

Cytoplasmic organization and symbiotic associations of *Didymocyrtis tetrathalamus* (Haeckel) (Spumellaria, Radiolaria)

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ABSTRACT: Cytoplasmic organization and symbiotic associations of *Didymocyrtis tetrathalamus* (Haeckel) were examined. The most distinctive cytological feature is the presence of an extraordinary, non-living wall structure (termed endocapsular wall) that lies immediately inside the outer medullary shell. It closely resembles the capsular wall, which is the most diagnostic feature for radiolarian cytoplasmic organization, but is completely enclosed by the latter wall. The capsular wall lies inside the cortical shell, except in the polar region where the wall is always outside the latticed skeleton of the bilocular cortex. Based on these observations, and previously reported skeletal changes during maturation, five ontogenetic stages are described. In the first stage, the test consists of the double medullary shell and the endocapsular wall may serve as the "first capsular wall." In the second stage, the equatorial girdle surrounding the medullary shell and the capsular wall is formed. A major portion of the cortical shell is constructed in the third stage, forming the nearly completed hour-glass-shaped shell. In the fourth and fifth stages, the cortical shell is completed and polar caps lying above each pole are deposited, associated with the expansion of the central capsule to partially encompass the bilocular shell. Two ultrastructurally different symbiotic dinoflagellates were observed in the extracapsular region around the cortical shell. One is identified as *Amphidinium* sp. and the other remains unnamed. They were never observed in the same host and appear to mutually exclude one another. In addition to dinoflagellates, there were also bacterial endobionts within vacuoles.

INTRODUCTION

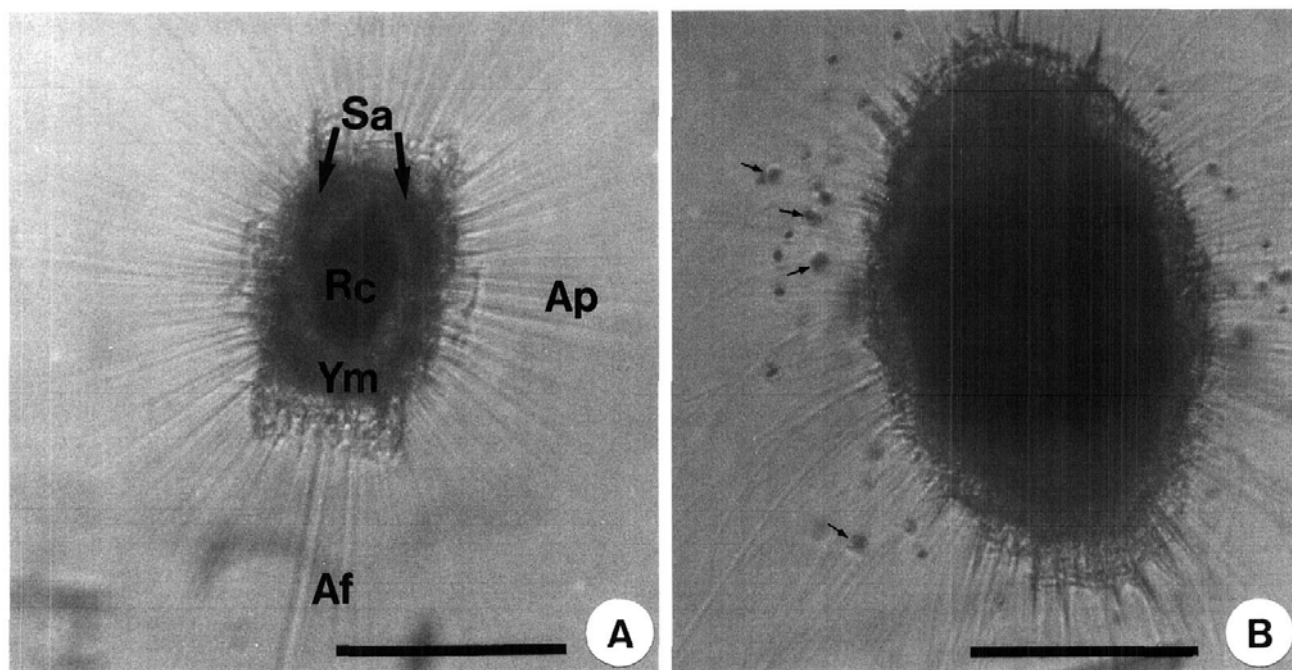
As is well known, radiolarian skeletons occur abundantly in pelagic sediments from the Paleozoic to recent times, and their important role as age indices has clearly been demonstrated even in the Middle Paleozoic (e.g., Noble 1994). Thus, the nearly continuous and well preserved microfossil record of radiolarian skeletons is a very good source of information to elucidate protozoan evolution and also to infer biological adaptations to environmental changes over long geological time spans. Clearly, a better understanding of the morphology and life processes of extant organisms is essential to validly reconstruct paleoenvironmental events. However, our knowledge on living radiolarians still remains limited and there appear to be many organic features that may have been acquired during an extensively long evolutionary history of this organism. Recently, Sugiyama and Anderson (1997) examined living and cytological features of three nassellarians *Eucyrtidium hexagonatum* Haeckel, *Pterocorys zancleus* (Müller) and *Spirocyrtis scalaris* (Haeckel), and reported that the last species has some very peculiar cytoplasmic features characterized by the eccentrically situated nucleus and an organic membrane (extracapsular wall) enclosing the more typical central capsule. They also discussed phylogeny and functional morphology of those nassellarians based on cytoplasmic features. That study demonstrated that morphological and cytological features of extant species can provide valuable information to better construct phylogenetic sequences and create more valid taxonomic categories of fossil species.

In this paper, we report some new findings on cytoplasmic organization and symbiotic associations of *Didymocyrtis tetrathalamus* (Haeckel) using light microscopy (LM) and

transmission electron microscopy (TEM). *Didymocyrtis tetrathalamus* has distinct shell characters, such as the porous bilocular shell surrounding a medullary shell, that make the primary species identification by LM definite, and we already have substantial information on its skeletal morphology and physiological ecology from previous extensive research (i.e., Anderson et al. 1986, 1990). Thus, some additional refinement in our knowledge of the skeletal-cytoplasmic relationship and organization of the cytoplasm in relation to symbiont distribution surrounding the shell may provide needed information to better establish the phylogenetic and taxonomic affinities of *D. tetrathalamus*.

MATERIAL AND METHOD

Plankton samples were collected using a 36µm mesh net at a location approximately 2km west of Holetown, Barbados, in short-duration tows (3-3.5 min.) during June to July, 1995. On return to the laboratory at the Bellairs Research Institute, St. James, Barbados, portions of the plankton samples were immediately distributed into 90mm diameter culture dishes for sorting. Fixation procedures for TEM and daily observations using LM follow published procedures (Sugiyama and Anderson 1997). For TEM observation, we cut the tip of epoxy blocks where specimens were settled in order to make a small chip prior to the ultramicrotomy. Then the tip was trimmed into a proper size and a part of skeleton was simultaneously cut off to expose siliceous matter outside the chip. Next the chip was put into HF solution to dissolve the whole skeleton. After drying in an oven, the chip containing the specimen was again infiltrated into epoxy resin that penetrate the opening. Through these procedures the skeleton was completely replaced by epoxy resin. Two mature specimens, collected on different dates, were examined for fine structural analysis based on observations of more



TEXT-FIGURE 1

Light micrographs of *Didymocystis tetrathalamus* showing living features. A: An individual with incomplete polar caps. B: Fully matured individual with developed polar caps and spongy layers surrounding the cortical shell. Ap, axopodia; Af, axoflagellum; Rc, reddish core; Ym, yellow mass; and Sa, symbiotic algae. Note numerous algae (small arrows) along the axopodia in text-fig. 1B. Scale bars indicate 100µm.

than one hundred grids. The observations by LM are based on 12 individuals cultured at ambient seawater conditions of temperature and salinity.

RESULTS

Light microscopy

The cytoplasm has three cytoplasmic zones based on color and location relative to the bilocular shell: (1) a pale-yellow to pale-green, dominant mass usually distributed inside the bilocular cortical shell (Ym), (2) a reddish core at the center of the cell, distributed along the major axis of the shell, and enclosing the medullary shell (Rc), and (3) yellow to orange symbiotic algae (ca. 10µm diameter) that are typically more than 20 in number and usually distributed along the margin of the pale-yellow mass immediately inside of the cortical shell (Sa) (text-fig. 1A). In mature individuals with polar caps, the pale-yellow and reddish masses further extend into the cavities within the polar caps. One individual had yellow algae (ca. 5µm diameter) outside the shell along axopodia (text-fig. 1B).

