JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 111, G03002, doi:10.1029/2005JG000158, 2006

An endobiont-bearing allogromiid from the Santa Barbara Basin: Implications for the early diversification of foraminifera

Joan M. Bernhard,[1] Andrea Habura,[2,3] and Samuel S. Bowser[2,3]

Received 31 December 2005; revised 24 March 2006; accepted 5 April 2006; published 19 July 2006.

[1] Our current understanding of paleoecology and paleoceanography is largely based on the superb Phanerozoic fossil record of foraminiferan protists. The early history of the group is unresolved, however, because basal foraminiferans (allogromiids) are unmineralized and thus fossilize poorly. Molecular-clock studies date foraminiferal origins to the Neoproterozoic, but the deep sea, one of Earth's most extensive habitats and presently hosting a significant fraction of basal foraminiferal diversity, was probably anoxic at that time and, until now, anaerobic allogromiids were unknown. Molecular, cell, and ecological analyses reveal the presence of a previously unknown allogromiid inhabiting anoxic, sulfidic deep-sea sediments (Santa Barbara Basin, California). The fact that the new foraminifer harbors prokaryotic endobionts implicates symbiogenesis as a driving force in early foraminiferal diversification.

Citation: Bernhard, J. M., A. Habura, and S. S. Bowser (2006), An endobiont-bearing allogromiid from the Santa Barbara Basin: Implications for the early diversification of foraminifera, *J. Geophys. Res.*, *111*, G03002, doi:10.1029/2005JG000158.

1. Introduction

[2] Many inferences concerning the evolution of early eukaryotes are based on molecular phylogenetic analyses of modern protists. The Rhizaria, a major protistan ''supergroup'' defined by molecular criteria [*Nikolaev et al.*, 2004; *Cavalier-Smith*, 2004], are of particular interest because certain members leave microfossils that can be used for micropaleontologic calibration of molecular clocks. These rhizarian protists therefore offer an unusual opportunity to use multiple lines of evidence to evaluate hypotheses regarding the diversification of eukaryotes during the Neoproterozoic.

[3] The Foraminifera are a major microfossil-generating group of rhizarians [*Adl et al.*, 2005], characterized by branching and anastomosing granular pseudopodia (i.e., reticulopods [*Bowser and Travis*, 2002]). Although the best-known foraminiferans are those with hard, multichambered calcareous or agglutinated shells (tests [*Sen Gupta*, 1999]), the basal, so-called ''allogromiid'' foraminiferans are naked or single-chambered thecate or agglutinated forms with a sparse-to-nonexistent fossil record. In lieu of fossil evidence, molecular-clock analyses reveal that these basal Foraminifera originated between ~0.7 and 1.2 Gya [*Pawlowski et al.*, 2003]. Unfortunately, the molecular data set used to infer the timing for foraminiferal origins could be considered biased, because it relies heavily on species inhabiting high-latitude settings. A more accurate molecular clock estimate may result with inclusion of additional (nonpolar) species [e.g., *Cranston and Rannala*, 2005]. A concentrated effort to identify allogromiids from nonpolar settings and to obtain their molecular-sequence data are, therefore, important aims of contemporary research.

[4] Modern allogromiids are known to occur in most aquatic environments, ranging from moist terrestrial soils to hadal trenches [*Gooday*, 2002]. Allogromiids are not known, however, to inhabit anoxic (i.e., lacking detectable oxygen), sulfidic (i.e., enriched in hydrogen sulfide) environments such as those thought to be widespread in the Neoproterozoic deep sea [e.g., *Canfield*, 1998; *Condie et al.*, 2001]. As allogromiids are considered to be the earliest evolving foraminiferans, the presumed absence of modern species in anoxic, sulfidic environments would seem to argue against an early origin of the group. A recent study, however, reported a single allogromiid specimen was collected from the anoxic zone of the Black Sea [*Gooday et al.*, 2006]. Unfortunately, this study lacked evidence that the specimen was indeed living at the time of collection and had not been recently transported into the anoxic zone. From a different perspective, studies have shown that the relatively fragile and small allogromiids are often overlooked in conventional ecological and micropaleontological screens of environmental samples [*Gooday*, 2002]. In environmental DNA screens, as many as 75% of the foraminiferal phylotypes detected are previously unidentified allogromiid taxa [*Habura et al.*, 2004]. It is therefore possible that allogromiids inhabit anoxic settings, but their presence has not yet been unequivocally documented.

[5] Here we use ecological, cellular and molecular approaches to identify an allogromiid inhabiting anoxic deep-sea sediments in the Santa Barbara Basin (SBB). Ultrastructural analyses demonstrate the presence of bacte-

[1]Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA.
[2]Wadsworth Center, New York State Department of Health, Albany, New York, USA.
[3]Also at Department of Biomedical Sciences, University at Albany, State University of New York, Albany, New York, USA.

0148-0227/06/2005JG000158$09.00

rial endobionts in the foraminifer, which implicates endosymbiogenesis as a driving force for the diversification of early Foraminifera.

2. Materials and Methods

2.1. Site Description and Water Column Sampling

[6] Samples were collected from the Santa Barbara Basin (SBB; 34°13.50N, 120°02.22W) on 3–4 October 2002 from 594-m water depth. The SBB is a silled seafloor depression off southern California (USA; eastern Pacific), which has a water column characterized by severe oxygen depletion. Presently, sediment deposition in the basin is manifest as laminations [e.g., *Thunell et al.*, 1995; *Bernhard et al.*, 2003], although, in the geologic past, laminites are interceded by bioturbated deposits [e.g., *Behl and Kennett*, 1996]. Dissolved oxygen concentration was determined using the microwinkler method (lower detection limit = ~0.1 μM O_2 [*Broenkow and Cline*, 1969]) on water collected ~20 m above the seafloor.

2.2. Sediment Sampling, Molecular and Microscopic Analyses

[7] Sediments were collected via Soutar box corer and processed in one of three ways. Samples from the surface centimeter and an interval ~10 cm below the sediment-water interface were preserved in 3 volumes of 95% ethanol. Total-sediment DNA was purified and tested for the presence of foraminiferal genomic DNA [*Habura et al.*, 2004]. Samples from both sediment horizons contained DNA, but only the surface sample contained template amplifiable with foraminifer-specific primers. Individual foraminiferal small subunit ribosomal RNA gene (SSU rDNA) sequences were obtained by cloning and sequencing as described by *Habura et al.* [2004]. A set of small subunit ribosomal RNA gene (SSU rDNA) sequences, representing all major foraminiferal phylogenetic groups [*Pawlowski et al.*, 2002] and two nonforaminiferans as an outgroup, was used to place the phylotypes. Sequences were aligned using CLUSTAL W [*Thompson et al.*, 1994]. Alignments were adjusted manually in SEAVIEW [*Galtier et al.*, 1996] to accommodate regions of variable length in the SSU consensus structures (see *Habura et al.* [2004] for a review). All unambiguously aligned positions were retained for phylogenetic analysis. Alignments are available for viewing at www.bowserlab.org/supplemental. For maximum likelihood (ML) analysis, the appropriate model of nucleotide substitution was selected via likelihood ratio test, as implemented in Modeltest 3.06 [*Posada and Crandall*, 1998]. A TVM + I + Γ model was selected as best for the data sets. The indicated substitution model was used to obtain ML trees using the method implemented in PAUP* 4b10 [*Swofford*, 2003], with tree bisection and reconnection, and 100 bootstrap replicates. Maximum parsimony (MP) and neighbor-joining analyses were also performed using PAUP*, with tree bisection and reconnection, and 1000 replicates.

[8] Syringe subcores (1.5-cm inner diameter), which were placed before other sediment collections were removed, were processed for life-position analyses using the Fluorescently Labeled Embedded Core (FLEC) method [*Bernhard et al.*, 2003]. FLEC material was examined with an Olympus Fluoview laser scanning confocal microscope (LSCM).

