

## Fine Structure of a Large Dinoflagellate Symbiont Associated with a Colonial Radiolarian (*Collozoum* sp.) in the Banda Sea

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### Abstract

A wide variety of algal symbionts has been reported in solitary and colonial radiolaria including prasinophytes, prymnesiophytes, and dinoflagellates. Among the symbiotic dinoflagellates the typical size is 6 to 12  $\mu\text{m}$ . During a plankton collection in the Banda Sea, we obtained a skeletonless colonial radiolarian (*Collozoum* sp.) with an unusually large dinoflagellate symbiont (c. 25  $\mu\text{m}$ ). We report the fine structure of the symbiont and possible correlates with function. The globose cell has a single layer of plastids distributed at the periphery of the cell, a mesokaryotic nucleus with puffy chromosomes characteristic of some dinoflagellates, a very large mass of osmiophilic matter that fills most of the cytoplasm, peripheral chloroplasts with lamina containing 2–3 thylakoids, and a very reduced chondriome. The relatively small amount of mitochondria, the large mass of reserve material, and the nearly continuous layer of plastids at the periphery suggest that this symbiont is maximally active as a photosynthetic unit with minimal respiratory activity, thus enhancing its role as a source of nutrients for the host. This is the first report of a dinoflagellate symbiont with these properties and raises the interesting question of

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why this host-algal symbiosis is so different from previously reported dinoflagellate associations with radiolaria.

Keywords: Algal symbioses, dinoflagellate, functional microanatomy, physiological ecology, plankton

## 1. Introduction

Algal symbioses occur widely in marine invertebrates including sponges, ascidians, coelenterates (e.g., corals), turbellaria (flat worms), benthic and planktonic foraminifera, acantharia, and radiolaria (Parke and Manton, 1967; Taylor, 1974; Anderson, 1976ab, 1978, 1983ab; Cachon and Caram, 1979; Febvre and Febvre-Chevalier, 1979; Trench and Blank, 1987; Spero and Angel, 1991; Douglas, 1992; Lee, 1992; Pardy and Royce, 1992; Wilkinson, 1992). Radiolarian symbioses are of particular interest since this group of planktonic sarcodines occurs relatively abundantly in widely diverse oceanic regimes including somewhat oligotrophic locations (e.g., Sargasso Sea). The presence of algal symbionts may account partially for their relative success since the host is less dependent on prey for nutrition. Radiolaria contribute substantially to the marine microfossil sedimentary record and factors accounting for their abundance and distribution are important in better interpreting modern and ancient marine environments (Casey, 1971, 1993; Anderson, 1983a). Consequently it is important to document the extent, kind and geographic distribution of algal symbioses in radiolaria. Since there have been few investigations of algal associations with radiolaria in the Banda Sea, we have explored some samples of radiolaria from plankton tows taken in a transect south of Indonesia in the eastern Banda Sea and describe an unusual association with a large dinoflagellate that has not been reported previously.

## 2. Materials and Methods

Collections were made during a brief cruise on the R/V Baruna Jaya IV. It departed from Ambon, Indonesia and sailed south into the Banda Sea. Plankton samples were taken on December 12, 1996, at a station in the eastern Banda Sea 5° S, 128° E at a depth of 1 m below the surface during brief tows of 5 minutes and a gentle velocity of 2 knots. Freshly collected samples from plankton tows were fixed in 2% glutaraldehyde buffered with 0.05 M cacodylate buffer (pH = 7.8). The fixed samples were refrigerated and returned by air to the Lamont-Doherty Earth Observatory laboratories. The *Collozoum* colonies were picked out photographed using a Nikon Diaphot inverted

