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Organelle loss in the endosymbiont of *Gymnodinium acidotum* (*Dinophyceae*)

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Summary. The freshwater dinoflagellate *Gymnodinium acidotum* is known to harbor a cryptomonad endosymbiont whose chloroplasts give the organism its blue-green coloration. Every cell examined from a wild population possessed chloroplasts, mitochondria, and other organelles which are of endosymbiotic origin. Transmission electron microscopy and fluorescence microscopy revealed that only 33% of these cells possessed the nucleus of the endosymbiont. The lack of a cryptomonad nucleus in some cells did not appear to affect the cells' ability to photosynthesize or move in response to varying levels of illumination. This represents the first report of a host/endosymbiont relationship in which a significant number of individuals from a given population lack a major endosymbiont organelle.

Keywords: Cryptophyceae; Dinoflagellate; *Dinophyceae*; Endosymbiosis; *Gymnodinium*; Ultrastructure.

Introduction

In the spring and fall of 1988 blooms of the freshwater dinoflagellate *Gymnodinium acidotum* Nygaard were collected from the surface waters of Amy Bayou (Henderson, Louisiana). *Gymnodinium acidotum* is one of several dinoflagellates known to contain cryptophytes which give the organisms a bluish-green coloration (Zhang et al. 1982; Wilcox and Wedemayer 1984, 1985; Larsen 1985, 1988). Although Larsen (1988) suggests that the presence of cryptomonads in *Amphidinium poecilochroum* is due primarily to selective feeding behavior, the internal cryptomonad of *G. acidotum* appears to be well incorporated into the host cell's cytoplasm and is most likely acting as an endosymbiont. In their initial study on the general ultrastructure of *G. acidotum*, Wilcox and Wedemayer (1984) reported that one of the two cells examined using transmission electron microscopy lacked an endosymbiont nucleus. Our survey of many hundreds of cells not only confirms this finding but suggests that this condition is typical of naturally occurring populations with only 33% of the cells having both a dinoflagellate and an endosymbiont nucleus. In this study we have reexamined the ultrastructure of *G. acidotum* with particular attention being given to organelles of the endosymbiont and how they might affect the relationship between the endosymbiont and the host dinoflagellate.

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Materials and methods

Gymnodinium acidotum Nygaard was collected from Amy Bayou (Henderson, Louisiana) in mid-October 1988 and was the dominant phytoplankter. Cells were concentrated by allowing samples to remain undisturbed for several hours under unidirectional light. The cells accumulated along the water surface nearest the light source. All cells were motile and appeared healthy prior to fixation.

Transmission electron microscopy

Approximately 5 ml of concentrated cells were fixed at 4 °C with 3.2% glutaraldehyde in 0.025 M PHEM buffer pH 6.9 and simultaneous exposure to osmium vapors. Cells were fixed on ice for 1 h, rinsed in 0.025 M PHEM buffer, post-fixed in 1% OsO_4 in buffer for 30 min, and dehydrated through a graded acetone series. Dehydrated samples were infiltrated with Spurr's resin (Spurr 1969) and polymerized at 70 °C for 8 h. Silver sections were cut on a RMC 6000 ultramicrotome and post-stained with uranyl acetate and lead citrate using an LKB Ultrostainer. Sections were examined using a Hitachi H-7000 transmission electron microscope.

Scanning electron microscopy

Cells were attached to poly-L-lysine coverslips, rapidly frozen by plunging into liquid propane, and substituted in − 80 °C acetone for 4 days. Samples were slowly brought to room temperature and critical point dried using liquid CO_2 as the intermediate fluid. Samples were

sputter coated with gold-palladium and examined in a Hitachi S-450 scanning electron microscope.

Fluorescence and light microscopy

Cells were fixed in a solution of 2% glutaraldehyde in 0.025 M PHEM buffer pH 6.9. Following several rinses in distilled water, samples were stained for the presence of DNA with either 4,6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI) and examined with a Zeiss Universal microscope using epifluorescence illumination. Living cells were examined with a Zeiss Universal microscope equipped with Nomarski Differential Interference Contrast optics and photographed using either Technical Pan Film 2415 (Kodak) or Fujichrome 100 DX (Fuji).

Results

The general ultrastructure of *Gymnodinium acidotum* has been previously described (Wilcox and Wedemayer 1984). The cell morphology of *G. acidotum* is similar to other gymnodinoid dinoflagellates in that the epicone and hypocone are nearly the same size and the cingulum is only slightly displaced (Fig. 1). The most unusual feature of *G. acidotum* is the presence of an endosymbiotic cryptomonad. Although a functional flagellar apparatus was not observed, the endosymbiont has retained its mitochondira (Figs. 14 and 17), chloroplasts (Figs. 6, 7, 10–15, and 19), Golgi apparatus (Fig. 12), and in some cases its nucleus (Figs. 2–6, 9, 10, and 13). The organelles of the endosymbiont are distributed throughout the host cell's cytoplasm (Figs. 6 and 7).

