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SEXUAL REPRODUCTION IN *STEPHANODISCUS NIAGARAE* (BACILLARIOPHYTA)[1]

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ABSTRACT

We observed sexual reproduction in a clonal culture of Stephanodiscus niagarae *Ehrenb. and used light and scanning electron microscopy to observe flagellated male cells, auxospore growth, initial valve structure and production, and subsequent daughter cell division. Free auxospores were spherical and nonsiliceous throughout growth, producing hemispherical initial valves devoid of spines and with nonfasciculate striae. Pregametangial cells averaged 43% of the diameter of the daughter cell population and were ⅛ the biovolume of initial cells. This paper is the first confirmed report of sexual reproduction in* S. niagarae, *although it appears that specimens of* Actinocyclus niagarae *H. L. Smith, described from Lake Erie in 1878, are actually initial valves of* S. niagarae.

Key index words: Actinocyclus niagarae; *auxospore; Bacillariophyceae; initial cell; sexual reproduction;* Stephanodiscus niagarae

Diatoms possess a cell wall of opaline silica that restricts their ability to increase cell size between vegetative divisions. In most diatom species, each mitotic division creates one cell of identical size to the parent cell and a second cell that is slightly smaller, as described by the MacDonald-Pfitzer Rule (MacDonald 1869, Pfitzer 1869, 1871). With each successive generation, constraints imposed by this division process theoretically lead to a population of continuously decreasing mean cell size while the standard deviation of cell size increases. Several taxa are known to possess interesting physiological and morphological modifications to overcome these constraints; however, most taxa regenerate populations of larger size cells via sexual reproduction (see Geit-

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ler 1932, von Stosch 1965, Reimann et al. 1966, Rao and Desikachary 1970, Round 1972b, Drebes 1977, Gallagher 1983 for exceptions).

The incidence of sexual activity is limited to a small size range window in a given taxon, typically 30–40% of a taxon's maximum size, and can be controlled by several environmental factors (Geitler 1932, Drebes 1977). Consequently, reproduction occurs among only part of any population, in a relatively isolated time period, with many years often separating sexual events (Nipkow 1927, Lewis 1984, Mann 1988). If environmental conditions are unfavorable, a population risks division beyond its inducible size window and eventual death.

Two classes of diatoms have very different forms of sexual reproduction. Centric genera produce small, motile, uniflagellate male cells that fertilize larger, nonmotile, female oocytes. Raphid pennate genera reproduce via modifications of isogamy or physiological anisogamy. Exceptions to this generalized view do exist, especially among a third diatom class, the araphid pennate diatoms. Several review papers give excellent treatments of the stages, types, and variability of sexual reproduction (Geitler 1932, Drebes 1977, Round et al. 1990).

Some of the most common planktonic freshwater taxa have seldom, if ever, been observed undergoing sexual reproduction (e.g. *Asterionella*; Mann 1988). *Stephanodiscus*, common worldwide in temperate regions, is such an example. Few reports have been published on its mode of reproduction. Bethge (1925) appears to be the first having identified the initial valves of *Melosira binderana* Kütz. This taxon was later correctly identified as *Stephanodiscus binderanus* (Kütz.) Kreiger (Round 1972a). Skabichevskii (1973) illustrated hemispherical initial valves of *Stephanodiscus astraea* (Ehrenb.) Grun.; *S. astraea* has since been found to be an invalid combination (Håkansson and Locker 1981), and unfortunately Skabichevskii's line drawings do not permit a taxonomic reassignment. Round (1982) described several stages of sexual reproduction in an unnamed *Stephanodiscus* species from Farmoor Reservoir. He provided the first observations of auxospores and their growth, centrifugal silicification of initial valves, loss of an organic auxospore wall, and division of the initial cell to form daughter populations. Initial cells in his population consisted of two hemispherical valves with nonfasciculate striae radiating from one or two central points. Missing from the initial valves were the marginal ring of spines characteristic of this genus, although a marginal ring of fultoportulae and several rimoportulae were present. The mantle was reduced and had a rough-toothed edge abutting the girdle. Similar morphological characteristics are common to the initial valves of *Stephanodiscus hantzschii* fo. *tenuis* (Hust.) Håk. and Stoermer (Kobayasi et al. 1985), *Stephanodiscus excentricus* Hust. (Håkansson and Stoermer 1987), *Stephanodiscus pseudoexcentricus* Håk. and Stoermer (Håkansson and Stoermer 1987), *Stephanodiscus rhombus* Mahood (Mahood 1981), and the related taxon *Cyclostephanos dubius* (Fricke) Round (Hickel and Håkansson 1987). Still lacking in our knowledge of *Stephanodiscus* reproduction is gametogenesis, sexual determination, the existence of flagellated male cells, fertilization, auxospore position and silicification, and cytological changes associated with sexual reproduction. Previous studies on the closely allied genus *Cyclotella* (Iyengar and Subrahmanyan 1944, Geitler 1952, Schultz and Trainor 1968, 1970, Rao 1970, Hoops and Floyd 1979) may be helpful in answering some of these problems.

Light and scanning electron microscope studies of a clonal *Stephanodiscus* culture allowed us to document some of these missing stages in the life cycle of *Stephanodiscus niagarae*, a common taxon in temperate North America (Theriot and Stoermer 1981). Our investigation also shows that a diatom described from Lake Erie as *Actinocyclus niagarae* H. L. Smith (1878) in fact represents only the initial cell stage in the life cycle of *S. niagarae*.

