

## ORIGINAL PAPER

# Morphological Variation of *Gephyrocapsa oceanica* Kamptner 1943 in Plankton Samples: Implications for Ecologic and Taxonomic Interpretations

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Submitted September 7, 2007; Accepted February 9, 2008  
Monitoring Editor: Michael Melkonian

**Morphological analysis of *Gephyrocapsa* spp. in plankton samples confirms the existence of five out of six morphotypes that were previously reported from Holocene sediments. Our data suggest a much higher diversity within the genus *Gephyrocapsa* than the currently accepted species circumscriptions. Furthermore, we confirm the morphological species delineations made by Kamptner that allow the separation of three morphological groups within the genus *Gephyrocapsa*: one group with large bridge angles (*G. oceanica* var. *typica* Kamptner 1943), a second group with small bridge angles (*G. oceanica* var. *californiensis* Kamptner 1956) and a third group of small coccoliths (*G. aperta* Kamptner 1963). However, a seemingly continuous transition from small to large coccoliths within *G. oceanica* var. *typica* along a temperature gradient points either to a high phenotypic plasticity of *G. oceanica* var. *typica* or numerous sibling species highly adapted to specific environmental conditions. Testing of these hypotheses is of utmost importance to understanding the diversity of marine plankton and its evolution, and to assessing the impact of future and past environmental change on primary producers such as coccolithophorids.**

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**Key words:** prymnesiophyte; coccolithophorids; morphospecies; phenotypic plasticity; marine protest; biodiversity.

## Introduction

The oceans cover about 71% of the Earth's surface and thus marine micro-organisms may play a major role in shaping the global environment. Coccolithophores represent a particularly

important group of marine micro-organisms because they constitute a significant part of the marine food web as primary producers and are important players in global biogeochemical cycles. Their production of dimethyl sulphide (DMS) and of calcite platelets, respectively, might have had a major impact on the global climate

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since their first occurrence in the late Triassic (Westbroek et al. 1993). Therefore, knowledge of the factors driving their distribution in today's ocean is essential to assessing the impact of future and past environmental change on the marine ecosystem and on marine plankton evolution. However, the biodiversity, biogeography, ecology and evolution of coccolithophores are still not well understood (Thierstein and Young 2004). One reason for our lack of understanding appears to be the morphology-based taxonomic species concept that currently does not account for small-scale morphological variability possibly reflecting species-level biodiversity.

The traditional taxonomic perception of homogeneous cosmopolitan species has become questionable in recent years because several cosmopolitan species exhibit remarkable fine scale morphological variations (Bollmann 1997; Bollmann and Herrle 2007; Hagino et al. 2000; Knappertsbusch et al. 1997; Quinn et al. 2005; Young and Westbroek 1991). These fine scale variations allow the identification of morphotypes that show a characteristic biogeographic pattern or environmental adaptation (Brand 1981). These results have led to questions regarding whether eco-phenotypic or genotypic variation within the cosmopolitan species such as *Gephyrocapsa oceanica* cause the fine scale morphological variation (Bollmann 1997).

### Species Definitions within the Genus *Gephyrocapsa*

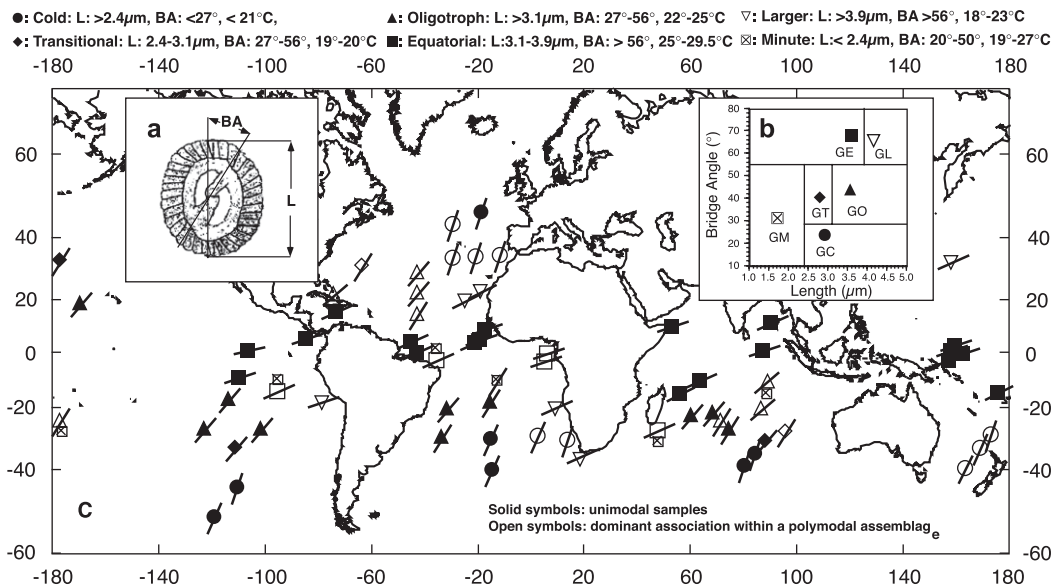
All extant species of the genus *Gephyrocapsa* form spherical to sub-spherical coccospheres of elliptical coccoliths with a diagonal bridge crossing the central area of the coccoliths (Kamptner 1943). Many variations of coccolith size, coccolith bridge angle and shape, the central collar, and the size of the central pore exist. In his original definition of the genus *Gephyrocapsa*, Kamptner (1943) included all coccoliths with a single bar (bridge) across the central area. Kamptner (1956) first used the angle between the bar crossing the central area and the short axis of the elliptical central area (bridge angle) to distinguish between *G. oceanica* var. *typica* Kamptner, 1943 (large bridge angle) and *G. oceanica* var. *californiensis* Kamptner, 1956 (small bridge angle), and the coccolith size to identify *G. aperta* Kamptner, 1963 (small size: 2–3 µm).

McIntyre et al. (1970) used similar morphological characteristics, but provided for the first time

precise morphometric boundaries to distinguish species within *Gephyrocapsa*. McIntyre et al. (1970) distinguished three species: *G. oceanica* (warm water species with a bridge angle greater than 45°), *G. caribbeanica* Boudreaux and Hay, 1967 (cold water species with a bridge angle smaller than 45°) and the small (2.2–1.9 µm) *G. ericsonii* McIntyre and Bé, 1967. Later, Pujos-Lamy (1976), Bréhéret (1978) and Samtleben (1980) distinguished several morphotypes within the genus *Gephyrocapsa* based on measurements of size and bridge angle of *Gephyrocapsa* coccolith assemblages from sediment samples. Since then, various combinations of size, bridge angle, roundness (ratio of width/length), pore width and other descriptive features led to the description of numerous morphospecies from the Miocene to the Holocene. A detailed overview is given by Perch-Nielsen (1985) and Bollmann (1997; Table 1).

