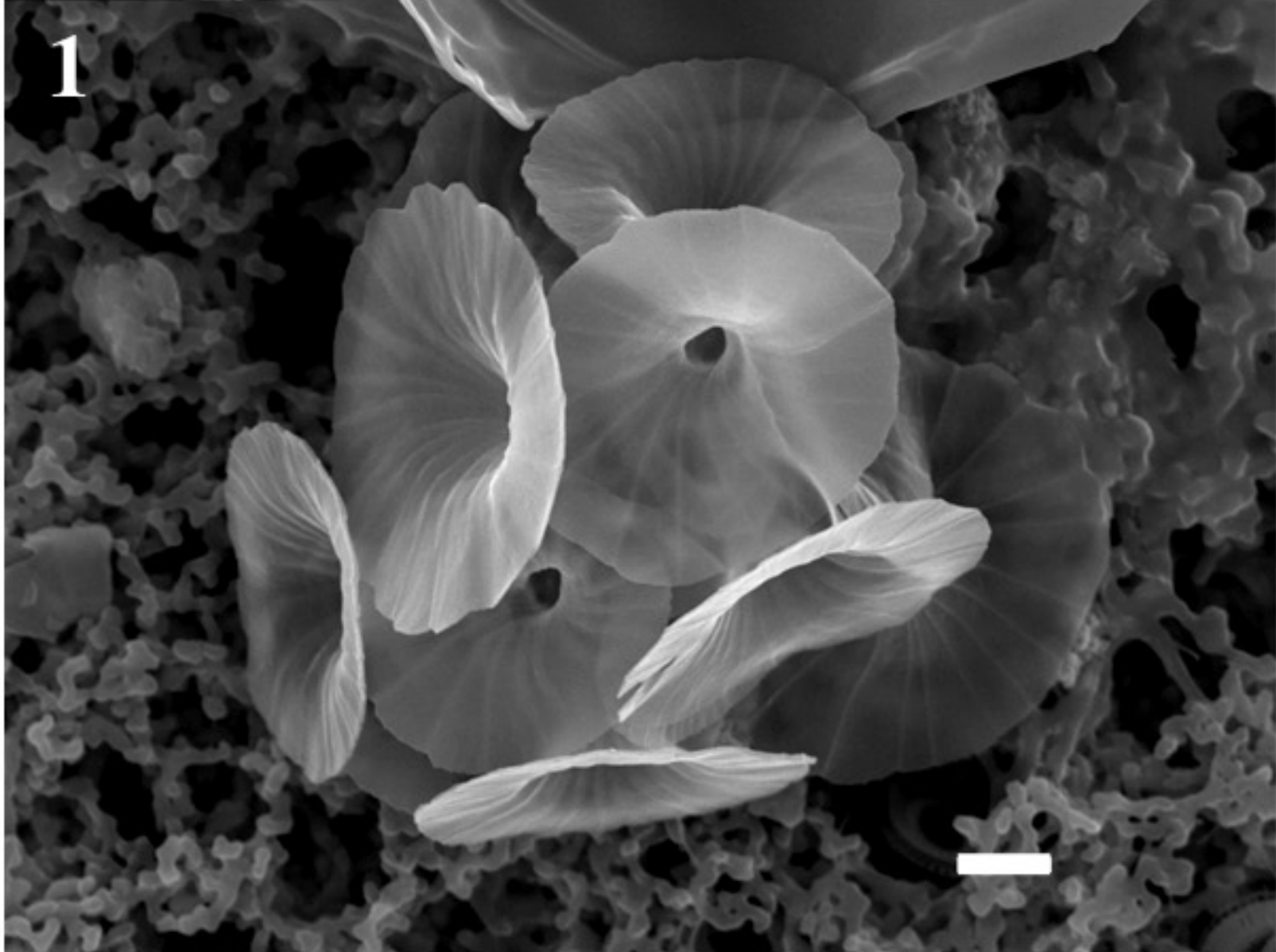


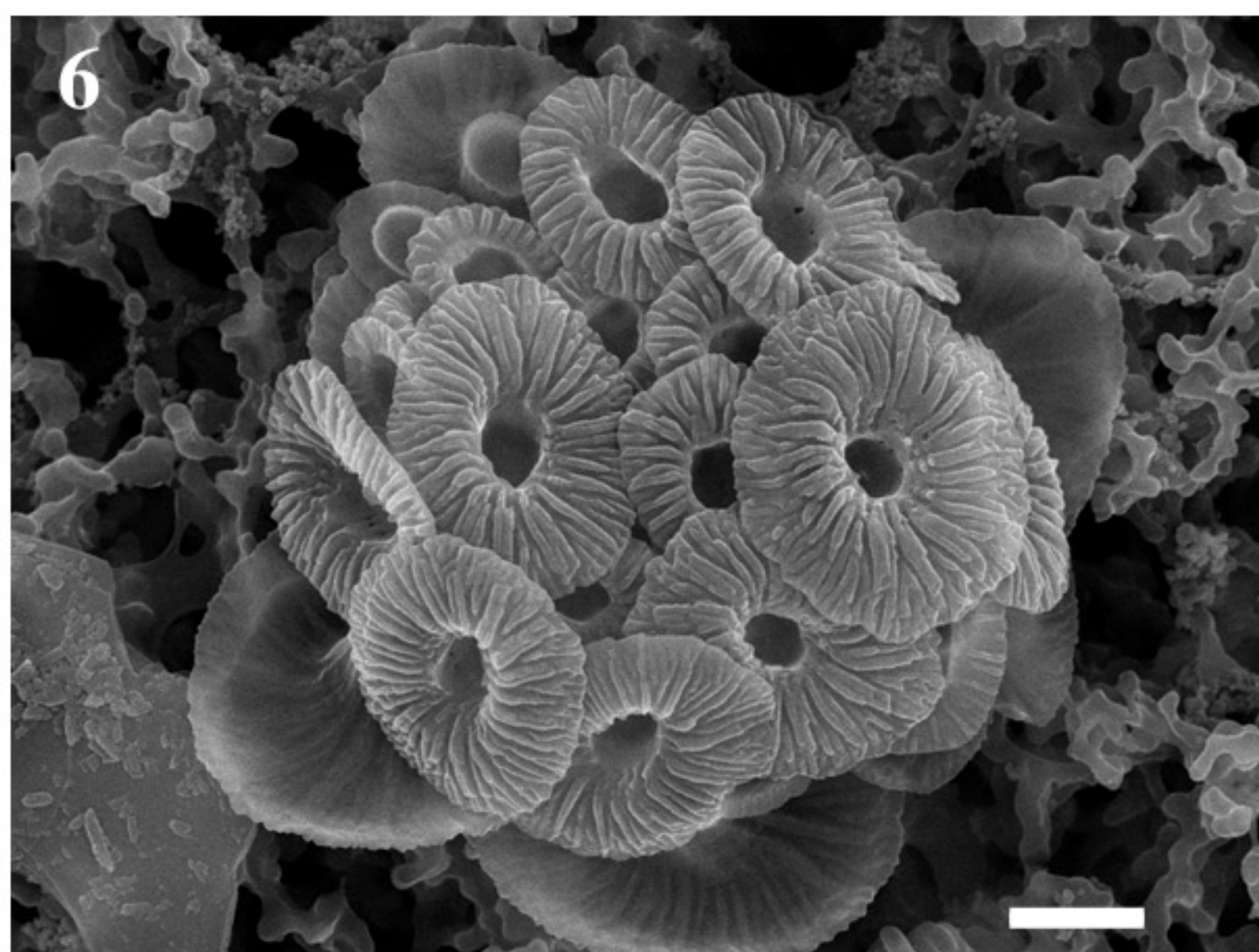
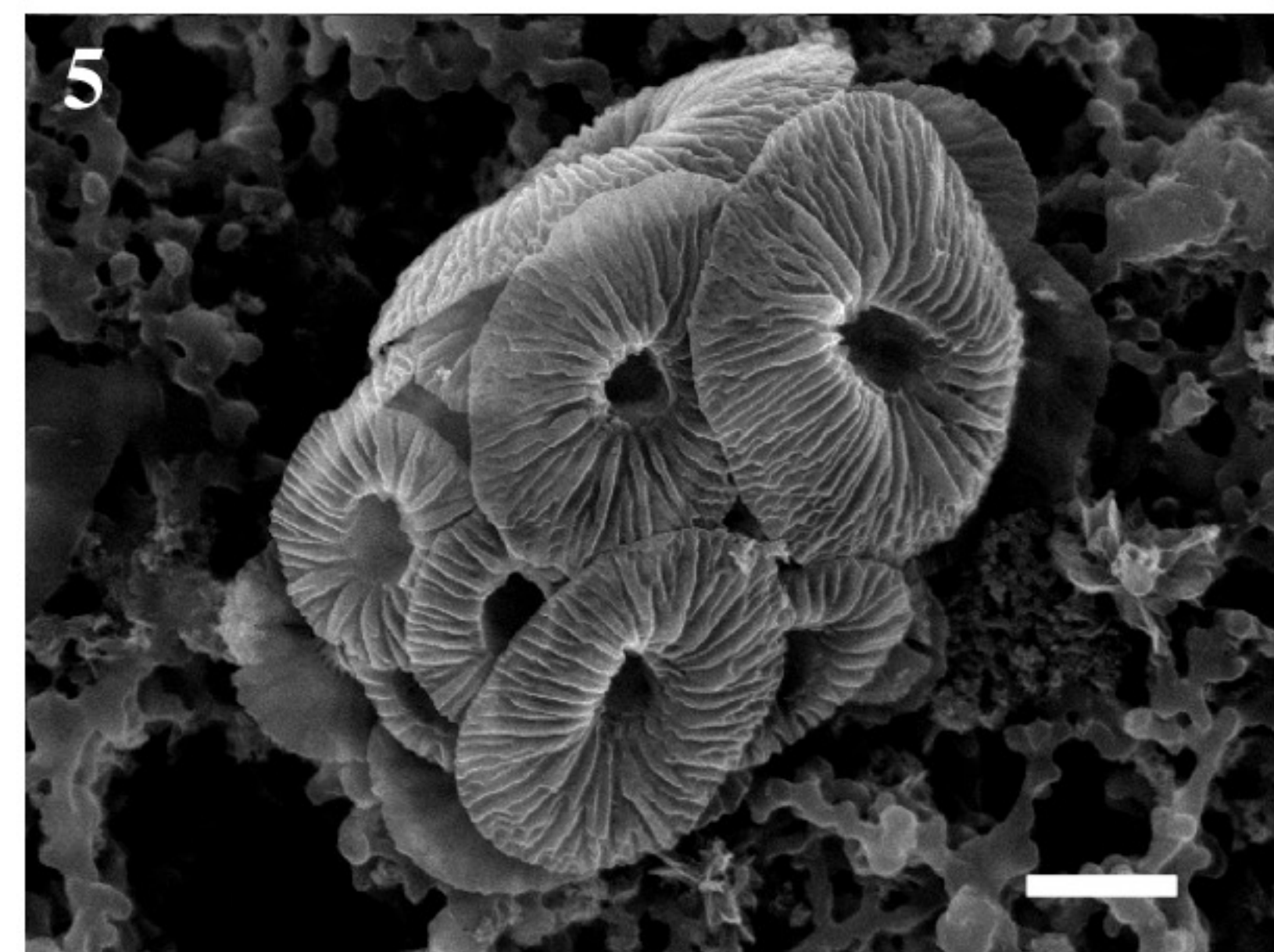
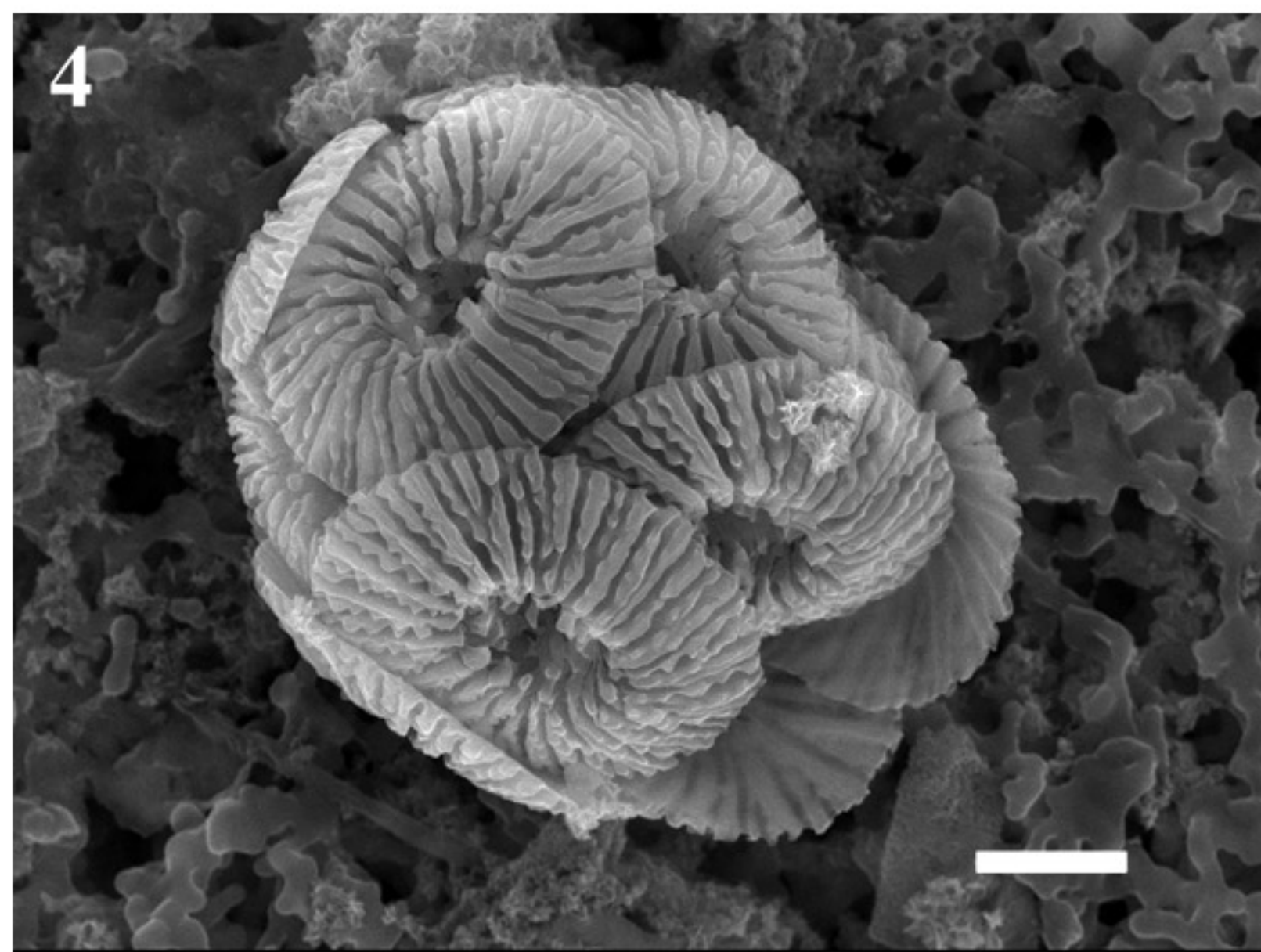
