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The Retention of Chloroplasts by the Foraminifer *Elphidium crispum*

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Abstract

Specimens of the foraminifer *Elphidium crispum* from Eilat, Israel, and Mombasa, Kenya, retain chloroplasts, probably derived from digested algae, in their cytoplasm. Some vacuoles containing chloroplasts also had additional algal remnants (e.g. pyrenoids, mitochondria and occasionally nuclei). Measurements of carbon uptake with ^{14}C tracer methods suggest that these chloroplasts are still capable of carbon fixation. Electronmicroscopical pictures of the cytoplasm indicate that, even through the chloroplasts may function as "symbionts", they are slowly but actively being digested by the host.

Keywords: Foraminifera, chloroplasts, carbon fixation, digestion

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1. Introduction

Endosymbiotic associations between algae and foraminifera are well known (reviewed in Lee, 1983, Lee and McEnery, 1983, and Leutenegger, 1984), but recently it has been shown that some foraminifera sequester the chloroplasts of the algae upon which they feed (Lopez, 1979; Leutenegger, 1984). Foraminifera are hardly unique in this respect. Sacoglossan molluscs have been shown to retain functional chloroplasts derived from their food (Greene and Muscatine, 1972; Trench et al., 1973; Hinde and Smith, 1974; Trench, 1975; Clark et al., 1981). Most of our knowledge on chloroplast retention in foraminifera is of 3 species, *Elphidium williamsonii*, *E. excavatum* and *Nonion germanicum* which were collected from relatively shallow surface sediments (0–0.5 and 6 m) in a Danish brackish water area, Limfjorden, where they were moderately abundant (10^5 individuals m^{-2}). Independent of season *E. williamsonii* contained about 4×10^6 chloroplasts per specimen (Lopez, 1979). It was estimated that to keep a steady state population of chloroplasts under light/dark cycles the foraminifer must acquire at least 65 chloroplasts per individual per hr. At light saturation $NaH^{14}CO_3$ uptake was proportional to chlorophyll content and in the range of 2.3×10^{-3}mg C $mg^{-1}hr^{-1}$ in *E. williamsonii* and 0.5×10^{-3}mg C $mg^{-1}hr^{-1}$ in *N. germanicum* (Lopez, 1979). Expanding on this original observation Leutenegger (1984) commented on chloroplast retention in *Elphidium* spp. from mostly shallow (1–5 m) depths in the Mediterranean, Red Sea and the Pacific (New Caledonia) (18 m), (Leutenegger, 1984).

From these studies it is not clear whether the chloroplasts retained by the foraminifer function as symbionts by transferring organic carbon to the host, or are slowly being digested. If indeed the turnover on a population of 4×10^6 chloroplasts is only 65 hr^{-1} (Lopez, 1979) then they are basically left intact. In the photomicrographs of the same study, however, they seem to be in an advanced state of degradation. Secondly, these observations were made on specimens from temperate waters and whether the conclusions have general validity is not certain. In this report we demonstrate that the chloroplasts of *Elphidium crispum* from tropical waters are being digested, even though they are still capable of carbon fixation after ingestion.

2. Materials and Methods

Collection

The 2 collections of *Elphidium crispum* in the Indian Ocean at, or near Mombasa, Kenya, were made during the Post-Congress Work Shop on Marine Protozoa (Lee and Röttger, 1986). Both collections were made at low tide and represented pooled samples gathered by the participants. One collection was made at a mud flat, the other collections were made in a back-reef environment. The wind and wave action on the reef in the latter habitat discouraged collection by SCUBA. All specimens were collected by reaching into or snorkling in water less than 2 m deep and mostly less than 1 m at low tide. The samples were returned less than an hour after collection to the Kenya Marine and Fisheries Research Institute in Mombasa where they were promptly picked, identified, and fixed for TEM as described below.