MATERIALS AND METHODS

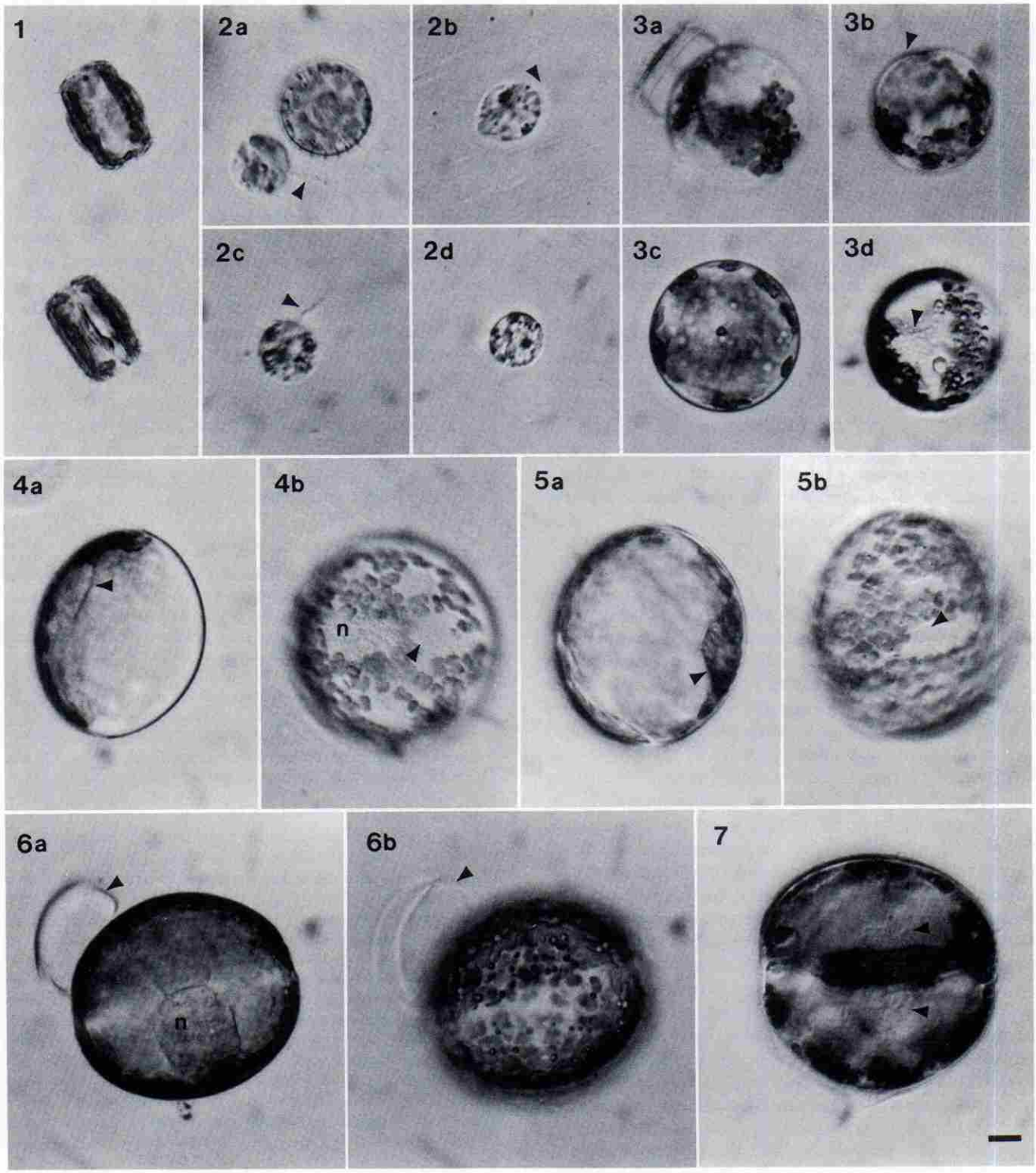
On 4 May 1989, a clonal culture of *S. niagarae* Ehrenb. was isolated using single cell micropipette technique (Pringsheim 1946). Material originated from a plankton tow collected on 2 May 1989 from Saginaw Bay of Lake Huron with a 35-μm-mesh net. The original isolate, designated ME184, and subsequent transfers were maintained in WC medium (Guillard 1983). Subcultures were incubated under various conditions including 15° and 20° C, 16:8- and 12:12-h LD cycle, and shaken and unshaken vessels. Illumination by General Electric Cool White fluorescent bulbs was adjusted to 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with screening. Observation of several transfers on 5 March 1990 revealed cells in many of the stages of sexual reproduction, and material was subsequently prepared for examination.

Light microscope observations were made on live specimens in Palmer counting chambers and on whole mounts of material fixed with 10% paraformaldehyde-glutaraldehyde (Lazinsky and Sicko-Goad 1979). For light microscopy of valve structures, cells were cleaned overnight in 30% H_2O_2 (Van der Werff 1955), rinsed six times with distilled water, dried onto coverslips, and mounted on slides in Hyrax®. All critical light microscope observations were made using brightfield optics of N.A. >1.30 on either a Leitz Ortholux or a Dialux 20 microscope. Samples were prepared for scanning electron microscopy using two techniques. To view valve ultrastructure, cells were cleaned as above, air-dried onto stubs, and coated with 20 nm AuPd using a Technics planar magnetron coating apparatus. Additionally, material fixed as above was dehydrated in a 25%, 50%, 70%, 95%, 3 × 100% ethanol series, transferred through two changes of hexamethyldisilizane (HMDS; Polysciences, Inc.), and dried onto stubs in a desiccator. Final sputter coating with 20 nm AuPd preserved the nonsiliceous cell membranes for SEM observation. A JEOL JSM-T100 SEM operated at 15 kV was used to view prepared stubs.

Terminology used in describing siliceous components of the diatom valve follows that recommended by Anonymous (1975) and Round et al. (1990).

RESULTS

Life cycle of Stephanodiscus niagarae. Maintenance of clonal culture ME184 for 10 months produced a monoecious population of cells with diameters of



FIGS. 1–7. Light micrographs of live *Stephanodiscus niagarae*, culture ME184, undergoing sexual reproduction. Scale bar = 10 μm (see Fig. 7). FIG. 1. Pregametangial cells in girdle view. Note central nucleus and parietal chloroplasts. FIG. 2a–d. Male gametes with few chloroplasts and 2–3 pigmented granules. a) Anterior uniflagellate (arrow) male gamete and vegetative cell that has divided beyond sexually inductive size range. b, c) Uniflagellate (arrow) male gametes (oblique illumination). d) Moribund nonflagellate male gamete. FIG. 3a–d. Nonsiliceous free auxospores. a) Auxospore with one mother valve still adhering to auxospore wall. b) Bulge (arrow) in auxospore envelope due to constriction by mother valve. c) Enlarging auxospore with parietal discoid chloroplasts. d) High focus of enlarged auxospore showing parietal nuclear (arrow) position. FIG. 4a, b. Formation of initial epivalve (oblique illumination). a) Girdle view of auxospore with all chloroplasts and nucleus (arrow) oriented parietally along one side of cell. b) Same specimen as Figure 4a, indicating nuclear position (n) at center of initial epivalve and striae (arrow) that have formed. FIG. 5a, b. Formation of initial hypovalve (oblique illumination). a) Girdle view of auxospore with chloroplasts oriented parietally around entire cell, and nucleus (arrow) with parietal orientation at center of initial hypovalve. b) Same specimen as Figure 5a, showing initial hypovalve striae (arrow). FIG. 6a, b.

30–35 μm (Figs. 1, 2a, 8). Although spermatogenesis or oogenesis was not observed, the first indication of sexual activity was the presence of anteriorly uniflagellate cells, presumably male gametes (Fig. 2a–c). Sperm cells were motile and approximately 15 μm in diameter, and contained a decreased complement of chloroplasts compared to vegetative cells and two to three dark pigmented granules. Sperm, presumably unable to complete fertilization, lost their flagella, became moribund (Fig. 2d), and degenerated. Fertilization was not witnessed; the next evident stage was auxospore production. Produced between the two mother thecae (Fig. 3a), the auxospores were "free." Mother valves were very quickly shed, leaving a spherical auxospore (Fig. 3c). Rarely the auxospore showed a bulge in the auxospore wall (Fig. 3b), from constriction by a mother valve. Auxospore enlargement proceeded isometrically, and the auxospore envelope remained organic, exhibiting no siliceous components reminiscent of the properizonia, epizonia, or scaly layers found in other centric genera (Crawford 1974, Ehrlich et al. 1982, von Stosch 1982). Auxospore growth was accompanied by a first metagamic nuclear division and then movement of one nucleus and all chloroplasts toward one side of the spherical cell (Figs. 3d, 4a). In live material we were unable to identify pycnotic nuclei following this metagamic mitosis (Geitler 1963). With the cell contents oriented parietally on one side of the auxospore, the initial epivalve was laid down centrifugally, beginning at the central area, which was located over the nucleus (Fig. 4b, arrow), and proceeded until fully silicified (Fig. 9). The epivalve was more heavily and crudely silicified than the hypovalve (Fig. 25). Following epivalve formation, the nucleus migrated toward the opposite side of the cell, underwent another metagamic mitosis en route, and was oriented adjacent to the auxospore wall. The chloroplasts became distributed parietally throughout the entire cell (Fig. 5a), and the initial hypovalve was then centrifugally deposited (Fig. 5b, arrow) beginning from a position over the nucleus. The auxospore envelope continued to surround the initial cell (Fig. 10) and still showed no siliceous components. External expressions of the fultoportulae and rimoportulae could be seen projecting through the auxospore wall (Fig. 11). Finally, girdle band silicification occurred, with concurrent cellular expansion along the pervalvar axis (Fig. 6a). During expansion, the auxospore wall split and was shed from the initial cell (Figs. 6a, b, 12), and the nucleus returned to the center (Fig. 6a) of the large, nearly spherical initial cell (Fig. 13). Initial cells then went through a first normal mitosis and produced two hemispherical cells, although cytokinesis was asymmetric (Fig. 7). The two new valves were of normal vegetative *S. niagarae* morphology, one being convex and one concave (Figs. 14, 18). The hemispherical cells continued to divide, producing the first daughter cells of normal vegetative morphology (lower cell, Figs. 15a, b). It appeared, however, that the hemispherical cells had only a limited division potential. Most hemispherical cells became moribund after only a few divisions (Fig. 16). The life cycle continued with a population of large vegetative *S. niagarae* cells 50–70 μm in diameter (Figs. 17a, b, 19). Evidence of sexual reproduction (i.e. motile cells and unsilicified auxospores) lasted for only 9 d following discovery, indicating sexual synchronicity within our populations. Accurate time scales for the stages in reproduction are unavailable because cells were unable to survive prolonged periods in Palmer counting cells.