Pseudopodia are of two kinds as observed in other spumellarians; numerous thin axopodia (Ap) radiating toward all directions, and a relatively thick axoflagellum (Af) extending from one pole (text-fig. 1A).

Skeletal-cytoplasmic relationships and general cytoplasmic features

The gross morphological relationship between the shell and cytoplasm is schematically shown in text-fig. 2. A capsular wall (CW) (0.06-0.08µm thickness) lies inside the cortical shell in

the equatorial region, and is penetrated by numerous fusules (F) and contains minor slits (e.g. text-figs. 3A, 3B and 4A). It follows the contours of the skeleton, lying internal to it in the equatorial region, but extending outside of the bilocular shell in the polar regions where it penetrates through the pores. Algal symbionts, surrounded by a perialgal vacuole, are nestled within infolded regions of the capsular wall in the equatorial region of the bilocular shell. In some parts, the capsular wall lies immediately adjacent to the double membrane system of the cytokalymma (Ck) that shrouds the skeleton (e.g., text-fig. 3B) or the thin perialgal cytoplasmic envelope (PE) enclosing symbiotic algae (e.g., text-fig. 9A). The intervening bars (IB) connecting the cortical and medullary shells are also immediately surrounded by the capsular wall (CW) that arises near the base of the intervening bars (arrows in text-fig. 4A). In addition to the capsular wall (CW) extending outside the cortical shell in the polar region (text-fig. 5A), each lattice of the cortical shell (CS) at the poles is completely surrounded by the sheath of the capsular wall (CW) lying outside the cytokalymma (Ck) (text-fig. 5B). There is another wall structure (termed endocapsular wall, EW) that lies immediately inside the outer medullary shell (OMS) and is distinct in position from the capsular wall (text-fig. 6A). The endocapsular wall (EW) is 0.06-0.08µm in thickness, pierced by minor slits (S) as observed in the capsular wall, and is attached to the cytokalymma (Ck) in most part (text-fig. 6C). It projects outward from the pores of the outer medullary shell as if it were emerging from the inside. The outer margin or rim of the tube-like projections of the endocapsular wall is reflexed, and always resembles a turtleneck collar (text-fig. 6B). The endocapsular wall (EW) extends inside the medullary shell along intervening bars (IB) connecting the outer and inner me-

dullary shells; therefore it also occurs in some parts of the surface of the inner medullary shell (IMS) (arrow in text-fig. 6A and text-fig. 6D).

The main part of the nucleus (N) is located within the outer medullary shell (OMS) (text-fig. 6A) but is likely to be complexly lobed, extending into the inner medullary shell, since electron-lucent zones are prominent even within the inner medullary shell and no skeletal matter interrupts the nucleus in this region. No axoplast was observed, and hence it is not so clear as with some Nassellaria and other Spumellaria how the axoplast may relate to the nucleus.

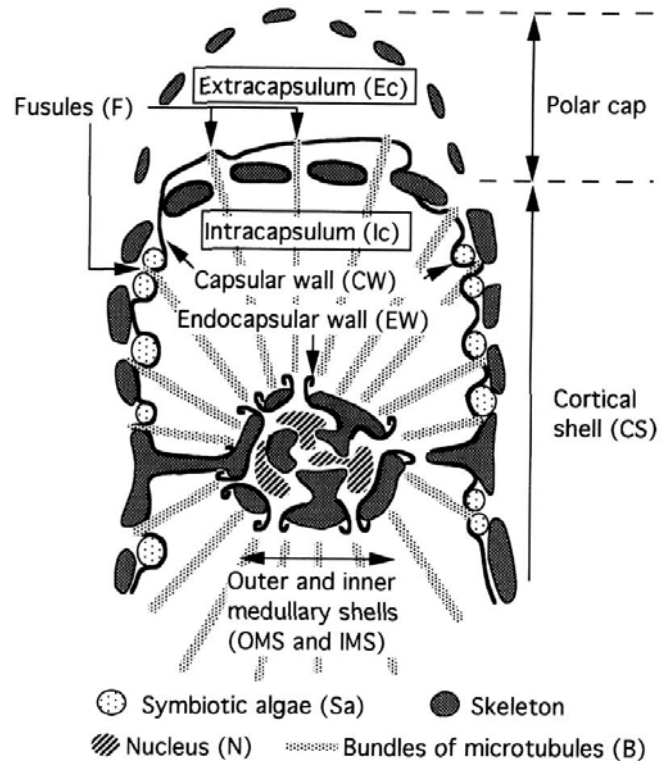
Numerous bundles of microtubules (B) radiate in all directions from the surface of the medullary shell (text-fig. 5C). Intracapsular lobes, separated by electron-lucent lacunae (La), are arranged more or less radially even near the capsular wall (CW) (text-fig. 5A). They contain numerous mitochondria (M) with tubular cristae, electron-dense peroxisomes (P), bow-shaped Golgi bodies (G), small pigment granules (PG), electron-dense reserve bodies (R) and electron-lucent vacuoles (V) (e.g., text-figs. 5C, 5D and 6A). The peroxisomes (P) are often closely associated with the mitochondria (text-fig. 5D) as has been reported in some planktonic foraminifera (Anderson and Tuntivate-Choy 1984). The vacuoles (V) become progressively more abundant peripherally (e.g., text-fig. 4A). Additionally, some single-membraned organelles with granular matrix (X) occur outside of the bundles of microtubules (B), and other more elongated organelles with electron-dense matrices (Y) occur abundantly inside the bundles (text-fig. 5C). Their function is not known, although they may be secretory vesicles transported along the microtubules passing from the intracapsular cytoplasm to the extracapsulum through the fusules.

The details of the fusules are shown in text-fig. 7. They are ca. 0.5 μm in diameter and are distributed all around the capsular wall. The intracapsulum (Ic) exhibits conspicuous constrictions (inner constriction, IC) at the region where it joins with fusules. The fusule membrane (FM) and peripheral electron-lucent zone (PELZ) are well developed. An inner osmiophilic tube, observed in nassellarian fusules (Sugiyama and Anderson 1997), is absent. The inner osmiophilic zone (IOZ) is ca. 0.2 μm in height, and approximately two times higher than the outer osmiophilic zone (OOZ) that is much more electron dense. There is a narrow, but sharp constriction (outer constriction, OC) in the outer osmiophilic zone. About five microtubules (Mt) pass through a longitudinal section of the fusule.

The extracapsulum (Ec) is highly alveolated, and contains numerous digestive vacuoles (DV) (less than 5 μm in diameter) enclosing varied organic matter as reported by Anderson et al. (1990) (text-fig. 3A). A considerably fine network of filamentous strands extends among the vacuoles, axopodia and rhizopodia, and forms a layer-like structure (LS) surrounding the cortical shell (text-fig. 3A). Bacterial endobionts (Bc) with elongated spheroidal shape (ca. 1 μm in major diameter) in longitudinal sections frequently occur in the extracapsular region, and are surrounded by thin filamentous strands and enclosed within vacuoles (text-figs. 3A, 3C and 3D).

