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Cryptic speciation in the living planktonic foraminifer *Globigerinella siphonifera* (d'Orbigny)

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Abstract.—Two living forms of *Globigerinella siphonifera* (d'Orbigny), presently identified as Type I and Type II, can easily be distinguished and collected by SCUBA divers because of differences in appearance, arrangement of the rhizopodial network, and the presence or absence of commensals. Additional biological differences are apparent from laboratory culture experiments; Type I individuals survive significantly longer than Type II under conditions of darkness and starvation and have significantly slower chamber formation rates. Stable isotopic analyses of Types I and II also reveal notable differences, with Type I consistently yielding more negative $\delta^{18}O$ and $\delta^{13}C$ values. Results of Mg/Ca ratio analyses indicate that Type II specimens precipitated their shells in slightly cooler (deeper) surface waters than Type I specimens. These observations and results from DNA sequencing unequivocally demonstrate that *G. siphonifera* Types I and II should be regarded as biological sister species.

Contrarily, biometric analysis of the empty shells reveals few significant differences between *G. siphonifera* Types I and II. Of all the features measured from X-ray and SEM images of serially dissected specimens, only shell porosity yields readily discernible differences, with Type I adult chambers averaging 10–20% porosity and Type II adult chambers averaging 4–7% porosity. Statistically significant differences between Type I and II populations are revealed in maximum test diameter (Type I is typically larger) and coiling (Type I is typically more evolute), but these differences do not justify species level distinction of Types I and II using traditional paleontological species concepts.

On the basis of the above evidence, and since all specimens were collected at the same location at ~ 3–8 m water depth, we conclude that *G. siphonifera* Types I and II are living examples of cryptic speciation, whereby biological speciation has occurred in the absence of discernable change in shell morphology. However, it is not clear when or where this speciation took place. Preliminary study of deep-sea cores from the Caribbean and Pacific sides of the Isthmus of Panama reveals a predominance of specimens with Type II porosity values, with rare occurrence of specimens yielding Type I porosity values. Systematic downcore measurement of shell porosity and tightness of coiling needs to be extended back to the middle Miocene, when *G. siphonifera* first appeared, to determine the timing of the Type I and II morphological divergence.

Postulated mechanisms for reproductive isolation and speciation of Types I and II include alloparapatric, depth parapatric, and sympatric speciation. These models could be tested if further analysis of fossil *G. siphonifera* shells allows determination of the timing of speciation, the preferred depth distribution, and the history of geographic distribution of Types I and II.

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Introduction

Modern planktonic foraminifera exhibit considerable morphologic variability due to some combination of environmental, physiological, and genetic factors. For example, at least three species and two genus names have been used for strikingly different forms of the modern species *Globigerinoides sacculifer* (Brady), but each of these phenotypes has been produced under varying conditions in laboratory cultures (Bé 1980; Bé et al. 1983). Biometric studies of fossil populations have also demonstrated that a number of taxa exhibit morphologies that intergrade with other "species" within or outside their clade (e.g., Olsson 1973; Malmgren et al. 1983; Wei 1987, 1994; Wei and Kennett 1988; Tabachnick and Bookstein 1990; Norris et al. 1993, 1996; Hodell and Vayavananda 1994; Huber 1994; Huber and Boersma 1994; van Eijden1995). Such morphotypic variability results in arbitrary taxonomic divisions

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and imparts an inevitable uncertainty in the placement of species datum events (Pearson 1993; Pearson and Chaisson in press).

The diagnosis of living foraminifera is based on test morphology, the network of granuloreticulose pseudopodia, and reproductive patterns (Tendal 1990). The latter two aspects have been investigated for only a few extant species of planktonic foraminifera, and no planktonic species has been observed to undergo a complete reproductive cycle in laboratory culture (Hemleben et al. 1989). Hence, all current taxonomic classifications of living planktonic foraminifera use a "paleontologic approach" to defining species since they are based on observations of adult shell morphology. A biologic approach, which incorporates features of ecology, ontogenetic morphology, cytoplasmic structure, behavior in laboratory culture, and genetics, helps to demonstrate the validity of morphologically based taxonomic groupings among extant species. A comprehensive understanding of biological factors that control shell morphology would provide a valuable foundation for recognizing ecophenotypic variation in the fossil record and help to avoid oversplitting of morphologically variable taxa.

In this study we attempt to compare the paleontologic and biologic species concepts for *Globigerinella siphonifera* (d'Orbigny), a species that occurs in the upper surface waters in tropical and subtropical regions. We focus on this species because previous studies have demonstrated distinct biological differences between two living forms (identified as Type I and Type II), but no consistent differences between their empty shells (Faber et al. 1988, 1989). Our principal goals are to (1) discuss the biological evidence for distinguishing *G. siphonifera* Types I and II as distinct species, on the basis of observations of living specimens and results from laboratory culture, pigment analysis, stable isotopic analysis, and DNA sequencing; (2) qualitatively compare the progressive changes in Type I and II shell morphology at different ontogenetic growth stages; (3) describe the metrics used to characterize the empty shell morphologies of Types I and II; and (4) discuss speciation mechanisms that may have led to cladogenesis in *G. siphonifera*.

Methods

Collection and Culturing

Type I and II morphotypes of *G. siphonifera* were identified individually by SCUBA divers and collected in glass jars about two miles off the west coast of Curaçao (Netherlands Antilles) within 3–8 m of the ocean surface. Ambient water temperature at the collection sites averaged 26.6°C. The samples were returned to the laboratory at the Caribbean Marine Biological Institute (CARMABI) and immediately transferred to flat-bottomed culture vials with 30 ml of seawater from the collection site filtered through a 0.45 μm Millipore filter. The collected specimens were observed under an inverted light microscope to ensure that they were correctly identified as Type I or II individuals, and some were cultured in filtered seawater at 23.5°C, 36.5‰ salinity, with blue-white light illumination of 50 $E/s/m^2$ for 12 hours/day, and a feeding frequency of one brine shrimp per day. Cultured specimens added up to five chambers in the laboratory before undergoing gametogenesis. Because of apparent differences with respect to the number of symbionts sequestered within each type of host, it was expected that physiological differences would become more pronounced at a low feeding frequency (one brine shrimp every two days), increased light intensity (white light of 150 $E/s,m^2$), and a higher nutrient concentration. The culture procedures have been described elsewhere in detail (Hemleben et al. 1987, 1989; J. Bijma et al.unpublished).

For DNA analysis, Caribbean (C) *G. siphonifera* Type I and Type II morphotypes were collected individually by SCUBA divers and cultured as described above. Type II morphotypes were also collected in plankton drift nets at between 3 m and 8 m depth off the Great Barrier Reef, Australia (A). The location was 0.8 nautical miles due east of Ribbon Reef 10 (14°40'S,145°39'E). They were cultured onshore, in a controlled environment culture chamber, at Lizard Island Research Station, in the Cairns section of the Great Barrier Reef. *Globigerinoides ruber* (d'Orbigny) "pink" (C) and "white" (A) morphotypes, *Globigerinoides conglobatus* (Brady) (A), *G. sacculifer* (C), and *Orbulina universa* d'Orbigny (C) were collected

by either SCUBA or drift net and also cultured as described.

All specimens were maintained in culture until they reached an advanced stage of the gametogenic process (spine resorption), just prior to gamete release. At this point 300,000–400,000 foraminiferal gametes are concentrated within the test (Spindler et al. 1978., Bé et al. 1983). The concentration of foraminiferal genomes is accompanied by a reduction in the level of contaminant genomes, significantly increasing the ratio of foraminiferal genomes to those of the contaminant (e.g., symbionts, prey). Using this approach, foraminiferal DNA can be amplified and sequenced (Darling et al. 1996a,b).

Stable Isotopic Analyses and Mg/Ca Ratios

Stable isotope analyses were measured at the Alfred Wegner Institute using a Finnigan MAT 251 mass spectrometer with a "Kiel Device." The precision for $\delta^{18}O$ is about 0.08‰ and for $\delta^{13}C$ is about 0.06‰. All measurements are reported relative to the Pee Dee Belemnite (PDB) standard.

Using an electron microprobe, the Mg/Ca ratio was determined for freshly collected Types I and II according to the method described in Nürnberg (1995). The calcification temperature was then calculated according to Nürnberg et al. (1996a,b) who have developed an empirical relationship between culture temperature and the Mg/Ca ratio in the shells of planktonic foraminifera.

DNA Sequencing

The extraction of foraminiferal genomic DNA and the amplification of an approximately 1000-base-pair fragment of the small subunit (SSU) ribosomal (r) RNA gene, located at the 3′ terminal, are described in Darling et al. (1996b). The amplified region was sequenced automatically using a direct enzymatic "cycle sequencing" approach (Leigh Brown and Simmonds 1994). Complementary strands were sequenced for cross-checking each base pair and each strand duplicated.

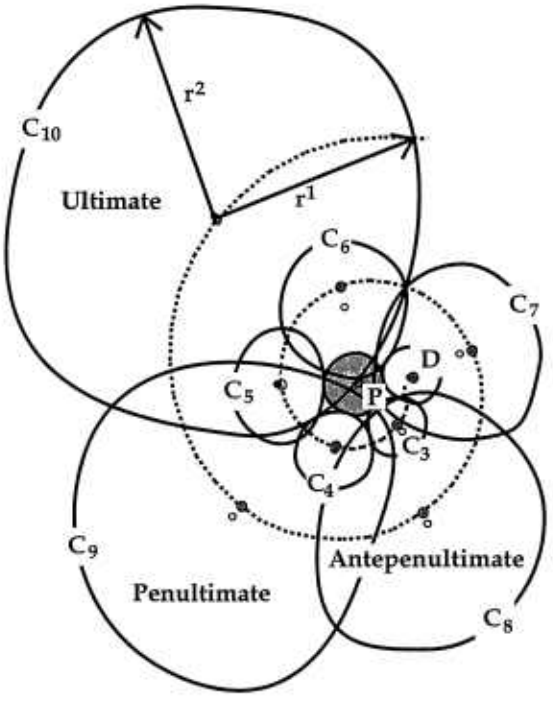
A within-species sequence investigation was also carried out on *G. siphonifera* Type I in order to determine the level of sequence variation among individuals within a morphotype collected from the same area. Five specimens, collected off Curaçao, were extracted, amplified, and sequenced separately and showed complete sequence identity among individuals.

Confirmation of the foraminiferal origin of the SSU rDNA sequences was made by comparison with 438 representatives of the diverse range of eukaryote taxa available within the SSU rDNA database (Maidak et al. 1994). Comparison of sequences representing the planktonic foraminiferal genera *Globigerinella, Orbulina, Globigerinoides,* and *Neogloboquadrina* with sequences of diverse eukaryote taxa, including symbionts and prey organisms, revealed that the planktonic foraminifera form a monophyletic group that is clearly distinct from any other protistan lineage (Darling et al. 1996b; Wade et al. in press).

Biometric Analysis

Most of the specimens used for biometric analysis were fixed in alcohol immediately after SCUBA collection. All specimens were ashed in a low-temperature asher to rid the shells of adhering cytoplasm. On the basis of previous studies (e.g., Brummer et al. 1987), the collected specimens were considered "adults" if they had more than 10–13 chambers in the test and the test diameter was greater than 175–250 μm.

The empty shell morphologies of Type I and Type II specimens were compared using analysis of X-ray images and serial shell dissections. X-ray images of specimens mounted in axial view were obtained on high-resolution film using methods outlined by Huber (1994). The X-ray images were magnified under a transmitted-light microscope with a mounted video camera linked to a video monitor and personal computer, and the images were captured and measured using Optimas™ image analysis software. Only specimen images that revealed all chambers in the ontogenetic series were used. Chamber outlines from the proloculus (= first chamber) to the final chamber, or penultimate chamber in kummerform specimens (specimens with diminutive final chambers), were digitized from the X-ray images of 68 adult Type I specimens and 42 Type II adult specimens. Metrics obtained from the X



LEGEND

P = proloculus
D = deuteroconch
r^1/r^2 = chamber equantness
C_n = chamber number
∘ = actual chamber center
⊙ = predicted chamber center
------- = predicted logarithmic spire

FIGURE 1. Graphic explanation of measurements and morphologic terminology used in the biometric study of *G. siphonifera*. See text for further explanation.

rays include chamber expansion rate, chamber number in the initial and final whorls, total chamber number, chamber roundness in the final three chambers, and maximum test diameter. Chamber equantness was approximated for the ultimate, penultimate, and antepenultimate chambers (Fig. 1) by dividing the chamber radius parallel to the logarithmic spire (r_1) by the chamber radius that is perpendicular to the logarithmic spire (r_2). The digitized tracings were also used to measure the logarithmic spire for each individual. This was calculated first by determining the center of form for each chamber, by measuring the cumulative distance (in arbitrary units) between the centers of consecutive chambers, and by calculating the cumulative number of whorls for each specimen.

A subgroup of the X-rayed specimens was mounted in thermoplastic on a Scanning Electron Microscope (SEM) stub (spiral sides down) and serially dissected using a free-standing micromanipulator (see Huber 1994 for more details). After each whorl dissection, the specimens were cleaned in an ultrasonic bath and then photographed in the SEM to reveal ontogenetic changes in pre-adult shell morphology, to determine proloculus diameters precisely, and to measure ontogenetic changes in shell porosity. Porosity values for each chamber in the ontogenetic series were obtained by capturing the SEM digital images at 2000×, using tools in image editing software to blacken the pores and lighten dark areas between the pores, adjusting to maximum contrast and brightness levels, and determining the percent area of black pixel density.

Biological Differences

Physiology

Several observations (Table 1) support the hypothesis that *G. siphonifera* Type I and Type II are sibling species (sensu Knowlton 1993: p. 190). In the field, the two morphotypes can readily be distinguished by SCUBA divers.

TABLE 1. Summary of differences in appearance of living *G. siphonifera* Types I and II.

Feature	Type I	Type II
Rhizopodial network	anastomosing, reticulate, woolly appearance	open network; distributed along spines
Spines	radiate in all directions; white	concentrated in two tufts; brown
Commensals	*Trichodesmium* "swarmers"	none
Endosymbionts	chrysophycophyte form A; distributed in vicinity of test, between spines; fewer chloroplasts	chrysophycophyte form B; evenly distributed along spines; more chloroplasts

Type I is rather conspicious, having a light brown test with whitish spines radiating in all directions, and the space between the spines is filled with a network of rhizopods that have a reticulate, anastomosing, woolly appearance (Fig. 2A,C). A filamentous blue-green alga, *Trichodesmium*, is usually, but not always, harbored between the spines (Fig. 2A) in commensal (extracellular) association with Type I hosts.

Type II is more difficult to discern in the water. The test has a dark, brownish appearance and the spines are generally radiate in two tufts on opposite sides of the test (Faber et al. 1988: Pl. 1, Fig. 2). The rhizopods are aligned along the spines and are organized in an open network (Fig. 2B,D). Type II individuals never associate with *Trichodesmium*.