Currently, the extant *Gephyrocapsa* species delineations used closely resemble those proposed by Kamptner (1956, 1963) based on bridge angle and coccolith size: *G. oceanica* var. *typica* (large bridge angle), *G. oceanica* var. *californiensis* (syn. *G. muelleriae* Bréhéret, 1978; syn. *G. caribbeanica* Boudreaux and Hay, 1967; small bridge angle) and *G. aperta* (syn. *G. ericsonii* McIntyre and Bé, 1967; distinguishable by its minute coccoliths). An additional distinction of *Gephyrocapsa* species with small coccolith size has been used based on ornamentations of the central collar or the shape of the distal shield (i.e. *Gephyrocapsa ornata* Heimdall, 1973; *Gephyrocapsa crassipons* Okada and McIntyre, 1977; *Gephyrocapsa protohuxleyi* McIntyre, 1970). In contrast to previous studies, the quantitative morphological analysis of *Gephyrocapsa* assemblages from globally distributed Holocene sediment samples revealed six dominant morphological associations related to distinct environmental conditions with respect to sea surface temperature and productivity (Figure 1; Bollmann (1997)). Furthermore, morphological parameters such as size or bridge angle appear to vary with environmental conditions such as temperature, showing seemingly continuous transitions between all morphological associations, suggestive of one global species with high eco-phenotypic plasticity (Fig. 1).

Bollmann (1997) described the different morphotypes informally because it is not evident from his sediment analysis whether the different morphotypes correspond to discrete species, or to one species showing a high phenotypic plasticity.



**Figure 1.** Morphological variation of *Gephyrocapsa* in Holocene sediments. (A) Morphological measurements determined from a single coccolith: BA, Bridge angle; L, Coccolith Length. (B) Six morphological associations of *Gephyrocapsa* determined in Holocene sediment assemblages. (C) Biogeography of the six different *Gephyrocapsa* morphological associations determined in Holocene sediments. The lines over the symbols indicate the mean orientation of the bridge within an assemblage (modified after Bollmann (1997)).

Furthermore, taxonomic and ecological data inferred from sediment samples might be biased by taphonomic effects such as selective dissolution of morphotypes, lateral transport, sediment mixing via bioturbation, or seasonal variations in the production of different morphotypes.

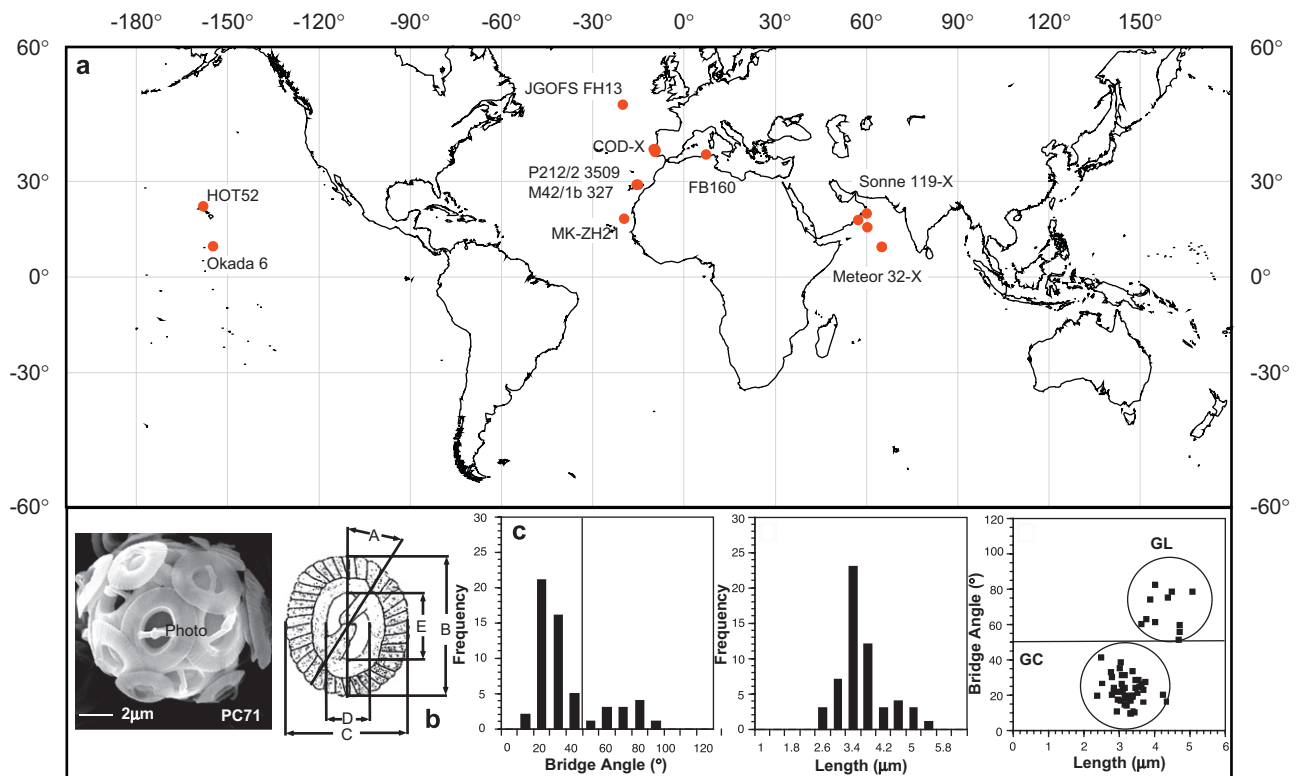
Several feasibility tests need to be conducted to verify whether the different Holocene morphotypes represent distinct species (Bollmann 1997). These tests include the following: (1) Studies of plankton and trap materials from time-series stations would reveal whether the Holocene morphotypes and morphological associations are present in the plankton, and might indicate whether morphological changes in coccoliths on living coccospheres along an environmental gradient are continuous (suggestive of phenotypic variation) or discontinuous (suggestive of successions of genetically distinct populations). (2) Culture experiments of monoclonal populations of *Gephyrocapsa* isolates from various regions grown under various environmental conditions would provide a test for phenotypic plasticity. A change in morphology of any cultured population into that of another morphotype would provide positive evidence for phenotypic plasticity. Consequently, populations of these different morphotypes would assume the taxonomic rank of subspecies. (3) Finally, genetic and morpho-

metric analysis of *Gephyrocapsa* from cultured strains and in the field (Iglesias-Rodríguez et al. 2006; Saez et al. 2003) are needed.

Here, we analysed the morphology of *Gephyrocapsa* spp. in plankton samples from all ocean basins covering a range of temperature and productivity gradients (Fig. 2, Table 1), to test whether (1) Holocene morphological associations and morphotypes of the genus *Gephyrocapsa* can be also identified in the plankton, and (2) different morphotypes represent discrete species or one species showing high eco-phenotypic plasticity.