The samples with *E. crispum* from the Gulf of Eilat, Israel, came from a calcareous mud flat seaward and just north of Wadi Taba at a depth of 38 m. *Operculina ammonoides* was quite abundant at this site. *Heterostegina depressa* was collected on stones at 40 m off the H. Steinitz Marine Biology Laboratory. The other species used in the comparative part of the study were also collected at Taba, but at shallower depths, 15–25 m. The mud was brought to the H. Steinitz Marine Biology Laboratory in Eilat immediately after collection. There a thin layer of sediment was placed in covered, deep petri dishes and overlain with filtered sea water. The dishes and their contents were left undisturbed on a shelf for approximately 18 hr during which the foraminifera crawled to the surface of the sediment. Although *E. crispum* was present, it was rare (about 20 per dish). The organisms were fixed for TEM as they were picked from the sediment (24–33 hr after collection from the sea). Some additional specimens were fixed 96 hr after collection.

Fixation, decalcification, embedding

The specimens were fixed in 6.5% glutaraldehyde in a 1:1 sea water: 0.1 M Na cacodylate buffer at pH 7.2 and at 4–10°C. They were post fixed in 1% OsO_4 in the same sea water-buffer mixture. They were decalcified in either EGTA or in Poly-No-Cal (Cat. 16865, Polysciences, Warrington, PA 18976), a propriatory agent. Dehydration was in graded series of ethyl alcohol. Embedding was in Spurr's low viscosity medium (Spurr, 1969). Sections were stained in uranyl acetate and lead citrate and observed in a Philips 300 TEM at 40 KV.

Inorganic carbon uptake experiment

This experiment was set up in Eilat the day following the harvest of the various species of foraminifera from the sea (Table 1). Two hundred organisms of each species were placed in sealed erlenmeyer flasks containing filtered sea water and labeled with $NaH^{14}CO_3$. Duplicate samples for specific activity were taken at the beginning and end of each incubation. Incubations were carried out for 48 hr in a 26°C waterbath placed near a north facing window so that the organisms were exposed to a light flux equal to approximately 10% of the light incident on the surface of the sea. The maximum light intensity was approximately 750 $\mu Em^{-2}s^{-1}$, a level equal to measurements made in the Gulf of Eilat at 15–20 m. The analytical methods described by Erez (1978) and slightly modified by ter Kuile and Erez (1987) were followed. Briefly: after harvest, animals were dried in a warm oven (60°C). The dried sample was crushed and thoroughly homogenized. A 5 mg sample was weighed on a Cahn electrobalance and transferred to a liquid scintillation vial. Twenty mg of reagent grade $CaCO_2$ was added to this vial which was then placed in a jar standing in an ice bath. Another vial containing 2 ml of Oxisorb (New England Nuclear) was placed in the same jar which was then sealed. Two ml of 8.5% H_3PO_4 was added by injection through a rubber port into the vial containing the $CaCO_3$. After an hour 10 ml Instagel (Packard) was added to the vial containing the Oxisorb. The vial originally containing the sample was washed over a preweighted Nuclepore filter. The filter was dried in a petri dish in a vacuum-dessicator and the wash water containing the acid was evaporated to 2 ml and then 10 ml Instagel was added. The dry filter was reweighed, transferred to the vial originally containing the sample and then 10 ml of Instagel was added. The samples so obtained were called respectively "skeletal fraction" "acid soluble organic matter" and "residual organic matter" (ter Kuile and Erez, 1987). The results were calculated according to the formula:

$$\frac{\text{Counts per minute}}{\text{Weight sample}} \times \frac{\text{Inorganic carbon content}}{\text{Specific activity}} = \mu g\ C mg^{-1}\ \text{foram}$$

The averaged standard deviation for the various fractions is 5.2% of the reported values (ter Kuile and Erez, 1987).