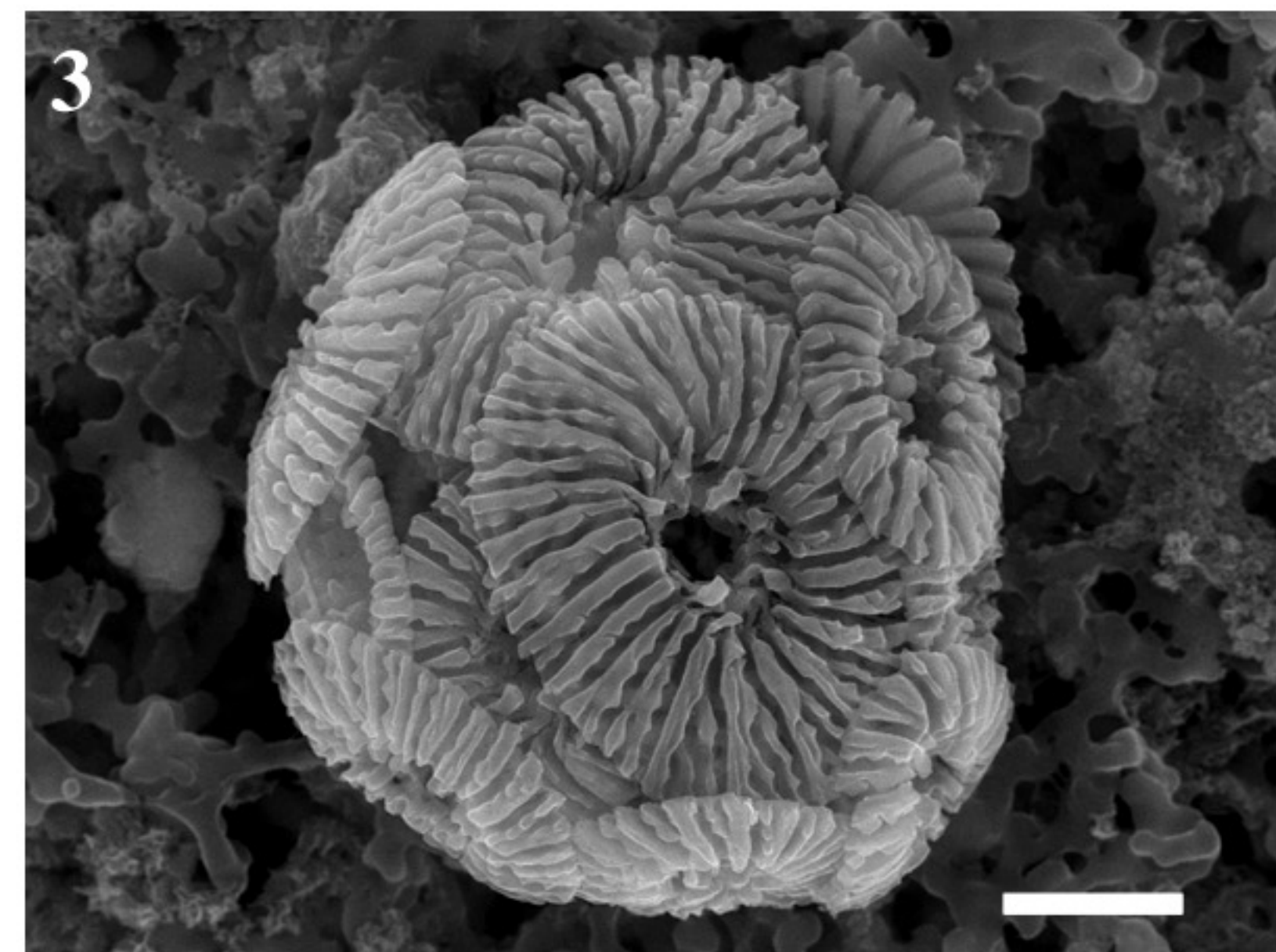
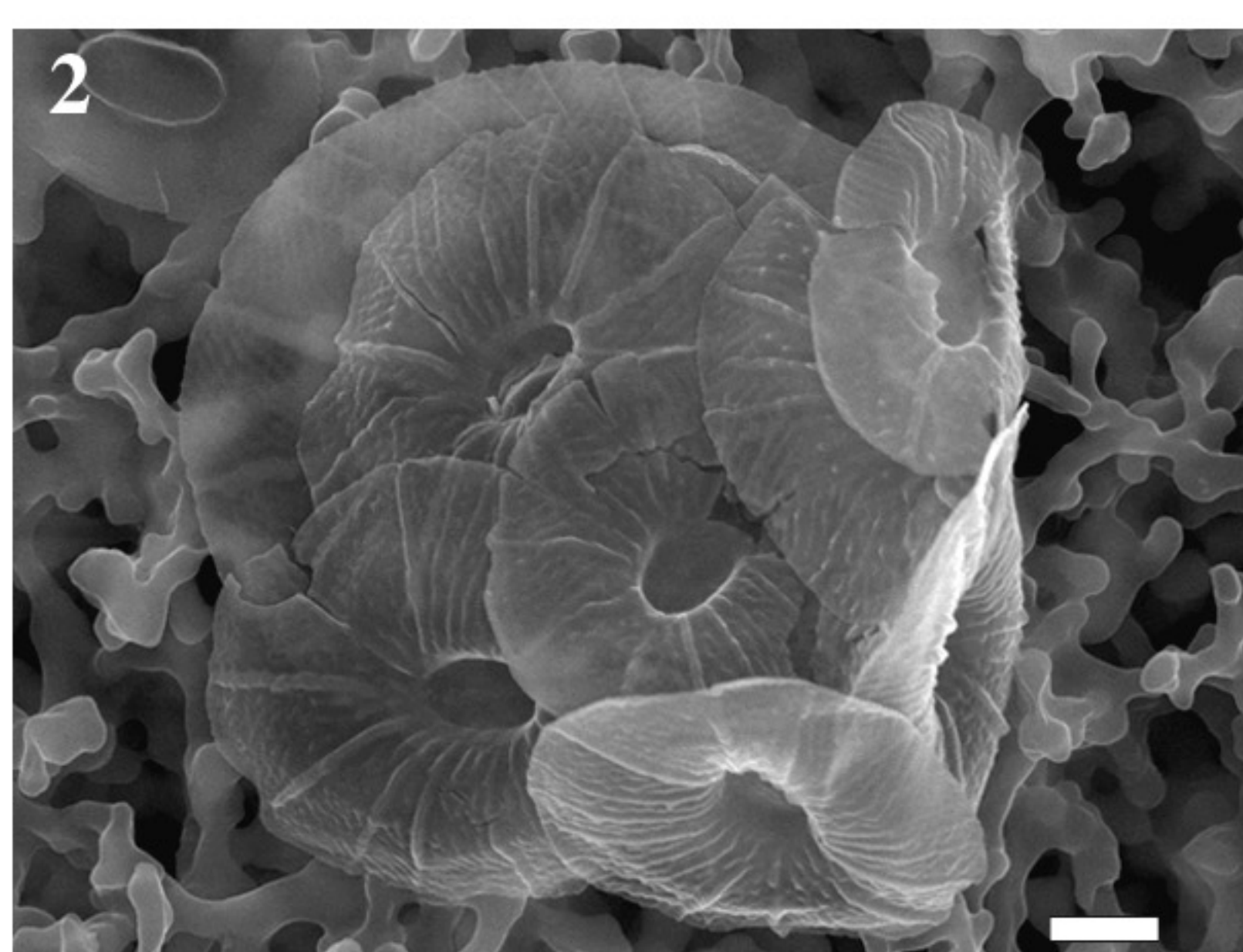
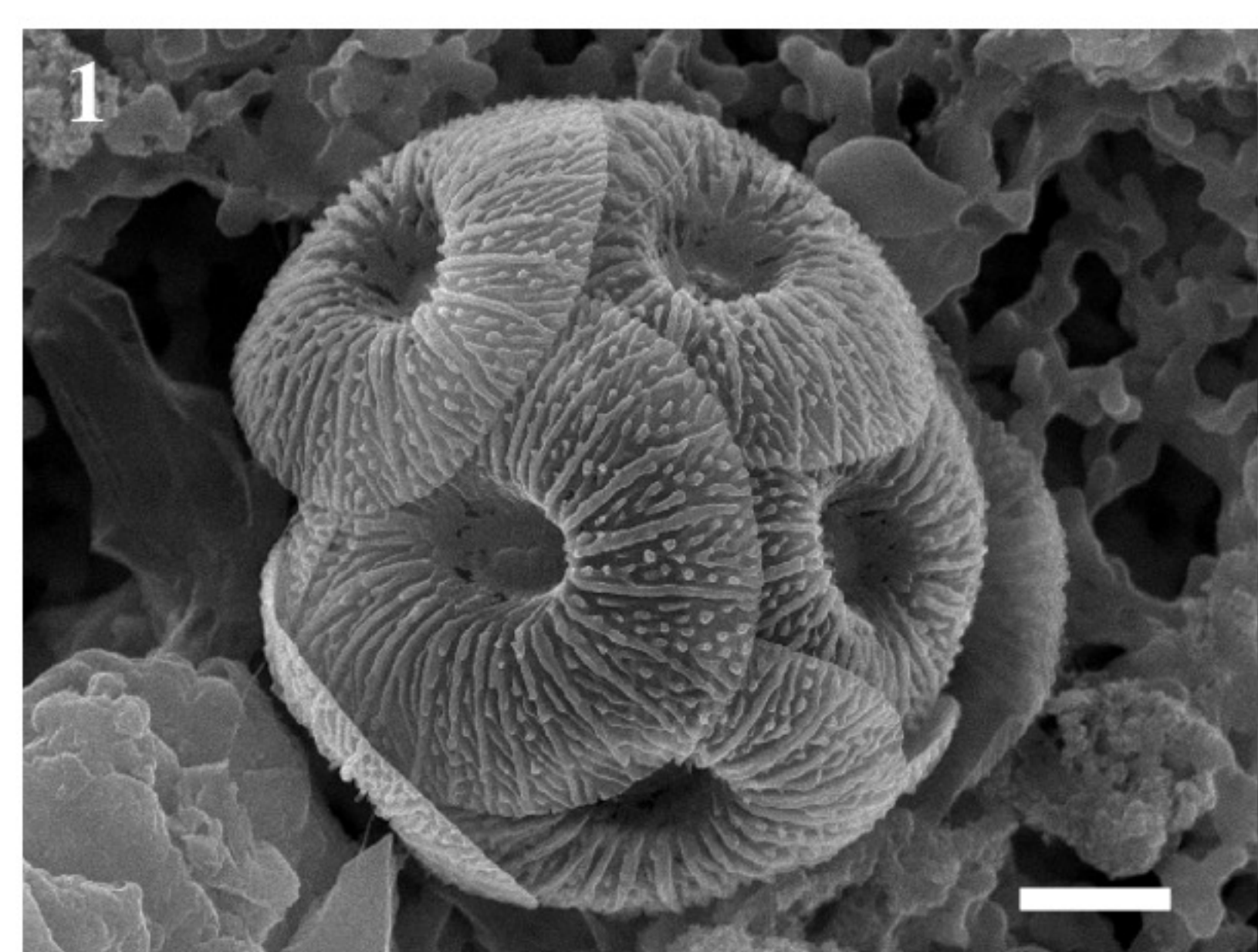
3