Morphology of initial valves. The postauxospore initial valve of *S. niagarae* was strikingly different from the valve of vegetative cells. Detailed descriptions of vegetative morphology have been published (Theriot and Stoermer 1981). Rather than the discoid cell typifying *Stephanodiscus* (Figs. 8, 19), the initial cell was nearly spherical (Figs. 13, 20b), composed of two hemispherical valves. Each valve possessed loculate areolae (Fig. 23) organized into striae originating at one or two sparsely areolate central areas (Figs. 20a, 21). The striae remained in single rows toward the valve margin, branched, and became subfasciculate only near and onto the mantle (Figs. 20b, 21). Mantle areolae were slightly more dense than those of the valve face. A marginal ring of spines was missing from initial valves, although fultoportulae were present in a ring above the mantle (Figs. 20b, 21, 22).

The loculate areolae were occluded interiorly by domed convex cribra (Figs. 23, 24). Marginal fultoportulae were fairly regularly spaced about the valve margin and possessed three or rarely four buttresses (Fig. 24). Scattered central fultoportulae were present on the valve face (Fig. 22). Several rimoportulae occurred near the margin, although often deposited more centrally on the initial valve face (Fig. 24).

External expressions of the fultoportulae and rimoportulae were similar short tubular projections on the initial valve mantle and girdle region (Fig. 25). Both external expressions were much reduced from those on vegetative cells. A marked difference occurred in the construction of the initial cell mantle

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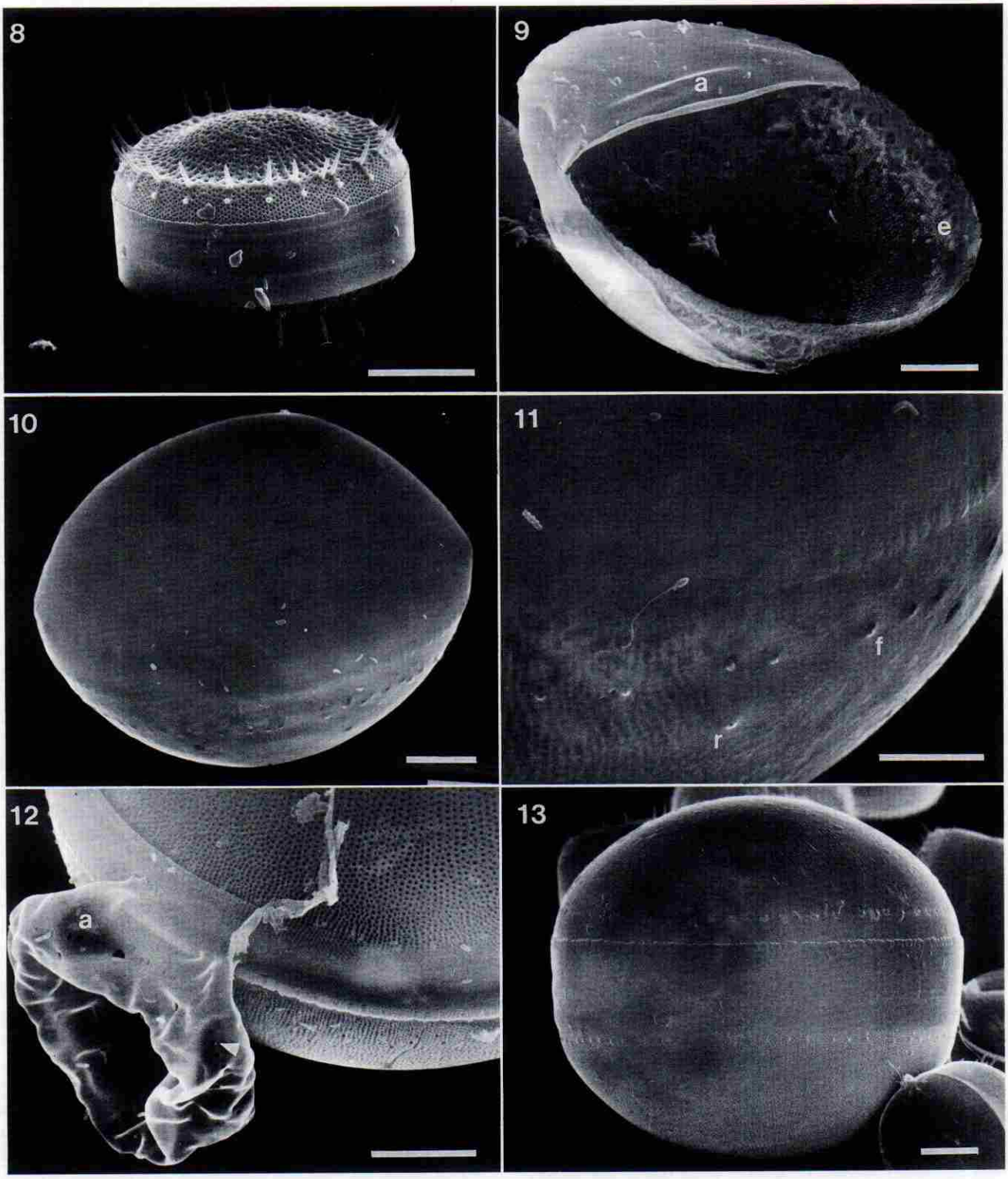
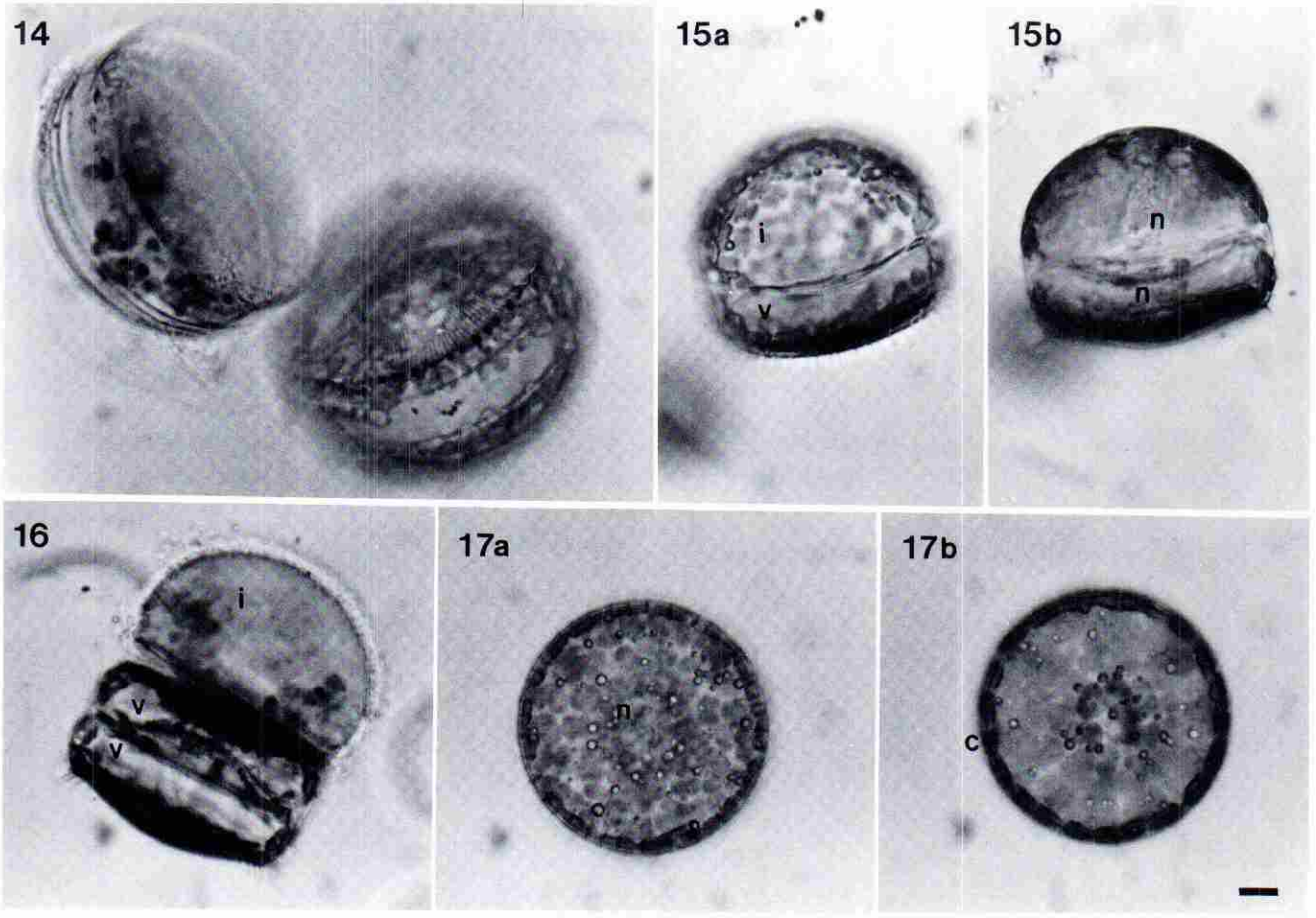
Pervalvar expansion and loss of organic auxospore wall from initial cell. a) Mid-focus girdle view of initial cell showing nucleus (n) returning to central location in cell and loss of auxospore wall (arrow). b) High focus of specimen in Figure 6a, indicating parietal discoid chloroplasts, expanded girdle region, and shed auxospore wall (arrow). FIG. 7. First mitotic division (nuclei arrowed) of initial cell to form two hemispherical daughter cells.