Description of algal symbionts

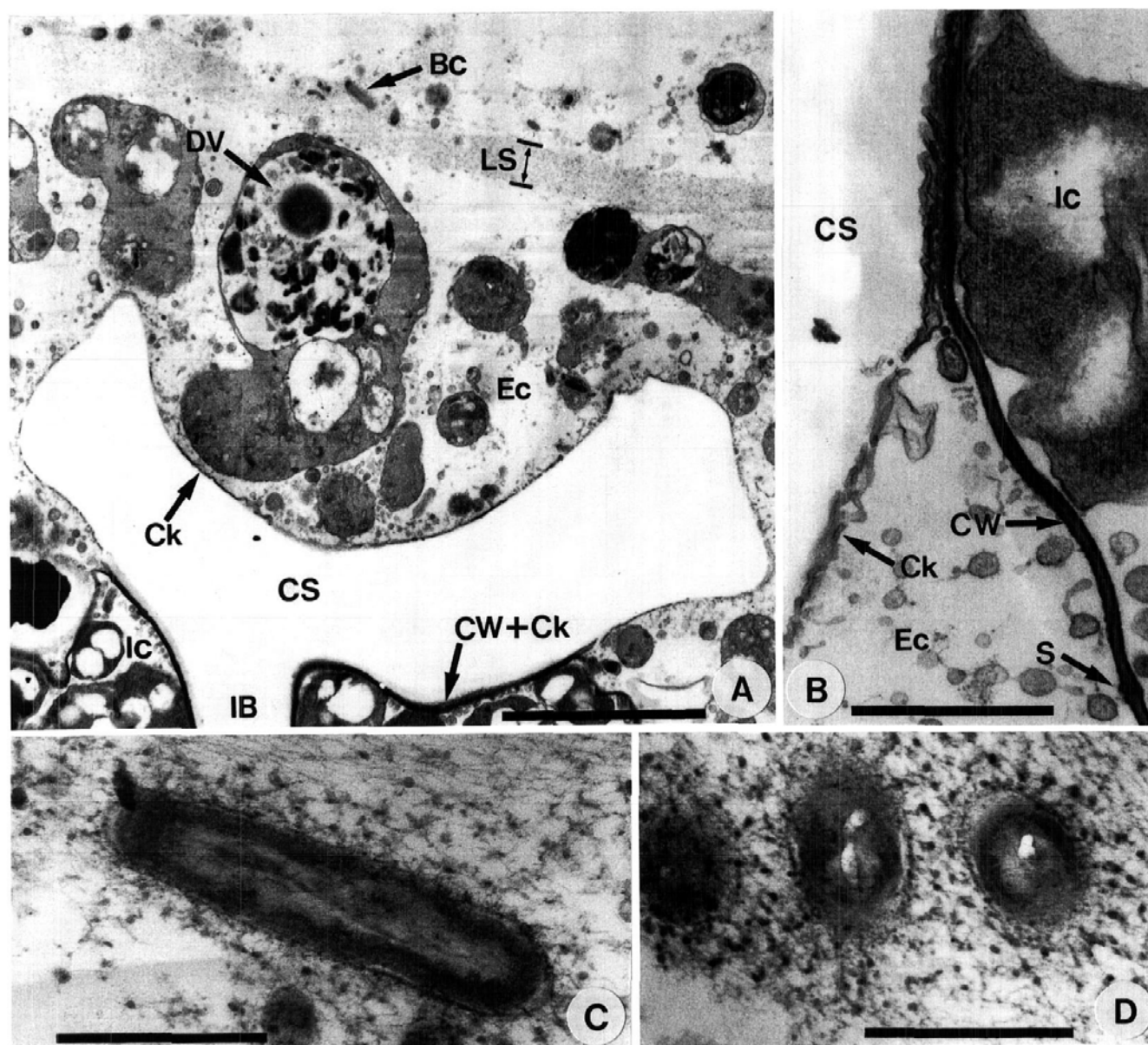
Two types of algae symbionts, both occurring in a single host, were found by fine structural analysis. They are described as Type I and Type II symbionts as follows.



TEXT-FIGURE 2

Schematic illustration showing the skeletal-cytoplasmic relationship of *Didymocyrtis tetrathalamus* in a longitudinal section.

Type I symbionts (text-fig. 8): The Type I symbionts are ovoid (10–15 μm in longest diameter) and closely enclosed by the perialgal envelope (PE) produced by the host (text-fig. 8E). The envelope is usually thin (ca. 0.02 μm thick), approximating a double membrane (OM and IM), and finely folded. The plasma membrane of the symbionts (PM) is distinct (less than 0.01 μm thick) and immediately surrounds an organic cell wall (OW) (ca. 0.02 μm thickness). Thecal vesicles are absent. The nucleus (N) is relatively large (6–8 μm diameter), and contains puffy, coiled chromosomes (Ch) characteristic of dinoflagellates (text-fig. 8C). The width/length ratio of the chromosomal mass is approximately 0.5 (ca. 0.7 μm width and 1.5 μm length). The peripheral chloroplast (Cl), comprising triple-thylakoid lamellae, is relatively narrow (ca. 1 μm width at the maximum) and irregularly reticulated. One of the chloroplast branches is attached to a single pyrenoid (Py) by a single stalk (text-fig. 8B). The pyrenoid is penetrated by numerous thylakoid-containing lamellae. Each lamella has two thylakoids (text-fig. 8D), and its peripheral part is covered by an electron-dense starch sheath (St) that is further surrounded by an electron-lucent zone (EL) (text-fig. 8B). Other cytoplasmic regions contain numerous mitochondria (M) with tubular cristae, juxtanuclear Golgi body (G), peroxisomes (P), abundant starch grains (St) and occasional reserve bodies (R) associated with the chloroplast (text-figs. 8C and 8F). Well developed endoplasmic reticula develop around the nucleus. Large vacuoles are absent.



TEXT-FIGURE 3

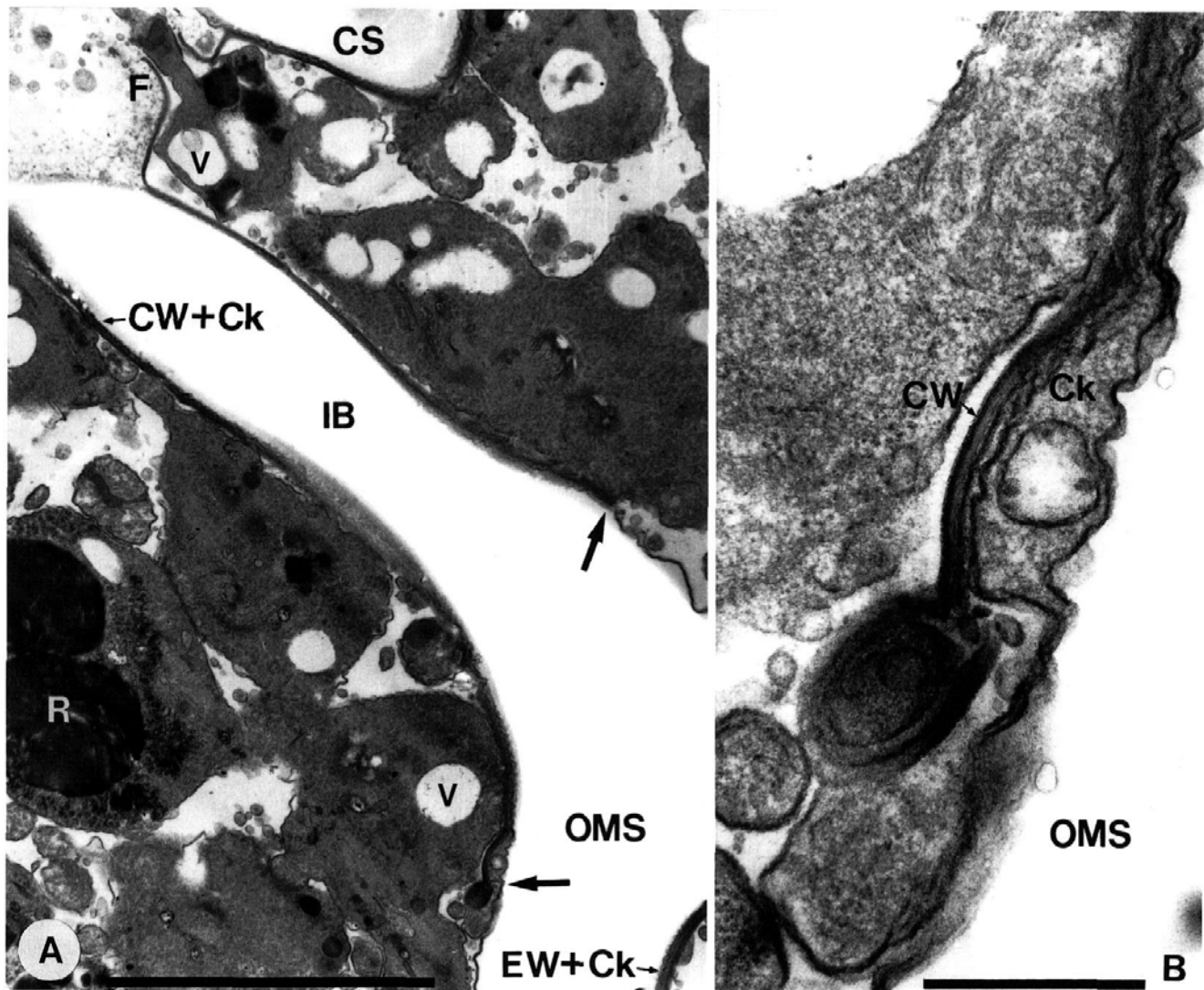
A: Cytoplasmic organization around a skeleton at the equatorial region (junction of the cortical shell (CS) and intervening bar (IB)). The intracapsulum (Ic) is attached to the inner surface of the skeleton. The extracapsulum (Ec) consists of numerous digestive vacuoles (DV) of various size. Fine network of filamentous strands forms a layer-like structure (LS) outside the cortical shell. There are numerous bacterial endobionts (Bc) in the extracapsular region. Ck and CW indicate cytotalkymma and capsular wall, respectively. Scale bar indicates 5 μ m.