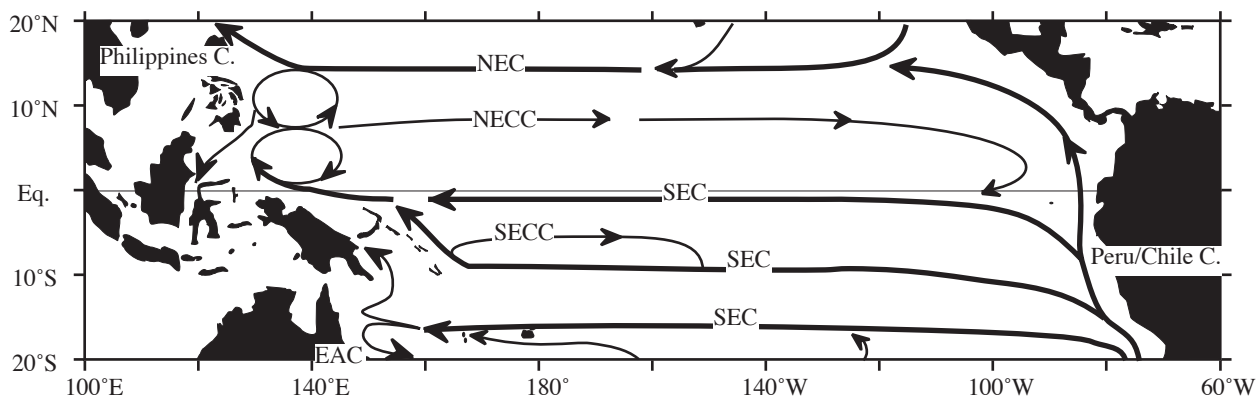
4



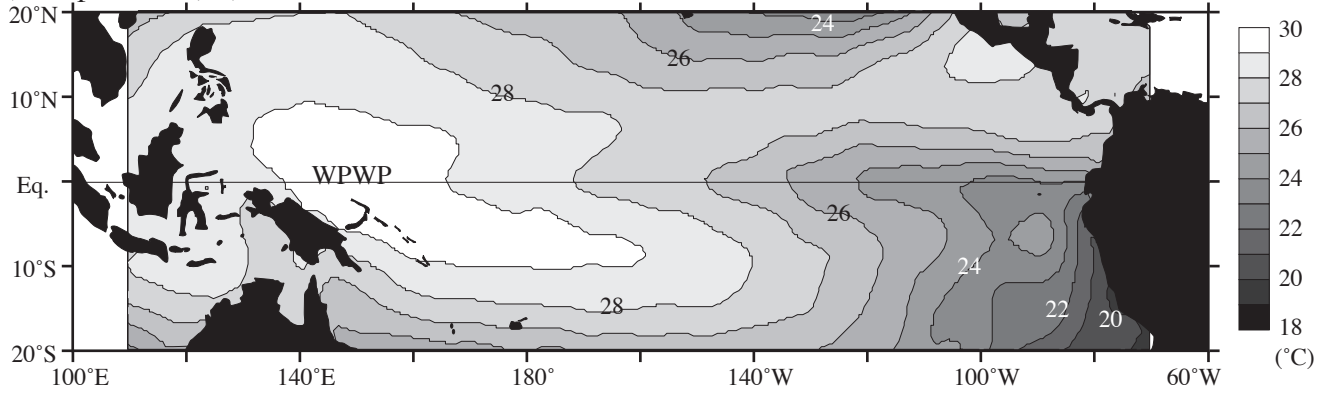




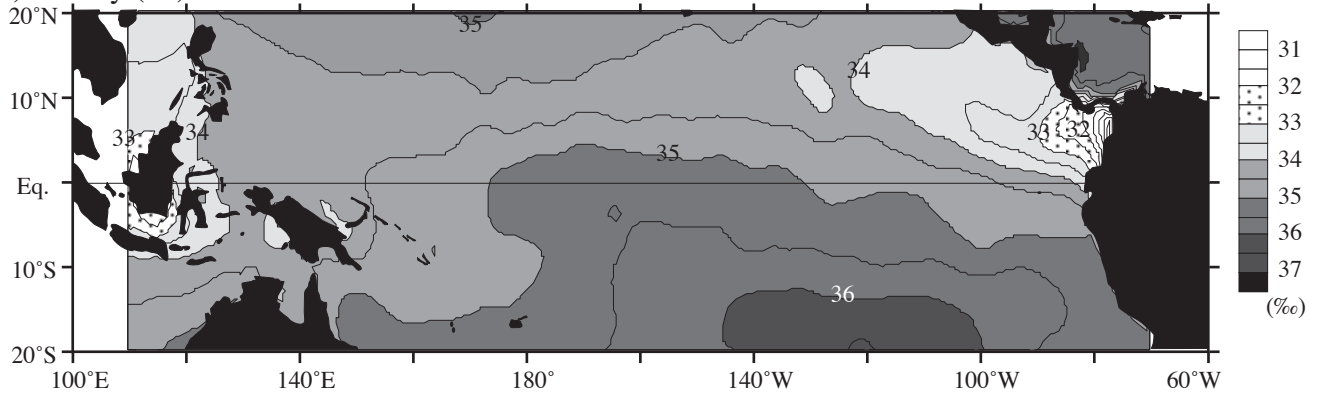
(a) Surface Currents in the studied area



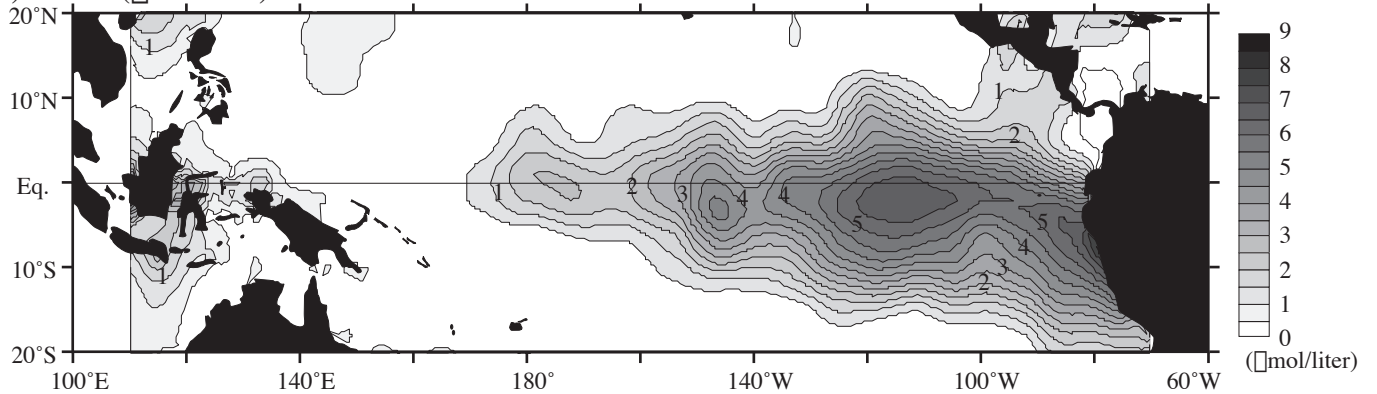
(a) temperature ($^{\circ}\text{C}$)



