Sphaeroplea robusta n. sp., a New Member of the Sphaeropleaceae (Chlorophyceae) from Texas[1,2]

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Abstract. *Sphaeroplea robusta*, a new species in the Sphaeropleaceae, is described from isolates of material collected in Texas. Like other species of *Sphaeroplea*, it is oogamous and produces long, unbranched vegetative filaments consisting of large coenocytic cells separated by crosswalls and plugs. *Sphaeroplea robusta* differs from all other species in the following features: (1) its relatively short antheridial cells (commonly 70–150 μm); (2) its large oospores (up to 71 μm); and (3) the distinct ornamentation of its oospore wall.

The genus *Sphaeroplea* comprises a group of fresh-water green algae occurring predominantly on periodically inundated ground (Fritsch, 1929). *Sphaeroplea* is characterized by the formation of unbranched filaments consisting of large coenocytic cells with numerous regularly spaced vacuoles that separate cytoplasmic regions containing nuclei and chloroplast components. Filaments propagate vegetatively by fragmentation; although zoospores have been reported to form occasionally in *S. wilmani* Fritsch et Rich (see Rieth, 1952), they are unknown in other species. Sexual reproduction is oogamous or, in *S. tenuis* Fritsch (see Fritsch, 1929), anisogamous.

The present paper describes a new species of *Sphaeroplea* that differs from all other known species in several vegetative and reproductive characteristics.

MATERIALS AND METHODS

Clonal isolates of *Sphaeroplea robusta* were obtained by L.R.H. from a sample collected by Drs. H. C. Bold and R. C. Starr in May 1979 from a roadside ditch at the intersection of Texas route 71 and Hamilton Pool road (FM 3238) in Travis County, Texas. Cultures were maintained in Modified Pocock's (MP) medium (Stanker & Hoffman, 1979) at 19–26°C and were illuminated with banks of cool white fluorescent lamps to provide a 12:12 h light-dark regime with a light flux of 180–240 μEin/m^2/sec.

Gametogenesis was obtained by inoculating filaments from 7–10-day-old cultures into a shallow layer (6–8 mm) of fresh MP medium in sterile, plastic Petri plates, which then were placed directly beneath the light source. Male and female gametangia formed 3–8 days later. Mature orange zygotes were collected and observed 20–30 days following inoculation.

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Photomicrographs were obtained with a standard Zeiss microscope (equipped with Nomarski interference-contrast optics), a Reichert Zetopan microscope (equipped with anoptral-contrast optics), and an Olympus BHS research microscope (equipped with bright-field and phase-contrast optics). Male gametes were fixed for photomicrography in 2% glutaraldehyde in MP medium prior to observation. Zygotes for scanning electron microscopy (SEM) were fixed in 2% glutaraldehyde, dehydrated in an acetone series, critical-point dried on circular glass coverslips, mounted on aluminum stubs, sputter-coated with gold or gold-palladium, and studied with a Cambridge Mark II or JEOL JSM-U3 SEM.

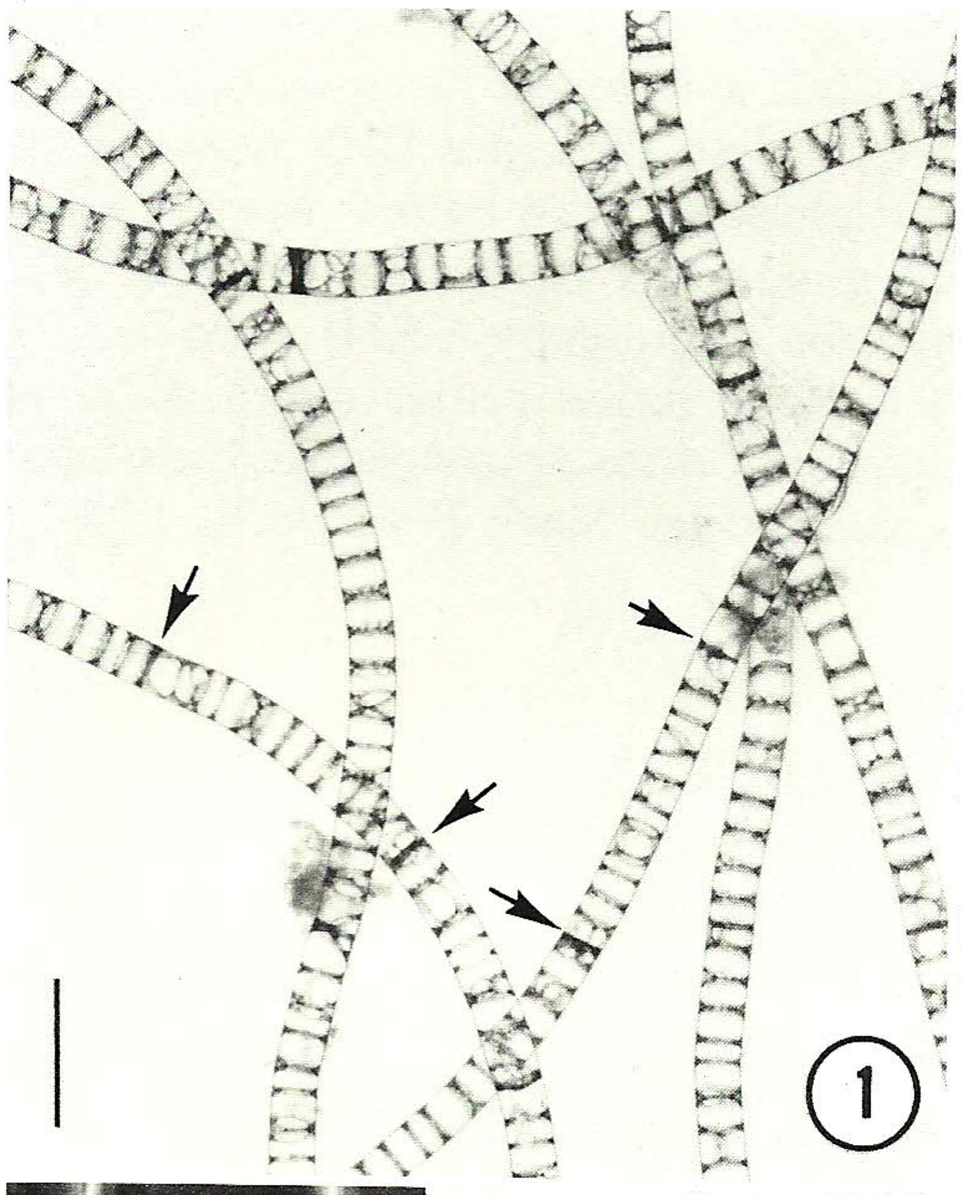
Taxonomic Account

Family Sphaeropleaceae Kützing, 1849
Genus *Sphaeroplea* C. A. Agardh, 1824
Sphaeroplea robusta n. sp.
(Figs. 1–20)

Diagnosis. Fila e cellulis coenocyticis longitudine valde variabilibus, 240–1,960 μm, plerumque 400–1,000 μm, composita, diametro 56–72 μm; chloroplasti variabiles, nunc reticula dissecta, nunc vittae annulares, a vacuolis magnis aequabiliter ordinatis separatae; pyrenoidum numerus variabilis, 2–5 in quaque zona cytoplasmica; generatio asexualis a fractura effecta; generatio sexualis oogama, fila bisexualia; ova sphaerica vel subsphaerica, 20–65 μm diametro; oogonia 140–1,200 μm longitudine; gametae masculae biflagellatae (haud typice quadri- vel pluri-flagellatae), ovoideo-elongatae, vel falcatae 8–15 μm longitudine; antheridia 32–520 μm, plerumque 70–150 μm longitudine; zygotae sphaericae, 29–71 μm diametro, a membrana primaria levi, tenui, denique fusa circumdatae; zygotae maturae tunica porcis prominentibus reticulata depressiones multas polygonas delineantibus in fundis prominentias humiles papillatas vel rugosas typicae proferentes.

