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The Black Band Disease of Atlantic Reef Corals.

I. Description of the Cyanophyte Pathogen

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With 8 figures and 1 table

Key words: Coral pathogen, *Cyanophyta, Cyanobacteria, Scleractinia, Phormidium corallyticum,* morphology, finestructure, pigments, systematics, new species.

Abstract. The cyanophyte *(Cyanobacterium)* that causes black band disease of Atlantic reef corals is described under the name *Phormidium corallyticum,* new species (family *Oscillatoriaceae*), and its generic placement is discussed from the standpoint of the GEITLERian (classical) and DROUET systems. Distinguishing characters include densely interwoven filaments that form a blackish mat and trichomes without significant cell wall constrictions, almost isodiametric cells (4.2 µm mean width, 4.0 µm mean length) tapering end cells, and thin (0.1 µm or less) mucilaginous wall coating. Transmission electron microscopy shows typical cyanophyte cell walls, sheath, nucleoplasm, and cytoplasmic inclusions, but an unusual thylakoid of straight, and, as seen in cell cross section, radiating lamellae. The dark coloration is due to a high concentration of phycocyanin and some phycoerythrin. The species is similar to *Oscillatoria (= Phormidium) submembranacea,* which differs in several morphological features and does not infect coral tissue. It is concluded that *Phormidium* should be used for this and related species that have external mucilage but not the distinct sheath found in *Lyngbya.*

Problem

Black band (or black line) disease affects many Atlantic reef corals, particularly of the *Scleractinia* genera *Montastrea* and *Diploria* (ANTONIUS, 1981). This common and widely distributed disorder was named for the darkly pigmented, interwoven filaments of a bluegreen alga *(Cyanobacterium)* that characteristically form a band (5–30 mm wide) separating live coral tissue from recently exposed, brilliant white skeleton (Fig. 1). Observations of this phenomenon by other workers are summarized in an ecological study of the black band disease by RÜTZLER *et al.,* 1983.

It has long been suspected that the blackish cyanophyte identified as *Oscillatoria submembranacea* ARDISSONE & STRAFFORELLO is not only associated with the disease but actually causes it (ANTONIUS, 1973; identification of the organism by D. L. TAYLOR; A. ANTONIUS, pers. comm.). Our experimental work at

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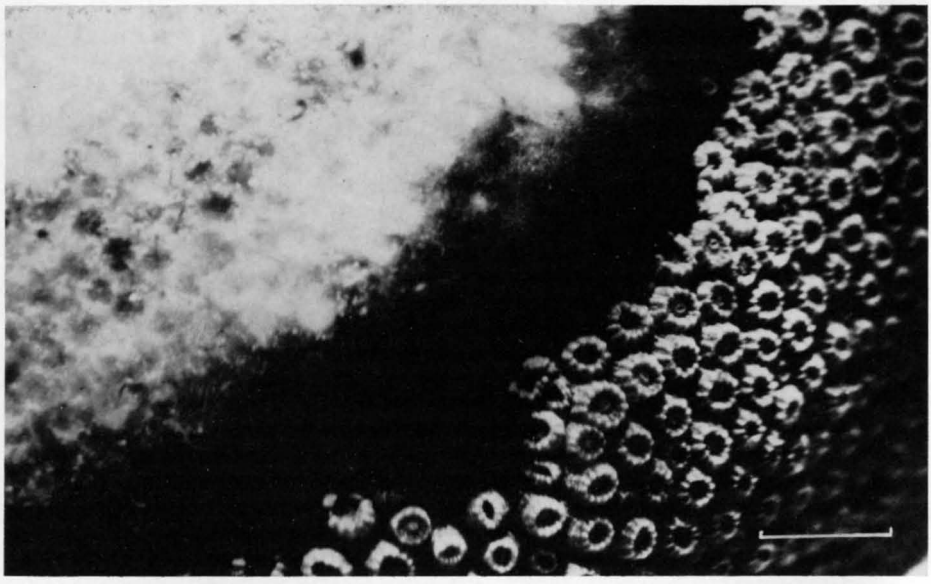

Fig. 1. Black band formed by intermeshed *Phormidium corallyticum* filaments advancing on *Montastrea annularis* (Belize); live polyps of the coral are seen to the lower right, recently exposed coral rock to the upper left. (Scale = 10 mm).

Carrie Bow Cay, Belize, and in Bermuda supports this earlier assumption but also raises some new ideas about the systematic status of the disease agent (RÜTZLER *et al.*, 1982). Although *O. submembranacea* (also reported as *Phormidium submembranaceum*) is recognized by both the classical and the DROUET systems (GEITLER, 1932; DROUET, 1968) and another specialist considers *Phormidium submembranaceum* the best suited available binomial (A. B. WHITTON, *in lit.*), most features of our pathogen do not correspond well with that diagnosis. Furthermore, we have collected an intertidal black cyanophyte at the Carrie Bow Cay study site (RÜTZLER & FERRARIS, 1982) – only tens of meters away from black band diseased corals – that was identified as *Oscillatoria submembranacea* (S. BRAWLEY, pers. comm.) and that agrees well with the published diagnosis. Our attempts to infect corals with the intertidal filaments was unsuccessful and confirmed our suspicion that the black band cyanophyte is taxonomically different.

After some hesitation – in view of the confusion over the systematic status of *Cyanophyta* – we decided to establish a new species because the organism has stable characters that are distinct from those described in related taxa and because it has a very unusual mode of life. Our description includes quantitative data, finestructure observations, and detailed illustrations to facilitate future revisional work, such as numerical taxonomic studies, proposed some time ago (GOLUBIĆ, 1969; WHITTON, 1969); such work should not rely on the subjective interpretations found in most of the literature.

Part I of our study of black band disease examines the systematic position of the new pathogen by means of traditional phycolocial methods as well as

electron microscopy. Parts II and III (TAYLOR, 1983; RÜTZLER *et al.*, 1983) will introduce complementary characteristics revealed by ecological observations, field and laboratory experiments and culture attempts.

Material and Methods

Tenths of black band samples were taken, observed alive, cultured under various conditions, and fixed or frozen for later study. Locations included shallow (1–3 m) back reef areas and patch reefs near Carrie Bow Cay and Curlew Bank (16°48'N, 88°05'W), Belize (RÜTZLER & MACINTYRE, 1982), and similar reef areas (2–4 m deep) north and east of St. George's, Bermuda, site of the Bermuda Biological Station. Samples were collected in May or June of 1980, 1981, and 1982 in Belize, and July of 1981, 1982 in Bermuda; one small, inactive tuft of the alga was collected in Bermuda in January 1982.

