In: *New Perspectives in Sponge Biology* (K. Rützler, ed.), 533 pp. Smithsonian Institution Press, Washington, D.C., 1990.

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Associations between Caribbean Sponges and Photosynthetic Organisms

Abstract

Five types of associations between sponges and endobiotic autotrophs were discovered in Belize and Bermuda and are currently under study. At least 21 species of common and large shallow-water sponges (genera *Ircinia, Aplysina, Verongula, Cribochalina, Xestospongia, Neofibularia, Ulosa, Geodia, Chondrilla*) contain *Aphanocapsa*-type unicellular blue-green algae (cyanobacteria). One spongiid, *Oligoceras violacea*, is permeated by filaments of a *Phormidium* species, an oscillatorian cyanobacteria. Two species of clionid sponges, *Cliona caribbaea* and *C. varians*, contain dinophycean zooxanthellae. *Mycale laxissima* (Mycalidae), *Igernella notabilis* (Darwinellidae), and at least one unidentified dendroceratid were found to harbor two species of filamentous algae embedded in the spongin fibers of their skeletons; one, *Ostreobium*, is a chlorophyte, the other, *Acrochaetium*, a rhodophyte. Two sponges substitute their skeletons with live calcified red algae. *Dysidea janiae* (Dysideidae) is supported by *Jania adherens*, *Xytopsues osbornensis* (Myxillidae) by *J. capillaria*. All reported cases have parallels in the Mediterranean Sea or in the Indo-Pacific region but are difficult to compare because important new data, on ultrastructure and pigment properties are subject to variables that are not yet fully understood. Observations and experiments provide evidence that both partners generally profit from the symbiosis—the sponges by obtaining food supplements or body support, the plants by gaining nutrients and protection from grazers.

For more than a century biologists have been aware of the association between sponges and photosynthetic organisms (cyanobacteria or Cyanophyta, several groups of eukaryotic algae) (see Feldmann, 1933, and the literature cited therein), but research on the types of relationships between them did not begin in earnest until the 1970s.

Pioneers in the Mediterranean were the Italian and French groups gravitating around M. Sarà and J. Vacelet (see citations in Sarà and Vacelet, 1973:532–537). Subsequently, researchers became interested in the coral reef environments of the Australian Great Barrier Reef (Wilkinson, 1978, 1980, 1983; Larkum et al., 1987), the Red Sea (Wilkinson and Fay, 1979), and New Caledonia (Vacelet, 1981). Until recently, however, few spent much time on the photosynthetic sponge symbionts of the subtropical and tropical Atlantic ocean, except to comment on them in passing in studies on sponge taxonomy (de Laubenfels, 1950:174–175).

This report represents an attempt to fill some of the gaps in our knowledge about sponge symbionts. In it I summarize the results of extensive work in Bermuda and Belize that began in the early 1970s. I hope that the inventory and electron microscope survey of cyanobacterial and algal sponge associates discussed here will provide a data base for ongoing and future ecological and physiological investigations into complex host-symbiont relationships.

Material and Methods

The sponges examined in this study were collected in three types of habitat: shallow coral reef (2–20 m), lagoon (0.5–8.0 m), and mangrove (0–2 m). The localities surveyed were Bermuda, Bimini (Bahamas), St. John (U.S. Virgin Islands), Dominica, Jamaica, and Carrie Bow Cay (Belize), but electron microscope studies were restricted to specimens from Bermuda and Belize. The presence of photosynthetic organisms in the tissues was ascertained in the field by examining fresh microscope preparations and alcohol-soluble (chlorophylls, and others) and water-extractable (phycobilin) pigments.

Material for electron microscopy was fixed in 1.5% glutaraldehyde in 0.2 M cacodylate buffer (with 0.1 M sodium chloride and 0.35 M sucrose), pH 7.2 (2–4 h, 29°C). Postfixation in 1% osmic acid in the same buffer solution (1 h, 4°C) and dehydration to 95% ethanol (5 steps) followed immediately. Where necessary, desilicification was accomplished by adding 5% hydrofluoric acid to the washing solution (buffer) before the final rinse and dehydration. The material was stored in the last (95%) ethanol stop (1–3 weeks) until 100% dehydration and resin embedding (Spurr low viscosity embedding media Polysciences, Inc., Warrington, Pennsylvania) for transmission electron microscopy (TEM). Sections (TEM) were stained with uranyl acetate and photographed by Philips 200 or Zeiss EM9 S–2 microscopes at 1,600–45,000 times primary magnification.

Estimates of tissue proportions (sponge:symbiont) are based on point counts using a Weigel graticule inside the ocular of a light microscope with 100× oil immersion objective and stained sections.

Results

ASSOCIATIONS WITH UNICELLULAR CYANOBACTERIA OF THE *Aphanocapsa feldmanni* TYPE

At least 19 species of the large and common shallow-water Demospongea of the West Indies contain small unicellular cyanobacteria of the *Aphanocapsa feldmanni* type (Feldmann, 1933; Sará and Liaci, 1964). This number constitutes 45% of the 42 conspicuous sponge species examined in Bermuda and Belize. Sponges hosting this type of microorganism belong to the orders Astrophorida (*Geodia*, 2 species), Hadromerida (*Spheciospongia*, 1 species; *Chondrilla*, 1 species), Dictyoceratida (*Ircinia*, 2 species), Verongiida (*Aplysina*, 4 species; *Verongula*, 3 species), Petrosiida (*Cribochalina*, 2 species; *Xestospongia*, 3 species), and Poecilosclerida (*Neofibularia*, 1 species). The cyanobacteria are concentrated in the peripheral regions of their sponge hosts, where they may reach a maximum density of 28% of the cellular tissue volume (*Chondrilla nucula* Schmidt). The average symbiont concentration in the species examined was 10%.

The fine structure of this symbiont (Figure 1) is close to that described for its Mediterranean relative (Sarà, 1971; Vacelet, 1971; Gaino et al., 1977). Most differences can be attributed to variations in fixation techniques or physiological state of the organism at the moment of fixation because they also show up in of our own material.

These cyanobacteria are ovoid and measure 1.1–2.0 μm × 0.6–1.0 μm in median TEM cross sections. Adult cells are always separate from each other. Dividing stages occur in only about 2–4% of the population. Division occurs by median constriction in a plane perpendicular to the longer axis, so that cells form a figure 8 just before the daughter cells separate (Figure 1*a*).