B: Enlargement of a skeleton of the cortical shell (CS) showing the details of a double membrane system of cytotalkymma (Ck) and capsular wall (CW). They form a triple membrane between the intracapsulum (Ic) and the skeleton in the upper half, whereas they are independent of one another in the lower half where extracapsulum (Ec) is present between them. Scale bar indicates 1 μ m.

C and D: Bacterial endobionts in longitudinal and cross sections. They are supported by a fine network of filamentous strands in the extracapsulum. Scale bars indicate 0.5 μ m.

Type II symbionts (text-fig. 9): The shape is subspherical (10–15 μ m in longest diameter) with a more or less folded margin, and there is a conspicuous depression along one side (arrow in text-fig. 9A). There is usually a wide electron-lucent zone (EL) between the perialgal envelope (PE) and symbiont. The symbi-

onts are sequestered in the perialgal envelope (PE) with undulating margins (text-fig. 9E), but it lacks the finely rippled surface as is observed in Type I. There is a very delicate plasma membrane (PM) overlying a very thin organic wall (OW) (text-fig. 9F). The nucleus (N) is relatively small (ca. 5 μ m diameter),



TEXT-FIGURE 4

A: Skeletal-cytoplasmic relationship around an intervening bar (IB) connecting the cortical shell (CS) and outer medullary shell (OMS). The capsular wall (CW) arises from the proximal part of the intervening bar (thick arrows), and extends along the intervening bar outside the cytolymma (Ck). The inner surface of the outer medullary shell is also rimmed by a triple membrane formed by cytolymma (Ck) and endocapsular wall (EW). The intracapsulum contains large reserve bodies (R) and electron-lucent vacuoles (V). A fusule (F) is visible in the uppermost part. Scale bar indicates 5 μ m.

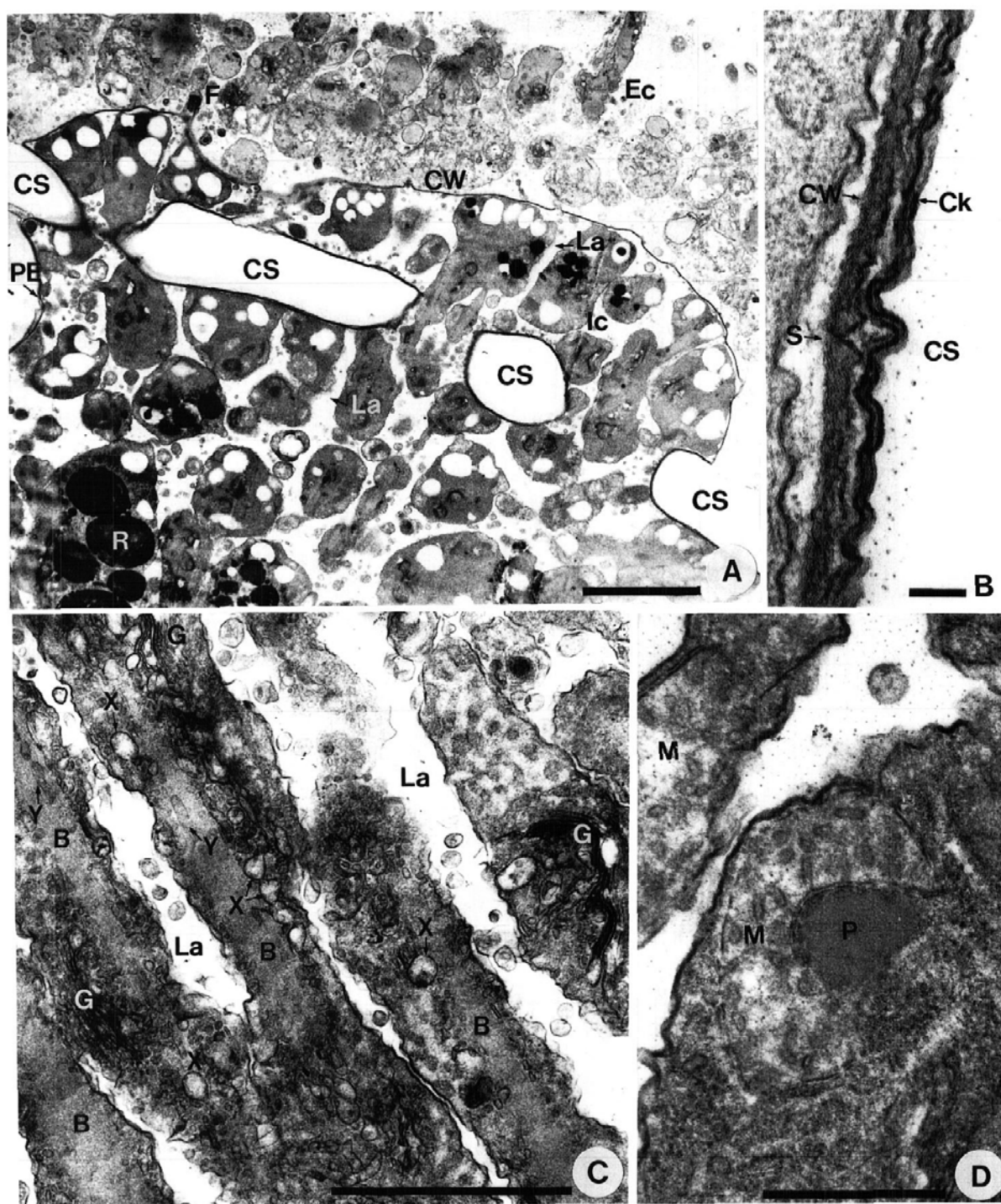
B: Enlargement of the starting point of the capsular wall (CW) showing a complicated structure, where cytolymma (Ck) enclosing the skeleton of the outer medullary shell (OMS) is relatively wide. Scale bar indicates 0.5 μ m.

and contains numerous, puffy chromosomes (Ch) but they are seemingly indistinct, some of which are very elongated with a width/length ratio of approximately 0.1 (ca. 1 μ m length and 0.1 μ m width) (text-fig. 9D). The peripheral chloroplast (Cl), containing triple-thylakoid lamellae as observed in the Type I, has a somewhat massive profile (ca. 2 μ m width at the maximum) and is not branched. In one individual, there are two pyrenoids that are attached to the chloroplast by one stalk and penetrated by four double-thylakoid lamellae (text-fig. 9B). Other cytoplasmic organelles contain numerous mitochondria (M) with tubular cristae, juxtanuclear Golgi body (G) (text-fig. 9C), numerous electron-opaque reserve bodies (R) (text-fig. 9A), peroxisomes and endoplasmic reticulum. A few starch grains may occur occasionally, but they are not conspicuous.

DISCUSSION

Skeletal-cytoplasmic relationship

As is well known, the test of *D. tetrathalamus* consists of a bilocular cortical shell enclosing a biconvex, double medullary shell (outer and inner medullary shells). In addition, mature individuals usually have a delicate, spongy, conical cap on each pole of the cortical shell. A further delicate spongy shell sometimes entirely surrounds the cortical shell. The ontogenetic development of the skeleton was fully examined by Anderson et al. (1986), who reported that the shell structures are successively developed, commencing with the medullary shell, progressing to the development of the bilocular shell initiated at the constricted region of the girdle, and extending to the polar caps de-



