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SYSTEMATICS OF THE ZYGNEMATACEAE (CHLOROPHYCEAE). II. ZYGOSPORE-WALL STRUCTURE IN *SIROGONIUM* AND A TAXONOMIC PROPOSAL[1]

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ABSTRACT

Zygospore-wall structure is described for nine species of Sirogonium *using the light microscope and scanning electron microscopy (SEM) on spores from cultures and on glycerin-preserved spores from field collections. Zygospores possess four distinctive wall layers with the innermost and outermost smooth and composed of cellulose. The two median wall layers are pigmented and the innermost of these may be conspicuously ornamented as in* Sirogonium ceylanicum *Wittrock,* S. hui *(Li) Transeau,* S. melanosporum *(Randhawa) Transeau,* S. ventersicum *Transeau,* S. pseudofloridanum *(Prescott) Transeau and* S. illinoisense *(Transeau) G. M. Smith. Inner median wall layers of* S. floridanum *(Transeau) G. M. Smith,* S. sticticum *(J. E. Smith) Kützing and* S. tenuis *(Nordstedt) Transeau lack distinctive ornamentation, but possess minor depressions or small raised areas on their surfaces. Types of ornamentation revealed by SEM are compared with earlier light microscope observations and the interpretations from SEM are used to construct improved taxonomic keys to* Sirogonium.

Key index words: green algae; sexual reproduction, Sirogonium; Sirogonium; *wall ornamentation, zygospore; Zygnemataceae; zygospore,* Sirogonium.

During the last three decades the family Zygnemataceae (*Spirogyra* and relatives) has been monographed three times (Transeau 1951, Randhawa 1959, Kadlubowska 1972). Even though these volumes with their descriptions of species and keys are indispensable frameworks for the taxonomy of the Zygnemataceae, they are mostly compilations which fail to reflect even the limited experimental taxonomic data dealing with species complexes, ploidy and hybridization. Because of their wide scope and total reliance on field collections, with data lacking from culture studies, these volumes prove to be, more and more, inadequate for even making identifications. Only experimental studies will reduce this inadequacy by adding to data already known from field collections.

Identification in the Zygnemataceae is typically a difficult task because sexual material with zygospores is required. Additional difficulty arises when an interpretation of the ornamentation, or lack of it, must be made for the median wall layer or layers of zygospores. Thus, published species lists often include only the generic name for zygnematacean species.

The taxonomic treatment in monographs of the Zygnemataceae is based on several vegetative and reproductive features. These include cell length and width, chloroplast structure and number, type of conjugation (scalariform or lateral), gametangial size and shape, and the length, width, color, number of wall layers and ornamentation of the median wall of the zygospore. The present paper demonstrates the taxonomic importance of zygospore structure in *Sirogonium* as it is viewed with the scanning electron microscope (SEM). Interpretations of wall structure are compared with earlier light microscope observations, and a proposal is described for using zygospore-wall structure as the primary taxonomic criterion for constructing keys to species of *Sirogonium.*

MATERIALS AND METHODS

Specimens with zygospores for examination were from unialgal cultures maintained in the Algal Research Laboratory at the University of Arizona and from glycerin-preserved filaments on slides in the Collection of the Zygnemataceae curated by E. N. Transeau (Transeau Coll.) of The Ohio State University, Columbus (Table 1). All unialgal cultures were isolated originally as clonal cultures in test tubes and later grown in one-half pint milk bottles of soil-water medium (Starr 1978). These strains were grown and manipulated, whenever direct light was required, un-