Live and fixed material was viewed and measured by light microscope (100 × oil immersion, regular or phase contrast). Fixation media for light microscopy included 4 % hexamine buffered formalin-seawater (pH 7.0–7.5) and 3.5 % glutaraldehyde followed by 1 % osmium tetroxide (see below). Stains used were 1 % aqueous anilin blue, 1 % aqueous methylene blue (for sheath detection, in particular), and Gram's iodine (iodine saturated in 1 % aqueous potassium iodite). Semipermanent slide mounts were embedded in 70 % corn syrup.

Material for electron microscopy was fixed in 3 % glutaraldehyde in 0.1 M phosphate buffer with the addition of 0.45 M sucrose (90 min. at 29 °C) and post-fixed in 1 % osmium tetroxide in the same buffer-sucrose mixture (60 min. at 29 °C). Fixation was immediately followed by dehydration in an ethanol series to 95 %, and the samples were then stored and transported to the laboratory from the field. Dehydration was completed ten days after fixation and the material embedded in Epon 812 resin. Sections were stained with uranyl acetate and examined and photographed through a Zeiss electron microscope EM 9 S–2.

Specimens for scanning electron microscopy (SEM) were fixed by the above EM technique, dehydrated in an ethyl alcohol series, and critical point dried (in liquid CO_2); they were coated by 20 nm of gold and photographed by a Cambridge Stereoscan Mark II A. Samples for pigment analysis were frozen and analysed by spectrophotometry in the laboratory of E. GANTT, Smithsonian Radiation Biology Laboratory. Experimental methods examining responses to salinity, light, temperature, nutrients, and antibiotics are outlined in another paper of this series (RÜTZLER *et al.*, 1983).

Results

1. Systematic position

a. Genus *Phormidium* KÜTZING

The family *Oscillatoriaceae,* order *Hormogonales,* includes filamentous blue-green algae lacking heterocysts and trichome ("true") branching. *Phormidium* is a typical representative with the following characteristics: Plants forming felt-like layers over the substrate; trichomes cylindrical, flexed but never spiraled; sheaths always present but thin, watery mucuous, never branched, at times confluent; end cells commonly tapering, outer membrane in some species thickened (GEITLER, 1932; COKE, 1967; HUMM & WICKS, 1980).

On the basis of its sheath, *Phormidium* is intermediate between *Oscillatoria,* without sheath, and *Lyngbya,* with distinct sheath (see discussion for proper definitions). BOURRELLY (1970), a modern proponent of the "classical" *Cyanophyta* system, follows THURET (1875) by including *Phormidium* in the

genus *Lyngbya*. We find, however, that *Phormidium* is a valid and useful taxon as long as it is properly defined.

b. *Phormidium corallyticum*, new species (Figs. 1–4)

Diagnosis. *Trichoma cylindricum non ramosum, atro-brunneum, 0.5–2.0 mm long.; vagina aquosa, gracilis sed definita (10–100 nm); cellulae 4.0–4.5 (4.2) µm lat., 2.5–5.0 (4.0) µm long. (2.5 µm post divisionem; 5.0 µm ante divisionem); cellula apicalis conica parum flexaque, sine calyptra; protoplasma homogeneum; filum non constrictum.*

Blackish brown, cylindrical, unbranched trichomes coated by a thin (10–100 nm) mucilaginous layer. Fast gliding movement with reversible polarity and oscillating free end but not rotation. One end cell tapering, the opposite one hemispherical; less commonly, both end cells hemispherical; rarely, both end cells tapering. Tip attenuation over 1–3 cells. Without thickened end walls, pronounced cross wall constrictions, or light microscopically conspicuous granules. Thylakoid membranes radiating outward from nucleoplasm, perpendicular to both outer cell wall and cross walls. Measurements (live): 0.5–2.0 mm trichome length; 4.0–4.5 (4.2) µm trichome (cell) width; 2.5–5.0 (4.0) µm cell length (2.5 µm after division, 5.0 µm before division). Width to length ratio (live mean) is 1.1 (Table 1).

Table 1. Cell measurements (µm) for samples of *Phormidium corallyticum;* means and standard error are given where 5 or more measurements (most commonly 10) are available (– = no data). Abbreviations are used to indicate localities (BDA = Bermuda, CBC = Carrie Bow Cay), preparation of material (L = live; F = formalin fixed, observed in water; EM = glutaraldehyde-osmic acid fixed, critical point dried), and method of measurement (LM = light microscope, SEM = scanning electron microscope); double cells are incomplete dividing stages (not included in the width : length index).

Sample No.;	Method	Width (all except end cell)			Lengh							Width: length ratio
					single cell			double cell			end cell	
		range	mean	±s.e.	range	mean	±s.e.	range	mean	±s.e.	range	
BDA 82.01;	L, LM	4.0–4.5	4.24	0.07	3.1–5.0	3.93	0.11	5.0	5.00	–	4.0–7.0	1.1
CBC 82.05;	L, LM	4.0–4.5	4.20	0.12	2.5–5.0	4.00	0.21	5.0	5.00	–	5.0–7.5	1.1
*BDA 81.08A;	F, LM	4.0–4.5	4.21	0.05	2.2–3.5	3.01	0.16	4.0–5.0	4.50	0.10	–	1.4
BDA 81.08B;	F, LM	3.5–4.5	3.95	0.12	2.3–4.0	3.20	0.11	–	–	–	3.5–9.0	1.2
CBC 80.AA;	F, LM	3.8–4.3	4.12	0.05	2.5–4.0	3.07	0.16	4.0–4.5	4.30	0.10	4.0–5.0	1.3
CBC 81.05;	F, LM	3.6–4.0	3.92	0.04	2.5–3.0	2.68	0.11	–	–	–	–	1.5
CBC 81–EM5;	EM,SEM	2.3–4.8	3.93	0.10	0.8–3.8	2.42	0.08	1.8–5.0	3.53	0.19	3.0–10.0	1.6
CBC 81–EM11;	EM,SEM	3.5–4.6	4.15	0.09	2.0–3.3	2.67	0.05	3.0–4.5	3.49	0.16	5.0–10.0	1.6
CBC 80–EMC;	EM,LM	3.5–3.8	3.71	0.05	3.0–3.8	3.48	0.10	3.8–4.5	4.02	0.10	6.0	1.1

* Holotype

Description. Single *Phormidium* filaments are best studied by letting a small tuft of the organisms spread out on a microscope slide, under a cover slip supported by wax feet (Fig. 2). The cylindrical, unbranched trichomes are blackish brown and are covered by a thin sheath that can only be discerned by staining (methylene blue). One end cell of a trichome is usually tapering, the other one

hemispherical (Figs. 2; 3; 4 a–c); however, two pointed or two hemispherical ends also occur. Tapering of attenuated tips is restricted mainly to the apical cell (Figs. 2, 3), but it can also take place gradually over two, three, or rarely four cells (Fig. 4 c). A tapering elongate apical cell can flex in all directions but never changes to hemispherical shape. Filaments appear to move preferentially towards the pointed end but direction is readily reversed and there are no obvious differences in gliding performance that could be correlated with apical cell shape.