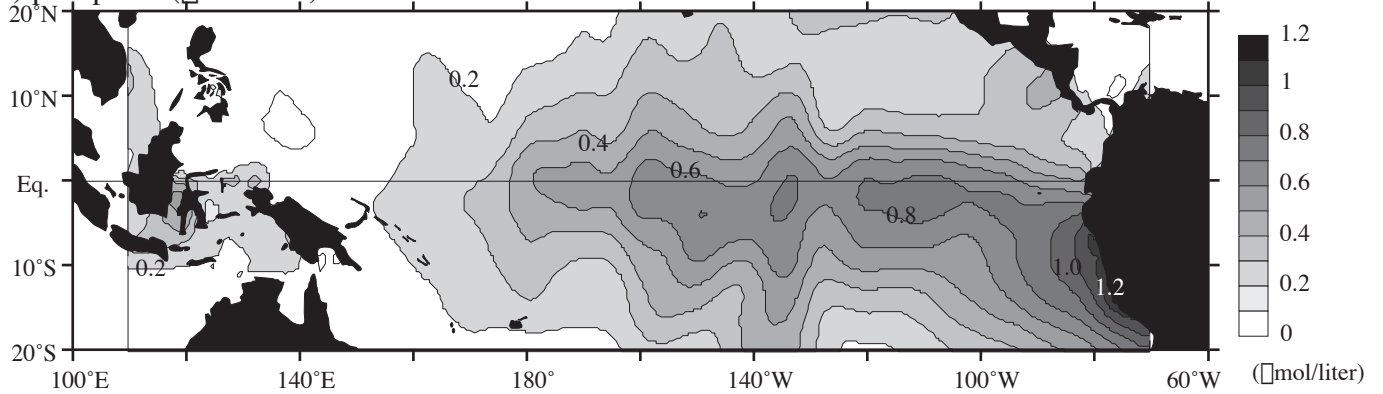
(b) salinity (‰)



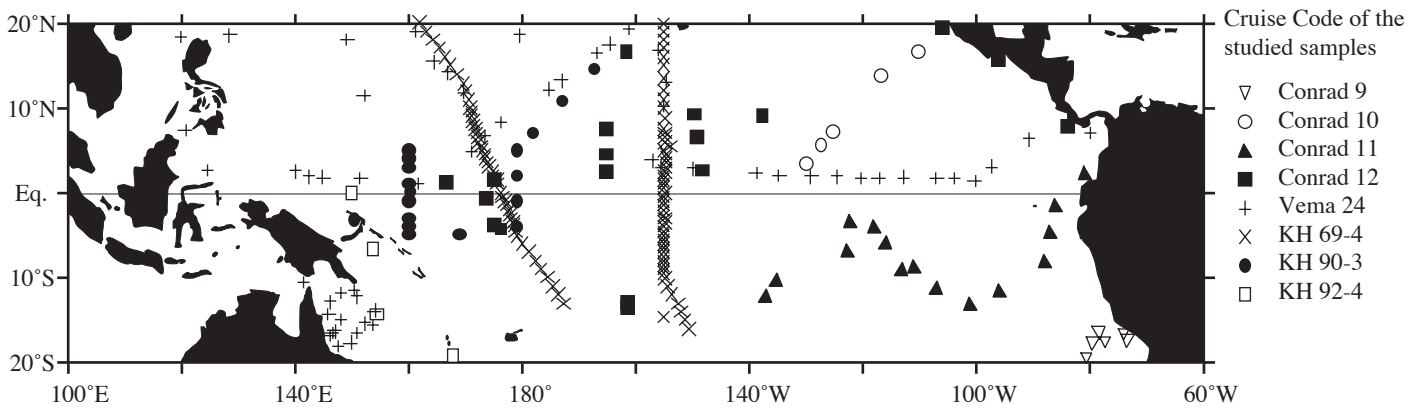
(c) nitrate ($\mu\text{mol/liter}$)



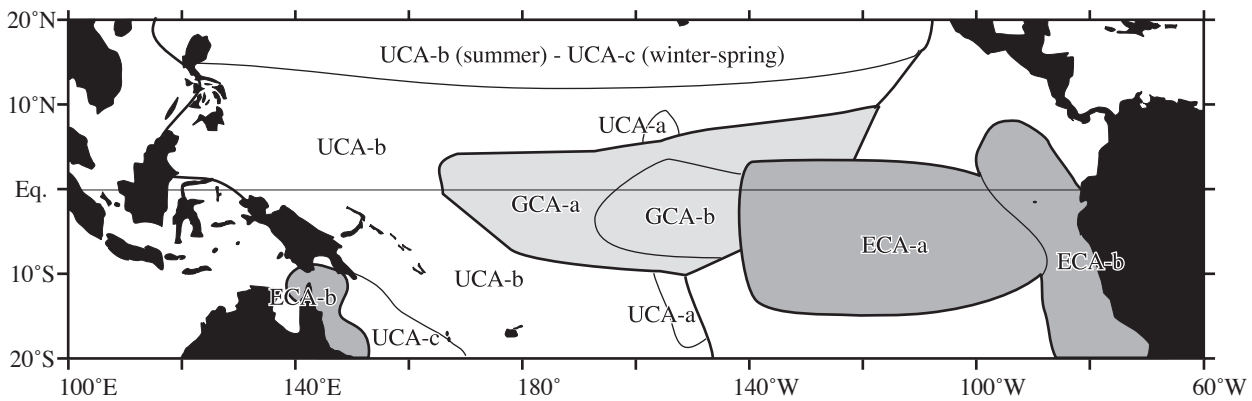
(d) phosphate ($\mu\text{mol/liter}$)



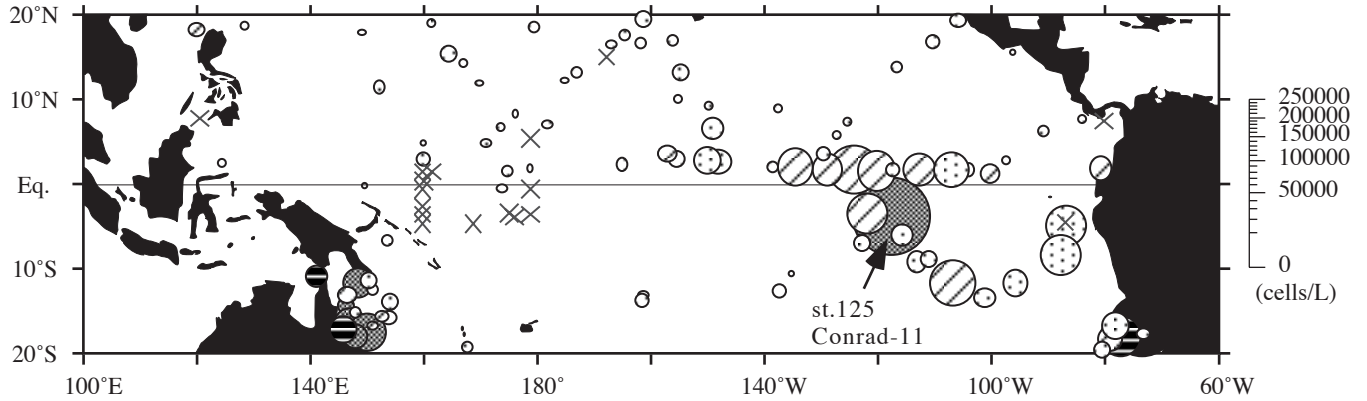
(a) Distribution of samples studied by Hagino and Okada (2004) and this study



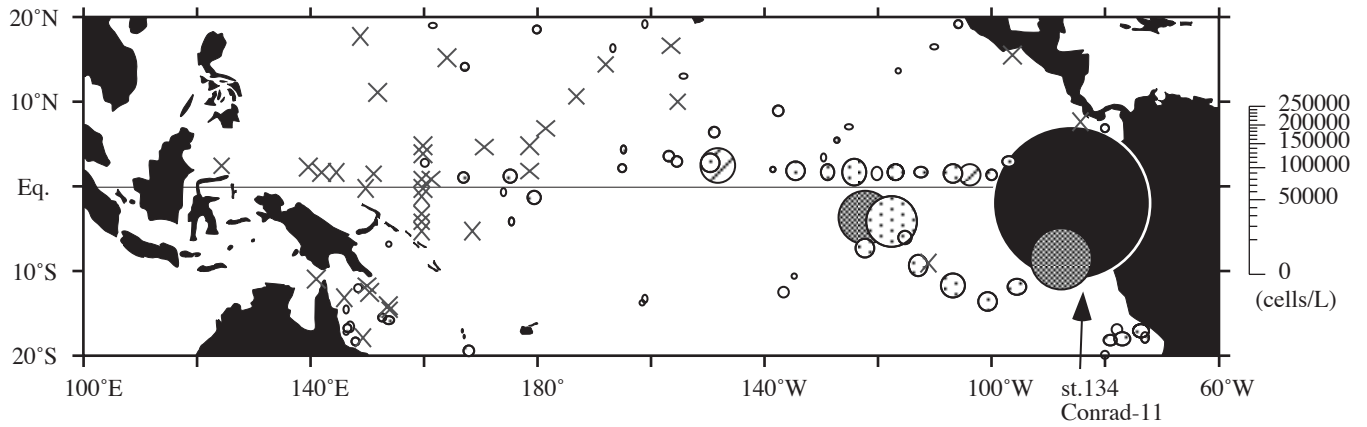
(b) Distribution of floral assemblages recognized by Hagino and Okada (2004)



