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Granular Reticulopodal Digestion — A Possible Preadaptation to Benthic Foraminiferal Symbiosis?

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Abstract

Foraminiferal digestion often begins outside of the organism's shell in the extended rhizopodia. It is reasoned that those algal species which avoid initial external digestion by the foraminifer can establish themselves as endosymbionts. Specimens of different species of foraminifera were assayed using Napthol AS-BL Phosphate for the presence of acid phosphatase (an indication of digestion), then prepared for light microscopy. Six of the species investigated harbor endosymbionts, five species husband chloroplasts, and three species have no known endosymbiont. The acid phosphatase was located around the periphery of the foraminifer, near the apertures, or in the last few chambers. This digestive enzyme, on the other hand, was never found near the location of the endosymbionts. Since algal endosymbiosis has arisen many times in the evolution of foraminifera, and it involves a wide variety of algal types, it is possible that extracameral initial digestion steps coupled with intracameral partitioning could be a fundamental foraminiferal property which underlies this phenomenon.

Keywords: Foraminiferan digestion, larger foraminifera, symbiosis in foraminifera, evolution of symbiosis, preadaptation for symbiosis

1. Introduction

Algal-bearing foraminifera (large and planktonic foraminifera) abound in the world's oceans, and in particular in the well illuminated tropical and semi-tropical seas. In the later, "living sands" literally carpet the sea floor in back

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reef habitats and there are pacific islands where beach sand is almost entirely foraminiferal. Since the Pennsylvanian (3×10^8 yBP), the fossil record indicates bursts of evolutionary lines of larger foraminifera (sizes up to 15 cm) from ordinary sized (0.5 mm) ancestors. Their abundances were not random but generally corresponded to polytaxic episodes (Fisher and Arthur, 1977) which were periods of global warming, relative drought, raised sea levels, expansion of tropical and semitropical habitats, and reduced oceanic circulation (Lee and Hallock, 1987).

When one reflects on the pulsed abundances in the fossil record and the fact that larger and planktonic foraminifera as a collective group are hosts for a greater diversity of algal types (dinoflagellates, chlorophytes, rhodophytes, chrysophytes, and diatoms) than any other group of organisms in the sea, one wonders if the biological properties of foraminiferal organization do not make them particularly good habitats for the establishment and maintenance of algal symbionts. One could argue that the diversity of algal symbionts itself is strong evidence. Additionally, some of the associations, those between diatom-bearing and dinoflagellate-bearing hosts and their algal partners, are not species specific (Lee et al., 1980a,b,c, 1983, 1985, 1989; Lee and Reimer, 1984; Reimer and Lee, 1988; Lee and Lawrence, 1990). The presence of rare or minor symbionts in some of the associations adds further evidence (Lee and Reimer, 1984; Reimer and Lee, 1984, 1988; Lee et al., 1983).

The complex life cycle of many foraminifera seems also to be a general preadaptation for symbiosis. Asexual reproduction of the agamont generation insures transmission of symbionts to their offspring. Although the life cycles of most larger foraminifera are not completely known, the sexual stages of some foraminifera are known to be gamontogamic (2 or more organisms fuse together or form a nuptual chamber around their umbilical surfaces). Gametes are released into this restricted space. If this happens in some of the larger foraminifera, the symbionts of the parents would be readily available to newly formed zygotes.

The survivability of symbiotic algae in a non-symbiotic species (one experiment: *Chlamydomonas hedleyi* in *Rosalina leei*, reported in Lee and Zucker, 1969) suggests that the general cameral structure of foraminifera serves preadaptation for symbiosis. Cameral organization subdivides protoplasmic streams and promotes regionalization of cellular activities and could separate algae into favorable microhabitats (e.g. under pores in *Amphistegina* spp.) (reviewed in Lee and Hallock, 1987). In fact the fossil record of larger foraminifera could be interpreted as showing that foraminifera have been quite plastic in their responses to the algal symbionts by evolving complex subdivisions (chamberlets) and organelles for channeling protoplasmic streams (Hottinger, 1982;

Lee and Hallock, 1987). This evidence led us to speculate that it was possible that the nature of digestion in foraminifera also could be a preadaptation to symbiosis.