[9] Other sediments were fixed in 3% glutaraldehyde (final concentration) buffered with 0.1 M Na-cacodylate (pH 7.2), from which specimens were isolated from the coarse residue after sieving with buffer over a 63-μm screen. Specimens were photographed using a Nikon Optiphot; some were processed for Scanning and Transmission Electron Microscopy (SEM and TEM, respectively) following our standard procedures [*Bernhard et al.*, 2000] with slight modification for TEM (i.e., an additional incubation in 2% OsO_4 for 4 hours). Sections were examined with a Zeiss EM910, Zeiss EM902A, and the Albany High Voltage TEM. SEM and X-ray microanalysis were conducted using a LEO 1550 VP SEM.

3. Results

[10] Water samples from two CTD bottle casts indicate that dissolved oxygen was undetectable in bottom waters at the time of sampling, and when both box cores taken from the site emerged above the sea surface, there was an unusually strong and immediate smell of hydrogen sulfide. Unfortunately, data are not available on the pore water hydrogen sulfide concentrations at the time of collection, but they certainly exceeded those reported for the same site at a different time (~50 μM at 1 cm depth [*Bernhard et al.*, 2003]).

[11] Replicate core surfaces were heavily veiled with the filamentous bacterium *Beggiatoa* (Figure 1), a sulfide oxidizer that can use nitrate rather than oxygen as its final electron acceptor [*Mußmann et al.*, 2003]. Although surface sediment visible between *Beggiatoa* tufts was dark brown, subcores revealed that this remnant oxidized layer was <1 mm thick; deposits below were black, indicating anoxic conditions beneath that horizon.

[12] PCR screening of environmental DNA from the top 1-cm SBB sediment interval identified numerous clones (n = 18) of a phylotype corresponding to the rotaliid *Nonionella stella* (Figure 2; deposited as Genbank accession number AY818727). Surprisingly, we also identified a second, unknown foraminiferal sequence (n = 2 identical sequences). The new phylotype, deposited as Genbank accession number AY818728, grouped consistently with others assigned to the basal foraminiferan lineage "Clade L" (Figure 2), a geographically widespread group of allogromiid foraminifers characterized by a finely agglutinated, ovoid to oblong single-aperture test [*Pawlowski et al.*, 2002]. A probe of the sample with a panel of PCR primers specific for individual clades of allogromiids also identified signal only from Clade L (not shown). Because the discovery of the endobiont-bearing allogromiid was unexpected, specimens were not available for FISH or immunocytochemistry.

[13] Careful wet picking of sediments revealed the presence of only two live foraminiferal morphologies: *Nonionella stella* and a small (230–610 μm; average = 438 μm, n = 14), white, ovoid-to-oblong allogromiid (Figures 3a and 3b). All specimens examined by light microscopy had a single, large nucleus (Figure 3d) and were covered by a ~2-μm-thick, finely agglutinated test (shell) with a single terminal aperture (Figure 3c). Electron

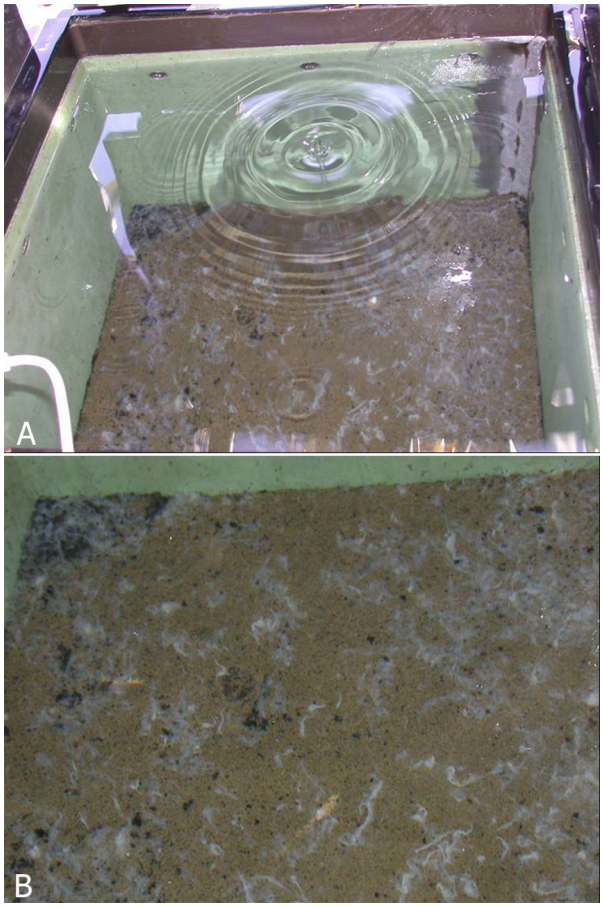

Figure 1. Photographs of a representative box core collected at the site showing the seafloor surface veiled with white filaments of *Beggiatoa*. Box core side is ~33 cm.

microscopy and X-ray microanalysis revealed that the wall is vested with aluminosilicate platelets (Figure 4) underlain by a granulofibrillar meshwork (Figure 5b). These combined characters distinguish the new morphospecies from other known allogromiids.

[14] Transmission electron microscopy revealed endomembrane features typical of other allogromiid foraminiferans, such as a well-developed Golgi system (Figures 5c and 5d) and numerous large (>10 μm diameter) vacuoles (Figures 3c, 3d, 5a, and 6), as well as the presence of prokaryotic endobionts (Figure 5e). The vacuoles did not contain phagocytosed material. Food vacuoles, which are typical in most other foraminifers (e.g., *Goldstein and Corliss* [1994], reviewed by *Anderson and Lee* [1991]), do not appear in the cytoplasm of the SBB allogromiid. The endobionts appeared adjacent to vacuoles but not at the allogromiid periphery (Figure 6) and displayed structures suggestive of elemental sulfur inclusions [*Vetter*, 1985; *Krieger et al.*, 2000; *Dubilier et al.*, 2001; *Pasteris et al.*, 2001; *Pflugfelder et al.*, 2005]. A count of 256 endobionts from 30 TEM negatives indicates that only two (<0.8%) were deemed visibly degraded. None of these micrographs showed evidence for phagocytosed material or other matter associated with the endobionts. Mitochondria with tubulovesicular cristae, occasionally grouped in large clusters (Figure 5f), are found throughout the cytoplasm. Allogromiids with intact mitochondria were also collected from a sediment depth of 2–3 cm (where anoxia certainly prevailed).

[15] The allogromiid was common in the surface centimeter (17.3–22.0 individuals cm^{-3}; n = 2 cores) and occurred in substantial densities to ≥2 cm depth (6.8 specimens cm^{-3} in the 1- to 2-cm layer; 3.4 specimens cm^{-3} in the 2- to 3-cm layer). In situ labeling with the fluorogenic probe CellTracker™ Green CMFDA [*Bernhard et al.*, 2003] demonstrated that the allogromiids were living down core in the anoxic zone (Figure 7), rather than in a surface veneer that could be interpreted as having trace concentrations of oxygen.