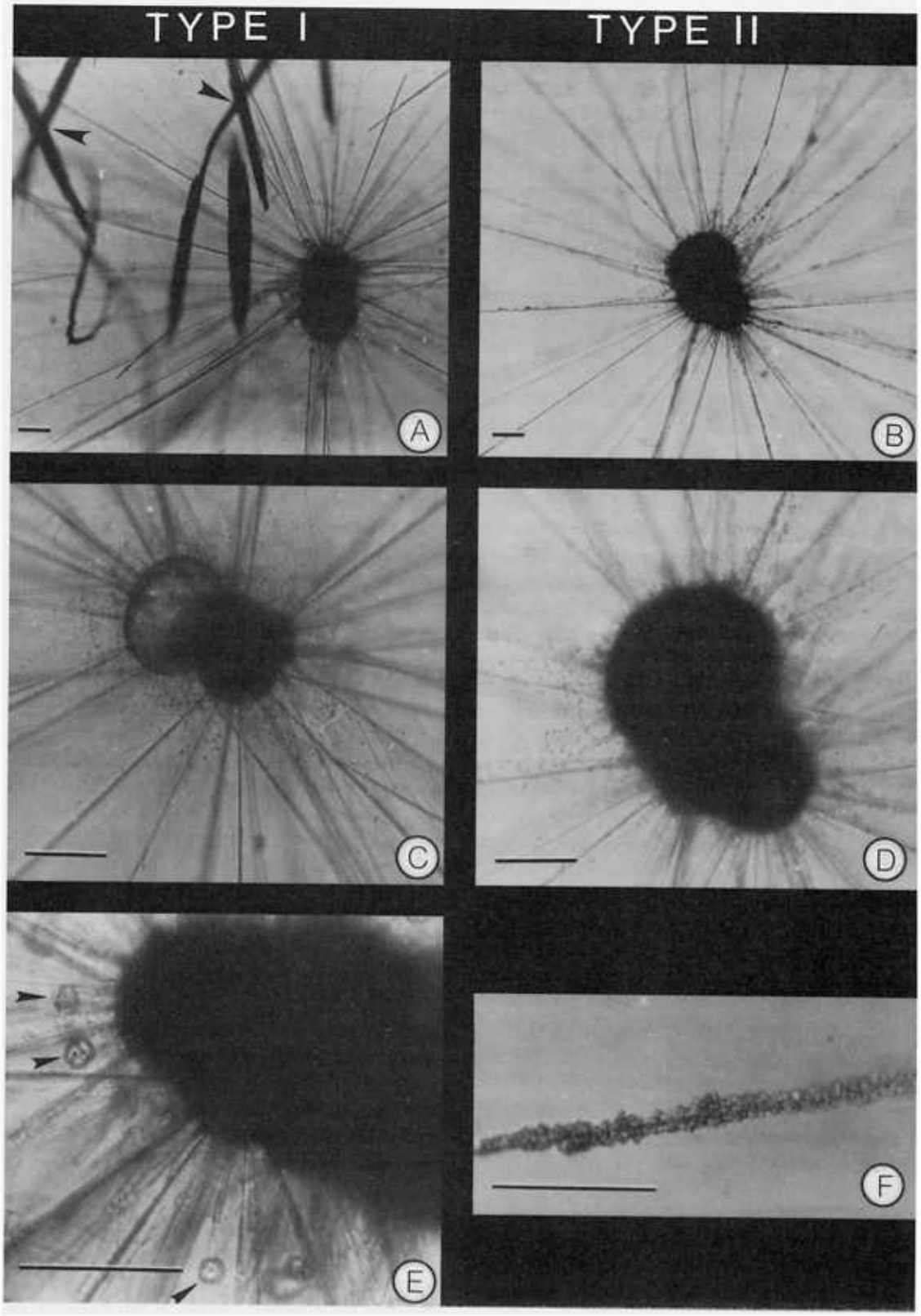
Observations from an inverted light microscope reveal additional differences. Type I is frequently found with commensal dinoflagellate "swarmers" between the spines (Fig. 2E), whereas Type II is not associated with swarmers. Endosymbiont distributions also differ between Types I and II. The brown color of the shell and the spines of Type II result from symbionts residing inside the shell and an even distribution of the endosymbionts along the spines (Fig. 2F), whereas the symbionts of Type I are normally dispersed between the spines (Fig. 2C; due to handling of the specimen the rhizopodia retracted and the symbionts were pulled towards the shell).

According to Faber et al. (1988), the endosymbionts possessed by both types of *G. siphonifera* belong to a group of golden-yellow pigmented algae, the Chrysophycophyta, which includes the Prymnesiida and Chrysomonadida. Transmission Electron Microscope (TEM) preparations by Faber et al. (1988) reveal that the symbionts in *G. siphonifera* Types I and II are ultrastructurally distinct, with significant differences in cell shape, plastid arrangement, lamellae organization, and location of vacuoles. These authors also noted that the Type II symbionts contain more chloroplasts than the Type I symbionts and the endosymbionts respond differently to culture conditions (Faber et al. 1989). This led Faber et al. (1988, 1989) to conclude that the endosymbionts in Types I and II may belong to separate species, and that the biologic features that distinguish *G. siphonifera* Type I and II are the result of possession of the different endosymbionts rather than genetic differences between the host foraminifera.

Laboratory culture experiments using differing light intensities and feeding frequencies reveal significant differences in survival time for the *G. siphonifera* morphotypes. In a study by J. Bijma et al. (unpublished), the survival time of Type I was almost 30% longer than that of Type II under low light intensity and a high feeding frequency, and about 50% longer under high light intensity and a low feeding frequency. This study also found that Type I populations had lower chamber formation rates (averaging 0.29 chambers/day) than the Type II populations (averaging 0.40 chambers/day). Faber et al. (1989) determined even greater differences in vital behavior between the Type I and II populations with as much as 3.1 times longer survival times and 5 times fewer chambers formed per unit time in Type I populations than Type II populations. The differences between these studies are mainly due to the fact that Bijma et al. (submitted) restricted their analysis to gametogenetic individuals whereas Faber et al. (1989) also included specimens that died in culture. Nonetheless, both studies suggest that Type I individuals have slower metabolic rates and may be better adapted to survive in highly oligotrophic conditions.

Pigment Analysis

Pigment measurements of SCUBA-collected specimens reveal significant differences between the Type I and Type II forms, as shown in Table 2 (from J. Bijma et al.unpublished). Although the mean size of the Type I population was almost 200 μm larger than that of the Type II population, the latter contained almost five times as much chlorophyll *a* and *c* per foraminifer. This may be the result of a higher quantity of pigment per symbiont, as suggested by the TEM study of Faber et al. (1988). It may also result from a greater symbiont density in the Type II cytoplasm. However, we were unable to confirm this as epifluorescent microscope counts of red-fluorescing cells in Type I and II specimens led to highly


TYPE I
TYPE II
A
B
C
D
E
F

variable results primarily due to problems with the technique used to prepare specimens for symbiont enumeration and differences in the distribution of the symbionts in the host foraminifer cell.

A pigment scan between 400 and 700 nm yields absorbance peaks at the same wavelengths for Types I and II (J. Bijma et al. unpublished). These results are consistent with the pigment analysis results (Table 2), confirming the presence of similar pigments in Types I and II and a higher chlorophyll *a*/carotenoid ratio in Type I.

The higher chlorophyll *a* content in *G. siphonifera* Type II may indicate predominant growth in lower light conditions at deeper levels in the water column. This is supported by the higher carotenoid content in Type II, which is a response to light absorption in the green part of the spectrum. However, we cannot exclude the possibility that the pigment differences could result from self-shading; a higher pigment concentration would be expected in specimens with symbionts that are closely packed or distributed inside the foraminifer shell.

TABLE 2. Results of pigment analysis for *G. siphonifera* Types I and II (from Bijma et al. unpublished). chloro. *a*/carot. = chlorophyll *a*/carotenoid ratio; *n* = number of specimens; avg. = average test diameter.

	Type I (n = 99; avg. = 521 μm)		Type II (n = 43; avg. = 338 μm)	
Pigment	μg/ml	ng/foram	μg/ml	ng/foram
Chlorophyll *a*	0.08	1.61	0.16	7.41
Chlorophyll *c*	0.02	0.45	0.04	2.06
Carotenoid	0.06	1.17	0.16	7.5
Chloro. *a*/carot.		1.38		0.99

Shell Stable Isotope Chemistry and Mg/Ca Ratios

Stable isotopic analyses of the shells of foraminifera provide insight into abiotic factors and the ecology of their ambient environment. This, in addition to their worldwide distribution, their good preservation potential and their long fossil record make these organisms ideal tools for paleoceanographic and paleoclimatic reconstructions. Because the $^{18}O/^{16}O$ ratio of calcite is primarily temperature dependent (Emiliani 1954), foraminifer shells grown in warmer surface waters are more depleted in ^{18}O than shells grown in cooler deep waters. This assumes that the shells were precipitated in equilibrium with the ambient water. The fractionation of carbon dissolved in the upper water column is controlled by photosynthesis rather than temperature. Dissolved inorganic CO_2 in upper surface waters tends to be enriched in ^{13}C because the lighter carbon isotope (^{12}C) is preferentially removed from the ambient water by phytoplankton. As the phytoplankton die and sink through the water column, the ^{12}C-enriched organic matter is oxidized and released. Thus, the shells of planktonic foraminifera that calcified in the upper surface waters are relatively enriched in $\delta^{13}C$, whereas those calcified at deeper levels are more depleted in $\delta^{13}C$ (e.g., Kroopnik 1974; Shackleton and Vincent 1978; Spero and Williams 1989). However, the $\delta^{13}C$ and $\delta^{18}O$ values of planktonic foraminifers generally covary with ontogeny where larger tests are typically enriched in $\delta^{13}C$ (shallow) and $\delta^{18}O$ (deep). This contradiction demonstrates that the interpretation of the isotopic composition in terms of equilibrium precipitation is problematic. Disequilibrium fractionation of $\delta^{18}O$ of $\delta^{13}C$ in planktonic foraminifera is caused by a variety of equilibrium and kinetic effects and largely depends on the metabolic rates of the host and their algal symbionts and on the

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FIGURE 2. Light micrographs of living specimens of *G. siphonifera* Types I (Fig. 2A,C,E) and II (Fig. 2B,D,F). 2A, Type I with bundles of the cyanobacteria *Trichodesmium* between the spines and some dinoflagellate "swarmers" close to the test. Due to disturbance cytoplasm containing the symbionts has been retracted within the test. 2B, Type II is never found with "swarmers" or *Trichodesmium*. The symbionts are sequestered within the rhizopodial network which mostly flows along the spines. The rhizopods are anastomosing and reticulate. 2C, Type I rhizopodia are strongly reticulate and therefore have a woolly appearance. 2D, Type II rhizopodia showing weakly reticulate arrangement and flow along the spines. 2E, Type I with dinoflagellate "swarmers" (arrows) in between the strongly reticulate rhizopodial network. 2F, Type II symbionts are sequestered within the rhizopodial network and are densly packed along the spines.

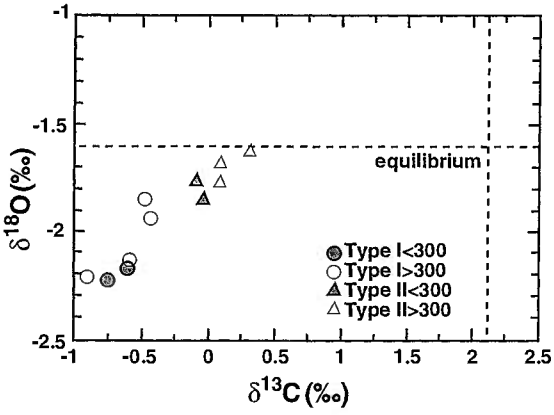


FIGURE 3. Oxygen and carbon isotope analyses of *G. siphonifera* Types I and II that were killed immediately after SCUBA collection. All specimens were collected as adults (>300 μm), but the final two to three chambers were amputated on some specimens for analysis of juvenile tests (<250 μm diameter). See text for explanation of calculation of $\delta^{18}O$ and $\delta^{13}C$ equilibrium values.

ambient carbonate system (for a more elaborate discussion consult Bijma et al. subm.).

Oxygen and carbon isotopic analyses of freshly collected specimens of *G. siphonifera* reveal that Type II is significantly and consistently more enriched in $\delta^{18}O$ and $\delta^{13}C$ than Type I (J. Bijma et al. unpublished). The differences are greatest between adult Type I and II specimens, with Type II $\delta^{18}O$ values averaging about 0.4‰ greater and Type II ^{13}C values averaging about 0.9‰ greater than for Type I adults (Fig. 3). Pre-adult specimens that had their final three chambers removed yield slightly lower differences in their $\delta^{18}O$ and $\delta^{13}C$ composition. In the analysis of SCUBA-collected specimens, Type II specimens reveal a much steeper ontogenetic (or ecotypic) trend in their stable isotopic composition (with the larger size fraction being about 0.3‰ more enriched in $\delta^{13}C$ and about 0.1‰ more enriched in $\delta^{18}O$) than Type I specimens. This is confirmed by Type I and II specimens grown in culture where a similar size-related stable isotopic fractionation was observed (J. Bijma et al. unpublished).

Using the paleotemperature equation of O'Neil (1969), equilibrium values for the oxygen isotopic composition are calculated as −1.33‰ at 26.6°C, assuming $\delta^{18}O = 0.93‰_{SMOW}$ at 36.3 ppt salinity (Fairbanks et al. 1992). Thus, adult Type II calcifies within ca. 0.2‰ of the estimated equilibrium calcite $\delta^{18}O$ value, whereas Type I is depleted in $\delta^{18}O$ by about 0.7‰. The more depleted $\delta^{18}O$ values of Type I specimens may reflect growth in a shallower surface-water habitat than Type II specimens and/or a greater degree of biological fractionation.

The $\delta^{13}C$ composition of calcite precipitated in equilbrium at the collection site should be about 2.25‰. This is based on a mean dissolved inorganic carbon $\delta^{13}C$ composition of 1.25‰ at the collection site and the assumption that calcite should be enriched in $\delta^{13}C$ by 1‰ relative to bicarbonate when secreted in isotopic equilibrium (Romanek et al. 1992). The >2.0‰ depletion of Types I and II in $\delta^{13}C$ relative to equilbrium calcite indicates a strong biological fractionation effect. A similar offset was reported by Kahn (1979) for *G. siphonifera* (undifferentiated) and for *G. praesiphonifera* (an ancestral morphospecies) by Pearson and Shackleton (1995). At first glance, the more enriched $\delta^{13}C$ composition of Type II relative to Type I specimens could be explained by the greater gross photosynthesis in Type II than in Type I and hence a more enriched calcification environment. However, this explanation has some severe drawbacks and the mechanism is more complicated (J. Bijma et al. unpublished). Recent experiments have demonstrated that "vital effects" are mediated by changes in the ambient carbonate system but reflect changes in the amount of respired CO_2 that is incorporated into the shell (H. Spero et al.unpublished). In this context, we believe that the reason for the heavier shells of Type II lies in the more effective uptake of light-respired CO_2 due to the higher photosynthetic rate and to the location inside the shell or at the shell surface of many Type II symbionts.

Measurements of the stable isotopic composition of *G. siphonifera* Types I and II at different feeding rates and light intensities also demonstrate significant differences. Bijma et al. (submitted) found that the $\delta^{13}C$ composition of Type I decreases by nearly 1‰ when the feeding rate increases from 0.5 to 2.0 brine shrimp per day, whereas the $\delta^{13}C$ fractionation of Type II did not respond systematically to changes in feeding rate. Exposure to contin-

uous darkness decreased the $\delta^{13}C$ composition of Type I shells, but the short survival time (two to three days) of Type II specimens kept in darkness prevented determination of any change in isotopic composition. Differences in the $\delta^{18}O$ composition of Types I and II were most apparent at low feeding rates, with Type I becoming slightly enriched and Type II becoming slightly depleted under a feeding scheme of one brine shrimp every two days.

The Mg/Ca molar ratios that were determined for Types I and II are 0.00489 and 0.00433, respectively. Using the relationship of Nürnberg et al. (submitted) and assuming that Type I mineralized its shell in equilibrium, the difference between Types I and II would indicate a 1.7°C colder precipitation temperature for Type II than for Type I. Assuming that Type II secreted its shell in equilibrium, this difference would indicate a 1.5°C warmer precipitation temperature for Type I than for Type II.

