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Host-symbiont associations of polycystine Radiolaria: epifluorescence microscopic observation of living Radiolaria

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Abstract

In all 29 polycystine radiolarian species were obtained from surface seawater on May 28, 1999, using a plankton-net at one station (Site 990528; 26°37'18"N, 127°47'35"E) approximately 5 km northwest of Okinawa Island, Japan. In most polycystine radiolarians of the orders Nassellarida and Spumellarida symbiotic algae were observed under light microscopy. The light microscopic (LM) images of the symbionts, however, varied in clarity among individuals because of the variations in microanatomy of the host radiolarian cells. On the other hand, epifluorescence microscopic (EFM) observation easily detected and confirmed the existence of the algal symbionts within the host cytoplasm even in radiolarians such as *Dictyocoryne truncatum* (Ehrenberg) that include algal symbionts in the depth of the cytoplasm. The chloroplasts of the algal symbionts emitted autofluorescence in ultraviolet irradiation and they appeared red. That is, the autofluorescence images of the chloroplasts can be used to recognize the existence of the algal symbionts within the host radiolarians. Moreover, staining of the symbiont cells with 4',6-diamido-2-phenylindole permitted visualization of the nucleus in the center of the symbiont cell, confirming the existence of living endosymbiotic algae within the polycystine radiolarians. Both the LM and EFM observations of eight polycystine radiolarian species revealed the specific patterns of various host-symbiont associations. (1) The investigated polycystine radiolarians all possess algal symbionts, except for one species, i.e. *Dictyocoryne profunda* Ehrenberg. (2) The size of the algal symbionts depends on the radiolarian species. The symbionts are largely classified into two types based on the size of their diameters, i.e. about 8–10 µm for the larger group and about 5 µm for the smaller one. (3) The algal symbionts show a variety of locations within the host radiolarian cytoplasm. The types of distribution of algal symbionts may be a useful characteristic for radiolarian taxonomy.

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1. Introduction

Polycystine Radiolaria (subphylum Sarcodia; class Polycystinea) are holoplanktonic protists, occurring exclusively in modern open ocean envi-

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ronments. They have a rich fossil record due to the presence of skeletal material comprised of silica which is preserved in marine sediments. Since Meyen's (1834) first report on polycystine Radiolaria, many studies of 'Radiolaria' have been published during the past seventeen decades ('Radiolaria' sensu lato includes the classes Acantharea, Polycystinea, and Phaeodarea). While many of these studies have been restricted to classification and morphology based on skeletal evidence from the sedimentary record or plankton samples (e.g. Haeckel, 1887), very little has been published on radiolarian physiology, ecology, and their fine structures (e.g. Cachon and Cachon, 1971; Anderson, 1976, 1983).

Among the physiologic and ecologic studies, in particular, the relationship between the radiolarians and their symbionts has been discussed as a subject of great interest by many researchers. The radiolarian symbionts were first observed by Huxley (1851) and Brandt (1881) who identified some of them as symbiotic algae. Early in the twentieth century, light microscopic (LM) analyses clearly revealed numerous algal symbionts associated with radiolarians (e.g. Haeckel, 1887).

Algal symbionts within radiolarians are generally yellow-green, spherical, minute cells, measuring several micrometers in diameter. They have been reported in a variety of radiolarian species, especially in the orders Nassellarida and Spumellarida. In all polycystines, each cell is physically divided into two major cytoplasmic regions by a porous capsular wall: the intracapsulum (endocyttoplasm) and the extracapsulum (ectocyttoplasm) (e.g. Anderson, 1983). The algal symbionts are generally found in the rhizopodial network of the extracapsulum and in most cases none have been observed within the central capsule (intracapsulum) (Anderson, 1976, 1983). However, there is one report in which intracapsular microalgae and bacteroids were observed in *Dictyocoryne truncatum* (Ehrenberg) using a transmission electron microscope (Anderson and Matsuoka, 1992).

Recently, fine structure evidence by transmission electron microscopy showed that polycystine radiolarians possessed one of the following algal species, i.e. dinoflagellate, prasinophyte, or prym-

nesiophyte (e.g. Anderson, 1976; Cachon and Caram, 1979). Furthermore, it has been clarified that the radiolarians and the microalgae form an ultramicrobial association in which the photosynthetically derived organic substances are transferred from the algae to the host radiolarians (Anderson, 1978).