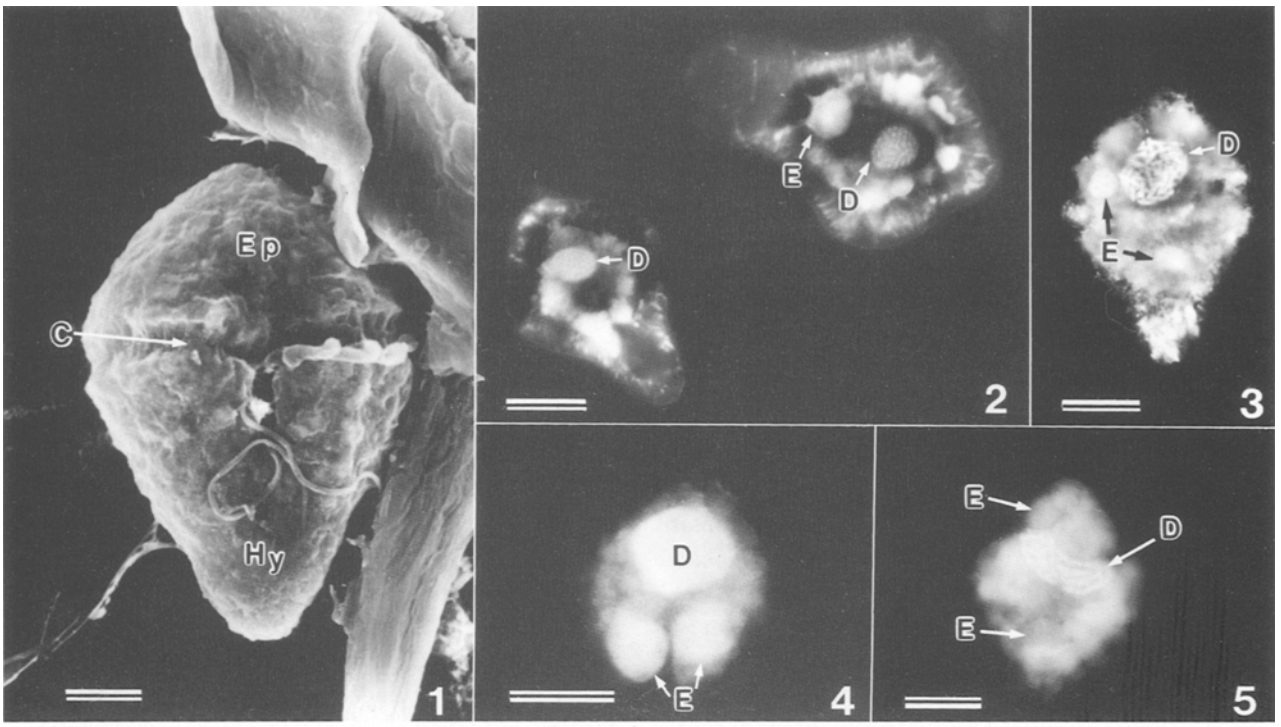
Each of over three hundred *G. acidotum* cells examined using epifluorescence had a dinoflagellate nucleus but only 33% also possessed an endosymbiont nucleus. When present the endosymbiont nucleus is located in the hypocone and is comprised of relatively diffuse chromatin with a prominent nucleolus (Figs. 6, 9, 10, and 13). In contrast, the dinoflagellate nucleus is situated in the epicone and is distinguished by the presence of permanently condensed, banded chromosomes (Figs. 6–8,16, and 19). Fluorescent images of PI and DAPI stained cells reveal the morphological differences between these two types of nuclei (Figs. 2–5). In a few cells the endosymbiont's nucleus had replicated whereas the dinoflagellate's had not (Figs. 3–5, and 10). Electron microscopy revealed that in at least one cell there was no trace of a mitotic spindle following karyokinesis (Fig. 10) and the two endosymbiont nuclei were found at opposite lobes of a single chloroplast (Fig. 11). Following karyokinesis, one of the endosymbiont daughter nuclei is located in the epicone and lies adjacent to the dinoflagellate nucleus (Fig. 3). The dinoflagellate nucleus subsequently divides thereby distributing one of each type of nucleus to the daughter cells (Fig. 5). Regardless of whether a cell contained an endosymbiont nucleus, a nucleomorph was found in the periplastidal space of each chloroplast (Fig. 12 and 13). Nucleomorphs were most often found adjacent to the endosymbiont nucleus (Fig. 13), or in the case of anucleate endosymbionts, in the region of the cell where the nucleus would normally reside (Fig. 12).

The endosymbiont chloroplasts are distributed along the periphery of the host cell (Figs. 6 and 7). The thylakoids are primarily grouped in pairs with electron-opaque lumens (Fig. 12) and electron dense lipid bodies scattered throughout the stroma (Figs. 13 and 15). Storage bodies are present both in the dinoflagellate cytoplasm (Fig. 16) and in the periplastidal space of the endosymbiont (Fig. 14). The chloroplast pyrenoids are traversed by thylakoids which in some cases form a highly convoluted reticulum (Fig. 15). In addition to the pyrenoids there are usually one to several chloroplast inclusions composed of closely packed particles (Figs. 19 and 20). The particles measure approximately 17 nm in diameter and appear identical in profile regardless of section plane. These chloroplast inclusions are exclusively found in the epicone, and most often lie adjacent to the dinoflagellate nucleus (Fig. 19).