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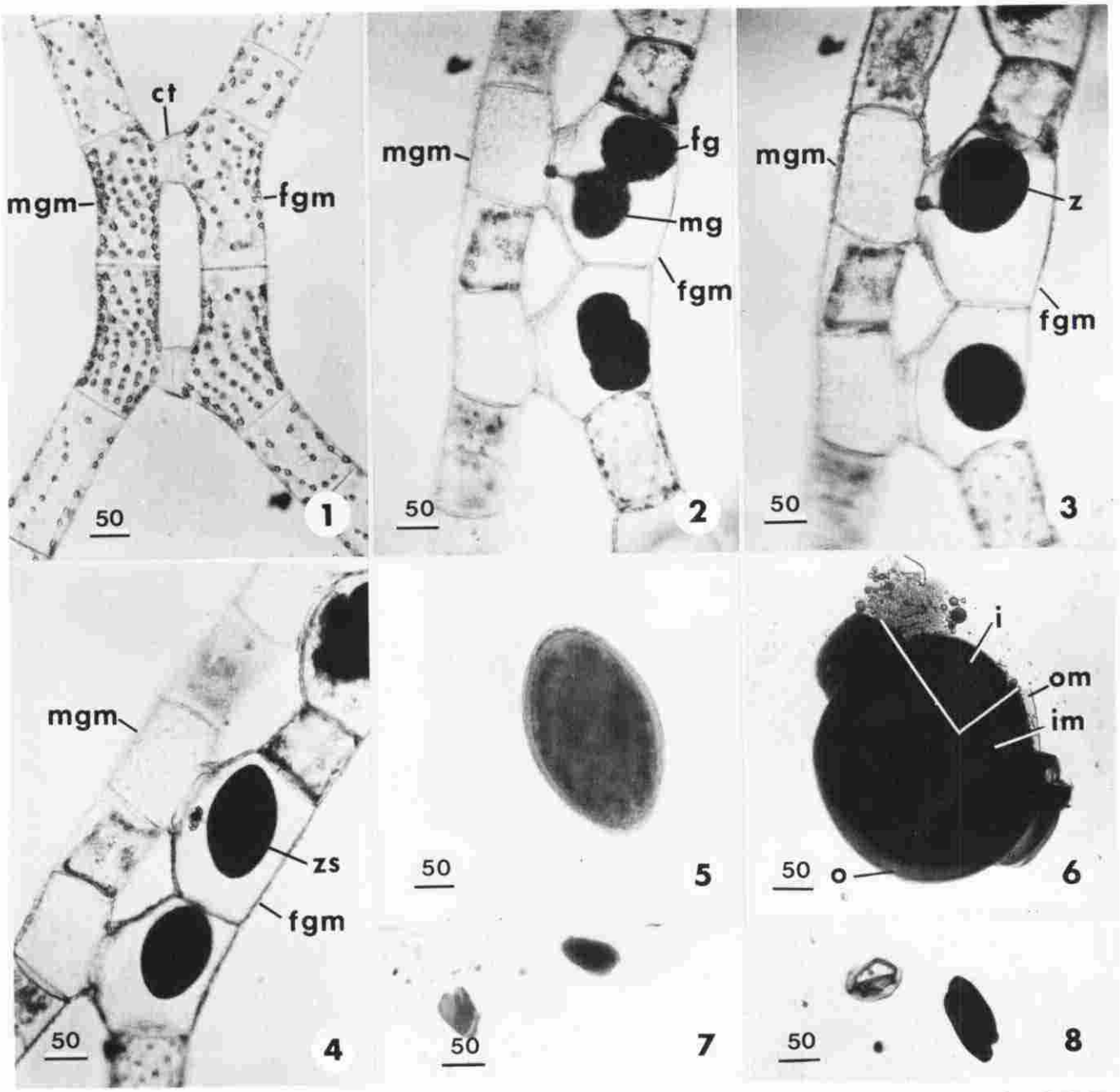
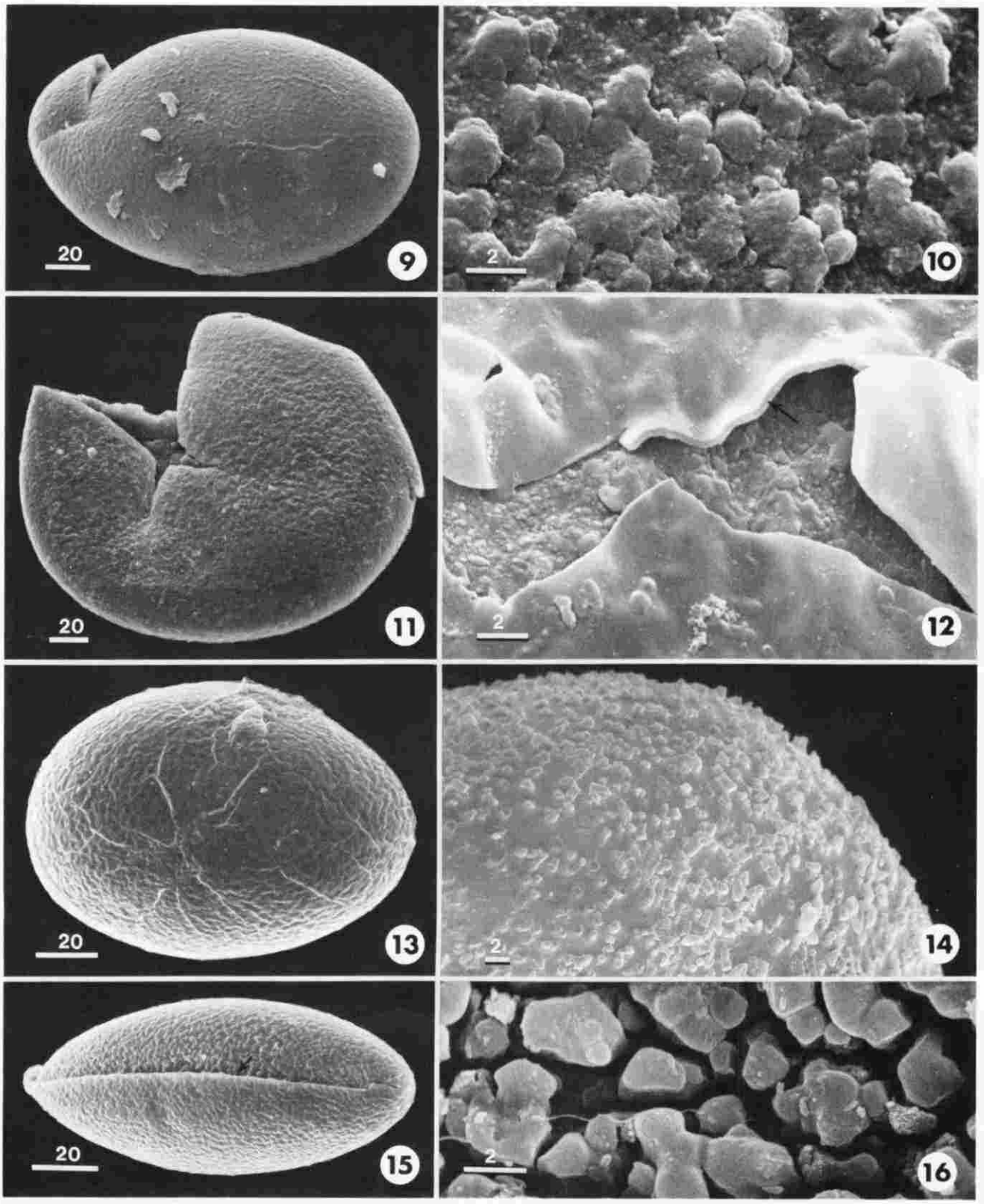