The $\delta^{18}O$ of Type II specimens larger than 300 m (−1.68‰ PDB) suggests a calcification temperature of 28.4°C whereas the larger fraction of Type I (−2.03‰ PDB) suggests 30.2°C (O'Neil 1969). Both types yield temperatures that are too high (collection temperature was 26.8°C), but the temperature difference between the two (1.8°C) compares well with the temperature difference calculated on the basis of Mg/Ca ratios (1.5–1.7°C).

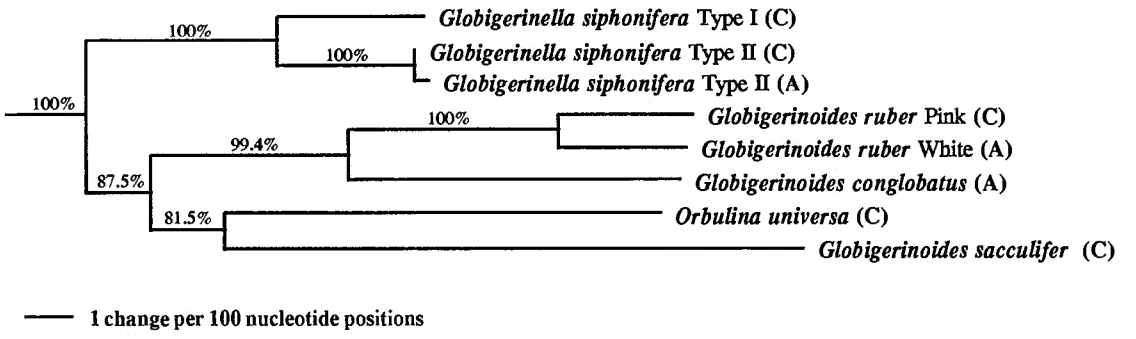
Genetic Differences

A recent breakthrough in sequencing the DNA of planktonic foraminifera has led to the recognition of species level differences between *G. siphonifera* Types I and II. This is based on the phylogenetic analysis of partial sequences amplification of the SSU rRNA gene, which so far has been accomplished for eight tropical planktonic foraminifer species (Darling et al. 1996b, 1997). Major difficulties in amplifying DNA from living specimens were overcome by extracting genomic DNA from gametogenic foraminifers.

Evolutionary Relationships between Three Spinose Foraminiferal Genera.—The phylogenetic sub-tree shown in Figure 4 forms part of a foraminiferal SSU rDNA phylogeny containing both benthic genera and spinose and nonspinose planktonic genera (Darling et al. in press). The three spinose genera, *Globigerinella, Globigerinoides,* and *Orbulina,* form a distinct group within the foraminiferal tree, supported in 100% of bootstrap replications. Strong bootstrap support is also provided for the branching pattern of the genera within the group. The *Globigerinella* genus, supported in 100% of bootstrap replications, is distinct from the other genera (Fig. 4). *Globigerinella siphonifera* Type I shows an evolutionary distance of 20.9% (20.9 changes per 100 nucleotide positions) from the grouping of *Globigerinoides ruber* and *Globigerinoides conglobatus,* 18% from *Orbulina* and 21.8% from *Globigerinoides sacculifer.*

Globigerinella siphonifera Type I is also significantly distinct from *G. siphonifera* Type II with an evolutionary distance of 6.2% between them. This is reflected in 30 base differences in the conserved regions (Fig. 5). In order to ascertain whether this level of variation is similar to that observed between other closely related but distinct foraminiferal species based on molecular data, two morphotypes of *Globigerinoides ruber* have been included in the analysis for comparison. The two morphotypes, *G. ruber* and *G. ruber* "pink," live in close proximity in the water column in the Caribbean, though the pink form is now extinct in the Pacific. The level of variation between these two morphotypes is 5.6%, which is very similar to the level of 6.2% observed between *G. siphonifera* Type I and Type II.

The two morphotypes of *G. ruber* were collected from the Caribbean (pink) and the Great Barrier Reef (white) and were therefore geographically isolated by the Isthmus of Panama. In order to test whether such isolation could account for the 5.6% difference observed between them, it would have been desirable to compare the pink and white forms from the Caribbean. However, *G. ruber* is difficult to keep in culture and only the pink form was successfully cultured to gametogenesis. As an alternative, *G. siphonifera* Type II collected in the Caribbean and Type II collected off the GBR were compared. Analysis showed that they vary by only 0.17% (Fig. 5), representing a single polymorphic change in 604 base positions. The same exercise has also



	G. siph I (C)	*G.siph* II (C)	*G.siph* II (A)	*G.ruber* (P) (C)	*G.ruber* (W) (A)	*O.univ* (C)	*G.sacc* (C)	*G.cong* (A)
(C) *G.siph* I	---	---	---	---	---	---	---	---
(C) *G.siph* II	6.17%	---	---	---	---	---	---	---
(A) *G.siph* II	6.04%	0.17%	---	---	---	---	---	---
(C) *G.ruber* (P)	22.11%	20.90%	21.34%	---	---	---	---	---
(A) *G.ruber* (W)	22.07%	20.35%	20.78%	5.52%	---	---	---	---
(C) *O.univ*	18.04%	18.09%	18.06%	23.41%	21.82%	---	---	---
(C) *G.sacc*	21.79%	23.70%	23.71%	27.76%	27.03%	21.58%	---	---
(A) *G.cong*	18.61%	19.04%	19.45%	14.63%	13.83%	20.36%	24.41%	---

FIGURE 4. Phylogenetic sub-tree showing the relationships between representatives of three spinose planktonic foraminiferal genera including *G. siphonifera* Types I and II. Branch lengths represent the evolutionary distance between species calculated from substitutional mutations/site. The scale bar corresponds to 1 change per 100 nucleotide positions. Bootstrap values, which provide an indication of the support for a particular branch, are expressed as a percentage (2000 bootstrap replications were performed). The sub-tree forms part of a phylogeny reconstructed from partial sequences (604 unambiguously aligned nucleotide sites) of the SSU rRNA gene of both benthic and planktonic foraminiferal genera (Darling et al. in press). The relative evolutionary distances between each of the planktonic species is shown expressed as a percentage in the accompanying table. Abbreviations: (C) = Caribbean, (A) = Australia, (P) = pink, (W) = white.

been carried out with *G. sacculifer* and *O. universa* (Darling et al. in press) and showed complete sequence identity in both species between the two regions.

Small Subunit rDNA Sequence Variations between and within G. siphonifera Types I and II.— Alignment of the amplified region of the SSU rRNA gene for four planktonic foraminiferal genera (Darling et al. 1996b) with representatives of the diverse range of eukaryote taxa within the SSU rDNA database (Maidak et al. 1994) has shown that the amplified regions consist of eight conserved nucleotide regions that are interspersed by seven variable regions. Comparison against all eukaryote sequences (438) presently within the database show that the variable regions fall into two categories. The amplified fragment contains four variable length expansion segments (V1–V4), which are present in most eukaryotes, and also three insertions unique to the foraminifera (F1–F3) (Fig. 5).

Thirty substitutional changes were observed between *G. siphonifera* Type I and Type II within the conserved regions, C1–C8 (Fig. 5). The changes were more commonly observed at the 3′ end of the amplified fragment, being primarily concentrated in C6, C7, and C8.

Considerable length and sequence variability was observed between *G. siphonifera* Type I and Type II within the foraminiferal specific insertions F1–F2 (Fig. 5B). In general, this made alignment within these regions impos-

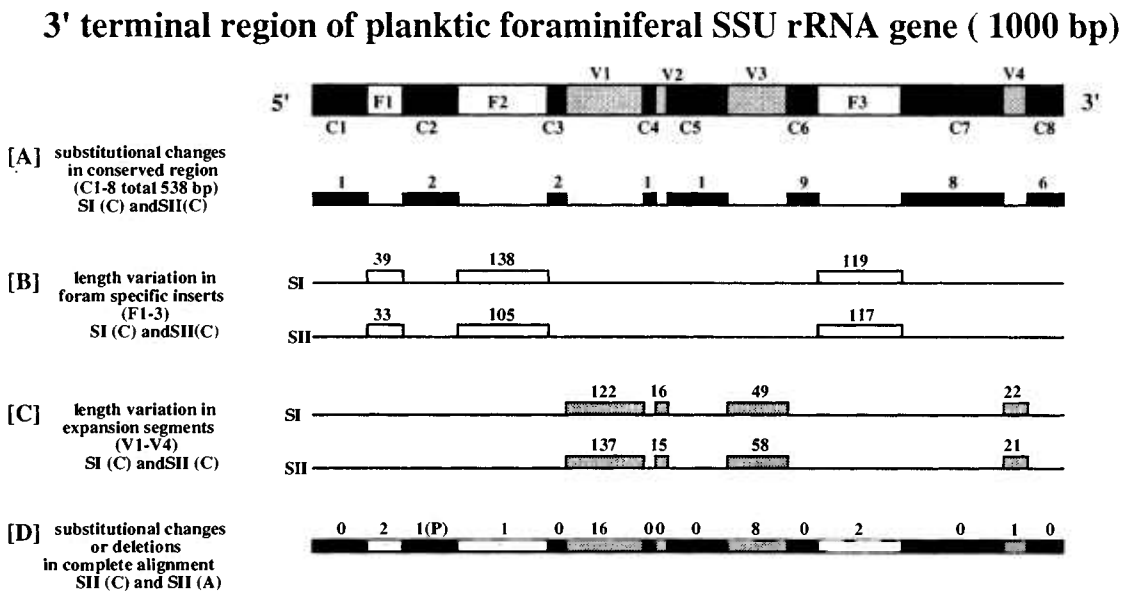


FIGURE 5. A schematic representation of the 3' terminal region of the SSU rRNA gene (approximately 1000 base pairs) used for phylogenetic analysis and sequence comparison. The fragment regions are divided into three categories: C1–C8 represent the highly conserved regions that are aligned relative to comparable regions present in all eukaryotes, V1–V4 represent variable length expansion segments present in most eukaryotes, and F1–F3 represent three insertions that are unique to the foraminifera. 5A, Substitutional mutations found in the conserved region between *G. siphonifera* Type I and II (30 in total). C6–C8 regions show the greatest variation. 5B, Length variation between *G. siphonifera* Types I and II in the foraminiferal specific insertions. Only F3 has a high degree of similarity, having only four substitutional changes and two deletions. Alignment within F1 and F2 was impossible. 5C, Considerable length variation was observed within the variable regions. This is also occurs between species within other planktonic foraminiferal genera (Darling et al. in press). 5D, The whole of the fragment of *G. siphonifera* Type II collected in the Caribbean could be unambiguously aligned against *G. siphonifera* Type II collected off theGreat Barrier Reef. Only one polymorphic substitutional change was seen in the conserved regions and little variation was observed in the foraminiferal specific insertions. However, a higher degree of variation was observed in the variable region, which should prove useful for further population studies.

sible. A high degree of similarity was observed, however, between Types I and II within insert F3. The F3 alignment showed only four substitutional changes accompanied by two deletions in Type II.

The expansion segments V1, V3, and V4 have a high level of base substitution and insertion/deletion events between Types I and II, making alignment within these regions also impossible (Fig. 5C). V1 and V3 have significant length variation, which is quite characteristic of the other planktonic foraminiferal genera (Darling et al. in press). The short region V2 has only one substitution and one deletion.

Comparison of *G. siphonifera* Type II, collected from the Caribbean and the GBR Australia, showed complete sequence identity between the conserved regions, C1–C8, except for one polymorphic position in C2 (Fig. 5D). The foraminiferal specific insertions had a surprising degree of conservation, having two substitutions in both F1 and F3 and only one in F2. The variable regions however, showed substantially higher variation.

Implications

The molecular data obtained in this study support the paleontologically based taxonomic schemes (e.g., Kennett and Srinivasan 1983) that place *Globigerina siphonifera* d'Orbigny in a separate genus (*Globigerinella*) from the other spinose species that were sequenced. If the evolutionary distances between *G. siphonifera* Type I and *O. universa* (21.82%) can be regarded as an example of separation at the genus level, then comparison among the other species analyzed suggests that *Globigerinoides sacculifer* should not be considered as congeneric with *Globigerinoides ruber* and *Globigerinoides*

conglobatus (Darling et al. in press). As additional molecular sequences become available, the relationships and progression of evolution within each of these lineages will be further clarified, and a revision of the current taxonomic scheme for modern planktonic foraminifera may be proposed.

Although *G. siphonifera* Type I and II are undoubtedly closely related, the high level of sequence diversity within the conserved region (6.2%) clearly shows that they are distinct species. Given that *G. siphonifera* Type I and II were collected from an identical location, at 3–8 m water depth, such substantial differences indicate that they are reproductively isolated from one another. The degee of variation is also similar to that observed between the pink and white forms of *G. ruber*. The fact that *G. siphonifera* Type II shows a remarkable degree of conservation between the Caribbean Sea and the western Pacific Ocean indicates that the sequence is entirely characteristic of the species and can be relied upon as a unique molecular marker for this species in future studies. It is highly probable that the *G. siphonifera* Type I sequence can also be used as a molecular marker, making the discrimination between the two species possible from individual specimens at any stage of their ontogeny.

Although the variability between the expansion segments and foraminiferal-specific insertions makes their use in phylogenetic analyses impractical at the species level, these regions will prove invaluable for analyses at the population level. The differences observed between *G. siphonifera* Type II from the Caribbean and Australia already indicate the potential for such investigations.

Ontogenetic Growth Stages

Type I and II shell morphologies and biometric data were analyzed to identify the prolocular, juvenile, neanic, adult, and terminal ontogenetic stages. The growth stages were originally defined by Brummer et al. (1986, 1987), who studied several extant tropical planktonic foraminifer species, including *Globigerinella siphonifera* (d'Orbigny) (= *Globigerinella aequilateralis* [Brady]). These authors included the first chamber (= proloculus) in the prolocular stage, the last chamber produced prior to gametogenesis in the terminal stage, and a variable, but species-specific, number of chambers in the juvenile, neanic, and adult stages. The juvenile stage was characterized by chambers showing log-linear growth (determined from plots of test diameter vs. chamber number) in the first 1.5 whorls, low trochospiral coiling, a low-arched, extraumbilical positioned aperture, and a nearly smooth surface texture with sparse, thin spines. During the juvenile stage, pores on the shell wall are initially concentrated near the chamber sutures and, in later juvenile chambers, are distributed throughout the spiral and umbilical chamber surfaces. Onset of the neanic stage was recognized by an abrupt increase in the log-linear growth rate, increase in chamber globularity, coiling spire height, and density and thickness of spines, spread of pores to the entire chamber surface, narrowing of the umbilicus, and migration of the aperture to a more umbilical position. Brummer et al. (1986, 1987) suggested that onset of the adult stage is characterized by minor but often distinct changes in morphology, such as migration of the aperture to its final position and attainment of mature pore and spine morphology. The terminal stage can be distinguished from the adult stage in some specimens if a diminutive (= kummerform) final chamber is produced just before reproduction. On the other hand, a final "normalform" chamber that is indistinguishable from earlier adult chambers may be built at this stage.