The cell wall exhibits the characteristic four zones of electron density. In many sections it is undulating and separated from the plasmalemma by a clear space of 20–50 nm; this condition is probably a fixation artifact here. A thin sheath (up to 40 μm thick) is present, although it is not clearly discernible in all specimens. The thylakoid consists of a simple flat lamella wound in a spiral around the nucleoplasm, oriented parallel to the longer axis of the cell. This arrangement is best seen in the three-dimensional reconstruction given by Vacelet (1971:fig.1). Each lamella consists of a 10-nm electron-transparent zone sandwiched between two dense layers of equal thickness (30 nm total thickness). There is no vacuolisation, branching, or stacking in the photosynthetic apparatus. The lamellar spiral takes one to two-and-a-half turns; the innermost edge often rejoins the preceding turn. The nucleoplasm occupies about 40–50% of the central cell and displays a characteristic reticulation of fine filaments.

The four most common cytoplasmic inclusions of cyanobacteria could be identified on the electron micrographs of this sponge symbiont. Cyanophycin granules,

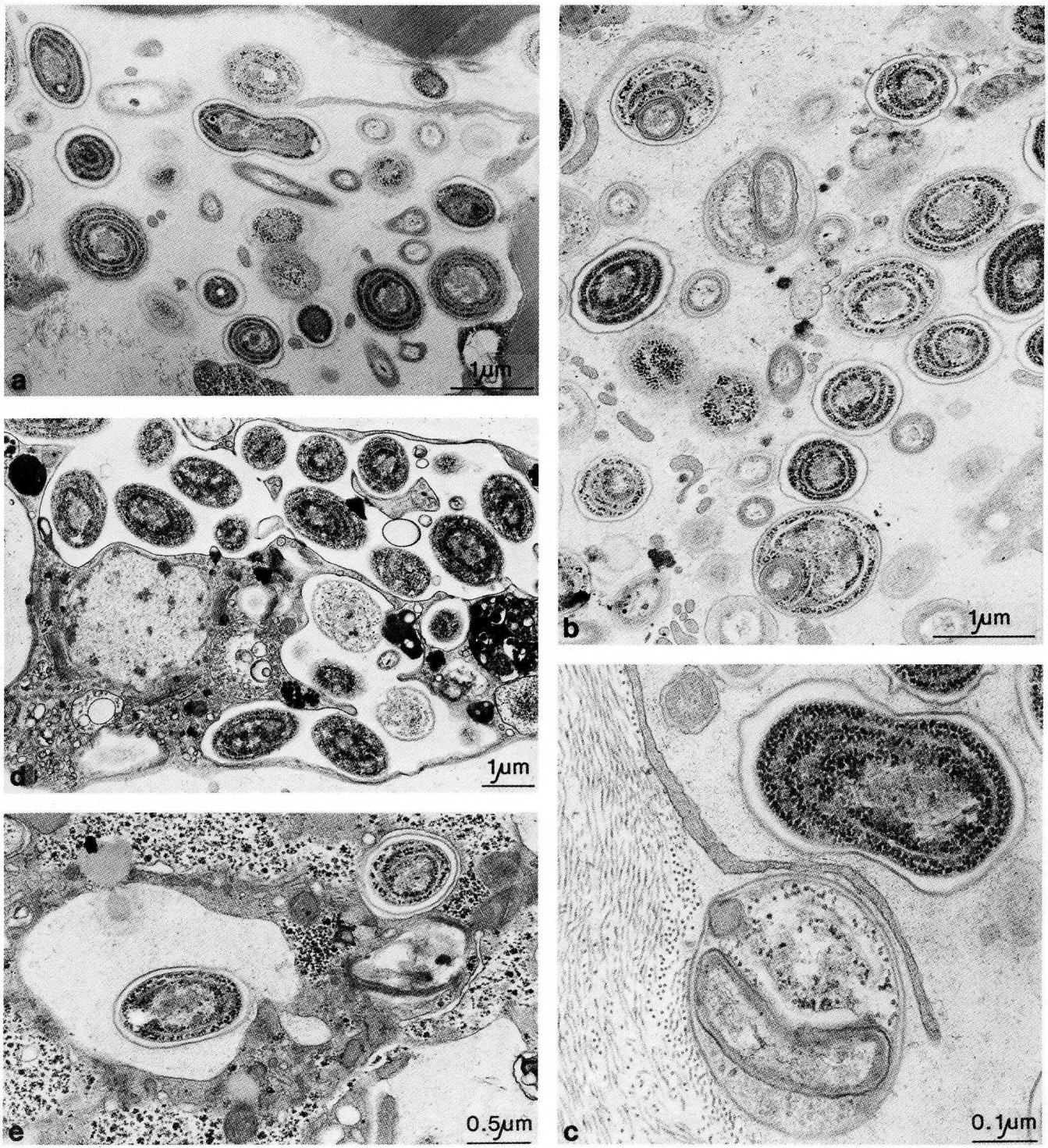

Figure 1. *Aphanocapsa feldmanni*-type cyanobacteria inside sponge hosts: *a–c*, in *Chondrilla* mesohyl; *d*, inside *Cribochalina* bacteriocyte; *e*, in stages of digestion inside *Chondrilla* archaeocyte. Note endoparasitic bacteria in *b* and *c*.

located primarily in the peripheral cell region, are electron dense (black) and measure 60–140 nm in diameter (cross sections). Polyglucan granules are also black, but only 15–20 nm in size. They occur between the lamellae of the thylacoids, often in great numbers. Clear areas 50–120 nm in diameter near the cell poles are presumed to be polyphosphate bodies. Polyhedral bodies are gray in outline, and measure 85–95 nm.

An interesting phenomenon is the endoparasitism of the symbiont by bacteria (Figure 1*b*,*c*) in associates of

Chondrilla nucula, both in the material of Belize as well as Bermuda. The afflicted cyanobacterial cell usually contains one rod-shaped bacterium, 0.6–0.8 μm × 0.3–0.4 μm in size; two bacteria were seen in only one specimen. These bacteria have a simple cell wall, a nucleoplasmic region filling 60–70% of the cell body, and no conspicuous inclusions. They look like many of the bacteria that occur freely in the mesohyle of the sponge. The pathogenic nature of the parasite is obvious from the progressive decay of the cyanobacterial host visible in the electron micrographs.

Cyanobacterial symbionts discussed in this section occur either free among the spongin fibrils of the sponge mesohyl or inside sponge cells. Most of the cyanobacteria are free. Intracellular symbionts are much rarer and occur either singly, inside small vacuoles of archaeocytes, or in groups, along with nonphotosynthetic bacteria, in bubble-shaped cells. In the former, the stages of disintegration indicate that the archaeocytes are digesting the symbiont. In the latter, all cells appear to be healthy and complete. They are inside a large (12–15 μm) bubble "vacuole" formed by a pinacocytelike cell (bacteriocyte). These cells have an ovoid (3.2 × 1.5 μm) anucleolate nucleus surrounded by the cytoplasm of a very small (5.0 × 1.8 μm) cell body that tapers to form a sheet 80–300 nm thick, the bubble wall. The lumen of the bacteriocyte contains not only populations of bacteria and cyanobacteria, but also cytoplasmic strands, apparently branched off the bubble wall, and fibrils of spongin identical to those surrounding the cell.