The constant bidirectional movement of particles in granular reticulopodia has fascinated many observers (e.g. Sandon, 1932; Jepps, 1942; Buchanan and Hedley, 1960; Hedley, 1964; Anderson and Be, 1976). Several modes of behavior have been observed in living foraminifera. Several carnivorous species have been observed to digest their trapped prey directly in their granular reticulopodial networks (Buchanan and Hedley, 1960; Anderson and Bé, 1976). Other species habitually collect food and form balls of algae near their apertures (e.g. Bowser et al., 1985). Sometimes the balls become so large that the foraminifera singly or in groups become embedded in them. Vacuoles with food have also been observed in cytological preparations of many species where they occur mainly in the youngest chambers (e.g. Muller-Merz and Lee, 1976; McEnery and Lee, 1981; Koestler et al. 1985). Only a few have attempted to detail the process of endocytic uptake and lysosomal fusion by cytochemical or ultrastructural methods. One of the first, Lengsfeld (1969) concluded that *Allogromia*, a monothalmus foraminifera, had a novel open digestive system consisting of intercellular anastomising channels of seawater, which could be traced from their origins in the aperture to sites of digestion within the cell. This lacunary system was interpreted as analogous to the open digestive tracts of several invertebrate phyla. This claim was disputed by further studies which suggested that the lacunary system was a fixation artifact (Anderson and Bé, 1976; Bowser et al., 1985). Interestingly, the latter two studies differed in their observations on the sites of lysosomal fusion with phagosomal vesicles. The former found fusion at sites of penetration of the reticulopodia into the naupliar prey. The latter found no lysosomes or acid phosphatase activity in the reticulopodia and concluded that the network in allogromids serves mainly as a food gathering and transport organelle. From the perspective of symbiont maintenance the site(s) of lysosomal fusion with phagosomes is a key issue, because symbionts are also surrounded by host vacuolar membranes. We began this study to broaden our knowledge of digestive processes in foraminifera. It is reasoned that if lysosomal fusion with phagosomes is extra-cameral in the group, as a whole, then this might be a very good biological feature for the maintenance of symbiosis once it becomes established. Algae which escape the initial digestive challenge would be relatively immune from further challenge even though their cell envelopes are greatly reduced when they are in their hosts.

2. Materials and Methods

Collections

Four species, *Amphisorus hemprichii* Ehrenberg, *Amphistegina lobifera* Larsen, *Peneroplis planatus* (Fichtel and Moll) Montfort, and *Planorbulina* sp. were collected between January and March, 1988 from the *Halophila* meadow near Wadi Taba, Gulf of Eilat, Israel, at depths from 10–25 meters. These specimens were brought to the H. Steinitz Marine Biological Laboratory-Interuniversity Institute of Eilat, and assayed about 24–48 hr after collection. *Baculogypsina sphaerulatus* (Lamark) Parker & Jones emend Sacco, *Calcarina gaudichaudii* d'Orbigny, and *Marginopora kudakajimensis* Gudmundsson were collected on July 15, 1989 from Kudaka Jima, Japan. The remaining species (*Allogromia laticollaris* Arnold, *Quinqueloculina costata* Walker & Jacob, *Elphidium incertum* Williamson, *E. translucens* Natland, *Protelphidium tisburyiensis* Butcher, *Haynesnia germanica* (Ehrenberg) Banner and Culver, and *Nonion* sp.) were collected from Lackey's Bay, Naushon Island,Woods Hole, MA in August, 1989. The Japanese and Woods Hole specimens were transported back to the laboratory in New York, and assayed 1–2 weeks after collection. Several smaller foraminifera were cultured in the laboratory, and several of their offsprings were assayed at a later time.

Acid phosphatase assay

The foraminifera were placed either in a 9-well spot plate or a small petri dish, with or without an agar base. All were offered a mixture of diatoms, *Dunaliella salina* and *Chlorella* sp. (AT), and allowed from 1 to 12 hr to extend their pseudopodia and feed.