Filaments composed of coenocytic cells of highly variable length, 240–1,960 μm, commonly 400–1,000 μm; cell diameter from 56–72 μm; chloroplasts variable from dissected reticulum to annular bands separated by large, regularly-spaced vacuoles; pyrenoid number variable, 2–5 occurring in each cytoplasmic zone; asexual reproduction by fragmentation; sexual reproduction oogamous, filaments bisexual; eggs spherical to subspherical, 20–65 μm in diameter; oogonia 140–1,200 μm in length; biflagellate male gametes (atypically four flagella or more) ovoid elongate to falcate, 8–15 μm in length; antheridia 32–520 μm in length, commonly 70–150 μm; zygotes spherical, 29–71 μm in diameter, surrounded by a smooth, thin primary membrane that eventually is discarded; mature zygote wall ornamented with a reticulate pattern of prominent ridges forming many polygonal depressions, the base of each typically bearing papillate to rugose projections of low elevation.

Holotype. A specimen with mature zygotes derived from a clonal culture isolated by L.R.H. from a collection obtained by Drs. H. C. Bold and R. C. Starr in May 1979 from a roadside ditch at the intersection of Texas route 71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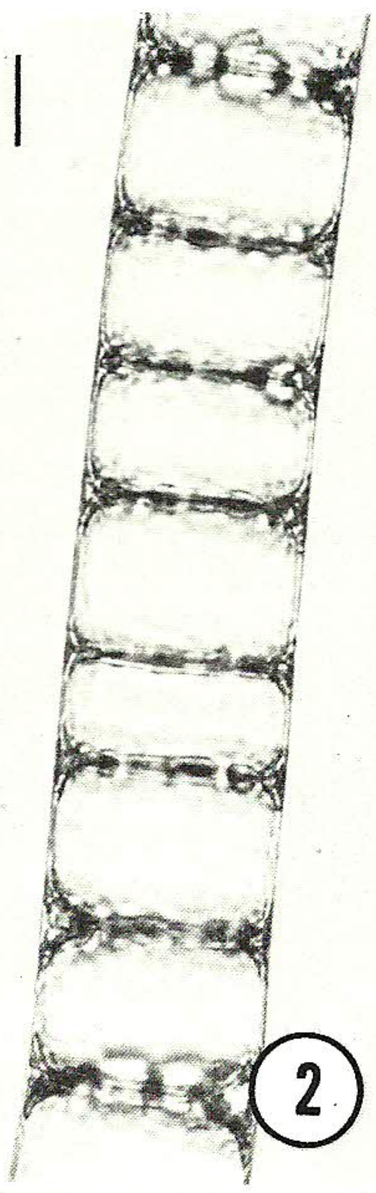
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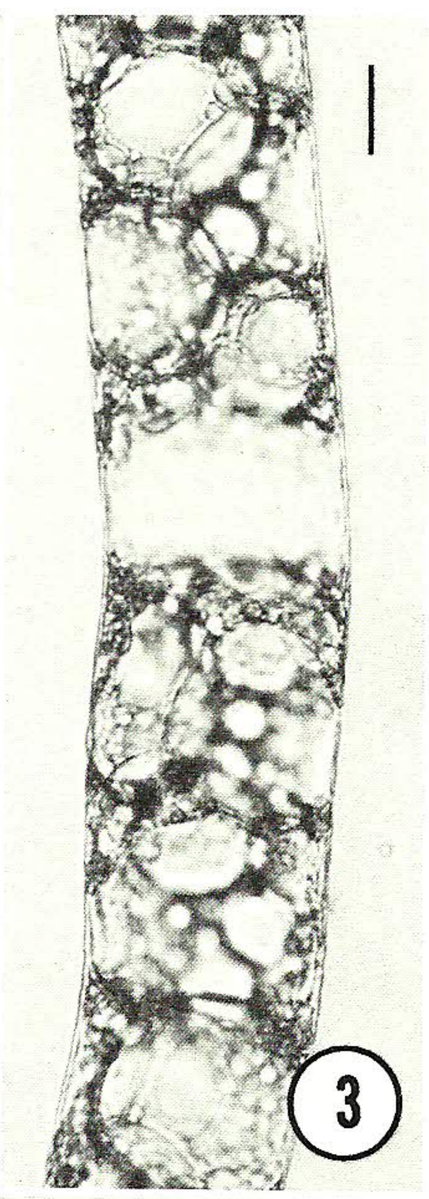
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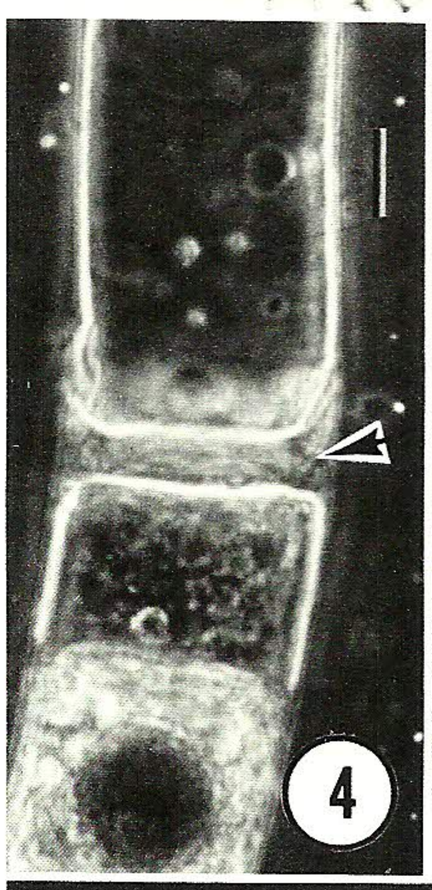
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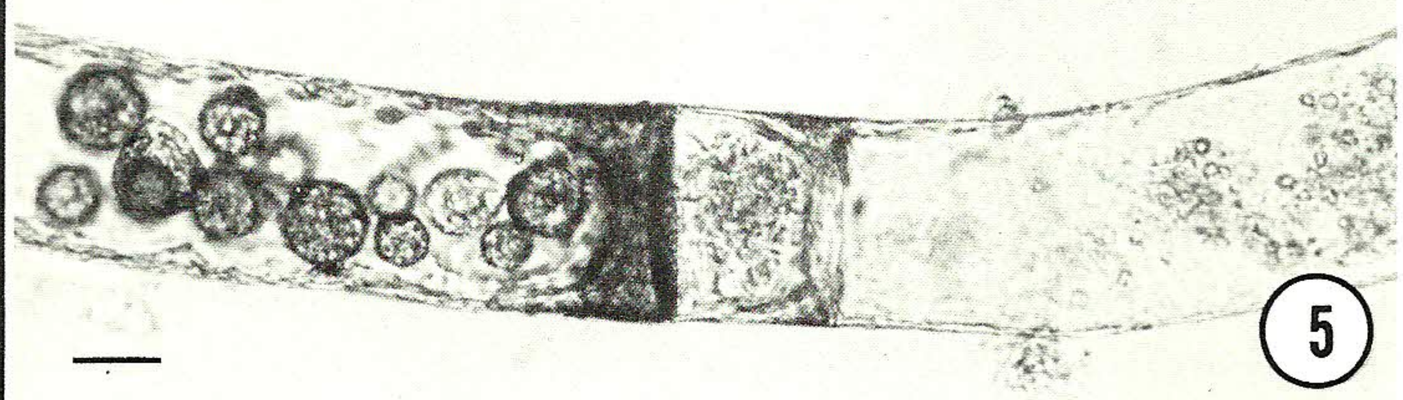
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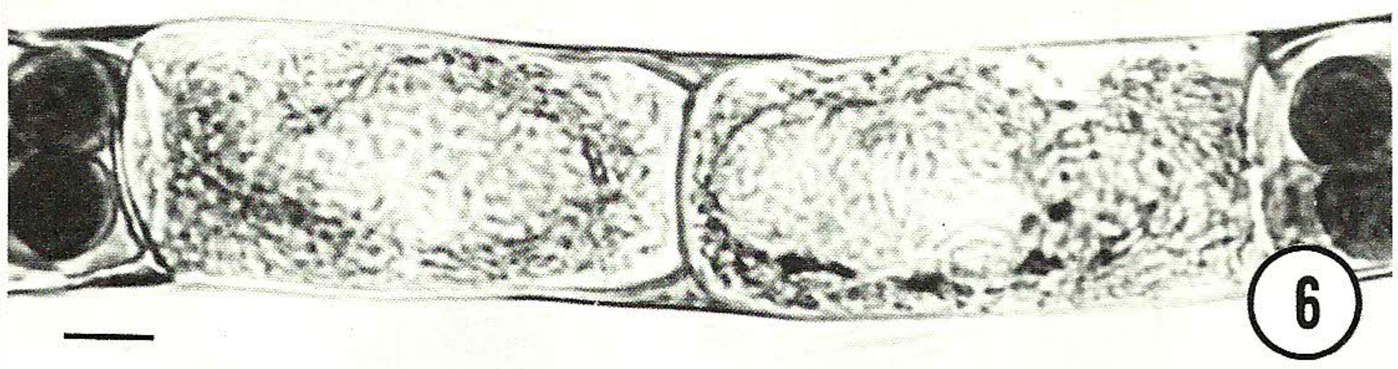
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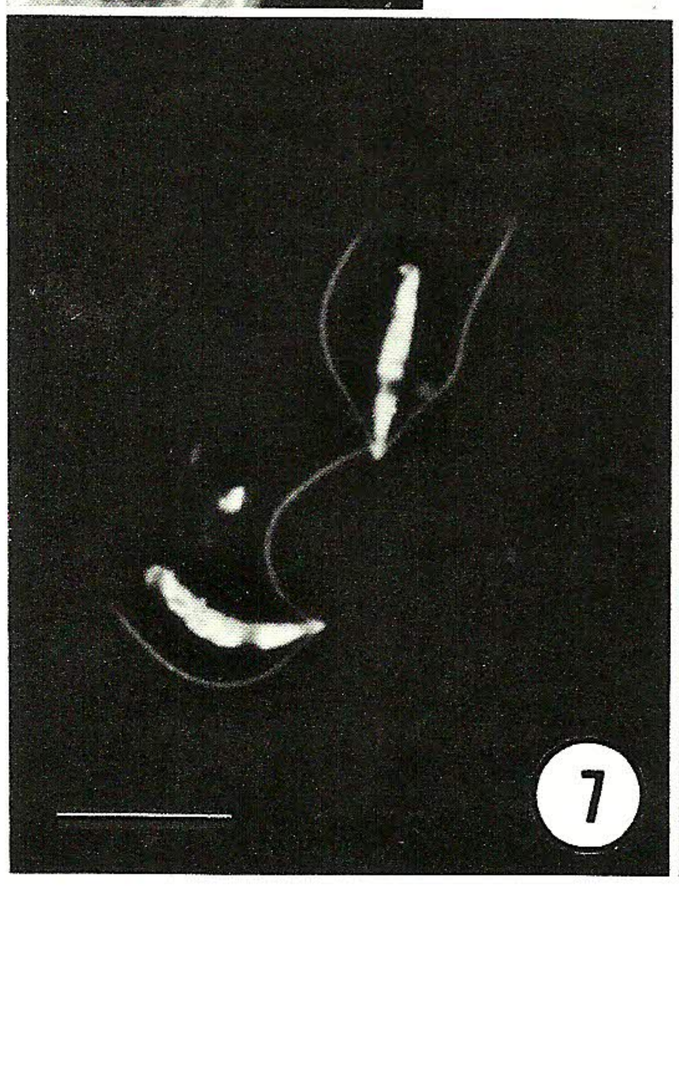
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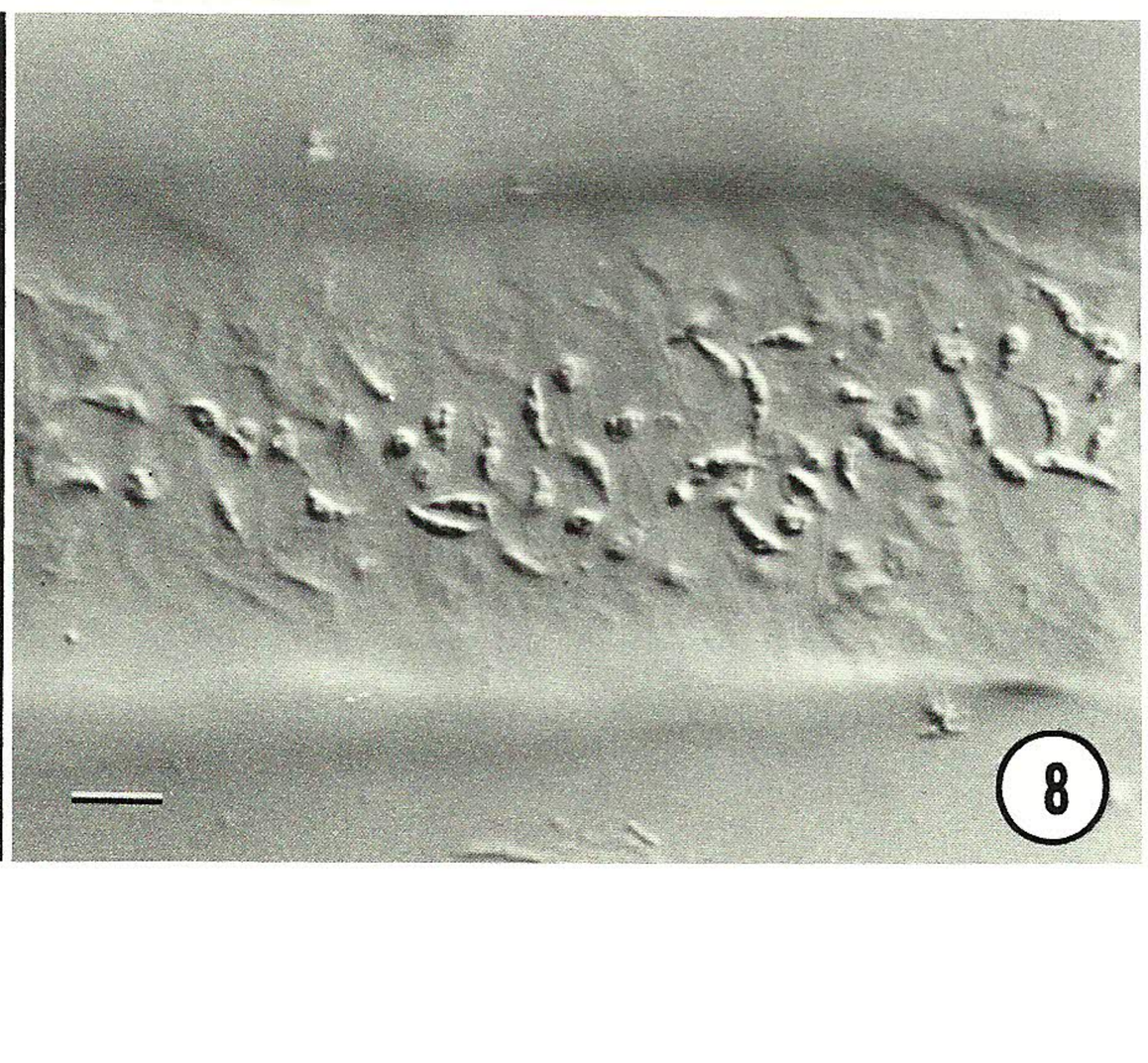
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and Hamilton Pool road (FM 3238), Travis County, Texas. Specimen deposited with the Field Museum of Natural History (No. 1054015). Isotypes (ILL). Cultures are maintained by the authors and the Culture Collection of Algae at the University of Texas at Austin (UTEX LB 2350).