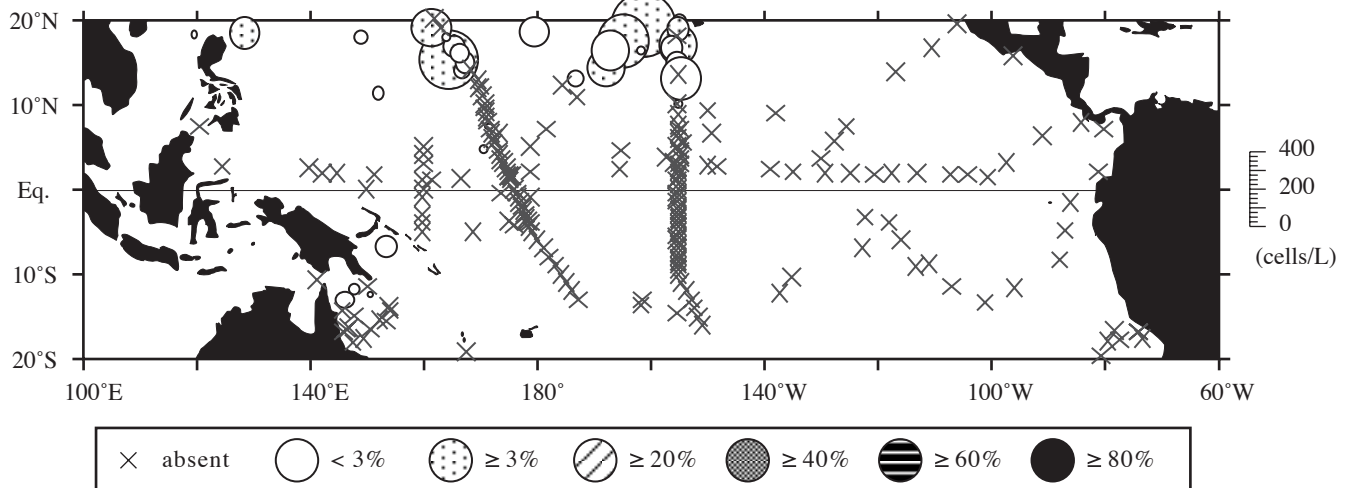
(a) *Emiliana huxleyi* Type A



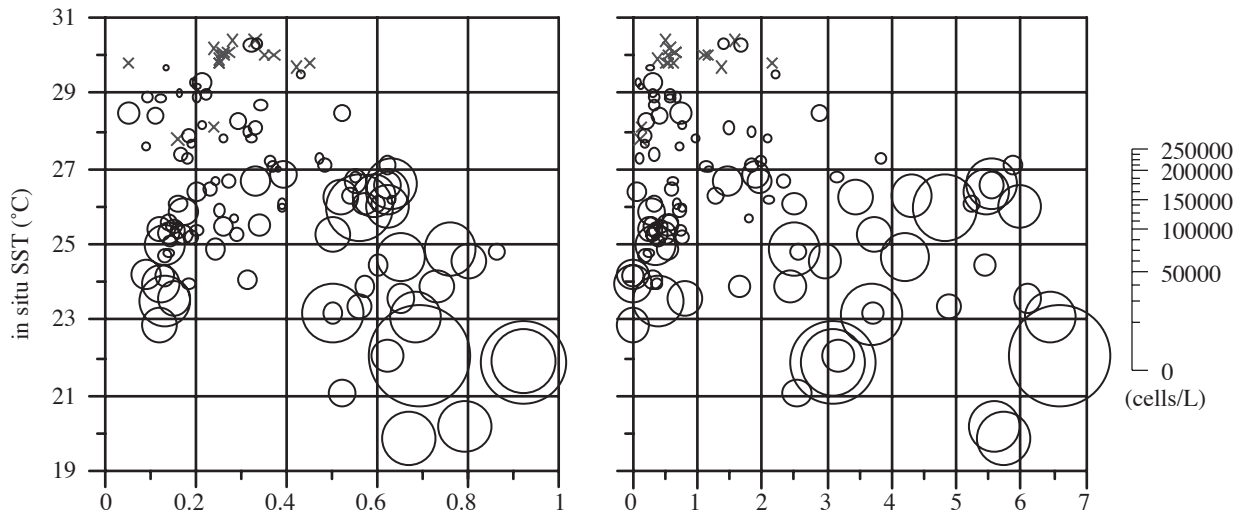
(b) *Emiliana huxleyi* Type C



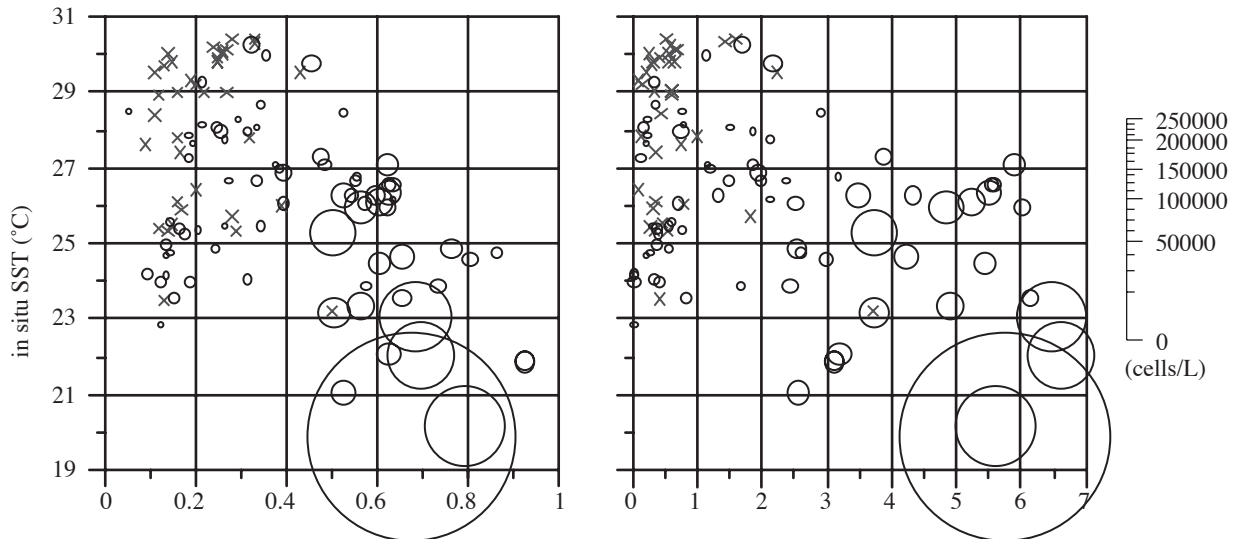
(c) *Emiliana huxleyi* var. *corona*



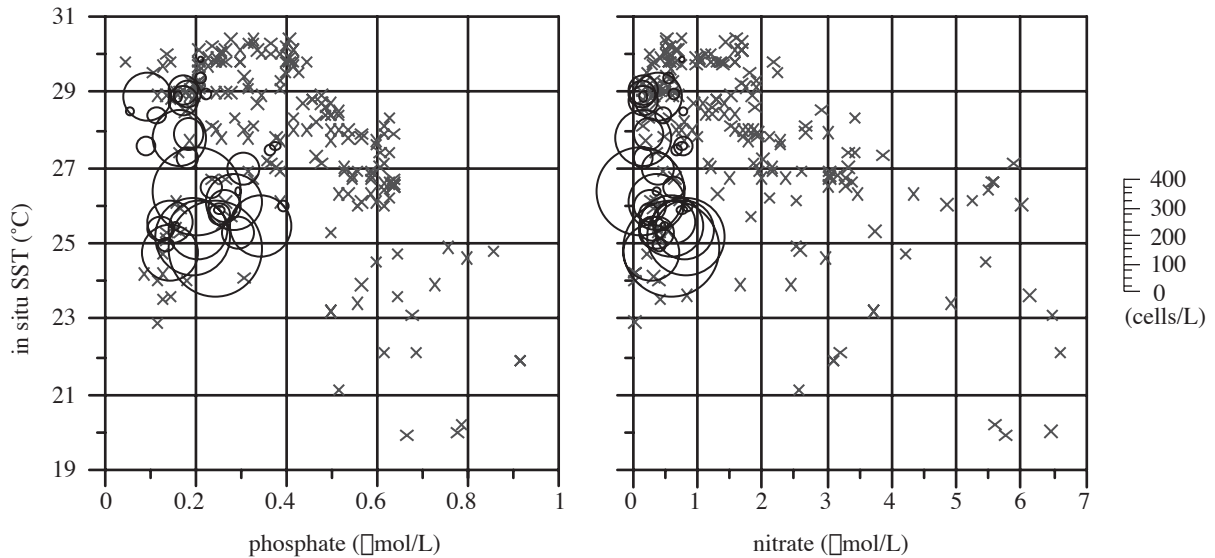
(a) *Emiliana huxleyi* Type A



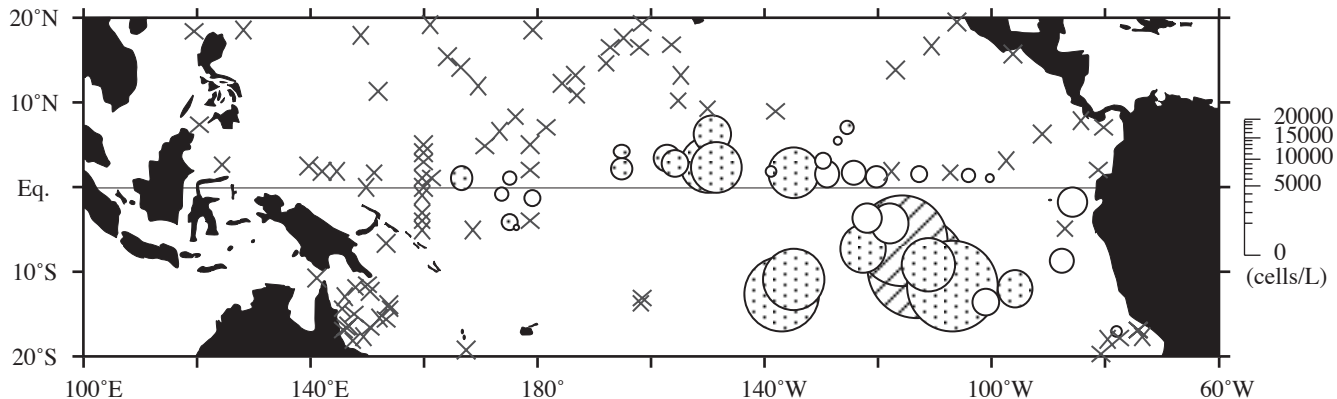
(b) *Emiliana huxleyi* Type C



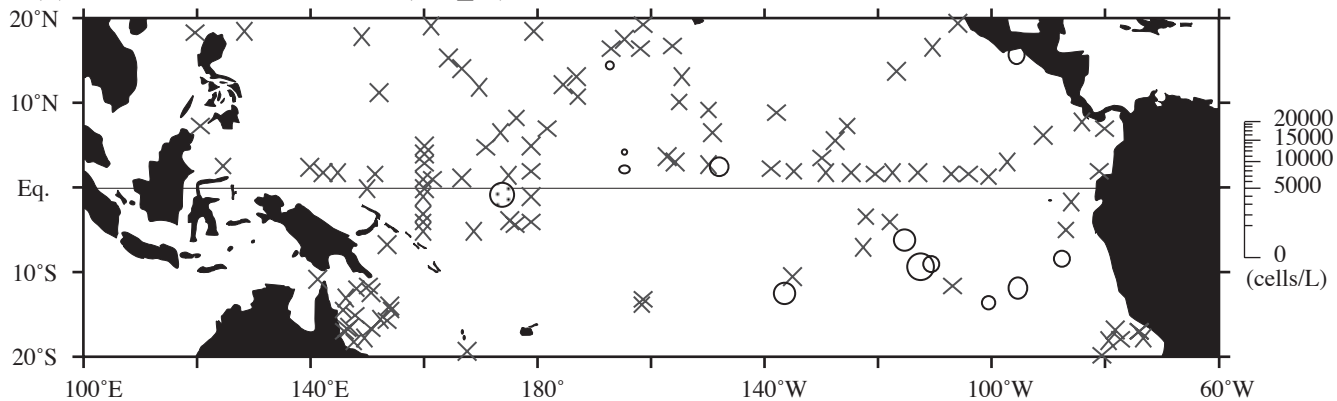
(c) *Emiliana huxleyi* var. *corona*



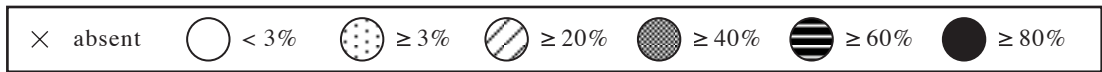
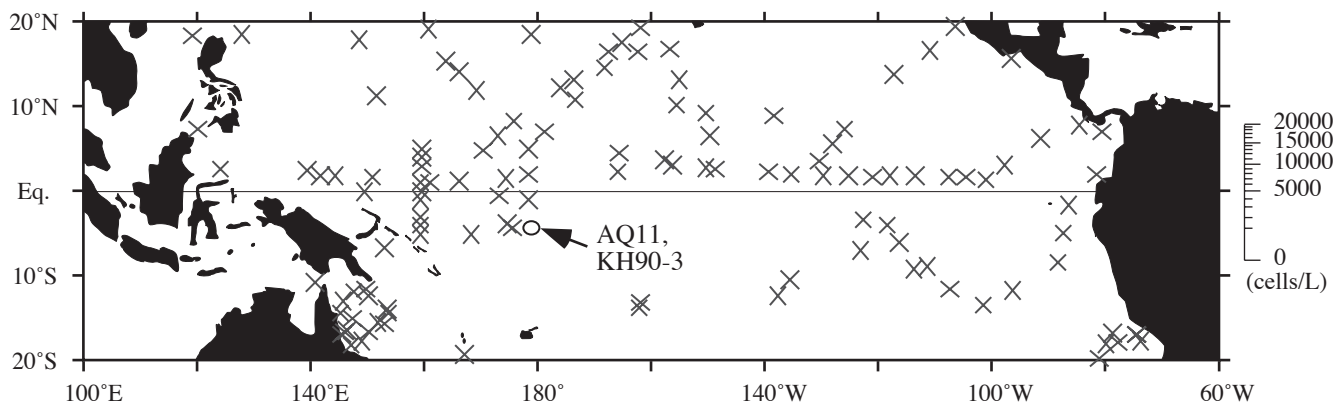
(a) *Calcidiscus* small (<5 μm)