## Results and Discussion

We analysed the morphology of about 500 coccoliths from 16 globally distributed plankton samples (Figs 3 and 4) and applied the same criteria as Bollmann (1997) to distinguish between unimodal and bi- or polymodal samples and to identify different morphotypes. Our analysis revealed that four of the six Holocene morphotypes reported by Bollmann (1997) were present in unimodally distributed plankton samples (Figs 3A–F, M; 4A–F, M, and 5A, B). These morphotypes are *Gephyrocapsa* Equatorial, *Gephyrocapsa* Larger, *Gephyrocapsa* Cold, and *Gephyrocapsa* Minute. The morphotype *Gephyrocapsa* Transitional (GT)



**Figure 2.** (A) Locations and code names of sampling sites for plankton samples analysed in this study; names with an X represent locations where various samples were taken (Table 1). (B) Left panel: SEM picture of a coccosphere of *Gephyrocapsa oceanica*. Right panel: dimensions measured from digitised SEM pictures of a single coccolith; bridge angle measured from the long axis of the central area (a), coccolith length (b), coccolith width (c), length of the central area (d), width of the central area (e). (C) Example of a bimodal assemblage consisting of two morphological associations. Left panel: frequency distribution of the bridge angle (interval size 10°). Middle panel: frequency distribution of the length (interval size 0.4 μm). Right panel: scatter plot of length versus bridge angle showing separation of the assemblage into two distinct morphological associations *Gephyrocapsa Cold* (GC) and *Gephyrocapsa Larger* (GL). The standard deviation of the assemblage is 19.91° for the bridge angle and 0.59 μm for length. After separating the two modes, the standard deviations of the dominant mode (GC) is 7.5° for the bridge angle and 0.39 μm for length.

could be identified only in one bimodal sample (Figs 3P, 4P, 5C) and the morphotype *Gephyrocapsa Oligotrophic* (GO) could not be identified in any plankton sample, although coccolith measurements on single coccospheres corresponded to this morphotype in several of our samples (Fig. 3). The near-absence of *Gephyrocapsa Transitional* and *Gephyrocapsa Oligotrophic* in the plankton samples is possibly due to the poor geographical coverage of our samples in the open ocean in general and in the oligotrophic gyres in particular. Hence, the existence of these morphotypes in the plankton cannot be ruled out.

The most common combination of morphotypes in polymodal samples was *Gephyrocapsa Larger* and *Gephyrocapsa Cold* (Fig. 3K, L, N, O and P), and to a lesser extent, *Gephyrocapsa Minute*

(Fig. 3H, K and O). A similar combination of morphotypes was found in Holocene sediment samples (Bollmann 1997). The co-occurrence of the morphotypes *Gephyrocapsa Larger*, *Gephyrocapsa Cold* and *Gephyrocapsa Minute* in the same plankton sample rules out the presence of one single cosmopolitan species with high phenotypic plasticity dependent on environmental conditions. Furthermore, this observation suggests that each of these morphotypes represents a separate biological species. Therefore, our data support the species delineation of Kamptner (1943, 1956, 1963), who distinguished between *G. oceanica* var. *typica* (large bridge angle) and *G. oceanica* var. *californiensis* (small bridge angle) as well as *G. aperta* (small size: 2–3 μm).

**Table 1.** Names and locations of plankton samples used in this study together with environmental parameters and morphometric data. (Sample) Sample name. (Date) Sampling date. (Depth) Sampling depth. (Temp) In-situ water temperature at the time and depth of collection. (Conc) *Gephyrocapsa* cell concentration. (AvN) Mean annual nitrate concentration averaged over the upper 100 m of the water column. (Phosp) Phosphate concentration at the time and depth of collection. (AvP) Mean annual phosphate concentration averaged over the upper 100 m of the water column. (Chla) Mean annual surface Chlorophyll-a concentration. (Angle) Sample average bridge angle. (Length) Sample average coccolith length. (Pore) Sample average pore size. (N) Number of coccoliths measured in the sample. (STDEV) Standard deviation. (NN) Not measured.

Sample	Latitude (°)	Longitude (°)	Depth (m)	Date	Temp. (°C)	Salinity	Conc. (cells ml <sup>-1</sup> )	Nitrate (µmol l <sup>-1</sup> )	AvN <sup>a</sup> (µmol l <sup>-1</sup> )	Phosp. (µmol l <sup>-1</sup> )	AvP <sup>a</sup> (µmol l <sup>-1</sup> )	Chla <sup>a</sup> (µg l <sup>-1</sup> )	Angle STDEV (°)	Length STDEV (µm)	Pore STDEV (µm)	STDEV N (%)			
COD1-3	38° 23.5'N	9° 14.9'W	43	20.04.99	13.8	NN	NN	NN	3.56	NN	0.21	0.27	53.84	17.61	4.49	0.87	43	3.9	42
COD1-1	38° 09.58'N	9° 29.45'W	50	20.04.99	14.4	NN	NN	NN	3.47	NN	0.19	0.28	38.09	18.77	3.89	0.98	41	4.3	43
COD2-5	38° 44'N	9° 32'W	8	15.06.99	14.5	35.85	NN	3.5	3.53	0.49	0.21	0.25	56.01	20.21	4.57	0.83	46	4.1	30
JGOF5 FH13	49° 40.9'N	20° 47.7'W	3	09.06.90	14.72	35.54	NN	1.7	5.65	0.2	0.27	0.55	23.16	6.73	3.38	0.25	45	3.9	28
FB160 ST-5	37° 29.0'N	07° 22.3'E	25	21.02.88	15	37.16	32.76	NN	1.99	NN	0.07	0.41	46.78	18.11	4.32	0.67	45	4.9	50
COD2-1	38° 40' N	9° 56'W	9	15.06.99	17.5	35.85	NN	0.5	3.46	0.3	0.20	0.24	42.07	20.30	4.22	0.94	52	4.6	38
Meteor 32-5-438	18° 31.8'N	57° 20.5'E	40	04.08.95	19.75	35.74	3.60	21.48	10.96	1.69	1.07	0.21	65.67	10.44	4.27	0.49	47	4.9	30
MK-ZH21	18° 51'N	19° 36.5'W	5	NN	19.9	NN	NN	NN	9.83	NN	0.62	0.67	62.41	11.94	5.12	0.57	44	3.8	33
M42/1b_327-72	29° 10.2'N	15° 30'W	25	01.07.98	21.17	36.79	0.90	0.09	1.81	0	0.12	0.26	28.70	12.68	2.22	1.10	58	7.8	21
HOTS52	22° 45'N	158°W	50	01.02.94	22.6	35.17	10.00	0.01	0.29	0.07	0.18	0.09	69.69	9.04	4.83	0.51	56	5.8	29
P212/2 3509-1	29° 05.35'N	15° 03.488'W	25	24.09.95	23.1	36.8	0.04	0.001	1.74	0.02	0.12	0.29	26.82	10.61	1.99	0.52	57	3.3	34
Sonne 119-37	20° 33.9'N	60° 03.1'E	20	03.06.97	25.99	36.25	5.50	NN	11.85	NN	1.09	0.56	64.80	10.77	4.50	0.56	49	3.3	30
Meteor 32-5-404	09° 58.8'N	65° 01.1'E	100	24.07.95	27.78	36.55	42.30	0.74	2.91	0.28	0.41	0.10	71.08	8.70	3.98	0.40	52	4.9	30
Meteor 32-5-404	09° 58.8'N	65° 01.1'E	20	24.07.95	27.9	36.55	48.80	0	2.91	0.19	0.41	0.10	71.59	7.87	3.94	0.51	57	4.9	30
OKADA-6	10° 3'N	154° 55.0'W	30	NN	28.5	NN	NN	NN	1.87	NN	0.48	0.07	67.63	13.06	3.91	0.37	45	5.1	58
Sonne 119-7	16° 12.1'N	60° 18.5'E	20	24.05.97	29.77	36.5	14.00	NN	6.77	NN	0.72	0.24	71.60	9.14	3.46	0.33	46	4.4	30