Etymology. The species epithet alludes to the vigorous appearance of *S. robusta*, particularly as evidenced by its large oospores (Latin *robustus*, strong, stout).

Observations

The long vegetative filaments of *Sphaeroplea robusta* are composed of coenocytic cells which vary in length from 240–1,960 μm, but most commonly are 400–1,000 μm (Fig. 1). The diameter of the cells varies from 56–72 μm. Senescent cells occasionally become barrel-shaped, with constrictions occurring at the crosswalls. Adjacent cells are delimited by two crosswalls which often are separated by dense material (Fig. 4), typical of *Sphaeroplea*, termed a "plug" (Fritsch, 1929). Preliminary transmission electron micoscopic (TEM) studies confirm that the plugs of *S. robusta* are essentially identical to those described in *S. annulina* (Roth) C. A. Agardh by Cáceres & Robinson (1980).

Chloroplast morphology varies considerably. When suspended in fresh medium, cells normally develop with evenly-spaced, annular plastids separated by vacuoles (Fig. 2). Cells from older cultures often contain chloroplasts that appear as diffuse reticula (Fig. 3).

Like most other species of *Sphaeroplea*, *S. robusta* propagates vegetatively by filament elongation and fragmentation, while sexual reproduction is oogamous and bisexual with a single filament producing both oogonia and antheridia (Figs. 5, 9, 13). During gametogenesis, numerous male gametes form in short antheridia (Figs. 5, 6) which range in length from 32–520 μm, but commonly are 70–150 μm. Antheridia may be separated by oogonia or vegetative cells, but often occur in adjacent pairs (Fig. 6) or, less frequently, in series up to six in number. The mature male gamete is a slender, ovoid elongate to falcate, biflagellate cell 8–15 μm in length (Figs. 7, 8). The flagella are 1.3–1.5 times the length of the cell.

Oogonia are 140–1,200 μm in length and contain 1–3 (usually 2) ranks of spherical eggs (Figs. 9–11). Eggs typically possess 2–5 pyrenoids (Fig. 11) and vary from 20–65 μm in diameter. As in other species of *Sphaeroplea* (with the possible exception of *S. tenuis*; see Fritsch, 1929), male gametes leave the

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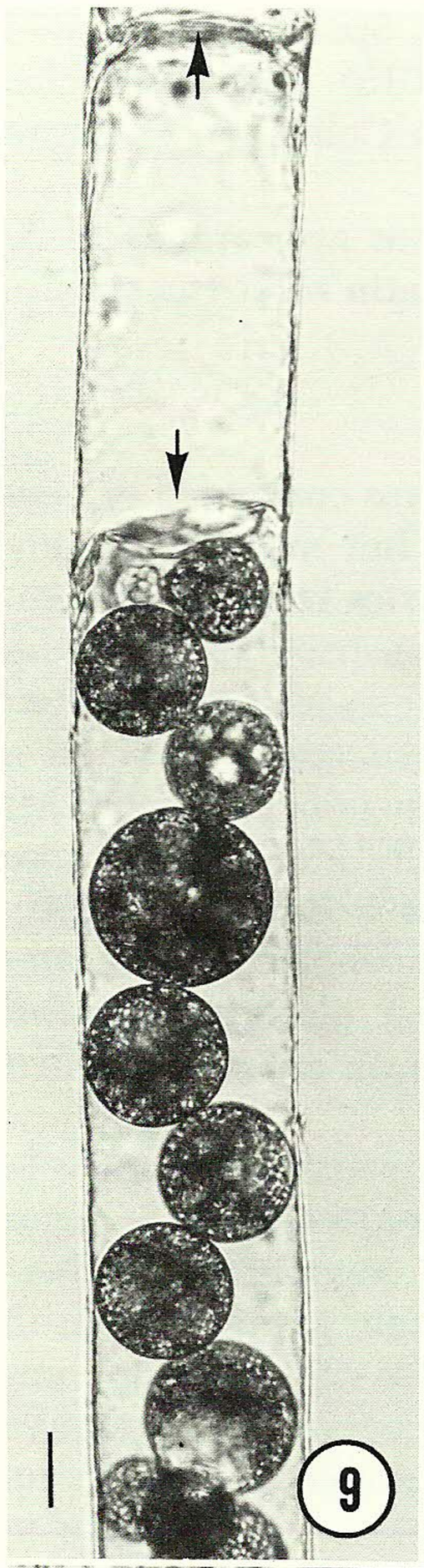
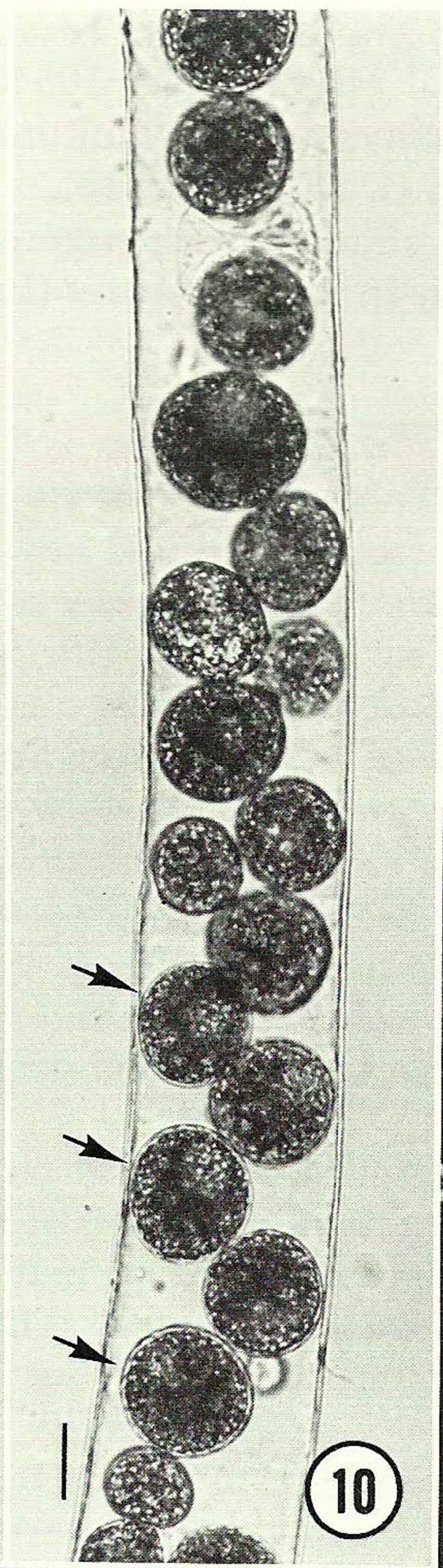
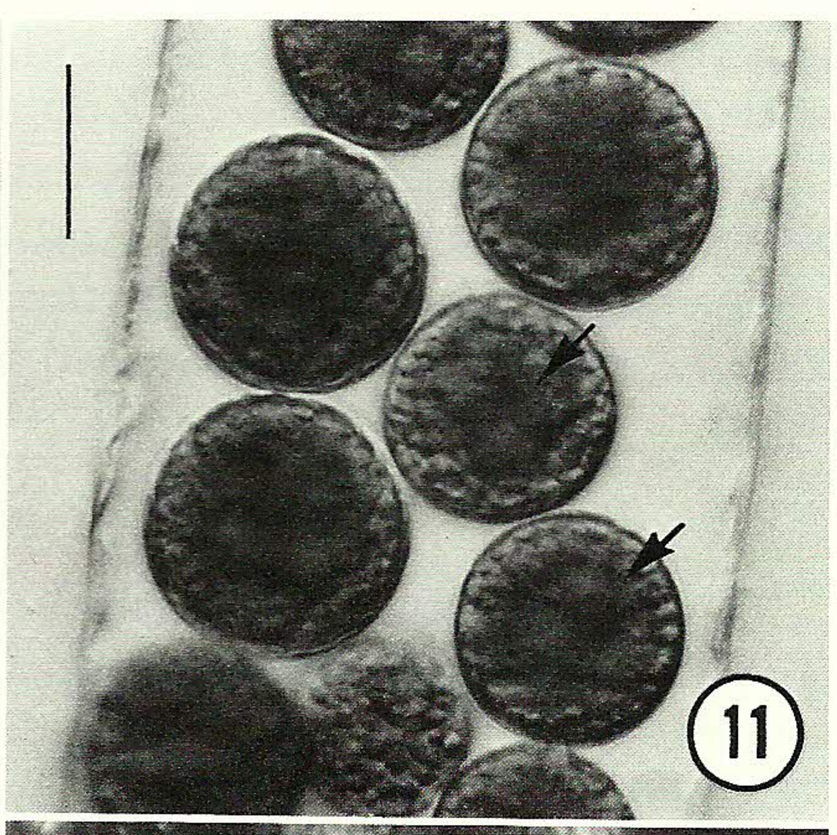
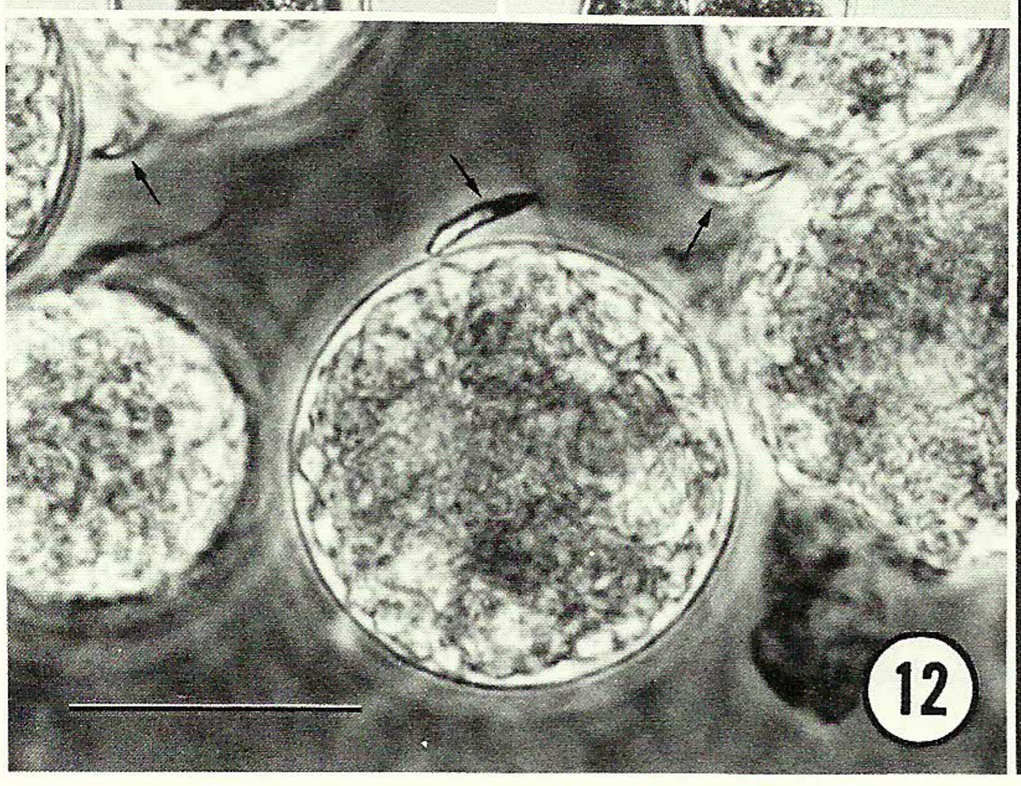
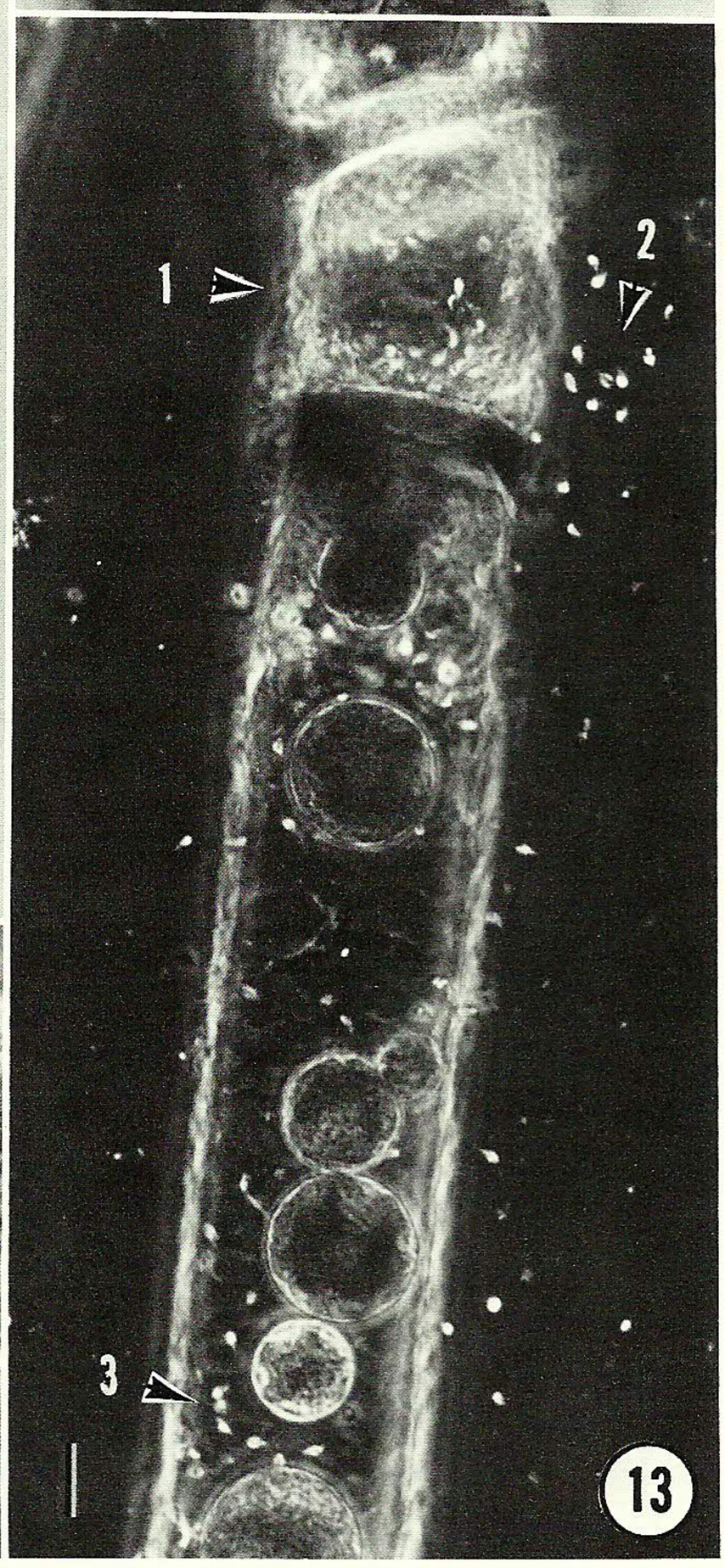
FIGS. 1–8. Vegetative and reproductive cells of *Sphaeroplea robusta*. Fig. 1. Vegetative filaments of *Sphaeroplea robusta*. Arrows indicate crosswalls and associated plugs. Fig. 2. Annular or band-shaped chloroplast components in a vegetative cell. Fig. 3. Reticulate chloroplast components in a vegetative cell. Fig. 4. Pair of crosswalls with intervening dense plug material (arrow) which separate adjacent cells. Anoptral contrast. Fig. 5. Extremely short antheridium containing mature male gametes. Crosswalls separate this antheridium from an oogonium on the left that contains eggs. Fig. 6. Two adjacent antheridia containing mature male gametes; adjacent oogonia contain eggs. Cells embedded in Spurr's resin. Fig. 7. Pair of liberated male gametes, one of which is curved (falcate). Anoptral contrast. Fig. 8. Mature male gametes within an antheridium. Nomarski. Scale bars represent 200 μm in Fig. 1, 20 μm in Figs. 2–6, and 10 μm in Figs. 7–8.











