J. Phycol. **36,** 172–177 (2000)

srDNA-BASED TAXONOMIC AFFINITIES OF ALGAL SYMBIONTS FROM A PLANKTONIC FORAMINIFER AND A SOLITARY RADIOLARIAN[1]

Rebecca J. Gast[2]

Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Tracey A. McDonnell

University of Texas, Austin, TX 78712

and

David A. Caron

Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Planktonic sarcodines (acantharia, radiolaria, and planktonic foraminifera) are oceanic amoeboid protozoa that often harbor a variety of microalgae as intracellular symbionts. The identity and function of these endosymbiotic algae have intrigued and perplexed biologists for more than a century. The most conspicuous and well-studied symbiotic algae of planktonic foraminifera and radiolaria are dinoflagellates, but a variety of nondinoflagellate taxa have also been reported. Ultrastructural features have been used to characterize some of these nondinoflagellate algae, but rarely has this led to clear taxonomic affiliations. We analyzed the nuclear small subunit ribosomal DNA (srDNA) isolated from the symbionts of the spinose planktonic foraminiferan *Globigerinella siphonifera* d'Orbigny (=*Globigerinella aequilateralis* Brady) and a solitary radiolarian (*Spongodrymus* sp. Haeckel) in order to determine the identity of these symbionts. The small coccoid algae isolated from *G. siphonifera* correspond to the Type I symbionts described by Faber et al. (1988). Phylogenetic analysis of the srDNA sequences places these symbionts within the prymnesiophyte (haptophyte) lineage, closer to *Prymnesium* Conrad than to *Phaeocystis* Lagerheim. To our knowledge, this is the first confirmed case of a symbiotic prymnesiophyte. In addition, we were able to examine the level of sequence heterogeneity between symbionts isolated from different individuals of a single host species. The three isolates in this study had srDNA sequences that were almost identical, indicating that the three were all of the same species. Very green symbiotic algae were isolated from three solitary radiolaria identified as species of *Spongodrymus*. The symbiont srDNA sequences from the three individual hosts were identical to each other, again implicating a single species of algae in that symbiotic association. These symbionts are prasinophytes most closely related to the clade containing *Tetaselmis convolutae* Norris, Hori et Chihara. *Tetraselmis convolutae* is the algal symbiont of the marine flatworm, *Convolutae roscoffensis* Graff.

[1] Received 10 August 1999. Accepted 25 October 1999.

[2] Author for reprint requests; e-mail rgast@whoi.edu.

Key index words: **molecular phylogeny; planktonic sarcodines; srDNA; symbiotic algae**

Foraminifera, radiolaria, and acantharia are marine protists commonly referred to as sarcodines. These protists are widely distributed, occurring in tropical, subtropical, and even polar marine environments. Members of the planktonic sarcodines contribute significantly to herbivory and carnivory in the communities that they inhabit (Caron and Swanberg 1990, Swanberg and Caron 1991). In addition to being voracious consumers, many of the tropical and subtropical surface-dwelling species harbor endosymbiotic algae and thus contribute to primary production as well. Although a wide range of algae have been reported as endosymbionts, the diversity of these algal symbionts is still poorly understood, due largely to the lack of reliable taxonomic features available for identification of the alga *in hospice.* Features of the cell wall, scales, flagella, and cell shape are lost or modified, and seldom have the free-living forms of these algae been cultured and examined. Most descriptions of the symbionts are based on ultrastructural features, such as plastid shape or nuclear structure (dinoflagellates), detected by electron microscopy. While helpful, these features can be variable or equivocal, and therefore limited in their ability to resolve taxonomic affiliations. In order to further assess the taxonomic relationships of symbionts of the planktonic sarcodines, to examine the symbiont diversity within a single host species, and to begin analyzing the variation in symbiont isolates of the same species, we have utilized the analysis of small subunit ribosomal DNA sequences.

Symbiotic algae present in most symbiont-bearing spinose planktonic foraminifera are dinoflagellates belonging to the species *Gymnodinium beii* (Gast and Caron 1996, Spero 1987). Previous descriptive studies of the small (3–4 μm) yellow-green symbionts of the spinose planktonic foraminiferan *Globigerinella siphonifera*, however, have resulted in their classification as members of the division Chrysophycophyta. The diverse collection of algae included in this division are the Chrysophyceae, Bacillariophyceae, and Prymne-

siophyceae. Gastrich (1988) reported that the *G. siphonifera* symbiont resembled a prymnesiophyte based on ultrastructural analysis. In another study based on ultrastructure, Faber et al. (1988) found that different individuals of *G. siphonifera* harbored one of two different endosymbionts (referred to as Type I and Type II). Neither symbiont type was classified further than the division Chrysophycophyta. The alga that Gastrich described appears to correspond to the Type I symbiont of Faber et al. (1988).