FIGS. 8–13. Scanning electron micrographs of *Stephanodiscus niagarae* sexual reproduction. Scale bars: Figures 8–10, 12, 13 = 10 μm; Figure 11 = 5 μm. FIG. 8. Pregametangial cell; peroxide-cleaned. FIG. 9. HMDS-prepared auxospore with wall (a) torn to reveal initial epivalve (e). FIG. 10. HMDS-prepared initial cell surrounded by auxospore envelope. FIG. 11. Same specimen as Figure 10, showing nonsiliceous nature of auxospore wall and external expressions of rimoportula (r) and fultoportulae (f) projecting through auxospore wall on initial epivalve. FIG. 12. HMDS-prepared initial cell with shed auxospore wall (a). Note holes in shed wall (arrow) from rimoportulae and fultoportulae. FIG. 13. Girdle view of peroxide-cleaned initial cell with completely silicified girdle region. The initial epivalve is the top valve.



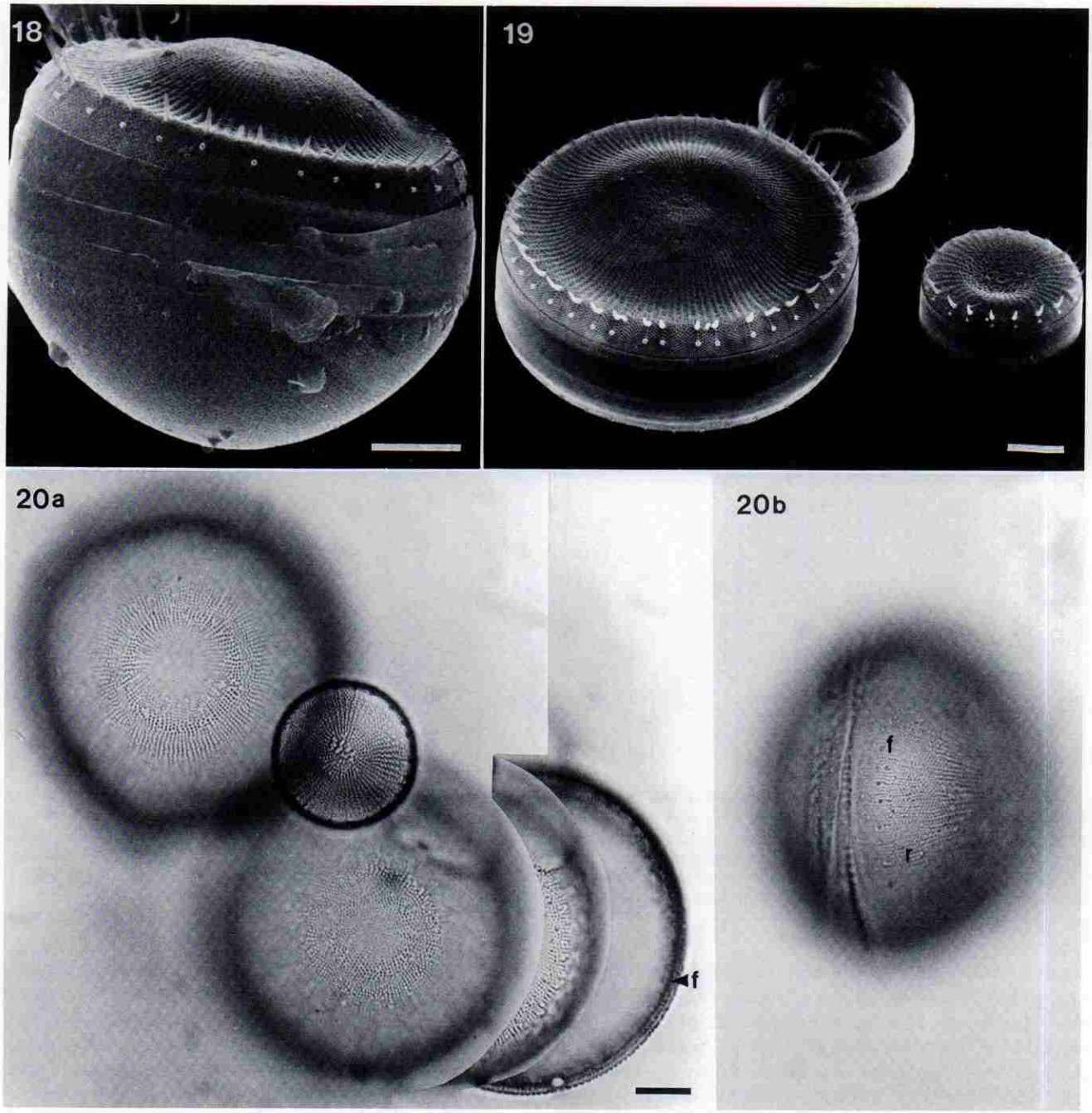
FIGS. 14–17. Light micrographs of post-initial cell reproduction in *Stephanodiscus niagarae*. Scale bar = 10 μm (see Fig. 17b). FIG. 14. Hemispherical daughter cells resulting from first mitotic division of initial cell. FIG. 15a, b. Division of hemispherical daughter cells (i) to form first vegetative daughter cells (v). a) High focus girdle view. b) Mid-focus of same specimen in Figure 15a with divided nuclei (n) and newly formed valve faces. FIG. 16. Additional mitotic divisions create multiple vegetative cells (v) but limited division potential results in moribund hemispherical daughter cell (i). FIG. 17a, b. Large vegetative cells produced by sexual reproduction. a) Mid-focus indicating central nucleus (n). b) High focus revealing parietal discoid chloroplasts (c) and striated valve face.