In addition to containing a single cryptomonad endosymbiont, each *Gymnodinium acidotum* cell contains a number of what appear to be ingested bacteria. These bacteria were most often found clustered together in membrane bound vesicles (Fig. 19). One to several red-pigmented bodies were also found in living cells and appeared to be food vacuoles (Fig. 13). A peduncle (a microtubule supported structure that is associated with phagotrophy in other dinoflagellates) was observed in the region of the flagellar openings (Fig. 18).

Discussion

Our examination of *Gymnodinium acidotum* supports the belief that the cryptomonad within the dinoflagellate is actually an endosymbiont. Previous reports of a dinoflagellate harboring a cryptophyte-like endosymbiont include *G. acidotum* (Wilcox and Wedemayer 1984), *G. eucyaneum* Hu (Zhang et al. 1982), and *Amphidinium wigrense* Woloszynska (Wilcox and Wedemayer 1985). A fourth dinoflagellate, *Amphidinium poecilochroum* Larsen, was originally described as being a phototroph with blue-green chloroplasts (Larsen 1985) but has since been shown to be a phagotrophic species that exists by ingesting cryptophycean prey (Larsen 1988). Unlike *A. poecilochroum* which can contain up to six ingested cryptomonads, every *G. acidotum* has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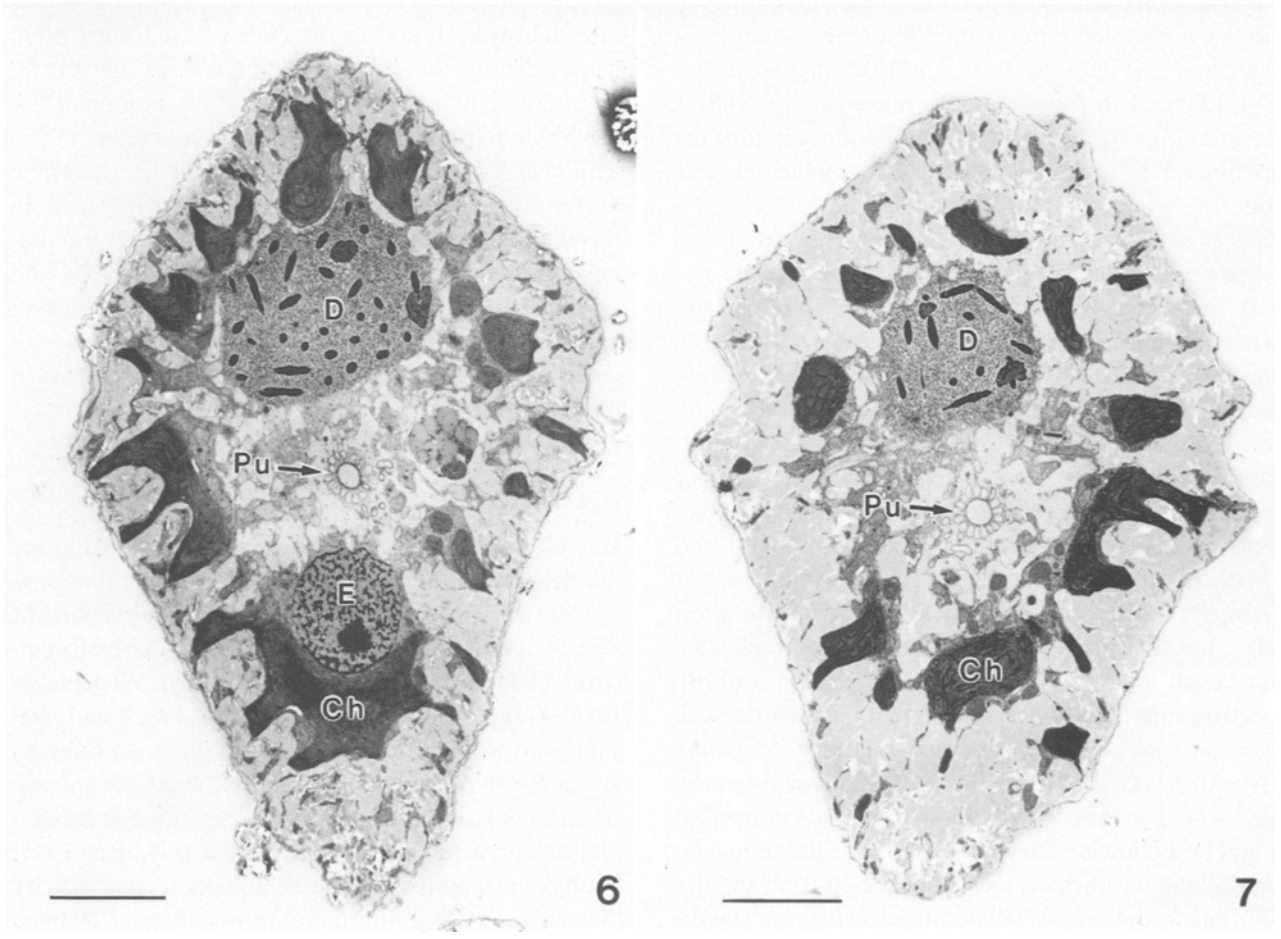
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but a single cryptomonad per cell. Furthermore the organelles of the cryptomonad are well distributed throughout the host cytoplasm of *G. acidotum* (see Figs. 2–7) and are not restricted to a phagocytic vacuole as they are in *A. poecilochroum* (Larsen 1988). The presence of a peduncle and food vacuoles supports the belief that *G. acidotum* remains capable of some phagotrophy (Wilcox and Wedemayer 1984). It is likely that nutritional demands are met by mixotrophy rather than relying solely on the endosymbiont. Attempts to culture *G. acidotum* were unsuccessful but may be due to factors other than the cell's ability to act efficiently as a phototroph. When it was collected *G. acidotum* was by far the dominant phytoplankter present.