Ultrastructural studies of radiolaria isolated from Bermuda and other Atlantic locations have led to the description of a diversity of algal symbionts in planktonic sarcodines (Anderson et al. 1983). Most radiolaria harbor dinoflagellate symbionts, but some radiolaria have been reported to have several different symbiotic algae. *Spongodrymus* is such a genus, and has been described as having three different symbiotic algae (not at the same time) (Anderson 1983). Identifying species of *Spongodrymus* is difficult, so it is possible that different species harbor the different symbiont types. This situation has yet to be resolved, but perhaps through the sequencing of srDNA from the hosts we will be able to determine whether the different individuals of *Spongodrymus* are the same species (Amaral-Zettler et al. 1997). We have previously analyzed the srDNA of the dinoflagellate symbiont from a *Spongodrymus* sp. and confirmed that the symbiont is identical to the dinoflagellate found in other radiolaria (Gast and Caron 1996). In this paper we discuss our identification of the prasinophyte symbiont from this radiolarian genus.

MATERIALS AND METHODS

Collection of sarcodines. Foraminifera and radiolaria were collected in October 1994 from the Sargasso Sea southeast of Bermuda. Three *Globigerinella siphonifera* and three solitary radiolaria (identified as species of *Spongodrymus*) were used for symbiont analysis. Symbionts were microdissected from individual hosts (except *Spongodrymus* 257) that had been rinsed three times in sterile sea water. Approximately half of each dissected symbiont sample (several hundred cells) was placed in a sterile 1.5-mL eppendorf tube and stored at −20° C until processed for DNA extraction. The other half of the dissected material was placed in 10 mL of Prov 50 culture media (Center for Culture of Marine Phytoplankton; CCMP) in order to culture the algae as free-living symbionts. We modified our Prov 50 by the addition of 5 mL nonalkaline soil extract (no NaOH was used in its preparation) instead of 10 mL, and included 1 mL of 0.054 M Na_2SiO_3 per liter. These cultures were placed in incubators at 25° C with 14/10 hour cycles of light/dark illumination. *Spongodrymus* 257 was transferred into sterile seawater, but died soon after collection. The released symbionts were retrieved by micropipetting and cultured as previously stated.

DNA isolation and amplification. Frozen cells were thawed on ice and spun briefly to form a pellet (3 min in a microcentrifuge at 12,000 rpm at 4° C). The supernatant was removed and the *G. siphonifera* symbionts were resuspended in 5% Chelex (Walsh et al. 1991) and vortexed vigorously. The mixture was incubated for 15 min at room temperature, vortexed, incubated for 15 min at 56° C, vortexed, incubated 8 min at 100° C, vortexed and then spun briefly in a microcentrifuge to pellet the Chelex. The amount of Chelex solution added ranged from 20 to 30 μL, depending on the size of the cell pellet. After lysis, the supernatant was removed from the Chelex resin and transferred to a new tube for storage at −20° C. The *Spongodrymus* symbiont samples were lysed by resuspending them in 30 μL of 1× PCR buffer (0.5 M KCl, 0.1 M Tris pH 8.3, 0.02 M $MgCl_2$, 0.01% gelatin, 0.5% NP40) followed by heating at 95° C for 10 min. The lysate was stored at −20° C. One microliter of either lysate was used in standard PCR amplification (Saiki et al. 1988) reactions using universal eukaryotic srDNA primers (A and B; Medlin et al. 1988). Forty cycles (1 min at 94° C, 1 min at 45°C, and 3 min at 72° C) were used to amplify the small subunit ribosomal RNA gene from microdissected samples.

Cultures of free-living algal symbionts were established from all six of the samples. One milliliter of a dense culture (>10,000 cells·mL^{-1}) was collected for DNA extraction. Cells were pelleted by centrifugation at 12,000 × *g* for 5 min at 4° C and DNA was extracted using 50 μL 5% Chelex. One microliter of the lysate was used in PCR amplifications as described above, except that only 30 cycles were used.