FIGS. 9–13. Gametangia and gametes of *Sphaeroplea robusta*. Fig. 9. Mature eggs within an oogonium adjacent to an empty antheridium (between arrows). Fig. 10. Oogonium with several young oospores that show early cell wall development (arrows). Fig. 11. Mature eggs with pyrenoids

antheridia and enter oogonia via pores formed in the gametangial walls (Fig. 14). Male gametes swim among the eggs prior to fusion (Figs. 12, 13).

The primary membrane and the sculpted oospore wall become apparent soon after fertilization (Fig. 14). As the oospore matures, the primary membrane is lost (Figs. 15, 16) and a bright orange-red pigment is produced. The oospore ranges in diameter from 29–71 μm, occasionally attaining a diameter almost equalling that of the oogonium (Fig. 15). The oospore wall is characterized by a network of raised ridges forming many polygonal depressions. Irregularly shaped, papillate to rugose projections typically occur in a cluster at the base of each depression (Figs. 17–19). Occasionally, a polygonal depression may be ornamented with only a few such projections or, rarely, they may be lacking altogether (Fig. 20). The projections vary in height and generally crest (Fig. 18, arrows) near the center of the depression, but rarely attain the prominence of the surrounding ridges. Rugose projections occasionally may connect directly with an adjacent ridge (Fig. 17, arrows).

Discussion

The most recent and comprehensive taxonomic treatments of the Sphaeropleaceae are those of Fritsch (1929), Palik (1950), and Ramanathan (1964). Both Fritsch and Palik present detailed synonymy and list references to most earlier literature on the group. Since Ramanathan's (1964) monograph, only one additional species of *Sphaeroplea* has been described (Sarma, 1974), bringing the total number of species in the genus to seven. The Sphaeropleaceae was considered a monotypic family until recently, when *Atractomorpha* with two new species was added (Hoffman, 1983, 1984).

