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Review article

Algal symbiosis in larger foraminifera

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Abstract

Foraminifera with endosymbiotic algae abound in shallow tropical and semitropical seas. Diverse groups of contemporary foraminifera are the hosts for a wide variety of endosymbiotic algae (diatoms, dinoflagellates, unicellular chlorophytes, unicellular rhodophytes and/or cyanobacteria) or their plastids suggesting that foraminifera are particularly good partners for the establishment of symbioses. The fossil record supports this idea. Since the Pennsylvanian there have been evolutionary bursts of symbiont bearing lineages of foraminifera in shallow, well-illuminated tropical and semi-tropical seas. Two factors predispose symbiosis in the group: 1) their general cameral subdivision (this compartmentalizes and separates different cellular activities: e.g. digestion is spatially separated from symbionts) and 2) asexual reproduction insures vertical transmission of symbionts. Host-symbiont specificity in diatom-bearing foraminifera is not finical; the same host species can harbor any one of several dozen diverse species of pennate diatoms. *Nitzschia frustulum symbiotica* is the most common of the diatom symbionts, being found in ~30% of the associations. *Nanofrustulum shiloi*, *Nitzschia laevis*, *Nitzschia panduriformis* and *Amphora* spp. are also more common than the other symbiont species. Often a second species of diatom can be isolated from the same host. Experiments demonstrate that some endosymbiotic diatom species can replace others. Red cyanobacteria have been found in dinoflagellate-bearing soritines. Specimens of *Marginopora vertebralis* from Lizard Island also host small numbers of prymnesiids. Many questions about host-symbiont relationships remain to be explored. Calcification of symbiont-bearing species is enhanced in the light. Foraminifera seem selective in the species of algae they assimilate. A number of species (*Archais angulatis*, *Sorites marginalis*, *Amphisorus hemprichii*, and *Amphistegina* spp) cannot grow if they are starved, even when incubated in the light, suggesting that algal photosynthesis alone does not satisfy their needs. Starved *Heterostigina depressa*, in contrast, grew in the light in the absence of obvious feeding on algae, but feeding on bacteria was not ruled out. Each host species grows within a range of light intensity. Symbiont-bearing foraminifera migrate toward or away from light sources if conditions permit them to do so. Both field observations and laboratory experiments suggest that larger foraminifera, as a group, grow best in oligotrophic conditions. Growth of hosts with their symbionts in the laboratory is balanced in illuminated chemostats that continuously supply low concentrations of nutrients.

Keywords: Larger foraminifera, *Archais angulatis*, *Sorites marginalis*, *Amphisorus hemprichii*, *Amphistegina* spp, *Heterostigina depressa*, algal symbionts, diatom symbionts, *Nitzschia frustulum symbiotica*, *Nanofrustulum shiloi*, *Nitzschia laevis*, *Nitzschia panduriformis*, *Amphora* spp, chlorophyte symbionts, *Chlamydomonas hedleyi*, *C. provasoli*, cyanobacterial symbionts, *Symbiodinium*, host-symbiont specificity, diatom surface antigen signaling, host bleaching, carbon budgets, calcification

1. Introduction

Perhaps fueled by Hedley's review (1964) on the biology of foraminifera that expressed concern over the lack of contemporary evidence of the phenomenon, there has been a burgeoning interest in symbiosis in foraminifera. In fact, there has been a broad acceptance of the hypothesis that symbiosis was the driving force in the evolution of certain groups of foraminifera (Lee and Hallock, 1980). Environmental degradation of tropical and semitropical seas, coral bleaching and global warming has also kindled general interest in the adaptive value and stability of algal-

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invertebrate symbioses in oligotrophic habitats and brings with it many fresh ideas and applicable comparative data (e.g. Hallock, 2000; Hoegh-Guldberg, 1999).

2. The Players

Symbiosis seems to have originated independently in a number of separate lineages of foraminifera. Today we recognize 11 families in three orders that host endosymbiotic algae (Lee, 1992). Four families, Alveolinidae, Amphisteginidae, Calcarinidae, and Numulitidae host diatoms (Lee, 1994; Lee and Correia, 2005; Lee et al., 1989, 1992,). One superfamily Soritacea has families and subfamilies that host a variety of different algal types: Peneroplidae host unicellular rhodophytes (Hawkins and Lee, 1990; Lee, 1990); Archaiasinae, host chlorophytes (Lee et al., 1974; Lee et al., 1979; Pawlowski et al., 2001); and Soritinae hosts dinoflagellates (Doyle and Doyle, 1940; Leutenegger, 1984; Lee et al., 1997, Pawlowski et al., 2001; Pochon et al., 2001, 2004) and to a lesser degree cyanobacteria (Lee et al., 1997) and haptophytes (Hawkins and Lee, 2001; Figs. 1A and C). Members of the planktonic family Globigerinidae host dinoflagellates and chrysophytes (Anderson and Be, 1976; Faber et al., 1988, 1989; Spiro, 1987). Members of four other planktonic families Candeinidae, Pulleniatinidae, Hastigerinidae, Globorotaliidae, are also the hosts for chrysophytes (Gastrich, 1988).

Diatom endosymbionts

A closer look at our present state of knowledge on the identities of the hosts and their symbionts suggests that there are many aspects of this topic that need further study. We know the most about the diatom-bearing genera because the identities of the symbionts are easiest to establish. The distinctive features of their frustules (siliceous cell envelopes) are used to identify diatoms. However, *in hospite*, endosymbiotic diatoms do not form frustules. Fortuitously, they can be liberated from their hosts and, in suitable media, they grow, divide and form diagnostic frustules. They are all small (<10 µm) pennate diatoms. To date >2,500 diatom-bearing hosts have been examined (Lee and Correia, 2005). One species, *Nitzchia frustulum symbiotica*, has been isolated in ~30% of the hosts. *Nanofrustulum shiloi*, *Nitzschia laevis*, *Nitzschia panduriformis* and several species of *Amphora* are also more common than the 20 other species that also have been isolated from hosts (Lee and Correia, 2005). Often a second species and rarely a third can be isolated from the same host.