4. Discussion

[16] The SBB eukaryotic assemblage present when oxygen is detectable in bottom waters was described previously [e.g., *Bernhard et al.*, 2000, 2003]; this is the first documentation of the SBB benthos during an anoxic episode. Although one allogromiid specimen was recently reported from an anoxic portion of the Black Sea [*Gooday et al.*, 2006], it remains to be demonstrated that it was living there. In addition, to date, allogromiids are occasionally found in microxic (i.e., having trace oxygen concentrations [*Gooday et al.*, 2000; *Bernhard and Buck*, 2004]) sediments, which are sometimes enriched in hydrogen sulfide [*Buck and Barry*, 1998; *Bernhard et al.*, 2000] and certain allogromiids survive experimentally induced anoxia [*Moodley et al.*, 1997]. This is the first documentation of allogromiid foraminifers living in a naturally anoxic setting. Because we demonstrate the presence of intact Golgi and mitochondria, as well as the positive label imparted by the esterase fluorogenic probe CellTracker Green CMFDA, it is certain that the allogromiids were living in SBB at the time of collection, i.e., while subject to anoxia. It is assured that the allogromiids were not transported from oxygenated upslope habitats because they were found living in laminated sediments as deep as 3 cm, which equates to ~4 years post deposition (assuming 0.75 cm/yr sedimentation rate before compaction [*Reimers et al.*, 1990]). In other words, the allogromiids exist at those depths as opposed to being transported and buried by mass sedimentation events such as turbidity flows.

[17] Molecular and cell biological analyses independently indicate that the foraminifer we illustrate here is an allogromiid. The morphology of the SBB allogromiid is similar to two other Clade L allogromiids (*Ovammina opaca* [*Dahlgren*, 1962, 1967]; *Cribrothalammina alba* [e.g., *Goldstein and Barker*, 1990]) and also the allogromiid *Vellaria* [*Gooday and Fernando*, 1992]. The SBB allogromiid is longer and more slender, has a thinner test wall, and has better developed cytoplasmic vacuoles than does *O. opaca*. The SBB allogromiid test has similar attributes to that of *O. opaca* and *C. alba* during gametogenesis. More specifically, both *O. opaca* and *C. alba* form test pores as an avenue for gamete release [*Dahlgren*, 1964; *Goldstein and Barker*, 1990]; we saw no evidence of gametogenesis other than pore formation (Figure 4a) in the SBB allogromiid, however. These morphologically similar allogromiid taxa are described from shallow environments (e.g., off Sweden;

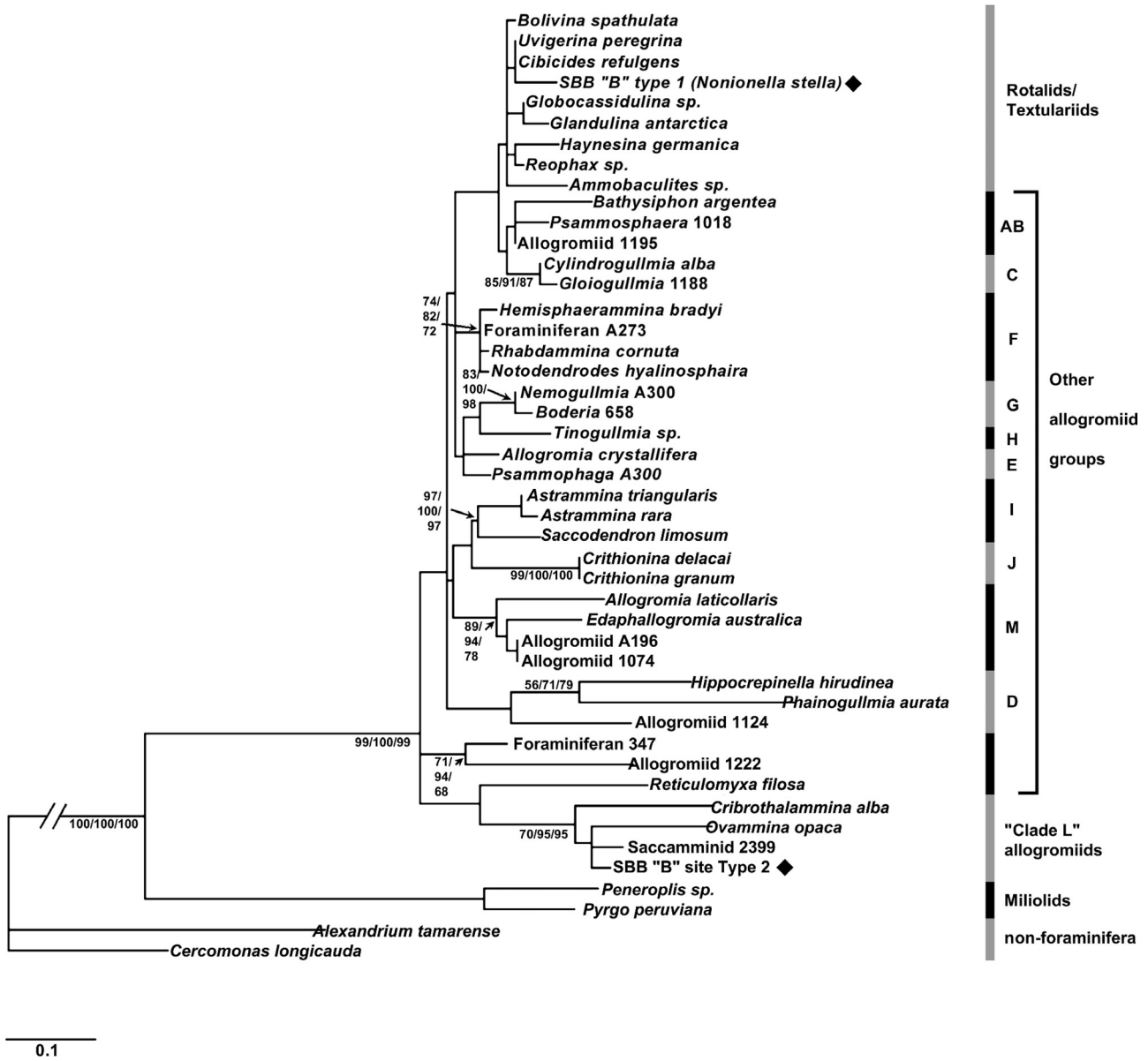

Figure 2. Phylogenetic placement of SBB anoxic-site environmental clones. A set of partial SSU rDNA sequences representing all known foraminiferal groups was used to place the novel sequences (diamonds) in context. One molecular phylotype (GenBank AY818727), identified as *Nonionella stella*, groups within the clade of rotaliids/textulariids. The other (AY818728) groups with phylotypes assigned to "Clade L," a geographically widespread group of allogromiid foraminifera. Maximum-likelihood (ML) tree is shown (406 nt, TVM + I + Γ, 100 replicates). Bootstrap values are given in order ML/distance/maximum parsimony and are shown for any node with a value >60. Branch at base of the foraminiferal clade is shown 1/10 actual length.

Georgia, USA; India; Antarctica [*Dahlgren*, 1962; *Goldstein and Barker*, 1990; *Gooday and Fernando*, 1992; *Sabbatini et al.*, 2004]), some of which are muddy. *Ovammina opaca* may at times inhabit anoxic sediments because it is occasionally abundant in mudflat settings, where a thin oxygenated surface is underlain by sulfidic sediments (S. T. Goldstein, personal communication, 2006). Unfortunately the distribution *Ovammina opaca* has not been examined on a fine scale in these sediments.