3. Results and Discussion

Carbon fixed

Specimens of *Elphidium crispum* fixed 1.5 μg carbon mg^{-1} foram d.w. in 48 hr (Table 1). This was slightly more than specimens of *Heterostegina depressa* (1.28 μg carbon mg^{-1} foram d.w.) a diatom-bearing species from the same depth (38 m). Another diatom-bearing species from the same depth, *Operculina ammonoides*, fixed carbon at twice the rate (3.22 μg C mg^{-1} foram d.w.) as did *Dendritina acicularia*, a red alga-bearing host, *Borelis schlumbergeri*, a diatom-bearing host, (2.7 and 3.16 μg C mg^{-1} foram d.w. respectively) and *Amphistegina lobifera* a diatom-bearing host (3.31 μg C mg^{-1} foram d.w.). The highest rates of fixation were found in *Peneroplis pertusus* (4.49 μg C mg^{-1} foram d.w.) a red alga-bearing host and in *Amphisorum hemprichii* (3.95 μg C mg^{-1} foram d.w.) a dinoflagellate-bearing host.

We are cautious about concluding too much from these comparative measurements based, as they were, on dry weight. The ratio of shell material to protoplasm (protein content) varies not only from one species to another, but also within the same species as a function of age and some environmental factors (depth, current). To make a more detailed comparison one would need data on protein content, chlorophyll content, as well as sizes and weights of the organisms. However, the data obtained clearly indicate that the retained chloroplasts of *E. crispum* are still actively fixing carbon, possibly at the same rate, with respect to organic fraction, as *O. ammonoides* and *H. depressa*, 2 species with whole diatom symbionts found at the same depth. The fixation rate seems lower than some of the foraminiferal symbiotic systems found at shallower depths (e.g. *A. lobifera, A. hemprichii, P. pertusus*).

Fine structure

Even a casual examination of the external morphology of *E. crispum* shows that this animal's surface is unusual (Figs. 1 and 3). Though the apertures, the principle opening(s) in the shells through which the pseudopodia emerge, vary greatly in foraminifera with respect to relative size, ornamentation, and location, the apertures of *E. crispum* are reduced to the size of the fossettes (Figs. 1 and 3). In fact if one did not know where the apertures should be they could not be distinguished from the more numerous fossettes. Closer examination of the septal pits leading to the fossettes, through which the pseudopods emerge, shows that the pits are bordered by "spikes" also called "tooth plate apertures") (Fig. 3). Knowledge of the chloroplast-husbanding habit of *E. crispum* leads us to speculate about the function of the tuberculate

borders of the septal pits. If digestion begins in the extrathalmic cytoplasm, as it does in *Amphistegina lessonii* (Koestler et al., 1985) then it is possible that the tubercules might act as the teeth in a comb, permitting small or flexible structures in partially digested algal cells to pass through without permitting larger inflexible structures, such as most diatom frustules, to pass into the test. Diatom frustules have not been observed internally in this species (see below) although they are commonly observed in other genera (e.g. McEnery and Lee, 1981, Koestler et al., 1985).

In lateral view, the chambers are arranged as a spiral (Fig. 2). The cytoplasm within the chambers of specimens from both Mombasa and Taba was filled with chloroplasts as well as numerous host organelles (Fig. 4). Judging from the uniform well-preserved state of the host's mitochondria (m) and golgi (g) in the cytoplasm near the envaculated chloroplasts, the chloroplasts themselves were in various states of disorganization (or degeneration). Some seemed fairly close to normal with respect to thylakoid organization (Figs. 4A,6,7 and 8), while the thylakoids in others (Fig. 4B and C) seemed progressively less parallel and shorter. A later stage may have been those chloroplasts in which the thylakoid integrity was lost in some regions of the vacuole (Fig. 5D). We recognize the possibility that optimal fixation for the host tissue might not be optimal or adequate for the chloroplasts and therefore what we are reporting might be artifactual. However, this seems less probable than degeneration since many chloroplasts appear normal. Some vacuoles in the cytoplasm contained a mixture of dense granules and less electron dense aggregates (e.g. Fig. 4D). These vesicles probably contain residues from digestion and are later egested (e.g. Koestler et al., 1985).