Due to the small amount of original microdissected sample available, and occasional difficulties in obtaining AB products, internal eukaryotic primers 373C and 1200RE (Weekers et al. 1994) were paired with B and A, respectively, to reamplify AB PCR products. These products were then used for direct sequencing and RFLP analysis. Microdissected symbiont DNA was used to generate templates for the comparison of RFLP patterns with cultured algae and the confirmation of variable region sequence information. Symbiont cultures were used to provide material for most of the ribosomal gene sequencing.

Restriction enzyme digestions. We used the enzymes *Hae*III, *Hin*fI and *Taq*I to digest srDNA PCR products obtained from algal symbionts. The RFLP patterns from cultured symbiotic algae were compared with those obtained with products from the corresponding microdissected symbionts. The suite of three enzymes provided a rough estimate on the similarity of the ribosomal sequences without the expense and time of sequencing. Fifteen microliters of PCR product were digested in a 30 μL reaction, and 15 μL was loaded onto a 1% agarose gel for separation and visualization. We were able to confirm that the cultures were similar to the original algae and proceed with sequence analysis.

TEM of cultured symbionts. Cultures at moderate to heavy densities (>1000 cells·mL^{-1}) were fixed for TEM by exposure to osmium vapor. Five microliters of live culture were placed on formvar-coated grids (300 mesh) in a disposable petri dish. One drop (~10 μL) of 2% osmium tetroxide was placed on the lid of the dish and the cells were exposed to the vapor for 30 seconds. Cells were then allowed to air-dry on the grids. After drying, the grids were gently dipped twice in distilled water to remove salt crystals. Cells were negatively stained for 5 min using uranyl acetate (1%). The grids were rinsed gently in distilled water and allowed to dry. A Zeiss 10 transmission electron microscope was used to view and photograph the cells.

Sequencing and phylogenetic reconstructions. We pooled and directly sequenced at least three PCR reactions for each symbiont sample, to reduce the effect of amplification errors and microheterogeneity among the genes on the resulting phylogeny. Both types of sequence variation appear as ambiguous sites; if not clarified by repeated sequencing, they were excluded from the phylogenetic analysis. The PCR products used for sequencing were purified using Wizard PCR Preps (Promega). Sequencing reactions were accomplished using the Epicentre long read cycle sequencing kit (Epicentre Technologies) and infrared dye-labeled primers (Table 1) that spanned the entire srDNA molecule. Reactions were run and detected by LI-COR 4000L automated sequencers (LI-COR), then analyzed and assembled by BioImage DNA sequence film reader software (Millipore). The entire srRNA gene was sequenced, and >80% of the molecule was sequenced on both strands, using three primers in the forward direction and three primers in the reverse direction (Table 1). Alignments were generated by eye in GDE (Steve Smith, University of Illinois). Small subunit ribosomal DNA sequences for prasinophytes, prymnesiophytes, chrysophytes, and chlorophytes were retrieved from GenBank (Bilofsky and Burks, 1988) and aligned with the symbiont sequences. Symbiont se-

TABLE 1. Small subunit ribosomal DNA sequencing primers. A, 514F, and 1055F prime in the 5′→3′ direction, whereas 536R, 1055R, and B prime in the 3′→5′ direction.

Primer	Sequence
A	5′-ACCTGGTTGATCCTGCCAGT-3′
536R	5′-WATTACCGCGGCKGCTG-3′
514F	5′-GTGCCAGCMGCCGCGG-3′
1055R	5′-CGGCCATGCACCACC-3′
1055F	5′-GGTGGTGCATGGCCG-3′
B	5′-GCTTGATCCTTCTGCAGGTTCACCTAC-3′

quences are available from GenBank using the accession numbers AF166376–AF166381.

Phylogenetic reconstructions were accomplished using PAUP 4.0.0d64 (final demo version, David Swofford). There were 1428 characters in the dataset, and 276 of those were parsimony informative. Maximum likelihood was run with the program default parameters, using the random addition of sequences and tree bisection–reconnection branch swapping. One thousand bootstrap replicates of maximum parsimony heuristic searches with tree bisection–reconnection and 10 random sequence additions per replicate were run to estimate the confidence level of the nodes. *Pavlova salina* was designated the outgroup in both analyses, but the trees are unrooted.