Although the pyrenoids of *Gymnodinium acidotum* are similar to those described for other cryptomonads (Gantt 1980) the unusual chloroplast inclusions merit some mention. The close packing arrangement of the particles and their uniform profiles suggests that they are globular in nature. Crystalline matrices have been found in the pyrenoids of the diatom *Achnanthes brevipes* (Holdsworth 1968) but with an average size of approximately 5 nm these particles differ significantly from the 17 nm particles found in *G. acidotum*. While it is possible that the chloroplast particles represent accumulations of the enzyme ribulose-bis-phosphate-carboxylase (Rubisco), they bear little resemblance to either the pyrenoids found in the endosymbiont of *G. acidotum* or other cryptomonad taxa (Gibbs, pers. comm.). Based on their size and distribution it is possible that they represent an accumulation of virus particles. If this is the case, their specificity to those regions of the chloroplast that lie adjacent to the dinoflagellate nucleus is perhaps significant. Until a more detailed investigation can be carried out the exact nature of the chloroplast particles remains open to speculation.

The presence of two endosymbiont nuclei in a few cells is best explained by the fact that nuclear division of the endosymbiont and that of the host are not synchronous. The data suggests that karyokinesis of the endosymbiont precedes dinoflagellate mitosis. Although no cells were observed to be at the metaphase or anaphase stage of the life cycle, the apparent lack of an endosymbiont mitotic spindle bears a similarity to the amicrotubular nuclear division described for the endosymbiont of another dinoflagellate *Peridinium balticum* (Tippit and Pickett-Heaps 1976). A disruption of this division sequence (i.e., host mitosis preceding endosymbiont karyokinesis) may account for the absence of endosymbiont nuclei in such a large proportion of *G. acidotum* cells.

The endosymbiotic theory suggests that the chloroplasts of many algae and higher plants are derived from a photosynthetic prokaryote that was ingested but not digested by a phagotrophic eukaryote (Raven 1970, Gray and Doolittle 1982). Alternatively, the presence of chloroplasts of many phytoflagellates is attributed to the secondary symbiosis of a photosynthetic eukaryote with a eukaryotic phagotroph (Gibbs 1978, 1981; Whatley 1981). In particular, it has been suggested that the chloroplasts of various dinoflagellate species are of either chrysophyte (Tomas and Cox 1973), cryptomonad (Wilcox and Wedemayer 1984, 1985; Schnepf and Elbrachter 1988), or even prasinophyte (Watanabe et al. 1987) origin. The endosymbionts of these dinoflagellates range from those which have retained a full complement of organelles (Tomas and Cox 1973) to those in which only the chloroplasts remain (Watanabe et al. 1987). It is presumed that these variations are due to the loss of the endosymbiont's organelles. This loss may occur if the functions of the endosymbiont's organelles are redundant with those of the host cell or if these functions are subsequently subsumed by the host's other organelles. An alternative theory suggests that chloroplasts alone may have been endocytized by

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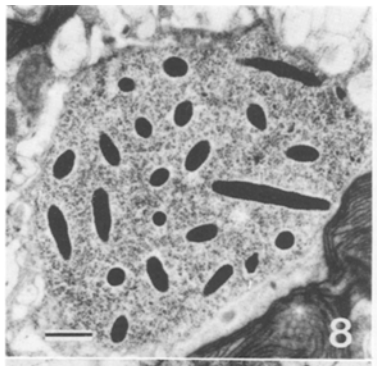
Abbreviations used in the figures: B bacteria; C cingulum; Ch chloroplast; D dinoflagellate nucleus; E endosymbiont nucleus; Ep epicone; FV food vacuole; G Golgi apparatus; Hy hypocone; l lipid body; Md dinoflagellate mitochondrion; Me endosymbiont mitochondrion; Nm nucleomorph; Nu nucleolus; Pe peduncle; Pu pusule; Py pyrenoid; S sulcus; St storage body