Live filaments are typically 0.5–2.0 mm long, but fragmented trichomes of only 3–20 cells are quite common during the stressed condition of microscope examination. The filaments are difficult to measure because they are densely intertwined. Teasing and gradual desiccation help to isolate them but also promote fragmentation; desiccation, increased salinity, and oxygen depletion cause dense coiling of the separated filaments. While experimenting with antibiotics to obtain axenic culture, we accidentally found that 12 h exposure to seawater treated with gentamicin sulfate (1 mg l^{-1}) resulted in wellstraightened unbroken filaments that can be accurately measured.

Living cells appear to be almost isodiametric, 4×4 µm in average, excluding apical cells. Close examination shows that filament width is quite constant, just over 4 µm, but that the length depends on the state of division (Fig. 4 d). Recently separated daughter cells are only about 2.5 µm long, whereas an almost divided mother cell ("double cell") measures close to 5 µm (Table 1). A few single cells were found to be as much as 5 µm long, but chances are that incomplete intercellular membranes are not always clearly discernible by light microscopy. Cell counts within a measured distance (50–100 µm) along a trichome convert to average cell lengths of just under 4 µm. The latter method was employed primarily in live preparations where filament movements make accurate individual measurements difficult. A tapering apical cell (Figs. 2; 3; 4 a, c) can reach a length of 10 µm, but cells of 5–7 µm are more common; thickening of the outer wall of terminal cells (calyptra) was never observed. Cell cross walls are only slightly constricted, except under adverse conditions, such as gradual desiccation, just before fragmentation occurs. The cell content appears quite homogeneous under a light microscope. Granules, where present, are not conspicuous enough in size or location within the cell to make them useful systematic characters. "Pseudovacuoles" (Fig. 2), too, are not diagnostic and do not appear clearly except under stress or certain undefined physiological conditions.

Fixation, dehydration, staining, and mounting in glycerin or corn syrup all influence the dimensions and appearance of the trichomes. Preparations in which these effects were obvious and strong were excluded from our measurements. Despite this precaution our data show an average shrinkage of 5 % in width and 23 % in length, in comparison with live and carefully fixed cells.

The blackish brown color of *Phormidium corallyticum* is a stable feature in the healthy organism. Bleaching to greenish or light brown is caused by adverse conditions and is due to loss of the phycobilin pigments. Aqueous extracts (0.1 M phosphate buffer, pH 6.8) that were examined with a spectrophotometer showed copious amounts of phycocyanin (618 nm peak) and lesser quantities of phycoerythrin (543 nm and 565 nm peaks) (E. GANTT, pers. comm.).

Remarks. *Phormidium corallyticum* can be considered close to *P. submembranacea,* as already pointed out. *P. submembranacea,* however, is diagnosed by: cells that are 5 μm wide and 4–10 μm long, constrictions at the trichome nodes, rounded terminal cells, and a depressed conical calyptra that develops

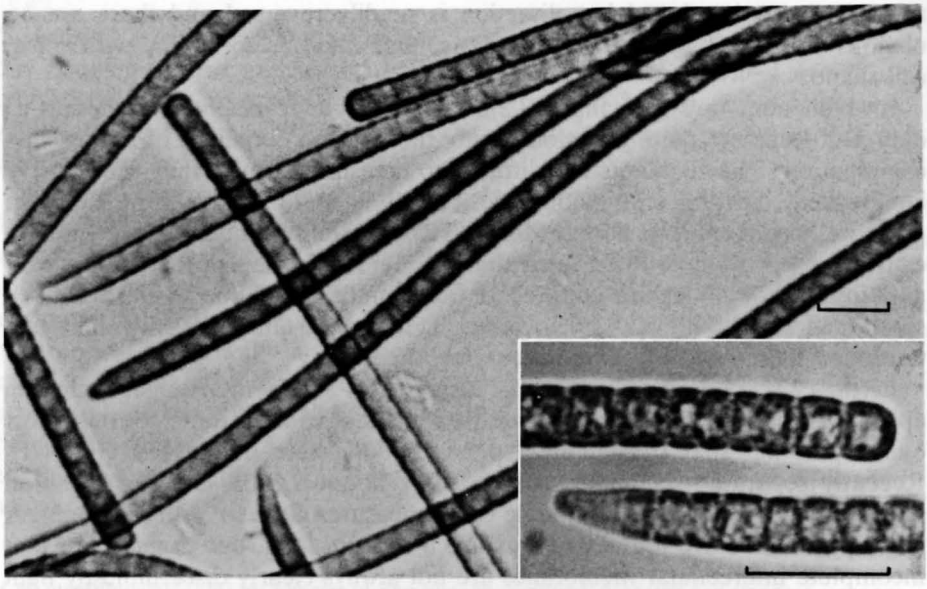

Fig. 2. *Phormidium corallyticum* filaments as seen by light microscopy, unstained. Inset: Enlarged (100 × oil immersion objective) view showing round and tapering end cells. (Scales = 10 μm).

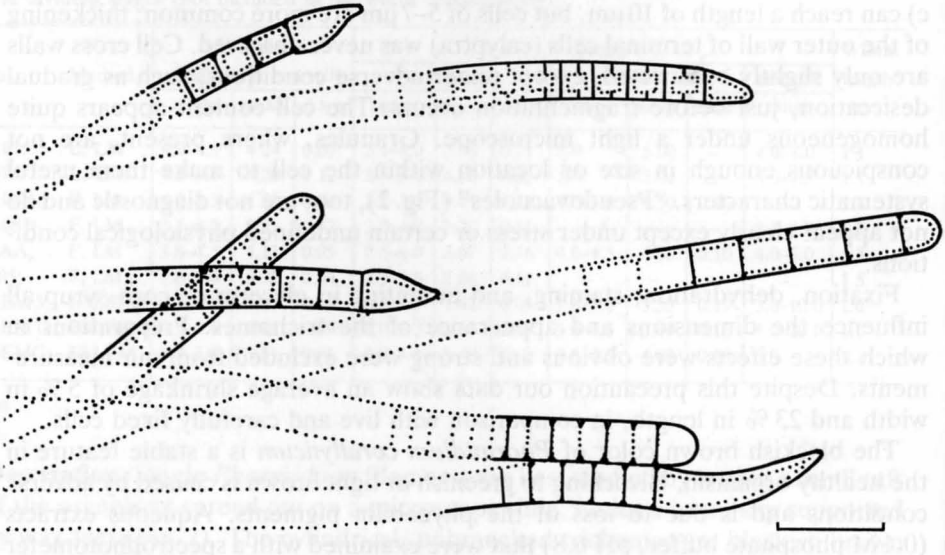

Fig. 3. *Phormidium corallyticum,* filament tip variations sketched from light photomicrographs. (Scale = 10 μm).

with age. It has been found only on rocky shores, never in association with live coral (GEITLER, 1932; HUMM & WICKS, 1980).