Sphaeroplea species are distinguished primarily on the basis of size, shape, and cell wall ornamentation of mature oospores (Fritsch, 1929). Of lesser importance are differences in vegetative morphology, in particular, vegetative cell width and length, chloroplast morphology, and septal thickness and ornamentation (Fritsch, 1929; Ramanathan, 1964).

Comparisons of vegetative cell width (Table I) show that *S. robusta* is similar to *S. annulina* and its varieties, *S. soleirolii* (Duby) Montagne ex Kützing, *S. africana* Fritsch, and *S. chapmanii* Sarma (see Ramanathan, 1964; Sarma, 1974). Only *S. africana* var. *crassa* Fritsch possesses filaments of larger diameter than *S. robusta*, while filaments of *S. wilmani*, *S. soleirolii* var. *crassisepta* (Rieth) Ramanathan, and *S. tenuis* generally are smaller in diameter (Ramanathan, 1964). Although cell width in *S. tricarinata* Gauthier-Lièvre is similar to that of *S. robusta*, cell length is proportionally much longer.

Chloroplast morphology differs among the various species of *Sphaeroplea* (Table I), with annular, band-shaped chloroplasts reported for some species

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(arrows). Nomarski. Fig. 12. Male gametes (arrows) swimming among mature eggs inside an oogonium. Phase-contrast. Fig. 13. Portion of a filament showing oogonium with eggs and short antheridium (arrow 1) with male gametes. Many male gametes have been liberated (arrow 2) and others have entered the oogonium (arrow 3). Anoptral contrast. Scale bars represent 20 μm.

TABLE I

Comparison of *Sphaeroplea robusta* with the seven recognized species of *Sphaeroplea* in four vegetative and two reproductive characters[a]

Species	Cell width (μm)	Cell length (μm)	Plastid shape	Septal thickening or ornamentation	Short antheridia[b]	Oospore diameter (μm)
S. robusta	56–72	240–1,960	annular-reticulate	absent	present	29–71
S. annulina	24–72	400–1,400	annular	absent	absent	11–36
S. annulina var. *multiseriata*	60–80	400–1,400	annular	absent	absent	15–27
S. wilmani	24–34	25 times breadth	annular	present	absent	23–27
S. africana	42–78	—	reticulate	present	absent	20–33 × 12–21
S. africana var. *crassa*	112–170	—	reticulate	present	absent	26–31 × 21–26
S. tricarinata	30–60	900–3,600	reticulate	present	absent	28–47 × 20–30
S. soleirolii	34–57	Up to 2,200	annular-reticulate	absent	absent	20–60 × 27–46
S. soleirolii var. *crassisepta*	18–38	85–770	reticulate	present	absent	20–60 × 27–46
S. tenuis	8.5–15	Up to 1,000	annular	absent	absent	12–15
S. chapmanii	44–71	440–1,012	annular	absent	absent	26–40

[a] After Ramanathan (1964) except for *S. chapmanii* (see Sarma, 1974).

[b] The regular occurrence of short antheridia has not been mentioned specifically in any other species and is presumed not to occur. However, in the absence of published data on antheridial length, this feature should be viewed with some caution.

and reticulate chloroplasts for others (Fritsch, 1929; Ramanathan, 1964). Annular chloroplasts are typical of *S. annulina* and its varieties, *S. wilmani, S. tenuis,* and *S. chapmanii* (see Sarma, 1974), while reticulate plastids are reported in *S. africana* and its variety, *S. tricarinata,* and *S. soleirolii* var. *cras-*