<sup>a</sup>From Levitus et al. (1998).



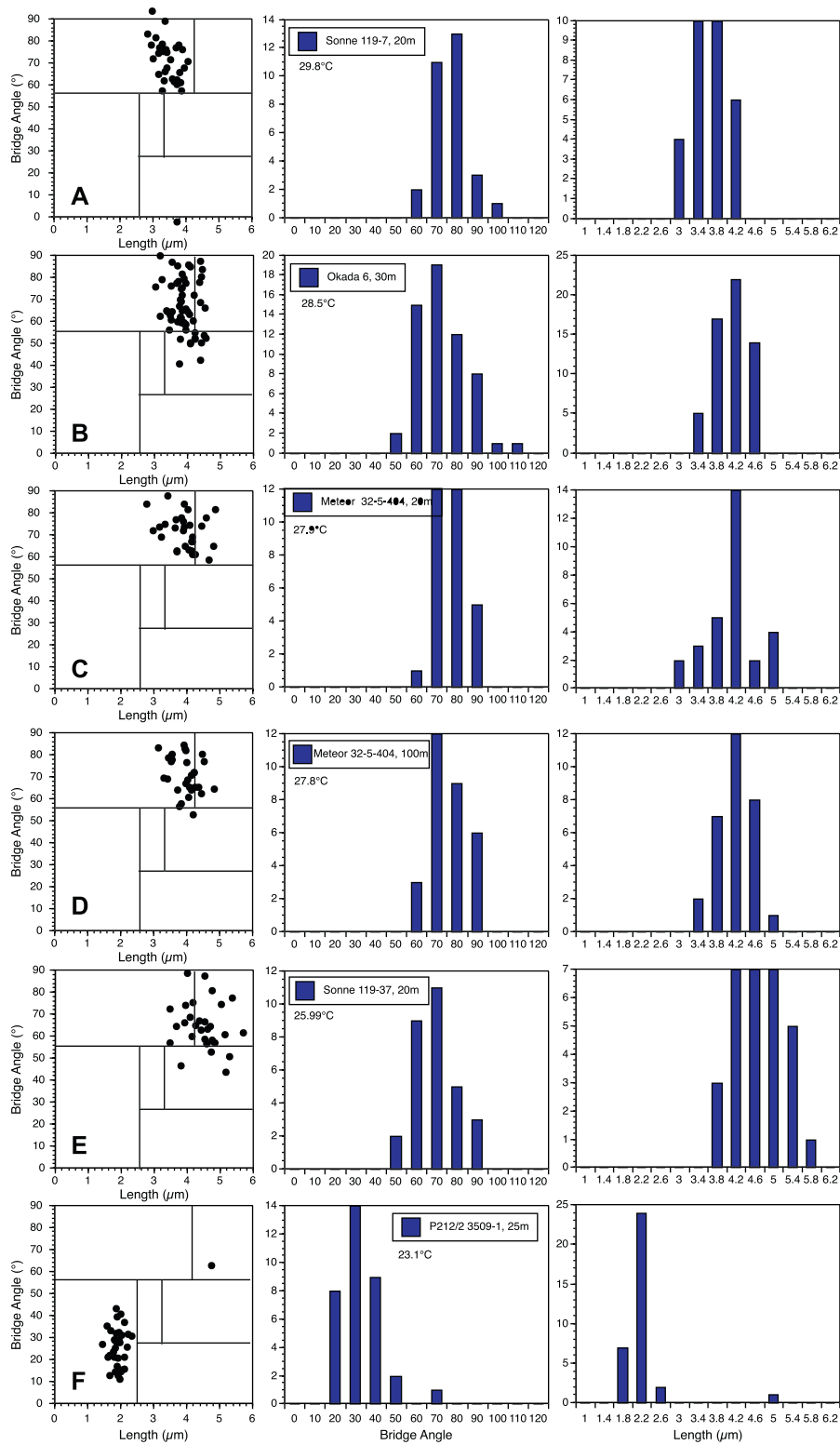


Figure 3.