Close examination of the vacuoles with more normal appearing chloroplasts showed that the vacuoles also contained pyrenoids (p) (e.g. Figs. 4–6) and mitochondria (m) (Figs. 6 and 7). Partial sections of nuclei (e.g. Fig. 7) were occasionally recognized. Thus we might more properly call the bodies husbanded within vacuoles "algal remnants". Two membranes are usually found encircling the algal remnants (e.g. Figs. 7 and 8). The outer one we would interpret as host phagolysosomal membrane and the other, which is not always entire, as the algal cell membrane. Within the latter is a third membrane belonging to the chloroplast (Fig. 8). We found some sections in which the outer enveloping membranes were not intact, but, in which smaller vacuoles were fusing to them (Fig. 6). Small vesicles reasonably interpreted as microbodies or lysosomes were seen at the periphery, suggesting that the host still actively digests the algal remnants and that their eventual total

Table 1. Relative rates of primary production in selected foraminiferal/algal symbiont systems from the Gulf of Eilat

Host species	Incorporation of ^{14}C* organic fraction	Skeleton	Total
Elphidium crispum	0.87	0.65	1.53
*Dendritina acicularia***	1.58	1.09	2.67
Amphistegina lobifera	1.91	1.40	3.21
Amphisorum hemprichii	2.14	1.80	3.95
Operculina ammonoides	0.96	2.25	3.22
Heterostegina depressa	0.72	0.56	1.28
Borelis schlumbergeri	1.84	1.31	3.16
Peneroplis pertusus	2.92	2.07	4.99

* μ g C/mg d.w foram/48 hr

** The authors are aware that others (e.g. Reiss and Hottinger, 1984) consider this organism to be a morphotype of *Peneroplis planatus*. We have tentatively followed the nomenclature of Hofker (1951) while the structure of this form is being studied.

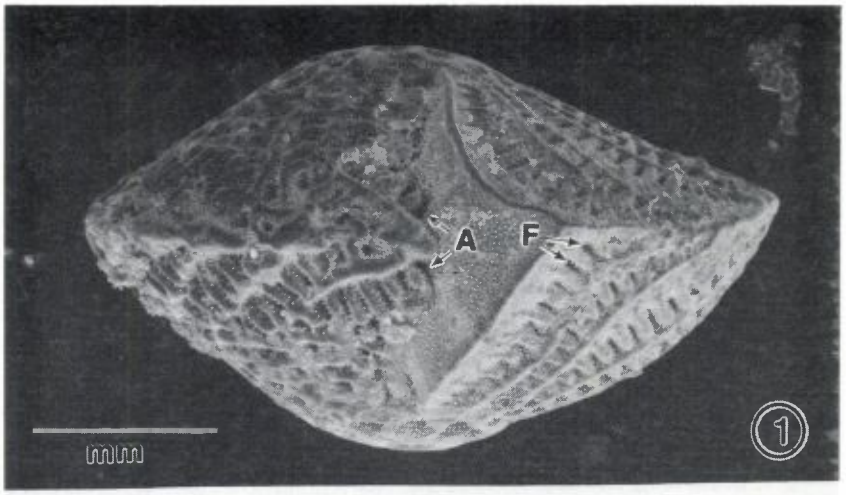
degradation is not entirely due to autolysis. This interpretation is further strengthened by the abundance and distribution of golgi in the cytoplasm of the host (e.g. Fig. 5).