FIGS. 1–8. Light micrographs showing conjugation and zygospore structure in cultured *Sirogonium melanosporum* (Hoshaw 716). FIG. 1. Double conjugation with male and female gametangia (*mgm*, *fgm*) connected by conjugation tubes (*ct*). FIG. 2. Male and female gametes (*mg*, *fg*) in contact in female gametangia; upper male gamete shown 9 min after beginning of flow into female gametangium. FIG. 3. Young zygote (*z*) in female gametangium 29 min after beginning of male gamete flow. FIG. 4. Young 24 h old zygospore (*zs*). FIG. 5. 28 day old zygospore after removal from female gametangium. FIG. 6. Four zygospore-wall layers: outer wall layer (*o*), outer median wall layer (*om*), inner median wall layer (*im*) large dark stained area, inner wall layer (*i*) outlined in V-shaped area; stained with IKI-H_2SO_4. FIG. 7. Inner wall layer (left) and outer wall layer (right); stained with IKI-H_2SO_4 and showing a positive test for cellulose. FIG. 8. Outer median wall layer (left) and inner median wall layer (right); stained with IKI-H_2SO_4 and showing a positive test for chitin. All scales in μm.

der 20-w cool-white fluorescent lamps of ca. 2,500 lx on 16:8 LD at 20 ± 2 C. Zygospores were identified as mature by their ellipsoid-ovoid shape and yellow-brown color. Prior to use for cytochemical tests or SEM, spores were removed from gametangia by means of No. 11 surgical blades.

For determination of cellulose and chitin in the wall layers of zygospores, the cytochemical tests described by Jensen (1962) were used.

Median spore-wall layers were examined by SEM using air-dried zygospores. Preparation of spores involved the following procedures: removal of outer wall layer and/or outer median wall layer by means of No. 11 surgical blades using a stereomicroscope; transfer of spores, following wall removal, to specimen holders with attachment by double stick tape; air drying for 24 h prior to carbon and gold-palladium coating (20–40 nm) in Denton DV515 vacuum evaporator. Specimens were examined and



FIGS. 9–16. Scanning electron micrographs of cultured and glycerin-preserved *Sirogonium* zygospores showing verrucose ornamentation of inner median wall layer. FIG. 9. *S. ceylanicum* (glycerin-preserved) with outer wall layer removed, exposing outer median wall. FIG. 10. Ornamentation of inner median wall layer of *S. ceylanicum* (glycerin-preserved) following removal of outer wall and outer median wall layers. FIGS. 11, 12. *S. hui* (glycerin-preserved): FIG. 11, outer wall layer removed and outer median wall exposed; FIG. 12, ornamentation of inner median wall showing through a break in outer median layer (arrow); outer wall layer removed. FIGS. 13, 14. Cultured zygospores of *S. melanosporum* (Hoshaw 716): FIG. 13, outer wall layer removed and outer median wall layer showing; FIG. 14,

TABLE 1. *Source of zygospores for 9 species of* Sirogonium *used for scanning electron microscopy.*

Species	Specimen type	Collection location, date	Collector; Isolator
S. ceylanicum	Glycerin-preserved	Sri Lanka (Ceylon), 1914	Transeau Coll.
S. floridanum	Glycerin-preserved	Africa: Cape Town, South Africa[a]	E. L. Stephens
S. hui	Glycerin-preserved	China: Yishan, Wu-ning, 1938	H. K. Teng
S. illinoisense	Glycerin-preserved	U.S.A.: Oklahoma, 1964	C. E. Taft
S. melanosporum	Culture (Hoshaw 716)	Mexico: Toluca, Mexico, 1964	A. E. Dennis; K. M. Kobriger
S. pseudofloridanum	Culture (Hoshaw 702)	U.S.A.: Kalispell, Montana, 1963	G. W. Prescott; R. W. Hoshaw
S. sticticum	Culture (Hoshaw 707)	U.S.A.: Ellettsville, Indiana, 1963	R. W. Hoshaw; R. W. Hoshaw
S. tenuis	Culture (Hoshaw 705)	Mexico: Toluca, Mexico, 1964	A. E. Dennis; R. L. Hilton
S. ventersicum	Glycerin-preserved	Africa: Ventersdorp, South Africa[a]	E. L. Stephens