region allowing further morphological separation of the more heavily silicified epivalve (Fig. 25) from the hypovalve. The initial epivalve mantle was bordered by a jagged toothed edge (Figs. 25, 26), whereas the initial hypovalve was bordered by a vertically grooved edge (Fig. 26) more similar to that found in vegetative cells (Fig. 8). The cingulum, similar to that in vegetative cells, was made of two or three closely appressed copulae or girdle bands (Fig. 26) that often appeared fused (Fig. 25).

DISCUSSION

Within the Stephanodiscaceae (sensu Glezer and Makarova 1986 *in* Round et al. 1990), sexual reproduction has been observed in *Cyclotella* and *Stephanodiscus*. To date no one has reported the complete sequence of gamete production, fertilization, auxospore production, growth, silicification, and division. Past work has focused on *Cyclotella*, but the variability encountered seems to raise more questions rather than to show a definitive reproductive plan for this family. Our study presents only the second observation of sexual reproduction, outside of initial valve features, within the common freshwater genus *Stephanodiscus*. While confirming many of Round's (1982) earlier observations, we have added many new aspects to knowledge of this process.

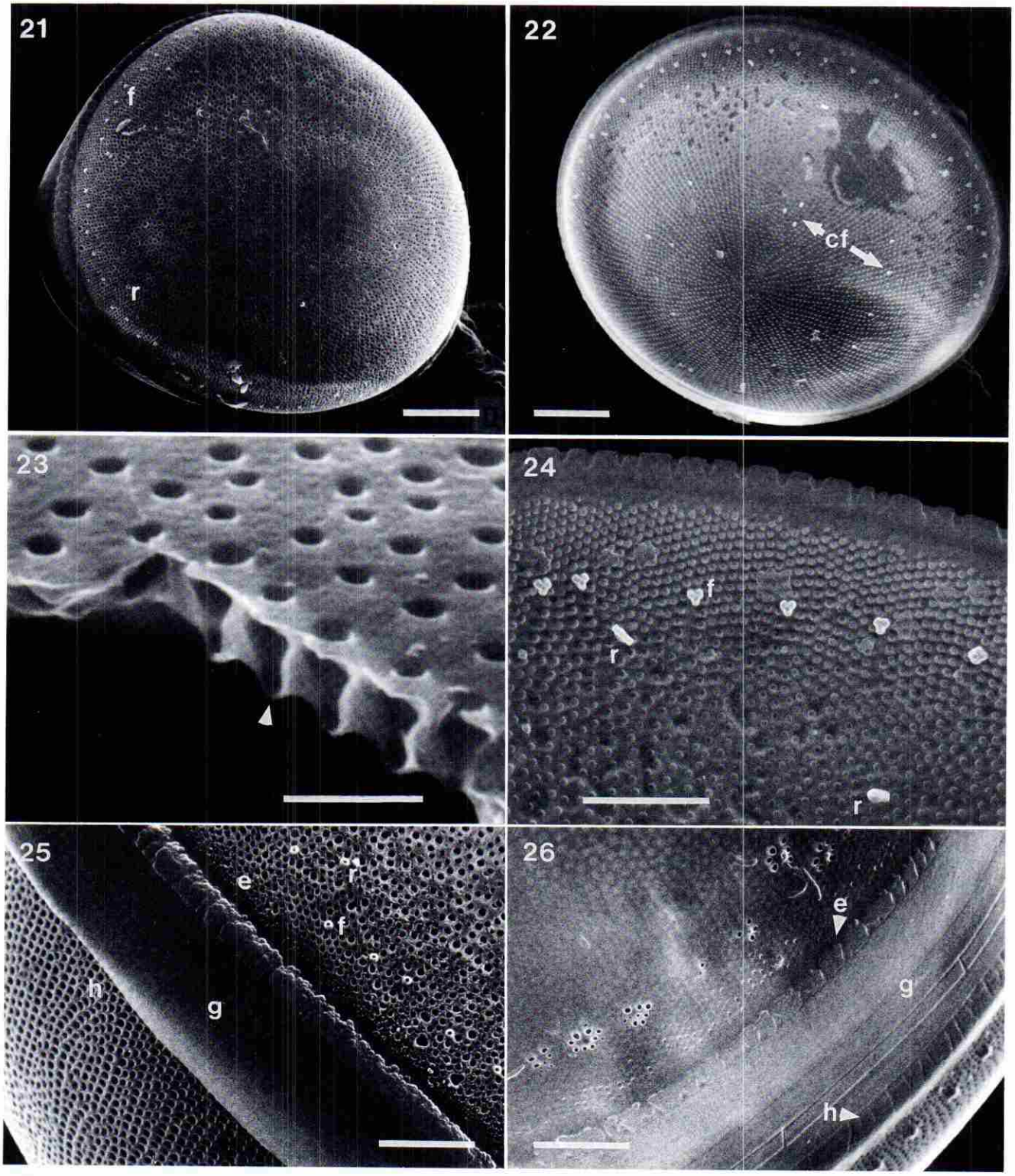
Motile cells have not been previously noted in *Stephanodiscus*. Round (1982) used preserved plankton collections and did not recognize any flagellated stages in his material. Motile cells in our material were anteriorly uniflagellate, and although we assumed them to be male gametes, we did not observe gametogenesis nor fertilization. Flagellated cells contained a reduced number of chloroplasts, which suggests hologenous origin (Round et al. 1990) similar to the uniflagellate sperm of *Cyclotella* (Schultz and Trainor 1968, 1970, Rao 1970, Hoops and Floyd 1979). Reports of spermatogenesis in diatoms occurring during the dark period (Furnas 1985) may explain our inability to identify gametogenesis in *S. niagarae*. Geitler's (1952) observations on meiosis



NOTE: Several photos taken from a stepwise focus series have been overlapped in Figures 20a, 27, and 30–36 in order to show both valve face and margin views.