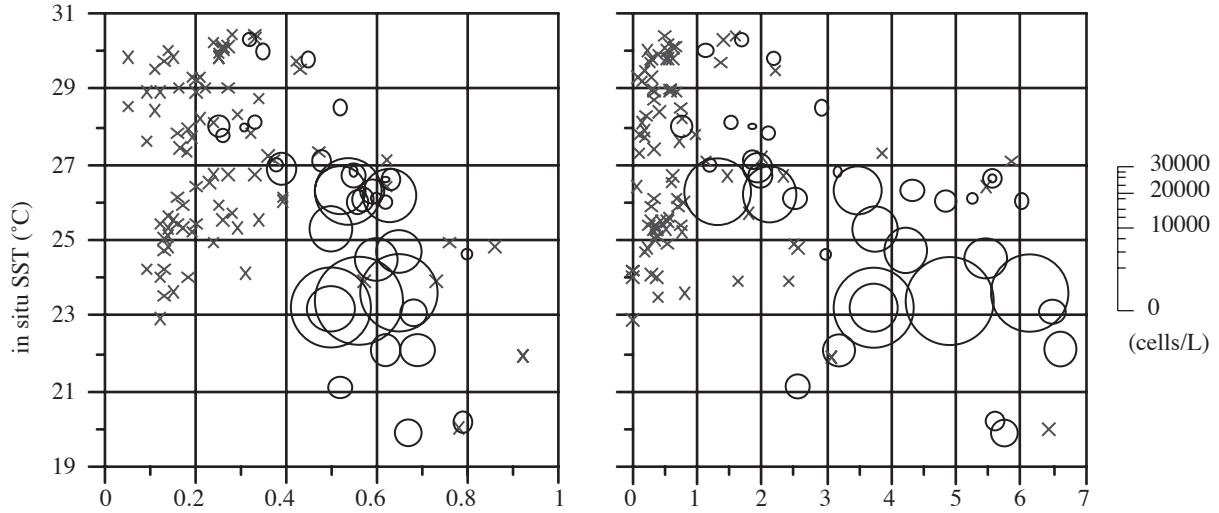
(b) *Calcidiscus* intermediate (5-8 μm)



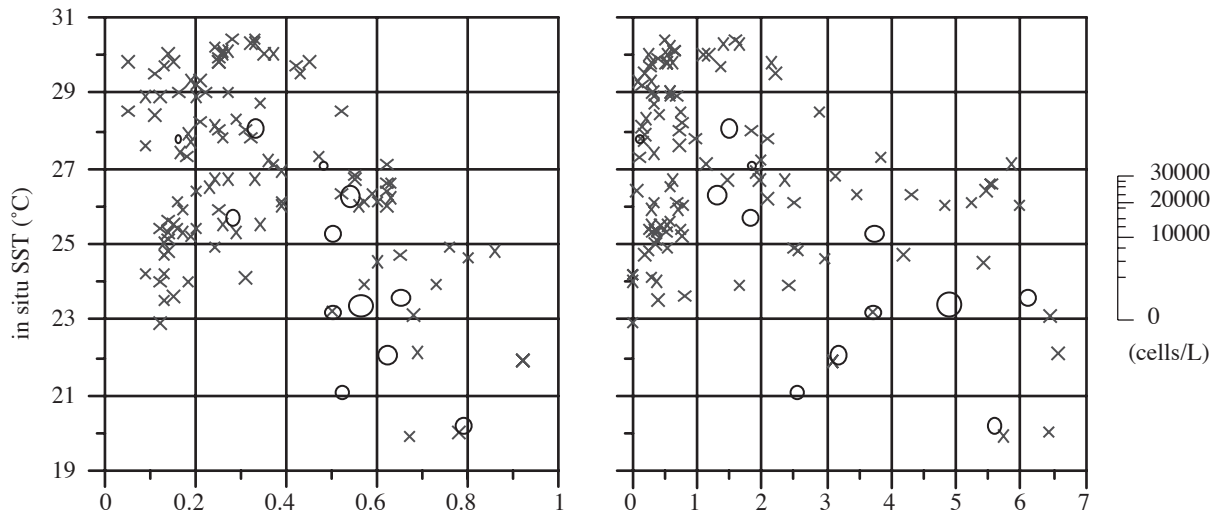
(c) *Calcidiscus* large (>8 μm)



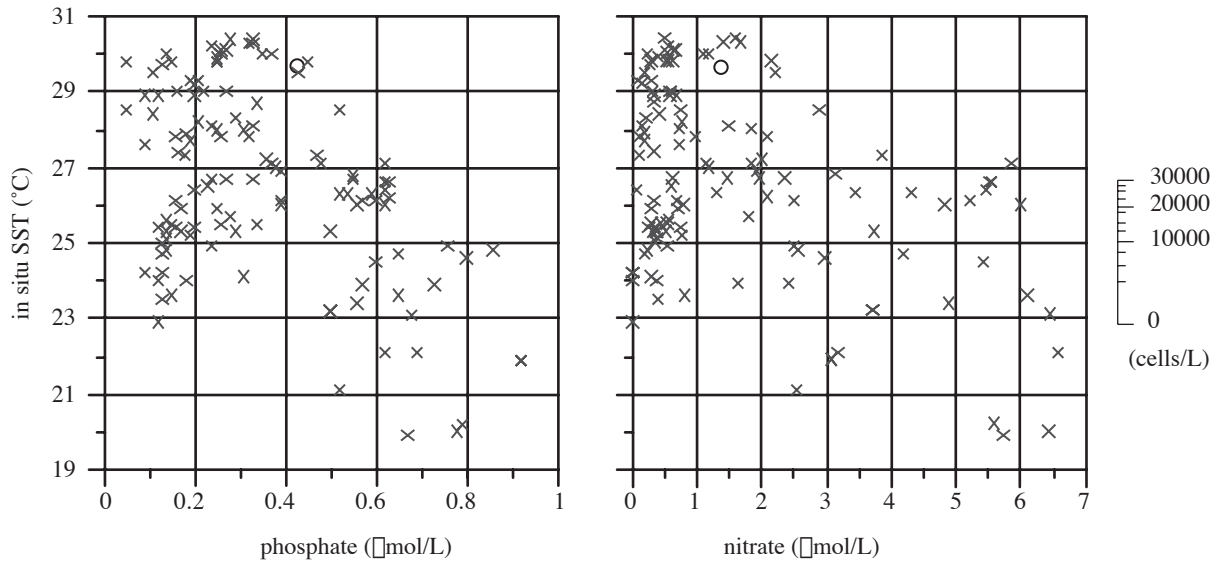
(a) *Calcidiscus* small (< 5 μm)



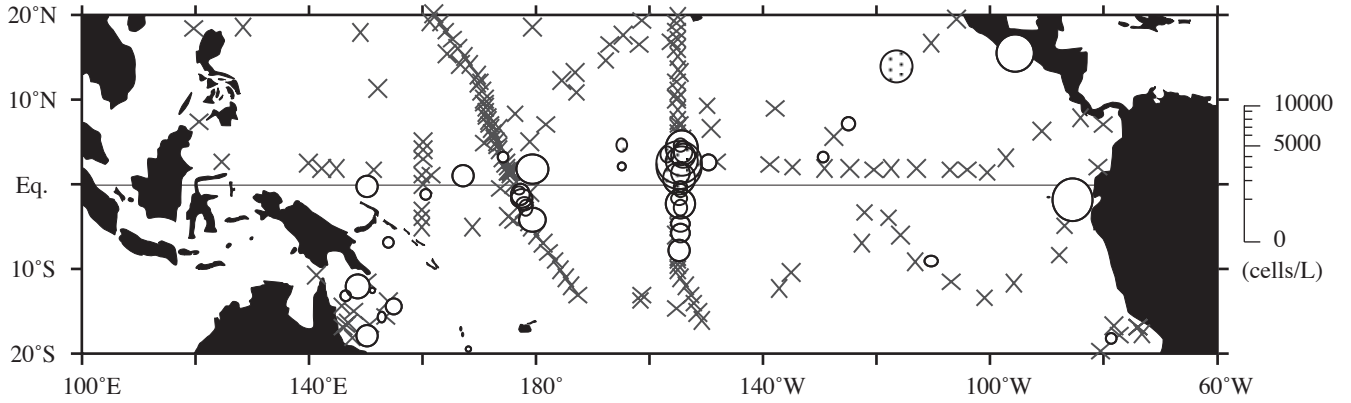
(b) *Calcidiscus* intermediate (5-8 μm)



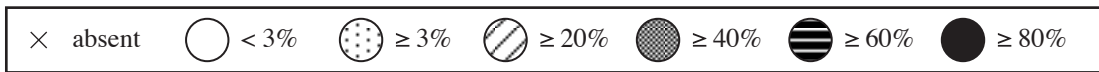
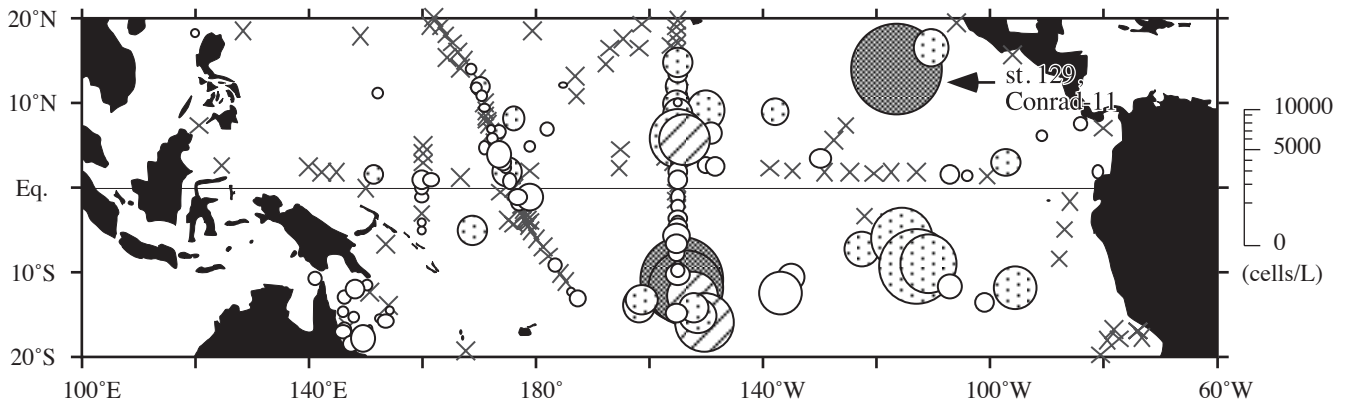
(c) *Calcidiscus* large (≥ 8 μm)