[18] The presence of specimens with intact organelles at least 2 cm below the sediment-water interface, which corresponds to an anoxic, sulfidic horizon even during aeration episodes when the basin is more oxygenated [*Bernhard et al.*, 2003], indicate that the SBB allogromiid is a facultative anaerobe. Anoxia and sulfide enrichment are environmental characteristics normally considered detrimental to aerobes. The existence of anaerobic protists is well documented, however [e.g., *Fenchel and Finlay*, 1995]. Although it has been reported that rotaliid foraminifers occur in oxygen-depleted to anoxic, sulfidic environments [*Bernhard*, 1993, 1996, 2003; *Grzymski et al.*, 2002; *Stoeck and Epstein*, 2003], and that foraminifera can survive

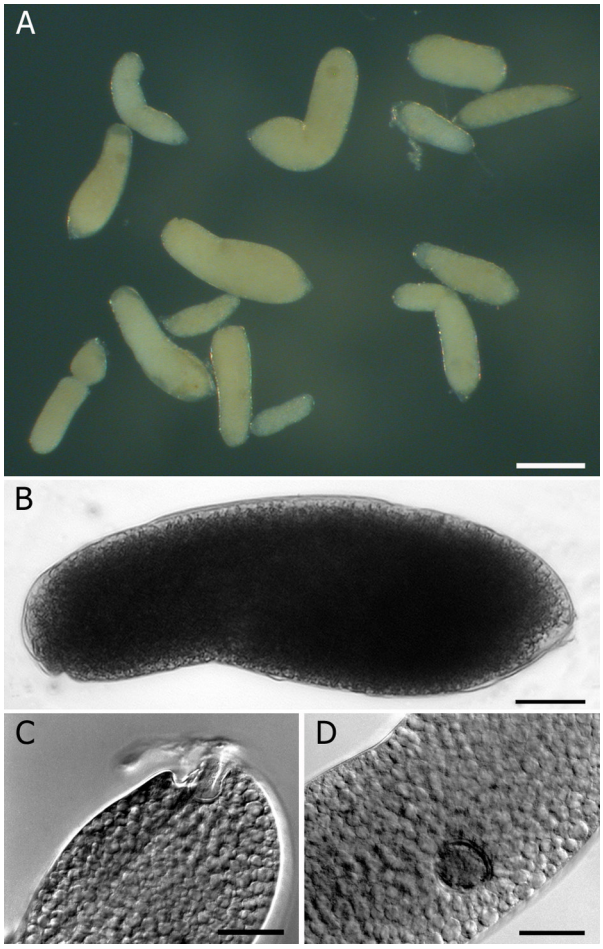

Figure 3. Santa Barbara Basin allogromiid. (a) Survey light micrograph of 14 specimens. Note oblong shape and white coloration. (b) Brightfield micrograph of representative specimen. Note vacuolated cytoplasm and thin test. DIC micrographs showing (c) entosolenian tube and (d) nucleus among vacuolated cytoplasm. Scale bars: Figure 3a, 200 μm; Figures 3b–3d, 50 μm.

anoxia or near anoxia for short periods [*Bernhard and Alve*, 1996; *Moodley et al.*, 1998], the occurrence of living basal (allogromiid) foraminifers in naturally occurring anoxia has not been previously documented.

[19] The presence of mitochondria in SBB allogromiid specimens collected from 2–3 cm depth implies that their mitochondria function anaerobically [*Tielens et al.*, 2002; *van Hellemond et al.*, 2003]. Although there was no evidence that the allogromiid consumes phagocytosed material, it is possible that nutrient uptake may be in the form of dissolved organic matter (DOM) like at least one other agglutinated foraminifer [e.g., *DeLaca et al.*, 1981], or that the allogromiid "reawakens" quickly after phytodetritus input, as shown by *Linke et al.* [1995]. Because we sampled in October, we would not expect to see material from the spring bloom in the foraminifer's cytoplasm. It may also be that the allogromiid's nutrition involves metabolic byproduct(s) of their endobionts. Because the endobionts displayed structures suggestive of elemental sulfur inclusions, we tentatively identify them as sulfur-oxidizing bacteria. Thus the endobionts may maintain the intracellular hydrogen sulfide at tolerable levels. Regardless of their specific functions, the high abundance of endobionts and the low percentage that were degraded advances a symbiotic function. In sum, the lack of food vacuoles and presence of putative symbionts implies that these allogromiids rely at least partly, or possibly completely, on chemoautotrophic symbionts, as is known for "gutless" symbiont-bearing metazoans [e.g., *Bright and Giere*, 2005]. Additional dedicated studies of the identity and functionality of the SBB allogromiid endobionts are necessary to elucidate details of the metabolic exchange between the host and endobiont.

[20] While it is possible that allogromiids evolved the ability to acquire endosymbionts in the Phanerozoic, the diversity of endobiont-bearing foraminiferans makes this possibility less likely. The acquisition of photosynthetic endosymbionts, such as diatoms, is well documented in both major lineages of modern calcareous foraminifera (reviewed by *Hallock* [1999]), which are of more recent evolutionary origin (~0.35 billion years before present [*Pawlowski et al.*, 2003; *Ross and Ross*, 1991]. Certain smaller calcareous foraminifers [*Bernhard*, 1993, 2003; *Bernhard et al.*, 2000], as well as a tree-like agglutinated species [*Richardson and Rützler*, 1999], harbor prokaryotic (nonphotosynthetic) endobionts. With the discovery of a basal foraminiferan that also has endobionts, the most

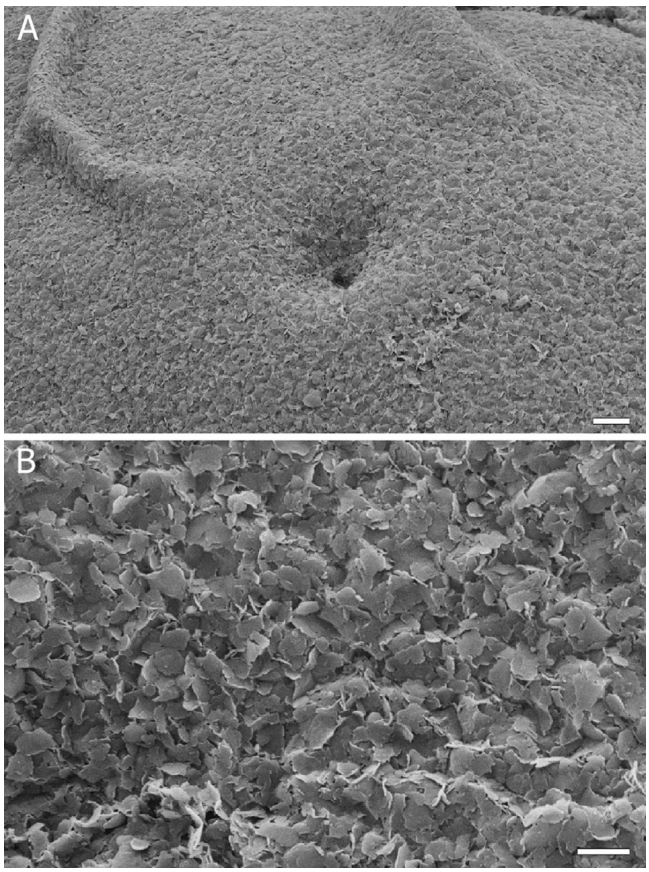

Figure 4. Scanning electron micrographs. (a) Low magnification view showing pore. (b) Higher magnification view showing test exterior. Scale bars: Figure 4a, 3 μm; Figure 4b, 1 μm.