RESULTS AND DISCUSSION

Observations of the cultured free-living symbionts. All the culture attempts yielded unialgal cultures, although often contaminated with bacteria. The *Spongodrymus* symbionts were very green and 6.5 to 7.5 μm in length. The cells possessed four flagella of equal length (10 μm). The free-living symbionts appeared similar in size and shape to descriptions of *Tetraselmis cordiformis.* Ultrastructural examination revealed the four hairy flagella characteristic of this organism (Fig. 1A).

Symbionts of *G. siphonifera* were much smaller than the *Spongodrymus* symbionts, approximately 2.5 μm in size, round, and greenish brown. Cultures were sporadically motile, and it was not possible to observe flagella using light microscopy. Careful fixation methods (described above) permitted the subsequent detection of two equal flagella and a haptoneme by TEM observation (Fig. 1B). The algae were also found to have two types of body scales (Fig. 1B), similar to scales produced by prymnesiophytes (Lee et al. 1985, Ostergaard 1998).

RFLP patterns. Previous studies of algal symbiont diversity have made use of restriction enzyme digestion patterns of the small subunit ribosomal gene for the identification of different symbiont types (Gast and Caron 1996, Rowan and Knowlton 1995, Rowan and Powers 1991). Very little DNA (<30 μL) was available from each microdissected sample, despite the overall number of symbionts present, and we were concerned about using all of this in our amplifications for sequencing. It seemed that the best alternative was to have a constant source of template and, since we were already growing the symbionts, we chose to utilize these cultures. We chose to use RFLPs to screen our algal cultures rather than proceed to sequence organisms that were not confirmed as being symbionts.

We digested small subunit ribosomal DNA amplification products from microdissected symbionts and compared the patterns obtained. Restriction fragment length polymorphism patterns (RFLPs) from the enzymes *Hae*III, *Hin*fI, and *Taq*I, indicate that all of the symbionts isolated from *G. siphonifera* were similar to each other, and that symbionts from *Spongodrymus* were also similar to each other. Both groups of symbionts had RFLP patterns that were distinct from each other as well as from the patterns obtained for dinoflagellate symbionts from foraminifera and radiolaria (Gast and Caron 1996). We then compared the microdissected symbiont RFLP patterns with those obtained from the corresponding cultured symbionts and confirmed that they were the same (Fig. 2).

srDNA sequences of symbiotic algae. The *Spongodrymus* symbiont srRNA gene was 1793 base pairs, and all three isolated symbionts had identical sequences. This identity indicates that they were most likely the same strain of algae. The analysis of the DNA sequence of the small subunit ribosomal RNA gene from the three *G. siphonifera* symbionts unambiguously indicated that they were the same species of alga, but minor sequence heterogeneity was observed among these isolates. The srRNA gene from these symbionts was 1793 (*G. siphonifera* 3), 1792 (*G. siphonifera* 1), and 1791 (*G. siphonifera* 4) base pairs. The larger size of *G. siphonifera* 3 was due to a single insertion in a series of C's, whereas the smaller size of *G. siphonifera* 4 symbiont was due to a single base deletion. There was a single base change shared by the *G. siphonifera* 4 and *G. siphonifera* 3 symbionts, which was included in the analyzed dataset, and another shared base difference between *G. siphonifera* 1 and 4 that was also included in the dataset. These single changes resulted in the small branch lengths for this clade shown in the maximum likelihood tree in Fig. 3. This heterogeneity is very small and within the error limit of PCR amplification, so it may not be real. Repeat sequencing of the regions confirmed the variation in these isolates, but this small amount of variability is probably not enough to preclude the description of these isolates as the same strain.

To additionally confirm that we had cultured and sequenced the algal symbionts, the DNA originally isolated from microdissected symbionts was PCR amplified using universal primers. The resulting band was partially sequenced and compared with the sequence obtained from the cultured symbionts. Both the radiolarian and the *G. siphonifera* cultured symbiont sequences correlated with the sequences obtained from the original samples. This result indicated that we had cultured and identified the symbionts.