Fig. 1. Ventral SEM of *Gymnodinium acidotum* showing relative proportion of epicone to hypocone and displacement of the cingulum. Bar = 5.0 μm

Fig. 2. Propidium iodide stained cells, one having both dinoflagellate and endosymbiont nuclei the other having only a dinoflagellate nucleus. Bar = 10.0 μm

Figs. 3–5. DAPI stained cells showing replicated endosymbiont nuclei prior to anterior migration (**Fig. 4**), after migration (**Fig. 3**), and at the onset of dinoflagellate karyokinesis – note elongated nucleus (**Fig. 5**). Bars = 10.0 μm

Figs. 6 and **7.** Longitudinal TEMs of comparable planes from a cell with both dinoflagellate and endosymbiont nuclei (**Fig. 6**) and from another having only a dinoflagellate nucleus (**Fig. 7**). Bar = 5.0 μm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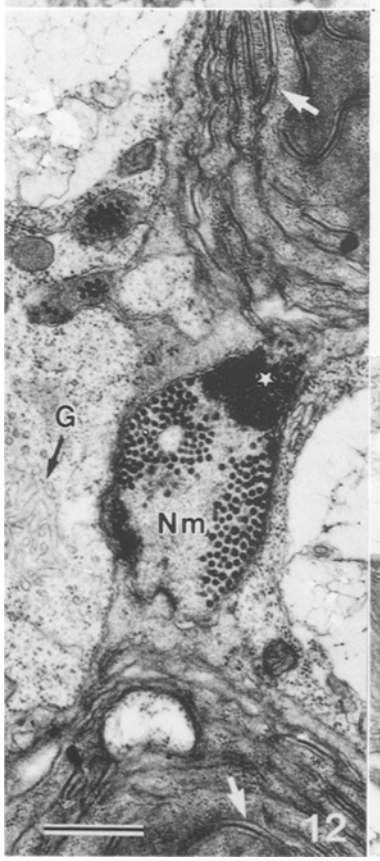
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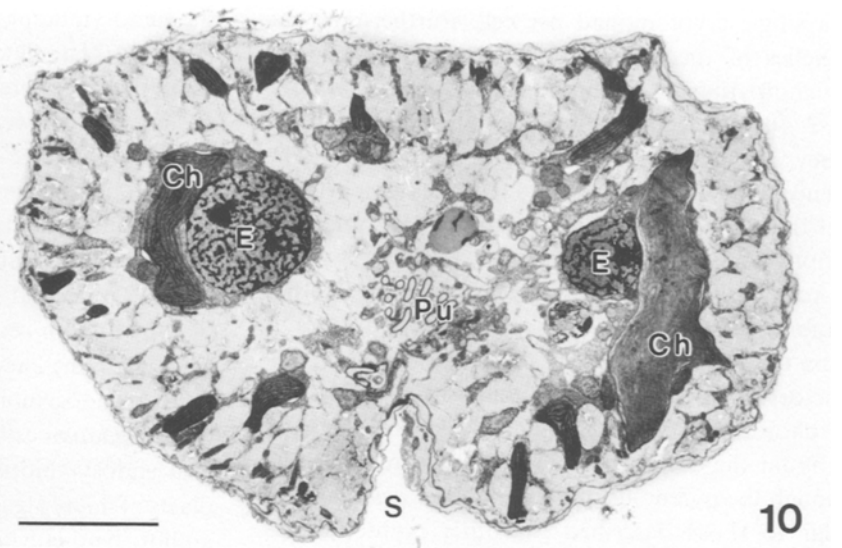
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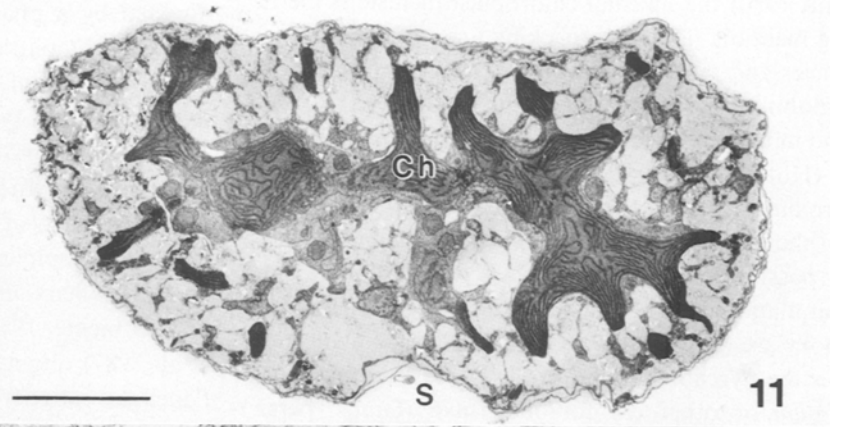
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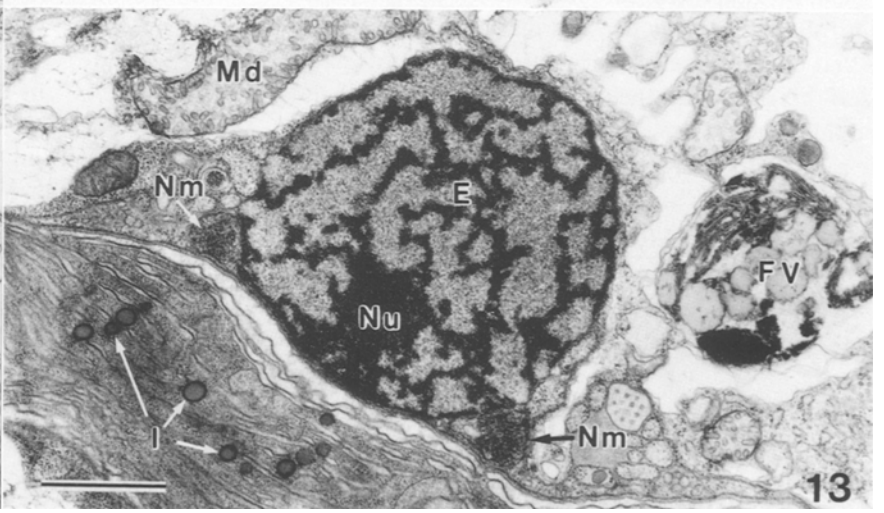
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myzocytotic dinoflagellates (Schnepf and Deichgräber 1984, Schnepf and Elbrächter 1988).

