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Review article

"Living Sands" – Larger Foraminifera and Their Endosymbiotic Algae

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Abstract

All of today's larger foraminifera ("living sands") are hosts to endosymbiotic algae. Members of various soritacean families (Peneroplidae, Archaiasidae, Soritidae, Alveolinidae) in contemporary tropical and semitropical seas are hosts for unicellular red, chlorophyte, dinoflagellate and diatom endosymbionts, respectively. Although the hosts seem to require the algae, because they die in the dark, even when they are fed, or in the light when their algal partners are inhibited by DCMU (3-3,4-dichlorophenyl)-1,1-dimethyl urea), the algal symbionts do not seem to require their hosts. They grow well in ordinary laboratory media when they are liberated by experimental manipulation. A cladistic analysis of the superfamily Soritacea, splits it into 3 clades (Gudmundsson 1994). Each clade is a host to a different algal type (rhodophyte, chlorophyte, or dinoflagellate). This symbiont diversity is in contrast to the corals, in the same warm, well illuminated seas, which are hosts only to many kinds of dinoflagellates. Since most of today's scleractinian families originated at various times in the Mesozoic, and since they are all hosts for dinoflagellates, it is reasonable to assume that the later evolving soritids acquire(d) their zooxanthellae from environmental pools contributed by the corals in their habitat. Diatom-bearing hosts are not finical in their relationships with their endosymbionts. Although any host can harbor any one of a score of

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taxonomically diverse pennate diatom species, six species, *Nitzschia frustulum* var. *symbiotica*, *N. panduriformis*, *N. laevis*, *Fragillaria shiloi*, *Amphora roettgerii*, and *A. erezii*, are the most common. These species occur in 75% of all the associations. In about 20% of the associations two diatom species were isolated from individual hosts. Polyclonal antibodies against symbionts show that these diatoms share in common a 104 kDa surface antigen not found on diatoms which are digested by the hosts.

Keywords: Larger foraminifera, algal endosymbiosis, diatoms, Peneroplidae, Archaiasidae, Soritidae, Alveolinidae, Calcarinidae, *Porphoridium purpurum*, *Chlamydomonas hedleyii*, *Symbiodinium*, *Archaias*, *Amphisorus*, *Amphistigina*, *Calcarina*, *Neorotalia*, *Marginopora*, *Nitzschia frustulum* var. *symbiotica*, *N. panduriformis*, *N. laevis*, *Fragillaria shiloi*, *Amphora roettgerii*, *A. erezii*

1. Introduction

Although they are not as prominent in undersea vistas as are the giant clams and corals, larger foraminifera are quite abundant in the same tropical and semitropical habitats. Once you know about them, they are quite conspicuous. Easily visible to the unaided eyes, they are spectacularly large, considering the fact that they are protists. "Larger foraminifera" is a collective, rather than a taxonomic, term. These foraminifera share in common two characters: they are large (0.1–6 cm), often 10 times larger than their ancestors, and they form associations with endosymbiotic algae. Modern larger foraminifera belong to seven families in two different orders. We also can recognize "larger foraminifera" in the fossil record from well illuminated seas going back to the late Paleozoic. The best known of these fossil deposits is the Eocene nummulitic limestone which was used to build the Egyptian pyramids. The fusulinids are extremely abundant fossils from the late Paleozoic and we presume from their size and complexity that they too were symbiont-bearing. Ross (1974) recognized larger foraminifera in at least 25 modern and fossil families, but taxonomic revisions to Foraminifera since that paper has increased the number (Loeblich and Tappan, 1988). The total number of families is really not the issue. The point is that many times in the past, when the seas were warm, shallow, well illuminated, and presumably oligotrophic, unrelated groups of foraminifera seemed to have established relationships with endosymbiotic algae. Descendants of these groups increased in size, with great surface to volume relationships, and developed complex internal structures, seemingly as adaptations to symbiotic relationships (Lee and Hallock, 1987). Endosymbiotic algae are also found in five modern families of planktonic foraminifera (Lee and Anderson, 1991).

Table 1. Diversity of symbionts and hosts in "Living Sands"

Family	Shape	Symbionts	No. of extant genera in family	Figures	Typical genus
Peneroplidae	Fan	Rhodophytes	3	5 & 6	*Peneroplis*
Archaiadae	Disc-fan	Chlorophytes	6	1	*Archaias*
Soritidae	Disc-fan	Dinoflagellate	3	23	*Marginopora*
Alveolinidae	Pencil	Diatom	2	–	*Borellis*
Amphisteginidae	Spheroid-hemispheric	Diatom	1	11	*Amphistegina*
Calcarinidae	Stars	Diatom	4	17	*Calcarina*
Nummulitidae	Coin	Diatom	4	–	*Heterostegina*

Diversity is also seen in the types of endosymbiotic algae (Table 1). Different families of modern foraminifera harbor chlorophyte (Figs. 1–4), unicellular rhodophyte (Figs. 5–7, 10), diatom (Figs. 8 and 9, 11–21), dinoflagellate (Figs. 23–29, 31 and 32) and cyanobacterial (Fig. 22) endosymbionts, respectively. In light of the diversity in both hosts and symbionts, it seems reasonable to conclude that foraminifera are particularly prone to form endosymbiotic relationships with algae.

2. What Biological Features Underlie the Predisposition of Foraminifera to Form Endosymbioses with Algae?

Foraminifera are separated from other amoeboid protists by their characteristic bi-directional granular pseudopodia which leads them to be placed into the Phylum Granuloreticulosea. It is easy to observe the granular two-way streaming in their reticular pseudopodal networks under a phase microscope at magnifications of 600 × and higher. Food is captured in the spider web-like pseudopodial network. Algal husbandry microhabitats are well separated from host digestive activities (Müller-Merz and Lee, 1976; Lee and Hallock, 1987). Cytochemical assays using napthol AS-BL phosphate for the presence of acid phosphatase (Sigma diagnostic kit #387A) on 14 species of foraminifera, some with symbionts, others without, showed that digestion begins in the pseudopodial web (Lee et al., 1991; Faber and Lee, 1991). Acid

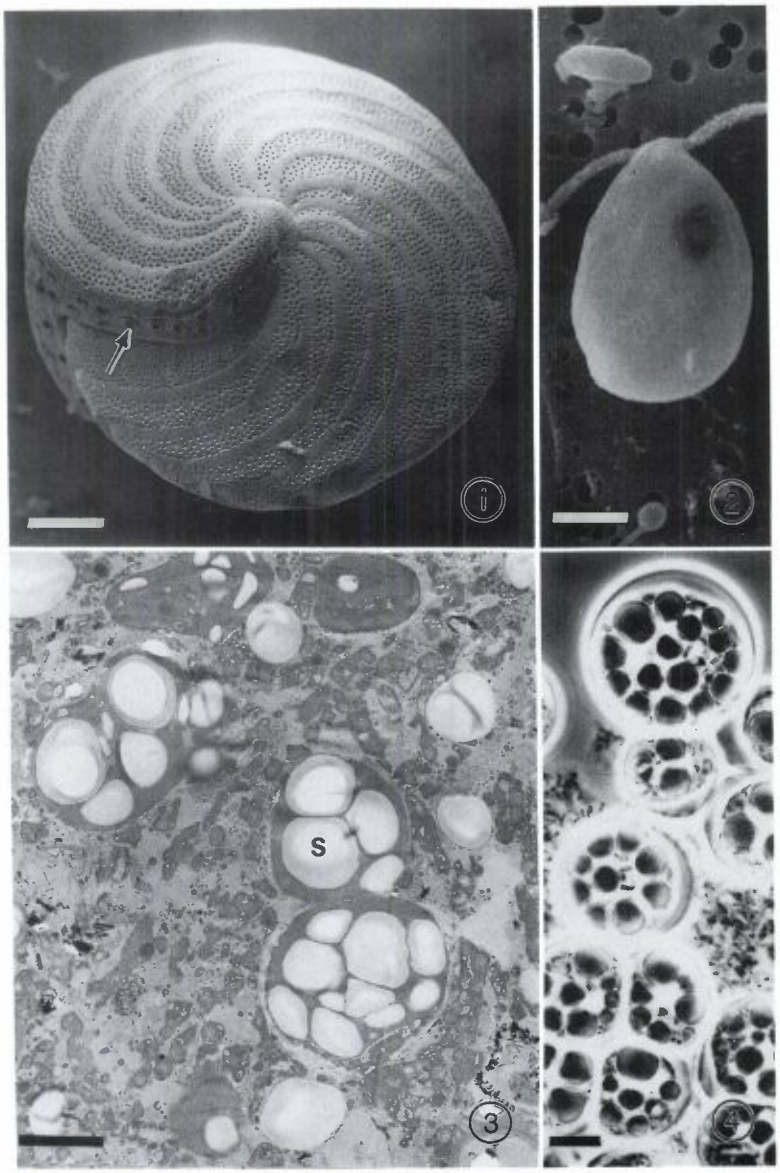

Figure 1. SEM of *Archaias angulatus*, a chlorophyte-bearing larger foraminifer showing apertures (arrow). Bar = 100 µm.