microscope, washed in cacodylate buffer (0.05 M, pH = 7.8), and post-fixed for 2 hours in 2% osmium tetroxide at 5°C in the same buffer as the glutaraldehyde fixative. Osmium-fixed samples were rinsed in distilled water, dehydrated in a graded acetone series, and infiltrated with and embedded in epon (Scipoxy 812™, Energy Beam Sciences, Agawam, MA). The blocks were hardened at 60°C overnight, and sectioned with a Porter-Blum MT-2 ultramicrotome fitted with a diamond knife. Sections were collected on copper grids, stained with Reynold's alkaline lead citrate and observed with a Philips 201 TEM operated at 60 kV. Stereological measurement of proportional cell volume occupied by a given structure in electron micrographs was according to Kubinová (1991). A grid of points is positioned over the electron micrograph. The proportion of points falling on a given structure relative to the total field delimited by the symbiont is an estimate of the proportional volume occupied by the structure.

### 3. Results

A light micrograph of a fixed preparation of *Collozoum* sp. collected from the Banda Sea (Fig. 1) illustrates the size and distribution of symbionts scattered among the larger central capsules (cell bodies) of the radiolarian host. The radiolarian is probably *Collozoum inerme* (Haeckel, 1887). This species is characterized by a spheroidal colony with central capsules surrounded by a thin membrane (c. 40–160 µm) without skeleton. Our isolate conforms to this description. *Collozoum inerme* has been reported from the Indian Ocean (Strelkov and Reshetnyak, 1971). Hence, it is not surprising to find it in the Banda Sea. The colony consists of a clear gelatinous globose matrix enclosing the host central capsules and symbionts. All the symbionts in these colonies are of approximately the same size (25 µm). The symbionts are enclosed within cytoplasmic sheaths connected to the extracapsular cytoplasm that forms a network of strands interconnecting the naked central capsules. A transmission electron microscopic view of a cross section through the symbiont (Fig. 2) shows the nucleus (6 × 4 µm) with puffy chromosomes containing coiled strands of DNA, a layer of chloroplasts at the periphery of the cell, a large deposit of electron dense storage material filling much of the central portion of the cell, vacuolar-bound dense matter, and starch grains (c. 3 µm diameter) surrounding the chloroplast pyrenoids and scattered throughout the cytoplasm. Much of the dense storage material may be osmiophilic lipids and some appears to have been leached out during dehydration. An enlarged view of the periphery of the dinoflagellate symbiont (Fig. 3) displays a thin, osmiophilic, non-living thecal membrane (arrow) characteristic of some coccoid symbionts. The chloroplasts (c. 0.3–0.4 µm wide) have lamina containing two to