According to Brummer et al. (1987), ontogenetic stage transitions in spinose planktonic foraminifera occur during distinct transitions in vital behavior. The prolocular stage is initiated by zygote formation and is probably very short-lived. The wall connecting the proloculus and deuteroconch (second chamber) is concave in both morphotypes of *G. siphonifera* (Fig. 6D), suggesting that calcification of the proloculus occurred prior to growth of the deuteroconch. The juvenile stage, which is initiated by deuteroconch formation, includes a growth phase where the trophic preference is algae and protozoa and infection by symbionts occurs. During the neanic stage, symbiosis increases and the diet changes to include

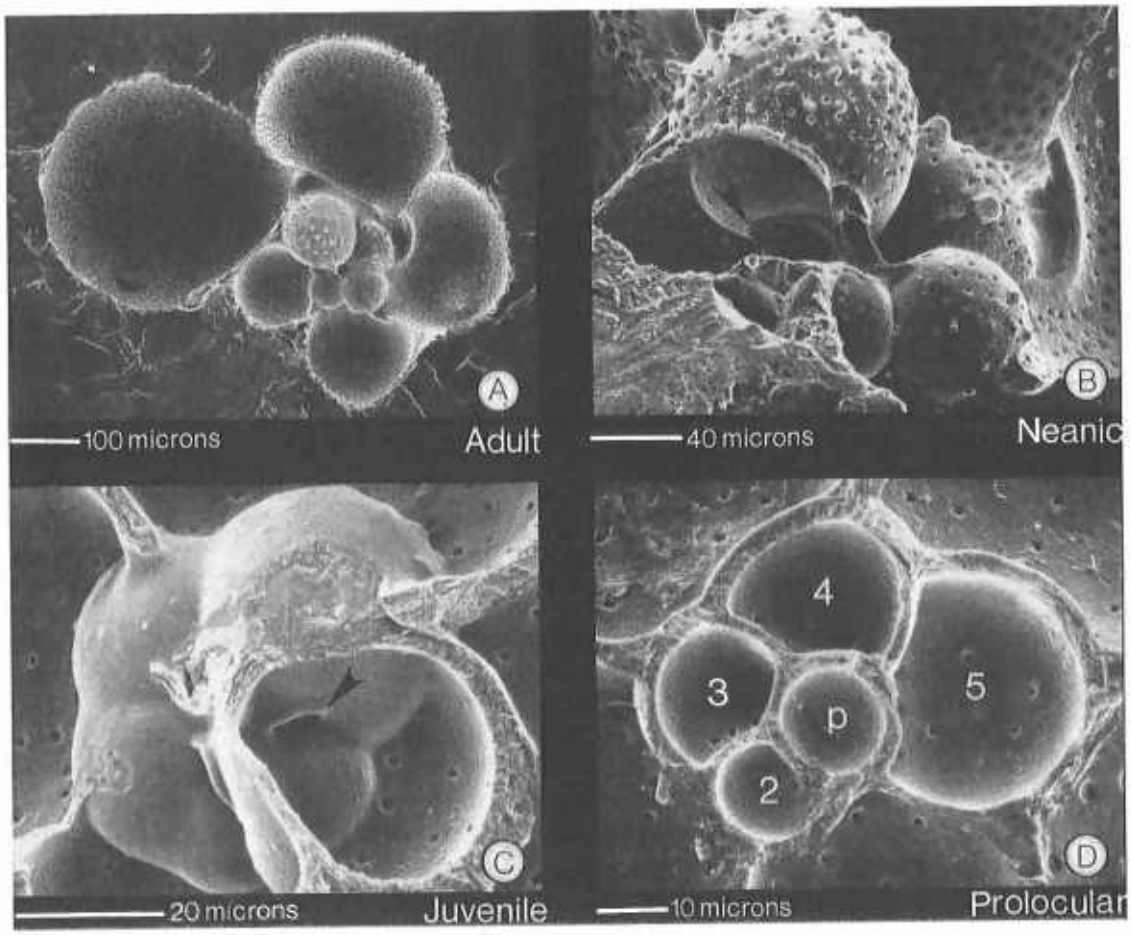


FIGURE 6. Scanning Electron Micrographs of serially dissected specimens of *G. siphonifera* Type I showing morphologic differences between the adult, neanic, juvenile, and prolocular growth stages. Specimens are embedded in thermoplastic and dissected using a micromanipulator. 6A, Adult shell with the outer whorl removed to reveal the penultimate whorl. 6B, Oblique view of neanic chambers shown in Figure 6A. Note that the aperture (see arrow) is extraumbilical in position and the wall texture becomes smoother earlier in the ontogenetic series. 6C, Specimen with two whorls removed to reveal chambers in the juvenile growth stage. Note the smooth surface of the chambers and nearly umbilical position of the small aperture (see arrow). 6D, Completely dissected specimen revealing the 4.5 chambers in the initial whorl surrounding the proloculus (p).

motile metazoans (primarily crustaceans) once the spines are sufficiently numerous and strong to prevent their escape. During the adult stage, reproductive maturity is attained, symbiosis reaches a maximum, and the diet is predominantly based on crustacean prey. As the foraminifer reaches the terminal stage, growth is terminated, symbionts are digested or expelled, and then gametes are released.

Type I and II specimens were not distinguished in the study by Brummer et al. (1987), so it is not clear what the proportional representation of these phenotypes was in their measurements. Their results indicate that (1) proloculus diameters average 18.9 μm and range from 12 to 30 μm, (2) transition from the juvenile to neanic stage occurs at test diameters ranging from about 70–90 μm and between the sixth and ninth chambers, and (3) transition from the neanic to adult stage occurs at test diameters ranging from 175 to 250 μm and between the tenth and thirteenth chambers.

Empty Shell Differences

Test Morphology

A detailed description of *Globigerinella siphonifera* (d'Orbigny 1839) can be found in

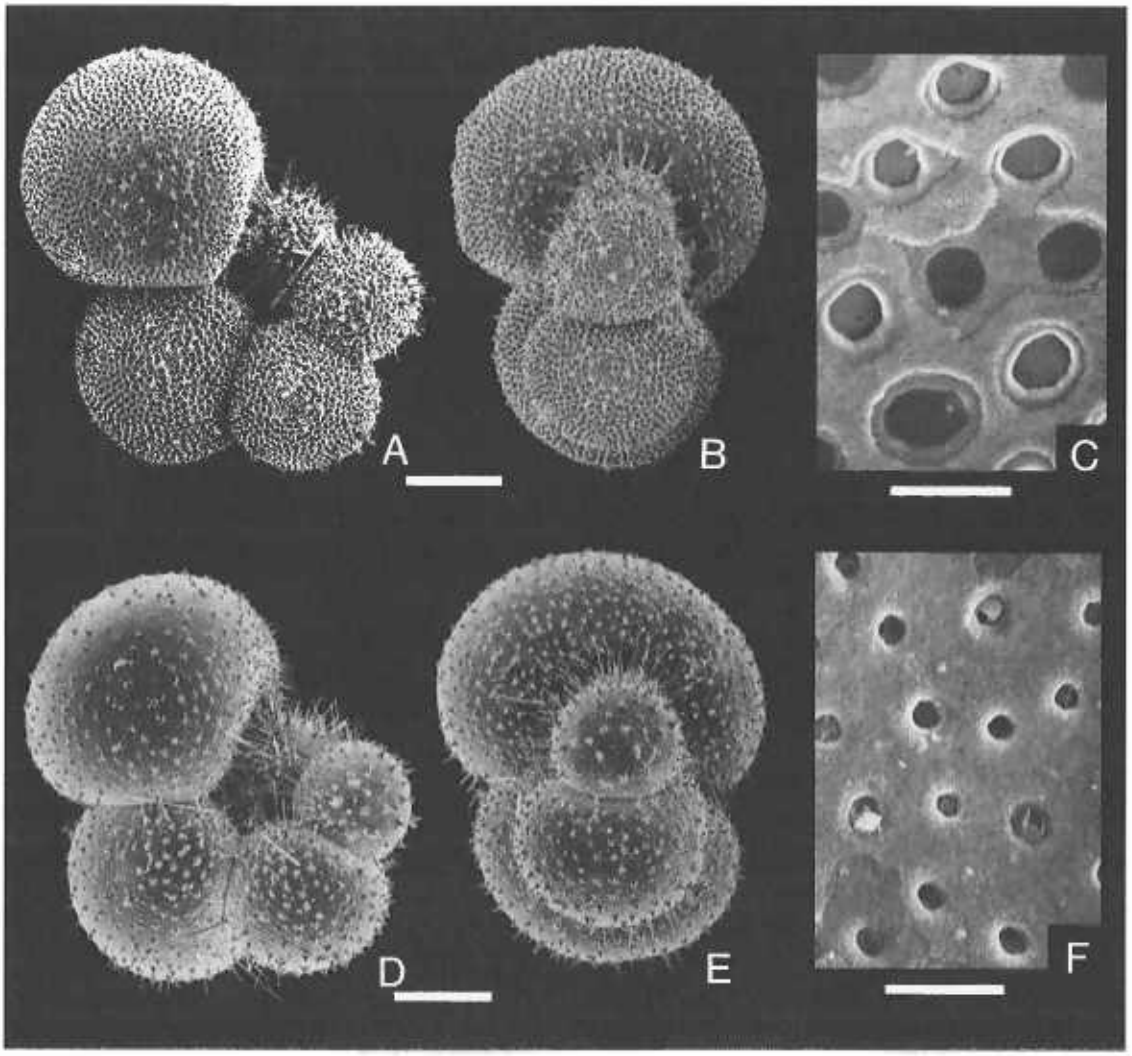


FIGURE 7. Scanning Electron Micrographs of SCUBA-collected *G. siphonifera* Types I and II from offshore Curaçao. Scale bars represent 100 μm for all figures except 7C and 7F, which represent 10 μm. 7A–C: Type I specimen. 7A, Umbilical view; 7B, Edge view; 7C, Interior view of pores on the 15th chamber showing 23% porosity and an average pore diameter of 4.5 μm. 7D–F: Type II specimen. 7D, Umbilical view; 7E, Edge view; 7F, Interior view of pores on the 15th chamber showing 5.3% porosity and an average pore diameter of 2.2 μm. Note that Type I specimens are characterized by a larger pore size and more open coiling than Type II specimens.

Banner and Blow (1960), who designated a lectotype for the species and, because of the Principle of Priority, recommended that the commonly used name *Globigerina aequilateralis* Brady 1879 be considered its junior synonym. The species is characterized by having a spinose test that appears to be planispirally coiled but actually coils in a low trochospire in the early ontogeny and becomes irregularly planispiral in the adult growth stage (Figs. 6, 7). Accordingly, the aperture is an extra-umbilically positioned, narrow, low arch in the juvenile stage (Fig. 6C), it migrates toward the equatorial periphery in the neanic stage (Fig. 6B) and then becomes equatorial and nearly symmetrical during the adult stage (Fig. 7B,E). Living specimens with fully grown spines are as much as 2 cm in diameter, but the spines break off upon death and the postmortem shells of adult specimens typically range between 300 and 800 m in maximum diameter.

Parker (1962) was the first to suggest that *G. siphonifera* could be subdivided into two morphologic groups. She characterized one group

as having more compressed chambers, more involute coiling, and possibly smaller proloculi than the other group. These observations were based on study of Recent samples from tropical Pacific cores rather than on living specimens. On the other hand, Faber et al. (1988, 1989) could easily identify the Type I and II morphologies among living SCUBA-collected specimens, but could not detect differences between the shells after the Type I and II specimens had died. Our observations suggest that while Type I shells tend to attain a larger size, the only feature that can be consistently used for light-microscopic discrimination of Type I from Type II forms is shell pore size, whereby adult chambers of Type I forms have much larger pore diameters than do the adult chambers of Type II (Fig. 7A–F). In fact, these differences are only easy to detect among SCUBA-collected specimens, as specimens from seafloor sediments have a coarser shell surface, probably due to gametogenic calcification, that obscures observation of shell porosity under the light microscope (Fig. 8A,B,D,E). Thus, we do not consider Parker's two morphogroups of *G. siphonifera* as having the same biological meaning as the Type I and II forms recognized by Faber et al. (1988, 1989). It is likely that Parker's Group I and II forms are ecophenotypes since she found latitudinal differences in their distribution.

Qualitative observations of serially dissected specimens reveal no recognizable differences between the Type I and II shells. Changes in wall texture, apertural morphology and position, and chamber arrangement that characterize the growth stages of both forms occur at somewhat variable chamber numbers in the ontogeny, and their morphologic expression is indistinguishable. Our observations generally confirm the size and chamber ranges for growth stage transitions in *G. siphonifera* that were outlined by Brummer et al. (1987). We found that for both Type I and II forms, onset of the juvenile stage (immediately following the prolocular stage) occurs at test diameters between 20 and 30 μm, onset of the neanic growth stage occurs between the sixth and eighth chambers, at test diameters between about 60 and 100 μm, and onset of the adult stage occurs between the tenth and thirteenth chamber, at test diameters between about 180 and 300 μm. Given the similarities in ontogenetic morphology of Types I and II and the considerable variability in test size at the growth stage transitions, the only hope of identifying morphological differences in what are clearly biologically distinct species lies with the biometric study of populations.

Biometric Results

Data from Shell X Rays.—Most of the measurements from X-ray images reveal a high degree of morphologic variability and little or no difference between adult populations of Types I and II. No significant differences were found in the number of chambers in the initial and final whorls, total chamber number, and ultimate/penultimate and penultimate/antepenultimate chamber diameter ratios (Table 3). However, populations of Type I have fewer kummerform chambers and the test diameters average 100 μm greater than those in Type II populations (Fig. 9). This observation is confirmed by laboratory studies (Faber et al. 1989; J. Bijma et al. unpublished). In addition, a greater frequency of sinistrally coiled forms was observed in Type I, but none of these is a unique feature that can be used to distinguish Type I from Type II individuals.

Also similar are plots of the relative increase in cross-sectional chamber area (Fig. 10). In all measured specimens, the deuteroconch is always smaller than the proloculus and the ensuing chamber growth is log-linear. These plots reveal a greater variability in chamber areas for the Type II population than for the Type I population. Inflections in the averaged chamber growth curves (Fig. 10C) indicate allometric growth for both forms, with the changes in slope corresponding to the morphologically determined transitions from the prolocular–juvenile, juvenile–neanic, and neanic–adult growth stages. The averaged chamber areas for each form are sinusoidally distributed around the calculated exponential regression line, and the slopes and correlation coefficients of those lines are also nearly the same (Fig. 11A).

