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Endocytoplasmic Microalgae and Bacteroids Within the Central Capsule of the Radiolarian *Dictyocoryne truncatum*

O. ROGER ANDERSON[1] and ATSUSHI MATSUOKA[2]
[1] *Biological Oceanography, Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964, USA*
[2] *Department of Earth Sciences, College of General Education Niigata University, Niigata, 950-21 Japan*
Tel. 1 (914) 359-2900 (452), Fax 1 (914) 359-5215

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Abstract

Micro-algae (c. 2.5 to 3.5 μm wide $\times$3.8 to 4.5 μm long) occur abundantly within perialgal vacuoles in the intracapsular cytoplasm of the radiolarian *Dictyocoryne truncatum*, a triangular-shaped, spongiose skeletal radiolarian. The fine structure of the microalgae resembles that of yellow-brown pigmented symbionts observed in larger spongiose skeletal radiolaria of the spongodrymid type. The density of microalgae in a typical ultrathin section is c. 4/100 μm^2. Bacteroids (0.2×0.5 μm) are present throughout the intracapsular cytoplasm. There is no evidence of a vacuolar membrane enclosing the bacteroids, but each is surrounded by an electron lucent zone. The central capsule of radiolaria contains the nucleus and cytoplasmic organelles and is bounded by a capsular wall. Previously, algae associated with radiolaria have been observed in the extracapsulum. The occurrence of intracapsular microalgae in *D. truncatum* is of interest since this indicates that the intracapsular cytoplasm, previously thought to be largely specialized for metabolism, storage of reserve substances and production of reproductive swarmers, can also be a site for host-algal interactions.

Keywords: fine structure, microalgae, bacteroids, endobionts, algal symbionts, radiolaria

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1. Introduction

Radiolaria are planktonic organisms found exclusively in open ocean locations. Among the several hundred extant species, sizes vary from less than 50 μm to over 1 mm. All are characterized by a zonal cytoplasmic organization (Anderson, 1984). There is a central zone known as the central capsule containing the nucleus or nuclei, mitochondria, Golgi bodies, reserve substances, endoplasmic reticulum, and other major organelles. The central capsule is surrounded by a capsular wall that separates it from the outer zone known as the extracapsulum. Strands of cytoplasm penetrate the capsular wall at specialized regions known as fusules and connect to an elaborate rhizopodial mass surrounding the capsule. Axopodia, each continuous with the cytoplasmic strand protruding through the fusule, radiate outward into the surrounding environment. Algal symbionts have been reported in a wide variety of radiolarian species in the Nassellarida and Spumellarida (Anderson, 1983). Dinoflagellate, prasinophyte, and prymnesiophyte algal symbionts have beenreported (Anderson, 1976, 1978, 1982; Cachon and Caram, 1979; Anderson et al., 1983a, 1986a,b). In all cases, these algal symbionts were found only within perialgal vacuoles in the extracapsulum, including large species such as *Thalassicolla nucleata* (dia. central capsule, c. 0.5 mm).

Dictyocoryne truncatum is a triangular skeletal radiolarian (c. 300 μm) occurring with maximum abundances of about 50 to 150 individuals per cubic meter of surface water near Barbados. We report the first data on its fine structure and the occurrence of intracapsular microalgae and bacteroids in individuals collected near Barbados.

2. Materials and Methods

D. truncatum were collected on July 29, 1991 using a plankton net (half meter square opening with 36 μm mesh net) at a location approximately 2 km from the western shore of Barbados. The plankton sample was suspended in 1 liter of seawater obtained at the sampling site and returned to the laboratory at the Bellairs Research Institute, St. James, Barbados. Individual specimens were collected from the plankton sample by drawing them into a pasteur pipette, and then transferred to a small vial for fixation. They were fixed for 2 hr (at 20:50 hr on the day of collection) in 2% v/v glutaraldehyde in cacodylate buffer (0.05 M, pH 7.8) prepared in seawater that had been filtered through Millipore filter (0.45 μm pore size), rinsed with filtered seawater, and post-fixed for 22 hr in 2% v/v osmium tetroxide prepared in the same cacodylate buffer as used for the glutaraldehyde. The fixed samples