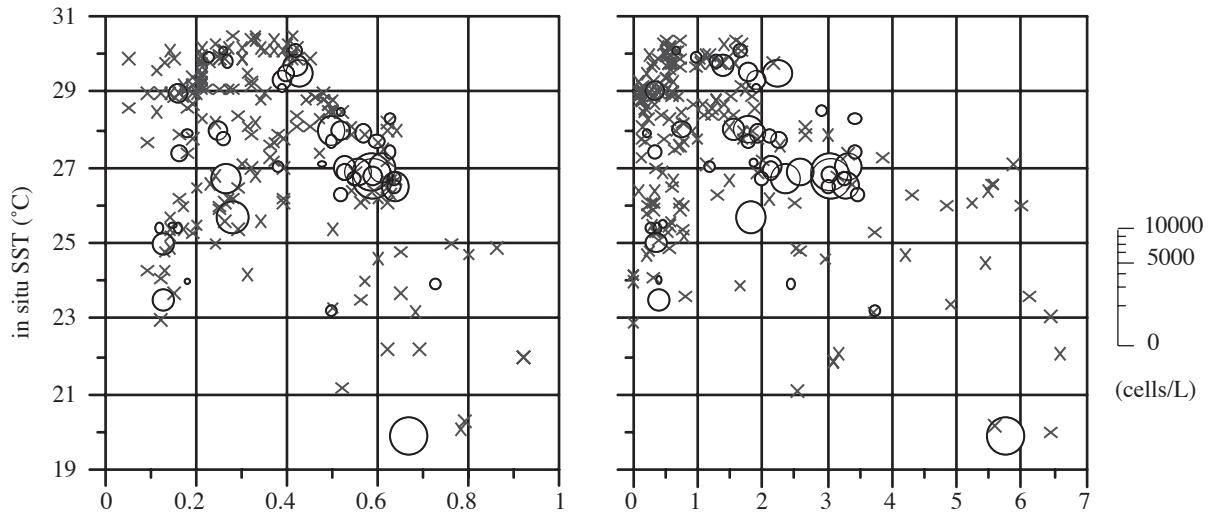
(a) *Umbilicosphara foliosa*



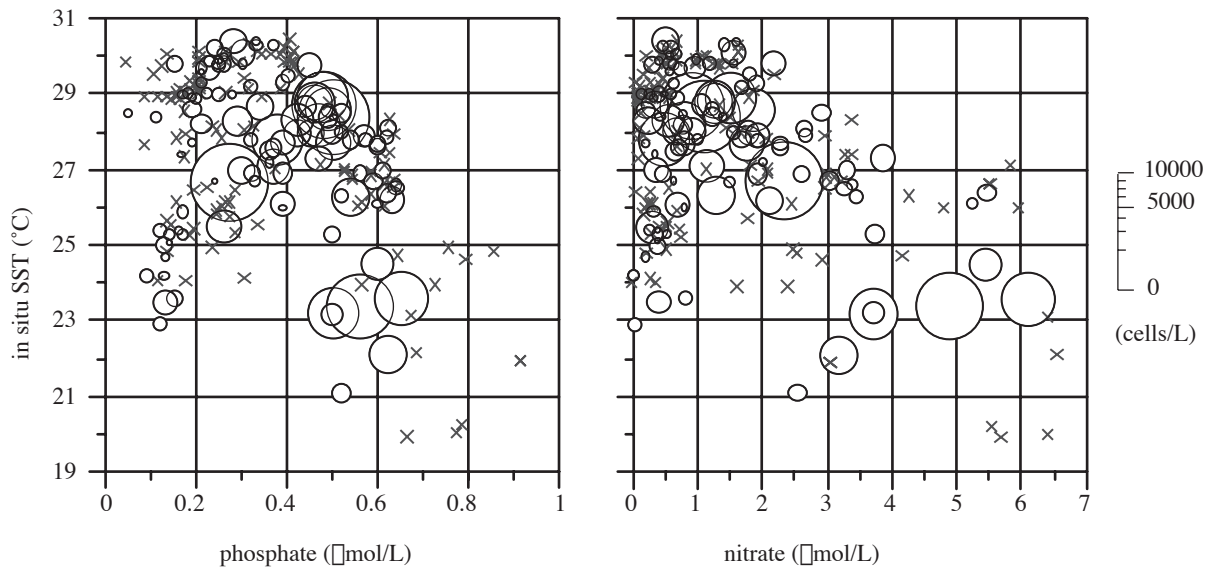
(b) *Umbilicosphaera sibogae*



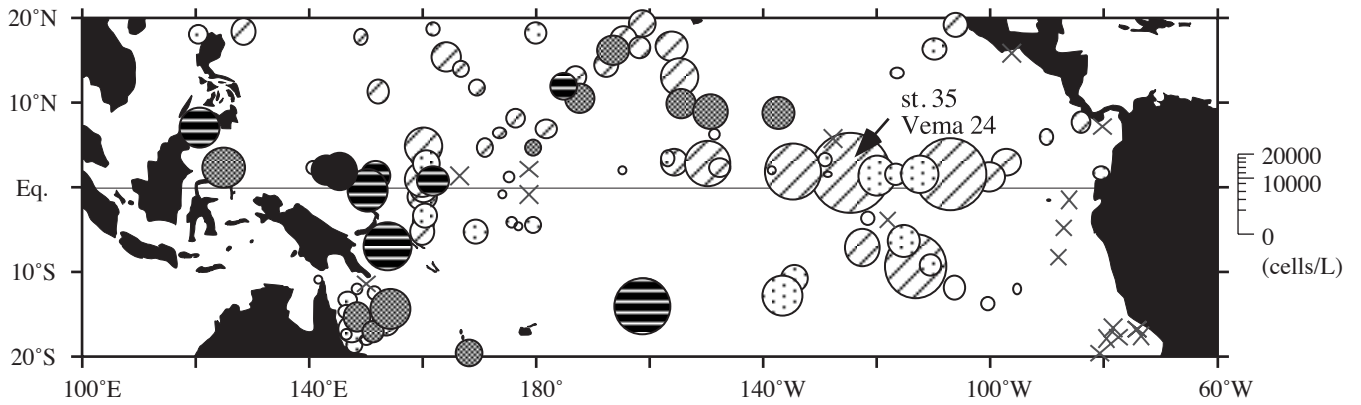
(a) *Umbicosphaera foliosa*



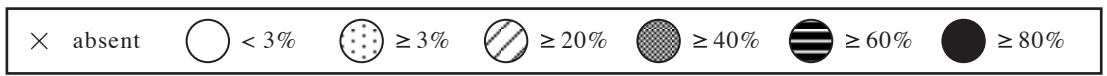
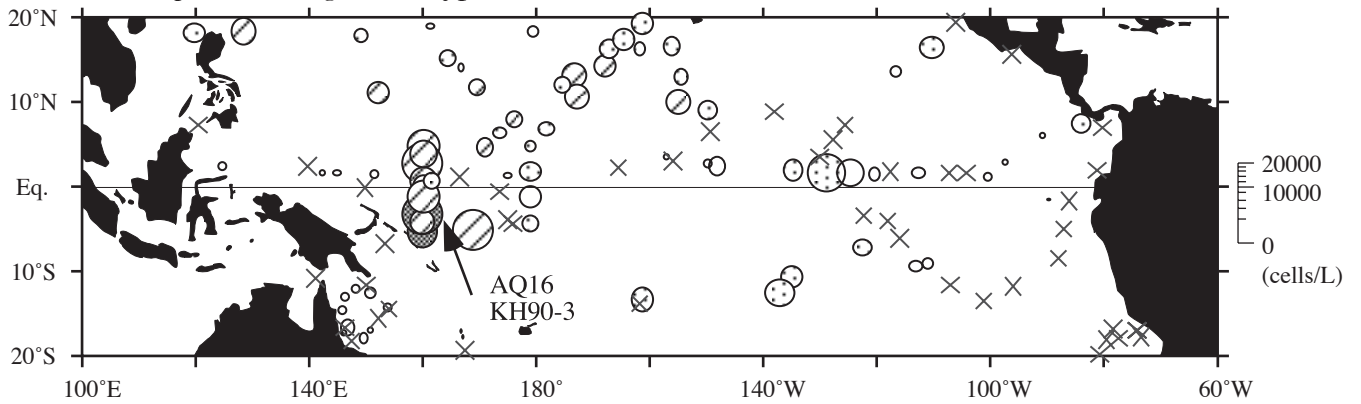
(b) *Umbicosphaera sibogae*



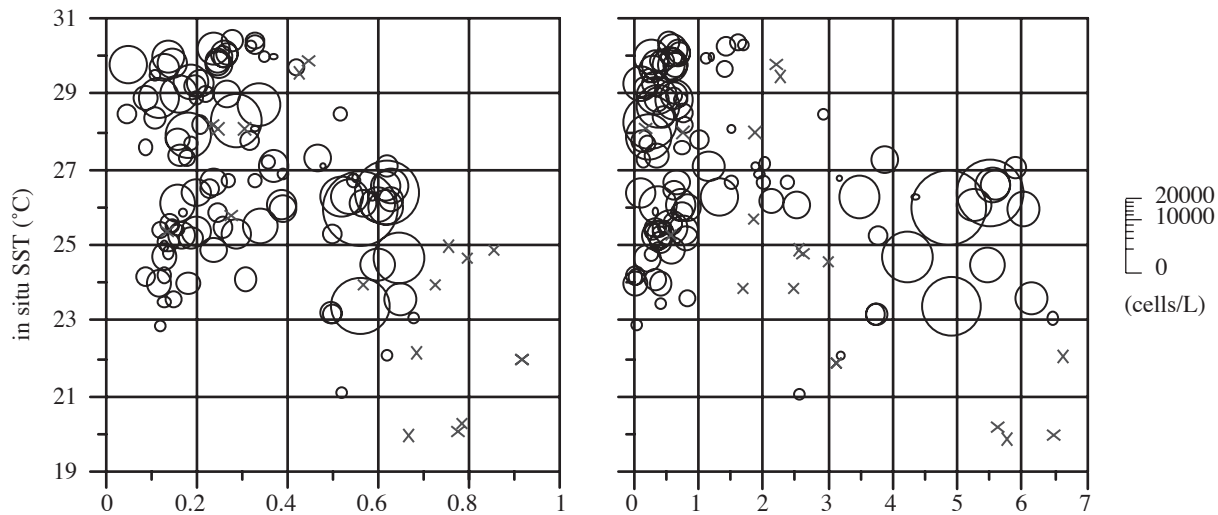
(a) *Umbellosphaera irregularis* s.s.