The new species is named for its coral tissue dissolving properties (Greek *lyo:* dissolve).

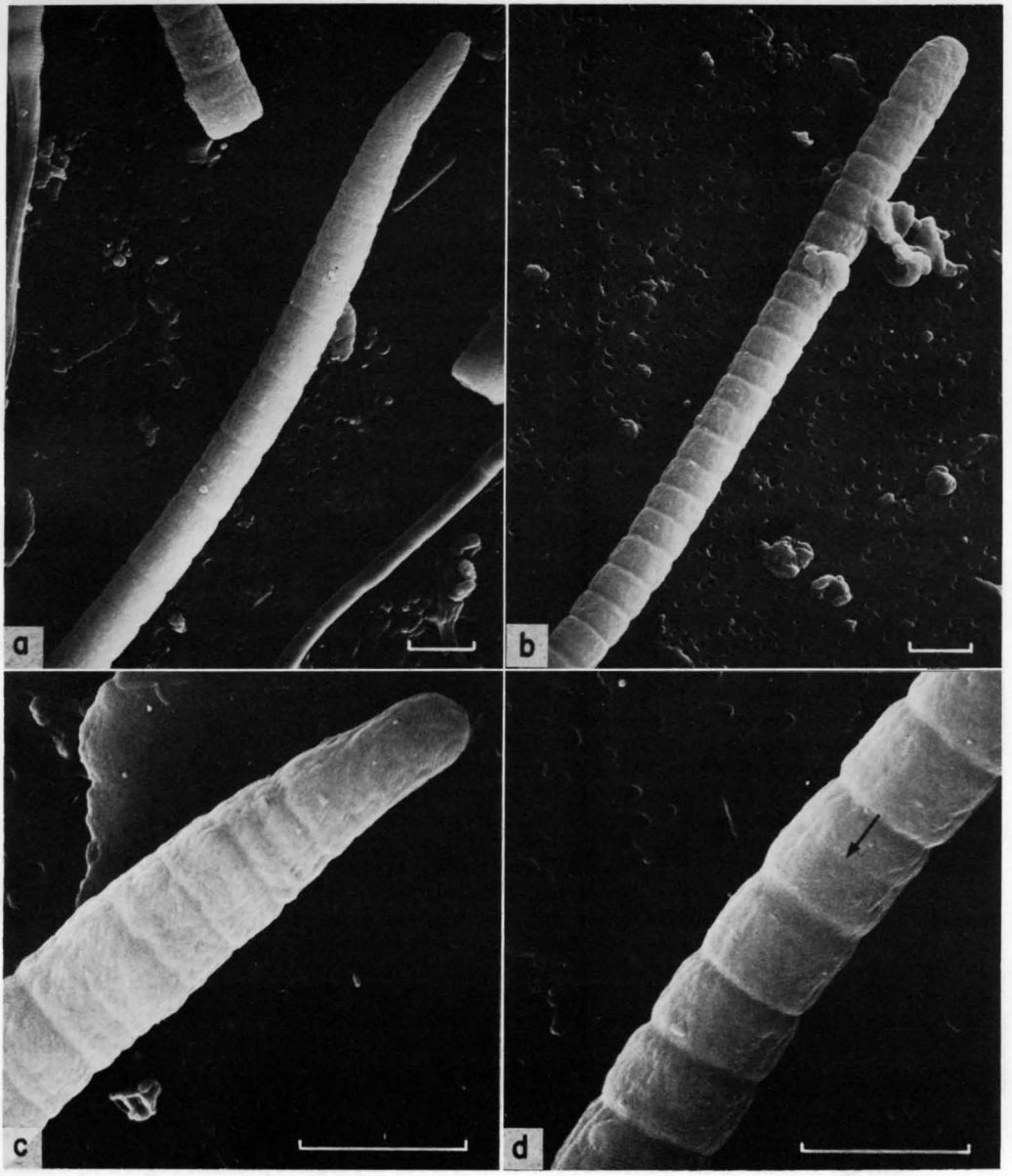

Fig. 4. *Phormidium corallyticum,* scanning electron micrographs. a: Filament with tapering end cell. b: Filament with rounded end cell. c: Tapering tip, enlarged. d: Trichome cells with mucus coating enlarged; arrow indicates new cross wall formation (Scales = 5 μm).

Material and distribution. Holotype BDA 81.08 A [microscope slide] on *D ploria strigosa* (DANA), 3 m, Three Hill Shoals, Bermuda, K. RÜTZLER, col. 2 July 1981; deposited in the U. S. National Herbarium, Smithsonian Institutio Washington, D. C., USA. Paratypes from Bermuda and Carrie Bow Ca Belize, are also deposited at the U. S. National Herbarium and in the Depa ment of Invertebrate Zoology (Lower Invertebrates) of the same institutio *Phormidium corallyticum* is also known from the reefs of Florida, the Flori Keys, the Bahamas, and Jamaica (A. ANTONIUS, pers. comm.; TAYLOR, 1983

2. Cellular organization

Cell walls and sheath. Outer walls are 35–40 nm thick and show the character tic sequence of four layers (L I–L IV) over the plasmalemma (Fig. 7). Layer I is covered by thin sheath material. Cross walls (septa) between cells a separated only by the two inner layers (L I–L II). Cell shrinkage during embe ding of the material is evident from the undulating appearance of wall layers a from clear spaces between cells (Figs. 5, 6 a, 7 b). New septa form one per c at a time, by symmetrical annular ingrowth (Figs. 5, 6 a, 7 b). Small circul (60–200 nm) electron transparent areas (granules?) appear regularly in cro sections on either side of the base of newly developing cross walls ("sept granule", Fig. 7 b); these are distinct from clear areas between cross walls a plasmalemma caused by shrinkage. Photosynthetic lamellae are cut by develo ing septa without changing their longitudinal trend. Neither plasmodesmata n junctional pores could be detected.