Relative rates of chamber size increase are

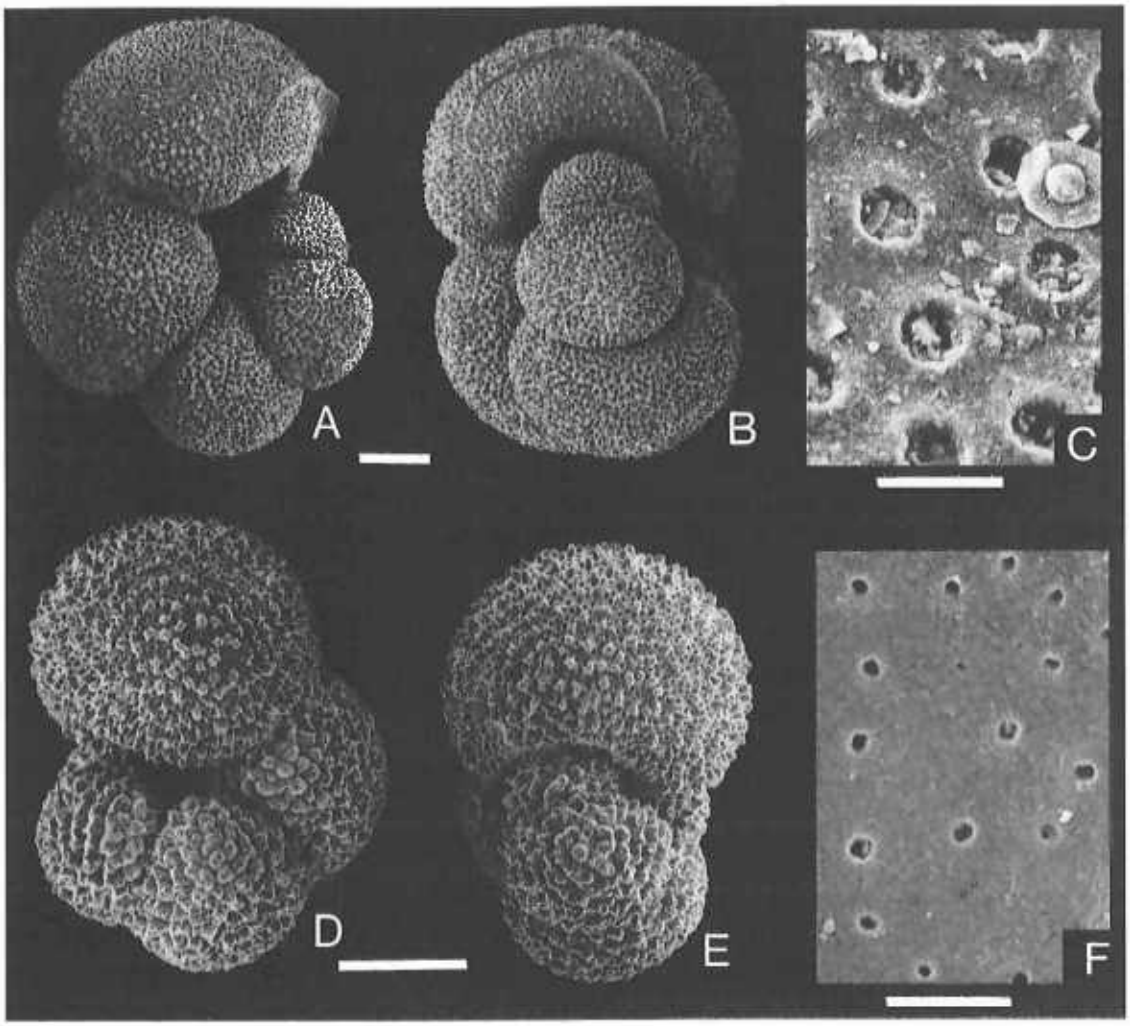


FIGURE 8. Scanning Electron Micrographs of Pleistocene *G. siphonifera* specimens from DSDP Site 154A (Sample 154A-1-1, 93–107 cm), located in the western Caribbean. Scale bars represent 100 μm for all figures except 8C and 8F, which represent 10 m. 8A–C, "Type I" specimen. 8A, Umbilical view; 8B, Edge view; 8C, Interior view of pores on the 15th chamber showing 16% porosity and an average pore diameter of 4.0 μm. 8D–F, "Type II" specimen. 8D, Umbilical view; 8E, Edge view, 8F, Interior view of pores on the 14th chamber showing 2.4% porosity and an average pore diameter of 1.2 μm. The specimens collected from Site 154 have a coarser surface texture than the SCUBA-collected specimens (Fig. 7), probably due to gametogenic calcification, which does not affect shell porosity as viewed from the chamber interior.

calculated by subtracting the mean cross-sectional area of each chamber from that predicted by the linear regression. The pattern of these residuals (Fig. 11B,C) is remarkably similar for Types I and II and their reversals in slope correspond with the ontogenetic growth stage transitions. Correspondence of residual patterns and growth stages was also observed by Brummer et al. (1987) in their plots of test diameter versus chamber number for *G. sacculifer* and *G. ruber* "pink."

The most significant differences measured from X-ray images of Type I and II specimens were obtained from analysis of the chamber coiling. Two examples of X-ray tracings with overlays of log-spiral growth curves illustrate these differences in Figure 12. The higher exponential regression slope and correlation coefficient for the Type I specimen reflect a more evolute and less variable coiling than is indicated by the slope and correlation coefficient shown for the Type II specimen. A similar re-

TABLE 3. Biometric results from analysis of X-ray images of specimens of *G. siphonifera* Types I and II. See text for explanation of metrics. 1σ = 1 standard deviation.

Measurement	Type I (n = 68)			Type II (n = 42)		
	Mean	1σ	Range	Mean	1σ	Range
Total no. chambers	14.52	1.26	12.00–17.00	14.38	1.47	13.00–18.00
Percent left coiled	68%	—	—	45%	—	—
Percent kummerform	4%	—	—	18%	—	—
No. chambers initial whorl	4.78	0.30	4.25–5.00	4.68	0.47	4.00–5.50
No. chambers final whorl	4.57	0.35	3.50–5.00	4.31	0.40	3.50–5.00
Max. test diam. (μm)	549.05	113.03	344.35–790.33	448.63	87.62	312.01–606.80
Penultimate/ult. chbr. diam.	0.80	0.12	0.68–1.30	0.82	0.13	0.68–1.10
Antepenultimate/penultimate chbr. diam.	0.77	0.07	0.56–0.93	0.78	0.06	0.67–0.94
Ult. chamber r1/r2	1.02	0.08	0.93–1.31	1.03	0.11	0.85–1.31
Penultimate chamber r1/r2	1.00	0.08	0.87–1.11	1.04	0.09	0.88–1.28
Antepenultimate chamber r1/r2	1.00	0.05	0.90–1.13	1.09	0.08	0.91–1.21

sult was obtained from an analysis of the total Type I and II populations. The data for both the regression slopes and correlation values, presented in Table 4, average more than one standard deviation value higher than those for the Type II specimens, although there is some overlap. We used a Mann-Whitney *U*-test to determine the significance of these values. This is a nonparametric approach that ranks the values for both populations for the variate being studied and tests for differences in the sum of the rankings for each of the two groups; the hypothesis to be tested is that the values were drawn from identical distributions. In both comparisons, the Type I population had significantly higher values than the Type II ($p << 0.001$), showing that the Type I specimens had higher slopes and higher correlations.

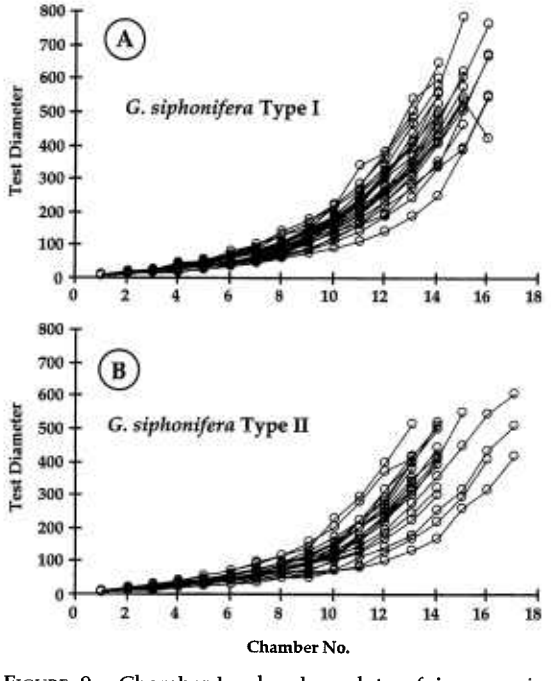


FIGURE 9. Chamber-by-chamber plots of increase in maximum test diameter for *G. siphonifera* Types I and II. Note that most Type I specimens attain a larger maximum test size and have lower variability in diameter per chamber number.

Proloculus Diameter.—Proloculus diameters measured from dissected specimens also revealed no significant difference between Type I and II populations (Table 5). The mean value for Type I specimens (13.38 μm) is within one standard deviation of the mean value for Type II specimens (15.62 μm), and their minimum and maximum values are within only 2 μm of each other. Brummer et al. (1987) found a greater range of variation in this measurement in *G. siphonifera* and suggested that it is not a very useful character for taxonomic distinction.

Shell Porosity.—Results of chamber-by-chamber measurement of the shell porosity in serially dissected specimens of *G. siphonifera* Types I and II demonstrate that adult Type I chambers on average attain more than two times greater shell porosity than Type II specimens (Table 6). Porosity values for both forms increase from none in the proloculus to less than 5% through the juvenile and neanic growth stages (Figure 13). By the adult stage porosity differences become very apparent. Type I adult chambers mostly range between 10% and 20% porosity and reach up to 30% porosity, whereas Type II specimens mostly range between

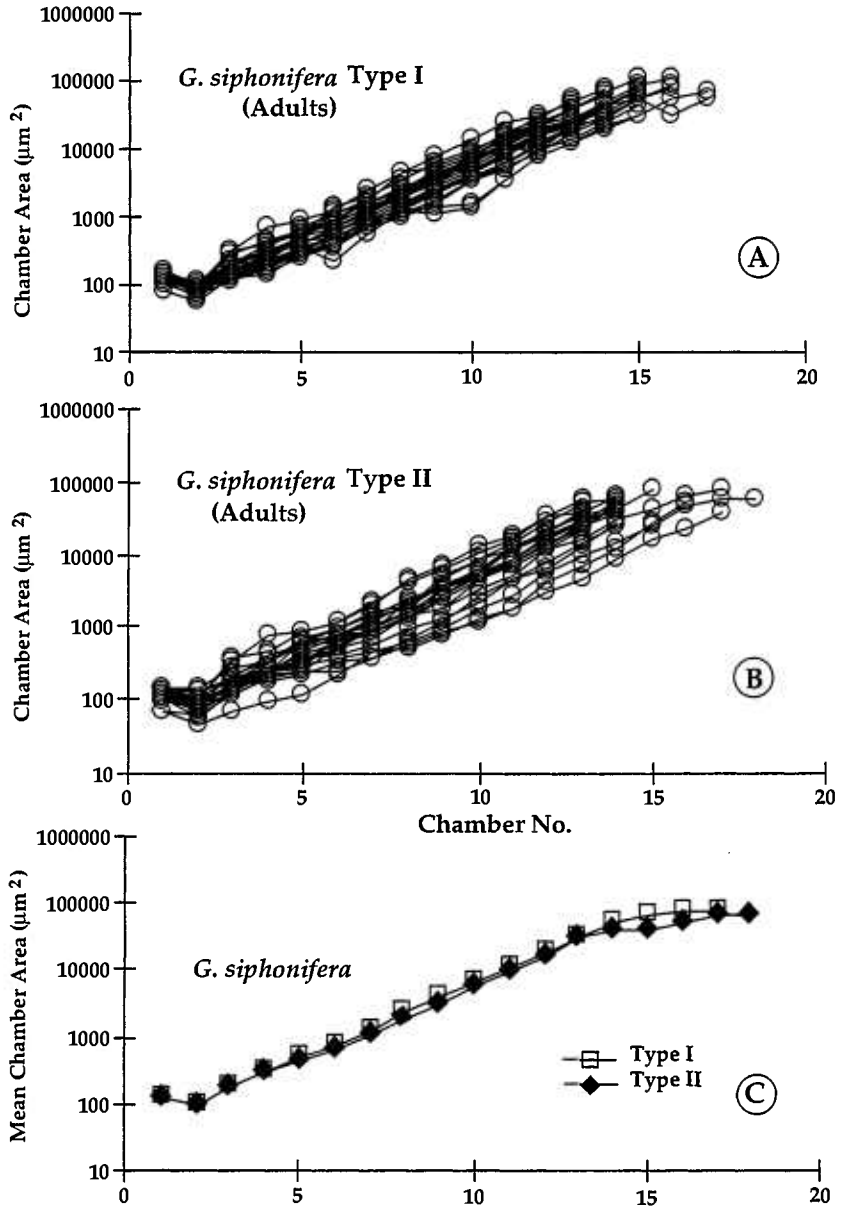


FIGURE 10. Chamber-by-chamber plots of cross-sectional chamber area for individual specimens and population means of *G. siphonifera* Types I and II. Measurements made from digitized high-resolution X-ray images. 9A, Ontogenetic trajectories measured from individual Type I specimens. 9B, Ontogenetic trajectories measured from individual Type II specimens. 9C, Mean values for Types I and II.

4% and 7% and reach up to 10% porosity. Maximum porosity values are consistently reached in either the ultimate or penultimate chamber of adult specimens, but chamber number cannot be used to predict porosity values directly, as there is considerable inter-specimen variation amongst the Type I and II populations.

The ontogenetic increase in shell porosity is primarily a function of increasing pore diameters, since pore concentrations show little ontogenetic change. Pore diameters in the ultimate and penultimate chambers of adult Type I specimens generally range between 4 and 5 µm, whereas those of Type II adult specimens range between 1 and 2 µm. Results

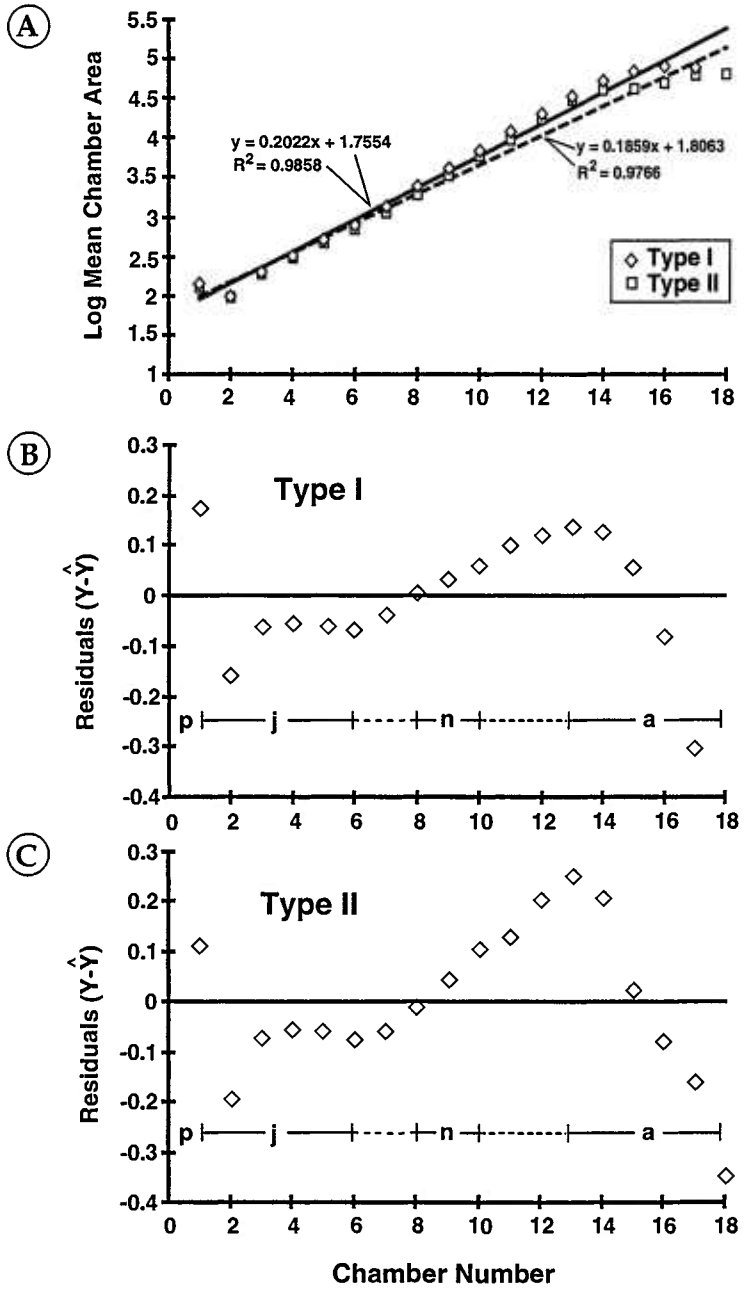


FIGURE 11. Cross-sectional chamber area increase during the ontogeny of *G. siphonifera* Types I and II. 11A, Composite relative growth curves; note that linear regressions and correlation coefficients are nearly identical for the Type I and II populations. 11B, Residuals calculated from the growth curve and linear regression of Type I. 11C, Residuals calculated from the growth curve and linear regression of Type II. Note that changes in the relative rate of increase in chamber area (inflections in the residuals curves) occur at the same growth stage for Types I and II. Morphologically determined ontogenetic growth stages are indicated as follows: p = prolocular, j = juvenile, n = neanic, and a = adult.