In this paper, we report that algal symbionts within polycystine radiolaria are readily detected using an epifluorescence microscope (EFM), even in such radiolaria as *Dictyocoryne truncatum* which includes algal symbionts in the depth of the cytoplasm.

2. Materials and methods

Radiolarians were collected from surface seawater (up to 3 m depth) on May 28, 1999, using a plankton-net (60-cm circle opening with 37- μ m mesh net) at one station (Site 990528; 26°37'18"N, 127°47'35"E) approximately 5 km northwest of Okinawa Island, Japan (Fig. 1). Plankton samples were diluted with seawater collected at the sampling site and returned to the laboratory at the Tropical Biosphere Research Center of the University of the Ryukyus, Sesoko Island, Okinawa Prefecture. Radiolarians were isolated from the plankton samples under an inverted microscope or a binocular stereoscopic microscope with a Pasteur pipette and observed using EFM with UV-excitation. For the epifluorescence microscopy, the radiolarians were fixed with 1% glutaraldehyde and stained with 4',6-diamido-2-phenylindole (DAPI) prepared in S buffer (Kuroiwa and Suzuki, 1980), and mounted on slides.

3. Observations and discussion

In all 29 polycystine radiolarian species were identified with LM: *Acanthodesmia vinculata* (Müller), *Callimitra annae* Haeckel, *Collosphaera macropora* Popofsky, *Dictyocoryne profunda* Ehrenberg, *D. truncatum* (Ehrenberg), *Didymocytis tetrathalamus* (Haeckel), *Euchitonia elegans* (Ehrenberg), *E. sp.*, *Eucyrtidium acuminatum* Ehren-