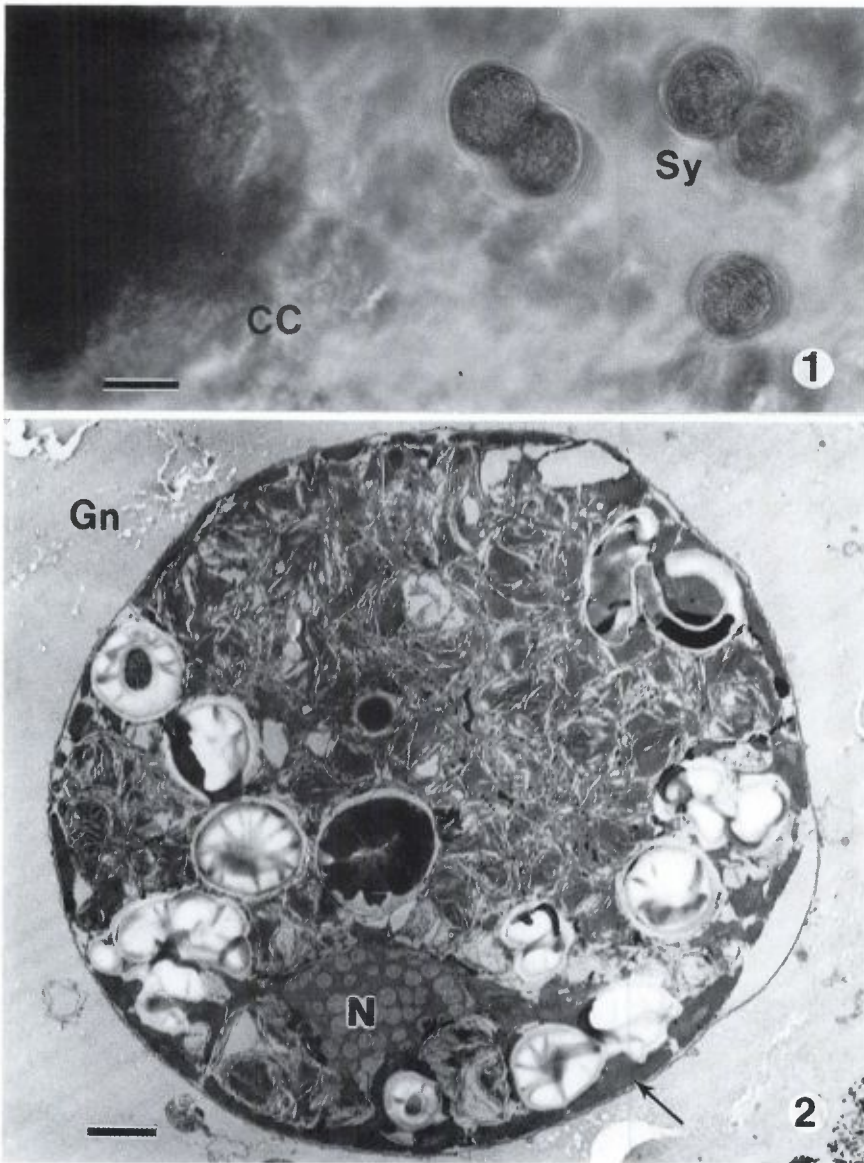


Figure 1. Light microscopic view of a portion of the *Collozoum* colony collected from the Banda Sea showing part of a central capsule (CC) and large dinoflagellate symbionts (Sy) in the surrounding gelatin. Scale bar = 20.0  $\mu\text{m}$ .

Figure 2. Transmission microscopic view of ultrathin section through a dinoflagellate symbiont contained in the gelatin (Gn) of the colony, showing the nucleus (N), peripheral chloroplasts (arrow, shown in greater detail in Fig. 3), starch grains, and massive deposit of osmiophilic reserve material filling the center of the cytoplasm. Scale bar = 2.0  $\mu\text{m}$ .