ASSOCIATIONS WITH UNICELLULAR CYANOBACTERIA OF THE *Aphanocapsa raspaigellae* TYPE

The relatively large blue-green symbiont *Aphanocapsa raspaigellae* has been found in association with sponges in only two cases in the Caribbean. The hosts are two closely related species of the Halichondriida genus *Ulosa.* The symbiont occurs thoughout the sponge body and makes up as much as 50% of the cellular tissue volume. It is never seen inside a sponge cell.

These cyanobacteria are conspicuous because of their bright green color and large (5–9 μm) size. They are spherical and divide by median constriction, and their thylacoids are unusual for their inflated sacs (Figure 2). The pigment composition of this organism is typical for cyanobacteria, although it has considerably less phycobiliprotein than other species in the group. This symbiont is described elsewhere in more detail (Rützler, 1981).

ASSOCIATION WITH A FILAMENTOUS CYANOBACTERIUM

The tissues of the dictyoceratid *Oligoceras violacea* (Duchassaing and Michelotti) are permeated by a filamentous

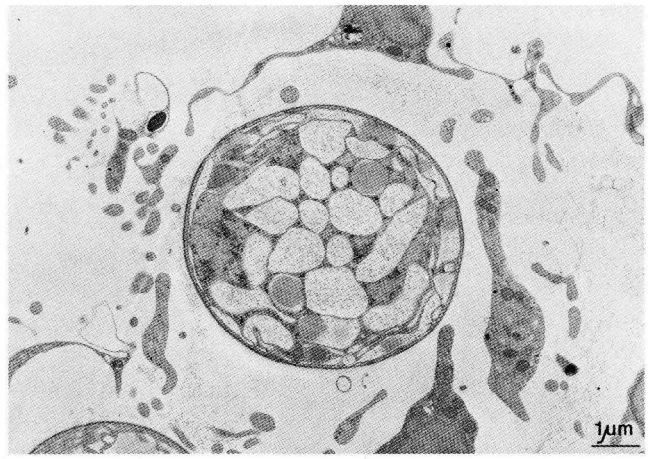

Figure 2. *Aphanocapsa raspaigellae*-type cyanobacterium inside sponge host *Ulosa.*

cyanobacterium (Figure 3). Although it contributes only about 13% to the total sponge volume , this procaryote may make up almost half of the cellular tissue volume in the host owing to the large quantities of sediment particles commonly incorported in *Oligoceras.*

One-cell stages of the symbiont are spherical and 7–8 μm in diameter. After division the daughter cells remain rounded, except for some flattening at the cross walls, and the trichome has the appearance of a string of pearls (Figure 3*a,b*). The average trichome is 40 μm long and is made up of about 10 cells. A 16-cell filament of 63 μm was the longest observed. Cells in a trichome are, on average, 7 μm wide and 4 μm long. The largest cells are usually found in the center of the trichome.

The cell wall (Figure 3*e*) is composed of the usual four layers, which together are about 28 nm thick. The outermost layer (L IV) is slightly undulated and supports a 250–nm sheath displaying three structural zones. The innermost sheath is 70 nm thick and medium dense, with bundles of fibrils oriented perpendicular to the cell wall. The middle layer measures only 10 nm but is very dense, with fibrils intertwined and oriented parallel to the cell wall. The outer zone is less defined than the other two, is 170 nm thick, and is composed of a loose reticulation of fibrils that are more or less prependicular to the cell surface.

The thylakoids are fairly flat and radiate from the central zone of the nucleoplasm toward the cell surface, where they terminate near the plasmalemma (Figure 3*c,d*). The membranes are 8 nm thick and separated by intrathylacoid spaces 20–160 nm (rarely as much as 300 nm) wide. The nucleoplasm occupies nearly 50% of the cell.

Angular dark gray cyanophycin granules (Figure 3*d*), 400–700 nm in diameter, are common in the outer cell region and along the cross walls. Smaller light gray bodies (190 nm) of similar shape occur closer to the inner