While this approach has given us the knowledge that any one of several dozen species can be a symbiont in a given host, many questions remain unanswered. Sampling thus far has been opportunistic and geographically quite random. Future studies of the symbiont should involve sampling the same population over the seasons and transects of habitats at various scales of distance. Many habitats have never been sampled at all. The distribution of diatom symbionts in relationship to light-depth has been barely been explored. Unlike the interest created in endosymbiotic dinoflagellates in corals and other marine invertebrates, there is a dearth of information about variation within the species of diatoms involved in symbiosis. Only *Nitzschia frustulum symbiotica*, whose description was broadened to reflect the range of morphological diversity found in isolates, has been studied in this respect (Lee et al., 2001).

Diatom plastids are also sequestered and function as temporary symbionts in a number of families of foraminifera (see review by Anderson and Lee, 1991; Correia and Lee, 2000, 2002a,b). Specimens of *Elphidium excavatum* retained approximately 3.7×10^4 diatom plastids in feeding experiments. Chlorophyte and dinoflagellate plastids were few in number and less than in starved controls (Correia and Lee, 2000). The half-lives of diatom plastids retained by starved *Elphidium excavatum*, incubated in the dark, was 9.5 weeks (Correia and Lee, 2002 b).

Chlorophyte endosymbionts

The archaiasines have been sparsely sampled. They all have green symbionts. To what taxon(s) do they belong? Our knowledge comes mainly from a collection of *Archaias angulatus* and *Cyclorbiculina compressa* from one field trip to Key Largo, Florida. The algal symbionts, *Chlamydomonas hedleyi* and *C. provasoli*, are very small and were described and distinguished from each other on the basis of the fine structure of their pyrenoids (Figs. 1B and D; Lee et al., 1979; Müller-Merz and Lee, 1976).

Molecular identities of the green symbionts in the other 5 genera of the subfamily have verified that all are species of *Chlamydomonas* belonging to the *C. eugametos* lineage and which cluster together suggesting a common ancestor. Their sequence divergence suggests that there may be more species than the two already described, *C. hedleyi* and *C. provasoli*, but this remains unresolved pending examination of their fine structure in the TEM or description of other attributes (Pawlowski et al., 2001). Much work remains before we understand the distributional parameters of symbionts in various hosts and localities in this subfamily.

Rhodophyte, cyanobacteria and haptophyte endosymbionts

A unicellular red alga, *Porphyridium purpurum*, has been isolated a number of times from *Peneroplis pertusus* and *P. planatus* collected at Taba, Red Sea (Lee, 1990; Hawkins and Lee, 1990). The simple fine structure of this organism made it easy to distinguish it as an already

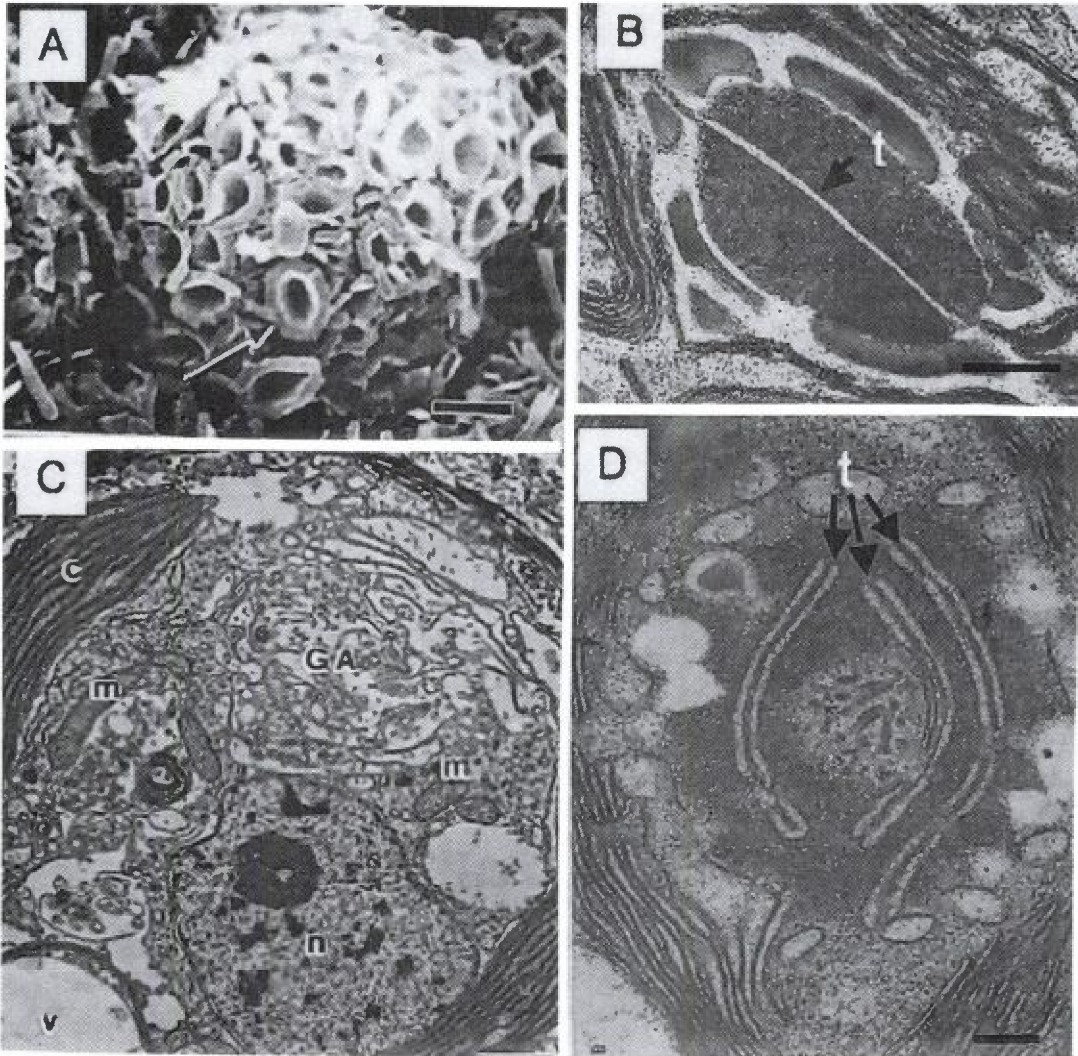

Figure 1. A. Whole cell of *Pleurochrisis* sp. Scale 1.5 μm. B. Thin section of *Chlamydomonas hedleyii* showing single thalakoid transversing the pyrenoid. Scale 500 nm. C. Thin section of *Pleurochrisis* sp showing nucleus (n), extensive Golgi apparatus (GA) mitochondria (m) and chloroplasts (c). Scale 800 nm. D. Thin section of *Chlamydomonas provasoli* showing multiple thalakoids transversing the pyrenoid. Scale 400 nm. (A is an SEM; B–D are TEMs).

described species, however, molecular genetic techniques have not yet given clues to the potential diversity of these unusual algal symbionts.