A thin mucilaginous layer coats the outer cell wall but does not form prominent sheath. Its fibrils are without noticeable orientation, and are inte meshed in a network that is much denser than that of the coral mucus that al appears on the photomicrographs (Figs. 5, 7 b). The mucous layer is 10–100 n thick, the average being 40 nm and thus similar in dimension to the cell wal

Thylakoid and nucleoplasm. The photosynthetic apparatus consists of thin fl lamellae radiating from a longitudinal axis or core – the nucleoplasm – towar the peripheral plasmalemma (Figs. 6 b, 7 a). In a cell cross section the me branes resemble the spokes of a wheel; although they do not penetrate t nucleoplasm, they are arranged as if they originated at a common axis. tangential longitudinal sections the thylakoids appear as parallel lines extendi from one cross wall to the next. On all sections we examined, the lamellae a flat, without intrathylakoid vacuolization, and oriented in one plane, except f a few locations where large granules cause deflections from the straight tre (Fig. 7 a). Stacking of membranes does not occur; branching or anastomoses a either rare or absent. The nucleoplasm occupies 30–40 % of the cell lume There are about 80 thylakoids per cell cross section; each is 10 nm thi (distance between membranes) and 65–80 nm from the next (at the circumf ence). Width of the lamellae, extending from the nucleoplasmic region to with 20–30 nm of the plasmalemma, is 0.3–1.0 μm, 0.7 μm on the average; no dir connection with the plasma membrane is evident. The membrane sheets are t same length as the cell (about 4 μm), although the general trend appe

Fig. 5. *Phormidium corallyticum,* transmission electron micrographs. a: Longitudinal median section of part of a filament. b: Longitudinal, nearly median section of a dividing cell, showing outer wall (ow), cross walls (cw; one septum being formed), thylakoid (th) with polyglucoside granules (pg), nucleoplasm (np), polyphosphate body (pp), cyanophycin granule (cg), polyhedral body (pb), and mucus of substrate coral (mc). (Scale = 5 μm for a; 1 μm for b).

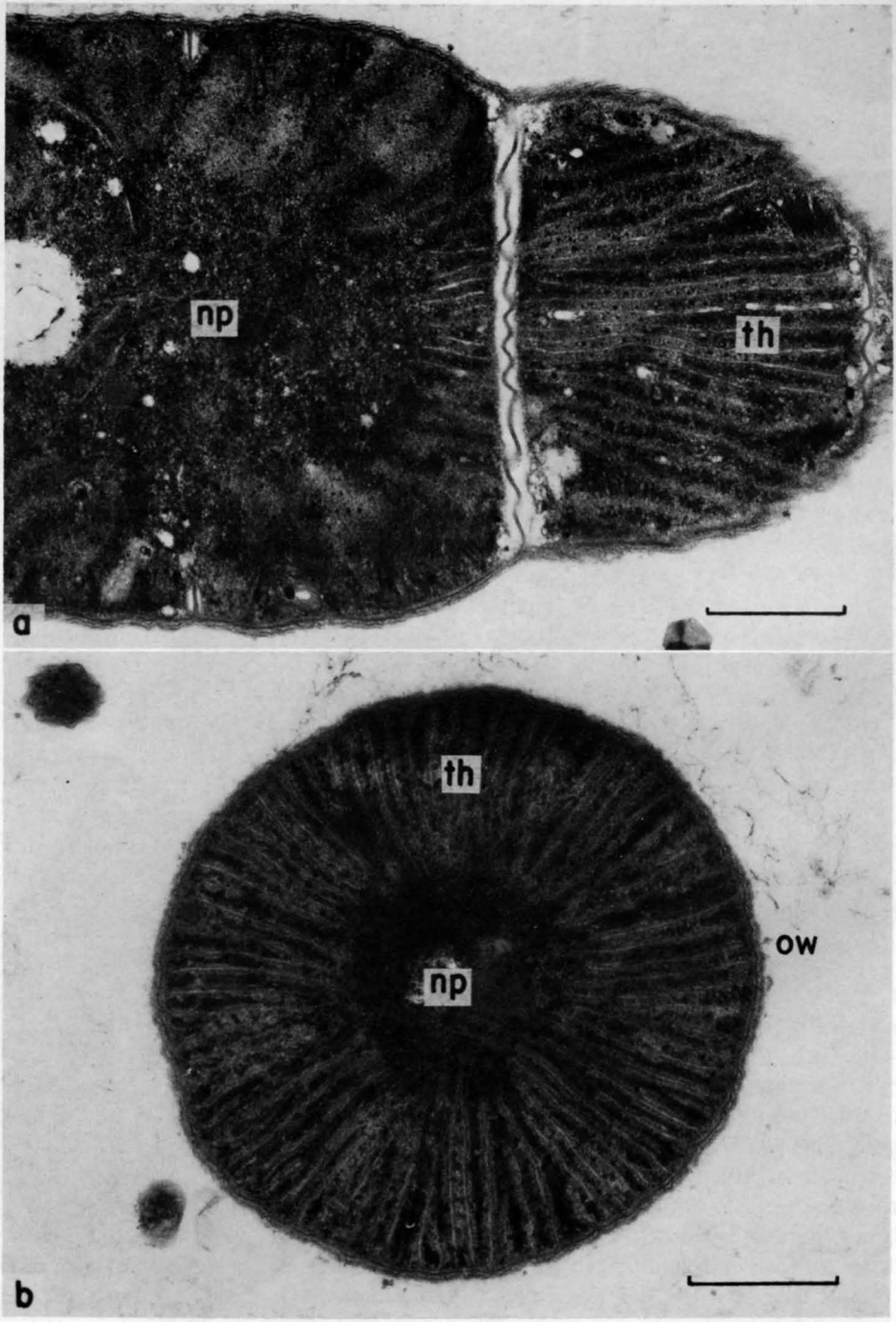

Fig. 6. *Phormidium corallyticum,* transmission electron micrographs. a: Longitudinal tangential section of two cells showing thylakoid (th) arrangement at peripheral chromatoplasm; trend of lamellae across septum is evident; nucleoplasm (np) is exposed in left cell. b: Cross section of cell depicting regular arrangement of thylakoid (th) membranes radiating from nucleoplasm (np) toward outer cell wall (ow). (Scales = 1 μm).