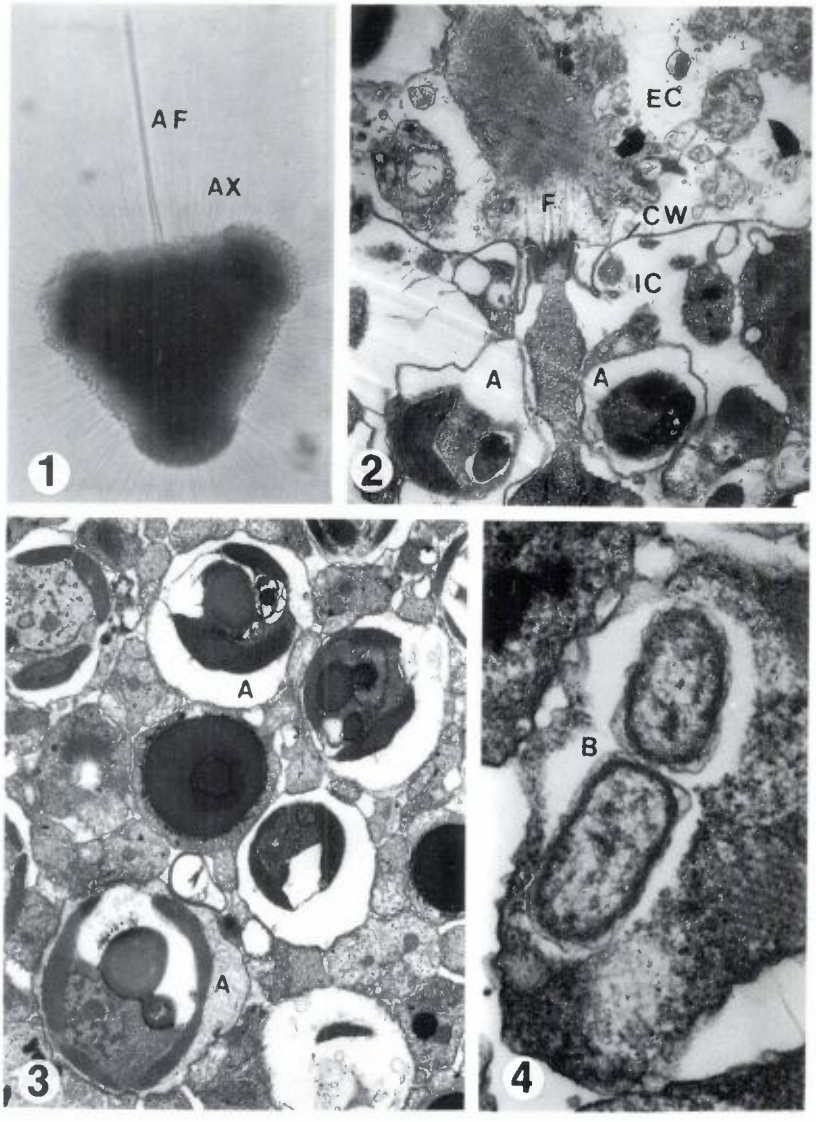
were rinsed in cacodylate buffer twice, stored, refrigerated, and subsequently dehydrated in aqueous-acetone series, infiltrated with a mixture of epoxy resin and acetone (1:1) and embedded in epoxy resin in BEEM® capsules (Ladd Res. Industries, Burlington, VT). Ultrathin sections, obtained with a diamond knife using a Porter-Blum MT-2 ultramicrotome, were collected on uncoated copper grids, stained with Reynold's lead citrate, and observed with a Philips TEM 201 electron microscope operated at 60 or 80 kV. Light micrographs of living individuals were made using a Nikon Diaphot inverted microscope.