We recovered small subunit sequences from GenBank in order to determine the phylogenetic affinities of our algae. Previous studies of the symbiotic algae of *G. siphonifera* reported that the symbiont was a chryso-

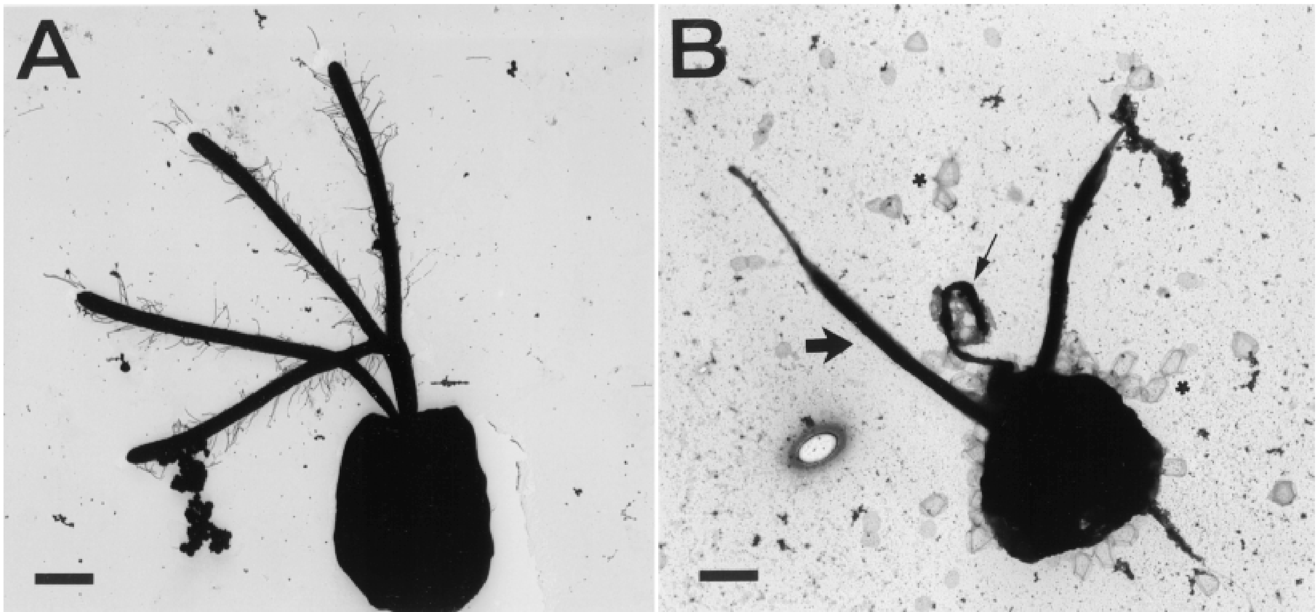

Fig. 1. (A) TEM micrograph of *Spongodrymus* symbiont at 6300× magnification. Note four flagella with hairs. Scale bar = 2 μm. (B) TEM micrograph of *G. siphonifera* symbiont at 10,000× magnification. Large arrow points to one of the two equal flagella, the small arrow points to the haptoneme. Asterisks indicate scales that have been dislodged and are distributed around and on the cell. Note the presence of two types of scales, one rounder and smaller than the other. Bar = 0.7 μm.

phyte or a prymnesiophyte (Faber et al. 1988, Gastrich 1988). We therefore retrieved representatives of these organisms from the database. As our observations of the *Spongodrymus* symbiont suggested that it is related to *Tetraselmis*, we included those sequences, and other prasinophytes and chlorophytes, in the alignment. We also performed Blast searches with both of our symbiont sequences to identify any other closely related sequences that might not have been retrieved using our search terms.

The results of the phylogenetic reconstructions for the srDNA from the symbionts are shown in Fig. 3. The maximum likelihood reconstruction shown is a strict consensus tree. Out of 89,407 rearrangements, nine trees were obtained. All nine were the same, except for the branch orders within the *Spongodrymus* and *G. siphonifera* symbiont groups. This result was expected, due to the overall high similarity of the sequences within each of those groups. The symbionts from *G. siphonifera* clustered within the prymnesiophytes rather than chrysophytes. To our knowledge, this species is the first prymnesiophyte to have been cultured and confirmed as an intracellular symbiont.

We have identified one of two species of symbionts observed in *G. siphonifera* (Faber et al. 1988). Identification of the second symbiont type is still of great interest to us for two reasons. First, there is the possibility that the Type II symbiont may represent a new symbiotic lineage, perhaps within the chrysophytes. Second, a recent srDNA phylogeny by Darling et al. (1997) suggests that the host, *G. siphonifera*, is actually two species. The presence of two different types of