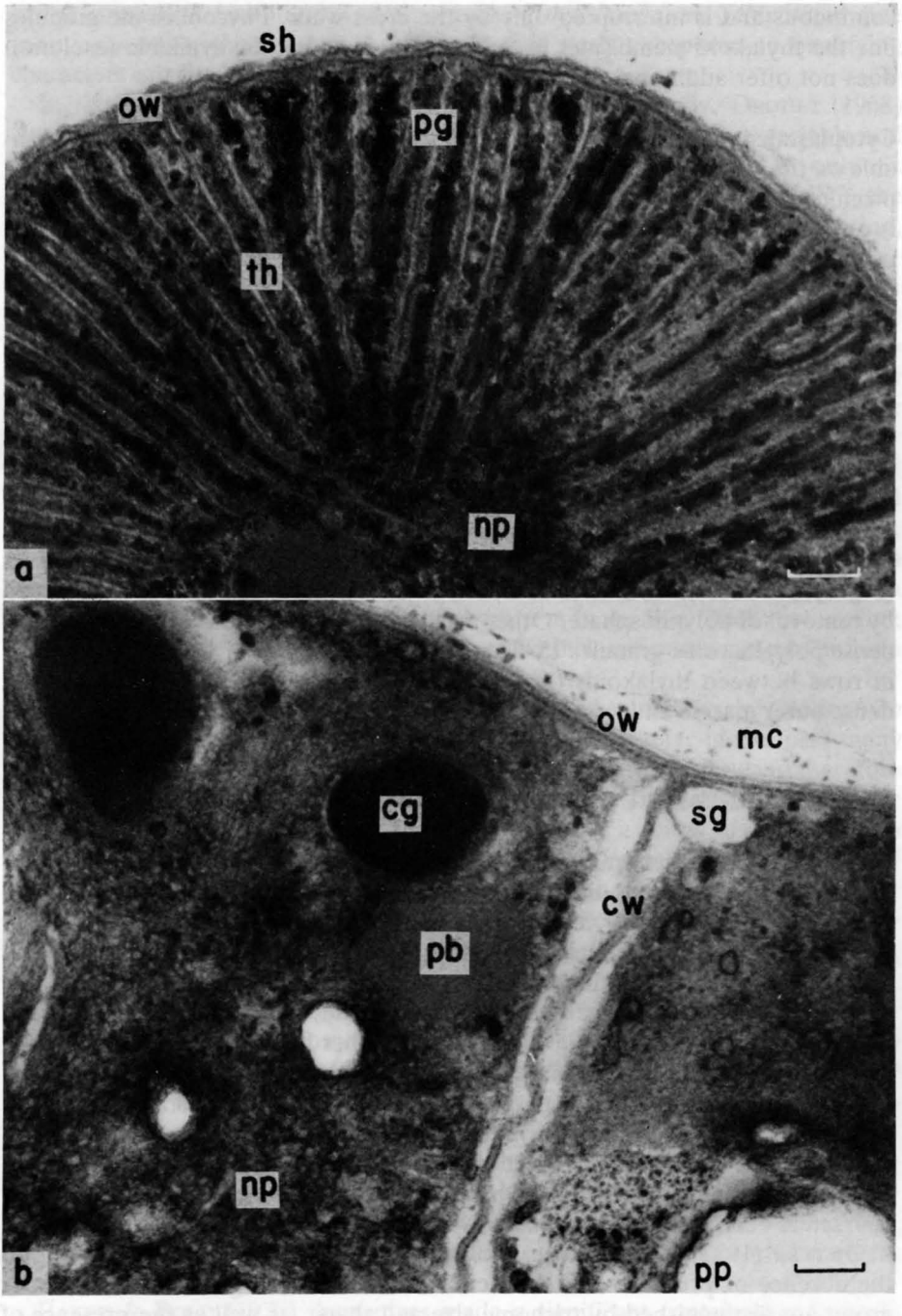

Fig. 7. *Phormidium corallyticum,* transmission electron micrographs. a: Cross section showing radial thylakoid (th) with polyglucoside granules (pg), outer cell wall (ow) with sheath (sh), and nucleoplasm (np). b: Longitudinal section illustrating outer cell wall (ow) with sheath and mucus from substrate coral (mc), cross wall (cw) with septal granule (?) (sg), cyanophycin granule (cg), polyhedral body (pb), polyphosphate body (pp), and nucleoplasm (np). (Scales = 0.1 μm).

continuous and is interrupted only by the cross walls. Phycobilisome granules line the thylakoid membranes in the usual manner but the available resolution does not offer additional detail.

Cytoplasmic inclusions. At least four types of inclusions are readily distinguishable on the basis of their shape, size, density, and position within the cell. Most prominent are the cyanophycin (structured) granules, although their abundance is quite variable among cells examined (Figs. 5, 7 b). They occur mainly in the peripheral cytoplasm, where these large granules influence the trend of the thylakoid membranes, and close to cross walls between cells. They have an angular outline and, although they are very electron dense, some show a faint radial pattern. Larger cyanophycin granules (cross sections) measure 300–500 nm in diameter. Inclusions of similar size (300–450 nm) that are distinctly polygonal in outline, medium gray and uniform in density, and distributed close to or within the nucleoplasmic region are polyhedral bodies (Fig. 7 b). Polyphosphate bodies are represented only by "holes" left behind after the actual granule disintegrated. These empty areas occur within the nucleoplasm of many cells, particularly in filaments collected from the active edge of the black band adjacent to diseased coral tissue; the holes measure 0.6–1.2 μm in diameter (Fig. 5). It is not known whether smaller "holes" (30–200 nm) were also caused by removal of polyphosphate. Other small spherical inclusions include electron-dense polyglucoside granules 15–30 nm in diameter, most of which are arranged in rows between thylakoids (Fig. 5, 7 a), and lipid droplets 30–50 nm, with a dense outer margin and less dense core, concentrated toward the cell periphery.

Discussion

To ensure proper systematic placement of the new organism within the *Cyanophyta,* we consulted the three major phycologic taxonomic reviews and revisions of the past fifty years as well as some specialized recent reports that would help clarify the value of commonly used systematic characters. There is general agreement that this organism belongs to the family *Oscillatoriaceae* (order *Hormogonales*) because it forms uniseriate, unbranched trichomes and lacks heterocysts. Generic allocation, on the other hand, requires reevaluation of morphological features whose significance and stability have been disputed by numerous authors over the past century. It should be emphasized that we are dealing with the traditional phycological classification of the group because ultrastructure and bacteriological methods have not yet been applied to a sufficient number of species, nor has the taxonomic significance of these new characters been tested enough to allow replacement of light optical observation.

GEITLER (1932) separates the *Oscillatoriaceae* into two groups according to the absence or presence of a distinct sheath. The seven genera of the former group are distinguished by trichome size and shape, as well as the presence of cell wall constrictions. The second group, comprising 18 genera, is recognized by the quality of sheaths, pseudobranching, number of trichomes per sheath, and shape and grouping of filaments. The difficulty of determining genera is evident from the fact that some genera are listed in two opposing parts of the

key (*Gomontiella:* with and without sheath; *Polychlamydum, Schizothrix:* one versus several trichomes per sheath) and that many exceptions to qualifying characters are listed in footnotes.

In the latest thorough phycological revision of the family, DROUET (1968) considers the sheath an environmentally influenced and therefore variable and taxonomically useless character and reduces the *Oscillatoriceae* to only six genera. Except for the aberrant *Spirulina* (without apparent cross walls[1]), the generic separation is based on just a few characters, such as trichome tapering, presence or absence of granules, and thickening of terminal cell walls.

DROUET's extreme views on oscillatoriacean variability and systematics are criticized by BOURRELLY (1970: 430, 431), who employs a classical systematic concept, but he concedes that numerous controlled cultures are still needed to clarify the validity of certain species. Eleven freshwater genera are dealt with by BOURRELLY and separated on the basis of sheath, number of trichomes per sheath, spores, trichome shape, cell shape, and membrane thickness.