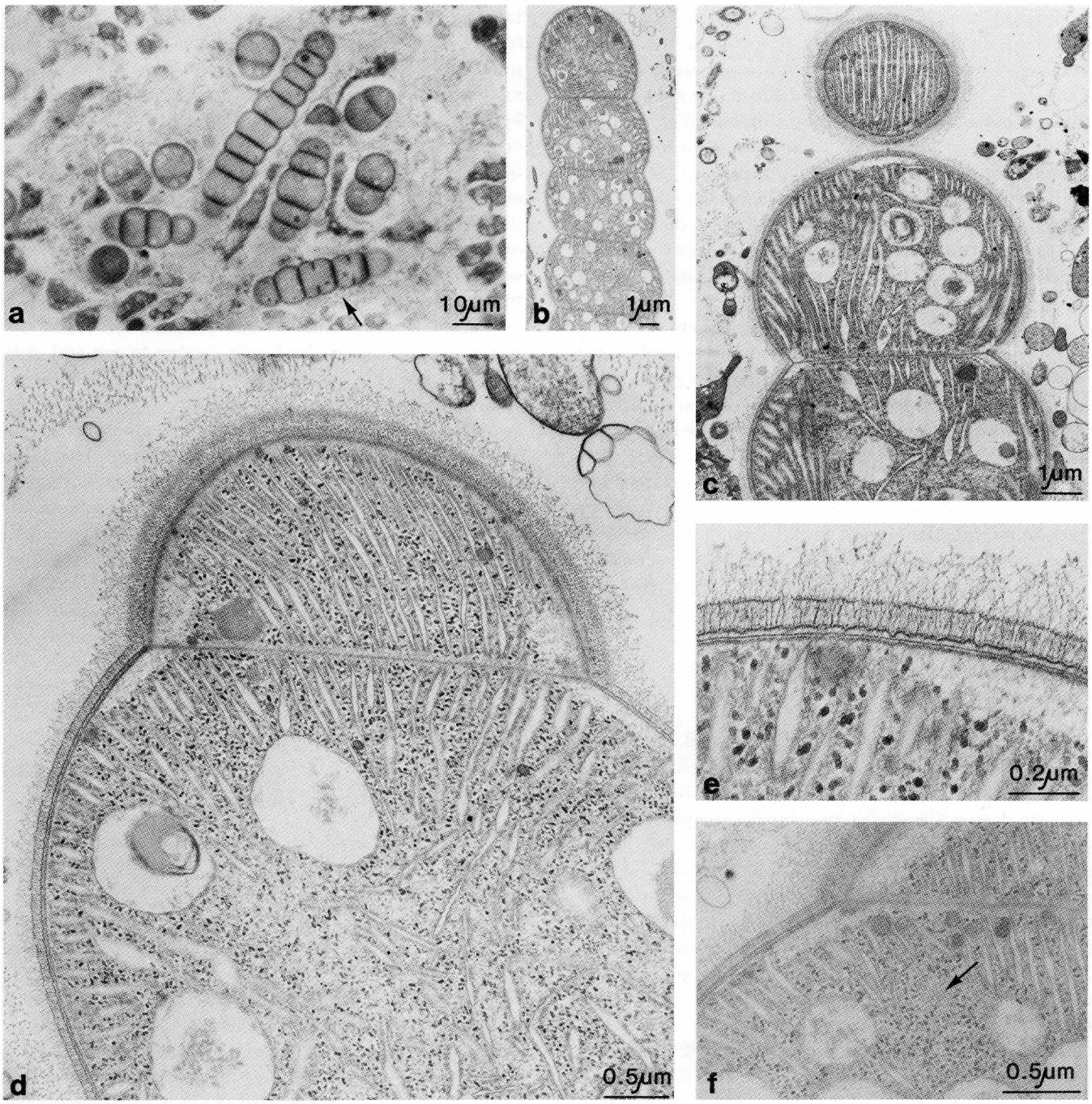

Figure 3. *Phormidium spongeliae*-type cyanobacterium inside sponge host *Oligoceras: a*, light micrograph of filaments in situ, some with dividing cells *(arrow); b*, TEM section of trichome; *c*, thylacoid arrangement in longitudinal section, one cell (top) cut tangentially; *d*, near-median longitudinal section showing cross wall and cell inclusions (note clear space separating symbiont from spongin fibrils, top left); *e*, cell wall and layered sheath; *f*, stellar body *(arrow)*.

cytoplasm. They are interpreted as polyhedral bodies, but they are rare and not as distinctive as in other cyanobacteria examined. Rod-shaped polyglucan granules (50 × 15 nm) are abundant between the thylakoids.

Many large circular clear spaces (4–50 or more per cell section) are present in the cytoplasmic region of each cell (Figure 3*d*). These spaces measure 300–800 nm (average 600 nm) in diameter, have the same electron transparency as the intrathylakoid spaces, and are bound by membranes strongly resembling thylacoid membranes. They were first interpreted as bubblelike inflations of the photosynthetic vesicles, but a connection with a thylacoid was

established in only one of the hundreds of these structures examined. As a rule, thylacoids approaching the bubble vesicle narrow to a point and terminate right at the bubble membrane, just as they reach the plasmalemma near the cell or cross walls. Unlike the intrathylakoid spaces, the lumina of many bubble vesicles contain inclusions, some of which consist of medium gray granules that almost fill the lumen or are smaller, occasionally occur in pairs, and may be with or without fibrous or loosely flocculent material. On some images the granules seem to consist of interwoven fibrils, on others they appear homogenous (Figure 3*d*).

A small number of star-shaped inclusions (260–320 nm) were observed in the peripheral region of only three cells (Figure 3*f*). They consist of a bright central region and about 18 thin electron dense rays. These structures correspond to the stellar bodies described by Berthold et al. (1982).

This filamentous symbiont is always in extracellular position. Occasionally, sponge cells are grouped closely or extend pseudopodia around the symbionts, but no direct interaction with sponge cells was seen. Spongin fibrils of the mesohyle always maintain a some distance from the cyanobacterial filaments (Figure 3d).

ASSOCIATIONS WITH UNICELLULAR EUCARYOTIC ALGAE

Two species or groups of closely related species of greenish-brownish-blackish clionid excavating sponges (Hadromerida, genus *Cliona*, including *Anthosigmella*) are associated with the symbionts commonly referred to as zooxanthellae. The symbionts are concentrated in the outer sponge zones (in the papillae of alpha-stage forms) where they may attain 50% of the cell biomass. Algae from *C. caribbaea* Carter (Figure 4*a*) are morphologically the same as zooxanthellae from *Cliona* species (Vacelet, 1981) and from many other invertebrates, such as protozoans, cnidarians, and mollusks (Taylor, 1968, 1974; Bishop et al., 1976; Deane and O'Brien, 1978; Schoenberg and Trench, 1980; Tripodi and Santisi, 1982). They belong to the dinophycean species *Gymnodinium microadriaticum* (Freudenthal). Fully grown cells are spherical and measure 8–9 μm in diameter, which increases to about 11 × 9 μm just before division by (binary fission). Freshly separated daughter cells are oval, approximately 8 × 6 μm. Zooxanthellae from *Cliona (= Anthosigmella) varians* (Duchassaing and Michelotti) are similar in appearance but smaller, 3.5–4.0 × 4.0–5.0 μm (Figure 4*b,c*). All are intracellular, either fully embedded in a host archaeocyte vacuole or encircled by host cell filopodia.

The zooxanthellae of *Cliona caribbaea* from Bermuda and *C. varians* from Belize have many of the same structural features. Both have a two-membrane (new daughter cells) to five-membrane periplast and a single pyrenoid attached to the chloroplast by one or (rarely) two stems but free of thylakoids (Figure 4*d*). They also have an oval nucleolate nucleus, displaying chromosomes and a double nuclear envelope penetrated by pores, and a prominent branching chloroplast curved along the perimeter of the cell. Both have the usual inclusions, such as accumulation body, ovoid starch grains, and angular calcium oxalate crystals. The two algae differ primarily in the structural details of the nucleus and chloroplast. A section of the *C. caribbaea* symbiont shows nucleus to have 13–25 chromosome sections that are fine-fibrillar, tightly coiled structures, 0.3–0.6 μm in diameter (Figure 4*a*). In the *C. varians* zooxanthella, fewer than 10 chromosomes are visible per nuclear sections and the fibrils appear coarse and only loosely coiled (Figure 4*b*). The chloroplast in the *C. caribbaea* alga is traversed by parallel and closely spaced lamellae of three opposed thylakoids along its entire length and it lacks inclusions (Figure 4*a,d*). The same structure in the *C. varians* symbiont shows lamellar bands of various lengths (0.1–1.6 μm), which occur in loose arrangement and usually in various directions, and in places includes electron-dense granules of unknown origin (Figure 4*b,c*). It should be noted that mitochondria with tubular cristae, Golgi stacks, endoplasmatic reticulum, and some unidentified vesicles occur in both algal forms but are particularly prominent in the *C. varians* symbionts. No flagellae were found, nor evidence such as kinetosomes or certrioles, that would indicate a rudimental flagellar apparatus.

