A PRELIMINARY INVESTIGATION OF THE PHYLOGENETIC RELATIONSHIPS AMONG THE FRESHWATER, APICAL PORE FIELD-BEARING CYMBELLOID AND GOMPHONEMOID DIATOMS (BACILLARIOPHYCEAE) [1]

John P. Kociolek[2] *and Eugene F. Stoermer*

Great Lakes Research Division, 2200 Bonisteel Boulevard, The University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT

Relationships among the apical pore field-bearing diatom genera Brebissonia, Cymbella, Gomphonema, Gomphoneis, Didymosphenia, Gomphocymbella, *and* Reimeria *and their close ally* Encyonema *were evaluated using cladistics. These biraphid genera are a polythetic group defined by chloroplast number and type, mode of sexual reproduction, valve orientation and presence of apical pore fields. Character states of valve as well as non-siliceous features were described and then polarized using the outgroup method, with the naviculoid genera* Anomoeoneis *and* Placoneis *serving as outgroups. The cladistic analysis suggests two groups of terminal taxa, corresponding to to a predominantly cymbelloid lineage and a gomphonemoid lineage.* Didymosphenia, *previously thought to be closely allied to gomphonemoid diatoms, is shown to be more closely related to* Cymbella. Gomphonema *appears to be without distinguishing characteristics. Relationships of the other genera are described. The systematic position of small, doubly-punctate* Gomphonema *species is discussed.*

Key index words: Bacillariophyceae; Brebissonia; *cladistics;* Cymbella; *diatoms;* Didymosphenia; Encyonema; *evolution;* Gomphocymbella; Gomphoneis; Gomphonema; *phylogeny;* Reimeria

Although diatoms have been observed with light microscopy for over 275 years (Anonymous 1703, Leeuwenhoek 1703) and with electron microscopy for over 50 years (Krause 1936), little is known about genealogical relationships in some groups, particularly at the generic level. Many authors have investigated relationships at this level of hierarchy; however, more recent works have concentrated almost exclusively on marine and freshwater centric diatoms.

Relationships among pennate diatom genera have only recently been explored through the cladistic approach. Williams (1985) based his view of relationships among members of the family Diatomaceae on results of a cladistic analysis, and Kociolek and Stoermer (1986a) used cladistics to evaluate relationships among monoraphid diatoms. In this report we attempt to evaluate phylogenetic relationships in one group of freshwater biraphid diatoms. Members of this group exhibit asymmetry in valve

shape and most assume a stalked growth habit via mucilage exuded through apical pore fields (APF's).

CLADISTIC ANALYSIS

Terminal taxa. Included in this group of diatoms are those taxa which are asymmetrical to the transapical axis (*Gomphonema* Ehrenb., *Gomphoneis* Cleve, *Didymosphenia* M. Schm.), asymmetrical to the transapical and apical axes (*Gomphocymbella* O. Müll.) and those taxa asymmetrical to the apical axis. Members of this latter group had previously been included in three genera, based on differences in growth habit. *Cocconema* Ehrenb. represented those cymbelloid diatoms which grow at the ends of mucilaginous stalks, whereas *Encyonema* Kütz. species grow within mucilaginous tubes. The genus *Cymbella* C.A. Ag. originally contained free-living species. These three genera were combined into *Cymbella* by Heiberg (1863) who argued that growth habit alone was not sufficient to base taxonomic differences at the genus level. This view predominated until recently when Krammer (1982b) showed the stalked forms (which have nomenclatural priority to the name *Cymbella*) differ substantially from those species which grow in tubes. Krammer (1982b) recognized *Cymbella* and *Encyonema* as distinct groups (subgenera of *Cymbella*); they are treated here at the level of genus. The remaining cymbelloid diatoms that fit neither into *Cymbella* nor *Encyonema* constitute a heterogenous group, called *Cymbopleura* by Krammer (1982b). Little is known about their ultrastructure. From this heterogenous group of cymbelloid diatoms Kociolek and Stoermer (1987) erected the genus *Reimeria*, which includes the former *Cymbella sinuata* Greg. and its allies. Thus, of the cymbelloid diatoms, *Cymbella sensu stricto*, *Encyonema* and *Reimeria* are recognized as natural groups and included as terminal taxa in the analysis.

Also included in the analysis is the genus *Brebissonia* Grun., a small group described originally as having naviculoid symmetry (Grunow 1860) but recently reported to be slightly asymmetrical to the apical axis (Mahoney and Reimer 1986).