Nucleomorphs are double membrane bound bodies that are associated with the periplastidal region of cryptomonads (Gillot and Gibbs 1980; Hansmann et al. 1985, 1986; Ludwig and Gibbs 1985, 1987; Hansmann 1988) and the amoebo-flagellate *Chlorarachnion* (Hibberd and Norris 1984; Ludwig and Gibbs 1987, 1989). The presence of both DNA (Hansmann et al. 1985, 1986; Ludwig and Gibbs 1985, 1987) and RNA (Hansmann 1988) in the nucleomorphs of cryptomonads has been demonstrated by a variety of techniques. It has been suggested that like many other phytoflagellates, the cryptomonads obtained their chloroplasts from a secondary endosymbiotic event and that the nucleomorph represents a reduced nucleus of a eukaryotic prey cell (Gillot and Gibbs 1980; Ludwig and Gibbs 1985, 1987).

The maintenance of chloroplasts in many green algae and higher plants is dependent upon nuclear encoded proteins that are translated in the cytoplasm and transported into the chloroplasts (Ellis 1981). The presence of a nucleomorph in *G. acidotum* may have some relevance as to how chloroplasts can be retained in those individuals which have lost their endosymbiont nucleus. The presence of both DNA and RNA in the nucleomorphs of cryptomonads raises the possibility that these organelles actively code for polypeptides. If this is the case, essential, non-chloroplast encoded proteins (Rubisco small subunit, etc.) may be coded for by genes within the nucleomorph rather than the cryptomonad nucleus and selective pressures for maintaining the cryptomonad nucleus within *G. acidotum* may be reduced. The presumed lack of fidelity in chromosome segregation that occurs during amitotic division of the endosymbiont nucleus suggests that this organelle is no longer essential to the viability of the host/endosymbiont relationship.

The complex relationship between the dinoflagellate host and its endosymbiont is underscored by the fact that *Gymnodinium acidotum* is positively phototactic. The cryptomonad endosymbiont (both those with and without nuclei) no longer produces a functional flagellar apparatus and is therefore dependent on the host for locomotion. Similarly, the motile, colorless dinoflagellate host apparently lacks its own photosensory structures. Red pigmented bodies found in the epicone are most likely food vacuoles and electron microscopic observations confirm that *G. acidotum* lacks a non-plastid associated eyespot similar to those found in other dinoflagellates (Spector 1984). Although the chloroplasts of *G. acidotum* do contain lipid granules scattered throughout the stroma, these granules lack the organization typical of eyespots found in other cryptomonads (Dodge 1969, Gantt 1980). Despite this, the dinoflagellate and its endosymbiont somehow act in concert to produce directional movement in response to light. The mechanism whereby this is accomplished remains unknown.