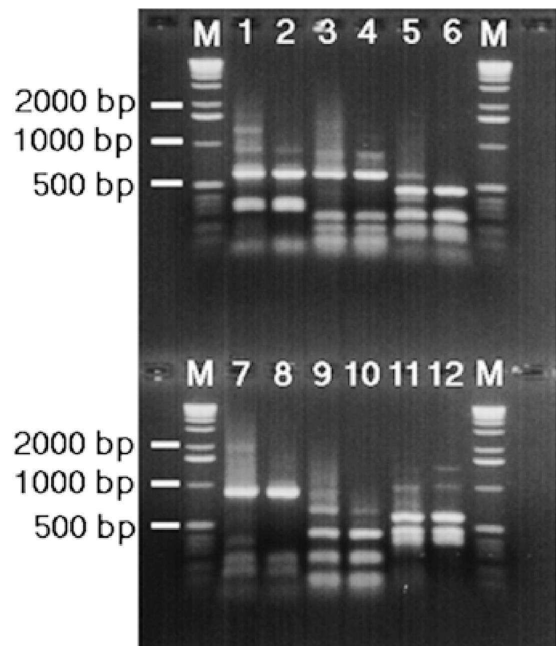

Fig. 2. Agarose gel stained with ethidium bromide. The restriction enzymes *Hae*III, *Hin*fI, and *Taq*I were used to digest PCR products amplified using the primers A and 1200R. Lanes 1, 3, and 5 are digests of PCR products from the microdissected *G. siphonifera* 3 symbiont; lanes 2, 4, and 6 are from the cultured *G. siphonifera* 3 symbiont. Lanes 7, 9, and 11 are digests of PCR products from the microdissected *Spongodrymus* 331 symbiont, and lanes 8, 10, and 12 are from the cultured *Spongodrymus* 331 symbiont. Lanes 1, 2, 7, and 8 are *Hae*III digests; lanes 3, 4, 9, and 10 are *Hin*fI digests; lanes 5, 6, 11, and 12 are *Taq*I digests. M = marker; 1 kilobase-pair ladder (Gibco/BRL).