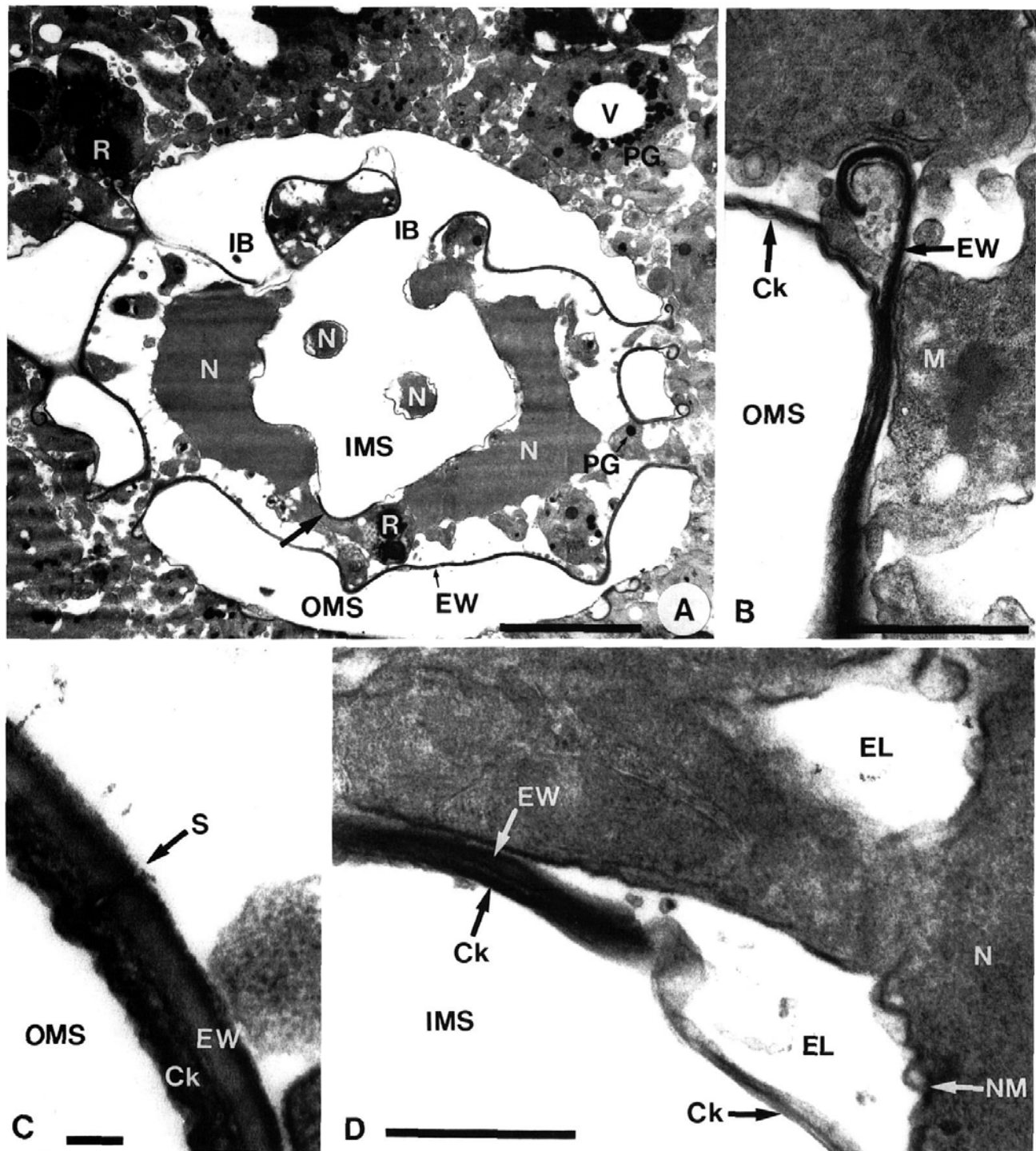
TEXT-FIGURE 5

A: Skeletal-cytoplasmic relationship around the polar region of the cortical shell (CS). The capsular wall (CW) with fusules (F) lies outside the lattices of the polar closure that are completely enclosed by the invagination of the capsular wall. Ec, Ic, La, PE and R indicate extracapsulum, intracapsulum, electron-lucent lacunae, perialgal envelope and reserve bodies, respectively. Scale bar indicates 5µm.

B: Enlargement of a lattice forming the polar closure of the cortical shell (CS), showing the details of the cytolymma (Ck) and capsular wall (CW) with a slit (S). Scale bar indicates 0.1µm.

C: The intracapsulum near the medullary shell that consists of bundles of microtubules (B) and electron-lucent lacunae (La). Golgi bodies (G) typically have a bow-shaped profile. Numerous single-membrane organelles with electron-lucent, granular matrix (X) and more elongated, more electron-dense organelles (Y) abundantly occur along and within the bundles, respectively. Scale bar indicates 2µm.

D: Mitochondria (M) with tubular cristae are closely associated with peroxisomes (P) having fine-grained matrix enclosed by a single membrane. Scale bar indicates 0.5µm.



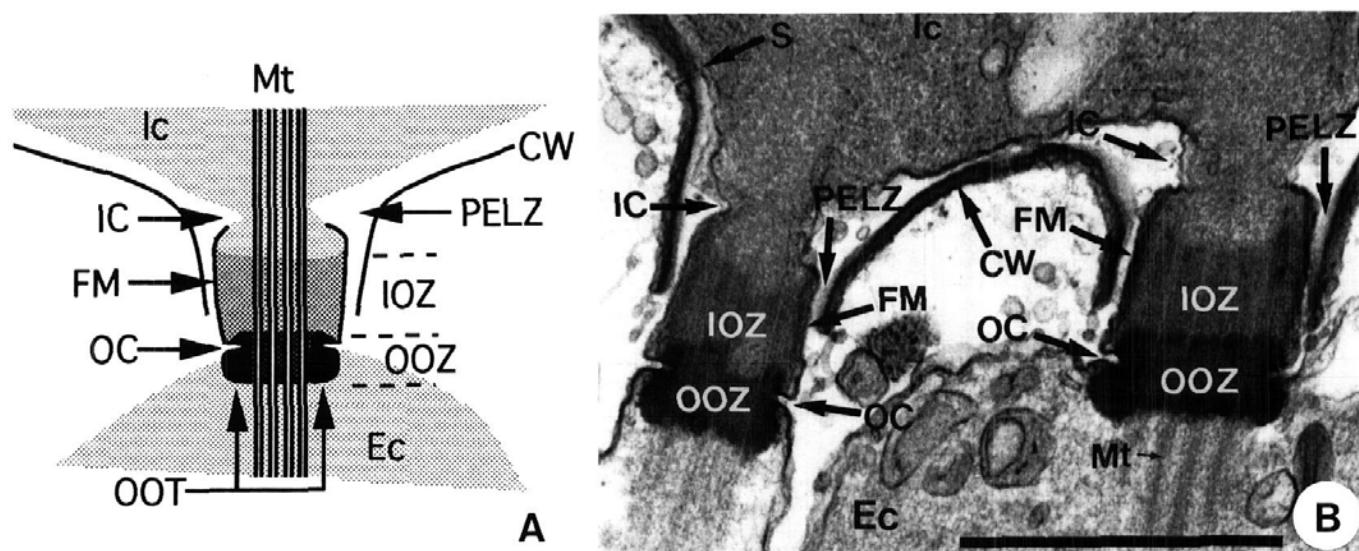
TEXT-FIGURE 6

A: Skeletal-cytoplasmic relationship around the medullary shell. The nucleus (N) is surrounded by the electron-lucent zone even in the inner medullary shell (IMS) and distributed within the outer medullary shell (OMS). The endocapsular wall (EW) lies immediately inside the outer medullary shell, and extends to the surface of the inner medullary shell (IMS) along the intervening bars (IB). Thick arrow points an occurrence of the endocapsular wall (EW) on the surface of the inner medullary shell (IMS). The endocapsular wall (EW) projects to the outside of the outer medullary shell through the pore, which forms characteristic turtleneck-like structure. Highly alveolate intracapsulum contains reserve bodies (R) and small pigment granules (PG).

B: Enlargement of turtleneck-like structure. The endocapsular wall (EW) extends outward along a skeleton of the outer medullary shell (OMS) with cytolymma (Ck), and is finally detached from cytolymma to be bent strongly. Mitochondrion (M) is visible near the endocapsular wall.

C: Enlargement of endocapsular wall (EW) with a slit (S) and cytolymma (Ck) surrounding the outer medullary shell (OMS).

D: Enlargement of a skeleton of the inner medullary shell (IMS) showing a starting point of the endocapsular wall (EW) that is attached to cytolymma (Ck). Electron-lucent zone (EL) develops around the nucleus (N) surrounded by nuclear membrane (NM).