[a] Collection date not on slide label of glycerine mount in Transeau Collection.

photographed with an ETEC Autoscan or Cambridge S4-10 electron microscope at 20 kV.

RESULTS

Zygospores are produced by the process of conjugation when filaments align and opposite cells become gametangia connected by conjugation tubes (Fig. 1). Figures 2–5 show the formation of zygospores in *S. melanosporum* (Hoshaw 716). Protoplasts of male gametangia form male gametes which flow as globular material through conjugation tubes. Male gametes reorganize in female gametangia and contact female gametes (Fig. 2) with fusion resulting in spherical zygotes (Fig. 3). Within 24 h zygotes become ellipsoidal and wall thickening has begun (Fig. 4). By 7 days zygospores possess thick, layered walls with a distinct yellow-brown color in the median wall layers (Fig. 5).

Zygospores of the nine species possess walls with four distinctive layers (Figs. 6–8). The outermost and innermost layers are colorless and composed of cellulose. The two median layers are yellow-brown with more of the pigmentation in the thicker, inner median wall layer. Ornamentation, when present, develops as part of the inner median wall. The median wall layers were negative for cellulose but positive for chitin as revealed by tests described in Jensen (1962).

Surface features of median wall layers of zygospores are revealed by the scanning electron micrographs in Fig. 9–25. Outer median wall layers of all species lack ornamentation but because of their thinness they may reflect the ornamentation of the inner median wall beneath. Six species (*S. ceylanicum, S. hui, S. illinoisense, S. melanosporum, S. pseudofloridanum, S. ventersicum*) possess zygospores with conspicuously ornamented inner median wall layers. In three species (*S. floridanum, S. sticticum, S. tenuis*) the wall surface lacks a distinctive pattern of ornamentation but does contain minor depressions or raised areas.

Median walls with verrucae. The inner median walls of *S. ceylanicum, S. hui, S. melanosporum* and *S. ventersicum* are verrucose with a dense arrangement of warty structures (Figs. 9–16). The outer median wall that covers these warty structures has an irregular wavy or rippled appearance (Figs. 9, 11–13, 15). Verrucae of *S. ceylanicum* (Fig. 10) are irregular in shape, rounded, rough-appearing, and often fused in groups of two or more. Major verrucae project above a surface densely covered with smaller verrucae. The large and small verrucae of *S. hui* (Fig. 12) are similar to those of *S. ceylanicum.*

Verrucae of *S. melanosporum* are irregular and mostly of one size class (Fig. 14). They vary from angular, flat-topped projections to elongate, roundish projections with pointed tips. Verrucae are densely scattered on the inner median wall as mostly single projections, but they occasionally touch to form groups of two or more. The verrucae of *S. ventersicum* (Fig. 16) are similar to *S. melanosporum* in being irregular, angular, and flattopped. However, the verrucae are more numerous and more closely spaced in *S. ventersicum* than in *S. melanosporum* and at high magnification verrucae show conspicuous connections by cylindrical structures (Fig. 21) that have also been seen in *S. melanosporum.*

Median walls with ornamentation other than verrucae. The inner median wall of *S. pseudofloridanum* is conspicuously ornamented, possessing a rough appearance of ridges and depressions with circular pits in the surface (Fig. 18). The spore wall is best described as scrobiculate when the wrinkled outer median wall is removed. The most uniquely ornamented inner median wall is that of *S. illinoisense* with its distinctively raised net-like reticulation and accompanying protuberances (Figs. 19, 20). Intermittently spaced along the reticulation are numerous protuberances with hollow crater-like centers (Fig. 22).

Nearly smooth median walls. Median walls of *S. floridanum, S. sticticum* and *S. tenuis* possess no distinct

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ornamentation of inner median wall following removal of outer wall and outer median wall layers. FIGS. 15, 16. *S. ventersicum* (glycerin-preserved): FIG. 15, outer wall layer removed and outer median wall layer showing; note suture (arrow); FIG. 16, ornamentation of inner median wall layer following removal of outer wall and outer median wall layers. All scales in μm.