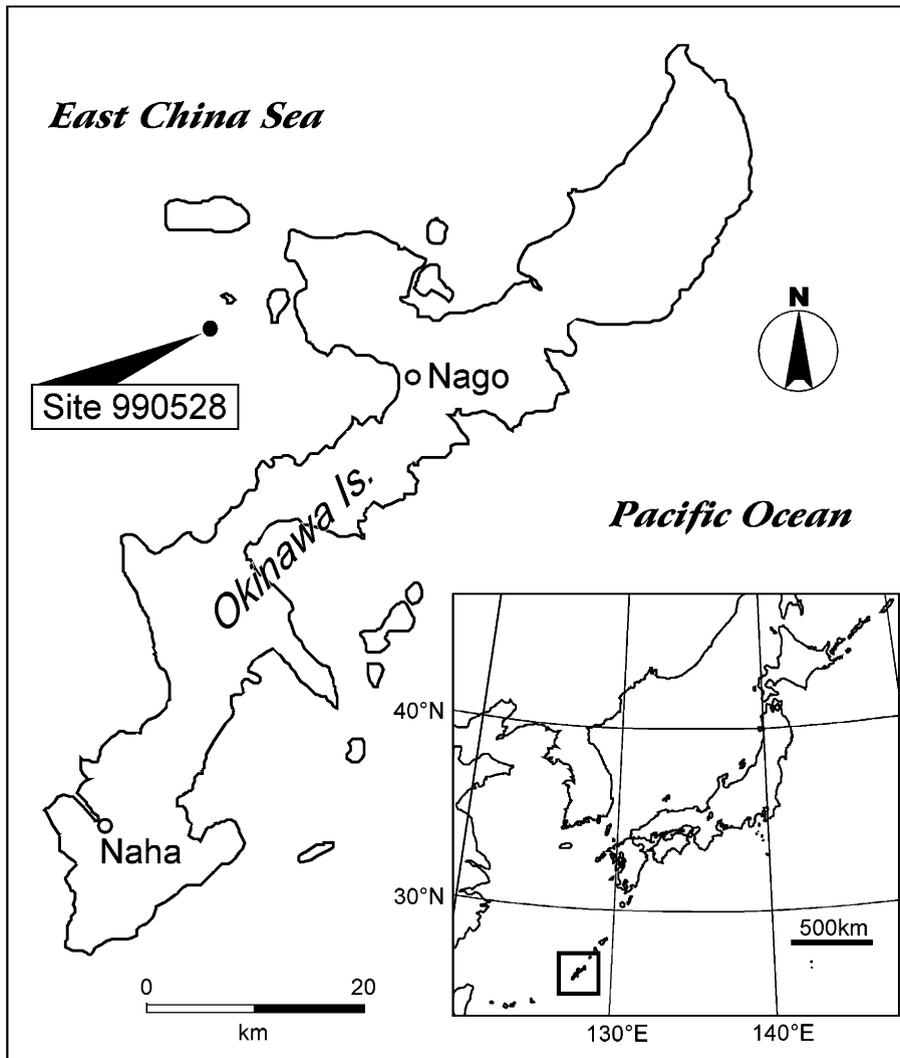


Fig. 1. Map showing the location of Site 990528, Okinawa Island, Japan.

berg, *E. hexagonatum* Haeckel, *Heliodiscus* sp., *Lipmanella dictyoceras* (Haeckel), *L. pyramidale* (Popofsky), *Lophophaena* sp., *Peridium spinipes* Haeckel, *Peromelissa phalacra* Haeckel, *Pterocanium praetextum* (Ehrenberg), *Pterocorys zancleus* (Müller), *Sphaerozoum fuscum* Meyen, *Spirocortis scalaris* Haeckel, *Spongaster tetras* Ehrenberg, *Spongodiscus biconcavus* Haeckel, *Tetrapyle octacantha* Müller, *Theocorythium trachelium* (Ehrenberg), *Theophormis callipilium* Haeckel, *Zygocircus productus* (Hertwig), and three gen. sp. indet.

Most of these polycystine radiolarians have been previously reported by Matsuoka (1993) near our sampling station.

Although symbiotic algae were observed in most of the 29 radiolarian species with LM (bright field illumination), the images of the symbionts varied in clarity among individuals because of the variations in microanatomy of the host radiolarian cells. However, we could in most cases detect the algal symbionts clearly within the host cytoplasm using EFM. The chloroplasts of the