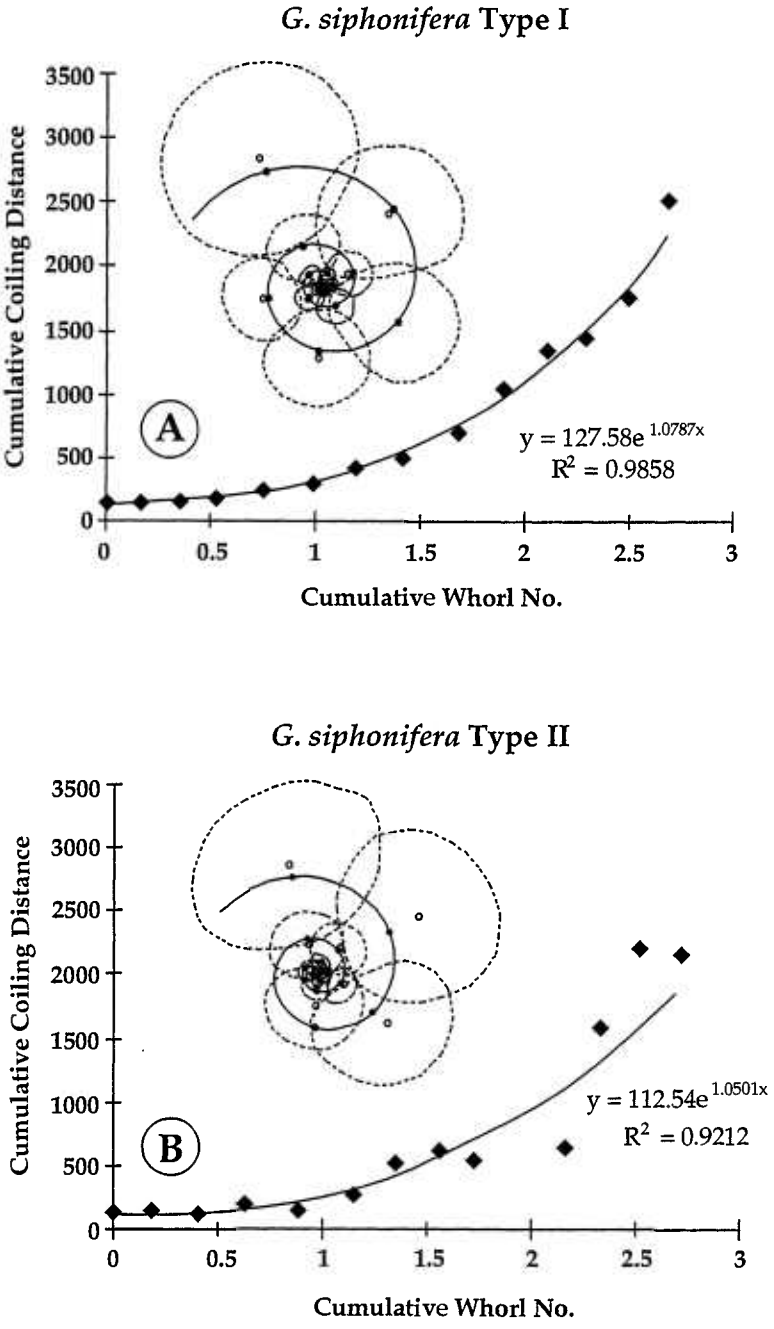


FIGURE 12. Comparison of the predicted logarithmic curve and actual coiling metrics for Type I and II specimens that were digitized from high resolution X-ray images. X-ray tracings are shown for Type I and II specimens with centers of form for each chamber and the predicted growth spiral. Y-intercept values and slopes for the exponential regressions and correlation coefficients are given for each example.

of previous planktonic foraminifer shell porosity studies could be taken to indicate that the lower porosity of *G. siphonifera* Type II is consistent with its growth in deeper (cooler) surface waters than Type I. Comparison of the shell porosity in a number of living tropical species (Wiles 1967; Bé 1968) and Recent tropical and subtropical species (Frerichs et al.

TABLE 4. Summary of shell coiling data obtained from X-ray images of *G. siphonifera* Types I and II, including slopes of the exponential regression curve, y-intercept values, and correlation coefficients. See text and Figure 12 for explanation of results. Specimen ID refers to identification codes used to locate X-ray images of each specimen.

Type I (n = 25)				Type II (n = 19)			
Specimen ID	Slope	y-value	r^2	Specimen ID	Slope	y-value	r^2
894la1	1.160	136.770	0.982	894iia1a	1.045	128.170	0.945
894la2	1.180	94.860	0.990	894iia1b	1.05	112.540	0.922
894la5a	1.120	114.700	0.984	894iia2a	0.991	153.950	0.841
894la6a	1.060	114.350	0.986	894iia2b	1.061	118.850	0.974
894la7a	1.058	98.720	0.990	894iia3a	1.039	129.530	0.887
894la9a	1.102	121.600	0.984	894iia10a	1.053	147.160	0.994
894ib1a	1.063	114.351	0.986	894iib1a	1.045	107.940	0.960
894ib7b	1.274	93.850	0.986	894iib1b	1.087	85.300	0.852
894ic1a	1.066	137.760	0.984	894iib2b	1.01	118.920	0.910
894ic5b	1.114	152.950	0.990	894iib5b	0.92	120.700	0.897
894ic7a	1.095	162.700	0.988	894iib6b	0.988	97.340	0.980
894id1a	1.227	113.020	0.966	894iib9b	0.879	117.500	0.931
894id2a	1.103	126.800	0.984	894iic8b	0.823	105.680	0.891
894id2b	1.181	107.890	0.992	492iiad4	1.04	161.060	0.937
894id3a	1.132	111.460	0.956	492iiae1	1.06	107.400	0.966
894id6b	1.079	127.580	0.986	492iibc5	1.03	141.870	0.951
894id9b	1.167	131.080	0.960	492iiad3	1.05	86.740	0.976
894id10b	1.085	138.420	0.976	492iibd3	0.945	125.290	0.984
894id11b	1.160	90.870	0.978	492iibe5	0.941	72.860	0.929
492iba4	1.080	140.550	0.992				
492iaa1	1.200	109.700	0.976				
492iba3	1.130	99.420	0.974				
492icb2	1.210	113.000	0.974				
492iba5	1.080	141.280	0.986				
492icb3	1.140	87.490	0.978				
Mean	1.131	119.247	0.981	Mean	1.003	117.832	0.933
Std. dev.	0.058	19.946	0.009	Std. dev.	0.0706	23.299	0.044
Min. value	1.058	87.490	0.956	Min. value	0.823	72.860	0.841
Max. value	1.274	162.700	0.992	Max. value	1.087	161.060	0.994

1972) with surface water temperature suggested these variables are directly proportional. For *G. siphonifera*, Frerichs et al. (1972) found that tropical specimens collected from sediment surfaces below ~28.5°C surface waters had porosities averaging 7.3%, whereas subtropical specimens from below ~22.5°C surface waters averaged 5.2% porosity. However, the laboratory culture study of Bijma et al. (1990) found that there is only a 2% difference between *G. siphonifera* (undifferentiated) specimens grown in 13°C water versus 30°C water. This would not account for the greater than 10% difference in porosity observed in SCUBA-collected populations of Types I and II from Curaçao (Table 6).

TABLE 5. Proloculus dimensions (in μm) from completely dissected specimens of *G. siphonifera* Types I and II. 1σ = 1 standard deviation.

	Type I (n = 18)	Type II (n = 12)
Mean	13.38	15.62
1σ	1.96	2.30
Range	11.04–18.40	13.10–20.50

Discussion

Multiple lines of biological evidence indicate that *G. siphonifera* Types I and II are separate species, but, in addition to differences in shell chemistry, the only readily discernible difference between the empty shells of SCUBA-collected Type I and II individuals is the much larger porosity in Type I specimens. Biometric analysis reveals that the Type I and II growth morphologies are indistinguishable for all measured characters except shell coiling, but the more evolute coiling of Type I specimens is apparent only among measured populations, not individuals. We conclude, therefore, that *G. siphonifera* Types I and II are living examples of cryptic speciation; their speciation would go unrecognized using the traditional paleontological species definition,

TABLE 6. Porosity values determined from completely dissected specimens of *G. siphonifera* Types I and II. Measurements obtained from SEM images by measuring pore diameters and pore concentrations of the shell interior at 10,000× magnification. 1σ = 1 standard deviation.

	Type I (n = 12)			Type II (n = 8)		
Chamber #	Avg.	1σ	Range	Avg.	1σ	Range
1	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
2	0.02%	0.01%	0.00–0.03%	0.23%	0.64%	0.00–1.81%
3	0.38%	0.30%	0.11–0.77%	0.43%	0.65%	0.00–1.87%
4	0.92%	0.80%	0.14–2.45%	0.56%	0.54%	0.00–1.50%
5	1.57%	1.22%	0.11–3.37%	0.93%	0.94%	0.00–2.51%
6	1.42%	1.06%	0.39–3.42%	1.54%	1.65%	0.00–4.04%
7	1.96%	0.75%	0.83–3.47%	1.88%	1.32%	0.00–3.86%
8	1.75%	0.56%	0.73–2.83%	1.80%	1.02%	0.82–3.76%
9	1.90%	0.43%	1.28–2.54%	2.02%	0.49%	1.10–2.61%
10	2.30%	0.99%	1.21–3.87%	2.09%	0.99%	1.01–4.16%
11	3.28%	1.39%	1.78–6.61%	2.68%	1.03%	1.28–4.70%
12	6.52%	3.62%	2.66–12.89%	2.66%	1.14%	1.57–5.18%
13	10.12%	4.63%	4.08–18.20%	3.54%	1.49%	1.39–6.01%
14	13.84%	4.79%	5.96–23.72%	4.50%	0.91%	2.83–5.53%
15	20.37%	5.02%	15.32–29.34%	5.35%	0.90%	3.98–6.41%
16	18.11%	0.60%	17.65–18.96%	6.80%	2.53%	3.90–10.11%
17				7.41%	0.00%	7.41%

which requires distinctive skeletal morphologies.

The biological significance of the porosity differences between Types I and II is not clear since shell pore size in planktonic foraminifera may be dependent on a combination of environmental and genetic factors. Beyond the constraint of genetic inheritance, shell porosity has been directly correlated to metabolic activity and foraminifer growth rates (Bijma, Faber, and Hemleben 1990). Such a correlation would suggest that specimens with the larger pore sizes (e.g., Type I) live in warmer surface waters (at shallower depths) and/or have a

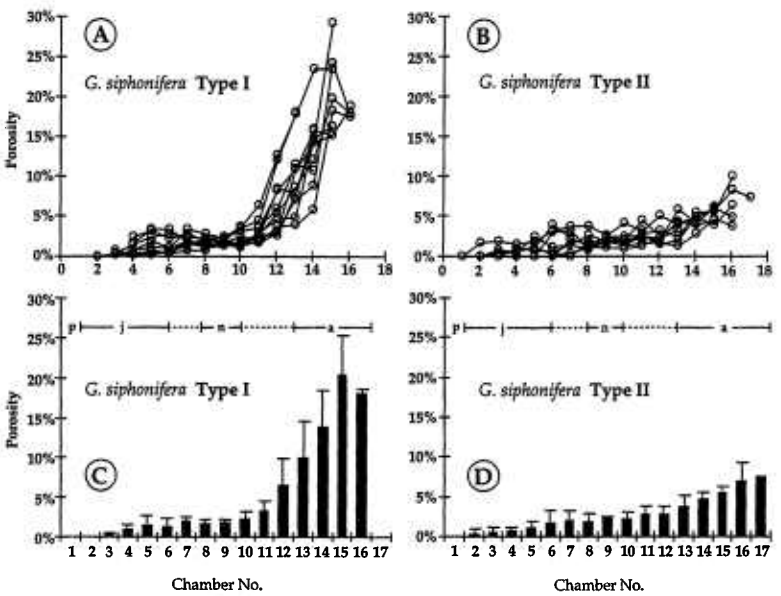


FIGURE 13. Shell porosity measurements from completely dissected *G. siphonifera* Types I and II specimens that were collected by SCUBA divers. 13A,B, Chamber-by-chamber plots of shell porosity for *G. siphonifera* Type I and II individuals. 13C,D, Histogram plots of mean chamber porosity with an error bar representing one standard deviation about the population means. Morphologically determined ontogenetic growth stages are indicated as follows: p = prolocular; j = juvenile; n = neanic; a = adult.

greater symbiont density than specimens with the smaller pore sizes (e.g., Type II). However, our observations do not entirely agree with this prediction. Oxygen isotopic, Mg/Ca ratio, and pigment analysis data suggest that Type I grows at shallower depths, but we have observed that its symbiont density is significantly lower than in Type II. Some simple culture experiments should illuminate the degree to which environmental factors can influence pore size in the Type I and II populations.

We are uncertain how much of the physiological and stable isotopic differences observed between *G. siphonifera* Types I and II result from genetic differences between the host foraminifer cells versus infection by genetically different endosymbionts. Nor can we ascertain whether the intracellular differences between the Type I and II symbionts observed by Faber et al. (1988) result from growth of genetically identical symbiont cells in genetically different hosts, or if the symbionts themselves are in fact genetically different. DNA sequencing of the Type I and II symbionts (K. Darling et al.unpublished) should help to clarify this uncertainty. In an effort to determine host-symbiont specificity, we starved Type I and II foraminifers to rid them of their symbionts (by digestion or expulsion from the cytoplasm) and then tried reinfecting them with symbionts from the opposite host. However, these experiments failed since the host cells died before completely ridding themselves of their symbionts.

Breeding experiments on *G. siphonifera* Types I and II are presently not possible since gamete fusion in planktonic foraminifera has never been documented definitively in the laboratory environment. Nonetheless, accomplishments in culturing planktonic foraminifera have begun to clarify some of the sophisticated physiological control mechanisms that ensure regular completion of the reproductive process (Hemleben et al. 1989). A better understanding of the reproductive mechanisms can, in turn, provide some insight into the speciation process in planktonic foraminifera.

At the onset of gametogenesis, spinose species resorb and/or shed their spines and the shell thickens by the addition of a patchy or continuous layer of calcite. Addition of this layer affects only the outside of the shell and does not influence the size of the shell pores. Simultaneously, the parent cell undergoes very rapid nuclear division. Within 20 hours of the beginning of nuclear division gametogenesis occurs and the parent cell dies. It is hypothesized that gametes from two different parent cells fuse to form a zygote in an exclusively sexual reproductive cycle, but this has never been observed. Although the odds against gametes of the same species ever coming into contact in the open ocean seem great, planktonic foraminifera have developed several adaptive strategies that help maximize the probability of gamete fusion. These include (1) release of high numbers of gametes (e.g, ~300,000–400,000 per parent cell), (2) production of motile gametes that contain sufficient food reserves for prolonged locomotion, (3) synchronization of gamete release with distinct phases of the moon (Spindler et al. 1979; Bijma, Erez, and Hemleben 1990, Erez et al. 1991, and (4) establishment of a depth preference for reproduction (Bijma et al. 1994).