It is difficult to compare our results with those of either Leutenegger (1984) or Lopez (1979). Of the six published micrographs none shows retention of organelles other than chloroplasts. Pyrenoids are visible in some specimens illustrated by both authors. Leutenegger's 2 micrographs, however, are at relatively low magnification ($\times 10,500$) and the chloroplasts illustrated are relatively advanced in their degradation. Two of Lopez's photomicrographs are sufficiently high in magnification ($\times 22,600$ and $\times 27,800$) to show mitochondria if they were present, they may, however, not be in the plane of sections. The specimens chosen, were also relatively advanced in their degradation. At present it cannot be determined whether the differences noted are observational, species specific, or due to some other factors. In this study we demonstrated that *E. crispum* retains chloroplasts, probably derived from partial digestional of algae outside of the shell, in its cytoplasm. Even though these chloroplasts are capable of carbon fixation and therefore may function as a source of organic carbon for the host, they seem to be digested on the long term.

Figure 1. Edge or apertial view of *Elphidium crispum* from Mombasa, Kenya. Note that the organism has very reduced apertures (A) similar in size to the fossettes (F).

Figure 2. Lateral view of *E. crispum* from Eilat, Israel, showing chamber arrangement and umbilical spiral canal system (c). Light micrograph of a specimen fixed and embedded for transmission electronmicroscopy.