FIGS. 18–20. *Stephanodiscus niagarae* post-initial cell reproduction. Scale bars = 10 μm. FIG. 18. Scanning electron micrograph of hemispherical daughter cell, possessing initial hypovalve, resulting from first mitotic division of initial cell. FIG. 19. Scanning electron micrograph showing size and morphological comparison of post- (left) and prereproductive (right) vegetative cells. FIG. 20a, b. Light micrographs illustrating morphology of initial valves. a) Stepwise focus valve views of two initial valve specimens and small vegetative valve. Note origin of striae from one (top specimen) or two (lower specimen) central points, crudely fasciculate striae, lack of spines, ring of marginal fultoportulae (f), and jagged toothed edge bordering the initial epivalve mantle. b) High focus girdle view of cleaned initial cell with ring of marginal fultoportulae (f) and centrally displaced rimoportula (r) indicated on initial epivalve. Note branching and poor fasciculation of striae.

during gametogenesis in *Cyclotella* supports the presence of flagellated male cells and oogamous reproduction in *Stephanodiscus*. We cannot, however, discount the possibility of autogamous reproduction. This process has been noted in several diatoms (Erben 1959, Drebes 1977) including *C. meneghiniana* Kütz. (Iyengar and Subrahmanyan 1944). Schultz and Trainor (1968) hypothesized that autogamy had



FIGS. 21–26. Scanning electron micrographs of peroxide-cleaned *Stephanodiscus niagarae* initial valves. Scale bars: Figures 21, 22 = 10 μm; Figures 24–26 = 5 μm; Figure 23 = 1.0 μm. FIG. 21. External view of hemispherical initial epivalve with striae originating from one central point and branching toward the mantle, no spines, a marginal ring of fultoportulae (f), and inwardly deposited rimoportula (r). Loculate areolae become more densely packed on the mantle. FIG. 22. Internal view of initial valve with scattered central fultoportulae (cf). FIG. 23. Fractured initial valve revealing loculate areolae occluded internally by convex domed cribra (arrow). FIG. 24. High magnification of specimen in Figure 22 illustrating rimoportulae (r) and marginal fultoportulae (f) internal expressions. Fultoportulae possess three or rarely four buttresses and rimoportulae are deposited irregularly onto the valve face. FIG. 25. Similar short tubular external expressions of both marginal fultoportulae (f) and rimoportula (r), and fused girdle bands (g) of initial epivalve (e). Initial epivalve is more heavily and crudely silicified than hypovalve (h). FIG. 26. Initial cell girdle or cingulum is made of two or three bands (g). A marked difference in construction of mantle area between epivalve and hypovalve exists. Epivalve mantle is bordered by a jagged toothed edge (e) and has a wide band of densely packed areolae. Hypovalve mantle is bordered by a vertically grooved edge (h) and a much more narrow band of areolae.

replaced oogamy in *C. meneghiniana*, perhaps as a facultative response when fertilization did not occur via oogamy, or that sperm production was simply no longer necessary within this taxon. These same interpretations might also apply to *Stephanodiscus* until evidence of fertilization via motile sperm can be presented.

As with most centric diatoms studied to date, our *S. niagarae* culture exhibited monoecious sexual determination. As the opportunity to observe spermatogenesis did not present itself, we were unable to identify whether cell size controlled diplophenotypic determination (Drebes 1977) in *S. niagarae*. Oogonial cells averaged 35 μm in diameter, approximately 43% the size of the largest daughter cell populations and in close accord with Geitler's (1932) observations. Because both sexes of cells were observed in the same culture vessel, we can assume that environmentally controlled diplophenotypes are not present in *S. niagarae*. In fact, the specific environmental conditions inducing sexuality in this taxon covered a wide range. Successful initial cell production occurred at 15° and 20° C, in 12:12- and 16:8-h LD cycles, in culture flasks ranging from 15 mL to 2.0 L in volume, and in both shaken and unshaken vessels. Cell size appears to be the most important induction factor, a conclusion seen with our taxon and commonly in other diatoms (Drebes 1977).

Although Round (1982) was unable to view live material, he proposed that auxospores produced in the *Stephanodiscus* population he studied were free, with no association to the maternal thecae. We observed shedding of the maternal thecae during production of free auxospores in live material of *S. niagarae*. This corroborates Round's hypothesis and may indicate that free auxosporulation is characteristic of *Stephanodiscus*. In *Cyclotella*, maternal valves often remain loosely associated with the auxospore (Iyengar and Subrahmanyan 1944, Hoops and Floyd 1979), although they do not produce any visible effect on formation of initial valves as seen in intercalary or semi-intercalary auxospores of other centric genera (e.g. *Melosira nummuloides* (Dillw.) C. Ag.; Crawford 1974).

Our findings indicate that *Stephanodiscus* is the first centric diatom genus investigated that does not produce any extraneous siliceous components, such as scales, hoops, bands, or plates (Crawford 1974, Ehrlich et al. 1982, von Stosch 1982, Schmid 1984), in association with the auxospore wall. Round (1982) did not recognize any scales in his *Stephanodiscus* specimens either, but was unsure of the age of his auxospores at the time of collection and considered that scales may have been shed earlier. As suggested by Round and Crawford (1981), scaled auxospores may represent retention of a primitive trait. The fact that the closely allied genus *Cyclotella* is known to have a scale-invested auxospore wall (Hoops and Floyd 1979) leads us to believe that an evolutionary loss of a scaled stage has occurred in *Stephanodiscus*. Support for this theory of evolutionary reduction can be found in the study of Krebs et al. (1987), which indicates that the genus *Cyclotella* appeared some 4.0 million years previous to *Stephanodiscus* in the fossil record from the western United States.

Concomitant with a lack of siliceous auxospore elements is our evidence of isometric enlargement of *S. niagarae* auxospores. Round (1982) illustrated several stages in expansion of *Stephanodiscus* auxospores, noting a very odd scalloped wall or "folding around the periphery" of the auxospore. He suggested a possible fixation artifact, and from our study, this appears to be the case. In live material, *Stephanodiscus* auxospores were nearly perfectly spherical throughout expansion and silicification, as might be expected in a diatom that does not possess a properizonium or epizonium (von Stosch 1982).

Initial cells from our clone had biovolume ratios approximately 9× those of the auxospore mother cells. This falls in the midrange of literature reports of volume ratios from 3:1 to 20:1 (Drebes 1977). The first division of initial cells produced two hemispherical cells, which continued to divide to produce morphologically normal daughter cells. Hemispheric cells in *S. niagarae* had a limited division potential, becoming mitotically inactive and moribund after a few divisions. This phenomenon has also been reported in *C. meneghiniana* (Iyengar and Subrahmanyan 1944) and *Cocconeis* (Geitler 1932). The apparent limited division potential of initial daughter cells is poorly understood, although it may be one reason why evidence of sexuality in *Stephanodiscus* and other genera is rarely reported, as initial valves would be quickly removed from a population. The extremely short time span of sexuality, the synchronicity of sex, and the often lengthy diatom life cycle (Mann 1988) also limit the observance of sexual reproduction.

Round (1982) provided a detailed study of the initial valve morphology of *Stephanodiscus*. Our specimens tend to corroborate his observations. Further analysis of reports on this genus (Bethge 1925, Skabichevskii 1973, Mahood 1981, Round 1982, Kobayasi et al. 1985, Håkansson and Stoermer 1987) shows several characteristic morphological trends in initial valve construction. All *Stephanodiscus* taxa investigated to date have hemispheric to strongly convex initial valves. Areolae are organized into striae near the valve center but become disorganized and only crudely fasciculate as they approach the valve mantle. The prominent spines on vegetative frustules are never present on initial valves. Most reports also indicate the regular presence of marginal fultoportulae delineating the more finely areolate initial valve mantle. These developments in initial valve morphology seem common to all members of the genus, and we suggest that initial valve construction may provide additional definitive evolutionary characters. A recent study (Kociolek and Stoermer 1989)

has successfully applied initial valve characters to pennate diatom phylogenetics.