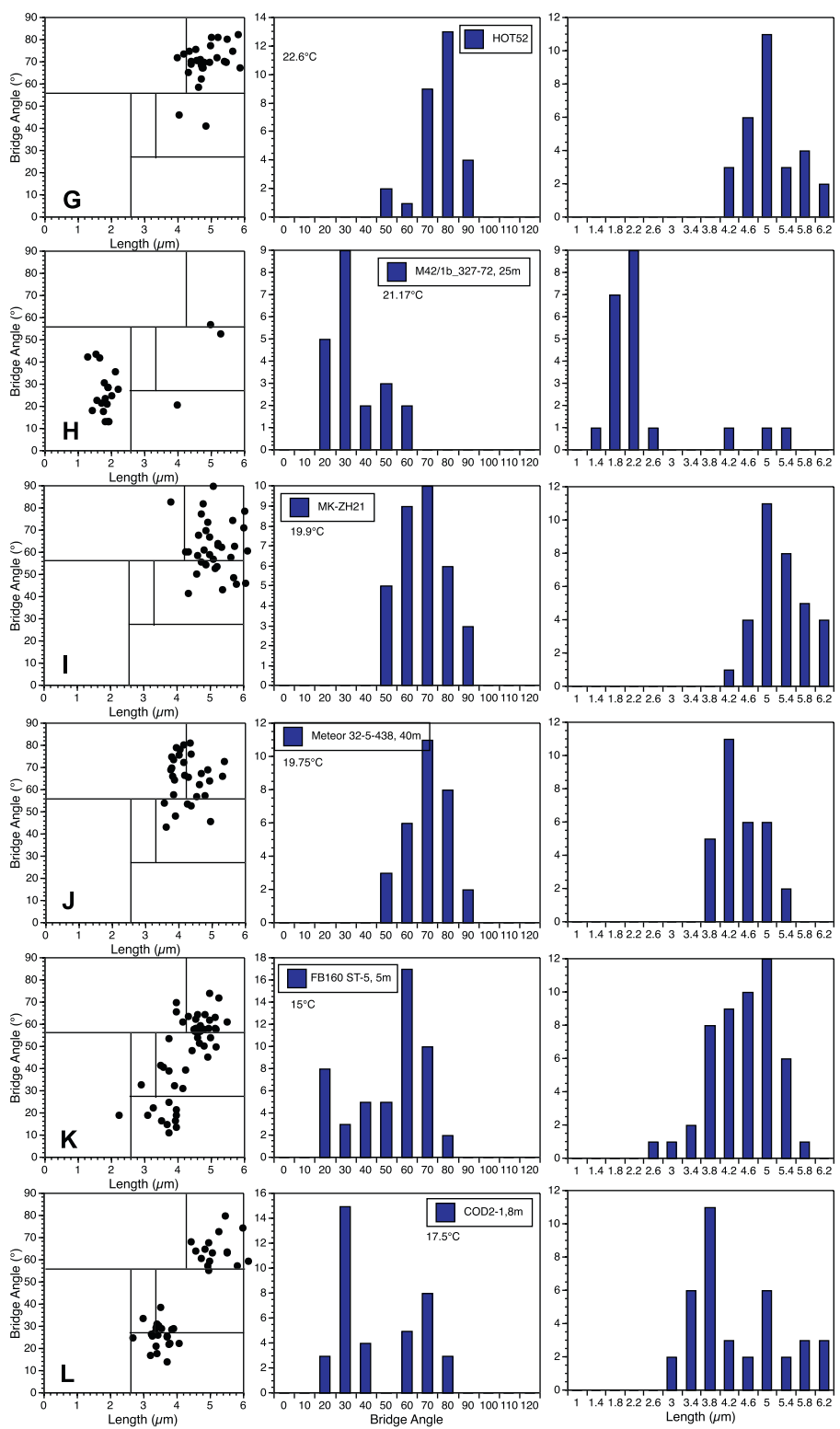
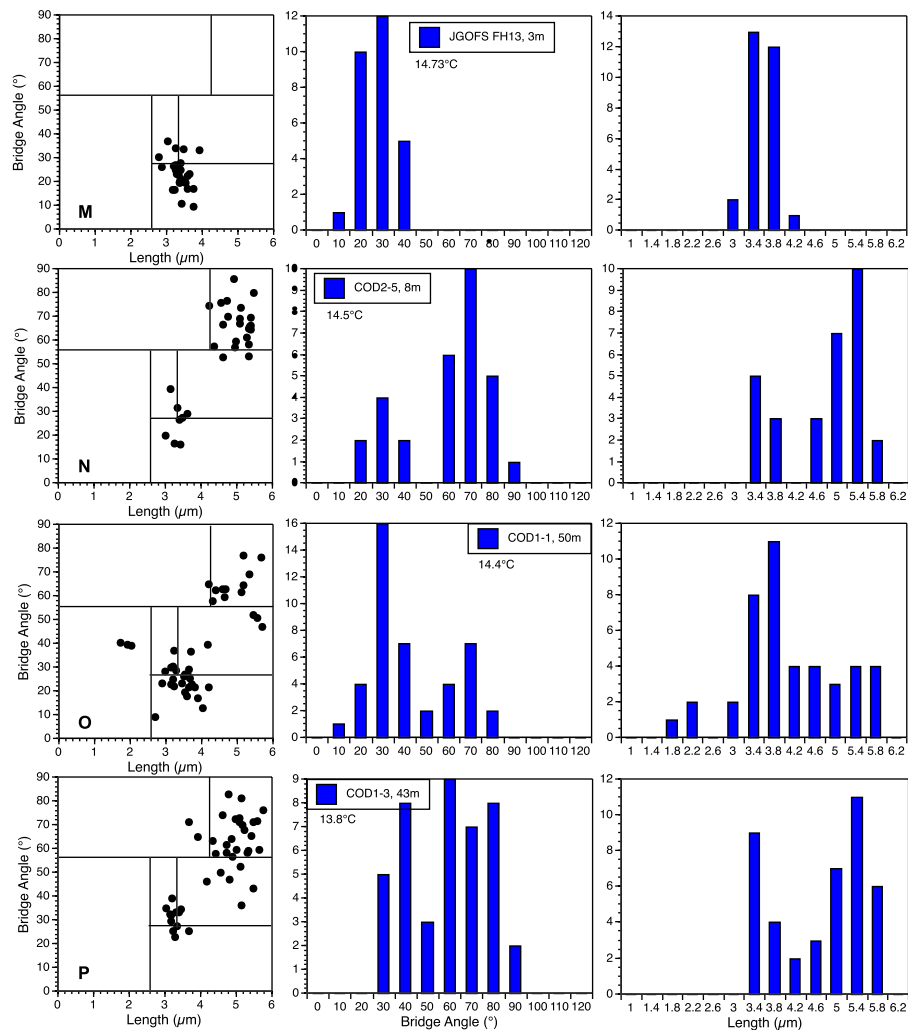


Figure 3. (Continued)