Diagrammatic interpretations of the eight genera included in the cladistic analysis are presented in Figure 1.

Two of the genera, *Cymbella* and *Gomphoneis*, are subdivided. The subdivisions include: (1) *Cymbella mexicana* (Ehrenb.) Cleve separated from *Cymbella s. s.* based on Krammer's (1982b) observations of dif-

BREBISSONIA

ENCYONEMA

CYMBELLA

REIMERIA

GOMPHOCYMBELLA

?

DIDYMOSPHENIA
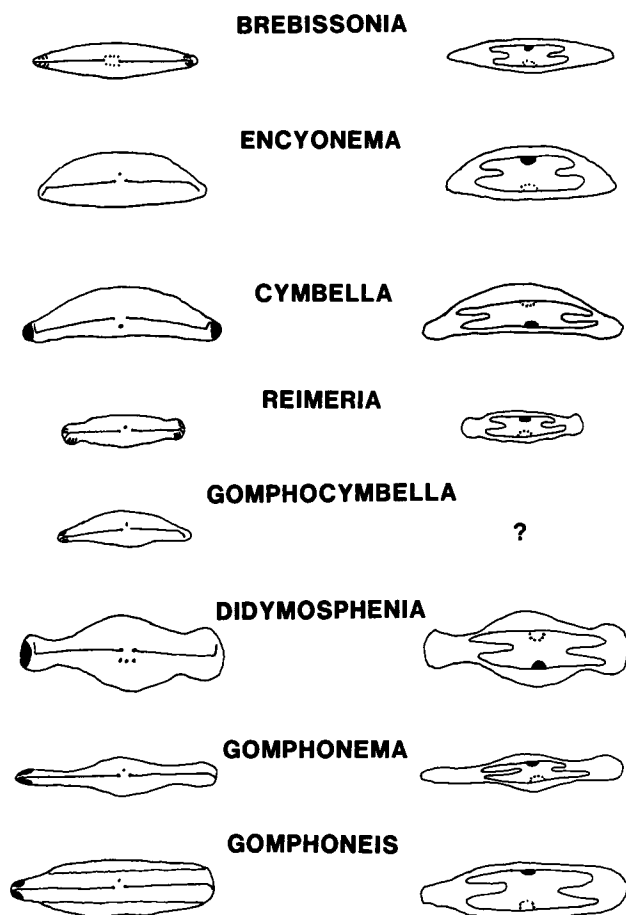
GOMPHONEMA

GOMPHONEIS

Fig. 1.   Schematic representation of valve (left) and cytological (right) features of the eight genera serving as terminal taxa in the cladistic analysis. At right, dark circles represent nuclei, dotted circles represent pyrenoids.

ferences in the structure of foramina, stigmata and APF's, and (2) separation of *Gomphoneis* into two groups, namely *G. elegans* (Grun.) Cleve and its allies and *G. herculeana* and its allies. Distinction between the two groups represents those species without and with an axial plate, respectively.

A complete listing of terminal taxa is presented in Table 1.

The freshwater APF-bearing cymbelloid and gomphonemoid diatoms and their close allies as a monophyletic lineage. The hypothesis that the terminal taxa form a monophyletic group is suggested by cytoplasmic, reproductive, valve and growth habit characteristics. As early as 1871 Pfitzer noted similarities in cytoplasmic features and modes of reproduction between species of *Cymbella s.l.* and *Gomphonema.* Mereschkowsky (1902–1903) grouped most of these genera together based on similarities of chloroplasts and, although *Gomphoneis* was not included in Mereschkowsky's treatment, unpublished observations indicate *G. herculeana* has a similarly-shaped chloroplast (Kociolek 1988). Hauptfleisch (1895), Steinecke (1931), and Geitler (1973) have noted similar-

ities between most species with regard to pattern of reduction division and sexual reproduction. Mann (1983a) observed that the genera *Cymbella s. l., Gomphonema* and *Didymosphenia,* among others, produce valves of *cis* orientation only, and similar observations have been made for *Gomphoneis* (Kociolek 1988). This provides further evidence that these genera are closely related. Dawson (1973b, c), Cox (1977), Kociolek and Rosen (1984), Mahoney and Reimer (1986) and Kociolek and Stoermer (1987) have noted similarities between most of these genera based on APF structure. Representatives from all genera except *Encyonema* form mucilaginous stalks. Thus, similarities of chloroplast shape, sexual reproduction, and APF structure and function suggest these genera form a monophyletic lineage.