The foraminifera were fixed with cold 3% glutaraldehyde in an acetate buffer. The acid phosphatase assay (#180) followed the method given in the Lymphocyte Enzyme kit marketed by Sigma Chemical Co. The specimens were rinsed in distilled water for 30 sec. A solution of Napthol AS-BL Phosphoric acid and Fast Garnet GBC with an acetate buffer was poured over the foraminifera, until covering the entire specimen, and placed in a 37°C oven or water bath for 1–2 hr. After incubation, the specimens were rinsed with distilled water and counterstained with methylene blue for 2 min. The foraminifera were gently decalcified with 5% poly no-cal (Polysciences, Inc. #16865) for about 2 hr, then dehydrated in an ethanol series, cleared with xylene, and embedded in tissuemat (Fisher Sci. #12-647C), a paraffin polyester resin. Thick sextions were cut at 7–10 μm. The sections were also counterstained with hematoxylin (Humason, 1962), since the methylene blue leeched

out of most of the specimens during the dehydration in ethanol. The sections were observed and photographed on a Zeiss Photomicroscope II.

3. Results

All the specimens had collected food balls and mats around their tests. Only a few specimens had extensive rhizopodia out along the agar base after feeding. This episodic gathering of food close to the foraminiferal shell seems to be a general behavior of all investigated foraminifera (Lee, 1974; Lee et al. 1988; Faber and Lee, in press, this volume), and can be observed *in situ* (Fig. 1). Often partially digested food particles and empty diatom frustules were found in these food mats (Figs. 2–3), and upon closer inspection, rhizopodia were seen intertwined (Fig. 4).

The acid phosphatase assay appeared a valid indication of digestion since every food vacuole observed within the foraminifera exhibited acid phosphatase activity, as noted by the red-diazo dye complex under light microscopy (Fig. 8). In all the species examined, acid phosphatase activity was observed in the external food mats (Fig. 12), and in the extended rhizopodia (Table 1, Fig. 18). Some species, such as *Planorbulina*, showed only acid phosphatase activity outside the shell of the foraminifera in these mats.

Acid phosphatase was noted in all areas where the rhizopodia protrude from the organism. Often the activity was found near the apertures (Fig. 17), along the outer edge of the organism (Figs. 5,7,10,15), or in the last few chambers (Figs. 19,20). In *B. sphaerulatus* and *C. gaudichaudii*, a strong reaction for acid phosphatase activity was present in the spines (Figs. 9–11, 13–15), and in the canal system along the tests (Figs. 10, 16).

In contrast, acid phosphatase activity was not seen in the vicinity of the endosymbionts (Table 1, Fig. 6). For example, *P. planatus* has a bilateral distribution of its endosymbionts throughout the foraminifera (Hallock, 1981). However, often in freshly collected *Peneroplis*, the outer few chambers appeared empty, lacking *Porphyridium* endosymbionts. The acid phosphatase assay showed activity only in these last chambers, which were devoid of endosymbionts (Fig. 19). Even in species which showed some food vacuoles distributed within the organism (e.g. *Amphisorus*, and *Marginopora*, Figs. 5,7), no enzyme activity was seen near the endosymbionts (Fig. 6).

The species lacking endosymbionts exhibited the same partitioning of cellular activity (Table 1). *A. laticollaris* showed a distinct internal compartmentation with acid phosphatase activity confined to the outer cytoplasmic streams, along the apertural regions, and in the external food balls. Whereas *Q. costata* showed acid phosphatase activity only in the outer chamber.