SPONGIN-PERMEATING FILAMENTOUS ALGAE

An unusual association was found in the spongin fiber skeleton of *Mycale laxissima* (Duchassaing and Michelotti) collected on shallow patch reefs in Belize. The rigid fibers here are 300 μm in diameter but fused in places to form 2–3 mm thick strands and are cored by staggered bundles of robust subtylostyle spicules, which occupy 30–50% of the fiber crossection. The fresh fibers are usually almost colorless, in contrast to the deep purple to blackish cellular tissue. At this location, however, most specimens have dark reddish to greenish fibers owing to the filamentous branching algae (at least two types) that are densely intertwined and fully embedded in the spongin strands (Figure 5*a*).

The algae have been identified as *Ostreobium* cf. *constrictum* Lucas (Chlorophyta) in the green fibers (Figure 5*b,d*) and *Acrochaetium spongicolum* Weber-van Bosse (Rhodophyta) in the red (M. J. Wynne, S. Fredricq; pers. comm.). The cells of *Ostreobium* measure 12–15 × 7–13 μm, those of *Acrochaetium* 8–21 × 3–9 μm; the latter are characterized by intercellular pit connections (Figure 5*c*). The filaments of both algae follow the direction of spongin layering of the host skeleton. On electron micrographs there is evidence of physical separation (tearing) of the

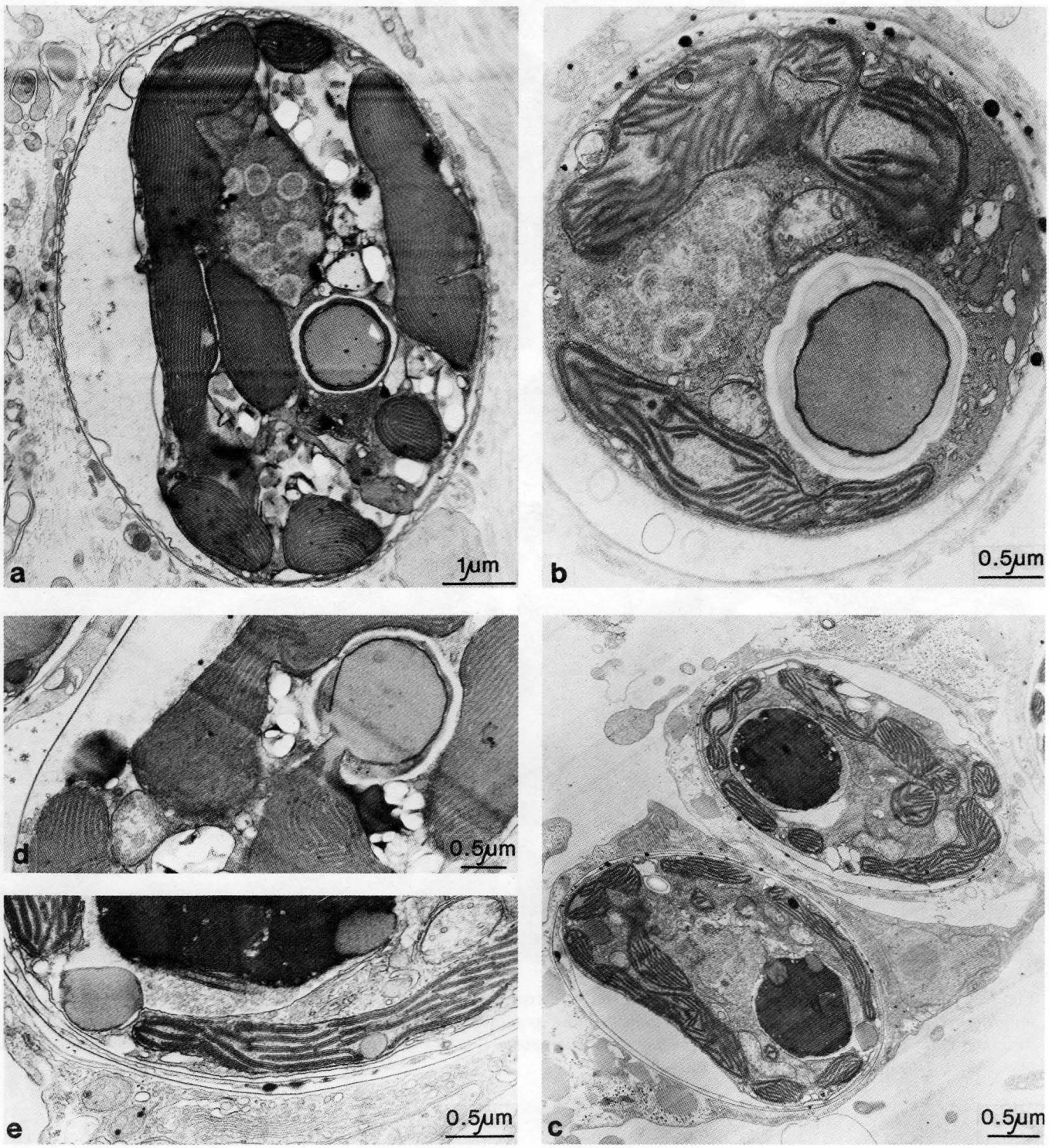

Figure 4. Zooxanthellae, *Gymnodinium*, inside sponge hosts: *a*, *G. microadriaticum* of *Cliona caribbaea; b,c*, *Gymnodinium* sp. of *C. varians* (note sponge cell enveloping recently separated daughter zooxanthellae in *c*); *d*, pyrenoid with stem attached to chloroplast, *G. microadriaticum; e*, chloroplast and accumulation body *(black)*, *Gymnodinium* sp.