Previous interpretations of phylogenetic relationships. Previous classification schemes for the cymbelloid and gomphonemoid genera considered here have traditionally been based on valve symmetry. Cymbelloid diatoms were previously classified together, and gomphonemoid diatoms were thought to form a separate natural group, based on their symmetry features (Müller 1905, Karsten 1928, Patrick and Reimer 1975). The genus *Brebissonia* has occupied a primitive place in this group, owing to its naviculoid symmetry, whereas *Gomphocymbella,* which is asymmetrical to both the apical and transapical axes, has been suggested to be intermediate between cymbelloid and gomphonemoid forms.

Very little evidence, other than symmetry considerations, has been offered to suggest phylogenetic relationships between the cymbelloid and gomphonemoid diatoms. Cleve (1894) and Yermoloff (1918) pointed to members of the genus *Navicula* Bory as possible ancestors of the genus *Cymbella.* The possibility of *Navicula*-like progenitors to the cymbelloid diatoms has been strengthened by the work of Cox (1979) and Krammer (1982b) which shows attributes of the *Navicula lineolatae* group in cymbelloid diatoms. Mann (1983b) has also suggested the possible origin of cymbelloid forms from *Navicula.* Some authors have suggested that gomphonemoid diatoms may have arisen from the cymbelloids. Cleve (1894) indicates that gomphocymbelloid diatoms may be intermediate between the two groups, while Krammer and Lange-Bertalot (1985:38) state that *Reimeria sinuata* (Greg.) Kociolek & Stoermer is, "eine(r) transitorische(n) Art zwischen *Cymbella* und *Gomphonema.*"

Characters and character polarity. Characters used in the cladistic analysis are described below. Definitions and distribution of character states are taken largely from previously published observations. Polarity of character states was determined by the outgroup method. This criterion for establishing character polarity assumes features found within the group under investigation (the ingroup) and in sister taxa (the outgroups) were also found in the ancestor of the groups and therefore are primitive. Features

TABLE 1. *Coding of character states for the ten terminal taxa. 0 = primitive, 1, 2 = derived, 9 = missing/unknown. * represents characters with unordered states. See text for description of features.*

Features	Brebissonia	Cymbella	C. mexicana	Didymos-phenia	Encyonema	Gompho-cymbella	Gomphoneis elegans	herculeana	Gomphonema	Reimeria
1.	0	1	1	1	1	1	0	0	0	1
2.	1	0	0	1	0	1	1	1	1	0
3.	0	0	0	1	0	1	1	1	1	0
4.	0	0	0	1	0	1	1	1	1	0
5.	0	1	1	1	0	0	0	0	0	0
6.	1	0	0	0	0	1	0	1	1	0
7.	0	1	0	0	0	0	0	0	0	0
8.	0	0	1	1	0	0	0	0	0	0
9.	0	0	1	1	0	0	0	0	0	0
10.	0	1	1	1	0	1	0	1	1	0
11.	0	0	0	0	0	0	0	0	0	1
12.	0	0	0	0	0	0	1	1	0	0
13.	0	0	0	0	0	0	1	1	0	0
14.	0	0	0	0	0	0	1	1	0	0
15.	0	0	0	0	0	0	0	1	0	0
16.	0	1	1	1	0	0	0	0	0	0
17.*	9	2	2	2	1	1	9	1	1	1
18.*	9	2	2	2	1	1	9	1	1	1
19.*	9	2	2	2	1	9	9	1	1	1
20.	0	1	1	0	1	0	0	0	0	0
21.	1	1	1	1	0	1	1	1	1	1

found in ingroup taxa only are considered derived, having evolved after the branching event that led to the ingroup and outgroup taxa (Watrous and Wheeler 1981, Maddison et al. 1984, Kociolek 1986). The naviculoid genera *Placoneis* Meresch. *emend* Cox and *Anomoeoneis* Pfitzer serve as outgroup taxa in this analysis. A close relationship between the outgroups and the terminal taxa is suggested by similarities in chromatophore structure (Schmidt 1876, Mereschkowsky 1902–1903, Heinzerling 1908) and the fact that species of both outgroups may possess isolated puncta in the central area (Patrick and Reimer 1975, Cox 1987), a feature found in most ingroup taxa.