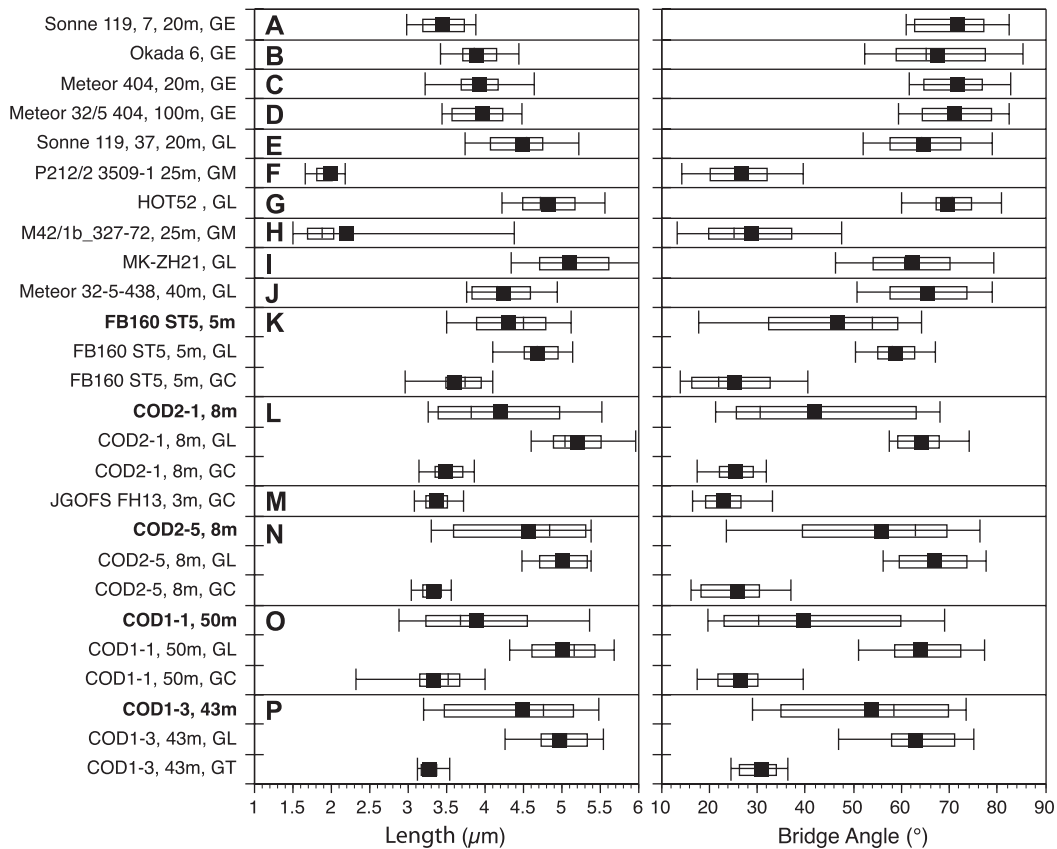


**Figure 3.** Bivariate plots (left panels) and frequency histograms of coccolith length (middle panels) and bridge angle (right panels) of coccoliths measured during this study in individual plankton samples (A-P). Sample name and temperature at the time of collection are given for each sample in the middle panel. Lines in the bivariate plots indicate morphological boundaries reported by Bollmann (1997).

The seemingly continuous change in morphological characteristics along a temperature gradient as reported from Holocene sediments (Bollmann 1997) was observed in the coccolith size of plankton samples (Fig. 5D) but not in the bridge angle. This is a reflection of the fact that only one GT assemblage and no clearly defined GO assemblages were found in plankton samples. However, in contrast to observations from Holocene sediments, the variation in coccolith length with temperature (or other associated environmental variables) is due to a continuous transition between morphotypes with a large bridge angle (*Gephyrocapsa* Larger and *Gephyrocapsa* Equator-

ial; Fig. 5B, D). Coccolith length of *Gephyrocapsa* Larger/*Gephyrocapsa* Equatorial assemblages increase with decreasing temperature, reaching maximum values at 20 °C. Below 20 °C no change in average coccolith length is observed (Fig. 5D). This can be explained by: (a) mixing between the two morphotypes *Gephyrocapsa* Larger and *Gephyrocapsa* Equatorial along the temperature gradient from 20 to 29.6 °C, where *Gephyrocapsa* Larger represents the “Cold” end member and *Gephyrocapsa* Equatorial the “Warm” end member; (b) the presence of numerous species highly adapted to a specific temperature; or (c) the presence of phenotypic plasticity within a single





**Figure 4.** Box plots of plankton samples analysed in this study. Error bars represent the range between smallest and largest value of corresponding morphological parameter in each sample. Lower quartile (lower boundary of open rectangle), median (line inside open rectangle), upper quartile (upper boundary of open rectangle) and average (solid squares) are shown. Note — Sample names in bold indicate bimodal or polymodal samples. In addition, plots of each morphological association of the bimodal or polymodal assemblages are shown. The separation of morphotypes within polymodal/bimodal assemblages is done based on the bivariate plots. GE = *Gephyrocapsa* Equatorial, GO = *Gephyrocapsa* Oligotrophic, GT = *Gephyrocapsa* Transitional, GC = *Gephyrocapsa* Cold, GL = *Gephyrocapsa* Larger, GM = *Gephyrocapsa* Minute.

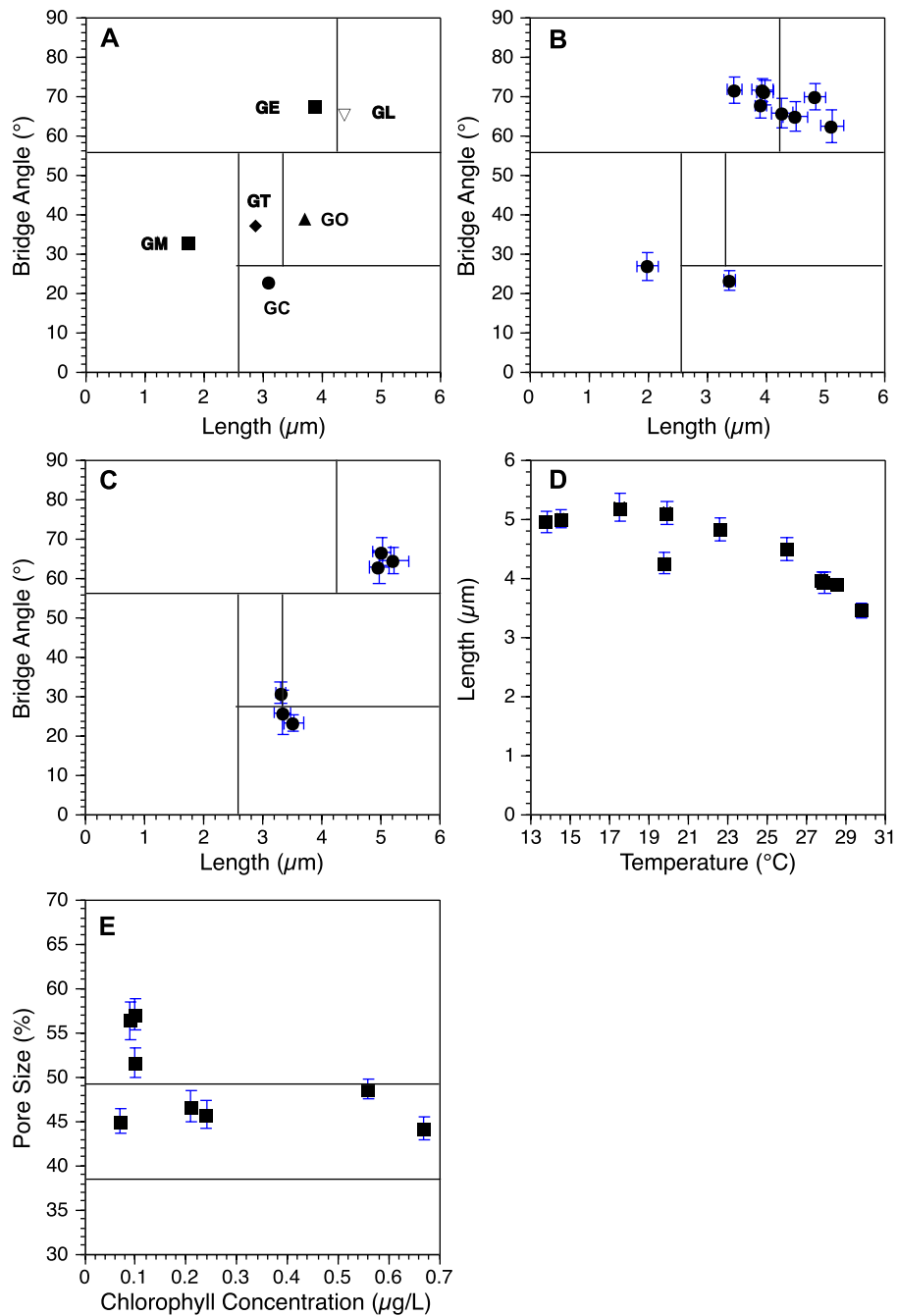
species in response to changes in temperature or co-varying environmental parameters.