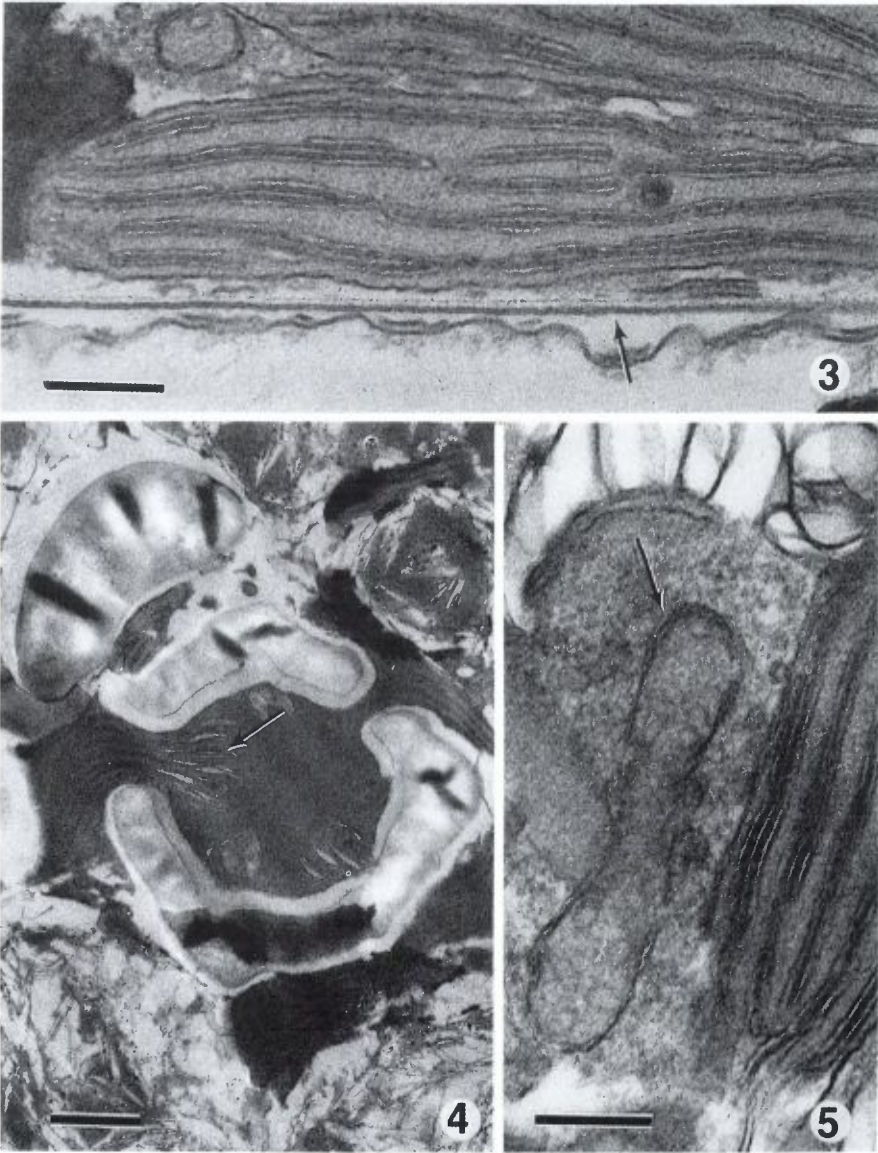


Figure 3. A higher magnification view of the periphery of the symbiont exhibits the thecal membrane of the coccoid symbiont (arrow), and a chloroplast situated immediately adjacent to the cell membrane. Scale bar = 0.2  $\mu$ m.

Figure 4. A multi-stalked pyrenoid of the symbiont contains intrusions of the plastid thylakoids (arrow) and abundant starch caps. Scale bar = 1.0  $\mu$ m.

Figure 5. A highly magnified view of a portion of the cytoplasm near the nucleus showing one of the few occurring mitochondria (arrow) with tubular cristae. Scale bar = 0.2  $\mu$ m.

Table 1. Symbiont characteristics in some solitary and colonial radiolaria with comparisons to the large dinoflagellate reported here from the Banda Sea.

Radiolarian group	Symbiont taxon	Symbiont size	Nuclear size	Plastid structure	Cell structural features	Literature source
Solitary species	Dino-flagellate <sup>1</sup>	4-6 µm	3-4 µm; meso-karyotic	Peripheral or folded toward center of cell, 3 thylakoids	Single-stalked pyrenoid + starch, mitochondria in central cytoplasm	Anderson, 1976a,b, 1977, 1978, 1983ab
	Prasino-phyte	5-8 µm	2 µm	Peripheral, lamina with 3 thylakoids	Pyrenoid with penetration by nucleus; starch in plastid lamina	Anderson, 1976a; Cachon and Caram, 1979
	Prymnesio-phyte	5-20 µm	5 µm	Peripheral, 3 thylakoids	Pyrenoid within plastid, large vacuole (4-12 µm) center of cell	Anderson, et al., 1983a
	Chryso-phyte	3-4 µm	1.5 µm	Peripheral enclosed in perinuclear envelope, 3 thylakoids	Posterior vacuole 2-3 µm or lacking, abundant reserve bodies surrounding vacuole	Anderson and Matsuoka, 1992
Colonial species	Chryso-phyte	3 µm	1-2 µm	Parietal, enclosed in perinuclear envelope	Small (1 µm) vacuole laterally located to nucleus, Golgi prominent	Anderson et al., 1986b
	Dino-flagellate	12-15 µm	4 µm; meso-karyotic	Peripheral, lamina with 3 thylakoids	Multiple-stalked pyrenoid + starch, mitochondria in central cytoplasm	Swanberg, 1979; Swanberg and Anderson, 1981
	Dino-flagellate	25 µm	6 µm; meso-karyotic	Peripheral, 2-3 thylakoids per lamina	Multiple-stalked pyrenoid, massive deposit of reserve material, few mitochondria	This publication