Not all diatoms have initial valves as different from the vegetative valves as does *Stephanodiscus* (e.g. *Gomphonema*; Hohn 1959). In these taxa, an obvious relation can be established between the initial and vegetative valves. However, in genera that have quite different initial valves, historic taxonomic misinterpretations have occurred. Synchronicity associated with sexual reproduction can create an abundance of morphologically and taxonomically confusing initial valves in any given collection. Misinterpretations often resulted in the application of incorrect specific epithets (Drebes 1967, Williams 1990) and even generic misnomers (Van Heurck 1896, Meunier 1915 *in* Drebes 1977). We believe that a generic taxonomic misinterpretation has also occurred in the identification of the initial valves of *S. niagarae*.

In 1878, H. L. Smith described a "puzzling" diatom in a "filtering" from Lake Erie near Cleveland, Ohio. The new diatom ". . . was associated with an abundance of *Stephanodiscus niagarae*," and he described it as "disc large, diam. .0038 [96 μm], valves very much inflated and densely packed with minute radiating punctae . . . scattered loosely and irregularly at the centre, and sometimes radiate from two central blank spaces . . . connecting membrane [cingulum] is broad . . . a characteristic circlet of minute spines, within the margins of the valves" (H. L. Smith 1878). Named *Actinocyclus niagarae*, it was thought to be the first freshwater representative of this genus and has been maintained as a valid taxon (G. M. Smith 1950, VanLandingham 1967).

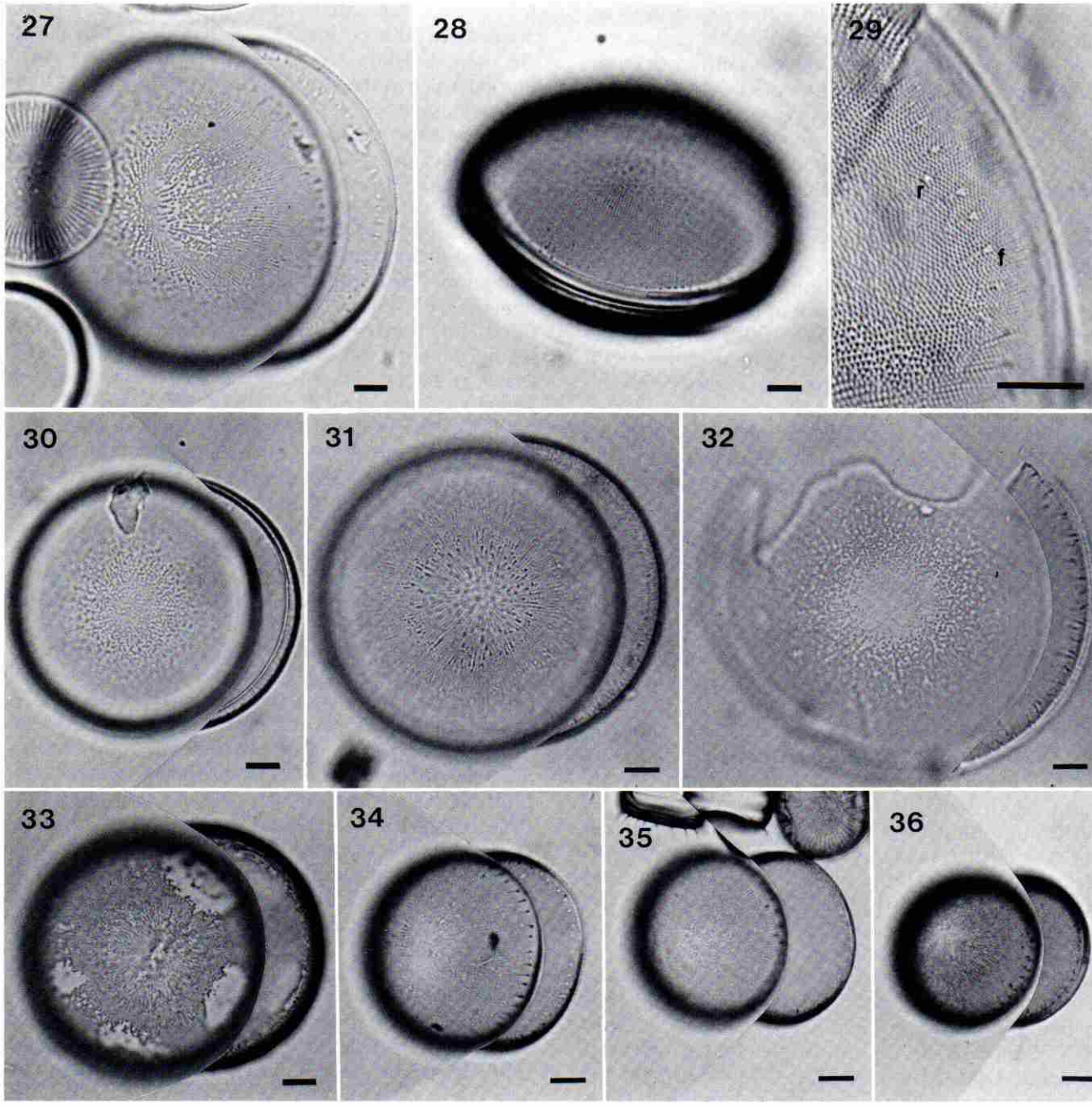
Although Smith did not designate a type for this new taxon, the Academy of Natural Sciences Philadelphia (ANSP) kindly provided us with the material from Smith's original working collection (H. L. Smith E-64, cross-referenced to H. L. Smith's Diatomacearum species typicae No. 701) as well as three other slides labelled "*Actinocyclus niagarae*, Lake Erie." Smith's material, although originally described in detail from Lake Erie at Cleveland, Ohio (H. L. Smith 1878), is confusingly cited as originating from Lake Erie at Buffalo, New York, in his collection notes and in his exsiccatae handbook. This confusion aside, it is believed that his working collection does, however, contain the collections that were used to describe his published taxa.

Smith's slide E-64 appears to be a nearshore plankton collection and is dominated by *S. niagarae* along with fewer representatives of *Cyclotella americana* (Ehrenb.) Kütz., *S. alpinus* Hust., *Aulacoseira granulata* (Ehrenb.) Simonsen, *Fragilaria intermedia* var. *fallax* (Grun.) A. Cl., *Cymatopleura elliptica* (Bréb. *in* Kütz.) Wm. Smith, *Entomoneis ornata* (J. W. Bail.) Reim., *Nitzschia sigmoidea* (Nitz.) Wm. Smith, and *Synedra ulna* var. *chaseana* Thomas. On the slide are 41 specimens that correspond to H. L. Smith's (1878) description of *Actinocyclus niagarae*. These hemispherical specimens are devoid of spines and have only crudely fasciculate striae originating at one or two central points (Figs. 27, 28). Evenly spaced along the valve borders are marginal fultoportulae with rimoportulae more centrally displaced (Fig. 29). Only 32 specimens were complete enough to measure morphometric characters, which included diameter and areolae and striae counts. The three other slides from ANSP (Boyer P-5-13, General Coll. 350, Febiger 961) were also examined. Collection information at ANSP indicates that material on these slides was also acquired from H. L. Smith's original collection. Four additional specimens meeting H. L. Smith's (1878) description of *A. niagarae* were identified. The total of 36 specimens ranged in diameter from 70.6 to 105.8 μm (mean 91.8 μm) with 12–14 striae in 10 μm, 12–13 areolae in 10 μm, and 3–4 marginal fultoportulae in 10 μm (Figs. 27, 30–32). Additionally, Wolle (1890) illustrated a specimen of *A. niagarae* that is 78 μm in diameter, and Boyer (1926–1927) provided morphometric characters for *A. niagarae* of 60–110 μm in diameter with 15–18 areolae in 10 μm.