Figure 2. SEM of *Chlamydomonas hedleyi*, the endosymbiotic alga found in *Archaias angulatus*. Bar = 1 µm.

Figure 3. TEM of *Chlamydomonas hedleyi* in *Archaias angulatus*. Note that the cells are filled with starch (S) and that there are similar grains in the cytoplasm. Bar = 5 µm.

Figure 4. Phase contrast of *Chlamydomonas hedleyi* freshly released from their host showing the abundance of starch grains. Bar = 4 µm.

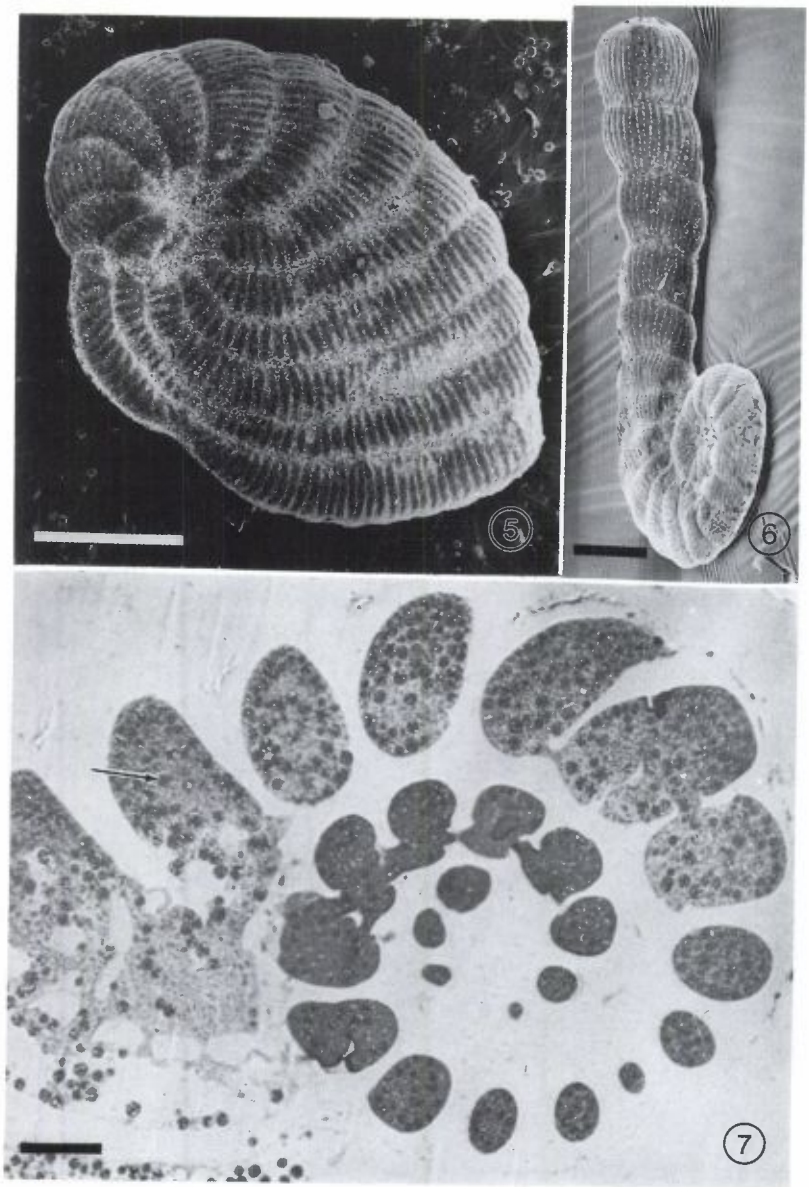

Figure 5. *Peneroplis pertusus*, a host of the rhodophyte, *Porphyridium purpureum*. Bar = 400 µm.

Figure 6. *Peneroplis arietina*, a host of the rhodophyte, *Porphyridium purpureum*. Bar = 400 µm.

Figure 7. A histological section through *Peneroplis planatus*, showing the fairly uniform distribution of the endosymbiotic *Porphyridium purpureum* throughout the host. Bar = 100 µm.

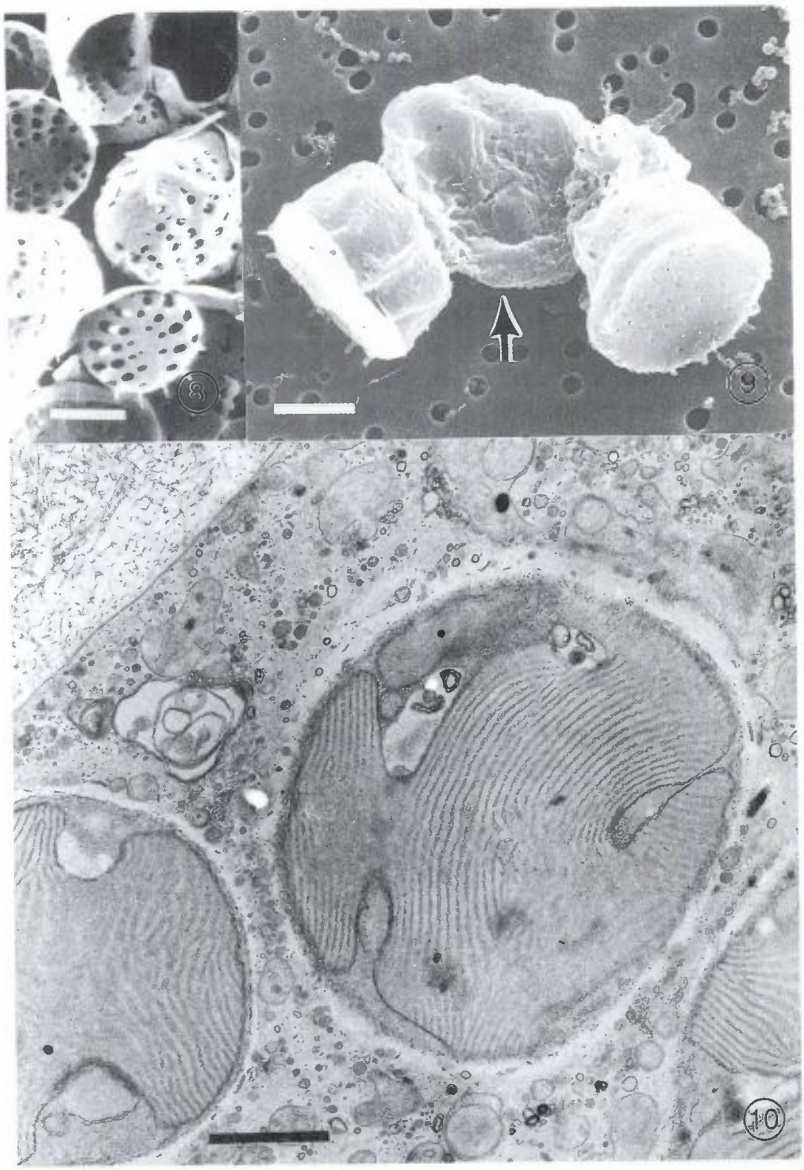

Figure 8. SEM of frustules of *Fragilaria shiloi*, a common endosymbiotic diatom. Bar = 4 µm.

Figure 9. SEM of frustules of *Fragilaria shiloi* growing in a medium to which host homogenate has been added. Note that no frustule has been built in the newly formed daughter cell (arrow). Bar = 2 µm.

Figure 10. TEM of *Porphyridium purpureum* in situ within its host. Note that no sheath is present; symbiont is not surrounded by a symbiosome membrane. Bar = 1 µm.

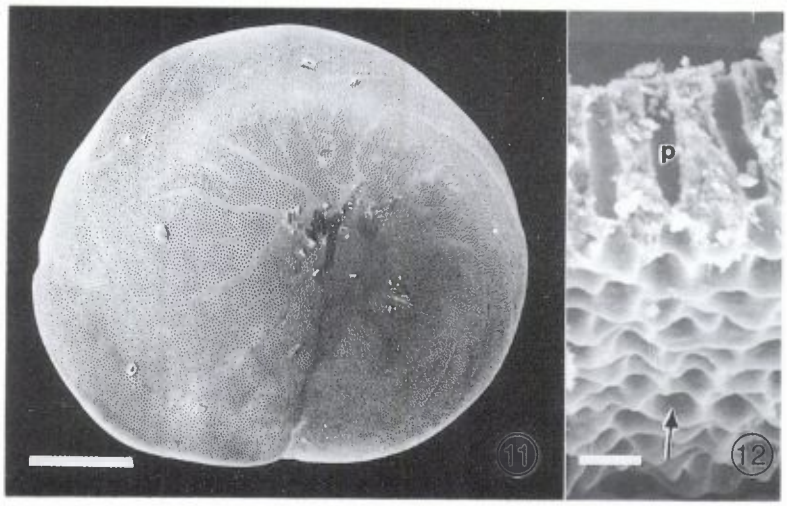

Figure 11. SEM of *Amphistegina lessonii*, a host for endosymbiotic diatoms. Note the fine pores on the surface of this host. Bar = 4 μm.