TEXT-FIGURE 7

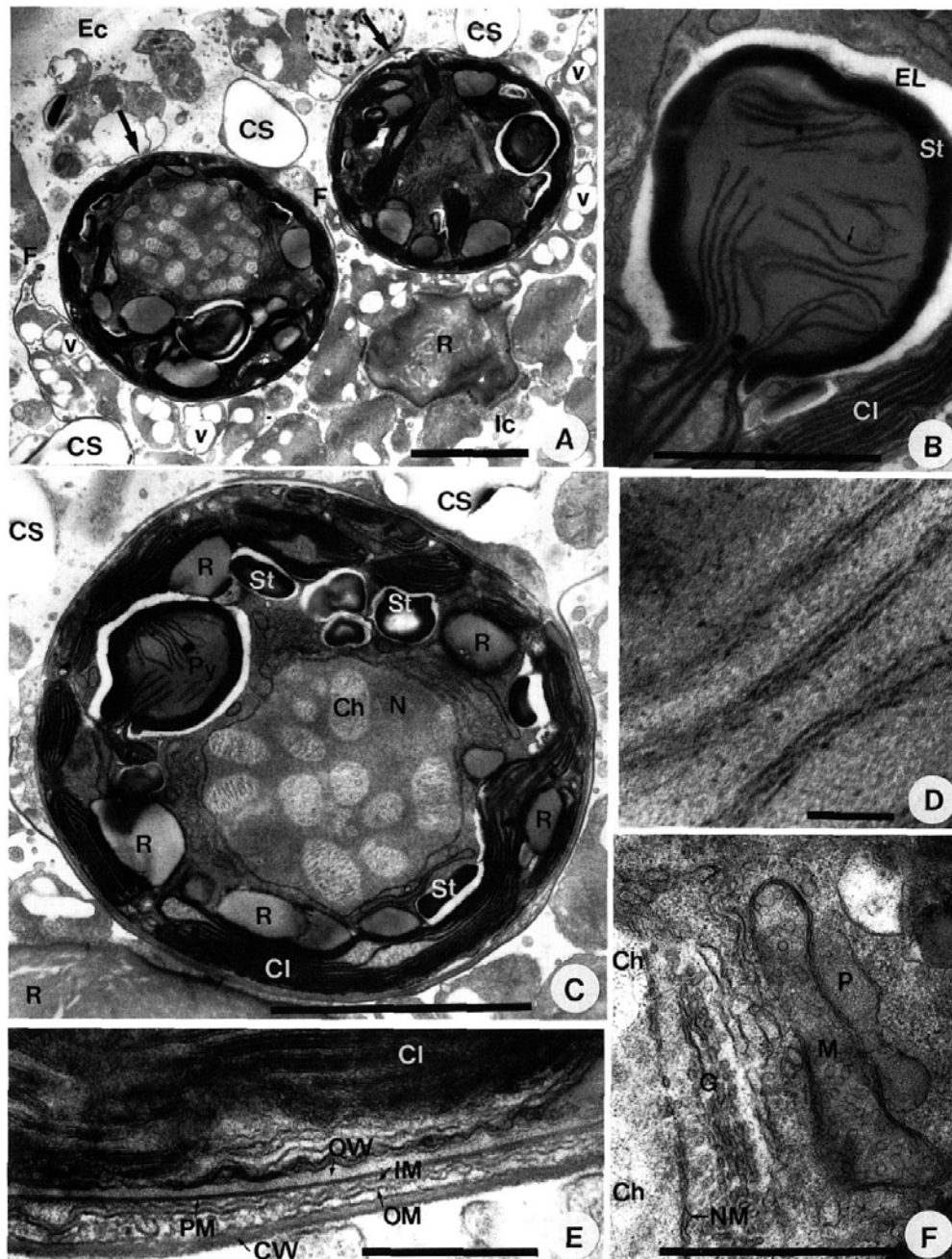
Fusule structures of *Didymocystis tetrathalamus*. A: Schematic illustration. B: Transmission electron micrograph (scale bar indicates 1 μ m). Abbreviations: Ec, extracapsulum; Ic, Intracapsulum; IC, inner constriction; IOZ, inner osmiophilic zone; CW, capsular wall; FM, fusule membrane; Mt, microtubules; PELZ, peripheral electron-lucent zone; OC, outer constriction; OOT, outer osmiophilic tube; OOOZ, outer osmiophilic zone. These terms follows Sugiyama and Anderson (in press).

posited at the ends of the shell. This sequence can easily be confirmed both in plankton and sediment samples since they usually contain various ontogenetic forms as illustrated by Anderson et al. (1986). Likewise, numerous species of spumellarians with spherical tests have cortical and medullary shells, and similar successive shell construction from a double medullary shell to cortex is known in some spherical spumellarians such as *Echinomma leptodermum* Jørgensen (Bjørklund 1974) and *Hexacantium enthacanthum* Jørgensen (Bjørklund 1976). However, the endocapsular wall, one of the most characteristic cytoplasmic features of *D. tetrathalamus*, is presently not known in other spumellarians (e.g., Hollande and Enjume 1960). This immediately suggests that cytoplasmic development of *D. tetrathalamus* during its ontogeny is quite different from other radiolarians.

Since there are some intracapsular lobes inside the medullary shell, the endocapsular wall is easily distinguished from the more centrally located nuclear membrane. On the contrary, owing to their similar thickness, it has a close similarity to the capsular wall, although it clearly differs from the latter in lacking the fusule structure. This peculiar cytoplasmic organization may be related functionally to the successive stages of test construction and, as suggested above, the endocapsular wall probably serves as the "first capsular wall" in an early ontogenetic stage as shown in text-fig. 10, in which five ontogenetic stages are schematically illustrated based on the combination of cellular fine structure and skeletal evidence.

The skeleton of the first stage (stage 1) is characterized exclusively by the two medullary shells with or without vestigial intervening bars from the equatorial region. In this stage, as mentioned above, the endocapsular wall may be the only wall enclosing the intracapsulum. Whether the fusules are present or

absent in the endocapsular wall at this stage is unfortunately not known, but it must be pierced by some kind of pores in addition to the narrow slits in order to sustain metabolic activity of the cytoplasm and allow exchange of matter with the surrounding environment. In the subsequent stage (stage 2), the intervening bars radiating from the outer medullary shell are developed on its equatorial border and, through elaboration of the skeletal matter tangentially at the distal ends of the bars, a well developed girdle ring is constructed surrounding the outer medullary shell. What is the relationship of the skeletal matter to the endocapsular wall at this stage? Based on the fine structural evidence that the intervening bars are rimmed by the capsular wall, it is hypothesized that the development of the intervening bars is associated with that of the capsular wall, and that the endocapsular wall is initially perforated by openings, which become elongated into the reflexed, collar-like extensions as the radial bars develop. During stage 3, the major part of the cortical shell is formed accompanied by the slight expansion of the central capsule space. According to light microscopy by Matsuoka (1993), algal symbionts in this stage are distributed around the outer medullary shell. This suggests that the central capsule is still closely enclosing the two medullary shells at this stage as is not observed in next stages 4 and 5. Moreover, it is likely that the algal symbionts have been sequestered within the host cytoplasmic envelope, as shown in text-figs. 2, 8 and 9, at this or possibly at a previous stage after the formation of the capsular wall, since no fine structural evidence indicates their occurrence in the intracapsular region. In stage 4, the central capsule begins to expand faster than shell growth and, finally, becomes invaginated at the more peripheral parts in the equatorial region where it encounters the already completed lattices of the bilocular shell. However, this expansion may precede the complete closure of the bilocular shell at the poles, and hence when completed the capsular wall lies peripheral to the polar surfaces of



TEXT-FIGURE 8

A: Typical occurrence of the Type I algal symbionts denoted by arrows. The intracapsulum (Ic) of the host, containing numerous vacuoles (V) and reserve bodies (R), is folded along the cortical shell (CS) and symbionts, and fusules (F) are distributed at the apex. Scale bar indicates 5 μ m.