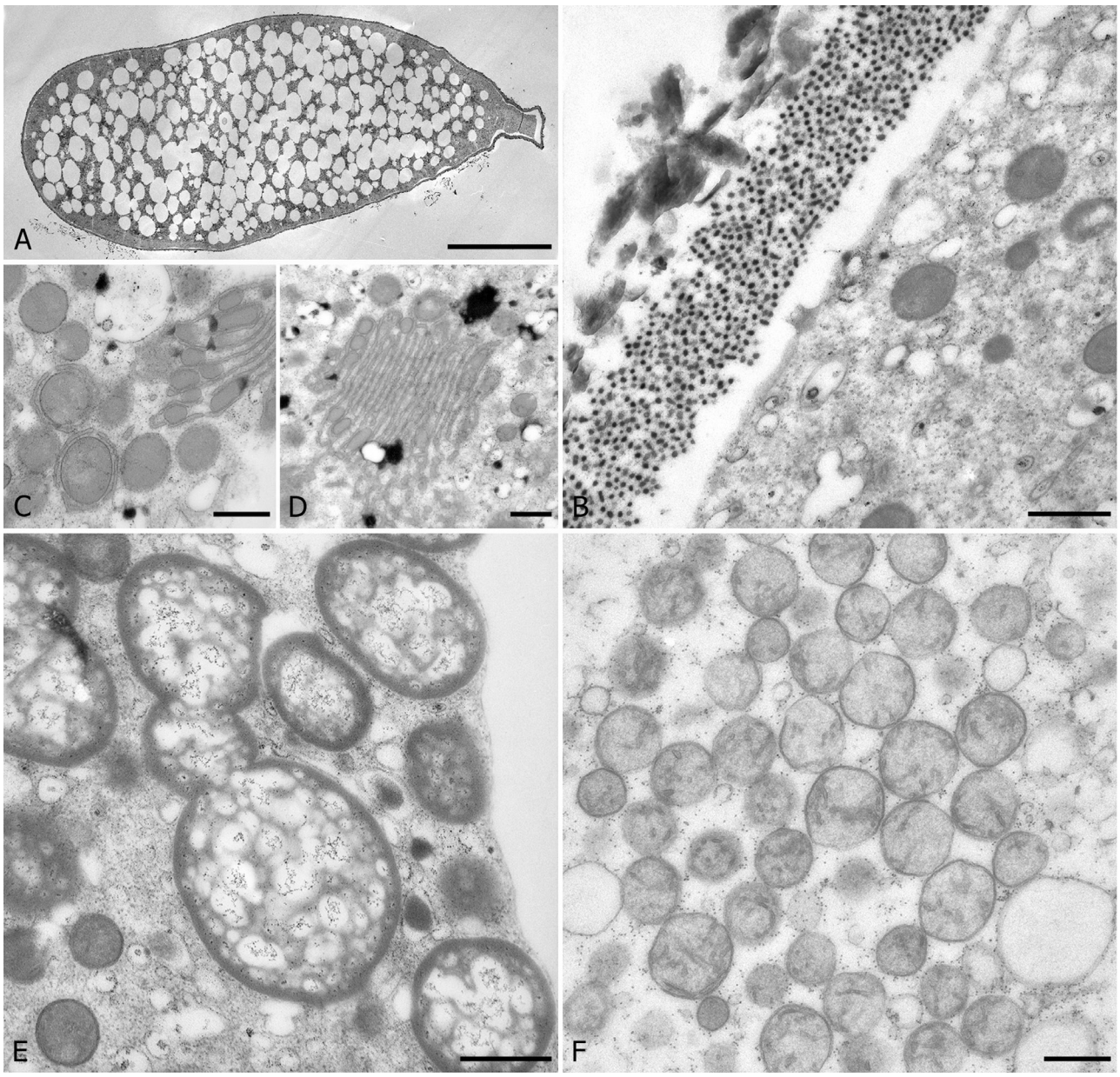

Figure 5. TEM micrographs. (a) Low magnification transmission electron micrograph showing vacuolated cytoplasm, necked aperture, and thin test. (b) View showing cytoplasm near test. Note granulofibrillar nature of the test and mineral platelets. (c, d) Golgi apparatus. (e) Vacuolated endobionts (asterisks). (f) Field of mitochondria with tubulovesicular cristae. Scale bars: Figure 5a, 50 μm; Figures 5b–5f, 0.5 μm.

parsimonious explanation is that symbiogenesis may be primitive to the group as a whole. If this assertion holds true, then the possible role of symbiosis in early foraminiferal evolution must be reexamined. Furthermore, our discovery of endobionts in a basal foraminiferan inhabiting anoxic sediments raises the exciting possibility that foraminiferans as a group have used endosymbionts as tools with which to invade inhospitable milieus for most of their history.

[21] Molecular clock analyses indicate that Clade L allogromiids, like many of the other allogromiid lineages, emerged during the mid-to-late Proterozoic (~0.7–1.2 billion years before present [*Pawlowski et al.*, 2003]). The deep oceans were likely anoxic and sulfidic during the Proterozoic [*Condie et al.*, 2001; *Kah et al.*, 2004; *Arnold et al.*, 2004; *Canfield*, 1998; *Shen et al.*, 2002] and did not attain modern oxygen levels until the Neoproterozoic-Cambrian boundary (0.54 Gya, [*Kah et al.*, 2004; *Canfield*, 1998]). Thus it can be concluded that at least some of the early evolution and diversification of eukaryotes like the Clade L allogromiids occurred in these anoxic, sulfidic conditions. The conditions of the SBB can be considered, therefore, as a modern analog of an environment within which early foraminifera diversified.

[22] The SBB allogromiid is morphologically similar to Proterozoic organic-walled microfossils of uncertain affinity

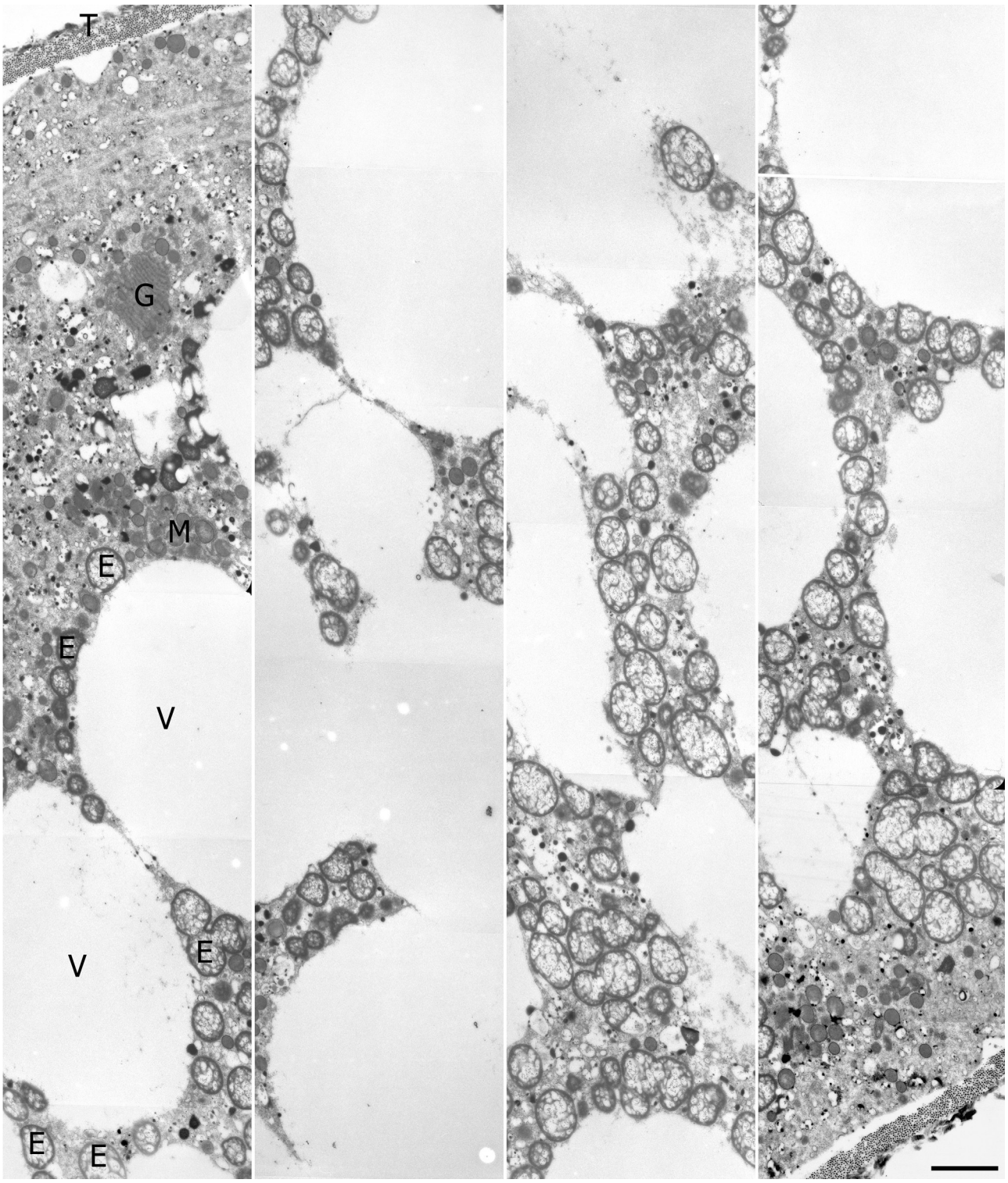

Figure 6. TEM montage of a cross-sectional transect through one specimen. T, test aluminosilicate platelets and granulofibrillar layer; G, Golgi apparatus; E, endobiont; V, vacuole; M, cluster of mitochondria. Scale bar = 2.5 μm.