Figure 3. Lateral view of *E. crispum* from Eilat showing the septal pits (SP) opening into the fossettes through which the pseudopods emerge. Note that the "comb-like" tuberculate borders of the septal pits ("spikes", "toothplate", "apertures") could possibly prevent the drawing of intact moderate sized (10 μm), rigid diatom frustules in food vacuoles into the test.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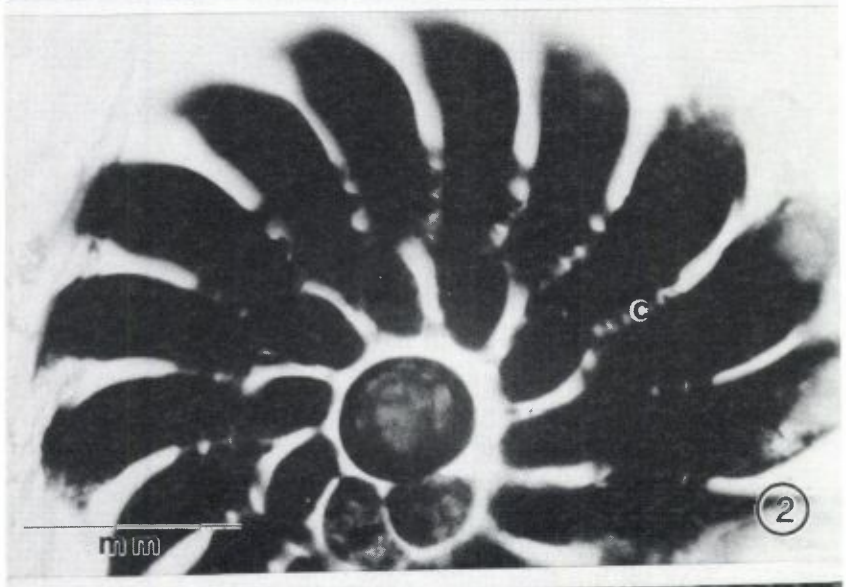
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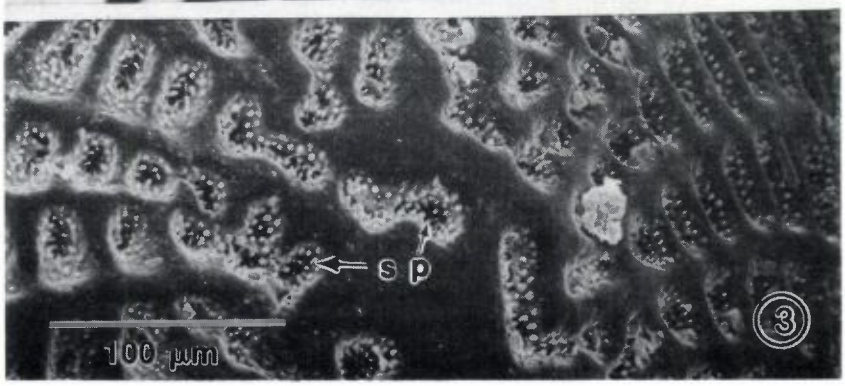
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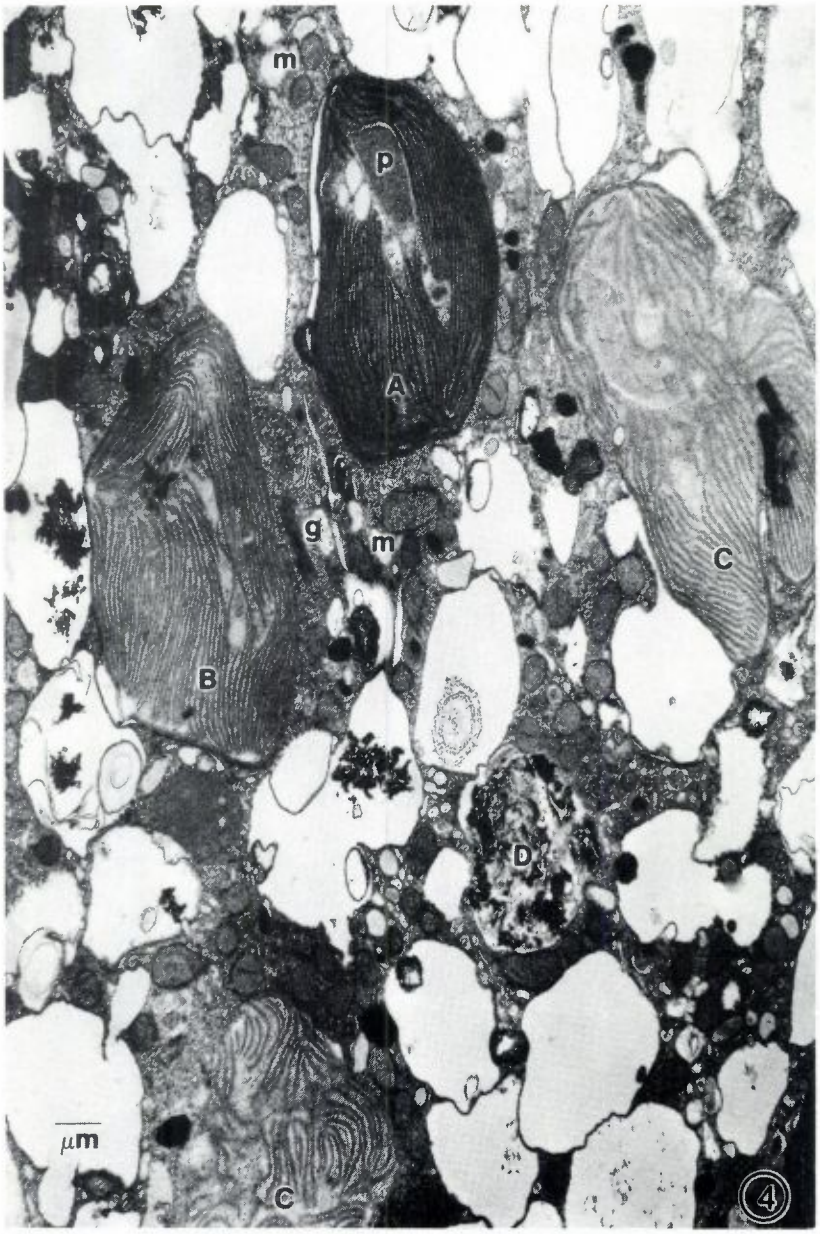

Figure 4. Section through a chamber of *E. crispum* fixed directly from the sea at Mombasa. Note the close juxtaposition of chloroplasts which appear close to normal in organization (A) and those that appear in various states of disorganization (B & C). A normal appearing pyrenoid (P) is in chloroplast (A). Food vacuole residua (D) are recognizable. Note the numerous mitochondria (M) distributed throughout the host cytoplasm. Golgi (g) are also numerous, but only one is visible in this section.