<sup>1</sup>This size symbiont is also found in some colonial species.

three thylakoids in a stack (0.03–0.05  $\mu\text{m}$  thick). The pyrenoid (2–2.5  $\mu\text{m}$  diameter) has multiple stalks with included, somewhat dilated, thylakoids that project into the center of the pyrenoid (Fig. 4). Scattered mitochondria (0.8  $\times$  0.1  $\mu\text{m}$ ), with tubular cristae (0.05  $\mu\text{m}$ , diameter), occur in patches of cytoplasm, especially near the periphery of the cell dispersed among the chloroplasts and starch grains (Fig. 5). There are few mitochondria relative to the cell volume that is composed largely of storage substances. Indeed, a stereological analysis of the symbiont cross section shows that approximately 80% of the cytoplasm is storage substances (e.g., dense matter, vacuolar bound material, and starch) and 60% of this (or 50% of the total cytoplasm) is the osmiophilic dense matter largely in the center of the cytoplasm.

#### 4. Discussion

A broad diversity of algal symbionts has been reported associated with radiolaria including dinoflagellates (Taylor, 1974; Anderson, 1976ab, 1977, 1978), prymnesiophytes (Anderson et al., 1983a), chrysophycophytes (Anderson and Matsuoka, 1992), and prasinophytes (Anderson, 1976a; Cachon and Caram, 1979). In some cases, the radiolarian is host to two different symbionts, for example *Physematium muelleri* contains dinoflagellates and yellow-green pigmented microalgae (Anderson, et al., 1986b). *Dictyocoryne truncatum* contains microalgae and bacteroid endobionts in the cytoplasm (Anderson and Matsuoka, 1992). Most of the algal symbionts are relatively small (c. 5–12  $\mu\text{m}$ ), although a presumed prymnesiophyte with large central vacuole described in a solitary radiolarian varied in size between 5–20  $\mu\text{m}$  diameter (Anderson et al., 1983a). Dinoflagellate symbionts, observed previously in colonial radiolaria, are also usually small (6–12  $\mu\text{m}$ ) including those of all *C. inermis* collected to date. A summary of current knowledge of symbiont types and sizes among a range of radiolarian taxa is presented in Table 1. In contrast, a much narrower range of symbiont types (mainly dinoflagellates and some chrysophycophytes, both typically 3–6  $\mu\text{m}$ ) has been reported in planktonic foraminifera, many inhabiting the same geographic locale as radiolaria (Anderson, 1983a; Lee and Anderson, 1991).

The finding of relatively large (25  $\mu\text{m}$ ) dinoflagellate symbionts with massive deposits of reserve material in *Collozoum* in the Banda Sea as reported here, adds further evidence of the diversity of forms observed in radiolaria. Since no other examples of this large dinoflagellate have been reported previously in radiolaria collected at other locations, it raises the interesting question of whether this association is peculiar to environmental conditions in the Banda Sea. It should be noted, however, that solitary