TABLE 2. *Surface character of inner median zygospore walls of* Sirogonium *as interpreted by light and scanning electron microscopy.*

Species[a]	Light microscopy (Transeau 1951)	Scanning electron microscopy
S. ceylanicum	Minute shallow depressions that have no distinct edges but are easily seen when viewed edgewise	Verrucose
S. floridanum	Smooth	Nearly smooth
S. hui	Verrucose	Verrucose
S. illinoisense	Scattered protuberances connected by a more or less prominent reticulum	Reticulate with crater-like protuberances
[*S. indicum*]	Scrobiculate	—
[*S. megasporum*]	Smooth	—
S. melanosporum	Verrucose	Verrucose
[*S. phacosporum*]	Finely reticulate-scrobiculate	—
S. pseudofloridanum	Finely corrugate and granulate	Scrobiculate
S. sticticum	Smooth	Nearly smooth
S. tenuis	Smooth	Nearly smooth
S. ventersicum	Densely and irregularly verrucose	Verrucose

[a] Species with names in brackets [ ] not available for investigation.

pattern of ornamentation. When viewed with the light microscope, the walls are difficult to interpret, but they appear almost smooth. Irregularly spaced depressions and raised structures are revealed by SEM (Fig. 23–25).

DISCUSSION

The number of zygospore-wall layers and the ornamentation of median walls in six species are at variance with earlier reports from light microscopy (Transeau 1951). *Sirogonium hui* is the only species previously reported to possess four zygospore wall layers and Transeau (1951, p. 235) states: "This is the largest species in the genus and the only one with a double median spore wall." All nine species considered here have four wall layers which are revealed by separation with surgical blades prior to SEM.

Six of the nine species examined have median walls with ornamentation different from the descriptions compiled by Transeau (1951). Light microscope and SEM observations are compared in Table 2. Four patterns of ornamentation occur rather than six. There are verrucose, scrobiculate, reticulate with craterlike protuberances and nearly smooth. The greatest differences occur in *S. ceylanicum* which is distinctly verrucose (Fig. 10), and in *S. pseudofloridanum* which is scrobiculate (Fig. 18). Previously undescribed protuberances of *S. illinoisense* can now be described as crater-like and empty. The surfaces of the inner median walls of *S. floridanum, S. sticticum* and *S. tenuis*, while reported as smooth in Transeau (1951), are interrupted by depressions and raised structures as revealed by SEM. This observation suggests that completely smooth inner median walls may not exist.

Four of the 12 species compiled by Transeau (1951) are reported with smooth median walls. Examination of spores for three of these species shows small depressions and raised structures which are difficult to identify as a pattern of ornamentation. However, these median walls are not entirely smooth (Figs. 23–25), and should be referred to as being nearly smooth. Transeau (1951, p. 16) recognized this problem when he states: "Some of the older species were described as having smooth median spore walls, although recent study of type specimens has shown them to be punctate. This circumstance is probably explained by the poor resolving power of the microscope lenses of the last century as compared with those available today." Another breakthrough has arrived with an improved interpretation of zygospore-wall ornamentation now provided by SEM.

Improved interpretations of spore-wall structure by SEM are useful for the revision of species descriptions and the construction of taxonomic keys. The number of wall layers and wall ornamentation,

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FIGS. 17–25. Scanning electron micrographs of cultured and glycerin-preserved zygospores showing various ornamentation of inner median wall. FIGS. 17, 18. Cultured zygospore of *S. pseudofloridanum* (Hoshaw 702): FIG. 17, outer wall layer removed and outer median wall layer showing, following removal of outer wall and outer median wall layers: FIG. 18, scrobiculate ornamentation of inner median wall following removal of outer wall and outer median wall layers. FIGS. 19, 20. Glycerin-preserved zygospore of *S. illinoisense*: FIG. 19, inner median wall layer showing reticulate ornamentation following removal of outer wall and outer median wall layers; FIG. 20, enlarged view of reticulate ornamentation showing crater-like protuberances. FIG. 21. Enlarged view of verrucae of inner median wall of glycerin-preserved *S. ventersicum* zygospores showing cylindrical structures (arrow). FIG. 22. Enlarged view of two empty crater-like protuberances on reticulate ornamentation of *S. illinoisense*. FIGS. 23–25. Glycerin-preserved zygospores of *S. floridanum, S. sticticum* and *S. tenuis*, respectively, showing minor ornamentation following removal of outer wall and outer median wall layers; note small depressions and raised structures (arrows). All scales in μm.