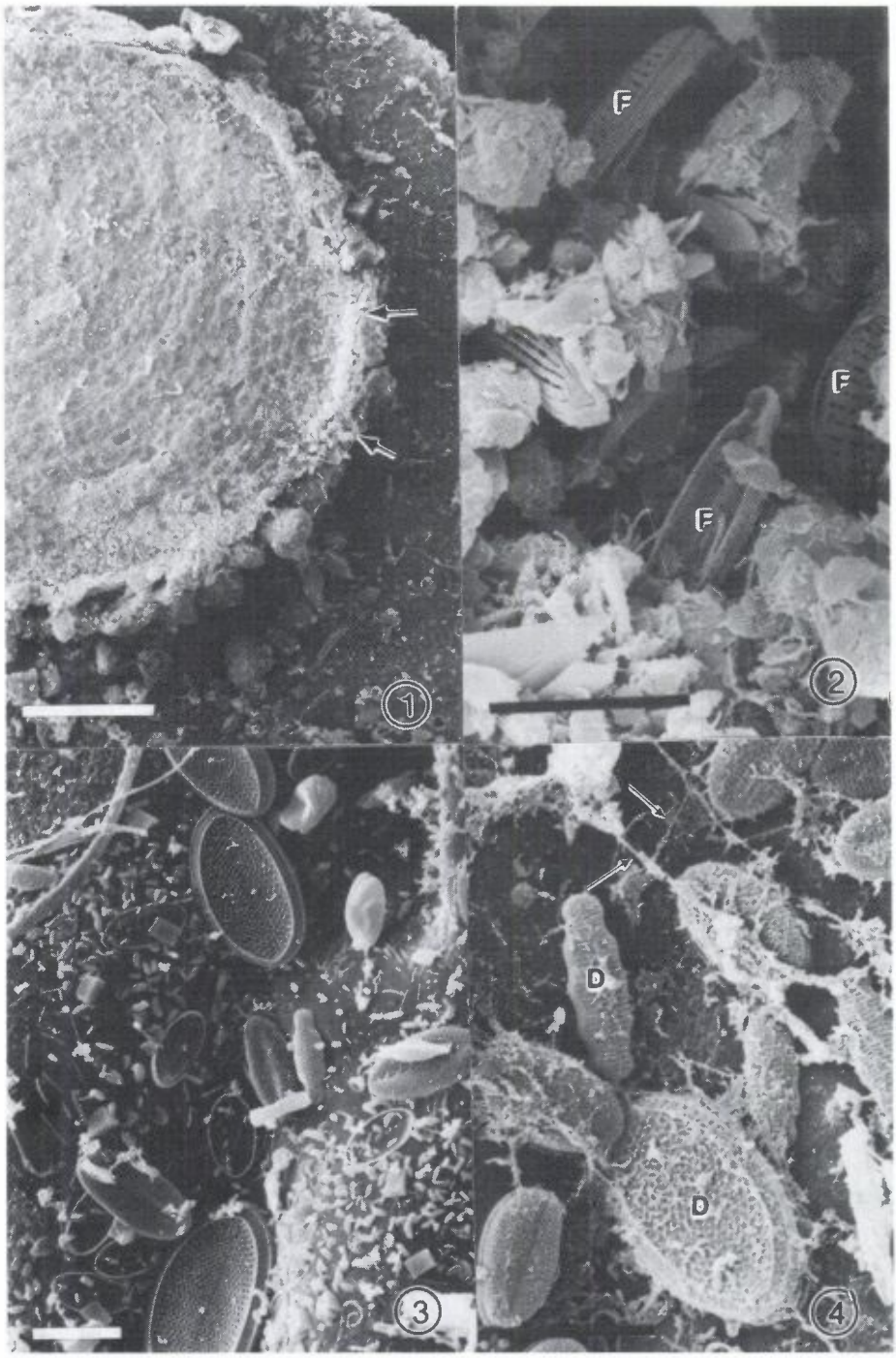
Figures 1–4. A microscopic approach to the food and potential food of *Amphisorus hemprichii*. All specimens were critical point dried and examined with SEM.

Figure 1. *Amphistegina hemprichii* on the surface of a *Halophila* leaf. Arrows point to residia ("midden heap") at periphery of animal. Scale bar = 600 μm.

Figure 2. A portion of the residium of the animal in Fig. 1. Note the empty *Amphora* frustules (F). Scale bar = 10 μm.

Figure 3. The surface of the *Halophila* leaf 1 cm from the animal. Note rich bacterial and diatom flora (*Cocconeis placentula, Cocconeis* sp., *Fragilaria* sp.). Scale bar = 10 μm.