3. Results

A light microscopic image of a living, mature *D. truncatum* (Fig. 1) shows the flattened, triangular skeleton, enclosing a more opaque central capsular cytoplasm, and radiating axopodia extending from the extracapsulum into the surrounding environment. A robust, elongate axoflagellum protrudes from the surface of the triangular skeleton near one side. The axoflagellum is a strand of cytoplasm (c. 9 μm in diameter and 230 μm long in a mature individual) that penetrates through a circular pore in the spongiose skeleton (Matsuoka, in press). The microanatomy of the axoflagellum in the vicinity of the central capsule was not determined.

Living *D. truncatum* maintained in culture, and observed with light microscopy, contain numerous, very small, brownish distributed along the axopodia. The symbionts are transported by cytoplasmic flow along the axopodia and move inward and outward with widely varying patterns during observational periods extending from early morning until evening. The radiolaria were not observed during the night, since we did not want to disturb their normal diel light/dark cycle while in culture. Sometimes the symbionts are observed gathered into a more dense patch on the vertex of the triangular skeleton opposite the side where the axoflagellum emerges. In other cases, the symbionts are distributed within the halo of axopodia radiating from the two sides of the skeleton lacking the axoflagellum, and in other cases are gathered together in small clumps on the axopodia distal to the central capsule. The cytoplasm internal to the skeleton is brownish-yellow, sometimes with a reddish hue.

A transmission electron microscopic view of an ultrathin section through a region of the central capsule near the capsular wall (Fig. 2) shows the organization of the fusule that connects the axopodia to the intracapsular cytoplasm. The diameter of the cytoplasmic strand where it enters the capsular wall is c. 0.5 to 0.6 μm. The extracapsulum consists of a frothy layer of cytoplasm next to the capsular wall. Digestive vacuoles, containing organic matter in



Figures 1–4. Morphology and cytoplasmic fine structure of *D. truncatum*.

(1) Light micrograph of a living *D. truncatum* showing the large axoflagellum (AF) and axopodia (AX) radiating from the extracapsular cytoplasm that surrounds the spongiose skeleton containing the more dense intracapsular cytoplasm. ×145.

(2) A transmission electronmicrograph of a section through the region of a fusule (F) connecting the intracapsular cytoplasm (IC) with the extracapsular cytoplasm (EC) that are separated by a capsular wall (CW). Microalgae (A) occur in vacuoles within the intracapsular cytoplasm. ×10,350.

(3) An overview of the microalgae (A) within intracapsular vacuoles. ×7,200.

(4) Bacteroids (B) occur scattered throughout the intracapsular cytoplasm separate from the microalgae and are suspended within an electron lucent zone in the cytoplasmic lobes. ×48,800.

advanced stages of lysis, were observed scattered throughout the extracapsulum. Only occasional perialgal vacuoles containing microalgae were observed in the extracapsulum in our preparations. The intracapsulum contains a loosely arranged mass of irregularly shaped lobes of cytoplasm (Fig. 3), forming a spongiose pattern in ultrathin sections, containing mitochondria, Golgi bodies, and prominent vacuoles each enclosing a microalga (c. 2.5 to 3.5 μm wide $\times$3.8 to 4.5 μm long). Bacteroids c. 0.2$\times$0.5 μm (Fig. 4) occur within hyaline regions in the cytoplasm within the same locations as the microalgae, though we have not observed bacteroids within the perialgal vacuoles. There is no evidence of a vacuolar membrane surrounding any of the bacteroids, as compared to a very distinct vacuolar membrane surrounding the microalgae (Fig. 3). An electron lucent zone immediately surrounds each bacteroid suspended within the intracapsular cytoplasm.