Red cyanobacteria are common in *Marginopora vertebralis* collected directly off shore from the Marine Station on Lizard Island on the Great Barrier Reef (Lee et al., 1997) (Lee, unpublished, see figure on the cover of this issue). They have been rarely observed as symbionts in *Amphisorus hemprichii* from the Gulf of Eilat (Lee, unpublished, see figure on the cover of this issue). Although they grew for a short time in culture they were never identified. Each of the *M. vertebralis* from the July 2000 collection from Lizard Island examined was also the host for ~20 haptophytes. These organisms were observed in histological sections (Lee et al., 1997) and were isolated in culture. Observations in the TEM and SEM allowed us to conclude that they were morphologically close to an already described species, *Pleurochrysis scherffelii* (Figs. 1A and C; Hawkins and Lee, 2001).

Dinozoan endosymbionts

Although a great deal of effort has been expended to gain an understanding of the dinoflagellate symbionts of the large subfamily soritinae, a large measure of uncertainty clouds the issue (LaJeunesse et al., 2003; Baker, 2003). Soritine symbiont sequences are quite diverse and mostly divergent from those found in cnidarians. Recently

published comparative sequence analyses placed soritine *Symbiodinium* within clades C, D, F, G and H. They dominated the latter 3 clades (Pawlowski et al., 2001b; Pochon et al., 2001, 2004). Several subclades are almost exclusively restricted to the soritines (Garcia-Cuetos et al., 2005). The *Symbiodinium* groups belonged to 3 clades and 5 subclades. Among the 22 soritine phylotypes found by Garcia-Cuetos et al. (2005), 14 showed strict symbiont specificity; they harbored only one group of *Symbiodinium.* Seven of the soritine phylotypes harbored 2 "groups" of symbionts and only one soritine was host for three "groups" of *Symbiodinium*. Although the types of *Symbiodinium* clades and subclades in soritines are restricted, present molecular systematic data does not provide strong evidence for co-evolution of soritines with their symbionts (Garcia-Cuetos et al., 2005).

Pochon and colleagues (manuscript in preparation) studied *Symbiodinium* haplotypes in soritines from 0–40 m depth in sites on Guam. Some *Symbiodinium* haplotypes had specific habitat preferences. The haplotype C91 was correlated with soritines collected at shallow depths (0–20 m) while the haplotype C92 was the symbiont in soritines living at 40 m. The C92 haplotype also dominated the deep water *Marginopora* (Mar III) from the Great Barrier Reef. Phylotype C91a was the symbiont in deep water (20–40 m) *A. kudakajimensis*. *Sorites*-specific haplotypes F5.1 and F5.1a were respectively correlated with deep and shallow depths. Pochon and colleagues (manuscript in preparation) also found significant seasonal variation in the symbionts of *Sorites* sp. they observed in Guam. Haplotype C91 was dominant between October 2002 and April 2003. That was followed by an increase in haplotype diversity in the summer.

There are currently eleven named species of the genus *Symbiodinium*: *S. microadriaticum*, *S. pilosum*, *S. kawagutii*, *S. goreaui*, *S. corculorum*, *S. californium*, *S. meandrinae*, *S. pulchrorum*, *S. bermudense*, *S. cariborum*, *S. linucheae* and *S. muscatinei* (Blank, 1992; Blanr and Trench, 1986). Several of the morphologically established species belong to the same genetic clade (eg. clade A) confounding boundaries that might be used to define how many "species" ("subspecies") should be recognized in this genus. The criteria previously used to separate and define species of *Symbiodinium* are presently being applied to a library of isolates of soritines (Lee and co-workers, in progress). None of the soritine symbionts has yet been assigned a specific epithet.

Soritine hosts

Until relatively recent times it was assumed that there was relatively little diversity among the soritines. Morphological (Gudmundsson, 1994; Lee et al., 2004; Cervasco and Lee, in progress) and molecular genetic (Holzmann et al., 2001; Garcia-Cuetos et al., 2005) studies have cast doubt on this assumption. During the course of our morphological studies we came to realize that the tests of soritines are fenestrated by pit lining tubules (Figs. 2B–D) and that there differences in wall structure among the soritines. Two new species have been described (*Amphisauris kudakajimensis* and *A. saurensis*), some species need amended descriptions, and several other forms are in the process of being examined for the possibility they too may be new species. Although Garcia-Cuetos et al. (2005) have molecular data suggesting host-symbiont specificity in this group, it seems prudent to reserve judgment on host-symbiont relationships in the sorintines until questions of host identity are resolved.

3. Specificity

There have been very few experiments on host-symbiont specificity in the foraminifera. Observations of specimens captured in the field make it clear that there is group specificity. Hosts that normally have diatom, chlorophyte, dinoflagellate etc. as endosymbionts have never been observed harboring different types of algae as major symbionts. Occasionally, our group has isolated *Chlorella* in diatom-bearing forms. This was tested experimentally as part of a study of the specificity of diatom symbionts (Lee et al., 1983, 1986), but broader experiments are feasible.

In re-establishment of symbiosis experiments, the hosts, *Amphistegina lessonii*, were rendered aposymbiotic by incubating them in seawater with DCMU (1×10^{-5} M (3-3,4-dichlorophenyl)-1,-dimethyl urea). They were then incubated in tissue culture flasks in the sea, at various depths, with randomized mixtures of diatoms and a chlorophyte (*Chlamydomonas provasolii*) which had been isolated as endosymbionts along with diatoms that had been isolated as free-living in the sea. After several weeks of incubation the experiment was terminated and the hosts with their re-established symbionts were examined. None of the free-living diatoms or *C. provasolii* was recovered from the "re-browned" hosts (Lee et al., 1983, 1986). Some endosymbiotic diatoms were recovered from the "rebrowned" hosts more frequently than others suggesting a "pecking order" of symbionts. *Nitzschia valdestriata* and *N. laevis* were the most successful and *Nanofrustulum shiloi* the least.