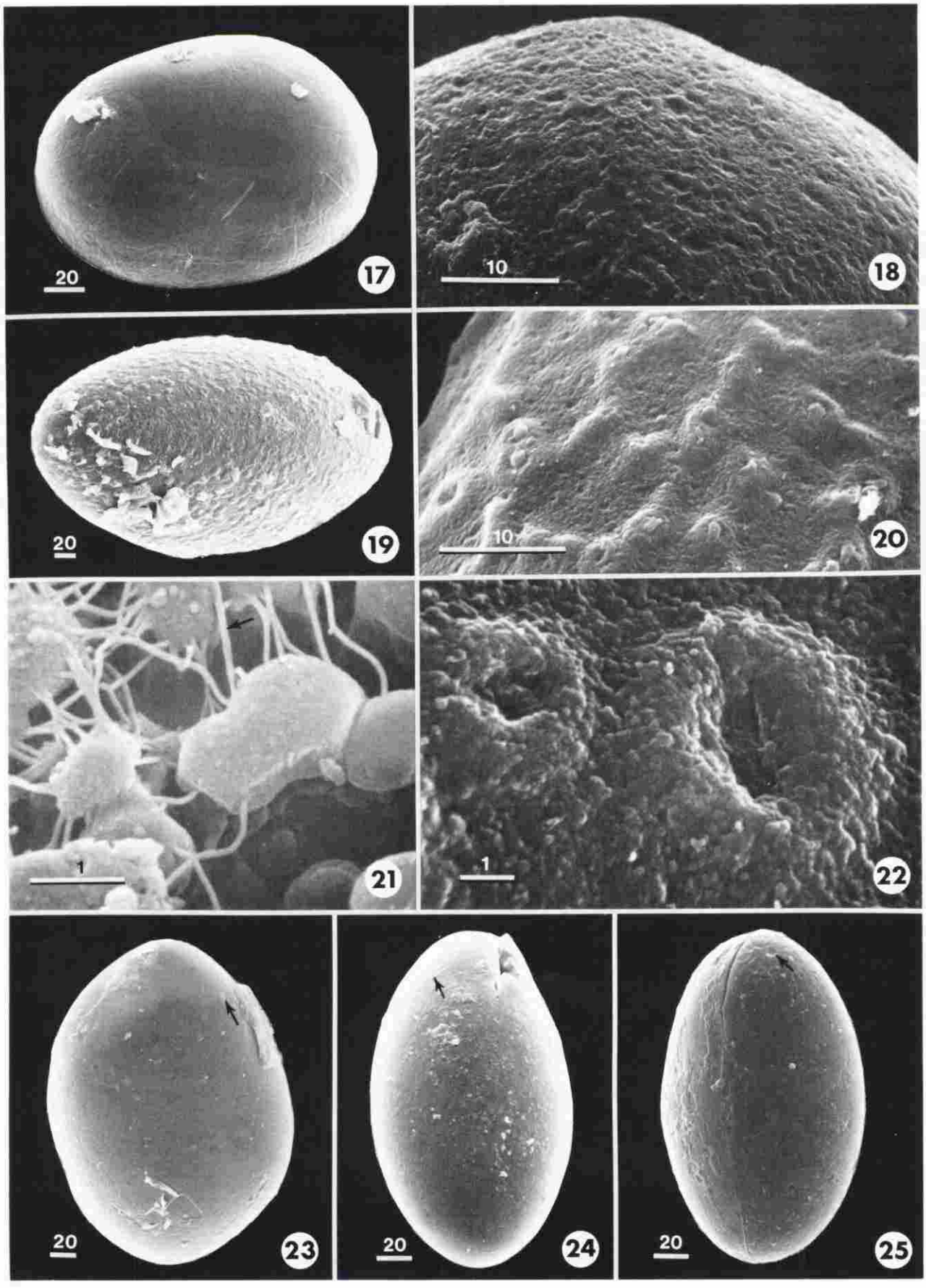
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TABLE 3. *Summary of four characteristics of eight species of* Sirogonium.

Species[a]	Characters			
	Vegetative cell width[c] (μm)	Inner median zygospore wall	Zygospore color[c]	Chromosome number
S. tenuis	32–36	Nearly smooth	Yellow	53 ± 2
S. sticticum	38–56	Nearly smooth	Yellow	53 ± 2
S. megasporum[b]	48–55	Smooth[c]	Yellow-brown	—
S. floridanum	56–66	Nearly smooth	Yellow	100 ± 2
S. ventersicum	65–72	Verrucose	Brown	—
S. ceylanicum	69–75	Verrucose	Yellow-brown	—
S. melanosporum	70–90	Verrucose	Brown to black	6
S. hui	82–108	Verrucose	Yellow	—

[a] Listed by increasing filament width.
[b] Species was not available for investigation.
[c] Data from Transeau (1951).

or the lack of it, are probably stable taxonomic characteristics compared to other vegetative and reproductive features. Characteristics that are variable and difficult to interpret include: filament width and length, chloroplast number, and zygospore length, width and color. Therefore, spore-wall structure should be relied on heavily as a major taxonomic criterion.

A proposal is made here on how species descriptions and keys may be revised. Although the two revised keys constructed here demonstrate the combination of certain species, these revisions do not propose formal combinations of taxa at this time. Needed yet are long-term culture studies to determine the variability of dimensions for vegetative cells and zygospores, and chromosome counts are needed to establish the relationships of species and the role of ploidy in speciation. Miller and Hoshaw (1974) have already demonstrated that cell width is not a valid taxonomic character for *Zygnema circumcarinatum* Czurda. Further, Allen (1958) found the occurrence of a species complex of three filament-width groups in a clonal culture of *Spirogyra pratensis* Czurda. She confirmed these width groups as being haploids, diploids and tetraploids and a polyploid series with the width groups showing conformity to the descriptions of *Sp. pratensis, Sp. parvula* (Transeau) Czurda and *Sp. catenaeformis* (Hassall) Kütz. These two cases demonstrate the uncertainties of speciation in zygnematacean algae, and suggest that additional studies should be conducted to establish the validity of presently described species.