1. *Asymmetry about the apical axis.* This character is found in *Cymbella*, *C. mexicana*, *Gomphocymbella* and species of *Didymosphenia* (Schmidt 1899, Skvortzow and Meyer 1928, Hustedt 1930). Lack of this feature in the outgroups suggests its presence is derived.

2. *Asymmetry about the transapical axis.* This feature is found in *Didymosphenia*, *Gomphonema*, *Gomphoneis* species, and *Gomphocymbella* but is lacking in the outgroups. Its presence is therefore considered derived.

3. *Asymmetry about the pervalvar plane.* This feature appears to be associated with asymmetry about the transapical axis. It is lacking in the outgroups, suggesting its presence is derived.

4. *APF restricted to one pole.* This feature is correlated with both [2] and [3] and is considered derived relative to a symmetrical positioning of porelli at both poles.

5. *Intermissio lacking.* As illustrated by John (1982) and Krammer (1982b: pl. 1061), continuation of the internal raphe fissure across the central area, accompanied by a hooded siliceous structure, repre-

sents lack of an intermissio. Most naviculoid diatoms, including the outgroups, have an intermissio, and its absence is considered derived.

6. *Internal stigmal openings slit-like.* An unoccluded, unspecialized pore in the central area is considered primitive, because such a condition is observed in *Placoneis* (Cox 1987) and certain members of *Anomoeoneis* (unpubl. observ.). This feature is also found in *Gomphoneis elegans* (Kociolek and Stoermer 1988). Any elaboration of the internal opening, such as the slit-like stigmal openings found in *Brebissonia* (Mahoney and Reimer 1986), *Gomphonema* (Dawson 1973c), *Gomphocymbella beccari* (Grun.) Forti (Krammer 1982a), and species closely allied to *Gomphoneis herculeana* (Kociolek and Stoermer 1986b, c) is considered derived.

7. *Dendritic stigmata.* *Cymbella* species exhibit a dendritic overgrowth of the stigmal opening (Cox 1977, Krammer 1982b, Schoeman and Ashton 1983), and this is considered derived relative to its absence in the outgroups.

8. *Stigmata covered by silica.* In *C. mexicana* (Krammer 1982b) and *Didymosphenia* (Dawson 1973a, b) the internal opening of the stigma is raised and conical with a siliceous covering. This stigma feature is considered derived since this feature is absent in the outgroups.

9. *Foramina complex.* The ornate, external siliceous structures of the foramina found in *C. mexicana* (Krammer 1982b) and *Didymosphenia* (Okuno 1954, Desikachary 1957, Dawson 1973a, b) are considered derived relative to the unoccluded puncta found in the outgroups. The possible significance of C-shaped foramina, as well as the number of rows of puncta composing the striae, has been discussed by several

investigators (Cleve 1894, Okuno 1974, Mann 1981b, Kociolek and Rosen 1984, Krammer and Lange-Bertalot 1985). Recent observations, however, suggest the type of puncta produced may vary within genera and perhaps even within a species (Krammer 1982a, Krammer and Lange-Bertalot 1985, Kociolek and Stoermer 1987). Also Krammer and Lange-Bertalot (1985) have suggested C-shaped foramina and doubly-punctate striae may be homologous (but see Kociolek and Stoermer 1987). Due to the confusion over this feature(s?) we have avoided using presence of C-shaped foramina or number of punctum rows in the striae as features.

10. *Differentiated APF's.* In most of the APF-bearing taxa, porelli of the APF's are physically separated from the striae by an unornamented strip of silica and structurally differentiated from the puncta (Dawson 1972, 1973a, b, c, Hufford and Collins 1972a, b, c, Schrader 1973, Gerloff and Helmcke 1977, Krammer 1979, 1982b, Kociolek and Stoermer 1986c). The alternative condition, that of undifferentiated porelli that appear as condensed striae, is found in *Brebissonia* (Mahoney and Reimer 1986), *Gomphoneis elegans* (Grunow *in* Van Heurck 1880, Patrick *in* Patrick and Reimer 1975, Kociolek and Stoermer 1988) and *Reimeria* (Kociolek and Stoermer 1987). The outgroup taxa do not have differentiated pores at the poles, thus differentiated porelli are considered derived.

11. *Unequal APF's.* The unequal distribution of APF porelli at the poles of *Reimeria* (Kociolek and Stoermer 1987) appears to be unique both within the ingroup and among the outgroup taxa; therefore, unequal APF's are considered derived.