4. Cell Signaling, Establishment of Symbiosis, and Maintenance of the Symbiotic Phenomenon

The region of physical contact between partners, through which they exchange a broad range of signals, is always of interest to symbiologists. The protein profiles of diatom frustrules from 11 endosymbiotic species and 5 non-symbiotic species were compared by immunoblotting them

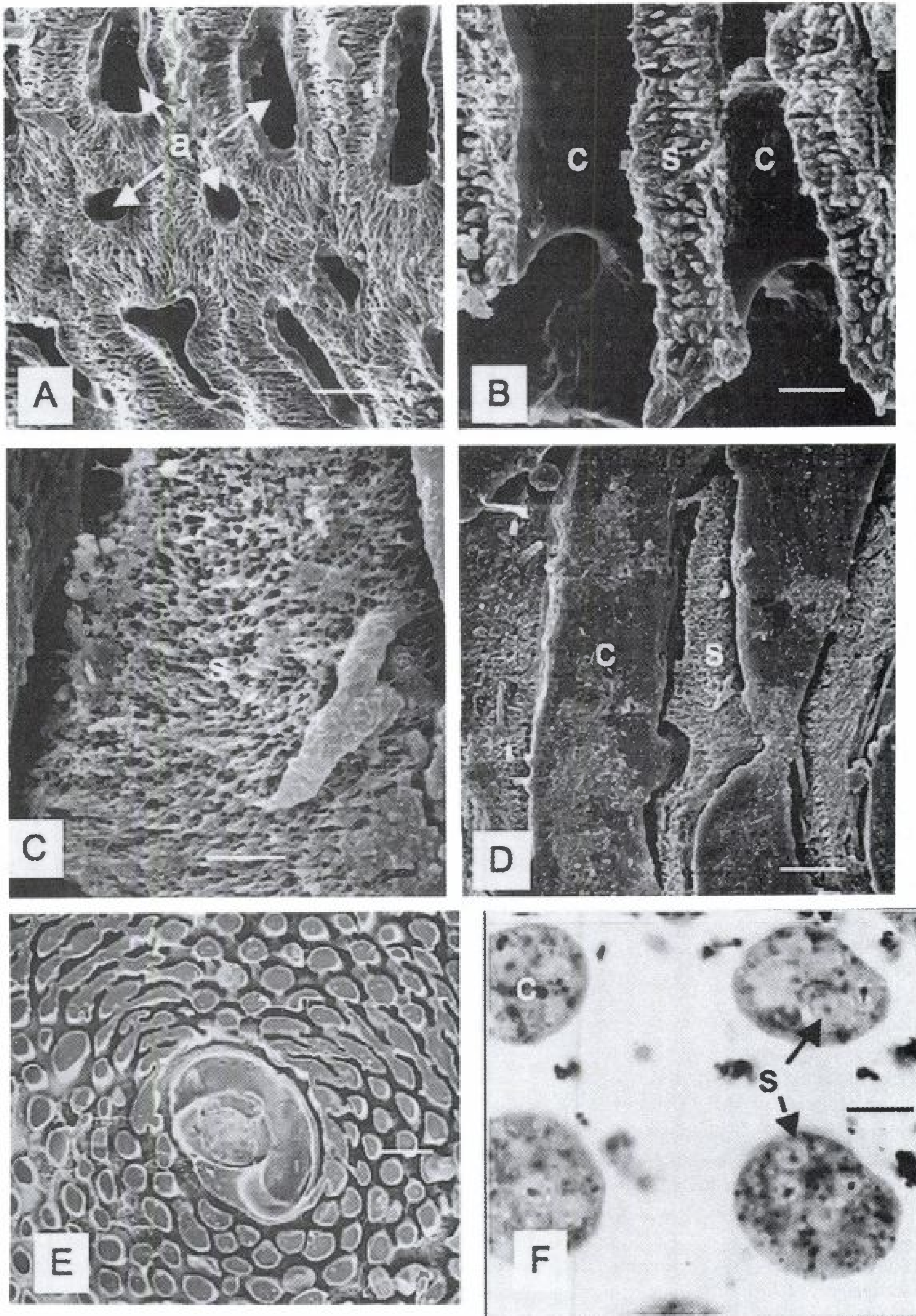

Figure 2. A. Apertural view of *Amphisorus hemprichii* from Taba, Gulf of Eilat, Red Sea, showing aperatures (a) and pitted test surface. Scale 25 µm. B. Fractured test of *A. hemprichii* showing that pits lead to tubules that infiltrate the septa (s) separating chamberlets (c). Scale 25 µm. C. Similar preparation of the test wall of another *Amphisorus* sp. collected seaward of the Interuniversity Institute showing that this species has finer tubules in its septa. Scale 5 µm. D. Hottinger (1979) cast of test wall showing the finer nature of the tubules at the same magnification as B. Scale 50 µm. E. Disc view of *Amphisorus hemprichii* showing chamberlets and their relative sizes compared to the thickness of the septa. Scale 100 µm. F. Histological section showing *Symbiodinium* sp. Symbionts (s) within the chambers. Scale 25 µm. (A–E are SEMs).

with polyvalent sera developed in rabbits against either *Nanofrustulum shiloi*, *Nitzschia frustulum*, *Nitzschia panduriformis* or *Amphora tenerrima*. A 104 kDa glycoprotein (CSSA, Common Symbiont Surface Antigen) was found on the surfaces of all the symbiotic species tested and was absent from the non-symbiotic species tested. (Chai and Lee, 1999a, 2000). Blocking this antigen with antibody caused a loss of the ability of the diatom to bypass digestion and be drawn into the test to become an endosymbiont within the foraminifera. Using immunocytochemical and fine structural techniques, they found that receptors for the CSSA were abundant on the pseudopodia making initial contact with the diatoms and on the primary organic lining of the test. Thus, it is clear that the initial recognition between the host foraminifer and the potential symbiotic diatoms is mediated by a cell signaling system involving molecules on the surfaces of diatoms and the pseudopods of the foraminifera. Soon after contact, the symbiotic diatom is phagocytosed and subsequently brought into the interior of the foram's test away from the active digestive processes (Chai and Lee, 1999b, 2000). The CSSA is produced by the diatom even after it has lost its normal cell envelope, and it seems necessary to maintain the association even after the association is established (Chai and Lee, 2000).