Figure 12. SEM of the pores (P) in the test of *Amphistegina lobifera*. Note the pore expands into a cup-like pore rim on inner surface of the test (arrow). Individual diatoms fit into these pore rims. Bar = 10 μm.

phosphatase activity was found in the web around the periphery of the foraminifer (Fig. 34), near the apertures (Fig. 33), or in the last few chambers. This digestive enzyme was never found near the location of the endosymbionts. If the basic multicameral (multiple chambers) nature of most foraminifera is regarded as a mechanism to separate cellular activities, (i.e. digestive functions and symbiosomes), then it is reasonable to argue that extracameral initial digestion, coupled with intracameral partitioning, could be a fundamental foraminiferal property. This has predisposed foraminifera toward the establishment and maintenance of those endosymbiotic algae which avoid initial external digestion. (We shall return to this point later in this paper.)

The diversity of symbiotic types and the non-finical, or looseness of fit, relationships shown in some of the associations, are evidence that foraminifera are generally potentially good habitats for the establishment of symbiosis (Lee and McEnery, 1983; Leutenegger, 1984; Lee and Anderson, 1991). The diatom-bearing hosts have been the most extensively studied in this respect. Including the diatoms isolated from Caribbean hosts (Lee et al., 1995b), the results of isolations from almost 3,000 hosts have been published (Lee et al., 1980a&b, 1989, 1992, 1995b). While two species, *Amphistegina lessoni* (681

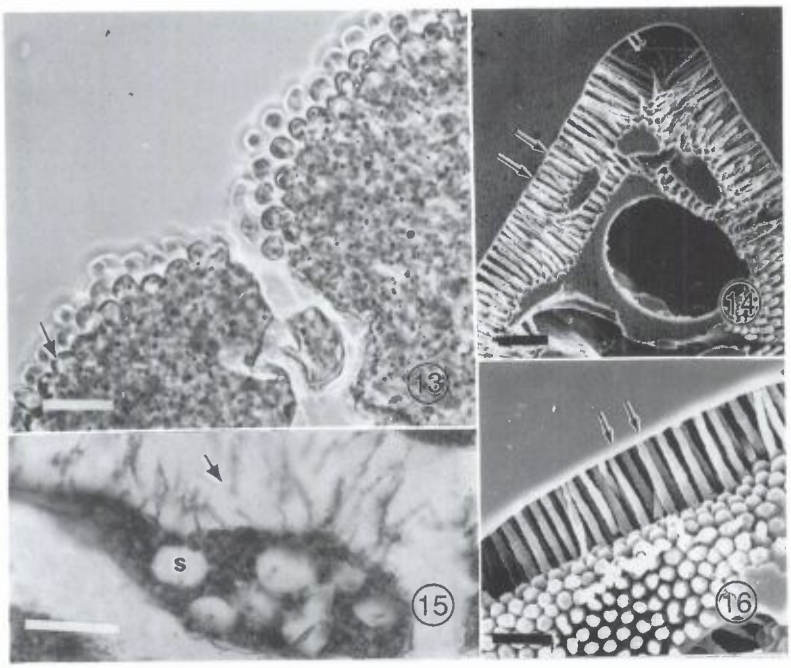

Figure 13. A histological section through of *Amphistegina lobifera*, showing the distribution of the endosymbiotic diatoms on the surface of the host cytoplasm. Compare with Fig. 12. The individual diatoms fit into the pore rims. Bar = 15 µm.

Figure 14. SEM of a cast of the test of *Amphistegina lobifera* prepared by Hottinger's technique which is of great aid in studying the spatial relationships of the pores (arrows) and the symbionts. Bar = 50 µm.

Figure 15. A histological section through *Amphistegina lobifera*, showing the organic lining of the pores. Compare with Fig. 16. The latter preparation is better for studying the spatial relationships. Bar = 20 µm.

Figure 16. A higher magnification of the above (Fig. 14) cast of the test of *Amphistegina lobifera* showing the spatial relationships of the pores (arrows) and their expanded rims which hold the symbionts. Bar = 20 µm.

individuals) and *A. lobifera* (975 individuals), made up more than half of those sampled (60.4%), significant numbers of 10 other diatom-bearing hosts [*A. gibbosa* (50), *Heterostegina depressa* (313), *H. antillarum* (18), *Borellis schlumbergi* (65), *Operculina ammonoides* (77), *Neorotalia calcar* (105), *Calcarina spengleri* (37), *C. defrancei* (51), *C. gaudichaudii* (167), and *Baculogypsina sphaerulata* (170)] were also sampled. The relationship between host species and endosymbiotic diatom species is not finical. Any of several dozen pennate diatoms were found in individual host specimens;

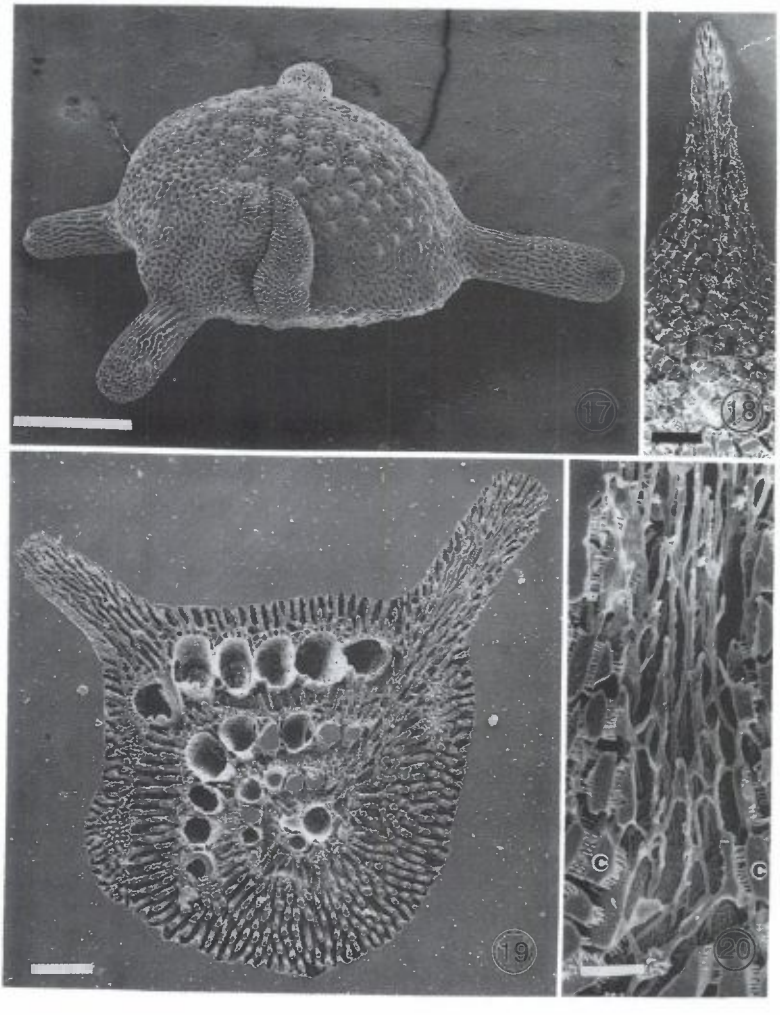

Figure 17. SEM of Calcarina *gaudichaudii*, a "star sand" host for endosymbiotic diatoms. Bar = 4 µm.

Figure 18. SEM of a cast of the test of *Calcarina gaudichaudii* prepared by Hottinger's technique showing the complex canal system and pores in a spine. Bar = 100 µm.

Figure 19. SEM of a cast of the test of *Calcarina gaudichaudii* prepared by Hottinger's technique showing the complex canal system and pores in this Foraminifer. Bar = 200 µm.

Figure 20. A higher magnification of the above (Fig. 14) cast of the spine of *Calcarina gaudichaudii* prepared by Hottinger's technique showing the complex canal system and chamberlets (C) which house the diatoms (arrows). Bar = 40 µm.