Figure 4. Surface of a leaf closer to the animal showing peripheral pseudopodial network (arrows) intermeshed on the surfaces of diatoms (D) and bacteria. Scale bar = 10 μm.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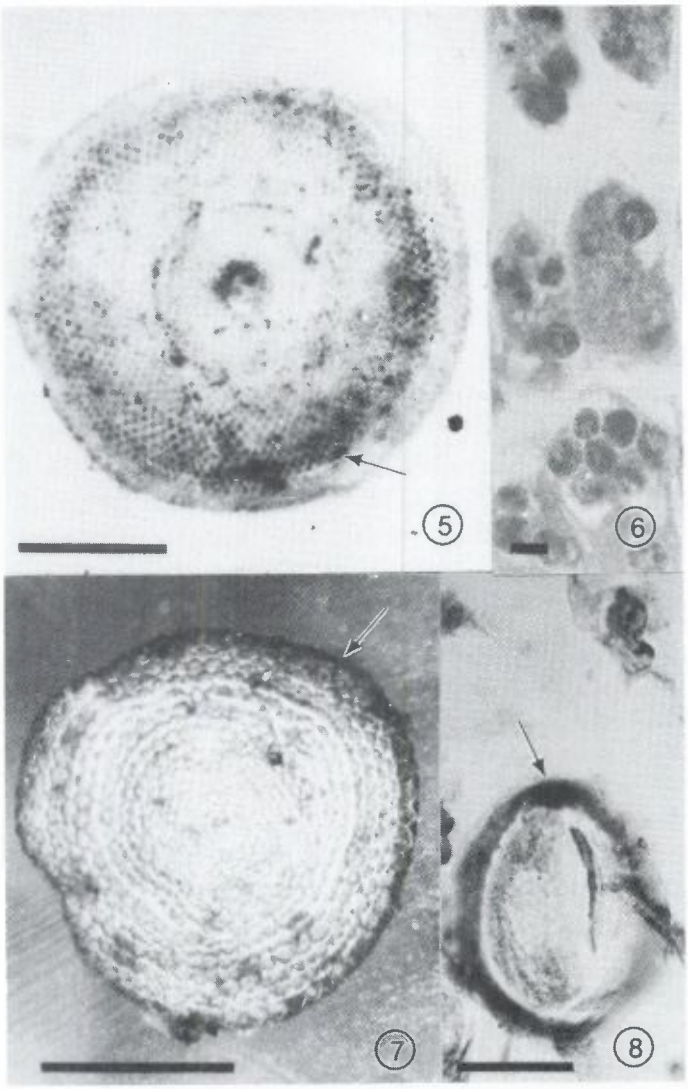

Figs. 5–20. All specimens after acid phosphatase assay, taken on a Zeiss photomicroscope II.

Figure 5. Decalcified *Amphisorus hemprichii* embedded in tissuemat prior to sectioning. Several large food vacuoles are seen (arrow). Scale bar = 100 μm.

Figure 6. Dinoflagelate endosymbionts of *Marginopora kudakajimensis*. Note the lack of any acid phosphatase activity in this region. Scale bar = 10 μm.

Figure 7. *Marginopora kudakajimensis*, prior to decalcification, showing an outer edge of acid phosphatase activity (arrow). Specimen photographed in distilled water rinse. Scale bar = 100 μm.

Figure 8. Close-up of a food vacuole in *Marginopora kudakajimensis*, with strong acid phosphatase activity (arrow). Scale bar = 20 μm.