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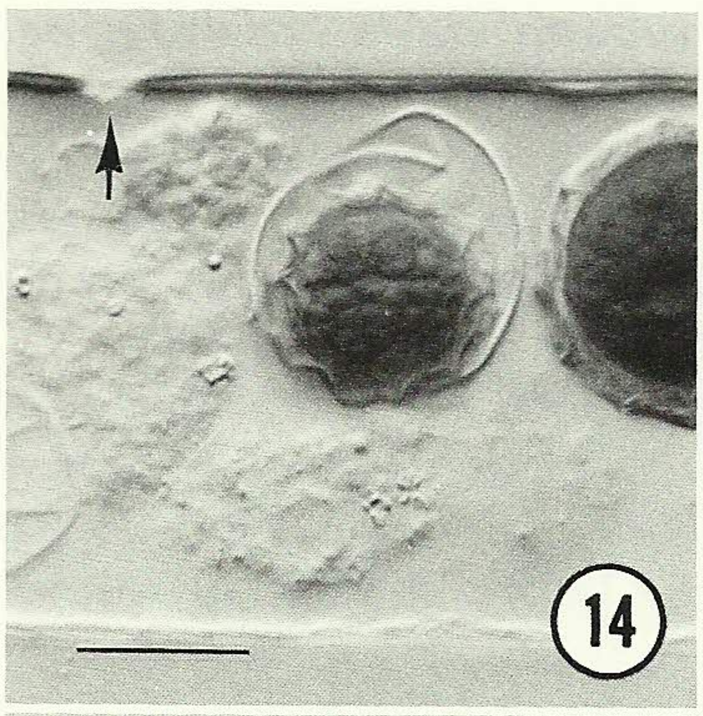
FIGS. 14–20. Oospores of *Sphaeroplea robusta*. Fig. 14. Young oospore with thickened cell wall and an intact primary membrane. Note pore present in the wall of the oogonium (arrow). Nomarski. Fig. 15. Portion of an oogonium containing oospores with thickened and ornamented walls. Adjacent are two sloughed primary membranes (arrows). Nomarski. Fig. 16. Portion of an oogonium with mature oospores of various sizes. Sloughed primary membranes are present between oospores. Fig. 17. Oospore exhibiting prominent ridges which form polygonal depressions ornamented with papillate to rugose outgrowths. Some of the rugose outgrowths connect with the prominent ridges (arrows). SEM. Fig. 18. Small oospore with clustered outgrowths which crest near the center of each polygonal depression (arrows). SEM. Fig. 19. Oospore with typical papillate