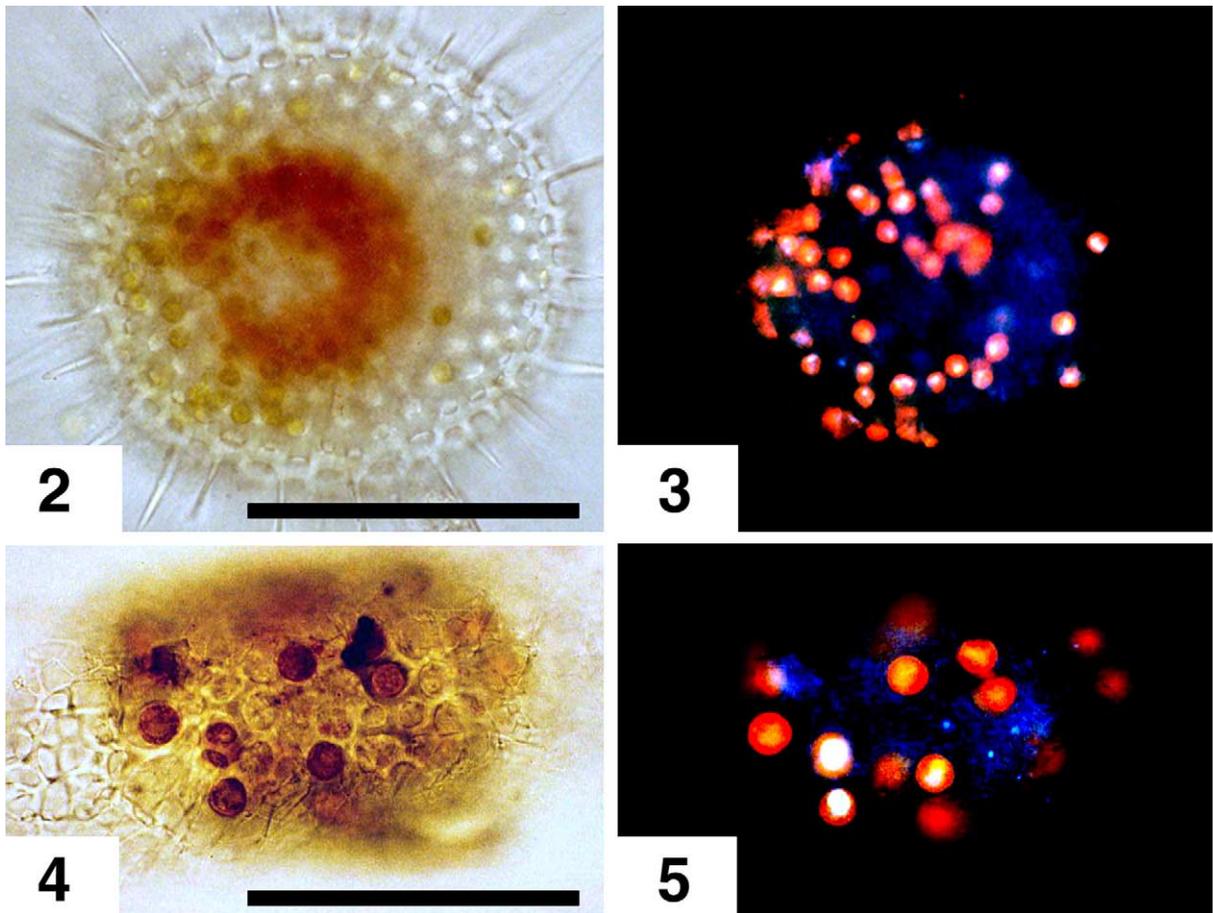


Fig. 2. LM photograph of *Heliodiscus* sp. Scale bar indicates 100 μ m.

Fig. 3. EFM photograph of *Heliodiscus* sp.

Fig. 4. LM photograph of *Didymocyrtis tetrathalamus* (Haeckel). Scale bar indicates 100 μ m.

Fig. 5. EFM photograph of *Didymocyrtis tetrathalamus* (Haeckel).

algal symbionts emitted autofluorescence in ultra-violet irradiation and appeared red. Moreover, symbiont cells stained with DAPI displayed the nucleus in the center of the cell (Figs. 2–5). The host cells include various substances (e.g. oil drops, bacteria and digestive vacuoles) which may emit autofluorescence, but the observation of the cells stained with DAPI confirmed the existence of ‘living’ endosymbionts within the polycystine radiolarians. The host radiolarians did not show the DAPI-fluorescence dots of nucleus-DNA. This could be due to the existence of an organic barrier within the central capsular wall of

the polycystine radiolarians secreted by the living envelope of membranes (Anderson, 1983).

Using both LM and EFM, we observed several specimens for every eight polycystine species: *Dicthyocoryne profunda*, *D. truncatum*, *Didymocyrtis tetrathalamus*, *Euchitonia elegans*, *Heliodiscus* sp., *Pterocanium praetextum*, *Sphaerozoum fuscum*, and *Spongodiscus biconcavus*. Then, we were able to examine the different patterns of occurrences of algal symbionts among the eight selected species of polycystine radiolarians as follows (see also Table 1):