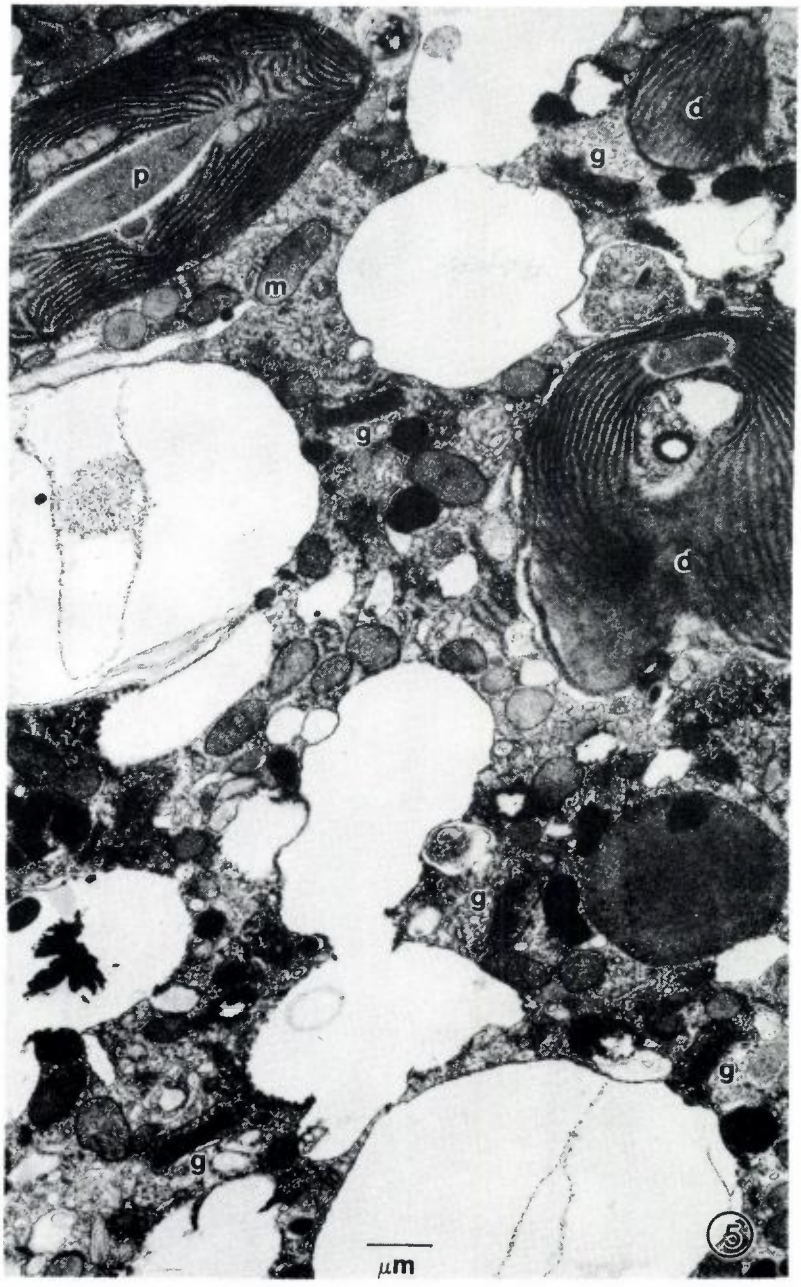

Figure 5. Section through a chamber of another specimen showing a chloroplast with a fairly normal appearing pyrenoid (P) and several considerably degraded chloroplasts (D). Note the abundance of golgi (g) and mitochondria (M) in the chamber.