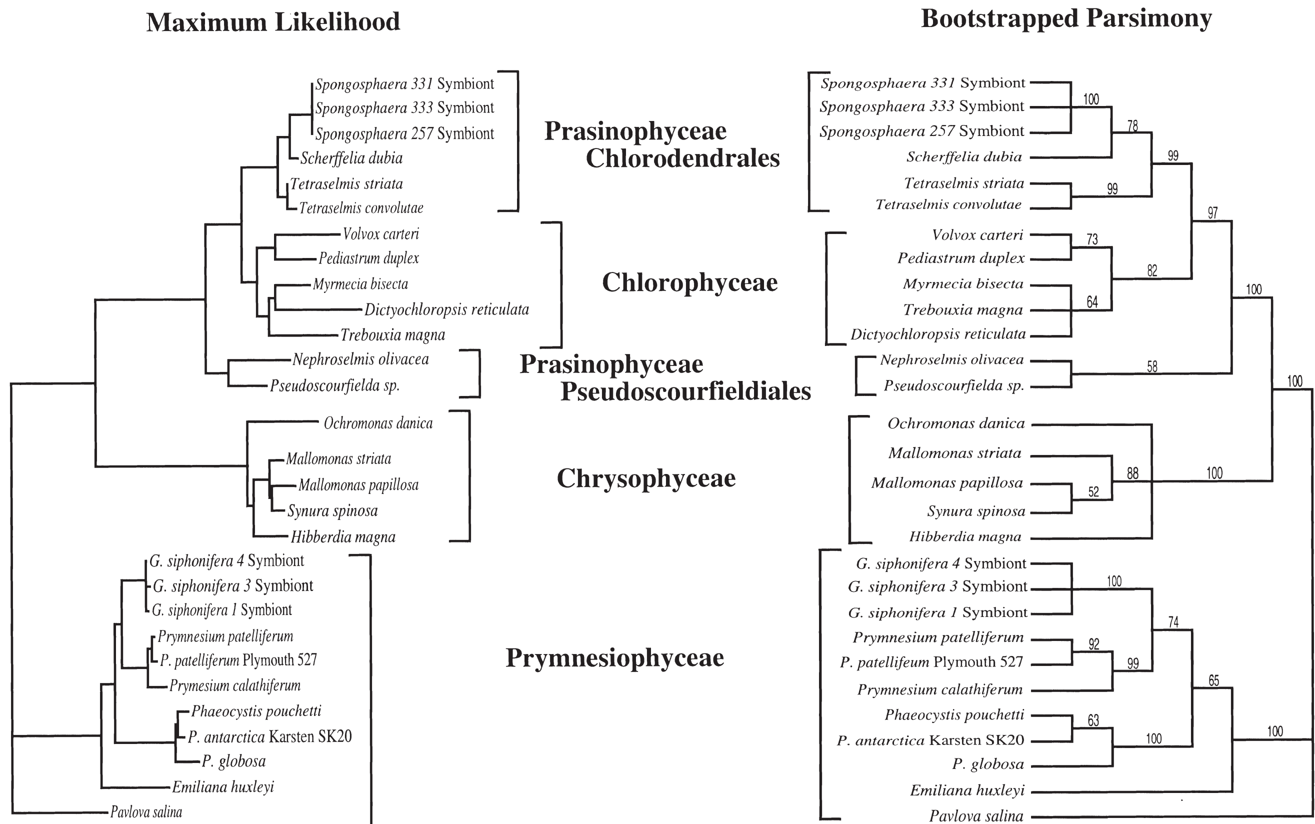

FIG. 3. Maximum likelihood and bootstrapped parsimony phylogenetic reconstructions. Bootstrap values are shown at the nodes on the parsimony tree. Both trees are unrooted.

symbionts would not be so unusual if they are associated with the two different *Globigerinella* species.

The *Spongodrymus* symbiont was very closely related to *Tetraselmis* and *Scherffelia*. *Spongodrymus* has been described as having at least three types of symbionts: the dinoflagellate *Scrippsiella* (Gast and Caron 1996), the prasinophyte described in this paper, and a third that is morphologically similar to the prymnesiophyte from *G. siphonifera* (Anderson 1983). It is unclear whether the different symbiotic algae are associated with different species of *Spongodrymus*, or whether one species can have different symbionts. If the same species does associate with different algae, this might be an interesting host in which to study the flexibility in choice of symbiont. In addition, the different algae are of great interest for examining the characteristics that make them all acceptable symbionts in a situation where we would normally see very strong specificity. Identifying species of *Spongodrymus* is difficult, but recent work on the molecular phylogeny of radiolaria has provided the tools to begin assessing speciation in these organisms (Amaral-Zettler et al. 1997).

Speculation on the evolution of the host and symbiont. We have been able to determine the phylogenetic relationships of four different algal symbiont types in planktonic sarcodines (this study and Gast and Caron 1996). Although sarcodines harbor a diverse collection of algal symbionts, there is very little genetic variation within a particular symbiont type. For example, dinoflagellate symbionts isolated from a variety of radiolaria were indistinguishable based on their srDNA sequences, regardless of the host species (Gast and Caron 1996). We now have evidence that the dinoflagellate symbionts of radiolaria (*Thalassicolla*) collected in the Pacific are similar to those in the Atlantic (R. J. Gast, unpublished data).

All sarcodine hosts must reacquire their symbiont from the environment during ontogeny. They are therefore exposed to a wide variety of algal species, but still exhibit a very strong selectivity for a particular one. This behavior is noteworthy given that photosymbioses in sarcodines appear to have arisen independently and repeatedly over evolutionary time (Norris 1996). Is there enough flexibility in symbiont selection to permit a host to acquire and maintain different symbiotic alga? Does this situation lead to the eventual speciation of the host, or vice versa? Both scenarios seem plausible at this time. Darling et al. (1997) established, based on srDNA sequence heterogeneity, that the planktonic foraminiferan species *G. siphonifera* could be considered two different species. We speculate that the Type I and Type II symbionts may differentially associate with these two host species. However, Darling et al. (1997) also showed that

the white and pink varieties of the planktonic foraminifera *Globigerinoides ruber* are just as diverse, but we found that they have the same symbionts as the rest of the *Orbulina–Globigerinoides* lineage. It appears that the flexibility for acceptance of alternative symbionts is present, but the role that it plays in speciation is still ambiguous.

The results from this research also provide interesting observations about the tendency of particular algae to establish symbiotic associations. All of these algal species share the distinction of being acceptable as symbionts and may help to provide insight into the basic genetic cues involved in symbiotic competence. There appears to be a predisposition of certain algal species towards being good symbionts. The most commonly studied algal symbionts are dinoflagellates, and there are several lineages that appear to be symbiotically competent. We have reported previously that a single dinoflagellate species is symbiotic with planktonic radiolaria and *Velella velella* (Gast and Caron 1996). A second dinoflagellate lineage includes the symbionts of the corals, anemones, benthic foraminifera (*Symbiodinium*), and planktonic foraminifera (*Gymnodinium beii*). In our current study we have established that, within the prasinophytes, *Tetraselmis* and the *Spongodrymus* symbionts appear to form a lineage of algal species that are also symbiotically competent. Whether this situation holds true for the prymnesiophyte symbionts is yet to be determined. Nondinoflagellate symbionts are common throughout the acantharia, nonspinose planktonic foraminifera, and radiolaria. Molecular analyses should be helpful for establishing the similarity (or dissimilarity) of these symbiont species to symbiont lineages already characterized.