outgrowths. SEM. Fig. 20. Atypical oospore showing polygonal depressions ornamented with only a few outgrowths; some lack ornamentation altogether. SEM. Scale bars represent 20 μm in Figs. 14–16 and 5 μm in Figs. 17–20.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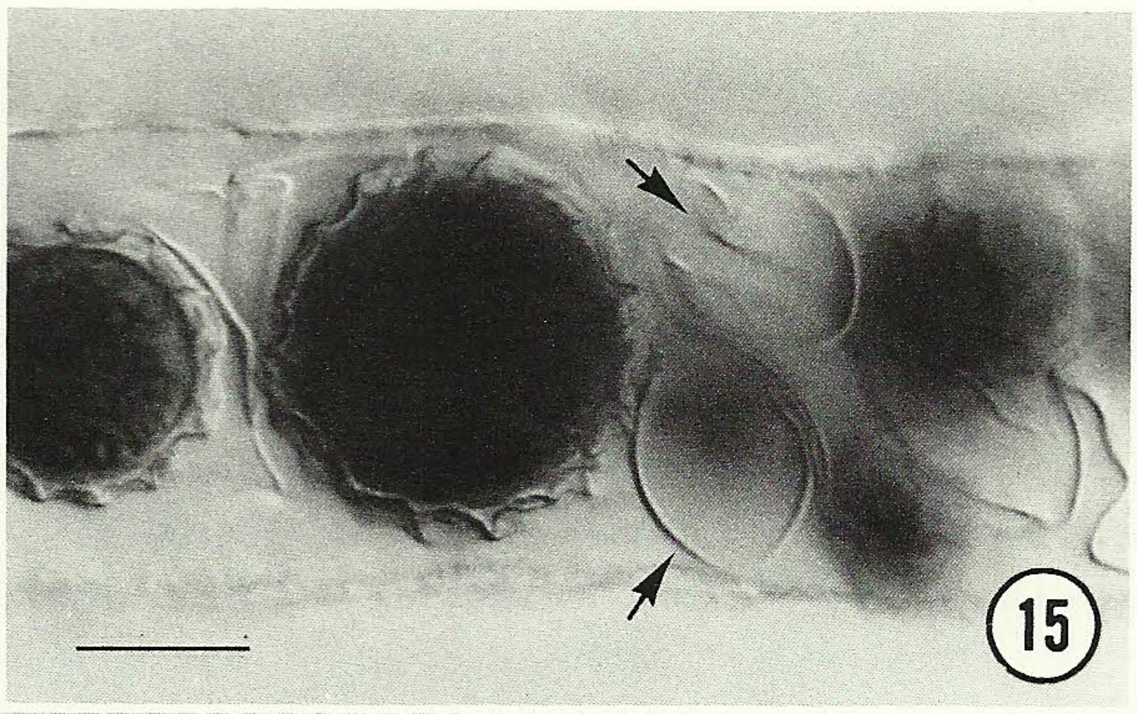
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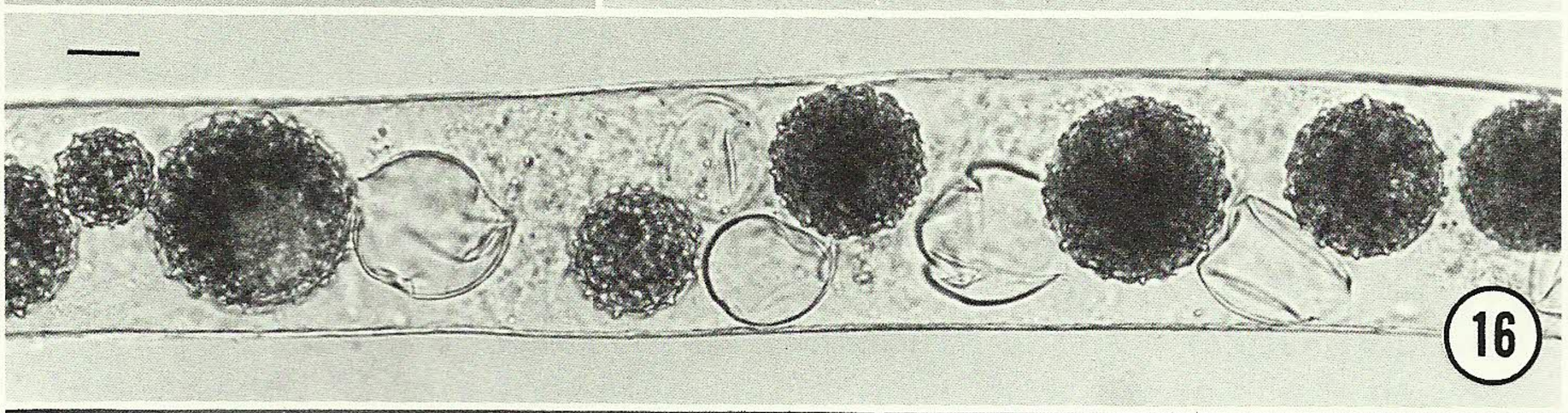
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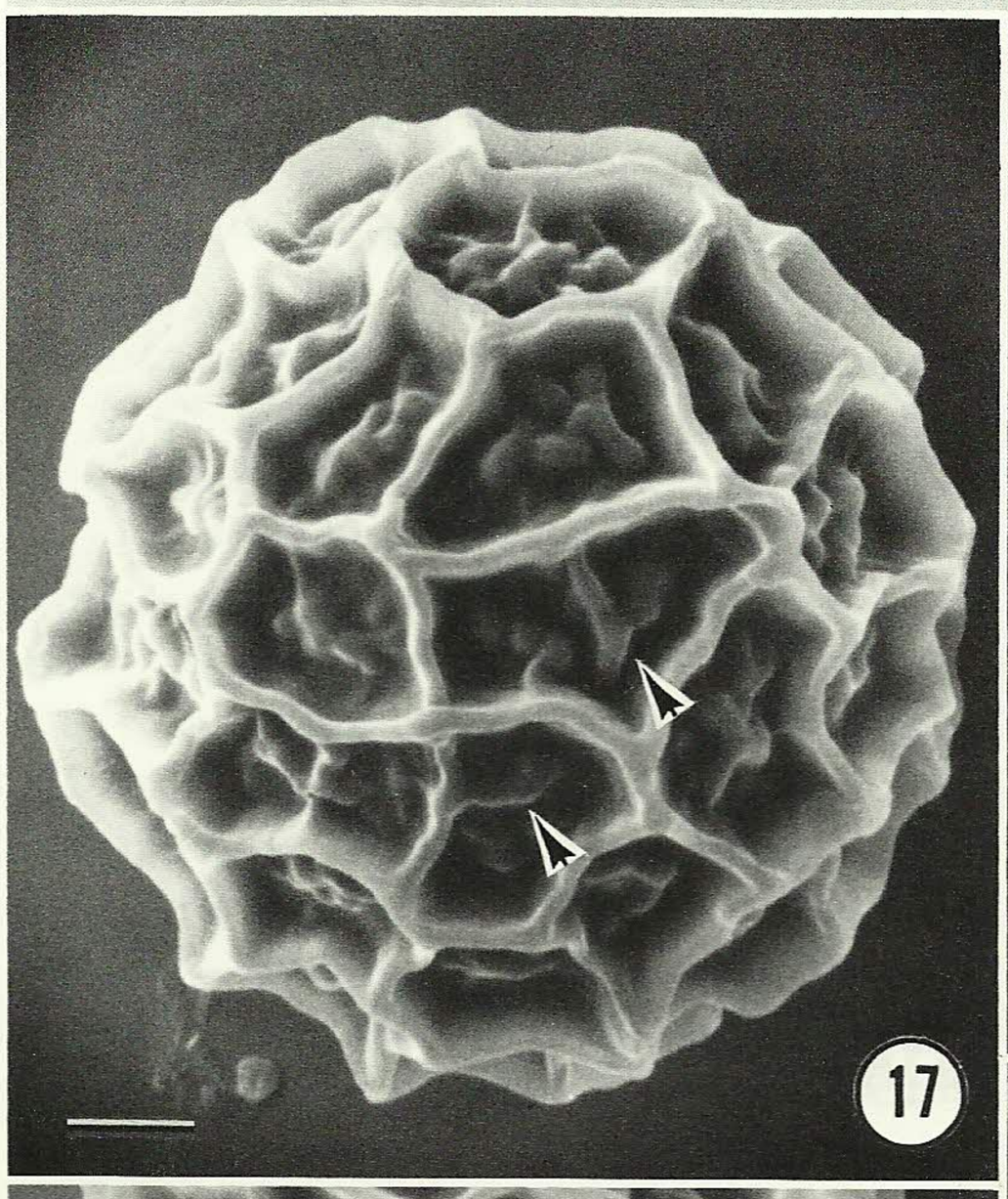
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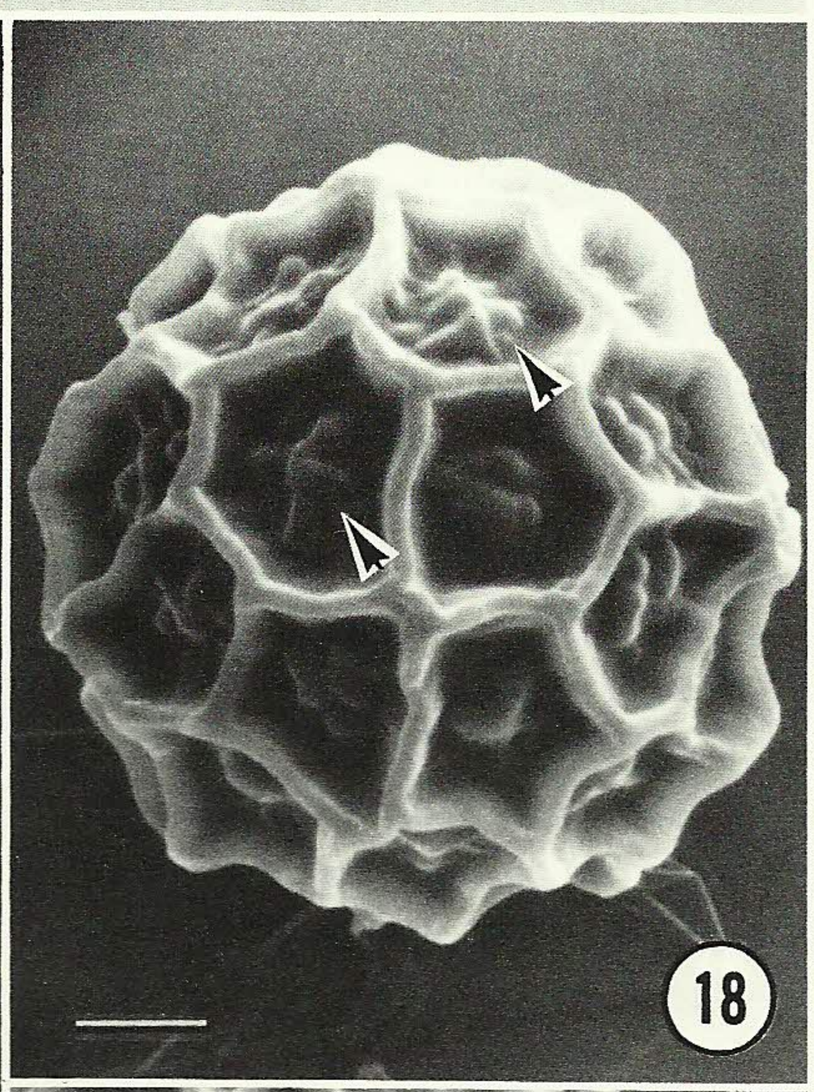
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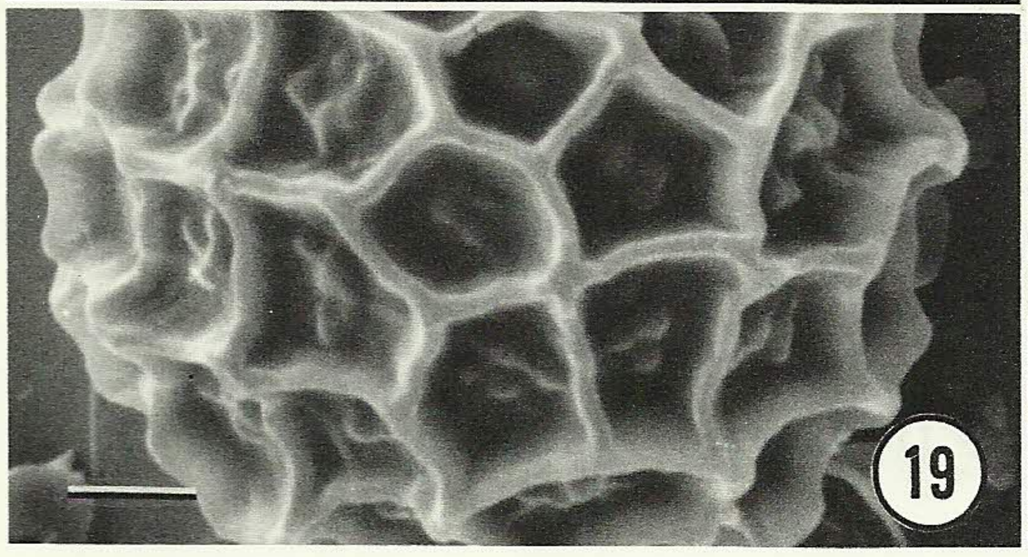
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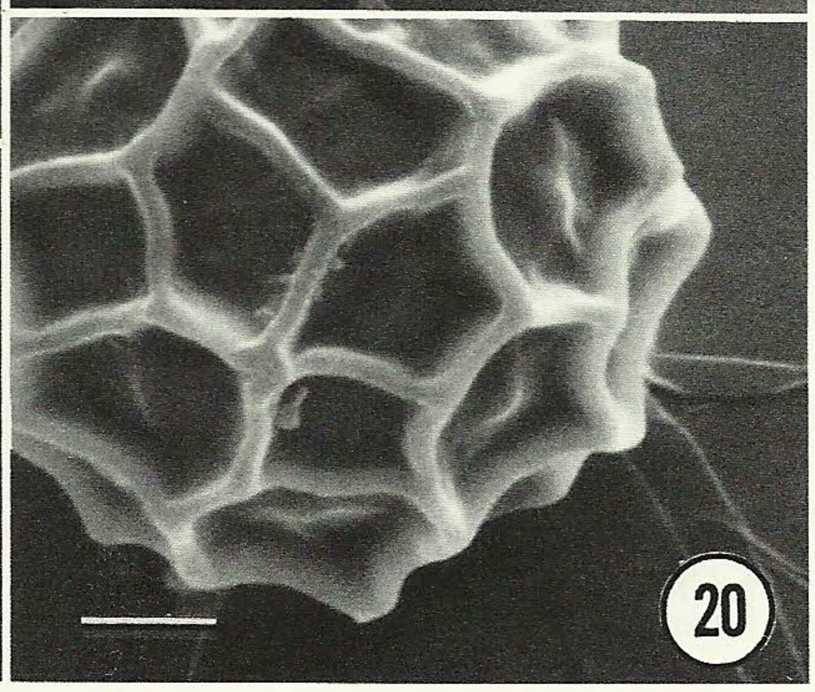
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sisepta. Both types of morphology occur in *S. robusta* and S. *soleirolii* (see Ramanathan, 1964).