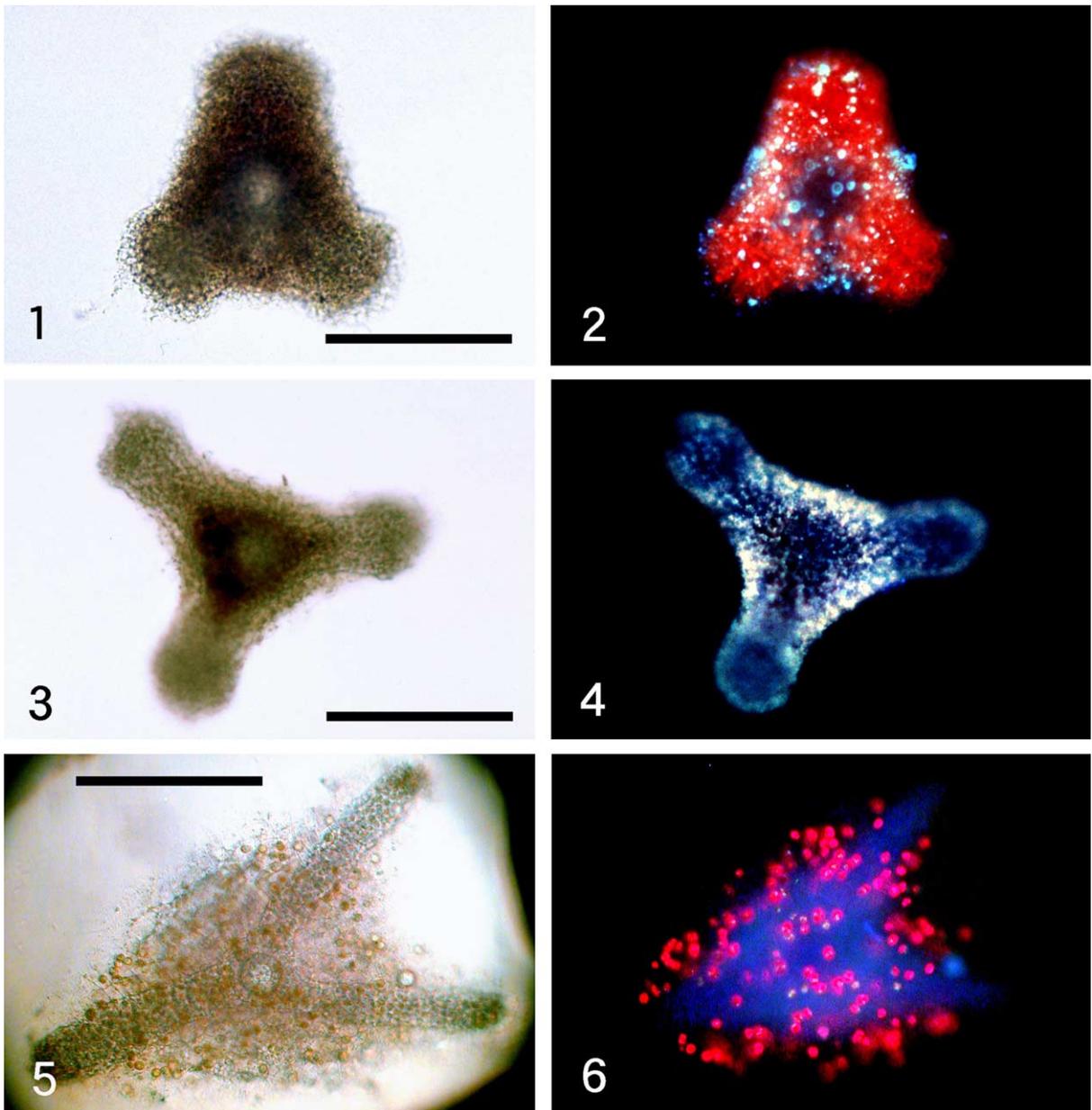


Plate I. LM and EFM images of living radiolarians. Scale bars indicate 200 μm .

1. LM photograph of *Dictyocoryne truncatum* (Ehrenberg)
2. EFM photograph of *Dictyocoryne truncatum* (Ehrenberg)
3. LM photograph of *Dictyocoryne profunda* Ehrenberg
4. EFM photograph of *Dictyocoryne profunda* Ehrenberg
5. LM photograph of *Euchitonia elegans* (Ehrenberg)
6. EFM photograph of *Euchitonia elegans* (Ehrenberg)

Dictyocoryne truncatum (Ehrenberg) (Plate I, 1,2). A light microscopic image of living *D. truncatum* shows a triangular, flattened shell with rounded apices, with the maximum length of the shell ranging from 200 to 300 μm . The surface of the shell has a spongy meshwork without ornamentation, enclosing light greenish-yellow to orange-colored cytoplasm (plus symbiotic algae, as described below) and radiating pseudopodia (axopodia and axoflagellum). We could not observe symbiotic algae within either the external or internal regions of the shell of *D. truncatum* using the LM. However, an EFM observation clearly showed their presence within the intra-shell cytoplasmic region (Plate I, 2). The sizes of the algal symbionts coincide with the results of Anderson and Matsuoka (1992), based on transmission electron microscopy; they are about 5 μm in diameter.

Dictyocoryne profunda Ehrenberg (Plate I, 3,4). Morphologic features of living *D. profunda* using LM observation closely resemble those of *D. truncatum*. *D. profunda* has a triangular, flattened shell with rounded apices ranging in length from 200 to 300 μm . The shell surface also has a spongy meshwork, enclosing brownish-orange to red-colored cytoplasm with radiating axopodia and an axoflagellum. The LM image of living *D. profunda* is distinguished from that of living *D. truncatum* in having rather dark-colored (brownish-orange to red) cytoplasm and rather rounded apices (Matsuoka, 1993). We examined several specimens of *D. profunda* with LM and EFM. The LM and EFM images show no algal

symbionts in either the internal or external regions of the shell of *D. profunda*. *D. profunda* is, therefore, clearly different from the other polycystine radiolarians in having no algal symbionts within its cytoplasmic body.