An important observation involving selective digestion by the marine amoeba *Trichosphaerium* Am1-7 suggests that symbiotic dinoflagellates may have a similar signaling system (Polne-Fuller, 1991; Rogerson et al., 1989). Trichosphaerium ingested *Symbiodinium* (strains #8, 45, 61 and 344), and surrounded them with a vacuole, but did not digest them. Later the *Symbiodinium* strains were egested by the amoebae in viable condition. We recently tested the diatom polyvalent antisera with the CSSA against the soritid *Symbiodinium* strains in our culture library and found that the antiserum did not have any affinity for the dinoflagellates cell envelopes (Lee and Reyes, 2006; see cover of this issue). Some different recognition molecule(s) must be involved in the *Symbiodinium*-soritine system.

Signaling must be involved in all of the foraminifera-algal symbioses because none of the endosymbionts form "normal cell" envelopes when they are within their host. In the case of *Porphyridium purpureum*, the alga has a thick viscous fibrillar sheath in culture but almost none in hospite (Lee, 1990).

In an experiment Lee and coworkers (1984) were able to show that an axenic homogenate of hosts (*Amphistegina*) could affect logarithmically growing symbiont cells. Firstly, the homogenate affected the formation of siliceous frustules as the cells grew and divided in culture. Secondly, the homogenate stimulated cells in culture to release ^{14}C labeled photosynthetate into the culture medium (Lee et al., 1984). The increase of release ranged from 190–9000%. A similar host homogenate effect was noted earlier in studies of zooxanthellae of cnidarians (Muscatine, 1967; Sutton and Hoegh-Guldberg, 1990).

Certainly the area of cell-to-cell signaling and interaction deserves attention in future research.

5. Nutritional Benefit – Cell Growth – Symbiont Control Experiments

Symbiologists raise many questions about the advantages of symbiosis to one or both partners in the association. The standard paradigm for corals and their zooxanthellae is that the latter, being photosynthetic, provide their hosts with a reliable source of fixed carbon. The animal, in turn, provides nutrients for the zooxanthellae through its catabolic pathways (Davies, 1984).

Hallock (1981a) developed an energetic model for algal symbioses in foraminifera and corals, predicting that the symbiosis provides such holobionts with literally orders of magnitude more energy than is available to non-symbiont animals living in nutrient-limited environments.

Symbiotic algae in axenic culture

One approach is to isolate the symbiotic algae in axenic culture and examine their nutritional needs. *Chlamydomonas hedleyi* isolated from *Archaias angulatus* grew best when urea (20 µM) was used as an N source in the medium. When NH_4 (2 µM) or NO_3 (20 µM) were used as N sources the total population growth was halved. Purines and pyrimidines did not serve as N sources for this alga. When urea was the N source, the optimum PO_4^{-3} was 0.1 µM, and was higher (1 µM) when NO_3^{-1} was used. No requirements for vitamins were demonstrated, however a supplement of thiamine boosted growth (Lee et al., 1974).

A similar study of *C. provasolii* from *Cyclorbiculina compressa* also showed that vitamins (B12, biotin and thiamine) stimulated growth. The alga grew well when ~200 µM of either NO_3^{-1} or NH_4^{+1} were the N sources. Maximum growth of the alga was obtained in media with 100 µM PO_4^{-3} (Lee et al., 1979). There is really no context for evaluating the high levels of N and P that stimulate the growth rates of these particular algae. The foraminifera hosting these symbionts are found in species-rich epiphytic microenvironments on leaves in meadows of *Thalassia*, which in turn, are bordered by lush mangrove habitats.

Comparative nutritional studies of endosymbiotic diatoms isolated from diatom-bearing hosts from the oligotrophic waters of the Gulf of Eilat can be evaluated in a different context (Lee et al., 1980). All 8 of the isolates tested required exogenous thiamine for growth. Biotin stimulated the growth of 6 clones and one clone of *Nitzschia frustulum symbiotica* required vitamine B12. The optimum concentration of NO_3^{-1} varied among the clones tested from 2 µM to 2 mM, which is considerably higher