Speciation Process

The co-occurrence of living specimens of Types I and II that were collected at the same location and water depth in the tropical ocean invites the question how their common ancestral population could have become reproductively isolated. As passive inhabitants of their environment, planktonic foraminifera are distributed wherever water currents, turbulence, and other hydrodynamic events carry them. Limits to their distribution are generally controlled by watermass boundaries, vertical oceanic gradients, and the arrangement of climatic belts (Bé 1977). Mixing of surface waters occurs on a global scale over a relatively short (~1000 yr) time interval (Broecker 1974) and is most intense in high-energy regions of the ocean such as downwelling or upwelling zones where dispersal of individuals occurs over a considerable volume of water. Thus, the oceanographic and climatic barriers to gene flow among the oceanic plankton tend to be diffuse and the strength and distribution of those barriers change over geological, rather than ecological, time scales.

The timing of separation of the Type I and

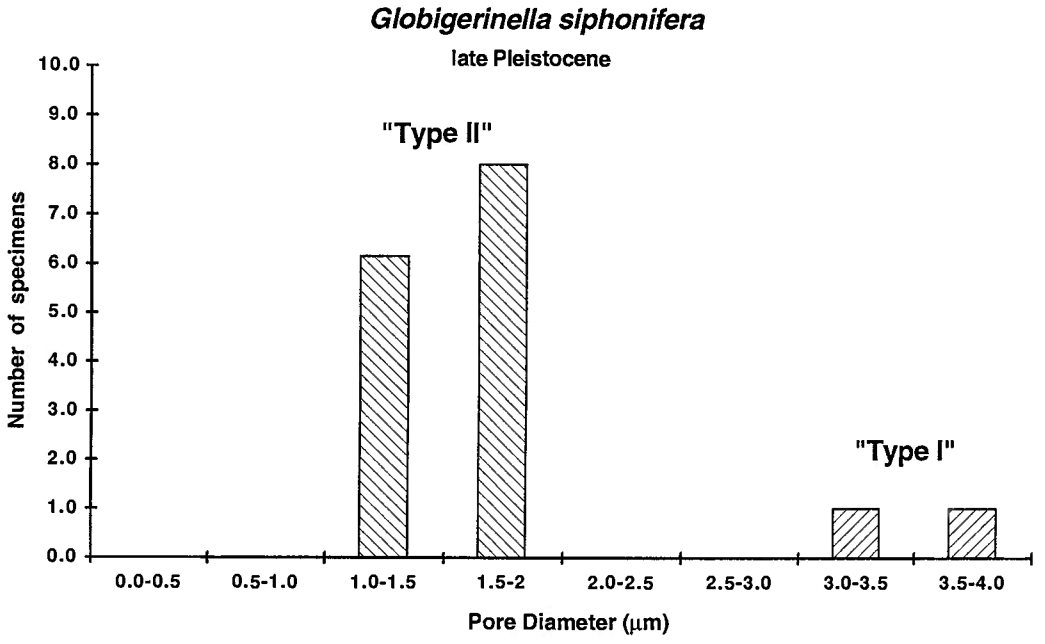


FIGURE 14. Pore diameters of Pleistocene specimens of *G. siphonifera* from a western Caribbean deep-sea sample (DSDP Sample 154A-1-1, 93–107 cm) obtained from SEM images of interior views of the final chambers from dissected adult specimens. Assignment to the Type I and II morphogroups is based only on comparative pore diameters among living populations.

II clades from their common ancestor is difficult to discern from the fossil record. Specimens of *G. siphonifera* from seafloor sediments have a more coarsely pustulose surface texture than SCUBA-collected specimens (Fig. 8), probably because the seafloor specimens added calcite to their shells just prior to gamete release (i.e., gametogenic calcification). As a result, differences in shell porosity can be determined only by dissecting adult chambers and measuring their porosity using from SEM images (note: the addition of gametogenic calcite is restricted to the outer chamber surfaces, and does not fill in the shell pores).

Hitherto, efforts to resolve when the Type I and II morphogroups diverged from their common *G. siphonifera* ancestor (which ranges back to the middle Miocene) have been limited to analysis of several deep-sea Pleistocene core samples from the Atlantic and Pacific sides of the Isthmus of Panama (DSDP Sites 154 and 155). A majority of the *G. siphonifera* specimens that have been picked thus far are tightly coiled and yield a unimodal distribution of pore diameters (averaging ~1.5 μm) and porosity values of less than 3%, which are consistent with the Type II morphology. However, one sample from the western Caribbean (DSDP Sample 154A-1-1, 93–107 cm) yields a bimodal distribution of pore diameters, with adult chamber pores of one group of specimens ranging from 1 to 2 μm and those of the other group ranging from 3 to 4 μm (Fig. 14). Measurement of shell porosity and tightness of coiling will need to be extended back to the middle Miocene, when *G. siphonifera* first appeared, to determine the timing of the Type I and II morphological divergence.

SCUBA-collection and identification of Types I and II have so far been done at relatively few localities, including marine stations in the Caribbean (e.g, Curaçao, Barbados, and Jamaica), northeast Australia, and Catalina Island (off southern California). By sequencing the DNA from specimens collected in plankton nets, we should be able to accurately identify whether Type I and II presently have a global distribution. Although downcore and global distributional information is limited, it is worth discussing which of the following speciation models is most likely to have contrib-

uted to the reproductive isolation of the Type I and Type II populations.

Alloparapatric Speciation.—Alloparapatric speciation occurs when populations of an ancestral species begin to differentiate after their geographic segregation, become sympatric over a limited area, and complete their divergence because of interactions between the differentiated populations in the zone of sympatry (Mayr 1942; Brooks and McLennan 1991). This model is more appropriately applied to the oceanic plankton than the allopatric model since no ocean basin is completely isolated by a continental barrier, and, therefore, differentiation must occur across a geographic cline.

One relatively recent tectonic event that has influenced biotic evolution in the Caribbean and eastern Pacific is the emergence of the Isthmus of Panama, which has been above sea level since about 3.5 Ma (Coates et al. 1992; Collins 1996). Clear evidence for allopatric speciation following this uplift has been obtained for a number of benthic marine groups (e.g., Woodring 1966; Herm 1969; Crouch and Poag 1979; Lessios 1981; Vermeij 1987; Cronin and Ikeya 1990). The rise of the isthmus may explain the different stratigraphic ranges of several planktonic foraminifer taxa on the Atlantic versus Pacific side beginning three to four million years ago (Parker 1973; Saito 1976; Keigwin 1982; Pearson 1995; Cronin and Dowsett 1996). Incongruence in these planktonic foraminifer distributions is probably related to changes in watermass conditions as well as to segregation of planktonic foraminifer gene pools following the isthmus uplift.

It is possible that populations of *G. siphonifera* became subdivided and reproductively isolated as the Isthmus of Panama emerged, resulting in the vicariant evolution of the Type I and II forms. The hypothesis that marine flooding has occurred across the isthmus after its initial uplift (Cronin and Dowsett 1996) could explain how the Type I and II populations merged following their initial segregation. This alloparapatric model would predict that the morphotypes initially varied clinally around the tropics, with an Atlantic-wide distribution of predominantly one genotype and an Indo-Pacific distribution of the other after 3.5 Ma. However, a majority of specimens observed from Miocene through Pleistocene deep sea cores from DSDP Sites 154 and 502 (on the Caribbean side of the isthmus) and Site 503 (on the Pacific side of the isthmus) reveal only one sample from the Pleistocene (154A-1-1, 93–107 cm) with two "Type I" specimens, which have pore diameters ranging from 3 to 4 μm. All other specimens have pore diameters of 2 μm or less, and are consistent with a "Type II" morphology. Investigation of more *G. siphonifera* from Miocene to Recent samples for shell porosity and coiling from these and many other deep sea sites will be required to adequately test the alloparapatric model globally.

Depth-Parapatric Speciation.—The pigment scan, oxygen isotope, and Mg/Ca ratio data cited above may indicate that Type II lives at a greater depth in the surface waters than Type I. SCUBA-collection of these two forms at the same depth could be explained if planktonic foraminifera are able to control their bouyancy for diurnal migration in the water column, as has been surmised by some authors (Berger 1969; Bé and Hamlin 1967; Holmes 1982). On the basis of seasonal and vertical sediment trap records, Deuser (1987) suggested that the preferred depth of reproduction for *G. siphonifera* is between 50 and 75 m. However, vertical segregation of Type I and II populations has gone unnoticed since these forms have only been discriminated among SCUBA-collected populations. If vertical segregation of Types I and II could be demonstrated in living populations, particularly at the time of gamete release, a depth-parapatric speciation model (sensu Lazarus 1983) may explain their speciation. Establishment of differences in depth habitats has been proposed as a possible mechanism for isolating the ancestral and descendent populations of the *Globorotalia crassaformis-Globorotalia truncatulinoides* lineage (Lazarus et al. 1995) and the fohsellid lineage (Norris et al. 1996), but much more detailed studies are needed to go beyond the present speculation.

Testing the depth parapatric model for Types I and II would first require daily plankton tows using vertically stratified nets over the course of several months at multiple lo-

cations to determine if the the two populations are reproductively isolated today. It would also require the abililty to differentiate Type I from Type II populations in the downcore record to determine the rate and timing of their differentiation. In short, "proving" depth parapatry in this group will be not be a simple task.

Sympatric Speciation.—Co-occurrence of adult Type I and II specimens in tropical surface waters today could indicate that cladogenesis of their common ancestor occurred without geographical or vertical segregation. The sympatric model has been cited as the most likely mode of speciation among the oceanic plankton (Lazarus 1983, 1986; Lazarus et al. 1995). Although this model is theoretically attractive, any mechanism proposed to lead to segregation of coexisting parent and daughter populations is hard to envisage in the open ocean environment.

One such mechanism is a divergence in the timing of the Type I and II reproductive cycle. Plankton tow and laboratory culture studies suggest that the reproductive cycles of spinose planktonic foraminifera are synchronized with distinct phases of the moon (Hemleben et al. 1989; Bijma, Erez, and Hemleben 1990; Bijma and Hemleben 1994). Most species are tuned to the full moon phase of the lunar cycle while some, including *G. siphonifera* (undifferentiated) have a secondary abundance peak tuned to the new moon phase (Bijma, Erez, and Hemleben 1990). It is not clear, however, if the semi-lunar component of the *G. siphonifera* reproductive cycle is consistent throughout the year or if it is geographically widespread, since the study of Bijma et al. (1990) spanned only one new moon phase in the Red Sea. Nor is it known which, if either, cycle is dominantly represented by Type I or II populations since no systematic effort has been made to discriminate these forms in a time series of plankton tows. Our qualitative observations from blue-water SCUBA dives in the Caribbean point toward lunar and semi-lunar segregation of reproductive cycles in the Type I and II populations, respectively, but these could easily have been biased by patchiness of population distributions in the surface waters.

Seasonal offsets of reproductive cycles could also have influenced segregation of the Type I and II genomes. Although seasonal changes in surface waters are currently not significant in most open ocean areas of the Tropics, this is not the case in tropical upwelling zones and this may not apply to the geological past. Seasonal cyclicity of planktonic foraminifer phenotypes has been demonstrated for *Neogloboquadrina pachyderma* from temperate regions (Reynolds and Thunell 1986), but living populations of these phenotypes have not been collected to determine if they differ genetically. Nonetheless, the presence of Type I and II forms throughout the year in areas where they have been identified would argue against seasonal offsets in their reproductive mode, and the high mixing rate in surface waters would likely overcome any seasonal isolating effects of an upwelling zone.

The sympatric speciation model requires not only observation that sister species are currently sympatric, but also that they were sympatric at the time of speciation (Brooks and McLennan 1991). As discussed previously, the fossil record of *G. siphonifera* so far has revealed specimens assignable only to the Type II form. Clearly a much more rigorous study of the deep-sea record will be required to test the possibility that cladogenesis occurred in sympatric populations of *G. siphonifera.*

Conclusions

Biological and geochemical evidence overwhelmingly suggest that *G. siphonifera* Types I and II are sister species, despite the morphological similarity of their empty shells. Biological justification for their separation includes (1) different arrangement of the rhizopodial network; (2) presence of chrysophycophyte endosymbionts with different cellular microstructures; (3) different responses to laboratory culture experiments; (4) greater amount of chlorophyll *a* pigment and higher carotenoid / chlorophyl *a* ratio in Type II symbionts; (5) presence of commensals in Type I specimens, but absence of commensals in Type II; (6) more positive oxygen and carbon isotopic composition and lower Mg / Ca ratios of Type II shells; and (7) significant genetic differences

determined from DNA sequencing of the SSU rRNA gene.

Most biometric data from SCUBA-collected shells of *G. siphonifera* indicate no significant differences between the Type I and II populations. Some subtle biometric differences that were identified include recognition that Type I adults (1) attain a larger test size; (2) have fewer kummerforn chambers; (3) have a greater frequency of sinistrally coiled forms; (4) are more evolutely coiled; and (5) show less coiling variability. On the other hand, shell porosity was found to be consistently different in the two forms, with Type I having a much greater porosity than Type II. But determination of porosity differences among specimens recovered from the seafloor requires SEM observation of dissected shells and detailed wall ultrastructure study since gametogenic calcification obscures observation of primary shell porosity in external view. This has prevented our determination whether or not the holotypes of *Globigerina siphonifera* d'Orbigny or *Globigerina aequilateralis* Brady (a junior synonym of *G. siphonifera*) are assignable to Type I or II since both were collected from sediments. In addition, the differences in shell porosity and shell biometry would not be considered sufficient to justify species-level distinction using a traditional "paleontological" approach to planktonic foraminifer taxonomy. We hesitate to name new species to accommodate Types I and II until we have a better understanding of the genetic versus ecologic control of their shell porosity, and until we have investigated the holotypes of *G. siphonifera* and *G. aequilateralis* further.

Our inability to distinguish Type I from Type II shells suggests that speciation in *G. siphonifera* is cryptic. Cryptic speciation has also been proposed for the *Fohsella* lineage of Neogene planktonic foraminifera on the basis of oxygen isotopic evidence for changes in reproductive ecology in the absence of morphological change (Norris et al. 1996). The results obtained from our study of *G. siphonifera* suggest that the genes that control nearly all aspects of shell morphology, at least in these instances, are conservative compared to the genes that control cellular physiology and metabolism. This implies that foraminifer shells are highly adapted to their ambient environment, such that there is no advantage to changing shell architecture when a shift in depth ecology occurs. This has important implications for foraminifer taxonomy, particularly regarding our understanding of the rates of evolution, survivorship, and species diversity of planktonic foraminifera.

Speciation mechanisms that could lead to segregation of once genetically homogeneous populations of planktonic foraminifera are presently poorly understood. The allopatric model of Mayr (1963, 1970) is an unlikely mode of speciation in oceanic microplankton since reproductive barriers separating small, isolate populations could not exist long enough in the open ocean to result in speciation (Lazarus 1983; Lazarus et al. 1995). The modern distribution pattern of *G. siphonifera* is compatible with the depth parapatric (Lazarus 1986) and sympatric (Maynard Smith 1966) speciation models, but development of discontinuities in the surface-water environment or offsets in the reproductive cycle seem insufficient to isolate the gene pools of organisms living in the surface mixed layer of the open ocean. Also viable is the alloparapatric speciation model (Mayr 1942), which may apply if the timing of the emergence of the Isthmus of Panama is proven to correlate with the divergence of the *G. siphonifera* phenotypes. Systematic downcore study of Neogene sediments from both sides of the Panama Isthmus and elsewhere in the Tropics should help constrain the timing and distribution history of the Type I and II taxa. Improved knowledge of the reproductive biology of planktonic foraminifera will also be critical to an enhanced understanding of speciation mechanisms in planktonic foraminifera.

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Literature Cited

Banner, F. T., and W. H. Blow. 1960. The taxonomy, morphology, and affinities of the genera included in the subfamily Hastigerininae. Micropaleontology 6:19–31.

Bé, A. W. H. 1968. Shell porosity of recent planktonic foraminifera as a climatic index. Science 161:881–884.