The principal question raised by these two opposing views is to what extent morphological features resolvable by light microscopy – such as sheaths, cell tapering, shape and membrane thickening of end cells, cell wall constrictions, granules, vacuoles, and pseudobranching – are genetically controlled and to what extent they are influenced by environmental conditions. Ambiguity as a result of nonquantitative evaluation of characters (*e. g.*, thin: thick; few: many; indistinct: distinct; more: less) is common to both classification systems and is a substantial element of misinterpretation.

The attempts to determine environment-induced variability in *Cyanophyta* are summarized by BAKER & BOLD (1970). Unfortunately, in the past many workers employed only field observations to determine variability and therefore could not improve upon taxonomic judgment. Supporting culture studies were rare or incidental, and were poorly controlled until a series of investigations during the 1960s – cited in BAKER & BOLD (1970) – examined the influence of environmental parameters (such as concentration of organic materials and other nutrients, moisture, pH, salt concentration, and media composition) on morphological features (including sheath structure and thickness, sheath coloration, trichome structure, cell shape and proportions, granulation, filament motility, and false branching, and plant mass configuration). In essence, all these experiments showed that some characters, particularly sheath formation, terminal cell shape, trichome structure, and cell proportions, are to be considered taxonomically important because they do not change beyond recognition within one taxon, despite their obvious variability. This observation supports GOMONT's (1892) view of almost a century ago that environmental modifications do not change one species into another (assuming a well-founded species concept). Examples of less reliable characters are numbers and types of false branching that seem entirely dependent (in those species considered) on moisture content of the substrate.

BAKER & BOLD (1970) themselves made a significant contribution to oscillatoriacean taxonomic methodology by culturing isolates from soil, air, marine,

[1] *Spirulina* does not strictly fit the diagnosis of the family because trichomes are multicellular by definition.

and freshwater samples under controlled conditions on nutrient agar. This work determined or confirmed that some morphological features, particularly the macroscopic structure of the plant mass, the sheath formation, and the shape of the terminal cell, are constant characteristics whose nature cannot be mistaken despite a certain degree of variability. Type of granulation and vacuolization, on the other hand, as well as cell size and shape, and thickening of the outer wall of the terminal cell – all considered important attributes in DROUET's (1968) classification – were found to fluctuate considerably with age and among different isolates of a monospecific population.

At the beginning of our investigation, we had difficulty deciding whether to call the delicate mucous polysaccharide layer enveloping the trichomes of the black band cyanophyte a proper sheath. To avoid the issue, we applied the DROUET system (DROUET, 1968; HUMM & WICKS, 1980), which places the organism in the genus *Schizothrix* because it lacks granules along the cross walls and thickening of the outer membrane of the terminal cell, and because, if trichome tapering occurs, only the terminal cell is attenuated. This characterization seems unsatisfactory as it is based mainly on characters (or rather absence of characters) that are experimentally proven to be unreliable. Furthermore, members of *Schizothrix* are known to have distinct, firm, even colored sheaths – although they can leave these and survive as naked trichomes, as DROUET (1963) demonstrated – and to form multiple trichomes per sheath and pseudobranches, characters that do not occur in our organism.

Pure culture material was used by RIPPKA *et al.* (1979) in an attempt to distinguish between phenotypic and genotypic expression in cyanobacteria. Forty-four strains of oscillatorians ("Section III genera") are examined and classified by characters seen with the light microscope. Nineteen of these strains are placed in three distinct sheathless genera *(Spirulina, Oscillatoria, Pseudanabaena),* the remaining twenty-five are assigned to the provisional LPP (*Lyngbya-, Phormidium-, Plectonema*-like) groups. Our organism falls in the heterogeneous LPP group B (possessing isodiametrical or cylindrical cells, high motility, unpronounced sheaths, cell width under 5 μm); neither physiological nor genetic testing of strains in this group by RIPPKA and colleagues allowed further meaningful breakdown into useful taxonomic units.

Encouraged by BAKER & BOLD's (1970) assessment, we reconsidered the classical systematics, particularly, the significance of the sheath. Accordingly, we noted that our organism has affinities to three genera, *Oscillatoria, Phormidium,* and *Lyngbya,* which are distinguished solely by the presence and development of sheaths. Like GOMONT (1892), we reserve *Oscillatoria* for species that do not develop a sheath, as judged by light microscope staining and electron microscopy of numerous specimens in different growth phases. *Phormidium,* as defined here, has a mucilaginous envelope that can be stained by methylene blue, contrasted by India ink, or made visible by electron microscopy; this envelope does not remain intact and distinct if separated from the trichome. *Lyngbya,* on the other hand, has a "distinct" sheath because the envelope is solid and discrete, allowing trichomes to glide back and forth, and remains intact even after the trichome has left (this quality is independent of possible layered structure); sheaths are visible without staining or contrasting by simple light microscopy.

It can be argued that our definitions are arbitrary and that borderline cases and transitions are likely to occur. However, we are focusing on one species that we have studied at two distant geographical locations and under many different ecological conditions and growth phases. In view of these observations and the literature based on light microscope studies it seems logical and convenient to place our organism in the genus *Phormidium*. A similar conclusion was reached by PEARSON & KINGSBURY (1966) in their experimental study of a "*Lyngbya* sp." that displays an indefinite and thin sheath under all culture conditions. The sheath of *Oscillatoria rubescens* DE CANDOLLE, studied under the electron microscope by JOST (1965), also corresponds closely to the mucus envelope of *Phormidium corallyticum* and would justify transfer of that species to *Phormidium*.

Our own electron microscope studies have limited value for this discussion because our observations could not be related to the finestructural morphology of a sufficient number of known oscillatoriacean species. Our findings can be used, however, as a data bank for future investigations in this direction (Fig. 8). In examining the finestructure of *Phormidium corallyticum*, we have paid particular attention to details that have already been identified as actually or potentially significant characters for taxonomic use (WHITTON, 1972). The cell wall is composed of four layers (L I–L IV), as is typical for bluegreen algae (JOST, 1965). Only the inner layers (L I, L II) participate in the formation of