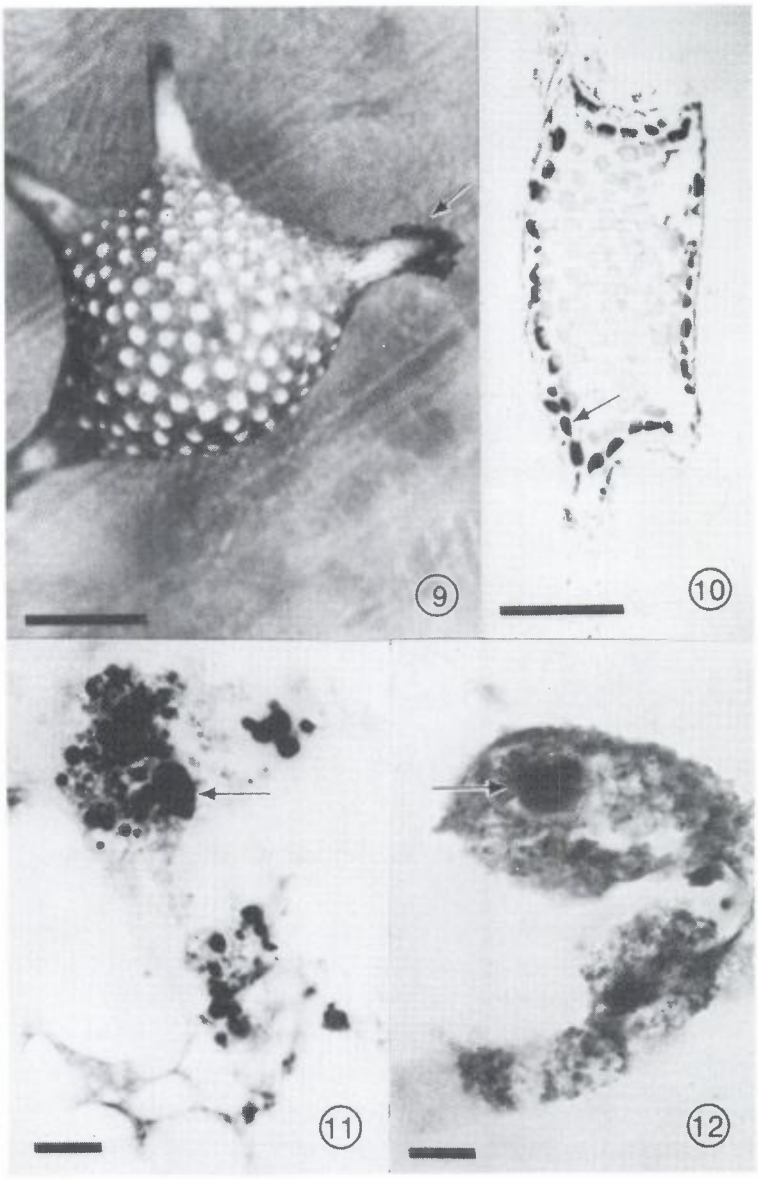

Figure 9. *Baculogypsina sphaerulatus*, prior to decalcification, showing attached food mat with acid phosphatase activity at end of spine (arrow). Specimen photographed in distilled water rinse. Scale bar = 200 μm.

Figure 10. Section of the specimen in Fig. 9, showing activity along the outer edge of the specimen. Scale bar = 200 μm.

Figures 11–12. Close-up of food vacuoles (arrows) within the spine of *Baculogypsina sphaerulatus*. Scale bar = 10 μm.