Figs. 8 and **9.** Nuclei of *Gymnodinium acidotum* showing permanently condensed, banded chromosomes of the dinoflagellate nucleus (**Fig. 8**) and the diffuse chromatin and nucleolus of the endosymbiont nucleus (**Fig. 9**). Bar = 1.0 μm

Figs. 10 and **11.** Transverse sections of a cell in which the endosymbiont nuclei have replicated showing both daughter nuclei (**Fig. 10**) and lobes of the chloroplast that connects them (**Fig. 11**). Note lack of a microtubular spindle between the daughter nuclei. Bar = 5.0 μm

Fig. 12. Nucleomorph with granular and fibrous (☆) regions associated with two lobes of a chloroplast. Note Golgi apparatus in endosymbiont cytoplasm and stacking of thylakoids into pairs (white arrows). Bar = 0.5 μm

Fig. 13. Endosymbiont nucleus with associated nucleomorphs. Bar = 1.0 μm

Fig. 14. Channel of endosymbiont cytoplasm within a pocket of the chloroplast. A storage body is located within the periplastidal space between the third (◀) and outermost (☆) chloroplast membranes. Bar = 0.5 μm

Fig. 15. A chloroplast pyrenoid with traversing thylakoids and lipid globules. Bar = 1.0 μm

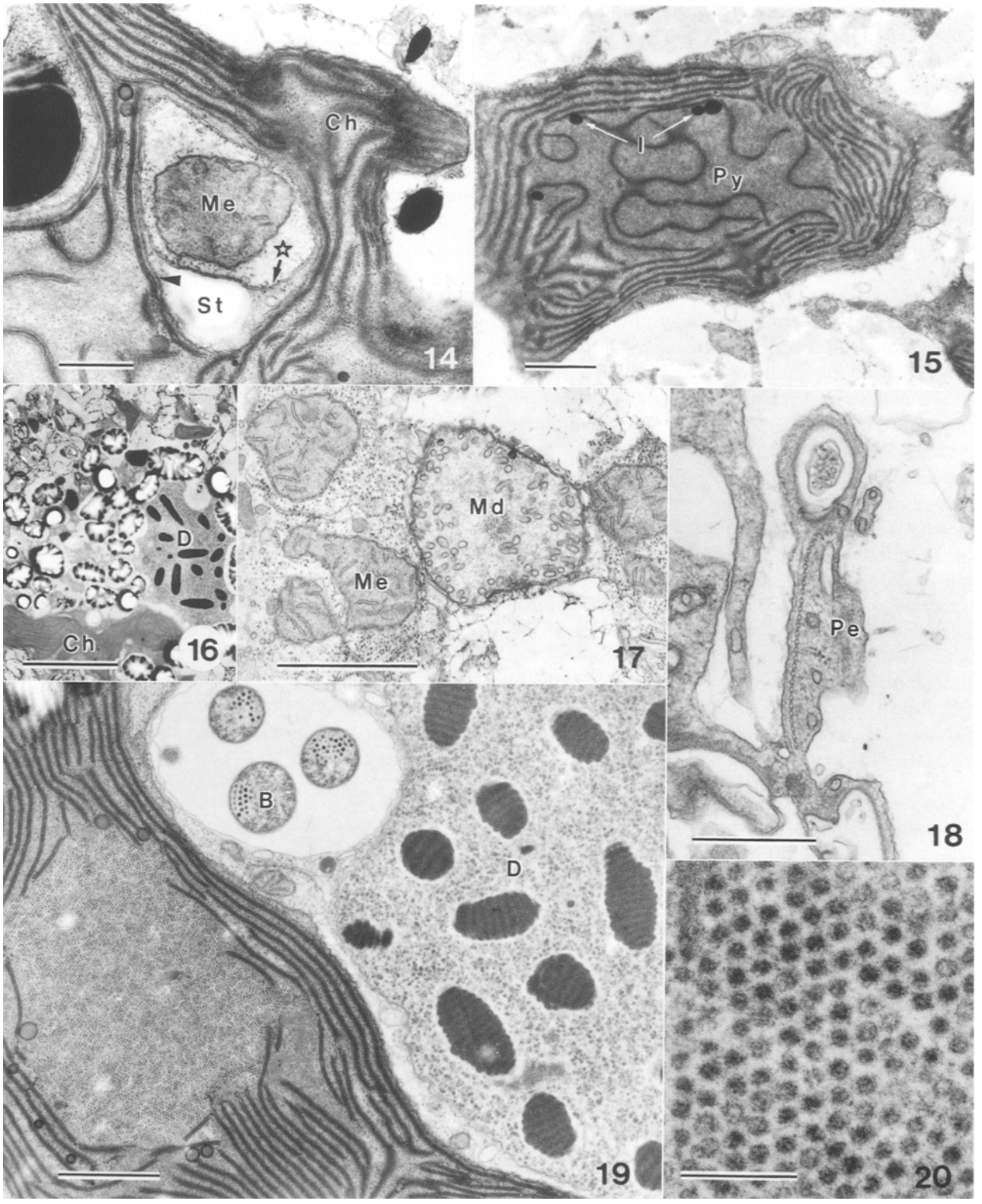
Fig. 16. Numerous storage bodies distributed throughout the dinoflagellate cytoplasm. Bar = 5.0 μm

Fig. 17. Close association of mitochondria of the dinoflagellate and its endosymbiont. Bar = 1.0 μm

Fig. 18. Microtubule lined peduncle. Bar = 0.5 μm

Fig. 19. Particle filled chloroplast inclusion adjacent to the dinoflagellate nucleus. Three bacteria within a vacuole. Bar = 1.0 μm