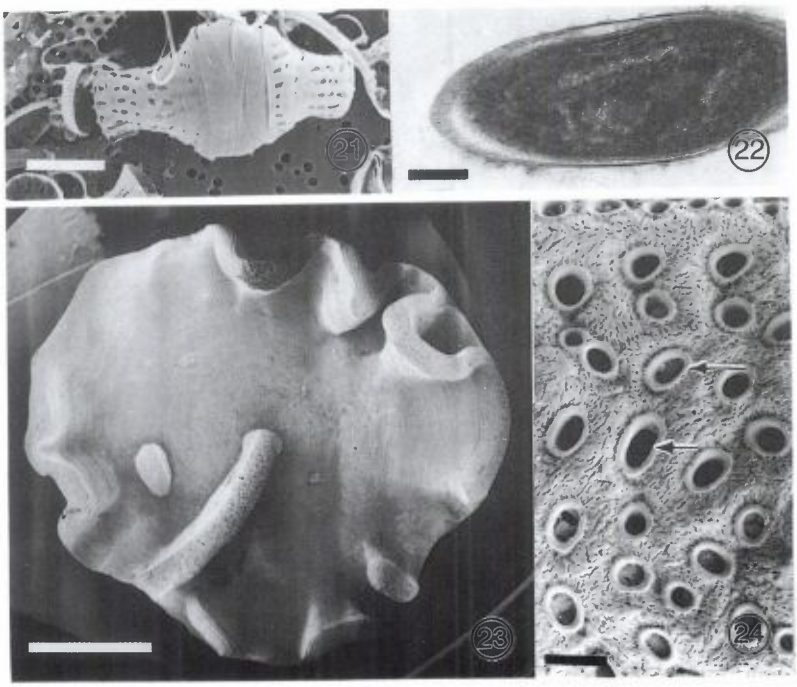

Figure 21. Infundibuliform cell of *Fragilaria shiloi* growing in primary isolation culture. Bar = 10 µm.

Figure 22. A cyanobacterium isolated from the foraminifer *Marginopora vertebralis*. Bar = 0.4 µm.

Figure 23. SEM of *Marginopora vertebralis*, a host for endosymbiotic dinoflagellates. Bar = 1 mm.

Figure 24 A higher magnification of the periphery of the above (Fig. 23) showing the many apertures (arrows). Bar = 50 µm.

however, 6 species, *Nitzschia frustulum* var. *symbiotica*, *N. laevis*, *N. panduriformis* var. *continua*, *Fragillaria shiloi*, *Amphora roettgeri*, and *A. erezi*, accounted for 75% of all of the associations in various hosts. If the number of most abundant endosymbiont diatom species is raised to 10, then they accounted for 90% of all associations. Many individual hosts harbored more than one species of diatom at the same time.

In the Gulf of Eilat, Red Sea, significant numbers of *N. frustulum* var. *symbiotica*, *N. laevis*, *N. panduriformis* var. *continua* were found in hosts harvested at every depth. However, other diatom species were more commonly collected from shallow waters (e.g. *F. shiloi*) or deeper waters (>25m) (e.g. *Achnanthes maceneryae*, *Protokeelia hottingeri*). We have done

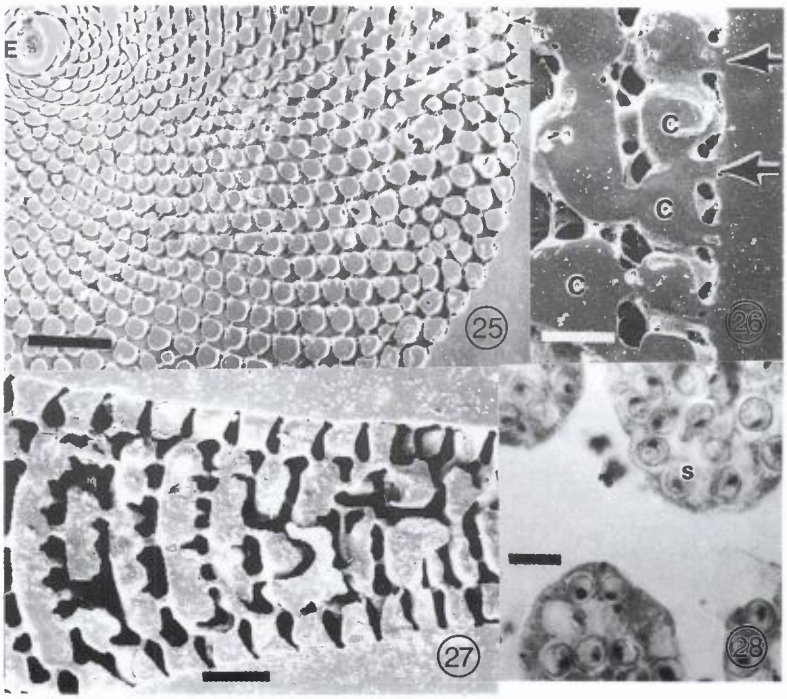

Figure 25. SEM of a cast of the test of *Marginopora vertebralis* prepared by Hottinger's technique showing the complex chamberlet arrangement. The embryonic apparatus (E, initial chambers), is at the center of the disc-shaped organism and the apertures (arrows), at the periphery of the cell. A line drawn between the two points would be a radius of the disc-shaped foraminifer. Bar = 200 µm.

Figure 26. A higher magnification of the figure to the left (Fig. 25). Cast showing the apertures (arrows) and the interconnections of the chamberlets (C) which house the dinoflagellates. Bar = 40 µm.

Figure 27. SEM of a cross-section cast of the test of *Marginopora vertebralis* prepared by Hottinger's technique showing that the complex chamberlet arrangement is cross-linked in three dimensions. The foraminifer expands from a single row of chamberlets at the embryonic apparatus which would be at the right of the section in the photograph and the apertures at the left of the section shown. Bar = 200 µm.

Figure 28. A histological section through *Marginopora vertebralis* showing the endosymbiotic dinoflagellates (S) in each chamberlet. Bar = 20 µm.

several searches of the habitats of larger foraminifera looking for endosymbiotic diatom species (Lee et al., 1989, 1992). One hypothesis was that the foraminifera were temporary hosts for the most abundant diatoms in their

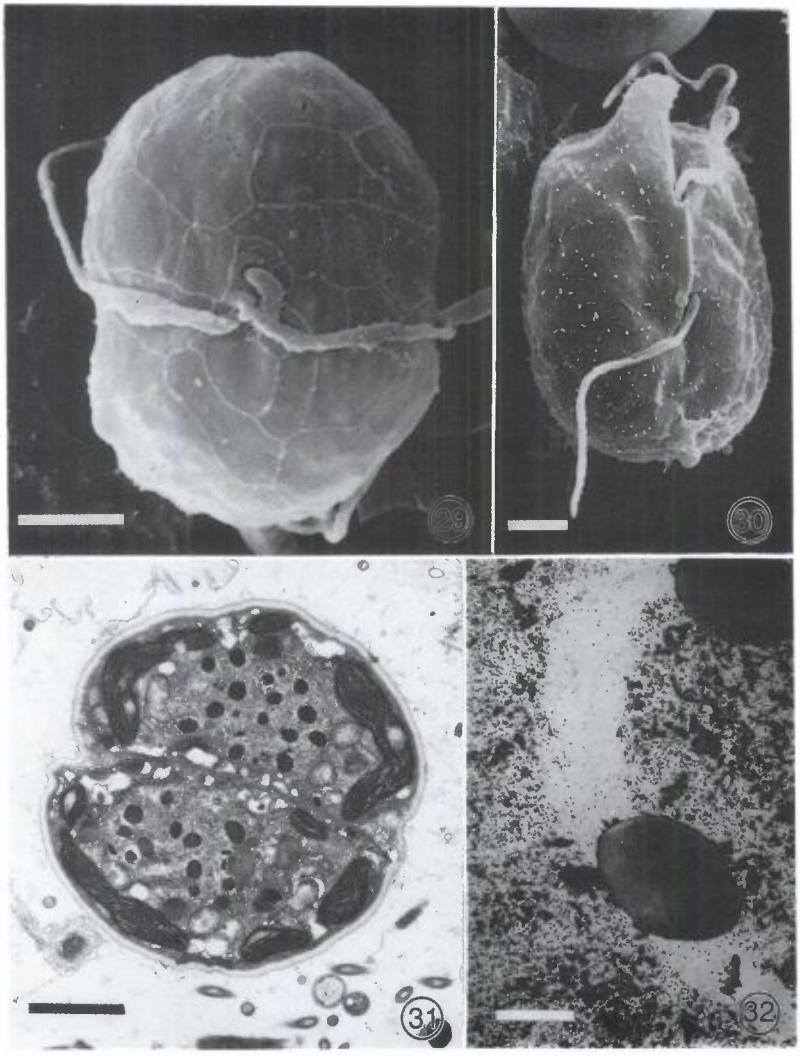

Figure 29. SEM of endosymbiotic dinoflagellate isolated from *Marginopora vertebralis*. Bar = 2 µm.
Figure 30. SEM of *Amphidinium* sp. isolated from *Amphisorus hemprichii*. Bar = 2 µm.
Figure 31. TEM of *Symbiodinium* sp. the endosymbiotic dinoflagellate isolated from *Marginopora kudakajimensis*. Bar = 1 µm.
Figure 32. Light micrograph of the grazing trail made by an *Amphistegina lobifera* in a lawn of diatoms. Bar = 1 mm.