A higher magnification view of the microalgae (Fig. 5) shows the perinuclear region, juxtanuclear Golgi body, massive reserve bodies, and arrangement of

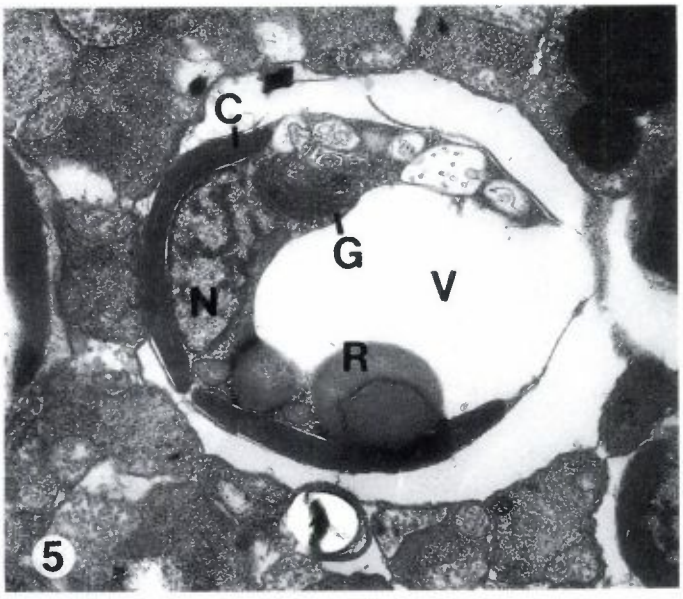


Figure 5. A transmission electronmicrograph of a section through a perialgal, intracapsular vacuole showing the microalga with nucleus (N), plastid lobe (C), Golgi body (G), reserve substance (R) and large vacuole (V). $\times$13,450.

the plastids. There are 7 to 8 lamellae (each, c. 30 nm thick, containing 3 thylakoids) per plastid. A schematic diagram showing the general organization of the microalga, derived from features observed in several micrographs, is presented in Fig. 6. Based on a count of microalgae within representative areas of the central capsular cytoplasm, we estimate a mean density of 4 microalgae per 100 μm^2 of area within an ultrathin section (e.g., Fig. 3).

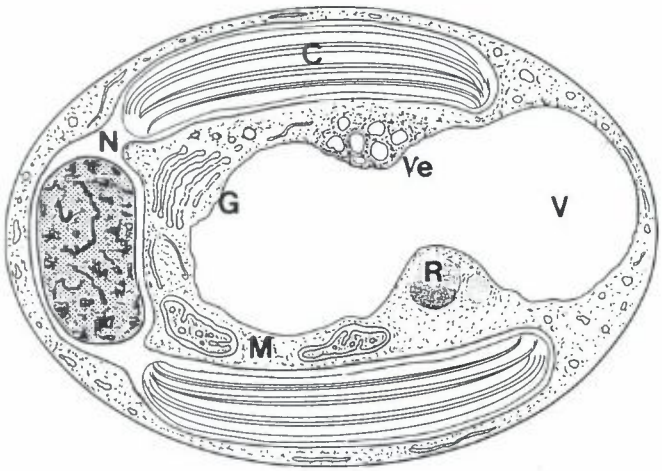


Figure 6. A composite diagram, based on data from several transmission electronmicrographs, showing the arrangement of the major organelles: nucleus (N) within an expanded perinuclear space formed by the cisterna of the nuclear envelope that also encloses the plastid lobes (c). The Golgi body is juxtanuclear outside of the nuclear envelope. The chondriome (M) reserve bodies (R), and vesicles (Ve) within a more granular portion of the cytoplasm occur throughout the cytoplasm surrounding a large vacuole (V). In some cases as shown in Figs. 3 and 5, plastid lobes occur anterior to the nucleus in addition to those distributed around the periphery of the cell. Each lamina of the plastid contains three thylakoids.