Table 3 summarizes four characteristics of species with smooth or nearly smooth walls and those with verrucose walls and provides data used to combine species. The four species now described with smooth or nearly smooth spore walls may be combined into a single species, *S. sticticum.* Transeau (1951, p. 233) recognized *S. sticticum* as a variable species when he states: "The numbers of chromatophores, cell diameters, and spore dimensions are highly variable." Chromosome data and hybridization studies conducted in our laboratory (Wells and Hoshaw 1971, Scalione 1974) support the decision to combine species. *Sirogonium floridanum, S. sticticum* and *S. tenuis* possess numerous (53 ± 2 to 100 ± 2), minute dotlike chromosomes (Wells and Hoshaw 1971). Since *S. tenuis* and *S. sticticum* have identical chromosome numbers, and Scalione (1974) obtained zygotes from matings of one strain of *S. tenuis* (Hoshaw 705) with three strains of *S. sticticum* (Hoshaw 703, 707, 712), *S. tenuis* appears to be a small form of *S. sticticum. Sirogonium floridanum* with 100 ± 2 chromosomes could be interpreted as a diploid form of *S. sticticum,* although here it is maintained as a separate species pending further study. Although specimens of *S. megasporum* have not been examined in the present study, this species seems to differ from *S. sticticum* primarily in having large diameter zygospores (Jao 1935). This earlier report described *S. megasporum* as a variety of *S. sticticum* so here this alga is referred to as *S. sticticum.*

The four species with verrucose median zygospore walls are a set of species with filaments of greater cell diameter than those with nearly smooth walls (Table 3). Further, they represent a series with increasing cell diameter starting with *S. ventersicum.* Chromosome and hybridization data are lacking for these species, except that it is known that *S. melanosporum* has six rod-shaped chromosomes (Hoshaw and Waer 1967). *Sirogonium melanosporum* and *S. ventersicum* appear closely related because they both have verrucae of one size class that are irregular in shape and mostly separate and distinct. Also, these species have verrucae connected by cylindrical structures like those shown in Figure 21 for *S. ventersicum.* Earlier Randhawa (1938) referred to *S. melanosporum* as *S. ventersicum* var. *melanosporum,* and Transeau (1951, p. 235) states for *S. melanosporum*: "Differs from *S. ventersicum* in being larger in all dimensions, and in having a black rather than a brown median spore wall." Two cultured strains of *S. melanosporum* (Hoshaw 700, 716) have consistently produced yellow-brown spore walls.

Sirogonium ceylanicum and *S. hui* differ greatly in cell diameter but appear closely related because they

possess similar median spore-wall ornamentation. In these species the verrucae are irregular in shape, rounded, rough-appearing and often fused in groups of two or more. In addition, the larger verrucae project above a surface densely covered with small verrucae.

Although two patterns of verrucae exist in the four species, the verrucose ornamentation does not differ sufficiently to distinguish separate species. Likewise, the progression of increasing cell diameters of the four species is considered less important than their verrucose spore-wall ornamentation. These four species may be combined into one with the priority of *S. ceylanicum* used to name them.

Since three of the 12 species (*S. phacosporum, S. indicum, S. megasporum*) compiled by Transeau (1951) have not been examined by the author, they are discussed here in only a cursory manner. *Sirogonium phacosporum* appears distinctive because of its lenticular shaped zygospores, all other species possessing zygospores that vary between ellipsoid and ovoid. Based on spore-wall ornamentation, *S. indicum* resembles *S. pseudofloridanum* but differs in having cells with greater diameter and more chloroplasts. Even though cell diameter and chloroplast number are highly variable characteristics, *S. indicum* should be maintained as a species until specimens can be examined. As discussed earlier, *S. megasporum* is best referred to *S. sticticum* since the large diameter zygospore is an inadequate character for species status.

The interpretations of spore-wall structure presented in this paper for nine of 12 species of *Sirogonium* described by Transeau (1951) may be used to revise his key to species in a conservative way as shown in Key A.