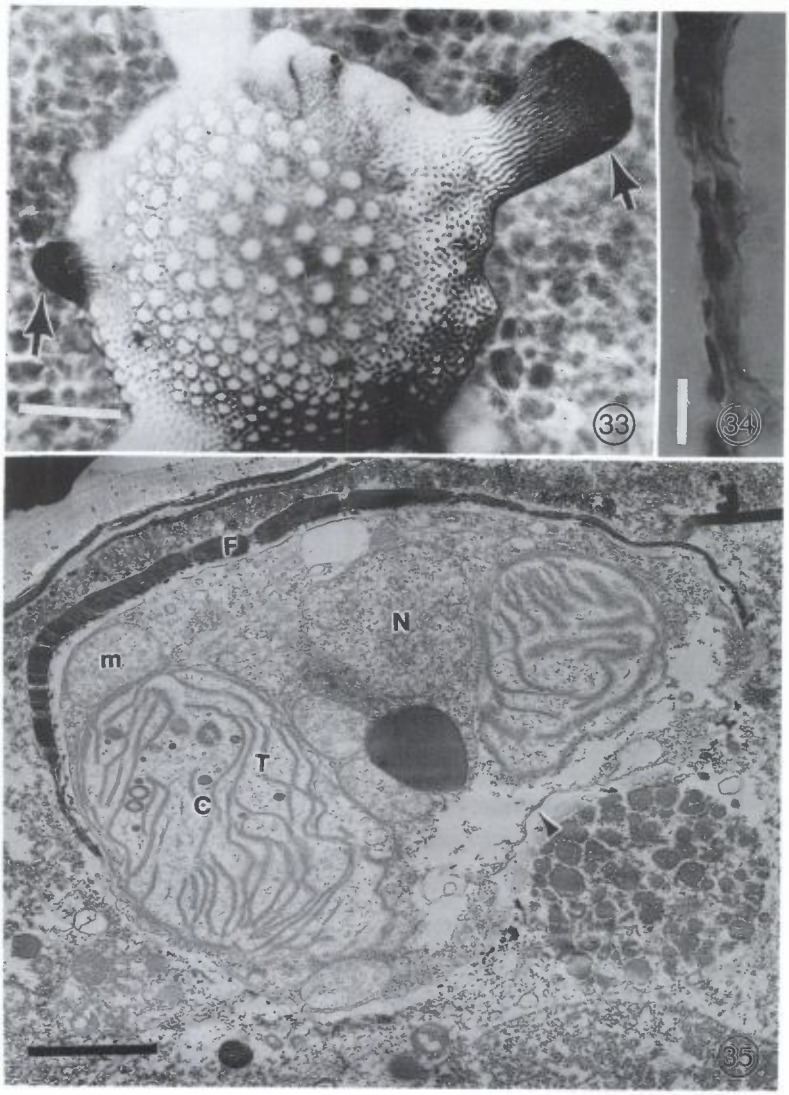

Figure 33. Light micrograph of a *Calcarina gaudichaudii* prepared to show the location of acid phosphatase(arrows). Bar = 400 µm.

Figure 34. Light micrograph of a part of the pseudopodal web prepared to show the location of acid phosphatase. Bar = 1 µm.

Figure 35. TEM of a newly ingested cell of the endosymbiotic diatom *Amphora roettgerii* showing the apparent resorption (arrow) of part of the frustule within the host *Amphistegina lobifera*. The nucleus (N) and the mitochondria (M) seem normal. Some thylakoids (T) seem missing from the chloroplast (C). Bar = 1 µm.

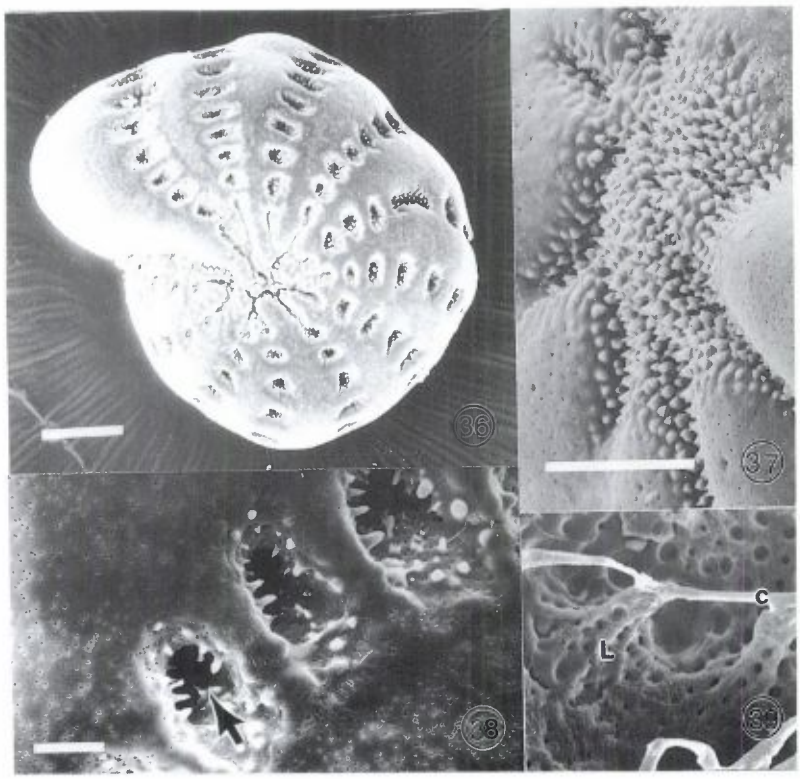

Figure 36. SEM of *Elphidium incertum*, a chloroplast husbanding foraminifer. Bar = 50 µm.

Figure 37. SEM of the umbilical region of *Haynesina germanica*, a chloroplast husbanding foraminifer. Bar = 40 µm.

Figure 38. A higher magnification of the *Elphidium incertum* above (Fig. 36) showing the fossettes (arrow) lined with denticles which comb the pseudopodia. Bar = 20 µm.

Figure 39. SEM of a cast of the test prepared by Hottinger's technique showing the funnel-like organic lining inside of the fossette (L) (Fig. 38 to the left) and their connection to the canal (C). Bar = 20 µm.

habitat. If that were true then we would expect a close correspondence between the endosymbionts isolated and the abundance of the same species in the habitat. That was not the case. Endosymbiotic diatom species (isolated from hosts harvested from the same place) were rare (<<1%) or absent from the habitat.

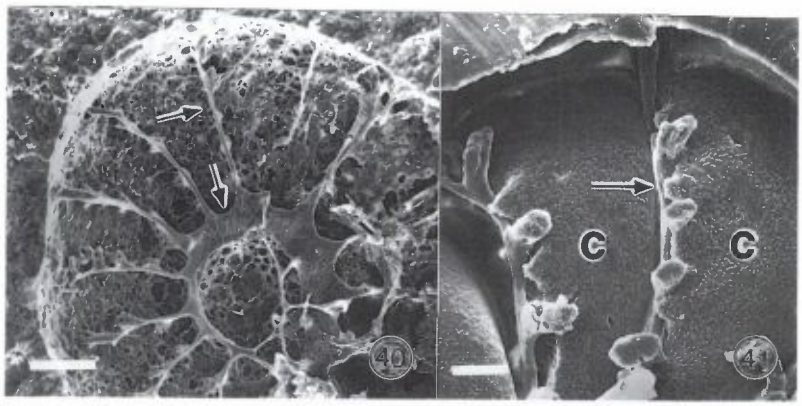

Figure 40. SEM of a cast of the test of *Elphidium incertum* prepared by Hottinger's technique showing the canal system (arrows). The chambers with the chloroplasts are not seen in this preparation. they would be located between the radial arms of the canal system. Bar = 125 μm.

Figure 41. SEM of a cast of the test of *Elphidium incertum* prepared by Hottinger's technique showing the canal system (arrow) and the chambers (C) with the chloroplasts. Bar = 20 μm.

Several experiments have been aimed at testing whether the symbiotic diatoms in a host could be replaced by other species of endosymbionts (Lee et al., 1983, 1986). Specimens of *A. lessonii* were rendered nearly aposymbiotic by incubating them in sea water with DCMU (10^{-5} M) in tissue culture flasks in the high light (at 5 m depth) in the Gulf of Eilat. After 5 days in the sea with DCMU, and no food, the foraminifera were fed mixtures of 10 different diatoms and chlorophytes. Each mixture of three species contained two species of endosymbiotic diatoms and a free living diatom isolate, or a chlorophyte. The foraminifera and the mixtures of algae were placed in flasks with fine membrane filters and incubated in the sea at normal depths (10 and 20 m). After a week most of the foraminifera regained color. The results of the isolations from treated "rebrowned" foraminifera showed that some endosymbiotic diatom species were selected over, or were more competitive, than others. Many introduced symbiont species replaced the endosymbionts previously established within the hosts. Bleached controls, which were not fed, but which were incubated in the light, also "rebrowned" due to the regrowth of their original symbionts. In histological preparations, we observed that as many as 15% of the diatoms were in cell division at the time of preparation. None of the free-living algae incubated with the foraminifera survived within

the hosts. At 10 m depth *N. laevis* was co-dominant with *N. valdestriata* in the "pecking order"; at 20 m *N. valdestriata* was dominant.