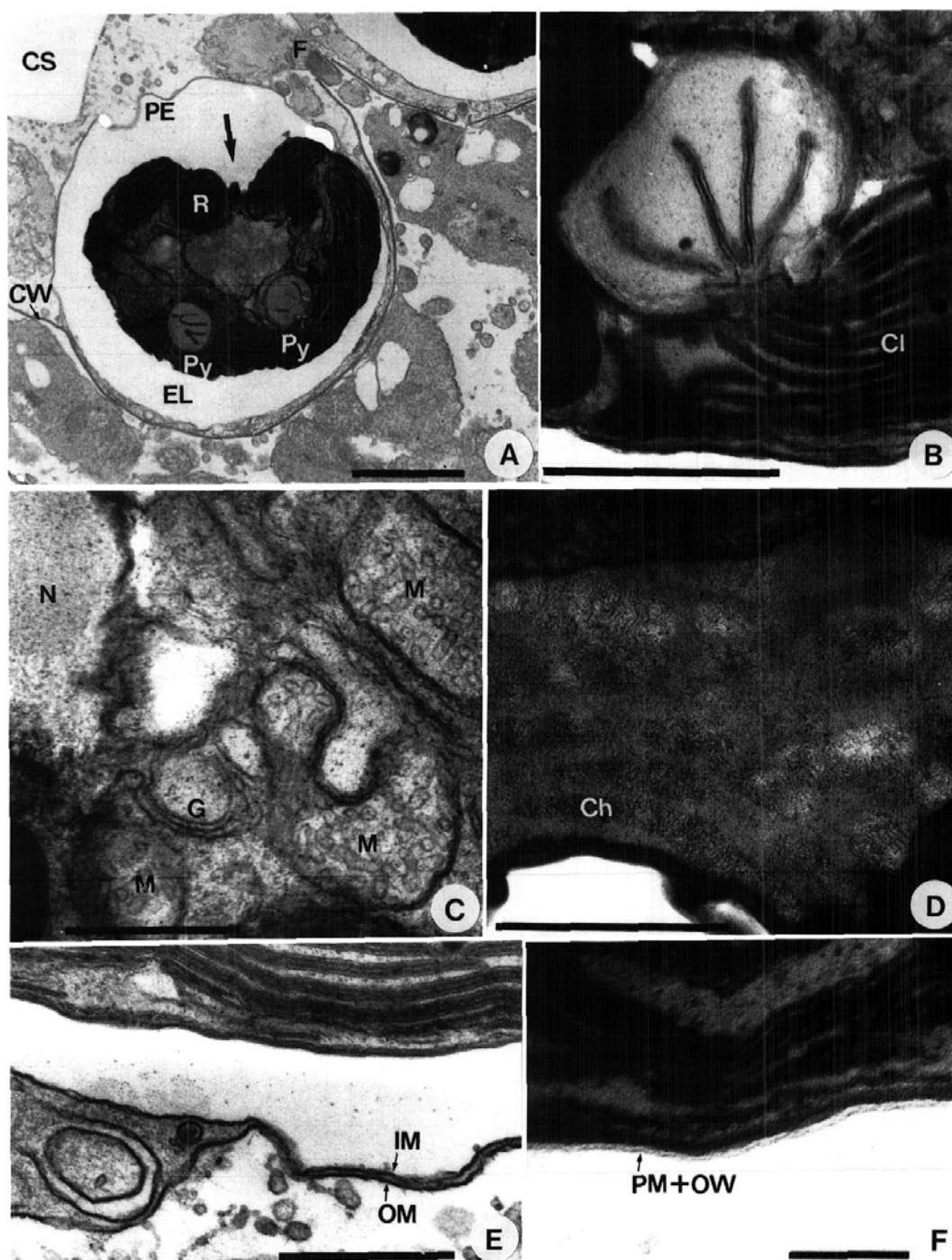
B: The pyrenoid of the Type I symbionts is attached to chloroplast (Cl) by a single stalk, and is characterized by having numerous plastid lamellae. It is surrounded by electron-dense starch (St) that is further surrounded by an electron-lucent zone (EL). Scale bar indicates 2 μ m.

C: The type I symbionts have prominent chromosomes (Ch) in the nucleus (N), and also possess many reserve bodies (R) and starch grains (St) associated with the peripheral chloroplast (Cl). CS in the right top and R in the left bottom indicate a skeleton of the cortical shell and an intracapsular reserve body of the host, respectively. Scale bar indicates 5 μ m.

D: Enlargement of the plastid lamellae of the pyrenoid; each has two thylakoids. Scale bar indicates 0.1 μ m.

E: Details of membrane system enclosing the symbionts. The thin perialgal envelope, consisting of outer and inner membranes (OM and IM), is finely folded and closely surrounds the plasma membrane (PM) of the algae overlying the thin organic wall (OW). Cl and CW indicate chloroplast of the symbionts and capsular wall of the host. Scale bar indicates 0.5 μ m.

F: Details of cytoplasmic organelles, showing puffy chromosomes (Ch), perforated nuclear membrane (NM), juxtanuclear Golgi body (G), mitochondrion (M) and peroxisome (P). Scale bar indicates 1 μ m.



TEXT-FIGURE 9

A: Typical occurrence of the Type II algal symbionts that have wide electron-lucent zone (EL) inside the perialgal envelope (PE) and two pyrenoids (Py). The capsular wall (CW) is folded and a fusule (F) often lies between two symbionts as observed in the case of the Type I. Scale bar indicates 5 μ m.

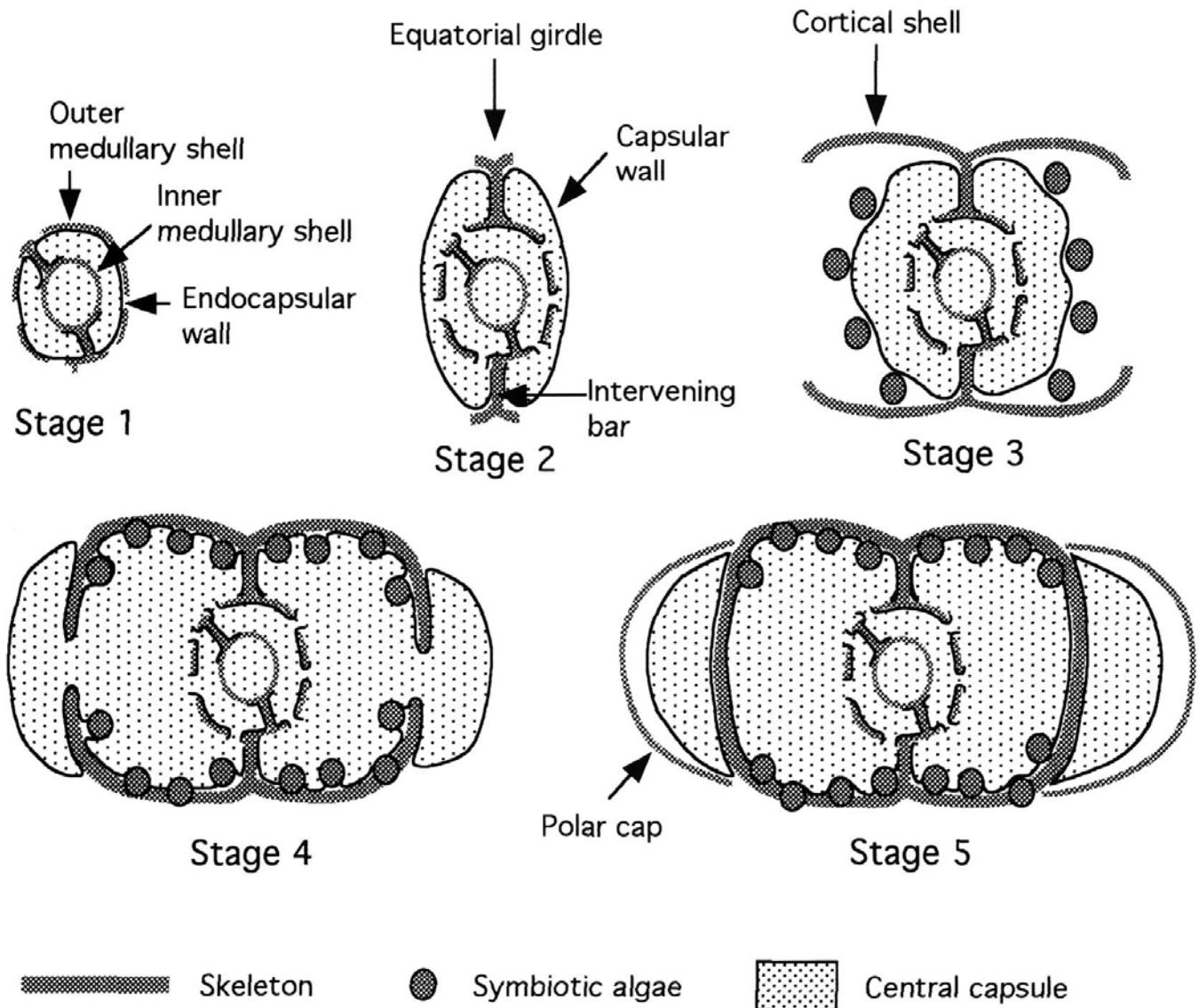
B: Enlargement of the pyrenoid that is attached to the chloroplast (Cl) by one stalk and possesses four two-thylakoid lamellae. Scale bar indicates 1 μ m.

C: Details of cytoplasmic organelles including nucleus (N), juxtannuclear Golgi body (G) and mitochondria (M). Scale bar indicates 0.5 μ m.