4. Discussion

Although radiolarian-algal associations have been known to exist from at least the late 19th century (Haeckel, 1887) and recent fine structural and physiological studies have provided additional evidence for a close structural and functional relationship (Anderson, 1982; Anderson et al., 1983b; 1986b), this is the first report to our knowledge of microalgae occurring within the intracapsular cytoplasm of radiolaria. The fine structure of the microalgae is similar to that of yellow-brown prymnesiad symbionts observed in the extracapsular cytoplasm of larger spongiose radiolaria (Anderson et al.,

1983a). Interestingly, they also resemble endobionts found in the endoplasm of Acantharia (Febvre and Febvre-Chevalier, 1979). The size of the endocytoplasmic microalgae in *D. truncatum* (3.8 to 4.5 μm long) is smaller than those reported by Anderson et al. (1983a) in the larger spongodrymid-type radiolaria (5 to 20 μm). However, there is a close resemblance between the smaller-sized algae in the larger radiolarian and those in *D. truncatum.* Both have 2 to 5 plasmid lobes surrounding the central vacuole, and a prominent Golgi body is located near the nucleus. There are approximately 7 lamellae, each with 3 thylakoids, in each plastid in the smaller microalgae in both radiolarian species. More lamellae were observed in some of the larger forms of the yellow-brown algae in the spongodrymid-type radiolarian (Anderson et al., 1983a). In the spongodrymid-type radiolarian the largest algal cells were 20 μm and contained a very large, ovoid, central vacuole. Apparently, there is some selectivity by *D. truncatum* to favor smaller individuals. Given the limited space in the central capsule which is somewhat smaller than the dimensions of the triangular skeleton (300 μm) and the large number of cytoplasmic organelles (nucleus, Golgi bodies, mitochondria, reserve substances, etc.), it is not surprising that only very small algae occur in the intracapsulum.

Moreover, we do not know how the microalgae become sequestered within the intracapsulum. Unless the fusules can dilate, the microalgae cannot pass from the extracapsulum into the intracapsulum through the fusules with diameter of c. 0.5 to 0.6 μm. The mature axoflagellum, however, is approximately 9 μm in diameter (Fig. 1). The skeletal pore where the axoflagellum emerges is 12 to 15 μm in diameter (Matsuoka, in press). This is sufficiently large to allow the microalgae to enter the central capsule assuming that the axoflagellum has cytoplasmic continuity with the intracapsulum in this species. We have no evidence, however, that the microalgae actually pass through the axoflagellum. Furthermore, additional research is needed to determine the overall morphology of the central capsular wall. There could be one or more major openings that we have not yet detected. Another possibility is that the microalgae are incorporated in the endoplasm at some point very early in the ontogeny of the radiolarian before the central capsular wall is developed, assuming that the capsular wall is a later development. But, we do not know the ontogenetic events during very early stages of development in these radiolaria, so this remains speculative. Axoflagella occur in other spongiose species of radiolaria, but no microalgae have been observed in their central capsules.

This raises the interesting question of why *D. truncatum* includes microalgae in the central capsule, and suggests that additional research is needed to determine how many other species if any possess intracapsular microalgae. It is also important to note that these specimens were fixed in the evening (20:50 hr).

D. truncatum may sequester the microalgae in the intracapsulum during the evening and night, and disperse them into the extracapsulum during the day. Other radiolaria have a diel cycle of distributing the algal symbionts into the peripheral regions of the axopodia during the day and retracting them into the pericapsular region near the outer surface of the capsular wall in the evening and during the night (Anderson, 1982). Algal symbiont-bearing planktonic foraminifera also have such a diel pattern, but many of the symbionts are withdrawn into the intrashell cytoplasm (Anderson and Bé et al., 1977; Anderson, 1984). Further observations are needed on the distribution off the microalgae in *D. truncatum* during a 24 hr period, particularly additional fixations for electron microscopy are needed at intervals throughout the day to determine changes in density if any, of the microalgae within the intracapsulum.