(vase-shaped microfossils, VSM [*Porter et al.*, 2003]) found in organically enriched, laminated facies. These facies also contain filamentous bacterial microfossils [*Dehler et al.*, 2001], which may represent prokaryotes similar to *Beggiatoa*. Thus, although the paleoecology of VSMs is equivocal [*Porter et al.*, 2003], available evidence supports the hypothesis that these organisms inhabited oxygen-depleted to anoxic environments, similar to that of present-day SBB. Although the oldest known foraminiferal fossils (Cambrian agglutinated forms) are from well-aerated shallow marine set-

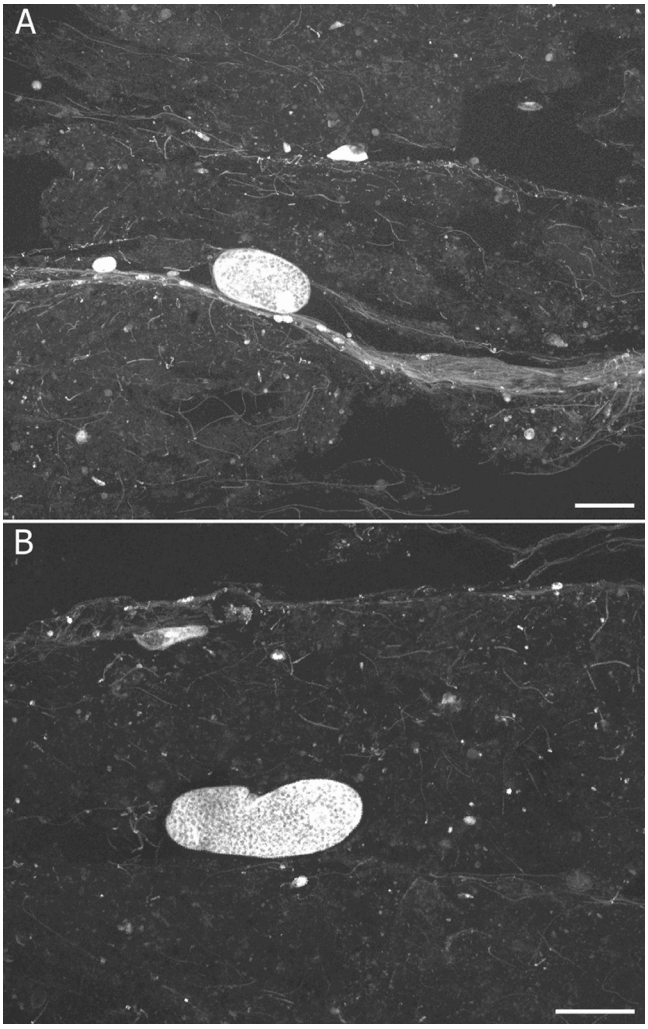

Figure 7. Laser Scanning Confocal Microscopy images of allogromiids in their life positions: (a) 6.2 mm and (b) 7.0 mm below the sediment-water interface. Also visible are flagellates, a ciliate, filamentous bacteria, and laminae bedding planes. Scale bars = 200 μm.

tings [e.g., *Culver*, 1991; *McIlroy et al.*, 2001; *Scott et al.*, 2003], our observations indicate that ancestral foraminifers could have inhabited both oxygen-replete and -depleted Proterozoic environments.

[23] We suggest that early allogromiid lineages, such as the one described here from SBB, could have competed well in prokaryote-dominated Neoproterozoic ecosystems, given that bacteria comprise a major portion of extant allogromiid diets [*Muller and Lee*, 1969; *Langezaal et al.*, 2005], and given also that certain modern allogromiids consume bacterial biofilms [*Bernhard and Bowser*, 1992], unicellular algae [*Gooday et al.*, 1995], and even metazoans [*Bowser et al.*, 1992]. Thus our combined observations raise the possibility that allogromiid foraminifers were pivotal players in benthic ecosystem structuring of Proterozoic sulfidic, oxygen-depleted oceanic sediments, and that, owing to their endobionts, they furthermore played a significant role in biogeochemical processes during that period of dramatic global oceanic and atmospheric change. The discovery of an anaerobic allogromiid provides further impetus to search for modern "extremophile" allogromiids, to analyze their fossilization potential, and helps alleviate the habitat bias in current molecular data sets. This finding also warrants a reexamination of the Proterozoic stratigraphic record, which should include a new microfossil search pattern similar to that used for multichambered agglutinates in Quaternary laminites [*Pike and Kemp*, 1996] but focusing on thin-shelled, fine-grained allogromiid foraminifers. In fact, similar constructs have recently been noted in Devonian deposits [*Schieber*, 2005; *Papazis et al.*, 2005].

5. Conclusions

[24] A previously unknown allogromiid foraminifer employs prokaryotic endobionts as a means to inhabit anoxic, sulfidic sediments, thereby strengthening the case for the Neoproterozoic origin of the group and suggesting a potential key role of symbiogenesis in early foraminiferal diversification.

[25] **Acknowledgments.** We thank the captain and crew of RV *Robert Gordon Sproul*, Scripps' Resident Technicians, and all members of the cruise scientific party; J. K. Blanks and M. J. Parmenter for laboratory assistance, J. Webber for X-ray microanalysis, and E. A. Richardson for sectioning and TEM assistance; and V. P. Edgcomb, J. P. Kennett, J. H. Lipps, S. M. Porter, and three anonymous reviewers for helpful manuscript comments. The Wadsworth Center's Electron Microscopy, Biochemistry, and Molecular Genetics Core facilities, as well as its National Biotechnology Resource for the Visualization of Biological Complexity (supported by a NIH BRTP/NCRR grant) are gratefully acknowledged. Funded by NASA Exobiology NRA-01-01-EXB-057 (to J. M. B.); W. Storrs Cole Memorial Research Award (Geological Society of America, to J. M. B.); NSF DEB0445181 (to S. S. B.).

References

Adl, S. M., et al. (2005), The new higher level classification of eukaryotes with emphasis on the taxonomy of protists, *J. Eukaryotic Microbiol.*, *52*(5), 399–459.

Anderson, O. R., and J. J. Lee (1991), Cytology and fine structure, in *Biology of Foraminifera*, edited by J. J. Lee and O. R. Anderson, pp. 7–40, Elsevier, New York.

Arnold, G. L., A. D. Anbar, J. Barling, and T. W. Lyons (2004), Molybdenum isotope evidence for widespread anoxia in Mid-Proterozoic oceans, *Science*, *304*(5667), 87–90.

Behl, R. J., and J. P. Kennett (1996), Brief interstadial events in the Santa Barbara Basin, NE Pacific, during the past 60 kyr, *Nature*, *379*(6562), 243–246.

Bernhard, J. M. (1993), Experimental and field evidence of Antarctic foraminiferal tolerance to anoxia and hydrogen sulfide, *Mar. Micropaleontol.*, *20*(3–4), 203–213.