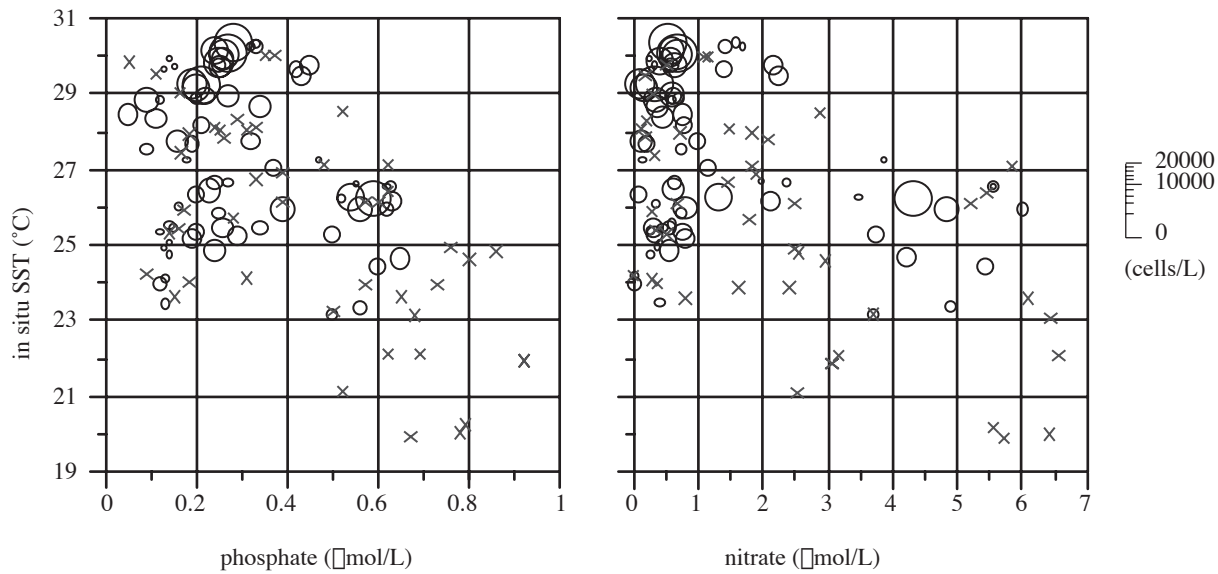
(b) *Umbellosphaera irregularis* Type 0



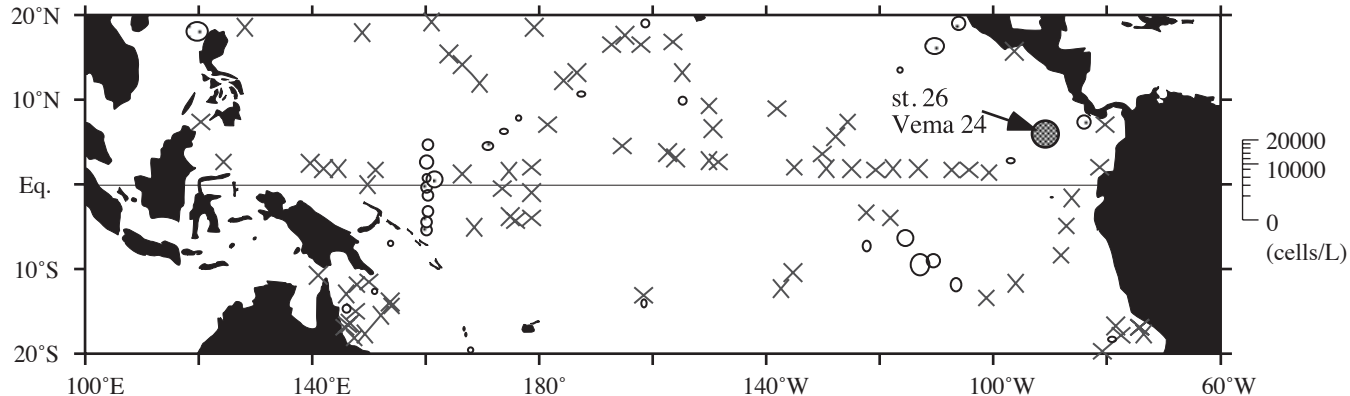
(a) *Umbellosphaera irregularis* s.s.



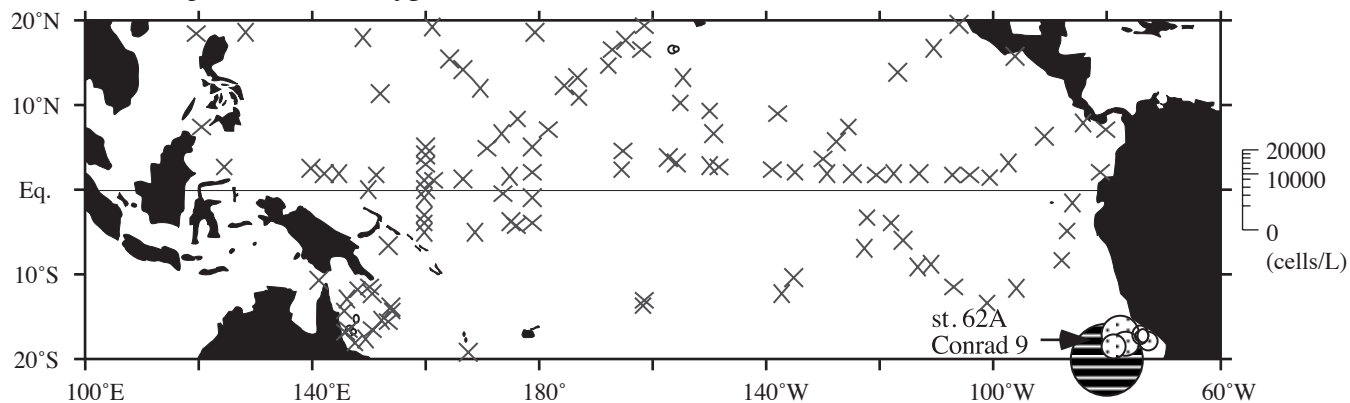
(b) *Umbellosphaera irregularis* Type0



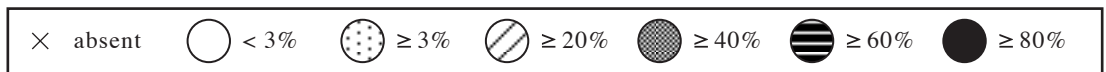
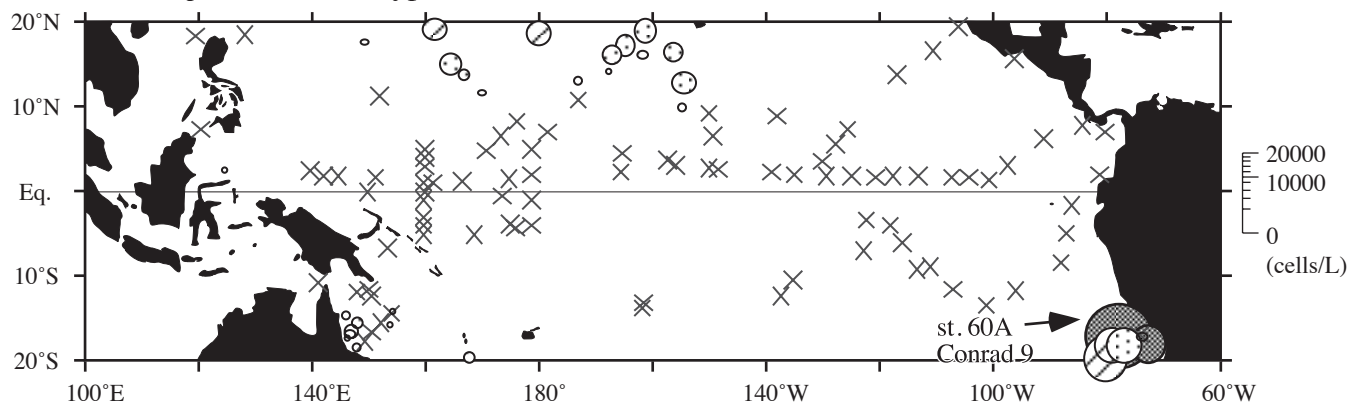
(a) *Umbellosphaera tenuis* Type I



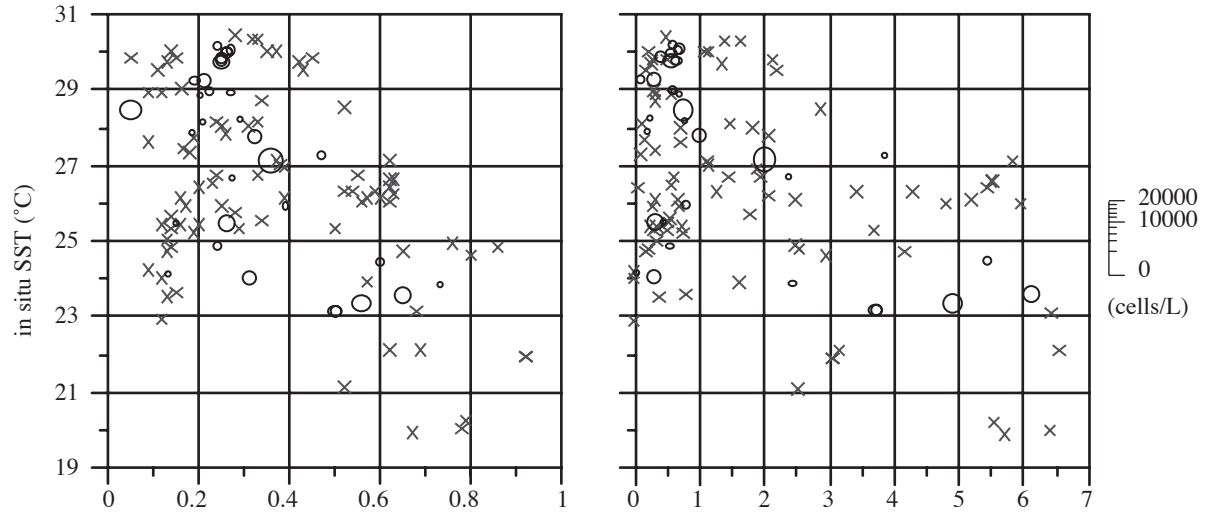
(b) *Umbellosphaera tenuis* Type III



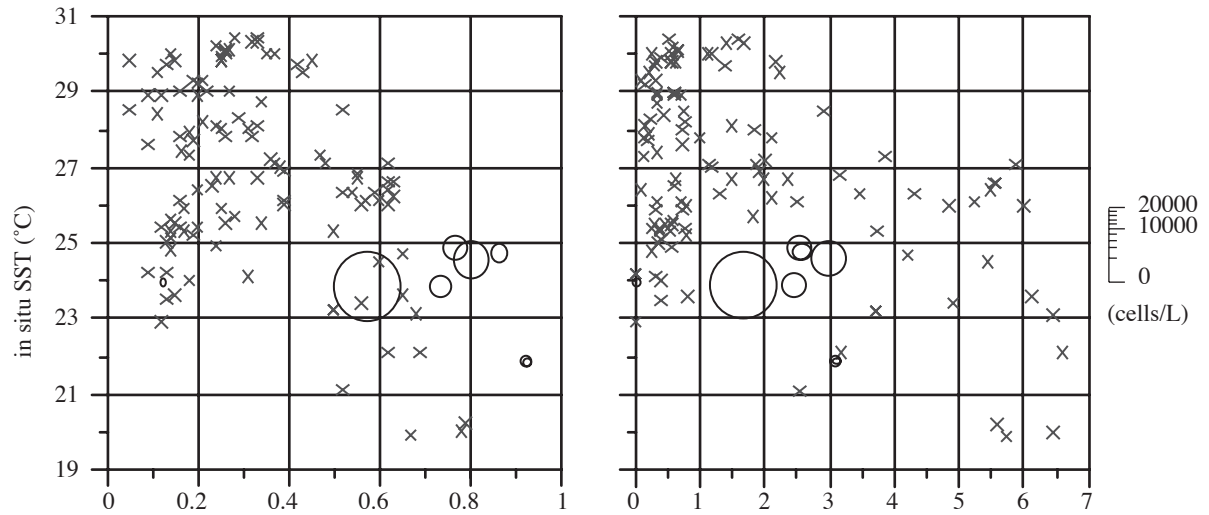
(c) *Umbellosphaera tenuis* Types II and IV



(a) *Umbellosphaera tenuis* Type I



(b) *Umbellosphaera tenuis* Type III



(c) *Umbellosphaera tenuis* Types II and IV