Although not as deeply explored, because taxonomic distinctions are more labor intensive, it is clear that there is considerable diversity in the algae in dinoflagellate- and chlorophyte-bearing hosts. The ssrRNA sequences we obtained from two different dinoflagellate-bearing hosts, *Amphisorus hemprichii* and *Marginopora kudakajimensis*, were not sister clades, but each was close to the endosymbionts from two very different coelenterate hosts (Lee et al., 1995a). A third sequence from a *Symbiodinium* sp. isolated from *Sorites orbiculus* was a sister clade to the *M. kudakajimensis* isolate (Langer and Lipps, 1994). Lee et al. (1995a) speculated that the later evolving soritids did not co-evolve with their zooxanthellae but acquired them from environmental pools contributed by other hosts with zooxanthellae. If they co-evolved with their zooxanthellae one would expect that the ribosomal sequences would be sister clades, closely related to each other. This does not imply that the soritids did not continually have zooxanthellae, only that some time in the past (perhaps in the present as well), they acquired new unrelated zooxanthellae. This is easy to imagine because foraminiferal zygotes have to acquire fresh zooxanthellae, or perish. Molecular comparisons of *Chlamydomonas hedleyii* and *C. provasolii*, two endosymbiotic-symbiotic chlorophytes from the family Archaiadae (our isolates), were also very distantly related (Bucheim, personal communication). This finding entreats further isolations of the symbionts in chlorophyte bearing hosts to see if they too have less finical relationships.

Belasky (1996) postulated that the same factor, temperature, seems to control the distribution of both the scleractinian corals and the larger foraminifera in the Indo-Pacific. If the present is a key to the past, then it is reasonable to believe that these two unrelated groups and their dinoflagellate endosymbionts have been similarly linked in the past.

Flexibility in acceptance of different potential endosymbionts by some larger foraminifera helps to explain the long and continued evolutionary success of the group. It is an adaptive mechanism to exploit new habitats, and remain in changing habitats. Our conceptual framework for the evolutionary development of the endosymbiotic phenomenon in the foraminifera is that they did not co-evolve with their endosymbiotic algae because: 1) foraminifera are generally predisposed to enter endosymbiotic relationships with algae (some evidence given above, more detail in reviews by Lee and Anderson, 1991; Lee and Hallock 1987); many different types of algae satisfy the needs of different foraminiferal hosts; 2) they have life cycles that insure transmission of endosymbionts after asexual reproduction; 3) by not having a finical relationship with their endosymbiotic algae they better their chances

of survival after sexual reproduction in cycles with more widely dispersed (e.g. flagellated) gametes; and 4) by not having a finical relationship with their endosymbiotic algae they better their chances for adapting to a broader range of environmental parameters such as illumination or temperature, by being able to enter relationships with alternative algae with differences in their abiotic niche factors.

If a flexible evolutionary model for dinoflagellate-bearing soritid (and possibly all larger) foraminiferan evolution is reasonable, it follows then that: 1) hosts may form stable symbiotic relationships with more than one member of the *Symbiodinium*-complex, or other dinoflagellates; 2) the opportunity for change of symbionts is present after every episode of sexual reproduction and is possible, because there are so many different types of zooxanthellae-bearing hosts in the same habitat, and because the selection pressure appears to be extremely high (survival or not) for endosymbiosis to be successful.

Another factor favoring endosymbiosis, in as yet unexplained way, is an in-place signaling system in foraminifera which seems to alter the surface of all the types of endosymbiotic algae involved in the phenomenon. The algae fail to form envelopes typical of their kind when they are in their hosts (Leutenegger, 1977, 1984; Müller-Merz and Lee, 1976; McEnery and Lee, 1981; e.g. Fig. 10). Fortunately, when the algae are released from their hosts into suitable culture media, they form envelopes. In the case of the diatom endosymbionts, they form frustules, which makes identification a relatively straightforward task.

3. New Characters Developed by Larger Foraminifera

It is hard to make generalizations which are appropriate for all larger foraminifera. Some living sands are quite simple externally and internally (e.g. *Peneroplis* Figs. 5–7). However, the morphological complexities of some taxa are remarkable! Certainly few would argue that these are among the most morphologically intricate protists known. In many cases, we observe test complexity which we interpret as adaptations to symbioses (reviewed in Hallock et al., 1991). For example, the interior of the pores of *Amphistigina* sp. are expanded to form little cups, each of which serves as a socket to hold a single endosymbiotic diatom (Figs. 12–15). Although there are membranes at the bottom of the pores, in addition to the cell and symbiosome membranes separating the diatoms and the sea, each symbiont has almost a direct pipeline to fresh supplies of sea water with its nutrients and dissolved gases. A marvelous casting technique developed by Hottinger has given us a tool to

study the cytoplasmic connections and test structures in three dimensions (Hottinger, 1979; Hottinger and Leutenegger, 1980). The casting technique has shown us that the shells of "star sands" are honeycombed with chamberlets and very complex canal systems (Figs. 18–20, 25–27). Although it is reasonable to believe that they serve to increase the circulation of sea water to and from the symbionts, carefully designed experiments to test this idea have not yet been done. The adaptations can also be viewed as mechanisms for compartmentalizing cellular activities. There is some variation even among genera of the same family. *S. marginalis*, for example, is quite regionalized. It is disc-shaped with concentric rings of chamberlets. The outer zone has digestive vacuoles, symbionts are restricted to the middle zone, and the micronuclei (generative nuclei), which give rise to the next generation, are found in the center of the disc (embryonic chambers) (Müller-Merz and Lee, 1976). In contrast, the symbionts of the even more complex M. vertebralis (Figs. 23–28), in the same family, are distributed throughout their host. The cyanobacteria (Fig. 22.) in this latter association are more medial.

4. Algal Characters

Light

Field observations and experiments with intact host-symbiont systems showed that there are ranges of light intensity which play an important role in the associations (Zmiri et al., 1974; Lee et al., 1980b&c; Röttger 1972, 1976; Hallock, 1981). The photosynthetic and growth responses of four symbiotic species grown in axenic culture, *N. valdestriata*, *N. laevis*, *N. panduriformis*, and *F. shiloi*, have been studied (Lee et al., 1982). Two of the species, *F. shiloi* and *N. laevis*, isolated from *A. lessonii*, grew best at high light intensity (312 μWcm^{-2}), while the other two isolated from *H. depressa*, a deep water species, or one which is found in shaded places, grew best at lower light levels (175 μWcm^{-2}). Photocompensation rates approximated 2% of the light level measured in spring in the sea at a depth of 1 m. If the algae were free, the photocompensation depth would be reached between 40–50 m in the sea at Eilat.

Nutrition

There have only been a few studies on the nutrition of the endosymbiotic algae isolated from foraminifera. In axenic culture seven species of endosymbiotic diatoms (*N. panduriformis*, *N. laevis*, *N. valdestriata*, *N. frustulum*, *A. tenerrima*, *Navicula reissii*, and *F. shiloi*) required thiamin (Lee

et al., 1980a). Biotin stimulated the growth of six species. Only one clone of *N. frustulum* required vitamin B_{12}. We speculate that the requirements for these vitamins is satisfied by the food organisms eaten by the host. Optimal concentrations of NO_3^- varied among the species tested (0.2 μM – 2 mM), but all grew best at levels of NO_3^- which exceeded by several orders of magnitude the levels found in the Gulf of Eilat (1 $\mu g l^{-1}$; Levanson-Spanier et al., 1979), where the hosts were captured.

Nutritional studies of *C. provasolii* from *Cyclorbiculina compressa*, *C. hedleyi* from *Archaias angulatus* and *Symbiodinium* sp. from *S. marginalis* gave very similar results (Lee et al., 1974, 1979). Growth of *C. hedleyi* was tripled in the presence of thiamin, but it did not have an absolute requirement for this vitamin. Biotin also stimulated growth when the medium was also supplemented with several amino acids. Growth was twice the level when urea (20 μM) was used as nitrogen source than when NO_3^- or NH_4^+ (20 μM) were used as nitrogen sources. Optimum phosphate concentrations (0.1–1 μM) varied with the nitrogen source used. The growth of *C. provasolii* was tripled in the presence of vitamin B_{12} and doubled in the presence of biotin. *Symbiodinium* sp. from *S. marginalis* had absolute requirements for vitamin B_{12} and thiamin. Both of these latter symbionts from Key Largo Sound, Florida, required very high optimum levels of nitrogen (NO_3^-/NH_4^+; 0.2 mM) and phosphorus (PO_4^{3-}; 0.1 mM).