The assumption of a single species is contradicted by the variability in growth rates of various strains of *G. oceanica* determined by Brand (1982) in culture experiments. Additional evidence for the presence of more than one species within *Gephyrocapsa oceanica* was reported by Hagino et al. (2000). Based on the proportion of the central area as compared to total coccolith area, Hagino et al. (2000) differentiated between two different morphotypes of *Gephyrocapsa oceanica* with morphotype 1 restricted to eutrophic areas and/or deeper layers in the water column and morphotype 2 occurring in oligotrophic regions. Although information on in-situ nutrient concentrations and primary productivity were not available

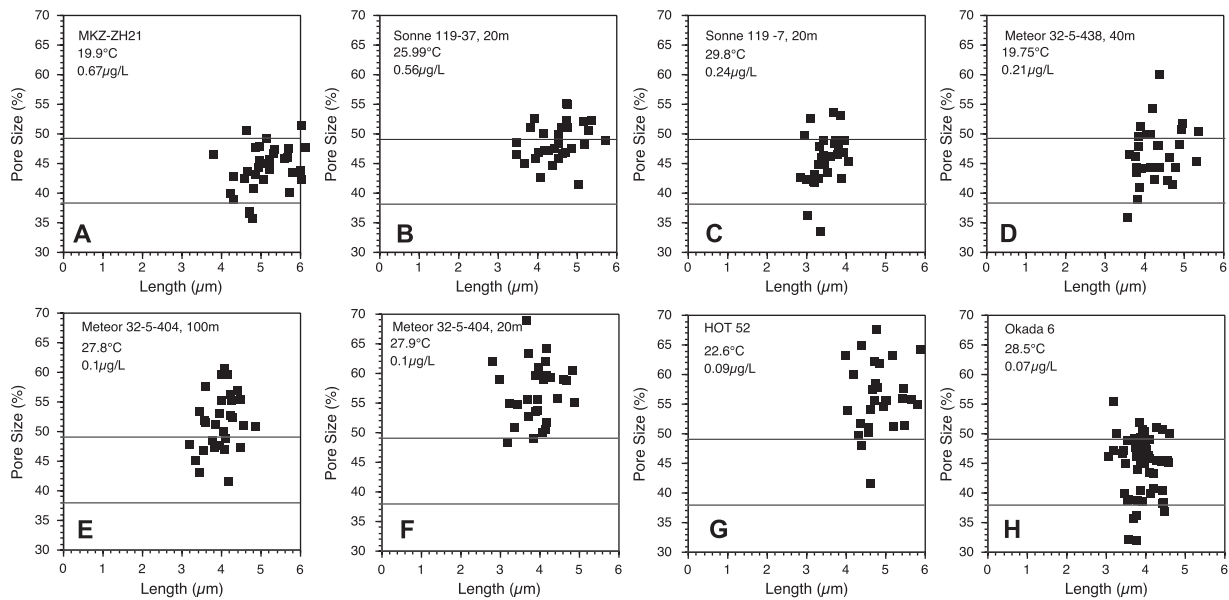
for several sampling sites in this study, our data essentially confirm the finding of Hagino et al. (2000) as *G. oceanica* coccoliths with a large central area seem to occur exclusively in areas with high SST (>20°C), low nutrient concentrations, and low Chlorophyll-*a* concentration (Figs 5E, 6). Based on the available data, however, we cannot rule out that the transition from a large to a small proportion of the central area is due to eco-phenotypic response to environmental conditions.

### Ecological Range

The morphotypic composition of the plankton samples indicates that the patterns observed in Holocene sediment samples are not obscured or



**Figure 5.** (A) Scatter plots of mean length versus mean bridge angle of morphotypes defined in Holocene sediment samples. *Gephyrocapsa* Equatorial (GE): mean bridge angle larger than 56° and mean length between 3.1 and 3.9 μm. *Gephyrocapsa* Oligotrophic (GO): mean bridge angle between 27° and 56° and mean length larger than 3.1 μm (characteristics of the subtropical central gyres). *Gephyrocapsa* Transitional (GT): mean bridge angle between 27° and 56° and mean length between 2.4 and 3.1 μm. *Gephyrocapsa* Cold (GC): mean bridge angle less than 27° and mean length larger than 2.4 μm. *Gephyrocapsa* Larger (GL): mean bridge angle larger than 56° and mean length larger than 3.9 μm. *Gephyrocapsa* Minute (GM): mean bridge angle between 20° and 50° and mean length less than 2.4 μm. (B) Mean bridge angle versus mean length of all unimodal plankton populations. (C) Mean bridge angle versus mean length of morphotypes within the polymodal plankton populations. (D) Mean length of all *Gephyrocapsa* Larger and *Gephyrocapsa* Equatorial morphotypes in plankton samples versus in situ temperature. (E) Mean relative pore size versus annual mean chlorophyll concentration. Lines indicate the mean values reported by Hagino et al. (2000). Note — B—E: error bars = 95% confidence limit of the mean.



**Figure 6.** Scatter plots of pore size versus coccolith length of all unimodal GE and GL associations. Lines indicate the mean values reported by Hagino et al. (2000). Each panel represents values for one plankton sample. In situ water temperatures ( $^{\circ}\text{C}$ ), annual mean chlorophyll concentrations ( $\mu\text{g Chl-a l}^{-1}$ ), sample code and sampling depth (m) are given in the panels.

**Table 2.** Comparison of temperature range of the six *Gephyrocapsa* morphotypes in Holocene sediments and plankton samples, respectively. (Name) Morphotype name. (SST range) Temperature range from Holocene samples based on mean sea surface temperatures. (In-situ temp.) In-situ temperatures at the time and depth of collection of plankton samples.