It is essential to first note that Smith's taxon does not belong to the genus *Actinocyclus*. Representatives of this genus are mostly marine. One freshwater taxon, *A. normanii* fo. *subsalsa* (Juhl.-Dannf.) Hust. (Hasle 1977), is presently found in the Laurentian Great Lakes, although it did not occur there until after approximately 1940 (Stoermer et al. 1987). More importantly, several morphological characters distinguishing *Actinocyclus* are not evident in Smith's material, including radially sectored valve markings, multiple large rimoportulae, and a pseudonodulus. Presence of fultoportulae (Fig. 29) definitively places Smith's taxon in the Thalassiosirales (Round et al. 1990). Morphological comparison of Smith's *A. niagarae* valves indicates a construction more consistent with initial valves of a *Stephanodiscus* species.

H. L. Smith's (1878) reported association of *A. niagarae* with *S. niagarae* in Lake Erie, and the morphological similarity to initial valves in our cultures suggests that *A. niagarae* represents only the initial valve in the life history of *S. niagarae*. We believe that the two taxa are synonymous. Fifty initial valve specimens, measured from our sexual material, had diameters ranging from 45.1 to 82.3 μm (mean 61.7 μm), 13–14 striae in 10 μm, 13–15 areolae in 10 μm, and 3–4 marginal fultoportulae in 10 μm (Figs. 33–36). We have plotted the frequency of diameters found in our material against those found in Smith's material (Fig. 37). The two populations have statistically different means (t-statistic = 18.140, $P = 0.00$) but overlap, which might counter our proposal for taxonomic synonymy. Two very interesting points are brought to light in the evaluation of these initial valve populations.

First, classical diatom taxonomy recognizes that initial valves are indicative of the largest size a taxon can achieve. This is usually reported as a maximum size or small range of sizes that is diagnostic of a



FIGS. 27–36. *Stephanodiscus niagarae* initial valves. Scale bars = 10 μm. FIGS. 27–32. Light micrographs of specimens from ANSP H. L. Smith slide E-64, *Actinocyclus niagarae*. FIG. 27. High and low focus of canted hemispherical valve showing striae originating from two central areas and branching toward mantle, and also ring of processes present above jagged valve mantle. Note similarity to initial valves of *S. niagarae* (Fig. 20). FIG. 28. Girdle view of hemispherical valve. FIG. 29. High magnification of valve margin identifies ring of processes as marginal fultoportulae (f) from their trigonal nature and suggests the inner deposited processes (r) are rimoportulae (oblique illumination). FIGS. 30–32. High and low focus of specimens illustrating size range of *A. niagarae*. FIG. 30. Specimen with diameter of 70.6 μm. FIG. 31. Specimen with diameter of 92.1 μm. FIG. 32. Specimen with diameter of 105.8 μm. FIGS. 33–36. High and low focus of specimens illustrating size range of *S. niagarae* initial valves from sexual reproduction in culture ME184. FIG. 33. Incompletely silicified specimen with diameter of 74.5 μm. FIG. 34. Specimen with diameter of 60.8 μm. FIG. 35. Specimen with diameter of 52.9 μm. FIG. 36. Specimen with diameter of 45.1 μm.

taxon (Geitler 1932, 1958b). Size ranges of initial valves covering greater than 40% of a taxon's size range might be considered a culture anomaly in our *S. niagarae* clone. However, Smith's plankton collection shows a similar trend (Fig. 37). Few incidences of this phenomenon have ever been mentioned in the literature. Kociolek and Stoermer (1989) reported a similar wide range of initial valve

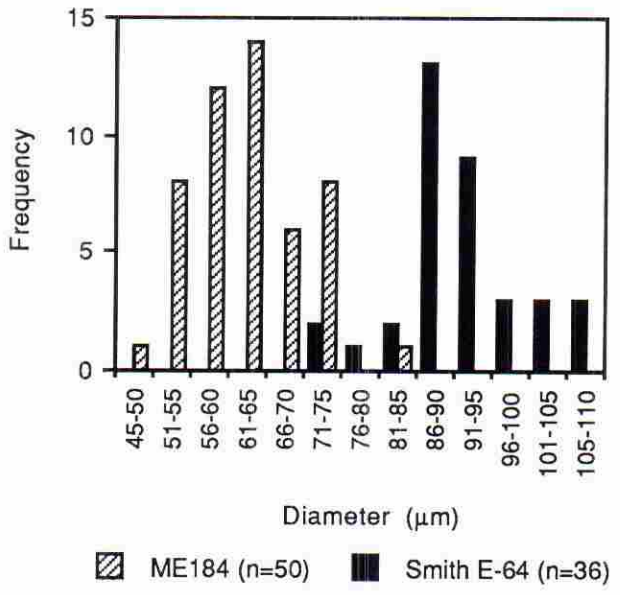


FIG. 37. Frequency histogram of initial valve diameters in two populations of *Stephanodiscus niagarae*.

sizes for *Gomphoneis eriense* (Grun.) Skv., and Bethge (1925) reported initial valve size variability in several *Melosira* species. The large range of initial valve sizes in each of these populations is contrary to the generally accepted concept of diatom life cycles and indicates a need for investigation of possible genetic or environmental factors controlling auxospore expansion. At minimum, a reiteration of Mann's (1986) call for detailed records of dimensions and characters of any diatom population studied seems appropriate.

Second, to establish a taxonomic association between *A. niagarae* and *S. niagarae* we must interpret and justify the evident statistical difference of initial valve sizes between these populations. Vegetative populations of *S. niagarae* do show large size differences (Theriot and Stoermer 1981), although the influence of this on sexual reproduction is unknown. However, Geitler (1932, 1958b, 1969) theorized that interbreeding populations and each clone of a diatom possess two characteristic points ("Kardinalpunkte") in their life cycle: the size of "Mutterzellen" or gametangia and the size of auxospore initial cells. Supportive studies by von Stosch (1965) showed that three clones of *Biddulphia mobilensis* (J. W. Bail.) Grun. *in* V.H. had different initial valve sizes. Additionally, Mann (1984) revealed that minor differences in the size and shape of populations within a classically defined "species" hindered interbreeding, indicating the presence of reproductive isolation mechanisms acting on populations. Therefore, two morphologically inseparable vegetative populations that have undergone a reproductive isolation could be identified only by the different size of the initial valves or gametangia. Taxonomic reevaluation or separation has not been required classically on these grounds, and we feel that the taxonomic synonymy of *A. niagarae* to *S. niagarae* is thus justified. Recent work on the *S. niagarae* species complex has revealed some taxonomic variability and apparent evolutionary volatility (Theriot 1987). Morphometric interpretation using principal component analysis has resulted in the separation of several new taxa from this complex (Theriot and Stoermer 1984, 1986). These new taxa are hypothesized to be reproductively/genetically isolated from the nominate variety, even thought they can co-occur with the nominate. Diatomists are cautioned to consider the taxonomic implications and the diatom varietal/species concept in light of these points. Can the biological species definition really apply to diatoms when morphologically inseparable, yet sexually isolated, vegetative populations exist? Or should the diatom species concept weigh morphological characters heavily even though sexual induction, determination, isolation, and hybridization (Geitler 1958a, 1969, Mann 1984) remain so poorly understood in the diatom life cycle?

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