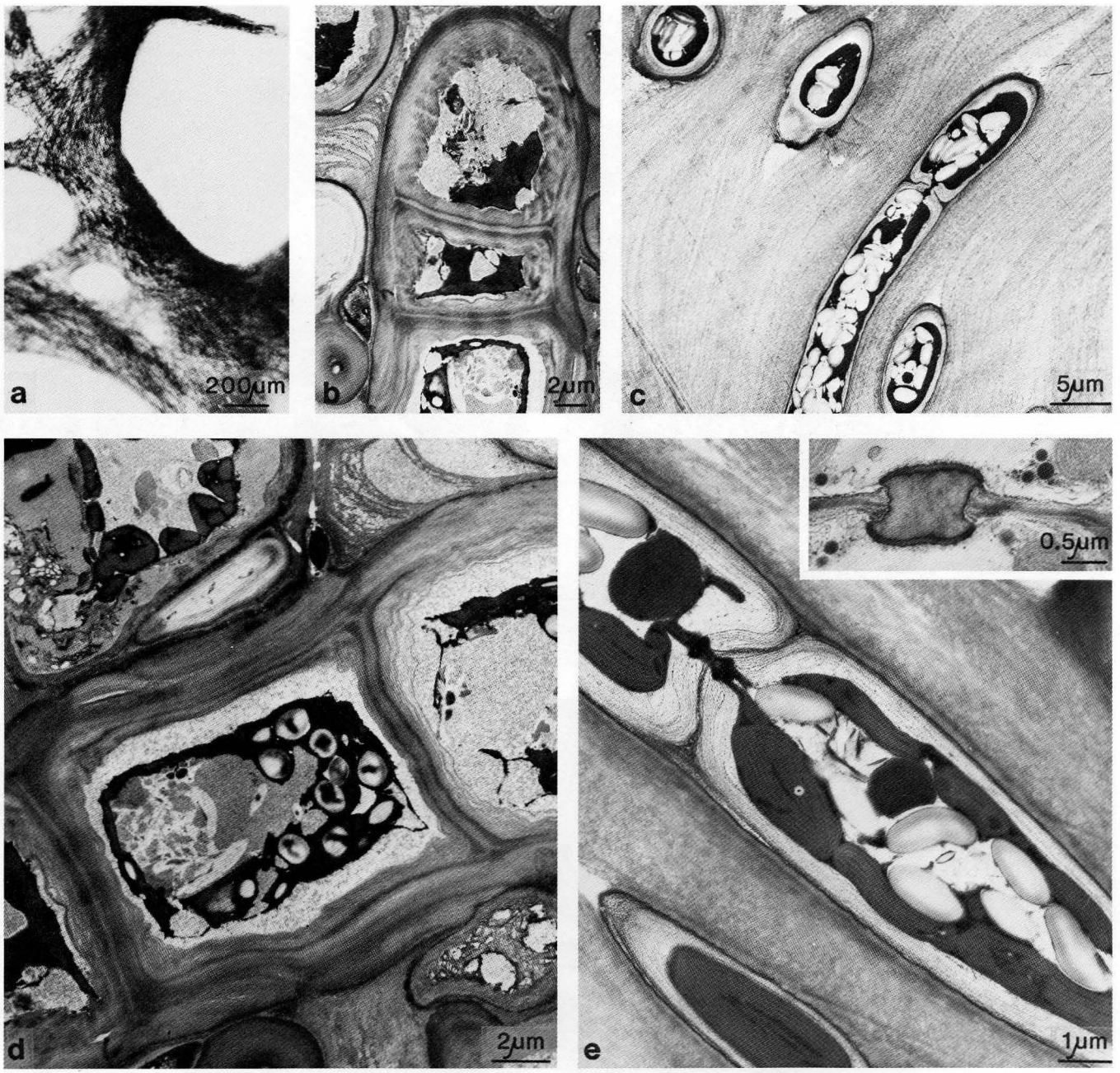

Figure 5. Spongin-permeating algae in *Mycale: a,* light micrograph of isolated spongin fibers riddled by algal filaments; *b,* longitudinal TEM section of filament tip of *Ostreobium* (note spongin layering left of top cell); *c,* sections of *Acrochaetium* embedded in spongin of *Mycale* fiber (note separation of spongin fibrils caused by algae); *d,* enlarged view of *Ostreobium* cell; *e,* enlarged view of *Acrochaetium* cell (insert: intercellular plug).

spongin layers by the growing algae (Figure 5*c*), but none of chemical dissolution (etching). On the other hand, spongin layering around some algal cells (Figure 5*b*) suggests that spongin was deposited onto the cell walls during simultaneous growth of sponge and alga, which is possible at the distal tips of the fibers that cause the conules on the sponge surface.

ALGAE AS SKELETAL SUPPORT

Two sponge species from the (sub-) tropical western Atlantic are known to substitute or reinforce their own skeleton by association with algae, the calcified Rhodophyta genus *Jania* (Figure 6). Both were found and studied in Bermuda. *Dysidea janiae* (Duchassaing and Michelotti) is