Unique morphological characters

In addition to the fact that many of the endosymbiotic diatoms are new species (e.g. *F. shiloi*, *N. hanseniana*, *A. roettgerii*), and new genera (*Protokeelia* and *Canopiophorum*), a number of the diatoms found as endosymbionts have unusual characteristics. Presumably, due to the fact that the very small sizes of these pennate diatoms fall below the generally accepted limits for sexuality and auxospore formation in the diatoms used as models for this phenomenon (Geitler, 1932).

N. muscatini which grows as a naked frustule-less zooxanthella in its foraminiferal host has an unusual life cycle in culture which includes large multinucleate cells. Lee and Xenophontos (1989) interpreted these to be autosporangia. This facet of a pennate diatom life cycle has not been encountered in any other species.

Some of the isolates of *N. frustulum* var. *symbiotica* from Caribbean hosts are also unusual (Lee et al., 1995b). The raphe-sternal systems in 40% of the isolates are extremely abnormal for the genus. The distribution and number of fibulae is quite irregular and the position and shape of the raphe-sternum varies on the valve face of different isolates. Many clones of this pennate

diatom are spherical. This, perhaps, is not so strange, when one considers that these symbionts are passed asexually from one host generation, to the next, without ever forming frustules. For these symbionts the characteristics of their frustule has no obvious adaptive value.

Infundibuliform frustules (Fig. 21) were formed in primary isolation cultures of *F. shiloi* isolated from hosts collected at two Pacific habitats (Lee, 1996). These unusual frustules are composed of three discrete elements: a normal valve; a perforated cylindrical internal valve; and a second funnel-shaped flaring internal valve. Unlike any other diatoms known, these diatoms grow larger as they divide the first time. This phenomenon suggests that there may be an asexual alternative, latent, size rejuvenation developmental program in diatoms which has been suppressed in almost all modern forms.

5. Host-Symbiont Interactions

Light-calcification/growth

How does light exert its effects on the host/symbiont system? The most obvious answer is to consider photosynthesis by the symbiotic algae. The potential is large. In some oligotrophic habitats (e. g. Gulf of Eilat, Red Sea) there is often more chlorophyll in a single 1–3 mm diameter larger foraminifer than in the phytoplankton in a cubic meter of sea water above it. ^{14}C tracer techniques have been used to study rates of primary production in two species of Amphistegina (Muller, 1978; Hallock, 1981). Rates of primary production were quite high ($2–3 \times 10^{-5}$ mg C/H/foraminifer). At Lizard Island, Great Barrier Reef, *M. vertebralis* fixed 0.05 ng C min^{-1} (Smith and Wiebe, 1977). One species of "living sand", *H. depressa*, survives and grows well in the light in the absence of any obvious concentration of food (Röttger, 1976). The other host/symbiont systems studied, *Peneroplis* spp., *Amphistegina* spp., *A. hemprichii* , require food, as well as light, for growth (Faber and Lee, 1991; Lee and Bock, 1976; Lee et al., 1988b).

Another advantage conferred by the symbiosis to larger foraminifera is enhancement of growth. For example, the calcification rates of three Caribbean soritid species, *A. angulatus*, *C. compressa*, and *S. marginalis*, are two to three times greater when incubated in the light than in the dark. In these species, calcification rates are proportional to light intensity in the range of 0–200 $\mu Em^{-2}\ s^{-1}$ (Dugay, 1983). At light saturation the total carbon fixed into the organic fractions of *A. angulatus* was 170 ng C mg dry $weight^{-1}\ h^{-1}$ (Dugay and Taylor, 1978). Erez, ter Kuile, and collaborators have studied the details of the uptake of inorganic carbon and calcium in *A. lobifera* and *A. hemprichii* (review, ter Kuile, 1991; ter Kuile and Erez, 1987; ter Kuile et al., 1987, 1989

a&b). They found evidence for differences in the uptake, kinetics, and internal cycling of carbon in these two larger foraminifera. Using pulse-chase tracer experiments they demonstrated the transfer of photosynthetically fixed carbon into the shell of *Amphistegina lobifera*, but not into the shell of *Amphisorus hemprichii*.

The importance of calcification in the overall carbon budget varies with the growth stage (ter Kuile, 1991). Young perforate foraminifera use a greater proportion of incoming inorganic C for building their tests than do older specimens. In *A. hemprichii*, which was used to model imperforate larger foraminifera, younger specimens had slightly higher ratios of calcification to photosynthesis. In older specimens the reverse was found (ter Kuile, 1991). Carbon budgets have been calculated for *A. lobifera* and *A. hemprichii* (ter Kuile, 1991) which serve, respectively, as good models for carbon cycling in perforate or imperforate species (ter Kuile, 1991).

Light-behavior

Several simple experimental studies have demonstrated phototaxis in larger foraminifera. *A. lessonii* was phototaxic at photonic fluxes of 10^{11-12} and unresponsive at lower light levels (Zmiri et al., 1974). *A. lobifera* was positively phototactic at an incident illumination of 0.1–1 klx and negatively phototaxic at higher light levels (Lee et al., 1980a). *A. hemprichii* was positively phototaxic at some point between 6 klx and 11 klx but photoinhibited above 22 klx. This latter organism, which sets up feeding territories in cultures, overcame its territorial behavior by its phototaxic responses (Lee et al., 1980a).

Nutrient transfer

There is very little evidence on nutrient transfer from symbionts to hosts. It was found that *C. hedleyi* freshly released from their host, *A. angulatus*, were filled with starch grains (Figs. 1 and 4.). By TEM examinations, similar starch grains were also found in the host cytoplasm. One could speculate that the algae undergo autolysis, or that the host causes the algae to be digested, or lysed, but static pictures have not yet given us an answer. It is also possible that in situ *C. hedleyi* releases soluble metabolites. In $H^{14}CO_3$-labeled media axenic *C. hedleyi* released large quantities of mannitol (Lee et al., 1974). More fixed carbon was found in the medium (57%) than in the cells (43%).

Kremer and co-workers (1980) also used tracer labeled ($H^{14}CO_3$) media to study the photosynthetates and their products in six larger foraminiferal associations. They identified floridoside and polyglucan in extracts of the

rhodophyte-bearing *P. arietina* (Fig. 6) and *P. pertusus* (Fig. 5). They found that 74% of the photosynthate in the dinoflagellate-bearing *Amphisorus hemprichii* was in unspecified lipids and 3.5% was in glycerol. In the diatom-bearing *A. lessonii, A. lobifera* (Fig. 11), and *H. depressa* a large percent of the label also was found in lipids (31, 51, 33%, respectively) and as glycerol (5, 6, 11%, respectively). More refined techniques would be needed to locate the labels in either the algae and/or their hosts.

In pulse chase experiments, radionuclide (^{14}C) labeled algae provided evidence of transfer of C ingested in food to the symbionts (Lee et al., 1988a). After feeding with tracer-labeled food the foraminifera, *A. lobifera* and *A. hemprichii*, were incubated for 24 h with cold food and then fixed and prepared by standard histological methods. The sections were stained, dipped in radioautographic emulsion, and incubated in the dark. Label was found in food vacuoles, in residua (feces), in the cytoplasm and in the symbionts. This type of experiment can be interpreted only in a general way. Carbon (in some form[s]) flows from the food organisms, through the host, to the symbionts. The label could have been catabolized and respired to CO_2 before it reached the algae and/or it could have been organic molecules released from food vacuoles and taken up from the host's cytoplasm by the algae.

Sterile host homogenates (Lee et al., 1984) increased the levels of photosynthate release by diatoms to their medium. *N. valdestriata*, released 76% of its photosynthate to the medium in the presence of host homogenate. The least affected species released about half (36%) as much. The concentration of photosynthates was too low in the medium to be identified by thin layer chromatography. Next steps would be separation and identification of the active factor(s). Without further work, we remain cautious about implying that the release of photosynthates, in the presence of homogenate in the experiment, indicates some kind of an integrating mechanism peculiar to the symbiotic relationship. We can imagine quite a number of physiological conditions and surface active molecules which could induce rapid leakiness by alteration of cell membrane properties. Even more subtle factors might be operating in the host milieu.

Envelope changes

In a series of experiments we exposed axenic log phase cells of endosymbiotic diatoms to filter sterilized homogenates of crushed *A. lobifera* and *A. lessonii* (Lee et al., 1984). To various degrees, depending upon species, the host homogenate affected the formation of new frustules of growing and dividing cells (Fig. 9). *F. shiloi* was the most sensitive species to the homogenate. New cells were spherical with no, or little, vestiges of a frustule. The inference is

that host "substances" are probably responsible for the maintenance of the frustule-less state in vivo and if ingested potential endosymbionts escape digestion, they could become frustule-less after growth and cell division. This hypothesis, however, needs a rigorous test. Some work in progress in our laboratory suggests that the frustules of ingested symbionts may be resorbed (Fig. 35).