Name	SST range ( $^{\circ}\text{C}$ )	In-situ temp. ( $^{\circ}\text{C}$ )
<i>Gephyrocapsa</i> Equatorial	25–29	27.8–29.8
<i>Gephyrocapsa</i> Larger	18–23	13.8–25.8
<i>Gephyrocapsa</i> Cold	<21	14.8
<i>Gephyrocapsa</i> Transitional	19–20	13.8
<i>Gephyrocapsa</i> Oligotrophic	22–25	22.6
<i>Gephyrocapsa</i> Minute	NN	23.1

biased by non-biological processes in the sediments. The comparison of ecological ranges of the different morphotypes as reported from Holocene sediments and our plankton data reveal no major differences except for the *Gephyrocapsa* Transitional morphotype (COD1-3, Table 2).

Furthermore, our data support the findings of Bollmann (1997) that *Gephyrocapsa* Cold/Transitional and *Gephyrocapsa* Larger morphotypes are found within similar temperature ranges but in different ecological provinces. The occurrence of unimodal *Gephyrocapsa* Cold association in plankton samples from the open North Atlantic and the combination of *Gephyrocapsa* Cold and

*Gephyrocapsa* Larger morphotypes in the Iberian coasts (Fig. 3L, N–P) suggests different habitats linked to nutrient conditions or other parameters associated with the transition from coastal to open ocean systems.

## Conclusion

Our results confirm the morphological species concept introduced by Kamptner (1943, 1956, 1963) within the genus *Gephyrocapsa* comprising one group with large bridge angles (*G. oceanica* var. *typica*), a group with small bridge angles

(*G. oceanica* var. *californiensis*) and a group with tiny coccoliths (*G. aperta*). Furthermore, we demonstrate the presence, in plankton samples, of five of six morphotypes reported by Bollmann (1997) from his analysis of Holocene sediment samples (*Gephyrocapsa* Larger, *Gephyrocapsa* Equatorial, *Gephyrocapsa* Cold, *Gephyrocapsa* Transitional and *Gephyrocapsa* Minute).

In addition, we show a gradual change in morphological characteristics between *Gephyrocapsa* Larger and *Gephyrocapsa* Equatorial along a temperature gradient which could result from the existence of several species with narrow environmental range, eco-phenotypic plasticity within a single species, mixing of the two morphotypes, or a combination of these factors. Testing of these hypotheses requires genetic and culture studies under varying environmental conditions using monoclonal strains from different ocean basins, and is of utmost importance to understanding the diversity of marine plankton and its evolution, and to assessing the impact of future and past environmental change on coccolithophorids.

Finally, our study demonstrates that quantitative analysis of coccolith morphology in the genus *Gephyrocapsa* and possibly in most extant coccolithophore genera is necessary for addressing key taxonomic, ecological and evolutionary issues.

## Methods

**Plankton samples:** Sixteen plankton samples were analysed from the Indian, Atlantic and Pacific Oceans (Fig. 2A, Table 1). Water samples were obtained from discrete depths (listed in Table 1) using Niskin and immediately filtered on individual membrane filters. After filtration, samples were dried overnight at approximately 50 °C and stored in separate containers for further analysis (for additional details see Bollmann et al. (2002)). Information on environmental parameters at the time and depth of collection are provided in Table 1, where available, together with climatic estimates for nutrient concentrations and Chlorophyll-a (Levitus et al. 1998).

**Morphometric measurements:** Morphometric measurements of plankton samples were carried out according to Bollmann (1997) (Fig. 1B). A minimum of 30 coccoliths were measured per sample. Isolated coccoliths as well as coccoliths on coccospheres were analysed, when possible. All measurements were collected using a Hitachi S2300 and a Philips XL30 Scanning Electron Microscope (SEM) at a magnification of 8000 × .

Because of instrument-related uncertainties when a SEM is used for geometric measurements (for details see ASTM Committee E-4 (1993)), the geometry and accuracy of size measurements in this study were controlled with measurements of ~30 calibration spheres (width and length) of 2, 5, 7 and 10 µm nominal diameter, respectively, before and after each plankton sample measurement series. Correction factors

were applied when average sizes for the calibration spheres measured before and after each plankton sample measurement series differed from the nominal sphere sizes. All measurements with an apparent size offset were corrected.

The comparison between measurements of identical calibration spheres (nominal diameter of  $1.98 \pm 0.1$  µm standard error) conducted by Bollmann (1997) and this study, respectively, revealed that all measurements reported by Bollmann (1997) are about 6.5% too small. The size of calibration spheres measured by Bollmann (1997) was about 1.85 µm rather than 1.98 µm (Bollmann, unpublished data). This small size offset was assumed to be negligible, as the measurements varied within the given statistical standard deviation of  $\pm 0.1$  µm for this type of calibration sphere. Therefore, the measurements were not corrected for the apparent size offset in Bollmann (1997). However, in this study, all measurements and the resulting morphological boundary values published by Bollmann (1997) were adjusted by a factor of 1.065 in order to avoid a biased sample set. All data are available at <http://www.ngdc.noaa.gov/mgg/geology>.

**Data treatment:** The data treatment was conducted according to Bollmann (1997) using the most important independent morphological characteristics of *Gephyrocapsa* coccoliths, namely, bridge angle and coccolith length (other measured morphological characteristics were not used because they tend to be correlated with coccolith length). Briefly, the mean value and variance of all morphometric measurements in each assemblage were calculated (Table 1). Box plots were used to compare the variance of coccolith size and bridge angle between single samples (Fig. 4).

Samples were separated into two subsets: one sample subset with low variance in length and bridge angle (unimodal assemblages), and a second sample subset with high variance in length and bridge angle (polymodal assemblages, see Fig. 2C, left and middle panel). Assemblages with standard deviations of less than 16° for the bridge angle or assemblages with standard deviations of less than 0.7 µm for coccolith length were considered to be unimodal. These limits were chosen after visual inspection of frequency histograms of bridge angle and length measurements for each individual plankton sample (example in Fig. 2C, left and middle panels). The remaining assemblages with standard deviations > 16° for bridge angle or standard deviations > 0.7 µm for coccolith length were considered bi- or polymodal. The polymodal assemblages were further subdivided into associations on the basis of the frequency distributions of bridge angle and length. Scatter plots of length versus bridge angle were also used in all cases to support the separations (example in Fig. 2C, right panel). The width of size classes in frequency histograms were 10° for the bridge angle and 0.4 µm for the coccolith length. In addition, a Kruskal–Wallis non parametric variance test (at a 99.9 probability level) of samples (*Gephyrocapsa* Equatorial *Gephyrocapsa* Larger ) along a temperature gradient from 20 to 29 °C revealed that median lengths are separable among this sample set.

## Acknowledgements

Michael Knappertsbusch, Hisatake Okada, Hans R. Thierstein and Ralf Schiebel provided plankton samples. Mara Y. Cortés, Rebecca R. Ghent and Jens O. Herrle made very useful comments on an

earlier version of the manuscript. The comments of three anonymous reviewers greatly helped to improve the manuscript. This research was funded by the Swiss National Fund Projects: No. 2053-053676: Plankton Ecology and Taxonomy, and JB's Natural Sciences and Engineering Research Council Canada Discovery Grant.

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