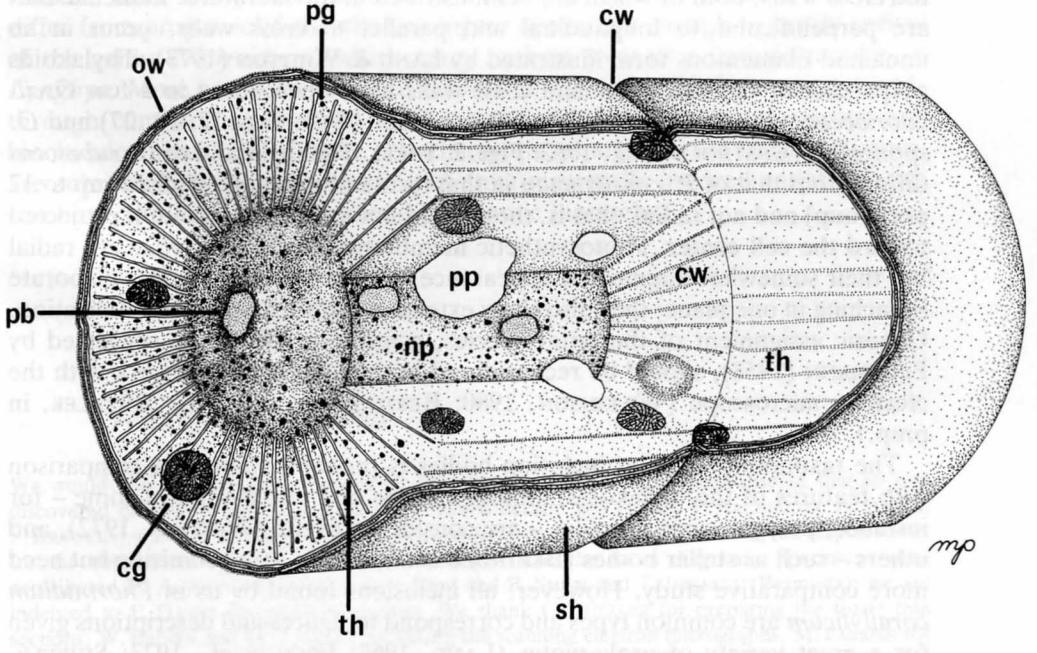

Fig. 8. *Phormidium corallyticum*, semischematic, three dimensional reconstruction of cell organization; parts of outer cell wall and thylakoid region are removed for clarity. Cell components shown are: Outer cell wall (ow), sheath (sh), cross wall (cw), thylakoid membranes (th), nucleoplasm (np), cyanophycin granules (cg), polyhedral bodies (pb), polyglucoside granules (pg), and polyphosphate bodies (pp).

cross walls, septa close in symmetrically, and there are no outer wall pores or plasmodesmata. Membrane undulations and other shrinkage phenomena were also noted by JOST (1965) and shown to take place during polymerization of embedding media as they are absent in freeze etching preparations of the same material. Freeze etchings by JOST also reveal the structure of mucilage or sheath material better than epoxy resin sections. The mucous coating of *Oscillatoria rubescens* in an epoxy thin section looks identical to that of *Phormidium corallyticum,* but in freeze etchings the fibrils (3 ×500 nm) of *O. rubescens* are clearly perpendicular to the cell wall; a high water content of the mucilaginous layer can also be demonstrated by this technique if antifreeze treatment is omitted (JOST, 1965: Figs. 2, 10–12, 20). A comparative study of solid *Lyngbya*-type sheaths, particularly by freeze etching, would be most useful because differences in microfibril orientation in some thick sheaths have already been demonstrated (LEAK, 1967: Fig. 4; LAMONT, 1969: Fig. 6c).

The thylakoid may be the most important taxonomic feature made visible by electron microscopy, although, as with any other character, a certain variability has to be anticipated with age, cell inclusions, and environmental conditions (WHITTON, 1972; LANG & WHITTON, 1973). From a list of eighteen cytoplasmic membrane characters considered systematically significant (WHITTON, 1972: Table I), only one applies directly to the organization of *Phormidium corallyticum;* that is, the thylakoids run at right angles to the [outer] wall. This diagnosis applies to membrane arrangement that is parallel or perpendicular to the cross walls, both of which are demonstrated in the literature. Lamellae that are perpendicular to longitudinal and parallel to cross walls, occur in an unnamed filamentous form illustrated by LANG & WHITTON (1973). Thylakoids perpendicular to both outer and cross walls have been noted in a few *Oscillatoriaceae,* such as *Oscillatoria rubescens* (JOST, 1965: Figs. 17, 19, 27) and *O. spongeliae* (BERTHOLD *et al.,* 1982: Figs. 2, 9–11). The thylakoid of *O. rubescens* differs from that of *P. corallyticum* in that its membranes are stacked (up to 17 per group) and not radial; that is, they would not meet at one point if projected toward the cell center. Photosynthetic membranes of *O. spongeliae* are radial but their somewhat fragmented appearance in cross section indicates elaborate branching in one plane; they also show extensive intrathylakoidal vacuolization. Generic assignment of the latter species, possibly to *Borzia* as suggested by FELDMANN (1958), should be reconsidered in the light of recent work with the electron microscope (WILKINSON, 1980; BERTHOLD *et al.,* 1982; RÜTZLER, in prep.).

The taxonomic value of inclusion bodies is probably limited in comparison with features of the thylakoids, although shape and distribution of some – for instance, polyglucoside granules – are considered useful (WHITTON, 1972), and others – such as stallar bodies (BERTHOLD *et al.,* 1982) – are promising but need more comparative study. However, all inclusions found by us in *Phormidium corallyticum* are common types and correspond to figures and descriptions given for a great variety of prokaryotes (LANG, 1968; FOGG *et al.,* 1973; SHIVELY, 1974).

These and other new characters are clearly needed before another revision of *Oscillatoriaceae* can be attempted. The ultrastructure of cell detail in these small organisms opens a new field. The improved method of visual examination at this

level of study could be supplemented by a series of biochemical tests, which have become standard procedure in bacteriology. Even the most advanced techniques will fail to clarify systematic relationships, however, unless variability of old and new characters is studied under carefully controlled conditions.

Summary

Phormidium corallyticum, a new species of the cyanophyte family *Oscillatoriaceae* found in shallow reefs of the subtropical and tropical western Atlantic, such as those of Bermuda, Florida, and Belize, is notable for its destruction of coral. Traditional phycological methods based on light microscopy alone make it difficult to classify the new organism within the existing oscillatoriacean systems. We have studied finestructure and responses to different ecological conditions in nature and in culture, but these characters have received no attention in the monographs on the group and will not assist in classification until an appropriate revision is made. Since generic allocation is unsatisfactory under the DROUET classification, the classical system employed by GEITLER and BOURRELLY should be reconsidered. Experiments show that several characters used in the classical system are more stable and reliable than field workers have interpreted them to be. The mucilaginous trichome envelopes differ from proper solid sheaths but appear distinctive enough to justify revival of the genus *Phormidium,* which many authors treat as a junior synonym of *Lyngbya.* More and better defined taxonomic features could be made available through comparative study of this species group by electron microscopy and biochemical reactions. If these new characters, along with the traditional ones, are submitted to controlled testing of environmentally induced variability, we can hope to arrive at an improved taxonomic hierarchy.

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