———. 1977. An ecological, zoogeographic and taxonomic review of recent planktonic foraminifera. Pp. 1–100 *in* A. T. S. Ramsay, ed. Oceanic micropaleontology, 1. Academic Press, London.

———. 1980. Gametogenic calcification in a spinose planktonic foraminifer *Globigerinoides saccullifer* (Brady). Marine Micropaleontology 5:283–310.

Bé, A. W. H., and W. H. Hamlin. 1967. Ecology of recent planktonic foraminifera, Part 3, Distribution in the North Atlantic during the summer of 1962. Micropaleontology 13:87–106.

Bé, A. W. H., O. R. Anderson, and D. A. Caron. 1983. Sequence of morphological and cytoplasmic changes during gametogenesis in the planktonic foraminifer *Globigerinoides sacculifer* (Brady). Micropaleontology 29:1–5.

Berger, W. H. 1969. Planktonic foraminifera: Basic morphology and ecologic implications. Journal of Paleontology 43:1369–1383.

Bijma J., and C. Hemleben. 1994. Population dynamics of the planktonic foraminifer Globigerinoides sacculifer (Brady) from the central red sea, Part I, Deep-sea research. Oceanographic Research Papers 41: 485–510.

Bijma, J., J. Erez, and C. Hemleben. 1990. Lunar and semi-lunar reproductive cycles in some spinose planktonic foraminifers. Journal of Foraminiferal Research 20:117–127.

Bijma, J., W. W. Faber Jr., and C. Hemleben. 1990. Temperature and salinity limits for growth and survival of some planktonic foraminifers in laboratory cultures. Journal of Foraminiferal Research 20:95–116.

Brady, H. B. 1879. Notes on some of the reticularian Rhizopoda of the "*Challenger*" expedition. 2. Additions to the knowledge of porcellaneous and hyaline types. Quarterly Journal of Microscopical Science. New Series 19:261–299.

Broecker, W. S. 1974. Chemical oceanography. Harcourt Brace Jovanovitch, New York.

Brooks, D. R., and D. A. Mclennan. 1991. Phylogeny, ecology, and behavior. University of Chicago Press, Chicago.

Brummer, G. J. A., C. Hemleben, and M. Spindler. 1986. Planktonic foraminiferal ontogeny and new perspectives for micropaleontology. Nature. 319:50–52.

———. 1987. Ontogeny of extant spinose planktonic foraminifera (Globigerinidae): A concept exemplified by *Globigerinoides sacculifer* (Brady) and *G. ruber* (d'Orbigny). Marine Micropaleontology 12:357–381.

Coates, A. G., J. B. C. Jackson, L. S. Collins, T. M. Cronin, H. J. Dowsett, L. M. Bybell, P. Jung, and J. A. Obando. 1992. Closure of the Isthmus of Panama: the nearshore marine record in Costa Rica and western Panama. Geological Society of America Bulletin 104:814–828.

Collins, L. S. 1996. Environmental changes in Caribbean shallow waters relative to the closing tropicl American seaway. Pp. 130–167 *in* J. B. C. Jackson, A. C. Coates, and A. Budd, eds. Environmental and biotic change in the Neogene of Central America. University of Chicago Press, Chicago.

Cronin, T. M., and H. J. Dowsett. 1996. Biotic and oceanographic response to the Pliocene closing of the Central American Isthmus. Pp. 76–104 *in* J. B. C. Jackson, A. C. Coates, and A. Budd, eds. Environmental and biotic change in the Neogene of Central America. University of Chicago Press, Chicago.

Cronin, T. M., and N. Ikeya. 1990. Tectonic events and climatic change: opportunities for speciation in Cenozoic marine Ostracoda. Pp. 210–248 *in* R. M. Ross and W. D. Allmon, eds. Causes of evolution: a paleontological perspective. University of Chicago Press, Chicago.

Crouch, R. W., and C. W. Poag. 1979. *Amphistegina gibbosa* d'Orbigny from the California borderlands: the Caribbean connection. Journal of Foraminiferal Research 9:85–105.

Darling, K. F, D. Kroon, C. M. Wade, and A. J. Leigh Brown. 1996a. The isolation and amplification of the 18S ribosomal RNA gene from planktonic foraminifers using gametogenic specimens. Pp. 249–259 in: A. Moguilevsky and R.C. Whatley, eds. Microfossils and oceanic environments. Proceedings of the International Conference on ODP and the Marine Biosphere, Aberystwyth, April 19–21 1994. University of Wales, Aberystwyth Press.

———. 1996b. Molecular phylogeny of the planktonic foraminifera. Journal of Foraminiferal Research 26:324–330.

———. In press. Planktonic foraminiferal evolution and the polyphyletic nature of their benthic origin. Marine Micropaleontology.

Deuser, W. G. 1987. Seasonal variations in isotopic compositon and deep-water fluxes of the tests of perennially abundant planktonic foraminifera of the Sargasso Sea: results from sediment-trap collections and their paleoceanographic significance. Journal of Foraminiferal Research 17:14–27.

Erez, J., A. Almogi-Labin, and S. Avraham. 1991. On the life history of planktonic foraminifera: lunar reproduction cycle in *Globigerinoides sacculifer* (Brady). Paleoceanography 6:295–306.

Faber, W. W., Jr., O. R. Anderson, J. L. Lindsey, and D. A. Caron. 1988. Algal-foraminiferal symbiosis in the planktonic foraminifer *Globigerinella aequilateralis*: I. Occurrence and stability of two mutually exclusive chrysophyte endosymbionts and their ultrastructure. Journal of Foraminiferal Research 18: 334–343.

Faber, W. W., Jr., O. R. Anderson, and D. A. Caron. 1989. Algal-foraminiferal symbiosis in the planktonic foraminifer *Globigerinella aequilateralis*: II. Effects of two symbiont species on foraminiferal growth and longevity. Journal of Foraminiferal Research 19:185–193.

Fairbanks, R. G., C. D. Charles, and J. D. Wright. 1992. Origin

of global meltwater pulses. Pp. 473–500 *in* R. E. Taylor, A. Long, and R. S. Kra, eds. Radiocarbon after four decades: an interdisciplinary perspective. Springer, New York.

Frerichs, W. E., M. E. Heiman, L. E. Borgman, and A. W. H. Bé. 1972. Latitudinal variations in planktonic foraminiferal test porosity, Part 1, Optical studies. Journal of Foraminiferal Research 2:6–13.

Hemleben, C., M. Spindler, I. Breitinger, and R. Ott. 1987. Morphological and physiological responses of *Globigerinoides sacculifer* (Brady) under varying laboratory conditions. Marine Micropaleontology 12:305–324.

Hemleben, C., M. Spindler, and O. R. Anderson. 1989. Modern planktonic foraminifera. Springer, New York.

Herm, D. 1969. Marines Pliozän und Pleistozän in Nord-und Mittel-Chile unter besonderer Berücksichtigung der Entwichlung der Mollusken-Faunen. Zitteliana 2:3–158.

Hodell, D. A., and A. Vayavananda. 1993. Middle Miocene paleoceanography of the western equatorial Pacific (DSDP Site 289) and the evolution of *Globorotalia* (*Fohsella*). Marine Micropaleontology 22:279–310.

Holmes, N. A. 1982. Diel vertical variations in abundance of some planktonic foraminifera from the Rockall Trough, northeastern Atlantic Ocean. Journal of Foraminiferal Research 12:145–150.

Huber, B. T. 1994. Ontogenetic morphometrics of some Late Cretaceous trochospiral planktonic foraminifera from the Austral Realm. Smithsonian Institution Contributions to Paleobiology No. 77.

Huber, B. T., and A. Boersma. 1994. Cretaceous origination of *Zeauvigerina* and its relationship to Paleocene biserial planktonic foraminifera. Journal of Foraminiferal Research 24:268–287.

Kahn, M. I. 1979. Non-equilibrium oxygen and carbon isotope fractionation in tests of living planktonic foraminifera. Oceanologica Acta 2:195–208.

Keigwin, L. D., Jr. 1982. Neogene planktonic foraminifers from Deep Sea Drilling Project Sites 502 and 503. In: W. L. Prell, J. V. Gardner, et al., eds. Initial Reports of the Deep Sea Drilling Project 68:269–288. U.S. Government Printing Office, Washington, D.C.

Kennett, J. P., and M. S. Srinivasan. 1983. Neogene planktonic foraminifera, a phylogenetic atlas. Hutchinson Ross, Stroudsburg, Penn.

Knowlton, N. 1993. Sibling species in the sea. Annual Review of Ecological Systematics 24:189–216.

Kroopnik, P. 1974. The dissolved O_2-CO_2-^{13}C system in the eastern equatorial Pacific. Deep Sea Research 21:211–277.

Lazarus, D. 1983. Speciation in pelagic Protista and its study in the planktonic microfossil record: a review. Paleobiology 9: 327–340.

———. 1986. Tempo and mode of morphologic evolution near the origin of the radiolarian lineage *Pterocanium prismatium*. Paleobiology 12:175–189.

Lazarus, D., H. Hillbrecht, C. Spencer-Cervato, and H. Thierstein. 1995. Sympatric speciation and phyletic change in *Globorotalia truncatulinoides*. Paleobiology 21:28–51.

Leigh Brown, A. J., and P. Simmonds. 1994. Analysis of HIV sequence variation. Pp. 161–188 *in*: J. Karn, ed. HIV—A practical approach. Oxford University Press, Oxford.

Lessios, H. A. 1981. Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the Isthmus of Panama. Evolution 35:618–634.

Maidak, B. L., N. Larsen, M. J. Mccaughey, R. Overbeek, G. J. Olson, K. Fogel, J. Blandy, and C. R. Woese. 1994. The ribosomal database project. Nucleic Acids Research 22:3484–3487.

Malmgren, B. A., W. A. Berggren, and G. P. Lohmann. 1983. Evidence for punctuated gradualism in the late Neogene *Globorotalia tumida* lineage of planktonic foraminifera. Paleobiology 9:377–389.

Maynard Smith, J. 1966. Sympatric speciation. American Naturalist 100:637–650.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

Norris, R. D., R. M. Corfield, and J. E. Cartlidge. 1993. Evolution of depth ecology in the planktonic foraminifera lineage *Globorotalia (Fohsella)*. Geology 21:975–978.

———. 1996. What is gradualism? Cryptic speciation in gradually evolving globorotaliid foraminifera. Paleobiology 22: 386–405.

Nürnberg, D. 1995. Magnesium in tests of *Neogloboquadrina pachyderma* sinistral from high northern and southern latitudes. Journal of Foraminiferal Research 25:350–368.

Nürnberg, D., J. Bijma, and C. Hemleben. 1996a. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. Geochimica Cosmochimica Acta 60: 803–814.

———. 1996b. Erratum on Nürnberg et al. 1996. Geochimica Cosmochimica Acta 60:2483–2484.

O'Neil, C. H. 1969. Oxygen isotope fractionation between CO_2 and waters, and the isotopic composition of marine atmospheric CO_2. Earth and Planetary Science Letters 5:285–295.

Olsson, R. K. 1973. Pleistocene history of *Globigerina pachyderma* (Ehrenberg) in Site 36, DSDP, northeastern Pacific. American Association of Petroleum Geology Bulletin 57:798 [Abstract].

Orbigny, A. D. 1839. Foraminifères. Pp. 1–224 *in* R. D. L. Sagra, ed. Histoire physique, politique et naturelle de l'île de Cuba. A. Bertrand, Paris.

Parker, F. L. 1962. Planktonic foraminiferal species in Pacific sediments. Micropaleontology 8:219–254.

———. 1973. Late Cenozoic biostratigraphy (planktonic foraminifera) of tropical Atlantic deep-sea sections. Revista Española Micropaleontologa 5:253–289.

Pearson, P. N. 1993. A lineage phylogeny for the Paleogene planktonic foraminifera. Micropaleontology 39:193–232.

———. 1995. Planktonic foraminifer biostratigraphy and the development of pelagic caps on guyots in the Marshall Islands group. *In* J. A. Haggerty, I. Premoli Silva, F. Rack, and M. K. McNutt, eds. Proceedings of the Ocean Drilling Program, Scientific Results 144:21–59. Ocean Drilling Program, College Station, Tex.

Pearson, P. N., and W. P. Chaisson. In press. Late Paleocene to middle Miocene planktonic foraminifer biostratigraphy, Ceara Rise. Proceedings of the Ocean Drilling Program, Scientific Results 154. Ocean Drilling Program, College Station, Tex.

Pearson, P. N., and N. J. Shackleton. 1995. Neogene multispecies planktonic foraminifer stable isotope record, Site 871, Limalok Guyot. *In* J. A. Haggerty, I. Premoli Silva, F. Rack, and M. K. McNutt, eds. Proceedings of the Ocean Drilling Program, Scientific Results 144: 401–410. Ocean Drilling Program, College Station, TX.

Reynolds, L. A., and R. C. Thunell. 1986. Seasonal production and morphologic variation of *Neogloboquadrina pachyderma* (Ehrenberg) in the northeast Pacific. Micropaleontology. 32: 1–18.

Saito, T. 1976. Geologic significance of coiling direction in the planktonic foraminifera *Pulleniatina*. Geology 4:305–309.

Shackleton, N. J., and E. Vincent. 1978. Oxygen and carbon isotope studies in Recent foraminifera from the southeast Indian Ocean. Marine Micropaleontology 3:1–13.

Spero, H. J., and D. J. Williams. 1989. Opening the carbon isotope "vital effect" black box 1. Seasonal temperatures in the euphotic zone. Paleoceanography 4:593–601.

Spindler M., O. R. Anderson, C. Hemleben, and A. W. H. Bé. 1978. Light and electron microscopic observations of game-

togenesis in *Hastigerina* pelagica (Foraminifera). Journal of Protozoology 25:427–433.

Tabachnick, R. E., and F. L. Bookstein. 1990. The structure of individual variation in Miocene *Globorotalia*. Evolution 44: 416–434.

Tendal, O. 1990. Why are Foraminiferida foraminifers? *In* C. Hemleben, M. A. Kaminski, W. Kuhnt, and D. B. Scott, eds. Paleocology, biostratigraphy, paleoceanography and taxonomy of agglutinated foraminifera. NATO ASI Series C 327:13–18. Kluwer, Boston.

Van Eijden, A. J. M. 1995. Morphology and relative frequency of planktonic foraminiferal species in relation to oxygen isotopically inferred depth habitats. Palaeogeography, Palaeoclimatology, Palaeoecology 113:267–301.

Vermeij, G. J. 1987. Evolution and escalation: an ecological history of life. Princeton University Press, Princeton, N.J.

Wade, C. M., K. F. Darling, D. Kroon, and A. J. Leigh Brown. In press. Early evolutionary origin of the planktonic foraminifera inferred from SSU rDNA sequence comparisons. Journal of Molecular Evolution 43.

Wei, K.-Y. 1987. Multivariate morphometric differentiation of chronospecies in the late Neogene planktonic foraminiferal lineage *Globoconella*. Marine Micropaleontology 12:183–202.

———. 1994. Allometric heterochrony in the Pliocene–Pleistocene planktonic foraminiferal clade *Globoconella*. Paleobiology 20:66–84.

Wei, K.-Y., and J. P. Kennett. 1988. Phyletic gradualism and punctuated equilibrium in the late Neogene planktonic foraminiferal clade *Globoconella*. Paleobiology 14:345–363.

Wiles, W. W. 1967. Pleistocene changes in the pore concentration of a planktonic foraminiferal species from the Pacific Ocean. Progress in Oceanography 4:153–160.

Woodring, W. P. 1966. The Panama landbridge as a sea barrier. Proceedings of the American Philosophical Society 110:425–433.