radiolaria (Spumellarida and Nassellarida) collected at the same time in plankton tows contained dinoflagellate symbionts of the more typical size (6–12  $\mu\text{m}$ ) indicating they were available in the Banda Sea. The large size of the symbionts reported here is not likely due to fixation artifacts, since the other radiolaria, containing normal sized symbionts, were fixed in the same way, simultaneously with the colonial radiolarian. Moreover, the symbionts show no signs of swelling, the periphery of the cell is lined by chloroplasts as expected in unaltered dinoflagellate cells. The peripheral membranes are intact and the host cytoplasm that closely encloses the symbiont has not been ruptured as would be expected if the cells had swollen during preservation. The large deposit of storage material and starch grains, consistently observed in all symbionts sectioned, suggest that the symbionts are photosynthetically competent. The organization of the cell should promote efficient gathering of light energy. Most of the chloroplasts are located immediately beneath the plasma membrane of the cell oriented with their long axis tangential to the cell membrane, and with the thylakoids running parallel to the long axis, thus presenting maximum surface area to intercept light passing across the cell surface. The relatively few mitochondria observed in the ultrathin sections suggest that the chondriome is limited relative to the photosynthetic apparatus. Previous fine structure research on radiolarian dinoflagellate symbionts shows widely distributed mitochondria occurring throughout the cytoplasm. Altogether, this may indicate that the symbiont has been structurally and physiologically altered to enhance photosynthesis and reduce respiratory loss of organic reserves, thus making the symbiont a more efficient source of nutrients for the host. The large deposit of osmiophilic reserve substances filling much of the central space and abundant starch grains (together occupying as much as 80% of the cytoplasm) further suggest the symbiont is photosynthetically efficient. Presence of robust starch caps on pyrenoids in radiolarian dinoflagellate symbionts is a common feature observed previously in solitary and colonial species (e.g., Anderson, 1983a). It is not unique to this large symbiont. Osmiophilic reserve materials, appearing as a dense granule, are also frequently seen in radiolarian symbionts. However, the massive deposits of osmiophilic substances reported here in this large dinoflagellate symbiont occupies much more of the cytoplasmic volume than observed previously and appear to be lipoidal in composition. Productivity at this location in the Banda Sea is relatively high. At the time of sampling gross  $\text{O}_2$  production was equivalent to  $1.6 \text{ g C m}^{-2} \text{ d}^{-1}$  and the net production was  $0.3 \text{ g C m}^{-2} \text{ d}^{-1}$  (C. Langdon, unpubl. obs.). In February 1994, productivity measured by the  $^{14}\text{C}$  method was  $1.3 \text{ g C m}^{-2} \text{ d}^{-1}$  (Kinkade et al., 1997). Hence, the large amounts of reserve material within the symbiont may



also reflect less demand for translocation of photosynthates by the host resulting in build up of starch and lipoidal material (e.g., Fig. 2).

Prior biochemical and isotopic tracer studies of dinoflagellate-radiolarian associations have shown that much of the photosynthate translocated to the host appears first in a lipid pool including triglycerides, fatty acids, and wax esters (Anderson et al., 1983b, 1986a). The storage of photosynthates as lipids in radiolarian symbionts may be a more efficient way of providing nutrient sources to the host. Since all of the symbionts observed in the colony were of the same size, and all of them observed in ultrathin sections contained the same cytoplasmic organization, we conclude that these large symbionts may be physiologically adapted to enhance the host-algal association.

The taxonomic identity of this large symbiont is not known. The multiple stalked pyrenoid with included thylakoids, mesokaryotic nucleus, and chloroplasts with lamina containing approximately three thylakoids is similar to the fine structure of dinoflagellate symbionts reported in other colonial radiolaria. Overall, the size of this symbiont is much larger, including the nucleus ( $6 \times 4 \mu\text{m}$ ) suggesting this is a different species of dinoflagellate symbiont, not simply an enlarged and physiologically altered cell. Further research is warranted to determine the geographic range of *Collozoum* sp. associated with these larger symbionts, to examine other species of colonial radiolaria for their presence, and to better document the structural and physiological relationship between symbiont and host.

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