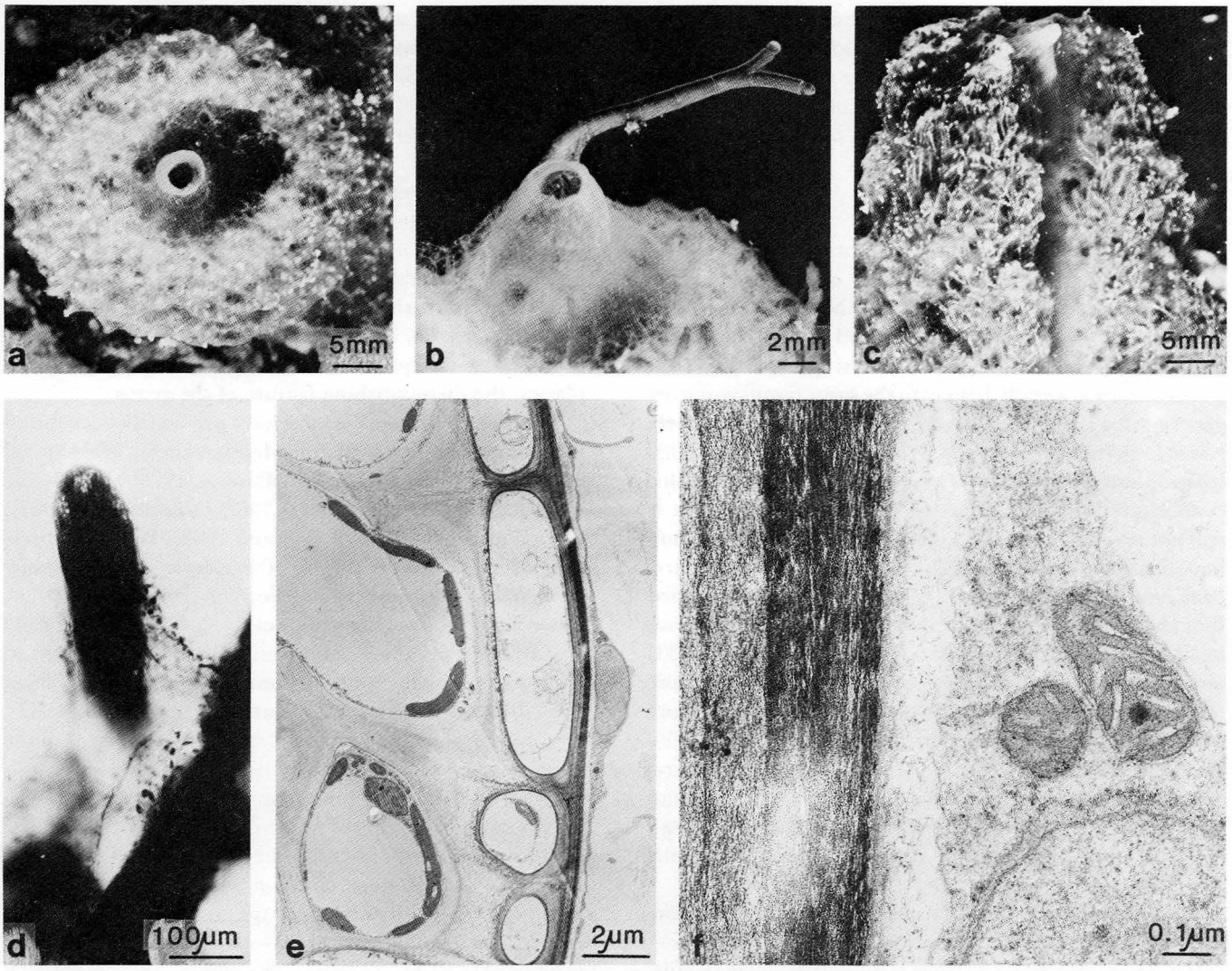

Figure 6. *Jania* as skeletal support: *a*, in *Dysidea janiae*, view of sponge chimney from above (osculum in center), algal growth tips appear as white dots; *b*, similar view as in *a* but one algal branch protruding from sponge; *c*, cutaway view of *Jania*-supported chimney of *Xystopsues* (principal exhalent canal in center leading to collapsed oscular cone on top); *d*, light micrograph of *Jania* branch enveloped by loosely organized cellular tissue of *Dysidea; e*, TEM cross section through *Jania* branch (chloroplasts and nucleus inside cells to left) coated by *Dysidea* pinacocyte *(right); f*, spongin-a fibrils in clear space between outer wall of *Jania* (blackish, striated), left, and *Dysidea* pinacocyte (with two mitochondria), right.

a keratose (Dictyoceratida) sponge that incorporates *J. adherens* Lamouroux instead of developing characteristic sediment-charged skeleton fibers. *Xytopsues osburnensis* (George and Wilson) is a myxillid (Poecilosclerida) full of the symbiont *J. capillacea* Harvey (W. Johansen, pers. comm.).

Dysidea janiae forms mounds or tubes 1–3 cm in diameter and 1–7 cm tall. The tissue is grayish tan but translucent, so the sponge takes the color of the underlying *Jania* algae, which varies from purplish red at the growth tips to greenish and whitish toward the base, which is often dead. The erect, branching algae have an average diameter of 100 µm. They constitute 60% or more of the sponge body and are fully contained by it. In other words, the sponge determines the shape of this compound organism, and algal growth stops just below the level of the ectopinacoderm; very rarely does a single algal branch break this pattern and protrude beyond the sponge surface. Sponge cells attach directly to the surface of algal branches, except for a 200 nm space filled with collagen (spongin-a) fibrils. None of the typical sand-filled spongin-b fibers of *Dysidea* were seen in the sections. This sponge grows among *Jania* turfs and has not been found without the endozoic algae in Bermuda or elsewhere.

Xytopsues osburnensis forms irregular masses 5–10 cm in diameter. Color, anatomy, and histology are much the same as in *Dysidea*, except that the *Jania* branches are more brownish red and slightly thinner (80 µm in diameter),

and the sponge develops its typical fiber strands with embedded spicules and sand grains. Judging from its pigmentation, *J. capillacea* seems healthier and more vigorous in its endozoic state than *J. adherens* of *Dysidea,* which is generally alive only in the distal part of its branches.

Discussion

Small (3 μm and less) ovoid chroococcoids are the most common photosynthetic associates of sponges in the Caribbean, just as they are in the Mediterranean Sea (Sará, 1966, 1971; Vacelet, 1971) and the Pacific Ocean (Wilkinson, 1978; Vacelet, 1981). Host sponges in the different oceans generally belong to the same families or even genera—for instance, *Chondrilla, Ircinia, Aplysina,* closely related petrosiids *(Petrosia, Calyx, Cribochalina, Xestospongia),* and *Neofibularia.* The systematic position of this cyanobacterium was discussed by Vacelet (1971) but is still not resolved because taxonomic revisions of the group have not taken into account the details of its fine structure. Sará and Liaci (1964) and several other authors assumed that the organism is identical with that from Mediterranean *Ircinia* and *Petrosia,* as described by Feldmann (1933) and therefore named it *Aphanocapsa feldmanni* Fremy. More recently, *Cyanothece* Komarek has been suggested as a more suitable generic allocation (Lafargue and Duclaux, 1979). Bacterial infection of symbiotic cyanobacteria has previously been observed in the host sponges *Neofibularia* and *Jaspis* from the Great Barrier Reef (Wilkinson, 1979a). This parasite is comparable in structure and size to the Australian organism classified as a bdellovibrio.

Large (5 μm or more), spherical, unicellular cyanobacteria from sponges are much less common than the *feldmanni* types, and published descriptions give few details. TEM micrographs were only taken on tissues of the Mediterranean keratose sponges *Ircinia* (Sarà, 1971) and *Aplysilla* (Duclaux, 1972). A Caribbean symbiont of this type described by Rützler (1981) is quite similar in size and structure but constitues as much as half of the biomass of the host sponge *Ulosa* (possibly better classified as genus *Dictyonella;* van Soest, pers. comm.). Another Caribbean cyanobacterial symbiont, described by Lafargue and Duclaux (1979) as *Synechocystis trididemni* from a didemnid ascidean, may be conspecific, but published TEM photomicrographs do not allow a positive identification. There may be more than one cyanobacterial species involved, although different fixation and processing techniques could account for the differences in structural details. On the other hand, we have evidence (Rützler and Muzik, unpublished) that crudely fixed and stored material may still retain taxonomically important details of the fine structure. Large (2-cm) chunks of coral rock coated by an undescribed encrusting sponge, *Terpios* sp., from Japan were fixed and stored in 2% cacodylate-buffered glutaraldehyde for more than three weeks before TEM processing. Even so, the TEM images show cyanobacteria structurally undistinguishable from the *Ulosa* symbiont in the Caribbean. Similarly, Cox et al. (1985) show TEM images of a symbiont from Great Barrier ascidians and sponges that closely resemble those of the *Ulosa* associates and identify it as *S. trididemni.* Again, without modern revisions, one needs to be careful about the use of taxonomic designations. This kind of sponge symbiont was first described as *Aphanocapsa raspaigellae* (Hauck) by Feldmann (1933). Lafargue and Duclaux (1979) would use the genus *Synechocystis* Sauvageau for this species, and for their new *S. trididemni,* although they admit to never having observed subsequent cell division in perpendicular planes, the distinguishing feature of the genus.