A fine structural examination of *Peneroplis* with their endosymbiotic *P. purpureum* seemed to suggest that the sheath of the red alga was digested as it was being formed (Lee, 1990; Fig. 10). These symbionts, however, are quite unusual because they are the only foraminiferan endosymbionts which lie naked in the cytoplasm of their hosts; they are not surrounded by a symbiosome membrane.

6. Chloroplast Husbandry

Three families of foraminifera, Elphididae, Nonionidae, and Rotaliellidae have members that retain large numbers ($\sim 1 \times 10^{2-4}$ cell^{-1}) of functional chloroplasts that they capture from partially digested food (Lopez, 1979; Lee and Lee, 1990). Tracer labeling studies using $^{14}CO_3$ to measure primary production by the chloroplasts suggested that *Elphidium williamsoni* was fixing carbon at a rate of 2.3 µg C mg ash-free dry weight^{-1} h^{-1} and *Haynesina germanica* fixed at a rate of 0.5. µg C mg ash-free dry weight^{-1} h^{-1} (Lopez, 1979). Specimens of *E. crispum* from the Red Sea fixed 1.5 µg C mg dry weight^{-1} 48 h^{-1} (Lee et al., 1988c). Fine structural studies and pigment analyses suggest that the chloroplasts are derived from diatoms (Lopez, 1979; Knight and Mantoura, 1985; Lee et al., 1988c).

Many questions arise about the nature of the chloroplasts which are retained by foraminifera. Are the chloroplasts of all algae equally viable in the foraminifera? If not, why? How long do chloroplasts last after they have been captured? Are there any differences of the phenomenon in different hosts? These questions have barely been explored. The results of a HPLC study of *H. germanica* suggested that there was very little digestion of the chloroplasts because little phaeophytin was found (Knight and Mantoura, 1985). They reasoned that if a large proportion of the chloroplasts were being digested, or autolysing, all the time, they would have detected this by the presence of a high percentage of degraded pigments (phaeophytin). In a study of *E. williamsoni* and *H. germanica* from Limfjörden, Lopez (1979) estimated that under normal light and dark conditions individuals of the former species needed to capture at least 65 chloroplasts h^{-1} while individuals of the latter needed to capture only 20 chloroplasts h^{-1}. Using an approach of feeding and

then starving, Lee and Lee (1990) made some calculations on the turnover time of the chloroplasts of *H. germanica* and several species of *Elphidium*. When *H. germanica* was starved and incubated in the dark it survived only 9 weeks, but when it was starved and incubated in the light it survived 13.5 weeks. In the dark there was a steady decline in the number of chloroplasts; the chloroplast half-life was estimated as 2 weeks. In the light the loss of chloroplasts in starved individuals was more gradual than in the dark. There was an initial drop in chloroplast number to half by 6 weeks, after which the loss became more gradual. In the dark starved *E. crispum*, from Drake's Island, rapidly lost chloroplasts ($T_{1/2}$ ~3 weeks), and all perished after 10 weeks. In the light there was a biphasic curve with an initial rapid loss ($T_{1/2}$ ~3.5 weeks) followed by a second slower phase ($T_{1/2}$ ~10 weeks). Feeding experiments with different species of diatoms suggested that there were differences in their retention time in the foraminifer. Obviously this is a topic for further research.

There seems to be an unresolved paradox about the morphology of the chloroplast husbanding foraminifera. The morphology of the tiny rotaliellids is quite ordinary. On the other hand, the elphidiids and nonionids are quite modified. Their apertures are highly modified as are their sutures (junction of chambers). Large funnel-like fossettes lined with denticles are found in the sutures of *Elphidium* spp. (Figs. 36, 38, 39). The pseudopodia emerge through these orifices in the test, and it is believed that the denticles act like sieves to hold back diatom frustules while permitting chloroplasts to be drawn into the shell. The umbilical region of *H. germanica*, through which its pseudopodia emerge is also lined with denticles which might have the same function (Fig. 37).

Casts prepared by Hottinger's method are quite useful in demonstrating the canal system which is connected to the ends of the fossettes (Figs. 39–41). The utility of this complex system needs an explanation, particularly since the rotaliellids are so simple.

7. Symbionts and Organelles

The last several decades have seen the development and general acceptance of ideas on the origin of eukaryotic cells through integration of endosymbiotic prokaryotic cells into new functional organellar units. Presumably the acquisition of new adaptive abilities is a powerful evolutionary leap which has very high selective power. In view of this, the question is why have the intracellular algal symbionts in larger foraminifera not become completely integrated with their hosts to become organelles? This would be of particular advantage to organisms which, although reproducing asexually, do have a

sexual phase in their life cycles. One could argue that the barrier to the next step of integration could be the presence of the symbiosome vacuolar membrane around each symbiont. When this membrane is lost, the potential for lysosomal fusion with the vacuole is lost and exchanges between compartments are facilitated. A possibility, yes, but not the whole answer. When we examined, for the first time, the red algal endosymbiont, *P. purpurum*, inside the foraminifer, *P. planatus*, we were surprised. The symbiont is not surrounded by a second membrane (symbiosome) outside its own cell membrane. The same is true in the other species of *Peneroplis* examined. Compared to its appearance in axenic culture, the envelope (sheath) of the alga in its host is drastically reduced to a thin layer of fibrils (Lee, 1990b). Since these algae lie independently in the cytoplasm of their hosts, as do mitochondria or chloroplasts, we had to ask ourselves whether these strains of endosymbiotic *Porphyridium* have achieved organellar status, or not. As is true for all larger foraminifera, the hosts cannot live very long, grow, or reproduce, without their photosynthetically functioning algal symbionts. But, the reverse is not true. When the hosts are broken open, and *Porphyridium* are inoculated into simple media they grow rapidly and show no loss of vigor over many serial transfers. When simple inorganic nutrients are given to them, they do not seem to need their hosts. Others may argue differently, but I would argue that the minimal evolutionary step for a symbiont to become an organelle, is loss of some genes required by the symbiont, but present in the host's genome, or transfer of symbiont genes to the host's genome. Thus I considered the red algae inside the peneroplids to be symbionts and not organelles. This, of course, obfuscates the answer to the question. Perhaps the time scale, >40 million years, is too short for greater genetic integration. Given the changes observed in other host characteristics (shell and behavioral) this is hard to believe. Are there advantages to maintaining cellular independence which outweigh greater cellular integration? The answer to this question may have been given in an earlier section in this review. Less finical relationships between the host and its symbionts may be more adaptive to changing or new habitats. What advantages would there be if there were more integration? It is clear that larger foraminifera represent lines of evolution which led to increasing cellular complexity rather than multicellularity. Does this fact underlie the lack of greater cellular integration? Our failure to satisfactorily answer this basic cellular question, challenges us to probe it in new ways.

8. Association Regulation

This is a topic which has not been studied extensively in foraminiferal

associations. Our studies of the mineral nutrition requirements of axenic cultures of endosymbiotic algae isolated from larger foraminifera suggested that the algae required levels of NO_3^- and PO_4^{-3} which are 100 to 1,000 times higher than they could get if they were growing free in the Gulf of Eilat where their hosts were harvested. This explains why they are not abundant there. Although this has not been tested experimentally, it is reasonable to suggest that there is a benefit to the algae in the association. As the host feeds on bacteria and algae in its habitat, the symbiotic system gains scarce nitrogenous and phosphorous compounds needed by both symbiont species. When we incubated several larger foraminifera (*A. lobifera* and *A. hemprichii*) in mineral nutritional experiments with levels of nitrate and phosphate which were optimal for their algae, the hosts and their symbionts died (Lee et al., 1991b). In batch culture, optimum growth of the foraminifera took place in the light, if the hosts were feeding, if the levels of nitrate and phosphate were low, and if the medium was changed weekly. Similar results were obtained in chemostats. These results lead us to speculate that the regulation of the numbers of algal symbionts in their hosts may be dependent on transfer of nitrogenous and phosphorous compounds to them. This speculation may tie in with some field observations made at stations where domestic, and similar wastes, enter seas. While remains in the sediment suggest that living sands were once abundant at these stations, living larger foraminifera (work in progress) are no longer present in seriously affected areas. Our present interpretation is that the excess of nutrients at these locations has upset the balance, or regulation, of the host-algal symbiont relationships in these larger foraminifera.

9. Conclusions

Larger foraminifera are, in some respects, a high point in the evolutionary development of protistan cells. Their cellular functions are compartmentalized by rather complex morphological adaptations whose functions are really not well understood. Because they are filled with endosymbiotic algae, and can not live without them, we could, perhaps, regard them as a type of algal colony. This however, is a very unsatisfactory conceptual frame work. Foraminifera have had a long and successful association with algae in well illuminated warm shallow seas. While we already know some aspects of these associations, most aspects of the phenomenon in these giant-sized protists remain unexplored in depth. The challenges are there waiting for us to explore them. The prospects of results of new probes are exciting to contemplate.

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