Bernhard, J. M. (1996), Microaerophilic and facultative anaerobic benthic foraminifera: A review of experimental and ultrastructural evidence, *Rev. Paleobiol.*, *15*(1), 261–275.

Bernhard, J. M. (2003), Potential symbionts in bathyal foraminifera, *Science*, *299*(5608), 861.

Bernhard, J. M., and E. Alve (1996), Survival, ATP pool, and ultrastructural characterization of benthic foraminifera from Drammensfjord (Norway): Response to anoxia, *Mar. Micropaleontol.*, *28*(1), 5–17.

Bernhard, J. M., and S. S. Bowser (1992), Bacterial biofilms as a trophic resource for certain benthic foraminifera, *Mar. Ecol. Prog. Ser.*, *83*, 263–272.

Bernhard, J. M., and K. R. Buck (2004), Eukaryotes of the Cariaco, Soledad, and Santa Barbara Basins: Protists and metazoans associated with deep-water marine sulfide-oxidizing microbial mats and their possible effects on the geologic record, in *Sulfur Biogeochemistry–Past and Present*, edited by J. P. Amend, K. J. Edwards, and T. W. Lyons, *Spec. Pap. Geol. Soc. Am.*, *379*, 35–47.

Bernhard, J. M., K. R. Buck, M. A. Farmer, and S. S. Bowser (2000), The Santa Barbara Basin is a symbiosis oasis, *Nature*, *403*(6765), 77–80.

Bernhard, J. M., P. T. Visscher, and S. S. Bowser (2003), Sub-millimeter life positions of bacteria, protists, and metazoans in laminated sediments of the Santa Barbara Basin, *Limnol. Oceanogr.*, *48*(2), 813–828.

Bowser, S. S., and J. L. Travis (2002), Reticulopodia: Structural and behavioral basis for the suprageneric placement of Granuloreticulosan protists, *J. Foraminiferal Res.*, *32*(4), 440–447.

Bowser, S. S., S. P. Alexander, W. L. Stockton, and T. E. DeLaca (1992), Extracellular matrix augments mechanical properties of pseudopodial in the carnivorous foraminiferan *Astrammina rara*: Role in prey capture, *J. Protozool.*, *39*(6), 724–732.

Bright, M., and O. Giere (2005), Microbial symbiosis in Annelida, *Symbiosis*, *38*(1), 1–45.

Broenkow, W. W., and J. D. Cline (1969), Colorimetric determination of dissolved oxygen at low concentrations, *Limnol. Oceanogr.*, *14*(3), 450–454.

Buck, K. R., and J. P. Barry (1998), Monterey Bay cold seep infauna: Quantitative comparison of bacterial mat meiofauna with non-seep controls, *Cah. Biol. Mar.*, *39*, 333–335.

Canfield, D. E. (1998), A new model for Proterozoic ocean chemistry, *Nature*, *396*(6710), 450–453.

Cavalier-Smith, T. (2004), Only six kingdoms of life, *Proc. R. Soc., Ser. B*, *271*(1545), 1251–1262.

Condie, K. C., D. J. Des Marais, and D. Abbott (2001), Precambrian superplumes and supercontinents: A record in black shales, carbon isotopes, and paleoclimates?, *Precambrian Res.*, *106*(3–4), 239–260.

Cranston, K., and B. Rannala (2005), Closing the gap between rocks and clocks, *Heredity*, *94*(5), 461–462.

Culver, S. J. (1991), Early Cambrian foraminifera from West Africa, *Science*, *254*(5032), 689–691.

Dahlgren, L. (1962), A new monothalamous foraminifer, *Ovammina opaca* n. gen., n. sp., belonging to the family Saccamminidae, *Zool. Bidrag Uppsala*, *33*, 197–200.

Dahlgren, L. (1964), On nuclear cytology and reproduction in the monothalamous foraminifer *Ovammina opaca* Dahlgren, *Zool. Bidrag Uppsala*, *36*, 315–334.

Dahlgren, L. (1967), On the ultrastructure of the gamontic nucleus and the adjacent cytoplasm of the monothalamous foraminifer *Ovammina opaca*, *Zool. Bidrag Uppsala*, *37*, 77–112.

Dehler, C. M., M. Elrick, K. E. Karlstrom, G. A. Smith, L. J. Rossey, and J. M. Timmons (2001), Neoproterozoic Chuar Group (~800–742 Ma), Grand Canyon: A record of cyclic marine deposition during global cooling and supercontinent rifting, *Sed. Geol.*, *141–142*, 465–499.

DeLaca, T. E., D. M. Karl, and J. H. Lipps (1981), Direct use of dissolved organic carbon by agglutinated benthic foraminifera, *Nature*, *289*(5795), 287–289.

Dubilier, N., et al. (2001), Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm, *Nature*, *411*(6835), 298–302.

Fenchel, T., and B. J. Finlay (1995), *Ecology and Evolution in Anoxic Worlds*, 276 pp., Oxford Univ. Press, New York.

Galtier, N., M. Gouy, and C. Gautier (1996), SEAVIEW and PHYLO_WIN, two graphic tools for sequence alignment and molecular phylogeny, *Comput. Appl. Biosci.*, *12*(6), 543–548.

Goldstein, S. T., and W. W. Barker (1990), Gametogenesis in the monothalamous agglutinated foraminifera *Cribrothalammina alba*, *J. Protozool.*, *37*(1), 20–27.

Goldstein, S. T., and B. H. Corliss (1994), Deposit feeding in selected deep-sea and shallow-water benthic foraminifera, *Deep Sea Res., Part I*, *41*(2), 229–241.

Gooday, A. J. (2002), Organic-walled allogromiids: Aspects of their occurrence, diversity and ecology in marine habitats, *J. Foraminiferal Res.*, *32*(4), 384–400.

Gooday, A. J., and O. J. Fernando (1992), A new allogromiid genus (*Rhizopoda: Foraminiferida*) from the Vellar Estuary, Bay of Bengal, *J. Micropaleontol.*, *11*(2), 233–239.

Gooday, A. J., J. M. Bernhard, and S. S. Bowser (1995), The taxonomy and ecology of *Crithionina delacai* (n. sp.), a dominant giant foraminifer in Explorers Cove, Antarctica, *J. Foraminiferal Res.*, *25*(4), 290–298.

Gooday, A. J., J. M. Bernhard, L. A. Levin, and S. Suhr (2000), Foraminifera in the Arabian Sea oxygen minimum zone and other oxygen-deficient settings: Taxonomic composition, diversity, and relation to metazoan faunas, *Deep Sea Res., Part II*, *47*(1–2), 25–54.

Gooday, A. J., O. V. Anikeeva, and N. G. Sergeeva (2006), *Tinogullmia lukyanovae* sp. nov.—A monothalamous, organic-walled foraminiferan from the coastal Black Sea, *J. Mar. Biol. Assoc. U. K.*, *86*(1), 43–49.

Grzymski, J., O. M. Schofield, P. G. Falkowski, and J. M. Bernhard (2002), The function of plastids in the deep-sea benthic foraminifer, *Nonionella stella*, *Limnol. Oceanogr.*, *47*(6), 1569–1580.

Habura, A., J. Pawlowski, S. D. Hanes, and S. S. Bowser (2004), Unexpected foraminiferal diversity revealed by small-subunit rDNA analysis of Antarctic sediment, *J. Eukaryotic. Microbiol.*, *51*(2), 173–179.

Hallock, P. (1999), Symbiont-bearing foraminifera, in *Modern Foraminifera*, edited by B. Sen Gupta, pp. 123–139, Springer, New York.

Kah, L. C., T. W. Lyons, and T. D. Frank (2004), Low marine sulphate and protracted oxygenation of the Proterozoic biosphere, *Nature*, *431*(7010), 834–838.