We would like to thank the editor and two anonymous reviewers for their invaluable comments on our manuscript. This work was supported by NSF Grant OCE-9314533 and the WHOI Summer Student Fellow Program (WHOI contribution #9992).

Amaral-Zettler, L., Sogin, M. L. & Caron, D. A. 1997. Phylogenetic relationships between the Acantharea and the Polycystinea: a molecular perspective on Haeckel's radiolaria. *Proc. Natl. Acad. Sci. USA* 94:11411–6.

Anderson, O. R. 1983. *Radiolaria.* Springer-Verlag, New York.

Anderson, O. R., Swanberg, N. R. & Bennett, P. 1983. Fine structure of yellow-brown symbionts (Prymnesiida) in solitary radiolaria and their comparison with similar acantharian symbionts. *J. Protozool.* 30:718–22.

Bilofsky, H. S. & Burks, C. 1988. The GenBank genetic sequence data bank. *Nucleic Acids Res.* 16:1861–64.

Caron, D. A. & Swanberg, N. R. 1990. The ecology of planktonic sarcodines. *Rev. Aq. Sci.* 3:147–80.

Darling, K. F., Wade, C. M., Kroon, D. & Brown, A. J. L. 1997. Planktic foraminiferal molecular evolution and their polyphyletic origins from benthic taxa. *Mar. Micropaleontol.* 30:251–66.

Faber, W. W., Jr., Anderson, O. R., Lindsey, J. L. & Caron, D. A. 1988. Algal–foraminiferal symbiosis in the planktonic foraminifer *Globigerinella aequilateralis*: I. occurrence and stability of two mutually exclusive chrysophyte endosymbionts and their ultrastructure. *J. Foraminiferal Res.* 18:334–43.

Gast, R. J. & Caron, D. A. 1996. Molecular phylogeny of symbiotic dinoflagellates from Foraminifera and Radiolaria. *Mol. Biol. Evol.* 13(9):1192–7.

Gastrich, M. D. 1988. Ultrastructure of a new intracellular symbiotic alga found within planktonic foraminifera. *J. Phycol.* 23:623–32.

Lee, J. J., Hutner, S. H. & Bovee, E. C. [Eds.] 1985. *An Illustrated Guide to the Protozoa.* Society of Protozoologists, Lawrence, KS.

Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 71:491–9.

Norris, R. D. 1996. Symbiosis as an evolutionary innovation in the radiation of Paleocene planktic foraminifera. *Paleobiology* 22: 461–80.

Ostergaard, J. 1998. A new method for fixation of unmineralized Haptophytes for TEM (whole mount) investigations. *J. Phycol.* 34:558–60.

Rowan, R. & Knowlton, N. 1995. Intraspecific diversity and ecological zonation in coral–algal symbiosis. *Proc. Natl. Acad. Sci. USA* 92:2850–3.

Rowan, R. & Powers, D. A. 1991. Molecular genetic identification of symbiotic dinoflagellates (Zooxanthellae). *Mar. Ecol. Prog. Ser.* 71:65–73.

Saiki, R., Walsh, P. S., Levenson, C. & Erlich, H. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–94.

Spero, H. J. 1987. Symbiosis in the planktonic foraminifer, *Orbulina universa*, and the isolation of its symbiotic dinoflagellate, *Gymnodinium beii* sp. nov. *J. Phycol.* 23:307–17.

Swanberg, N. R. & Caron, D. A. 1991. Patterns of sarcodine feeding in epipelagic oceanic plankton. *J. Plankton Res.* 13:287–312.

Weekers, P. H. H., Gast, R. J., Fuerst, P. A. & Byers, T. J. 1994. Sequence variations in small-subunit ribosomal RNAs of *Hartmannella vermiformis* and their phylogenetic implications. *Mol. Biol. Evol.* 11:684–90.

Walsh, P. S., Metzger, D. A. & Higuchi, R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10(4):506–13.