Intracytoplasmic bacteroids have been observed in a wide variety of protista (Lee et al., 1985) including amoebae (Chapman-Andresen and Holter, 1971; Anderson, 1977), ciliates (Kirby, 1941; Ball, 1969; Preer et al., 1974), dinoflagellates (Gold and Pollingher, 1971; Spero and Angel, 1991), kinetoplastid flagellates (Guttman and Eisenmann, 1965), and termite flagellates (Kirby, 1941). The bacteroids found in the cytoplasm of *D. truncatum* do not appear to be parasitic. In spite of the large numbers of these bacteroids, the host cytoplasm appeared to be fully healthy. Beyond the electron lucent region immediately surrounding the bacteroids, the cytoplasm appeared normal, and the intracapsular lobes containing the bacteroids were fully formed and none showed signs of lysis or necrosis. The role of these endobionts remains to be determined. The bacteroids are not enclosed within vacuoles as would be the case if they were being digested, and they consistently occur in the intracapsulum, a region lacking digestive vacuoles (Anderson and Botfield, 1983). Digestive vacuoles were consistently observed in the extracapsulum as is typical of radiolaria (Haeckel, 1887; Anderson, 1983).

The presence of intracapsular endobionts adds an additional qualification to the cytoarchitectural classification pattern devised by Anderson (1984) to describe the organization of sarcodinina protists. There are three classes in this scheme: (1) diffuse organized cells such as amoebae that lack major specialized regions delimited by membranous or skeletal barriers, (2) transitional organized cells as occur in the foraminifera where a shell forms a partial barrier segregating intrashell cytoplasm from the more frothy external cytoplasm, but with continuity through pores or apertures, and (3) zonal organized cells as in the radiolaria where a "permanent" specialized barrier (capsular wall) separates the intracapsulum from extracapsulum. Cytoplasmic continuity occurs through specialized structures such as the fusules, and perhaps the axoflagellar apparatus. Current evidence indicates that zonal organized organisms

such as radiolaria possess very different structural and functional characteristics for the intracapsulum and extracapsulum (Anderson, 1983; Anderson and Botfield, 1983). The research reported here, adds further evidence of differentiation between intracapsular and extracapsular cytoplasmic organization; namely, the intracapsulum in *D. truncatum* consists of a spongiose-like systems of lobes containing mitochondria and Golgi bodies while the extracapsulum is more frothy and vacuolated with digestive vacules. The presence of algal and bacteroid endobionts within the intracapsulum is a new feature and suggests a degree of structural and functional continuity between the two zones not found previously. We do not know to what extent the microalgae move between the intracapsular and extracapsular cytoplasmic compartments, nor if there is a different host-algal relationship when the microalgae are held within the central cytoplasm compared to the peripheral cytoplasm. Additional information is needed on the photosynthetic activity of intracapsular microalgae, their possible role as sources of nutrition for the host, and their fate at the time of radiolarian reproduction. In other radiolarian species examined thus far, the symbionts are ejected during swarmer production and the reproductive swarmers (c. 10 μm diameter) are too small to carry algal symbionts. Their cytoplasm is largely filled by the nucleus and a large strontium sulfate, crystal-containing vacuole (Anderson, 1984; Anderson et al., 1990).

It is interesting to ask if the presence of intracapsular microalgae in *D. truncatum* represent a more primitive or more recent phylogenetic advance for radiolaria. The presence of microalgae within the central capsule increases the complexity of this region compared to those species that do not have intracapsular algae. If this provides a mechanism for protecting the microalgae possibly against predation at night, and if they are symbionts providing a nutritional source for the host, this could represent a more advanced characteristic for this zonal organized organisms. Further examination of other related radiolarian species for the presence of intracapsular microalgae is needed in addition to more detailed observational data on the behavior of the host-algal association in order to assess the phylogenetic significance of the intracapsular endobionts.

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