Euchitonia elegans (Ehrenberg) (Plate I, 5,6). A light micrograph of a living *E. elegans* reveals a triangular, flattened shell with maximum shell length of about 400 μm in the adult stage. The surface of the shell is a spongy meshwork with veil-like ornamentation. The cytoplasm is generally colorless or rarely brownish-red with radiating pseudopodia (axopodia and axoflagellum). Many algal symbionts, more than 100, were observed, but only on the surface of the shell with LM. The algal symbionts are yellow-green in color with size ranging from 8 to 10 μm in diameter. The EFM image of living *E. elegans* shows that the algal symbionts are within the meshwork of the veil-like ornamentation around the surface of the shell and no symbiotic algae were observed in the intra-shell cytoplasmic region. *E. elegans* thus has a pattern of host-symbiont associations different from that of *Dictyocoryne truncatum* and *D. profunda*. This finding presents an interesting problem from the viewpoint of the taxonomic relationships among these three species.

Cytoplasmic features of living *Didymocyrtis tetrathalamus* (Haeckel) and *Pterocanium praetextum* (Ehrenberg) are simply described as follows. *D. tetrathalamus* (Figs. 4 and 5) possesses a bilocular, porous shell, about 200 μm in length, enclosing a porous spheroidal medullary shell. Light microscopic image of living *D. tetrathalamus* shows

Table 1
Relationship between locations and sizes of symbiotic algae among eight polycystine radiolarians

Species	Symbiotic algae	
	Sizes (μm)	Locations
<i>Dictyocoryne truncatum</i> (Ehrenberg)	Ca. 5	I
<i>D. profunda</i> Ehrenberg	–	–
<i>Didymocyrtis tetrathalamus</i> (Haeckel)	Ca. 10	I+O
<i>Euchitonia elegans</i> (Ehrenberg)	8–10	O
<i>Heliodiscus</i> sp.	8–10	I
<i>Pterocanium praetextum</i> (Ehrenberg)	8–10	I
<i>Sphaerocozium fuscum</i> Meyen	Ca. 5 / Ca. 10	–
<i>Spongodiscus biconcavus</i> Haeckel	Ca. 5 / Ca. 10	I+O

Abbreviations: I, inside of shell; O, outside of shell.

brownish-orange to red-colored cytoplasm and yellow-green algal symbionts, about 10 μm in diameter. The algal symbionts are commonly found between the central capsule and the bilocular shell. *Pterocanium praetextum* (not shown) is classified into the order Nassellarida. It has a conical, segmented porous shell with an apical horn and three feet. The cytoplasm of living *P. praetextum* is brownish in hue and occupies the bell-shaped thorax. Numerous thin axopodia radiate from the shell surface. *P. praetextum* possesses algal symbionts generally in the distal end (near the basal opening) of the shell which are yellow-green in color ranging in size from 8 to 10 μm .

4. Concluding remarks

From the LM and EFM observations of eight selected polycystine radiolarian species, which revealed various patterns of host-symbiont associations, the following generalizations can be made.

(1) The investigated polycystine radiolarians all possess algal symbionts, except for one species, i.e. *Dictyocoryne profunda* Ehrenberg. (2) The algal symbionts are variable in size for depending on the host radiolarian species. They are largely classified into two types based on the size of their diameters, i.e. about 8–10 μm for the larger group and about 5 μm for the smaller one. (3) The algal symbionts show a variety of locations within the host radiolarian cytoplasm. The types of distribution of algal symbionts may be a useful characteristic for radiolarian taxonomy.

These variable algal symbiont characteristics raise the question whether the patterns of host-symbiont associations should be used as a means of classification or for studying diversity and/or evolution among polycystine Radiolaria. What kinds of evolutionary innovations are of major significance in this regard? When did the host-symbiont association appear in evolution or when did it diversify? These questions will be resolved only upon further biological analysis of polycystine radiolarians. Both molecular and fine structural examinations are necessary to examine this hypothesis.

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