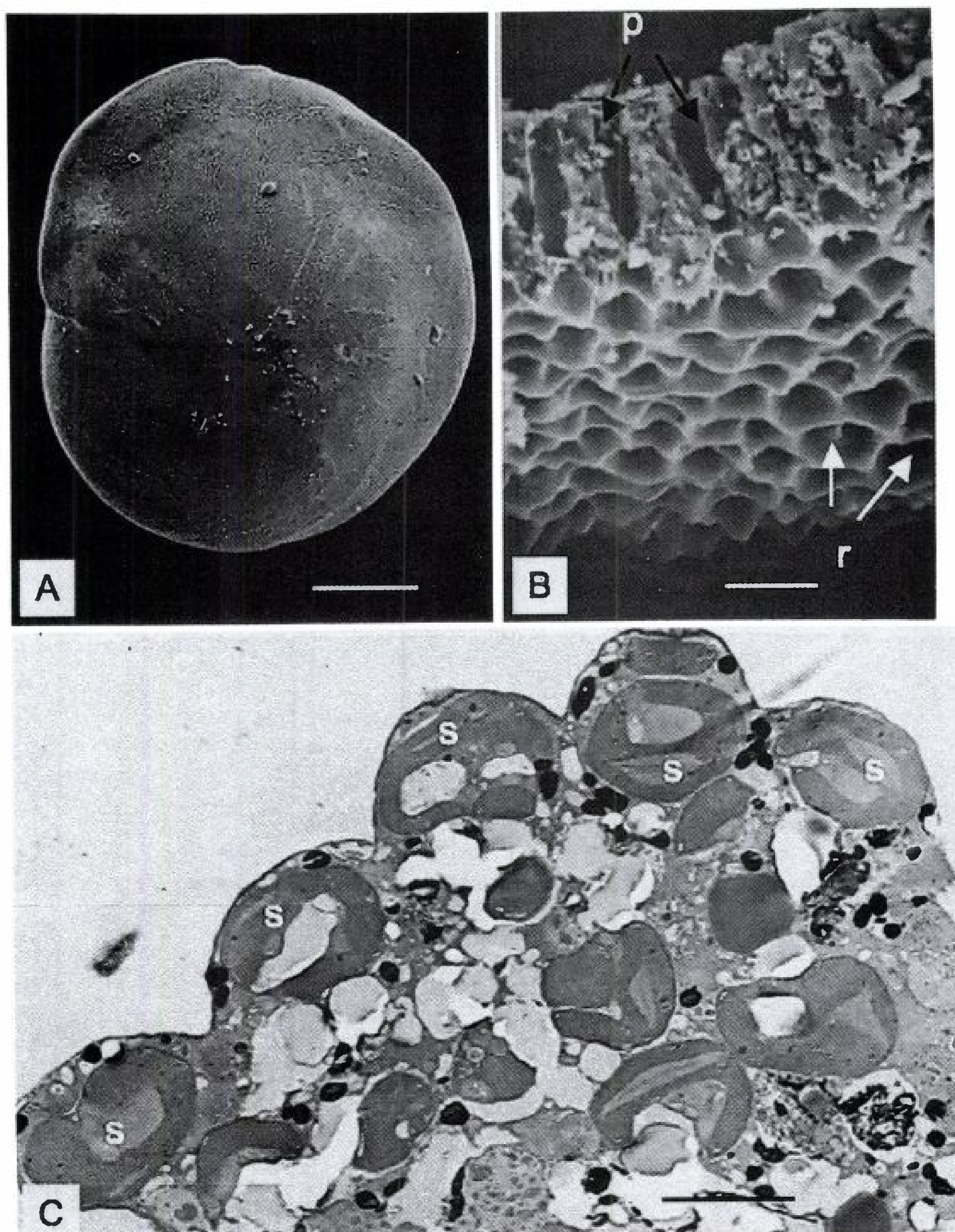

Figure 3. A. Whole cell view of *Amphistegina lessoni*. Scale 500 μm. B. Broken piece of the test wall of *Amphistegina lessoni* showing the pores through the test wall and the cup-like pore rims on the interior of the test wall (r). Scale 25 μm. C. Thin section of the peripheral cytoplasm of *Amphistegina lessoni*. The test has been removed in this preparation and the cytoplasm bulges where the individual symbionts (s) are pressed into the pore rims of the test. Scale 9 μm. (A and B are SEMs; C is a TEM).

than the values measured in the Gulf (1 μg/l) (Levanson-Spanier et al., 1979) at the depth where the foraminifera were captured. A similar result was observed when P was the limiting nutrient. Values for maximum growth varied among clones from 1 μM to 100 μM also exceeding the average value of P (0.3 μg/l) in this Gulf. This suggests that the growth of the symbiotic algae in their hosts is always nitrogen and phosphorus limited and indicates why the species of algae that serve as symbionts are rarely found free-living in the Gulf.

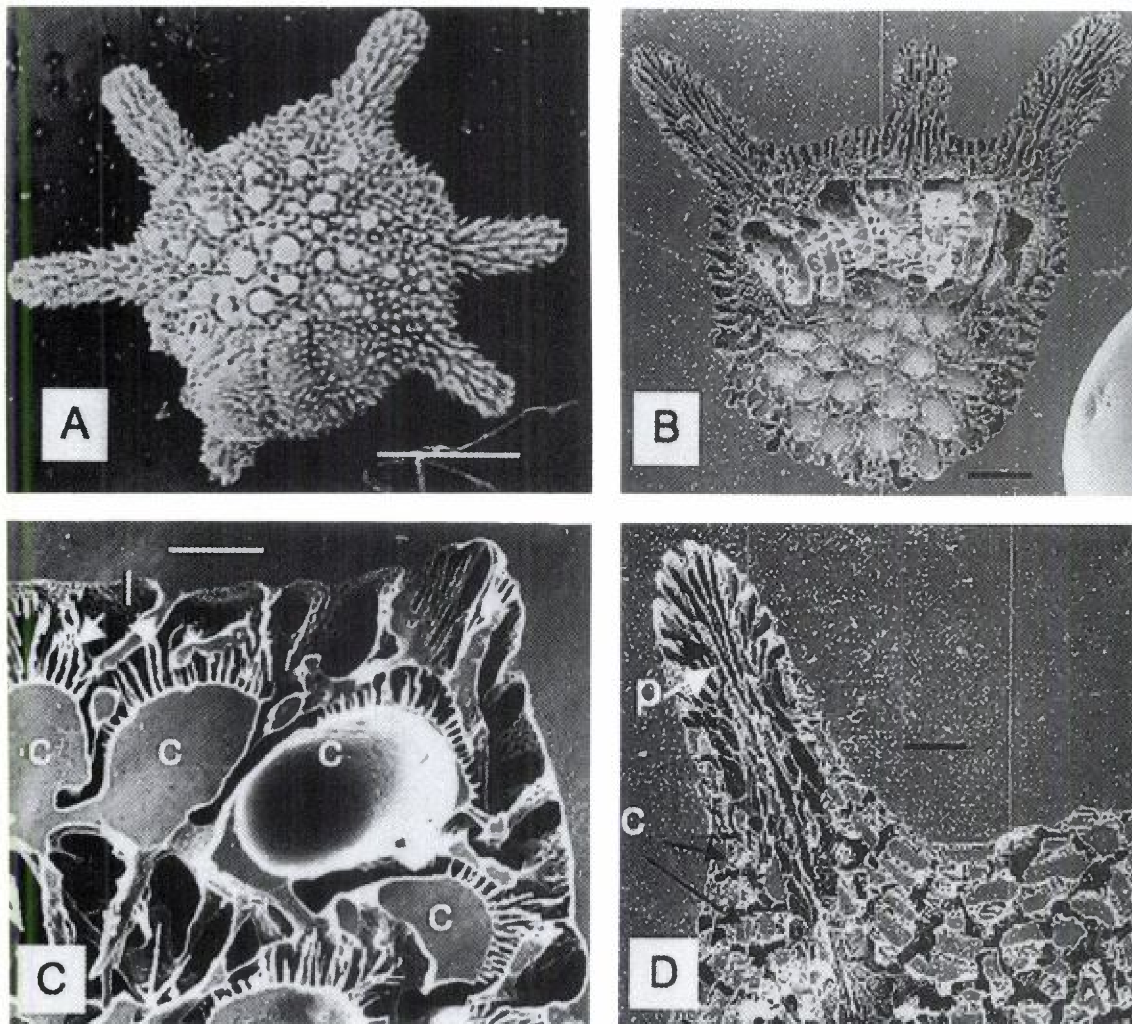

Figure 4. A. Whole organism view of *Calcarina hispida* form *spinosus*. Scale 500 µm. B–D. Sections of the interior of *Calcarina hispida* form *spinosus* prepared by the Hottinger casting method showing chamberlets (c), pore liners and pore canals leading to chamberlets (p). B. Scale 500 µm. C. Scale 100 µm. D. Scale 200 µm. (All figures are SEMs).