KEY A TO SPECIES OF *SIROGONIUM*

1. Zygospores lenticular ..... *S. phacosporum*
1. Zygospores ellipsoid or ovoid
   2. Inner median zygospore wall smooth or nearly so
      3. Vegetative cells 36 μm or less diam .... *S. tenuis*
      3. Vegetative cells greater than 36 μm diam
         4. Vegetative cells 38–56 μm diam
            5. Zygospore width 70 μm or less .. .. *S. sticticum*
            5. Zygospore width greater than 70 μm ..... *S. megasporum*
         4. Vegetative cells greater than 56 μm diam ..... *S. floridanum*
   2. Inner median zygospore wall reticulate, scrobiculate or verrucose
      6. Inner median zygospore wall reticulate or scrobiculate
         7. Inner median zygospore wall reticulate ..... *S. illinoisense*
         7. Inner median zygospore wall scrobiculate
            8. Vegetative cells 51–60 μm diam; chloroplasts 4 to 5 .... *S. pseudofloridanum*
            8. Vegetative cells 65–80 μm diam; approximately 7 chloroplasts .... *S. indicum*
      6. Inner median zygospore wall verrucose
         9. Zygospores yellow or yellow-brown
            10. Vegetative cells 69–75 μm diam; zygospores yellow-brown .... *S. ceylanicum*
            10. Vegetative cells 82–108 μm diam; zygospores yellow ..... *S. hui*
         9. Zygospores brown or brown to black
            11. Vegetative cells 65–72 μm diam; zygospores brown .. *S. ventersicum*
            11. Vegetative cells 70–90 μm diam; zygospores brown to black .... *S. melanosporum*

If the variability of cell width is considered along with known chromosome numbers and earlier taxonomic interpretations, a major revision of the key may be constructed to include only 7 of the 12 species as shown in Key B.

KEY B TO SPECIES OF *SIROGONIUM*

1. Zygospores lenticular ..... *S. phacosporum*
1. Zygospores ellipsoid or ovoid
   2. Inner median zygospore wall smooth or nearly so
      3. Vegetative cells 56 μm or less diam .... *S. sticticum*
      3. Vegetative cells greater than 56 μm diam ..... *S. floridanum*
   2. Inner median zygospore wall reticulate, scrobiculate or verrucose
      4. Wall reticulate ..... *S. illinoisense*
      4. Wall scrobiculate or verrucose
         5. Wall scrobiculate
            6. Vegetative cells 51–60 μm diam; chloroplasts 4 to 5 .... *S. pseudofloridanum*
            6. Vegetative cells 65–80 μm diam; chloroplasts approximately 7 .... *S. indicum*
         5. Wall verrucose ..... *S. ceylanicum*

Because of its conservative nature, zygospore-wall ornamentation has been treated as a major criterion for speciation in *Sirogonium*. Likewise, chromosome number and structure are major criteria, but their use in delimiting species is now limited by a lack of data. Few specimens exist for cytological study because even though *Sirogonium* is worldwide in distribution, collections are rare except for *S. sticticum*.

Before additional species of *Sirogonium* and other zygnematacean algae are described, long-term cul-

ture studies should be conducted. Although adequate examination of zygospore-wall structure by SEM is possible for spores from field collections, an evaluation of the role of ploidy and hybridization in speciation requires the use of cultures. Whenever possible, investigators should attempt to identify specimens with existing species descriptions until long-term culture studies are completed.

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MORPHOLOGY AND CHEMICAL COMPOSITION OF A NATURAL POPULATION OF AN ICE-ASSOCIATED ANTARCTIC DIATOM *NAVICULA GLACIEI*[1]

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ABSTRACT

During winter (1973), a very pure natural population of the diatom Navicula glaciei *Van Heurck occurred in dense concentrations (up to 244 mg·m^{-2} chlorophyll a) in the sea ice at Signy Island, South Orkneys, Antarctica. Samples of algal material were collected for subsequent chemical analysis. The diatom had a composition of 33.77% ash, 21.81% lipid, 25.38% crude protein, 19.04% crude carbohydrate and an intact calorific value of 15.384 KJ·g^{-1}. Carbon, hydrogen, nitrogen and phosphorus formed 34, 5.3, 4.1 and 0.52% dry wt respectively. The material was analysed for the trace elements Na, K, Fe, Ca, Mg, Al, Zn, Cu, Pb, Mn, ^{137}Cs. Fatty acid composition was dominated by 16:0 palmitic acid (20.46%), 16:1 palmitoleic acid (32.86%), and 20:5 docosahexaenoic acid (19.33%). To supplement a very scanty original description, a full taxonomic description is given in the text.*

Key index words: Antarctic diatom; chemical composition, diatom; ice-associated microalgae; Navicula

The habitats associated with sea ice exhibit considerable diversity and great structural complexity. Earlier authors distinguished two basic microalgal habitats, "snow" communities (8, 24), and "ice" communities (5,7). However, several distinct but confluent sympagic habitats have subsequently been recognised (37). A series of parallel tide-cracks through which seawater can pass is formed where fast ice adjoins land (Fig. 1). Snow and frozen seawater accumulate and depress the ice in this region and form the basis of a dynamic seawater environment of slush or semi-frozen infiltration ice, termed the coastal tide-crack overflow area.

At Factory Cove, Signy Island (60°42.5′S, 45°36′W), cores through the algal layer of this region into solid sea ice contained up to 244 mg·m^{-2} chlorophyll *a* with maximum concentrations of 7.5 mg·l^{-1} in melted samples of substrate. The community consists almost entirely of *Navicula glaciei* Van Heurck with the commonest contaminant alga *Nitzchia curta* (Van Heurck) Hasle present as less than one cell in 10^7 (37).

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