Figure 6. An algal cell remnant showing a fairly normal chloroplast with pyrenoid (p) and numerous algal mitochondria (M; host mitochondria also labeled). Note that phagosome vacuole is intact at many places and absent or degraded at others (arrows). A microbody (mb) seems in close juxtaposition to a vacuole associated with the chloroplast.

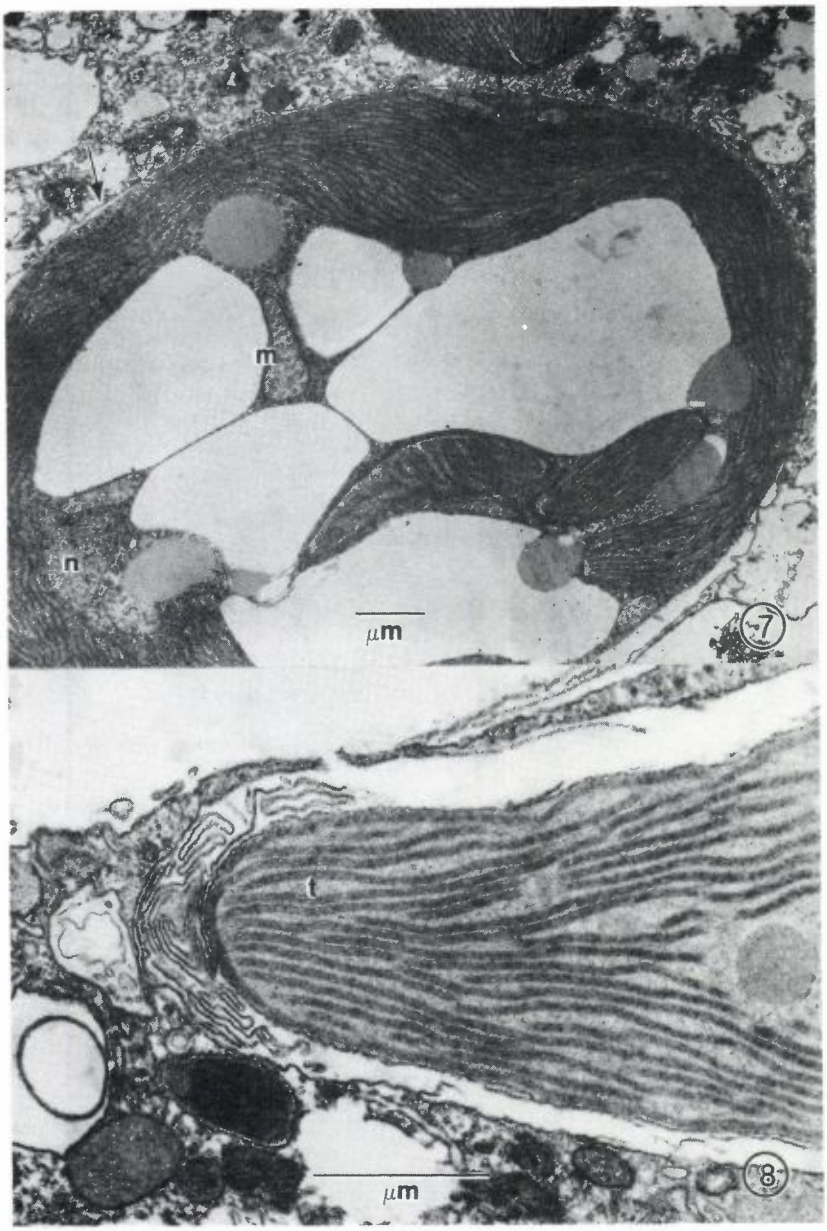

Figure 7. An algal cell remnant showing well preserved mitochondria (M), a portion of nucleus (n) as well as chloroplast. Arrow points to phagosome membrane which was complete in this specimen.

Figure 8. A portion of a chloroplast with fairly well preserved thylakoid structure (T) typical of a diatom.

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