Holobiont dissolved nutrient studies

Researchers working with corals have concluded that zooxanthellate corals are successful because they are effectively closed systems with respect to dissolved inorganic nitrogen (Falkowski et al., 1993; Hallock, 2001). The population density of the zooxanthellae is controlled by systematic N limitation within the host. When the level of external N is elevated, as when a habitat becomes eutrophic (Hallock and Schlager, 1986; Falkowski, 1993; Hallock, 2001), the zooxanthellae outgrow their hosts and the host loses control over its symbiotic algae. These ideas seem applicable to the relationships of larger foraminifera and their algal symbionts. The idea that the host can lose control of the growth of their symbiotic algae was also proposed by Hallock (2000). Talge and Hallock (2003) reported consistent increased densities of diatom symbionts in *Amphistegina gibbosa* maintained in nutrient-enriched media, as compared with symbiont densities in specimens recently collected from the field.

Holobiont feeding, nutrients and light

Simulating conditions at 25 m in the Gulf of Eilat, *Amphisorus hemprichii* grew as fast as 37 µm per week when they were fed mixed species of algae isolated from their normal habitat, incubated in the light and in media that were changed weekly (Lee et al., 1991b). They did not grow when incubated in the dark and all were dead after 8 weeks of incubation in the dark. In a similar experiment, *A. lobifera* survived longer (13 weeks). Growth rates dropped dramatically when the medium was changed less frequently

(every 3 weeks). In experiments in which the foraminifera were incubated in chemostats, *A. lobifera* species and *Marginopora kudakajimensis* withdrew nitrate and phospate from the medium. Enrichment of the media in the chemostats with either nitrogen, phosphate or both to levels of above 8 μM NaH_2PO_4 and 1.5 mM $NaNO_3$ led to algal overgrowth and eventual death of the hosts.

Isolated diatom symbionts show considerable differences in their growth rates or photosynthetic rates when they are incubated at different light intensities (Lee et al., 1980; Lee et al., 1982). A number of researchers have attempted to evaluate the relative contributions of light (photosynthesis of symbionts) and/or feeding to growth and survival of the hosts. Röttger et al. (1980) found that *Amphistegina lessonii* would not grow in the dark even when it was fed autoclaved mashed *Cladophora socialis*, detritus or yeast. Both *Amphistegina lessonii* and *Heterostegina depressa* grew best 600–800 lux and were inhibited at higher light levels. Hallock (1981b, 1986) found that *Amphistegina* grew much better at 2600 μW cm^{-2} than it did at 300 μW. Hallock et al. (1986) compared the growth of *Amphistegina gibbosa* and *A. lessonii* at several light levels. Additional light experiments done by Hallock's group (e.g. Hallock et al., 1995; Williams and Hallock, 2004) are mentioned below in a different context.

Nutritional and other physiological experiments are hard to judge in absolute terms because so many of the researchers have used traditional nutrient enriched media (e.g. Erdschrieber) and, for practical reasons, local mesotrophic sea water rather extreme oligotrophic natural sea water from the natural habitats where the larger foraminifera are found. Of course, some experiments have been done in marine laboratories (e.g. IUI [InterUniversity Institute] in Eilat) near the sites of collection. Studies with artificial sea water formulations have not been reported. But even this issue is not a clear one because, even though the sea water in the natural habitats may be oligotrophic, the larger foraminifera are actually living and feeding in rich epiphytic or epilithic microbial communities. Hallock et al. (1991) reasoned that the high surface-to-volume ratio of larger foraminifera could be quite advantageous in taking up nutrients from plant or sediment surfaces, thereby providing the potential for the holobiont to live essentially autotrophically.

Feeding and carbon budgets

The role of feeding in the carbon budget of larger foraminifera and the comparative nutritional value of different species of food have been the focus of a number of different studies (Lee and Bock, 1976; ter Kuile et al., 1987; Lee et al., 1988; Faber and Lee, 1991b). Though it would seem that it would be simple to measure feeding rates, several factors make it difficult to make accurate assessments of feeding rates. First, feeding in foraminifera is episodic. Second, everything that is captured is not ingested, digested or assimilated. Third, there is a great deal of recycling of nutrients between host and its symbiotic algae, a factor that needs to be carefully considered when using radionuclide tracer methodology.

Early studies by Röttger (1972a) with *Heterostegina depressa* suggested that this species can grow without feeding if it is incubated in the light. The protocol he used did not rule out the possibility that this foraminifer could have been feeding on bacteria. Radionuclide tracer and respirometric studies of *Archaias angulatus* and *Sorites marginalis* suggested quite the opposite was true for these species (Lee and Bock, 1976). Feeding was the more important process even at midday. The ratio of carbon gained by feeding to primary production was >10:1. The rate of primary production was generally higher in *A. angulatus* than in *S. marginalis*. Depending on age (size) of the experimental specimens, juveniles of both species deposited ~4% of dry weight Ca in their tests (shell) per day.

Because of their abundance near the Inter-University H. Steinetz Biological Laboratory on the Gulf of Eilat, Red Sea, some of the most detailed studies on feeding, carbon budgets and calcification have used *Amphistegina lobifera*, *Amphisorus hemprichii* and *Peneroplis planatus* as experimental organisms. Selective feeding was found in *P. planatus*. It ingested five times more ^{14}C labeled *Cocconeis placentula* and *Amphora* sp than other algal species tested (Faber and Lee, 1991b). *P. planatus* did not grow if starved. It grew slowly when fed, but incubated in the dark. This organism was unusual in that its assimilation rates for some algal species was very high (~100%) for the first 24 hrs. The data suggested that even though light is necessary for growth of *P. planatus*, it acquires most of its carbon and energy for growth from food and cannot grow solely on carbon compounds fixed, transformed, and released by its endosymbiotic algae (Faber and Lee, 1991b).