Krieger, J., O. Giere, and N. Dubilier (2000), Localization of RubisCO and sulfur in endosymbiotic bacteria of the gutless marine oligochaete *Inanidrilus leukodermatus* (Annelida), *Mar. Biol.*, *137*(2), 239–244.

Langezaal, A. M., N. T. Jannink, E. S. Pierson, and G. J. van der Zwaan (2005), Foraminiferal selectivity towards bacteria: An experimental approach using a cell-permeant stain, *J. Sea Res.*, *54*(4), 256–275.

Linke, P., A. V. Altenbach, G. Graf, and T. Heeger (1995), Response of deep-sea benthic foraminifera to a simulated sedimentation event, *J. Foraminiferal Res.*, *25*(1), 75–82.

McIlroy, D., O. R. Green, and M. D. Brasier (2001), Palaeobiology and evolution of the earliest agglutinated foraminifers: *Platysolenites*, *Spirolenites* and related forms, *Lethaia*, *34*(1), 13–29.

Moodley, L., G. J. van der Zwaan, P. M. J. Herman, L. Kempers, and P. van Breugel (1997), Differential response of benthic meiofauna to long-term anoxia with special reference to foraminifera (Protista: Sarcodina), *Mar. Ecol. Prog. Ser.*, *158*, 151–163.

Moodley, L., B. E. M. Schaub, G. J. van der Zwaan, and P. M. J. Herman (1998), Tolerance of benthic foraminifera (Protista: Sarcodina) to hydrogen sulphide, *Mar. Ecol. Prog. Ser.*, *169*, 77–86.

Muller, W. A., and J. J. Lee (1969), Apparent indispensability of bacteria in foraminiferan nutrition, *J. Protozool.*, *16*(3), 471–478.

Mußmann, M., H. N. Schulz, B. Strotmann, T. Kjær, L. P. Nielsen, R. A. Rosselló-Mora, R. I. Amann, and B. B. Jørgensen (2003), Phylogeny and distribution of nitrate-storing *Beggiatoa* spp. in coastal marine sediments, *Environ. Microbiol.*, *5*(6), 523–533.

Nikolaev, S. I., C. Berney, J. F. Fahrni, I. Bolivar, S. Polet, A. P. Mylnikov, V. V. Aleshin, N. B. Petrov, and J. Pawlowski (2004), The twilight of Heliozoa and the rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes, *Proc. Natl. Acad. Sci. U. S. A.*, *101*(21), 8066–8071.

Papazis, P. K., K. L. Milliken, S. J. Choh, and J. Schieber (2005), The widespread occurrence of agglutinated foraminifera in black shales, paper presented at Annual Meeting, Geol. Soc. of Am., Salt Lake City, Utah.

Pasteris, J. D., J. J. Freeman, S. K. Goffredi, and K. R. Buck (2001), Raman spectroscopic and laser scanning confocal microscopic analysis of sulphur in living sulfur-precipitating marine bacteria, *Chem. Geol.*, *180*(1–4), 3–18.

Pawlowski, J., M. Holzmann, C. Berney, J. Fahrni, T. Cedhagen, and S. S. Bowser (2002), Phylogeny of allogromiid foraminifera inferred from SSU rRNA gene sequences, *J. Foraminiferal Res.*, *32*(4), 334–343.

Pawlowski, J., M. Holzmann, C. Berney, J. Fahrni, A. J. Gooday, T. Cedhagen, A. Habura, and S. S. Bowser (2003), The evolution of early foraminifera, *Proc. Natl. Acad. Sci. U. S. A.*, *100*(20), 11,494–11,498.

Pflugfelder, B., C. R. Fisher, and M. Bright (2005), The color of the trophosome: Elemental sulfur distribution in the endosymbionts of *Riftia pachyptila* (Vestimentifera: Siboglinidae), *Mar. Biol.*, *146*(5), 895–901.

Pike, J., and A. E. S. Kemp (1996), Silt aggregates in laminated marine sediment produced by agglutinated foraminifera, *J. Sed. Res.*, *66*(3), 625–631.

Porter, S. M., R. Meisterfeld, and A. H. Knoll (2003), Vase-shaped microfossils from the Neoproterozoic Chuar Group, Grand Canyon: A classification guided by modern testate amoebae, *J. Paleontol.*, *77*(3), 409–429.

Posada, D., and K. A. Crandall (1998), MODELTEST: Testing the model of DNA substitution, *Bioinformatics*, *14*(9), 817–818.

Reimers, C. E., C. B. Lange, M. Tabak, and J. M. Bernhard (1990), Seasonal spillover and varve formation in the Santa Barbara Basin, California, *Limnol. Oceanogr.*, *35*(7), 1577–1585.

Richardson, S. L., and K. Rützler (1999), Bacterial endosymbionts in the agglutinating foraminiferan *Spiculidendron corallicolum* Rützler and Richardson, 1996, *Symbiosis*, *26*(4), 299–312.

Ross, C. A., and J. R. P. Ross (1991), Paleozoic Foraminifera, *Biosystems*, *25*(1–2), 39–51.

Sabbatini, A., J. Pawlowski, A. J. Gooday, S. Piraino, S. S. Bowser, C. Morigi, and A. Negri (2004), *Vellaria zucchellii* sp. nov.: A new monothalamous foraminifer from Terra Nova Bay, Antarctica, *Antarct. Sci.*, *16*(3), 307–312.

Schieber, J. (2005), Agglutinated foraminifera in Devonian black shales of the eastern US: Implications for paleoenvironmental reconstructions, paper presented at Annual Meeting, Geol. Soc. of Am., Salt Lake City, Utah.

Scott, D. B., F. Medioli, and R. Braund (2003), Foraminifera from the Cambrian of Nova Scotia: The oldest multichambered foraminifera, *Micropaleontology*, *49*(2), 109–126.

Sen Gupta, B. K. (1999), *Modern Foraminifera*, 371 pp., Springer, New York.

Shen, Y., D. E. Canfield, and A. H. Knoll (2002), Middle Proterozoic ocean chemistry: Evidence from the McArthur Basin, Northern Australia, *Am. J. Sci.*, *302*(2), 81–109.

Stoeck, T., and S. Epstein (2003), Novel eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen-depleted marine environments, *Appl. Environ. Microbiol.*, *69*(5), 2657–2663.

Swofford, D. L. (2003), *PAUP*. Phylogenetic Analysis Using Parsimony and Other Methods*, version 4 b10, Sinauer Assoc., Sunderland, Mass.

Thompson, J. D., D. G. Higgins, and T. J. Gibson (1994), CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice, *Nucl. Acids Res.*, *22*(22), 4673–4680.

Thunell, R. C., E. Tappa, and D. M. Anderson (1995), Sediment fluxes and varve formation in Santa Barbara Basin, offshore California, *Geology*, *23*(12), 1083–1086.

Tielens, A. G. M., C. Rotte, J. J. van Hellemond, and W. Martin (2002), Mitochondria as we don't know them, *Trends Biochem. Sci.*, *27*(11), 564–572.

van Hellemond, J. J., A. van der Klei, S. W. H. van Weelden, and A. G. M. Tielens (2003), Biochemical and evolutionary aspects of anaerobically functioning mitochondria, *Philos. Trans. Soc, R., Ser. B*, *358*(1429), 205–215.

Vetter, R. D. (1985), Elemental sulfur in the gills of three species of clams containing chemoautotrophic bacteria: A possible inorganic energy storage compound, *Mar. Biol.*, *88*(1), 33–42.

J. M. Bernhard, Department of Geology and Geophysics, MS 52, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA. (jbernhard@whoi.edu)

S. S. Bowser and A. Habura, Wadsworth Center, New York State Department of Health, PO Box 509, Albany, NY 12201-0509, USA.