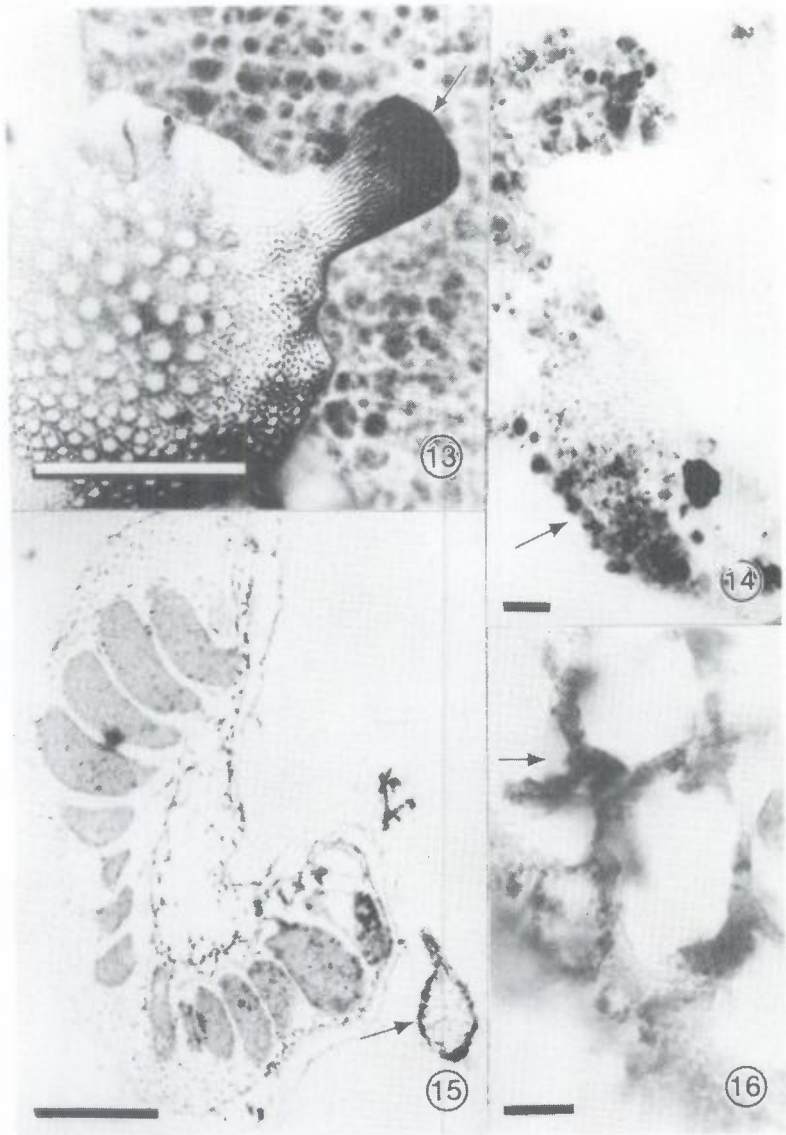

Figure 13. *Calcarina gaudichaudii*, prior to decalcification, showing acid phosphatase activity at spine. Specimen photographed in distilled water rinse. Scale bar = 500 μm.

Figure 14. Cross section of *Calcarina gaudichaudii* spine showing food vacuoles (arrow). Scale bar = 25 μm.

Figure 15. Section of *Calcarina gaudichaudii* showing spine (arrow), and along the edge of the shell. Scale bar = 200 μm.

Figure 16. Close-up of the acid phosphatase activity (arrow) following the canal system along the edge of the shell of Fig. 15. Scale bar = 20 μm.

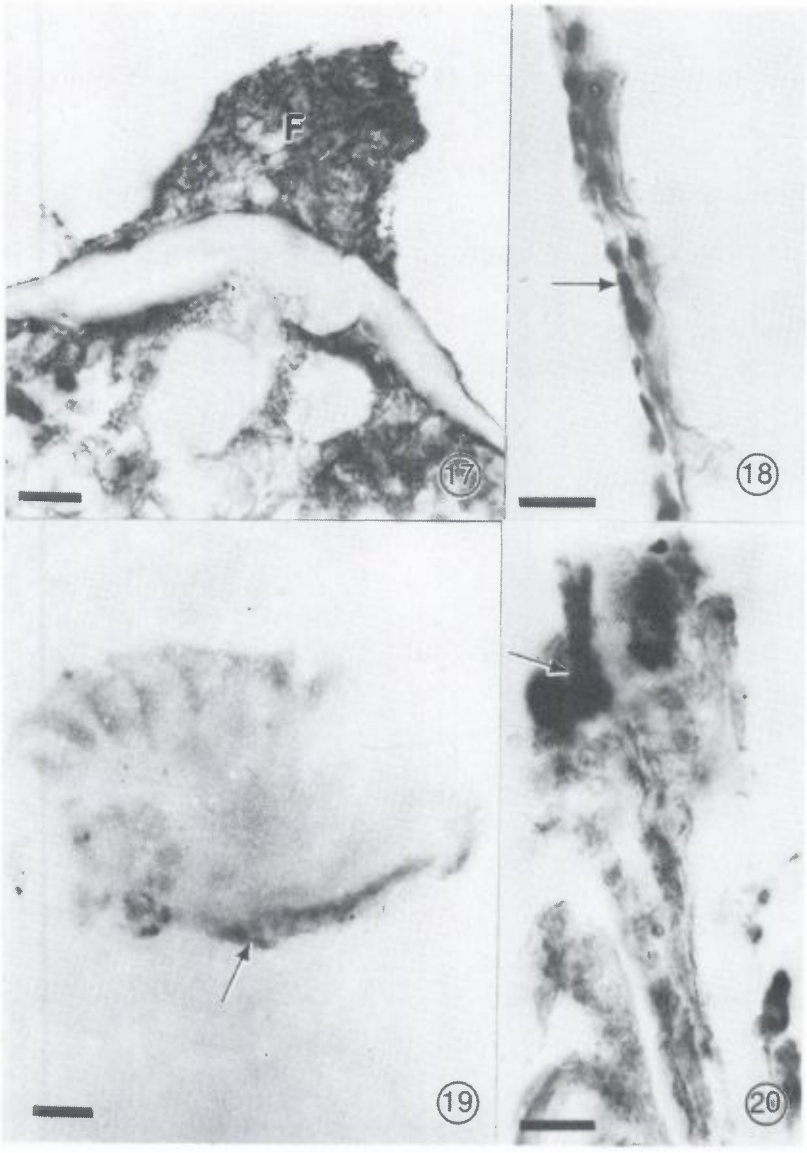

Figure 17. Apertural region of *Allogromia* sp. with attached food mass (F). Scale bar = 10 μm.