Fig. 20. High magnification view of chloroplast particles showing their close packing arrangement. Bar = 0.1 μm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References

Dodge JD (1969) The ultrastructure of *Chroomonas mesostigmatica* Butcher (Cryptophyceae). Arch Mikrobiol 69: 266–280

Ellis RJ (1981) Chloroplast proteins: Synthesis, transport and assembly. Ann Rev Plant Physiol 32: 111–137

Gantt E (1980) Photosynthetic cryptophytes. In: Cox ER (ed) Developments in marine biology, vol 2, phytoflagellates. Elsevier North Holland, New York, pp 381—405

Gibbs SP (1978) The chloroplasts of *Euglena* may have evolved from symbiotic green algae. Can J Bot 56: 2883–2889

– (1981) The chloroplasts of some algal groups may have evolved from endosymbiotic eukaryotic algae. NY Acad Sci Ann 361: 193–208

Gillott MA, Gibbs SP (1980) The cryptomonad nucleomorph: its ultrastructure and evolutionary significance. J Phycol 16: 558–568

Gray MW, Doolittle WF (1982) Has the endosymbiont hypothesis been proven? Microbiol Rev 46: 1—42

Hansmann P (1988) Ultrastructural localization of RNA in cryptomonads. Protoplasma 146: 81–88

– Falk H, Sitte P (1985) DNA in the nucleomorph of *Cryptomonas* demonstrated by DAPI fluorescence. Z Naturforsch 40c: 933–935

– – Scheer U, Sitte P (1986) Ultrastructural localization of DNA in two *Cryptomonas* species by use of a monoclonal DNA antibody. Eur J Cell Biol 42: 152–160

Hibberd DJ, Norris RE (1984) Cytology and ultrastructure of *Chlorarachnion reptens* (Chlorarachniophyta divisio nova, Chlorarachniophyceae classis nova). J Phycol 20: 310–330

Holdsworth RH (1968) The presence of a crystalline matrix in pyrenoids of the diatom *Achnanthes brevipes*. J Cell Biol 37: 831–837

Larsen J (1985) Algal studies of the Danish Wadden Sea. II. A taxonomic study of psammobious dinoflagellates. Opera Bot 79: 14–37

– (1988) An ultrastructural study of *Amphidinium poecilochroum* (Dinophyceae), a phagotrophic dinoflagellate feeding on small species of cryptophytes. Phycologia 27: 366–377

Ludwig M, Gibbs SP (1985) DNA is present in the nucleomorph of cryptomonads: further evidence that the chloroplast evolved from a eukaryotic endosymbiont. Protoplasma 127: 9–20

– – (1987) Are the nucleomorphs of cryptomonads and *Chlorarachnion* the vestigial nuclei of eukaryotic endosymbionts? Ann NY Acad Sci 503: 198–211

– – (1989) Evidence that the nucleomorphs of *Chlorarachnion reptens* (Chlorarachniophyceae) are vestigial nuclei: morphology, division and DNA-DAPI fluorescence. J Phycol 25: 385–394

Raven PH (1970) A multiple origin for plastids and mitochondria. Science 169: 641–646

Schnepf E, Deichgräber G (1984) „Myzocytosis", a kind of endocytosis with implications to compartmentation in endosymbiosis. Observations in *Paulsenella* (Dinophyta). Naturwissenschaften 71: 218–219

– Elbrächter M (1988) Cryptophycean-like double membrane-bound chloroplast in the dinoflagellate, *Dinophysis* Ehrenb.: evolutionary, phylogenetic and toxicological implications. Bot Acta 101: 196–203

Spector DL (1984) Unusual inclusions. In: Spector DL (ed) Dinoflagellates. Academic Press, Orlando, pp 365–390

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 31–57

Tippit DH, Pickett-Heaps JD (1976) Apparent amitosis in the binucleate dinoflagellate *Peridinium balticum*. J Cell Sci 21: 273–289

Tomas RN, Cox ER (1973) Observations on the symbiosis of *Peridinium balticum* and its intracellular alga. I. Ultrastructure. J Phycol 9: 304–323

Watanabe MM, Takeda Y, Sasa T, Inouye I, Suda S, Sawaguchi T, Chihara M (1987) A green dinoflagellate with chlorophylls *a* and *b*: morphology, fine structure of the chloroplast and chlorophyll composition. J Phycol 23: 382–389

Whatley JM (1981) Chloroplast evolution – ancient and modern. NY Acad Sci Ann 361: 154–165

Wilcox LW, Wedemayer GJ (1984) *Gymnodinium acidotum* Nygaard (Pyrrophyta), a dinoflagellate with an endosymbiotic cryptomonad. J Phycol 20: 236–242

– – (1985) Dinoflagellate with blue-green chloroplasts derived from an endosymbiotic eukaryote. Science 227: 192–194

Zhang X, Liu M, Liu Q, Wang H, Li S (1982) Preliminary separation and characteristics of phycocyanin in blue-green *Gymnodinium*. Kexue Tongbao 27: 1000–1003