The photobiological effect on foraminiferal growth and calcification has been demonstrated many times (Lee and Zucker, 1969; Dugay and Taylor, 1978; Duguay, 1983; ter Kuile and Erez, 1987; Muller, 1978; Hallock, 1981b; Röttger et al., 1980). Ter Kuile and coworkers (1987) starved their experimental organisms, *Amphistegina lobifera* and *Amphisorus hemprichii*, before beginning their feeding experiments. Under these experimental conditions, which were attempting to model the episodic behavior observed in the microscope, feeding was initially voracious and then slowed down after 8–24 hours. Less than 5% of the carbon taken up as food ended up being incorporated into the test (shell).

Using *Amphistegina lobifera* in an experiment to test whether dissolved inorganic phosphorous or nitrate in the medium could be a substitute pathway for these nutrients gained by feeding, both enhanced growth for at least two weeks. Growth was five times greater in fed, or medium-

enriched organisms, than it was in starved ones. Fed organisms grew slightly faster than medium enriched ones. The growth of *Amphisorus hemprichii* was stimulated only two-fold in a parallel experiment. The researchers concluded that their observations indicated that *A. lobifera* uses feeding mainly as a source of nitrogen and phosphorus, while *A. hemprichii* relies on food to satisfy its energy and carbon requirements, as well as nitrogen and phosphorus.

Feeding, light and calcification

Ter Kuile (1991) concluded that *A. lobifera* and *Amphisorus hemprichii* differ also in their calcification mechanisms. Experiments using DCMU and carbonic anhydrase suggested that there is a competition for inorganic carbon between photosynthesis and calcification in *A. lobifera*, while he found none in *A. hemprichii*. Observations led him to conclude that the symbionts in *A. lobifera* take up inorganic C in the form of CO_2 from the seawater and the CO_2 deposited in the test comes from an internal pool destined for this purpose. *Amphisorus hemprichii* does not have an internal pool. The CO_2 uptake is not energy dependent and is more easily modeled by diffusion.

Transfer of photosynthates

The nature of the photosynthate product(s) released by the symbionts to their hosts has not yet received much attention. Wilen, as part of team (Lee et al., 1984) studying the effects of host homogenate on the growth of endosymbiotic diatoms, found that mannitol was the principle radionuclide labeled metabolite. In a more detailed study Kremer et al. (1980) used ^{14}C to follow the photosynthates in 6 intact algal-foraminifera associations. They identified floridoside (2-0-D glycerol-D-galacto pyranoside) and polyglucan in extracts from *Peneroplis arietina*, and *P. pertusus*. They found 74% of the ^{14}C label in extracts from *Amphisorus hemprichii* was in lipids and 3.5% was in glycerol. In *Amphistegina lessonii* (31%), in *A. lobifera* (51%), and in *Heterostegina depressa* (33%) of the label was also found in lipids and glycerol. Other methodology would be necessary to demonstrate the pathway from symbiont to host, but it is reasonable to speculate that glycerol is the key metabolite transferred in these associations. Clearly this aspect of the symbiotic phenomenon in foraminifera needs more research attention.

Global change

Hallock and her students (Hallock, 2000; Talge and Hallock, 2003; Williams and Hallock, 2004) have looked at light-nutrient interactions from the perspective of global change. Hallock (2000) feels that progressive eutrophication of coastal systems is a serious issue for all symbiont-bearing benthic organisms including the larger foraminifera. In her view the species of Calcarinidae, Soritidae and Amphistigidae that are restricted to the shallowest reef-flat and reef-margin habitats are most at risk not only because of eutrophication, but also from increasing biologically damaging UVB. Talge and Hallock (2003) studied the intracellular damage associated with bleaching of natural populations on the Florida Reef tract. Field-collected, normal-appearing *Amphistegina gibbosa* had 5 times more viable symbionts and one third as many apoptotic symbionts as did partially bleached specimens. Experimental foraminifera exposed to light intensities >13 µM photon m^{-2} s^{-1} were similar in fine structure to partially bleached field-collected specimens. Depending upon intensity and water temperature, photic-stress induced cytological changes within days to weeks. ATP concentrations were higher in partially bleached, field-collected and experimentally photic-stressed specimens than they were than in normal freshly collected specimens. In the laboratory, Williams and Hallock (2004) studied the influence of spectral quality of photosynthetically active radiation (PAR) and UV on the growth rates and bleaching of *Amphistegina gibbosa*. They grew when PAR was >5 µmol photon m^{-2} s^{-1} and were saturated at 6–8 µmol photon m^{-2} s^{-1}. Growth rates increased in blue light and were not influenced by 0.0162 W m^{-2} UVB. However, when the UVB was increased tenfold (0.105 W m^{-2}), growth was significantly inhibited. Bleaching increased with increased PAR photon flux densities and with exposure to shorter wavelengths. Photoprotective darkening was seen in specimens which were exposed to UVB to PAR ratios >0.003.

6. Test Structure and Symbiont Location

We have noted that one of the characters which predisposes foraminifera to be hosts for algal symbionts is that their digestion begins in the granuloreticular network just after the pseudopods contact their prey (Faber and Lee, 1991a). Once the alga, in a phagosome, escapes initial digestion and its surrounding membrane is converted to one of a symbiosome, it is drawn into a foraminiferal test and is spatially separated from most digestive activity. Larger foraminifera are so morphologically modified from their ancestors that it is most likely that the evolutionary changes were driven by adaptation to symbiosis (Lee and Hallock, 1987). The functional anatomy of larger foraminifera has been a subject that has attracted a number of researchers (reviews by Hallock, 1985; Hallock et al., 1991; Hottinger, 1978, 2000). The greenhouse nature of the transparent tests and the canals and pores of many larger foraminifera are seen as adaptations to symbiosis. Experimental evidence on carbon fixation and calcification has shown differences in

how the test structure must affect the mechanisms of these processes in foraminifera with imperforate and perforate tests (e.g. Kuile ter, 1991; Kuile ter and Erez, 1987) but for the most part there have been few other experimental attempts to probe the physiology of pore or canal function (i.e. Leutenegger and Hansen, 1979). In some perforate larger foraminifera (e.g. *Amphistegina* spp.) it has been noted that the symbiotic algae are located at the periphery of the cytoplasm just under expanded pore rims (Figs. 3A–C). The cytology of other genera (e.g. *Calcarina* spp. Figs. 4A–D) have not been studied and we do not know the relationships of their symbionts to the specialized structures that presumably serve to increase nutrient and gas exchanges. This topic is obviously a ripe target for future research.

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