D: Enlargement of the nucleus showing the details of the chromosomes. Labeled chromosome (Ch) is extremely elongated (ca. 0.1 μ m wide and more than 1 μ m long). Scale bar indicates 1 μ m.

E: The perialgal envelope bounded by the inner and outer membrane (IM and OM) is usually thin and gently folded (right half) but is partially thickened with extracapsulum (left half). Scale bar indicates 0.5 μ m.

F: Enlargement of the plasma membrane (PM) and very thin organic wall (OW) surrounding the Type II symbiont. Scale bar indicates 0.1 μ m.



TEXT-FIGURE 10

Schematic illustration showing five ontogenetic stages of skeleton and central capsule of *Didymocirtis tetrathalamus*. Detailed cytoplasmic features such as fusules are not illustrated in this figure. The skeleton of stage 1 is characterized exclusively by the two medullary shells with or without vestigial intervening bars from the equatorial region. In this stage, the endocapsular wall may be the only wall enclosing the intracapsulum. In stage 2, the intervening bars radiating from the outer medullary shell are developed on its equatorial border and a well developed girdle ring is constructed surrounding the outer medullary shell. The intervening bars are rimmed by the capsular wall, so that the development of the intervening bars appear to be associated with that of the capsular wall. It seems that the endocapsular wall is initially perforated by openings, which become elongated into the reflexed, collar-like extensions as the radial bars develop. During stage 3, the major part of the cortical shell is formed with the slight expansion of the central capsule space. The central capsule is still closely enclosing the two medullary shells at this stage. It is likely that the algal symbionts have been sequestered within the host cytoplasmic envelope at this or possibly at a previous stage. In stage 4, the central capsule begins to expand faster than shell growth. The polar caps develop in stage 5. For further detailed explanation refer to the text.

the bilocular shell (text-fig. 2, 5A, and 10). The polar caps develop last (stage 5), finally enclosing the completed poles of the bilocular shell and surrounding the capsular wall.

Unfortunately we did not collect younger individuals at this time, and the earliest stages of development remain to be determined. Additional research is also necessary to elucidate further the detailed events that produce the characteristic perforations

of the endocapsular wall and the more exact timing of events during formation of the capsular wall.

Comparison of the fusule structures

Comparative studies of spumellarian fusules have been done by Cachon and Cachon (1972) and Anderson (1982). Among hitherto investigated taxa, *Cyrtidosphaera reticulata* Haeckel illustrated by Cachon and Cachon (1972), which is one of the representatives of the Periaxoplastidies (Hollande and Enjume

1960), has fusules most similar to those of *D. tetrathalamus*. The fusules of *C. reticulata* are also characterized by having well developed peripheral electron-lucent zones and a similar fusule membrane, and two kinds of osmiophilic zones, although there are a few minor differences such as the development of constrictions at the outer osmiophilic tube (outer constriction) in the fusules of *D. tetrathalamus*.

Anderson (1982) clearly demonstrated that colonial spumellarians have certain features in common with one another. Nassellarian (Cyrtida) fusules also have some common features with one another as shown by Sugiyama and Anderson (1997). It is important to specify which fine structural features of the fusules are common to all radiolarians and which are specific to a given taxon; however, from these results we conclude that fusule structure may be a useful taxonomic characteristic in setting higher taxonomic categories, and that *D. tetrathalamus* can be included in the Periaxoplastidies in the sense of Hollande and Enjume (1960), based on the several common features mentioned above.

Symbiotic Associations

As described in the preceding chapter, the two types of algal symbionts have many different cytoplasmic and morphological features, which apparently indicate that they represent different dinoflagellate species. The two species were never observed to co-occur in a single host; therefore, they are considered as being mutually exclusive. Although it is known that the large spongiolate skeletal spumellaria have varied symbionts associated with different hosts including dinoflagellate, prasino-phycean and prymnesiid symbionts (Anderson et al. 1983), this is the first report confirming the occurrence of two mutually exclusive endosymbionts in Radiolaria at the species level. Although our examined materials were from the same water mass off Barbados, this ecologic feature immediately indicates that *D. tetrathalamus* can be infected by various species of dinoflagellates living in different water masses, which may be related to the relatively wide geographic distribution of *D. tetrathalamus* (Lombardi and Boden 1985). Similarly more than one symbiotic species of algae infect different individuals of the planktonic foraminifer *Globigerinella aequilateralis* (Faber et al. 1988) and also some benthic foraminifera such as *Amphisorus hemprichii* (Lee and Lawrence 1990). *Globigerinella aequilateralis* harbors two species of chrysophytes and *A. hemprichii* possesses two species of dinoflagellate symbionts *Symbiodinium* sp. and *Amphidinium* sp.

Among hitherto known radiolarian dinoflagellate symbionts (e.g., Anderson 1976a, b, c, 1977; Swanberg and Anderson 1981), the Type I symbionts are closely similar to those of *Sphaerococcus punctatum* Müller reported by Anderson (1976c) except for the reticulation of the chloroplast. Both symbionts have relatively narrow peripheral chloroplasts, prominent chromosomes, numerous starch and reserve bodies and a one-stalked pyrenoid penetrated by double-thylakoid lamellae. Based on these features, the Type I symbionts are confidently identified as *Amphidinium* sp. (Dodge 1973; Taylor 1974). In contrast, the taxonomic position of the Type II symbionts remains uncertain due to their characteristically invaginated outline. However, it should be noted that each plastid lamella in the pyrenoid of the Type II has two thylakoids, which is consistent with the characteristic features of the genus *Amphidinium* (Dodge 1973; Taylor 1974). At least, they do not belong to the genus *Symbiodinium* Freudenthal judging from the pyrenoid with invasive plastid thylakoids (Trench and Blank 1987). The

Type II symbionts closely resemble symbiotic dinoflagellates of the nassellarian *Pterocorys zancleus* from the same locality (Sugiyama and Anderson 1997) which also has an invaginated shape. One may suggest that such a fine structural profile of the symbionts, each surrounded by a wide electron-lucent zone, has resulted from digestion by the host. However, we have never observed evidence indicating digestion, such as partial degradation of the symbiont cytoplasm (Anderson 1976b). Further studies on isolated symbionts from the host are needed to more accurately identify their taxonomic position.

Faber et al. (1988) found that the peripheral cytoplasmic layer of extrashell cytoplasm and web of rhizopodia in *G. aequilateralis* have different organizations, including the position of the symbionts, in response to the type of symbiont. In *D. tetrathalamus*, we found two types of symbiont distribution in the extrashell cytoplasm; one is restricted around the cortical shell, and the other is dispersed from the shell along the axopodia. The latter type was, however, found only in one individual thus far and we did not confirm its identification by fine structural analysis. Consequently, the explanation for the differences in arrangement of the endosymbionts observed in *D. tetrathalamus* remains to be determined.

Moreover, further questions arise with respect to the individual illustrated in text-fig. 1B, since it has a well developed spongy shell around the cortical shell with symbionts enclosed inside it as well as distributed along the axopodia on the outside. The pore diameter of the spongy shell is approximately 5 µm based on previously published data (e.g., Anderson et al. 1986, Plate I, 1), which is apparently smaller than the diameter of the examined symbionts 10–15 µm. This raises the interesting question of whether the symbionts observed outside the spongy shell were able to pass through the pores. If they are physically segregated from those inside, they simply may be ones that were excluded from the interior of the spongy shell during its development, or possibly they may be a different symbiotic alga that has been captured subsequent to the construction of the spongy shell. Alternatively, the algae may not be endosymbiotic, but may be commensals as observed in some colonial radiolarians (Spero and Angel 1991). This peculiar association as shown in text-fig. 1B deserves further investigation.

Bacterial endobionts have been known in some spongiolate spumellarians (Anderson and Matsuoka 1992; Matsuoka 1992, 1993). Interestingly, we did not observe clumps of bacteria distributed along the host axopodia as is observed in some spongiolate spumellarians (Matsuoka 1992, 1993). It is not known whether this is due to differences between the species of bacterial endobionts or due to differences in axopodial activity of the host. Furthermore, our knowledge of radiolarian bacterial endobionts in general is unfortunately still very restricted. Since bacteria are among the most ancient forms of life and have been available well before radiolarian evolution began, it is possible that physiological interactions with bacteria may have played an important role since radiolaria first appeared in the Cambrian (e.g. White 1986). Much additional research is needed to clarify the functional significance of the radiolarian - bacterial interrelationship.

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