Septal thickness and ornamentation also vary among species (Table I). *Sphaeroplea robusta* does not possess remarkably thickened or ornamented septa which, on the other hand, occur in *S. wilmani*, *S. africana* and its variety, *S. tricarinata*, and *S. soleirolii* var. *crassisepta* (see Ramanathan, 1964).

In the preceding comparison of vegetative characters, *S. robusta* clearly differs from four of the seven species, but cannot be easily distinguished from *S. annulina*, *S. soleirolii*, and *S. chapmanii*. However, when reproductive traits are compared, *S. robusta* may be distinguished readily from all other species.

Oospores of *S. robusta* are distinctive in size, shape, and ornamentation. They are larger than those of any other species, attaining a diameter up to 71 μm. Only in *S. soleirolii* do oospores approach the maximum size observed in *S. robusta*; however, its oospores differ greatly in shape and ornamentation.

Sphaeroplea robusta is easily distinguished from all but *S. annulina* (and its varieties) and *S. chapmanii* on the basis of oospore shape and the pattern of ridges on the surface of the oospore[2]. Each of the three species produce spherical to subspherical oospores ornamented with ridges that form a polygonal pattern on the oospore wall; however, only oospores of *S. robusta* and *S. chapmanii* (see Sarma, 1974) produce elaborate papillate to rugose projections within the polygonal depressions formed by the ridges. Oospores of *S. annulina* either lack such projections altogether, or demonstrate only very rudimentary projections (Hoffman, unpublished observations). Comparison of oospores of *S. robusta* and *S. chapmanii* shows that the latter possesses more prominent ridges that occasionally are almost wing-like. Moreover, the ridges in *S. chapmanii* ordinarily follow a more sinuous course than those of *S. robusta*.

One of the most distinctive features of *S. robusta* is the short length of its antheridia, a condition not reported for any other species of the genus. Antheridia of *S. robusta* are 32–520 μm (commonly 70–150 μm) long and thus are much shorter than adjacent vegetative cells (less than 5–50% of the length of most vegetative cells). Fritsch (1929), Ramanathan (1964), and Smith (1950) all noted that *Sphaeroplea* is unique among oogamous green algae in that gametangia arise from vegetative cells without any structural modification[3]. The antheridia of *S. robusta* may, however, be considered an exception since they are conspicuously shorter than the vegetative cells, and cannot be considered to arise directly from a typical vegetative cell. Although the details of antheridial development in *S. robusta* remain to be elucidated, it is obvious that cytokinesis must be involved in their formation.

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[2] Oospores of *Sphaeroplea annulina* have been described as possessing spines in addition to surface ridges (Fritsch, 1929; Ramanathan, 1964). However, when these oospores are examined with SEM, it can be demonstrated that what appear to be spines with the light microscope are, in fact, optical sections through ridges (Hoffman, unpublished observations).

[3] Other oogamous green algae now known to share this feature include the sphaeropleacean alga *Atractomorpha* (see Hoffman, 1983) and a number of chlorococcalean genera (e.g., *Eremosphaera*, see Kies, 1967; *Golenkinia*, see Korshikov, 1937 and Starr, 1963; and *Oocystaenium*, see Gonzalves & Mehra, 1959).

Fritsch (1929) states that oospore structure is the most variable feature within the genus *Sphaeroplea* and serves as the primary criterion for species distinction. We have shown that *S. robusta* produces oospores distinctive in both size and ornamentation. Moreover, *S. robusta* regularly produces remarkably short antheridia. These reproductive characteristics clearly distinguish this Texas species from all others in the genus.

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