Filamentous cyanobacteria have previously been found as sponge symbionts in the Mediterranean (Feldmann, 1933, Sarà, 1966), Red Sea (Wilkinson, 1979b), the Pacific (Vacelet, 1981; Berthold et al., 1982; Larkum et al., 1987), as well as the Caribbean (Wilson, 1902). Wilson's observations were made on his *Cacospongia (= Oligoceras) spongeliformis,* a species close to *Oligoceras violacea* (= *O. hemorrhages* de Laubenfels of authors). Only *Dysidea herbacea* Keller from the Pacific seems to harbor symbiont in close to the great numbers numbers found in *O. violacea* (30–40% of the sponge cell volume; Berthold et al., 1982). The systematic postion of the cyanobacterium is, as in the cases above, still open to question. *Phormidium* or *Oscillatoria spongeliae* (Schulze) have often been used in the literature because this organism was first observed in the 1870s in Mediterranian *Spongelia (= Dysidea).* The generic allocation varied, depending on whether the author detetected a sheath coating the trichome. However, other important taxonomic criteria—such as the number of cells per trichome, trichome branching, and presence of hormogonia and necridia—vary from one author to another, which suggests that different species may be involved. Morphologically, even with respect to the fine structure, the *O. violacea* symbiont compares best with recently described Pacific forms (Vacelet, 1981; Berthold, 1982; Larkum et al., 1987). The most significant difference seems to be that the Caribbean organism displays a distinctive, layered sheath.

It is curious that the classical "zooxanthellae" known to be common symbionts of many carbonate-secreting organisms such as corals occur only with limestone-excavating sponges, the clionids. This phenomenon was recently discussed by Vacelet (1981), who also noted that the zooxanthellae-bearing Pacific sponge *Spirastrella inconstans* should actually be classified as *Cliona.* This is a parallel case to our present observations on *Anthosigmella varians,* which has always been considered a spirastrellid. The delicate type and relative rarity of microscleres and the boring habit of early stages suggest that this sponge is a typical member of Clionidae. Observations on the fine structure of the *C. caribbaea* symbiont *Gymnodinium micro-*

adriaticum agree with Vacelet's (1981) findings for Pacific sponges. In particularly, the algae are alway located in intracellular postion, the chloroplast is denser than in cnidarians or in *Tridacna*, and flagellar structures are never seen. Structural differences in the nucleus and chloroplast of *G. microadriaticum* and the symbiont of *C. varians* are significant and indicate systematic differences on the species level or above. Some features of *G. simplex* (Lohmann) in TEM photomicrographs published by Dodge (1974) are similar to *C. varians* zooxanthellae, particularly the chloroplast structure, but the former is only known as a free-living form. The isolated alga will have to be cultured and more detailed taxomonic study before systematic relationships can be clarified.

It is remarkable to find algae flourishing inside the spongin skeleton of a densely pigmented, dark purple sponge such as *Mycale laxissima* at a water depth exceeding 5 meters. However, the body of this tubular species is fairly thin (10 to 20 mm), and light can reach the skeleton from both the outer and inner (atrial) surfaces. Both algae, as well as some close relatives, have been reported from similar cryptic habitats and even from sponge fibers. At least six *Ostreobium* species are known to occur as boring or endolithic algae in limestone substrates such as corals, mollusk shells, and calcified algae (Lukas, 1974). One member of the genus was once found with *Acrochaetium spongicolum* inside the skeleton of an unidentified keratose sponge in the Pacific (Weber-van Bosse, 1921). The photosynthetic efficiency of one of these algae (endolithic in the coral *Porites* in Hawai) was found to reach its peak at light levels between 10 and 100 Lux (Franzisket, 1968). Illumination at the main growth site of the alga, 10 mm below the coral surface, was measured at 15 Lux, which was only 0.03% of ambient light at the habitat of the host coral in a depth of 2 m. *Acrochaetium spongicolum* was first described from a keratose sponge fiber, as mentioned above. A similar form *(Rodochorton)* was subsequently reported from the skeletons of Mediterranean Keratosa, genera *Spongia, Cacospongia, Dysidea*, and *Aplysilla* (Sarà, 1966). Among our own samples that were not studied by TEM are *Acrochaetium*, such as *Igernella* from Belize, *Pleraplysilla* from Bermuda, *Niphates* from the U.S. Virgin Islands, and *Callyspongia* from the eastern Pacific (Chile; courtesy R. Desqueyroux). Some authors consider *Audouinella* to be the principal genus of this complex of filamentous Rhodophyta, but taxonomic studies are still inconclusive (Woelkerling, 1983).

Both cases of symbiosis with *Jania* algae were previously described from Bermuda (de Laubenfels, 1950: *Dysidea* as *D. fragilis* f. *algafera*, p. 22; *Xytopsues* as *X. griseus*, p. 75). *Dysidea janiae* is a species characterized by its obligatory relationship with *Jania*, and not just an algal-infested form, as de Laubenfels (1950) assumed. On the other hand, *Xytopsues osburnensis*, is known from its type locality off North Carolina as a thin encrustation without algal symbionts and with proper skeleton fibers filled with sand grains (George and Wilson, 1919). Similar associations have been described from Pacific waters (e.g., *Gellius-Ceratodictyon*: Vacelet, 1981; Price et al., 1984), but there the algae determine the shape of the compound organism, whereas in our case the opposite occurs. It can be assumed that microclimatic conditions inside the sponge, a balance of growth-stimulating and impeding factors, control algal growth, but no details of such mechanisms are known. *Jania* is obviously capable of living without a host, unlike the Pacific *Ceratodictyon* symbiont, which is unable to survive by itself (Price et al., 1984).

Conclusions

Symbioses between shallow-water sponges and photosynthetic organisms are just as common and varied in the Caribbean as in other tropical and subtropical seas. More experimental work is needed to determine the benefits of the symbiotic life style for each partner, but numerous studies confirm that sponges receive phototrophically produced metabolic energy from associated cyanobacteria or algae (Vacelet, 1971; Wilkinson, 1979b; Wilkinson and Fay, 1979; Rützler, 1981; Wilkinson, 1983) and that phototrophic symbionts use animal waste for nutrients and a habitat protected from grazers (de Laubenfels, 1950; Price et al., 1984). An ecologicaly less significant but curious phenomenon is the skeleton-support function provided by certain algae. An interesting question is whether any of these associations could be harmful to one partner or could lead to parasitism. In one case under study, chroococcoid symbionts are able to damage the *Geodia* host tissue (Rützler, 1988). In addition, spongin-boring filamentous algae as seen in *Mycale laxissima* fibers could be considered parasitic, as they doubtlessly damage the skeleton needed to support this sponge.

Acknowledgments

Algal identifications and valuable suggestions were provided by M. J. Wynne, S. Fredricq, and W. Johansen. I thank P. Riordan and C. Rützler for TEM sectioning, R. Summers for TEM instructions, and K. Smith for field assistance and darkroom work. The Bermuda Biological Station and Philips Company provided invaluable assistance by allowing me to use their facilities. Financial support for this study was provided by the Smithsonian Fluid Research Fund, the Smithsonian Research Opportunities Funds, and a grant from the Exxon Corporation (for work in Belize). This is contribution no. 178, Caribbean Coral Reef Ecosystems (CCRE) program, National Museum of Natural History, Washington, D.C.

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