Figure 18. Food vacuoles (arrow) seen in the extended rhizopodia of *Amphistegina lobifera*. Scale bar = 10 μm.

Figure 19. Decalcified *Peneroplis planatus* embedded in tissuemat prior to sectioning. Acid phosphatase activity (arrow) seen along last chamber. Scale bar = 100 μm.

Figure 20. Strong enzyme activity (arrow) seen in the outer chamber of *Quinqueloculina costata*. Scale bar = 20 μm.

Table 1. Location of acid phosphatase activity (an indication of digestion), and of endosymbionts (when present) of various species of benthic foraminifera.

Species	Collection site	Acid Phosphatase Activity					Endosymbiont location
		Rhizopodial web	aperture	spines	Outer chambers	Canal system	
Amphisorus hemprichii	Elat	+	+		+		In concentric band around early chambers below the lateral surface
Marginopora kudakajimensis	Kudaka Jima	+	+		+		In wider concentric band around early chambers below the lateral surface.
Peneroplis planatus	Elat	+	+		+		Bilaterally throughout the shell
Amphistegina lobifera	Elat	+	+				Below lateral chamber walls of last 1 or 2 whorls
Calcarina gaudichaudii	Kudaka Jima	+	+	+		+	Throughout the shell (not in the spines)
Baculogypsina sphaerulatus	Kudaka Jima	+	+	+		+	In layers of outer chamberlets
Elphidium incertum	Woods Hole	+	+				Along outer edges of chambers
E. translucens	Woods Hole	+	+				Along outer edges of chambers
Protelphidium tisbuyiensis	Woods Hole	+	+				Along outer edges of chambers
Haynesnia germanica	Woods Hole	+	+				Along outer edges of chambers
Nonion sp.	Woods Hole	+	?				Along outer edges of chambers
Quinqueloculina costata	Woods Hole	+	+		+		–No known endosymbionts–
Planorbulina sp.	Flat	+	+				–No known endosymbionts–
Allogromia	Woods Hole	±	+				–No known endosymbionts– (some acid phosphatase activity at food plaque nodes)

4. Discussion

The results of this study seem to support the hypothesis that the initial steps of digestion are generally extra-cameral in some benthic foraminifera. Therefore if living undigested algae are drawn into the shell they have potential to develop as endosymbionts. Very little is known about the digestive processes in symbiont-bearing forms. We found differences in the amount of carbon egested after *A. hemprichii* and *A. lobifera* had fed on different species of algae (Lee et al., 1988). In particular we noted that the cell wall of *Chlorella* sp. (AT) was egested intact. The digestive enzymes of both hosts were unable to degrade this potential energy source during its residence time within the host. In an experimental study with nearly aposymbiotic *Amphistegina* Koestler et al. (1985) found several interesting facts. Isolation techniques showed that various species of axenically cultured endosymbiotic diatoms could escape digestion and repopulate the host. Although free-living species were fed to the foraminifera in mixtures containing symbionts none escaped digestion. Fine structural studies revealed two additional facets of the relationship. Some of the diatoms in a chain of *Fragilaria shiloi*, an endosymbiotic species, were digested, others were not. This requires much more detailed examination. The other facet was shown in experimental groups of *Amphistegina*, which were starved and incubated in the dark, and specimens in groups starved and incubated in the light with DCMU. The cytoplasm, as time progressed, became greatly vaculated, less granular and eventually the pore rim cups, which ordinarily are the sites for endosymbionts, gradually became vacant. Microbodies were seen in the vicinity of the symbionts but the study was not detailed enough to discriminate between possible autolysis of stressed symbionts and digestion by host lysosomal fusion with symbiont vacuoles. Further studies on the digestive process in algal-symbiont-bearing foraminifera and chloroplast husbanding foraminifera by fine structural examinations are now in progress.

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