12. *Septa present.* Members of the genus *Gomphoneis* possess a septum at the headpole and the footpole (Schmidt 1899, Kociolek and Rosen 1984, Krammer and Lange-Bertalot 1985, Kociolek and Stoermer 1986b, c, Kociolek et al. 1986); lack of septa in the outgroups suggests their presence is derived.

13. *Pseudosepta present.* Pseudosepta have been reported and illustrated at the headpole and footpole of *Gomphoneis* species (Schmidt 1899, Kociolek and Rosen 1984, Kociolek and Stoermer 1986b, c, Kociolek et al. 1986). The outgroups lack these features, suggesting their presence is derived.

14. *Marginal laminae present.* The internal siliceous covering along the valve mantle and margin that coalesces with interstriae before reaching the girdle bands is known as the marginal laminae. They are present in species of the *G. herculeana* subgroup (Kociolek and Stoermer 1986b, Kociolek et al. 1986, Kociolek 1988) and *G. elegans* subgroup (Krammer 1982a, Krammer and Lange-Bertalot 1985, Kociolek and Stoermer 1988). This feature is lacking in the outgroups, which suggests its presence is derived.

15. *Axial plate present.* This internal siliceous structure is expanded from the axial area and is responsible for the image of longitudinal lines in the *G. herculeana* subgroup (Kociolek and Rosen 1984, Kociolek and Stoermer 1986b, c, Kociolek et al. 1986). None of the other terminal taxa or the outgroups possesses this feature, thus its presence is considered derived.

16. *APF not bisected by external raphe ends.* In most of the terminal taxa, as well as the outgroups, the raphe bisects the terminal group of pores. In most *Cymbella* species (Krammer 1979, 1981, 1982b) as well as *C. mexicana* (Krammer 1982b) and *Didymosphenia* (Dawson 1973b, c) the distal raphe ends curve abruptly and do not bisect the APF's. The latter condition is considered derived.

17. *Placement of stigmata or isolated pores.* Stigmata are placed along the ventral margin in *Cymbella, C. mexicana* (Krammer 1982b) and *Didymosphenia* (Müller 1905). In the latter genus this condition is most evident in species asymmetrical to the apical axis [*D. curvirostrata* (Temp. & Brun) M. Schm., *D. sibirica* (Grun.) M. Schm.; Schmidt 1899, Skvortzow and Meyer 1928]. Stigmata or isolated pores are placed along the dorsal margin in *Encyonema* (Krammer 1982b, Mann 1983b), *Reimeria* (Reimer *in* Patrick and Reimer 1975, Schoeman and Archibald 1978) and *Gomphocymbella* (Fricke 1902, Müller 1905, Hustedt 1930). That stigmata are placed dorsally in *Gomphonema* and *Gomphoneis herculeana* is evidenced by specimens asymmetric to the apical axis (Müller 1905, Mayer 1928; pers. observ.) that have stigmata placed along the more highly arched (dorsal) margin. The condition in *G. elegans* and *Brebissonia,* which have stigmata on either side of the central area, cannot be determined. Because these conditions are lacking (or at least cannot be determined) in the outgroups, the dorsal (a) and ventral (b) positions are each considered derived and unordered.

18. *Deflection of external distal raphe ends.* Two conditions exist for the terminal taxa. One group of taxa (*Cymbella, C. mexicana,* and *Didymosphenia*) possess distal raphe ends that are deflected dorsally. The second condition, ventrally deflected raphe ends, is found in *Encyonema* (Krammer 1982b), *Reimeria* (Kociolek and Stoermer 1987) and *Gomphocymbella* (Krammer 1982a), as well as *Gomphonema* and *Gomphoneis herculeana* as determined in [17]. The condition for *G. elegans* and *Brebissonia* cannot be determined. Because both ventrally deflected (a) and dorsally deflected (b) raphe ends are lacking in the outgroups (or cannot be determined), both states are considered derived and unordered.

19. *Position of the chloroplast, pyrenoid and nucleus.* This feature has been discussed relative to the segregation of *Encyonema* and *Cymbella* species (Geitler 1958, 1981, Mann 1981a). Species of *Cymbella* and *C. mexicana* possess a dorsal chloroplast with a dorsal pyrenoid and ventral nucleus (Cholnoky 1929, Geitler 1981). A ventrally rotated chloroplast with ventral pyrenoid and dorsal nucleus is observed in *Encyonema* (Cholnoky 1929, Geitler 1981, Mann 1983b)
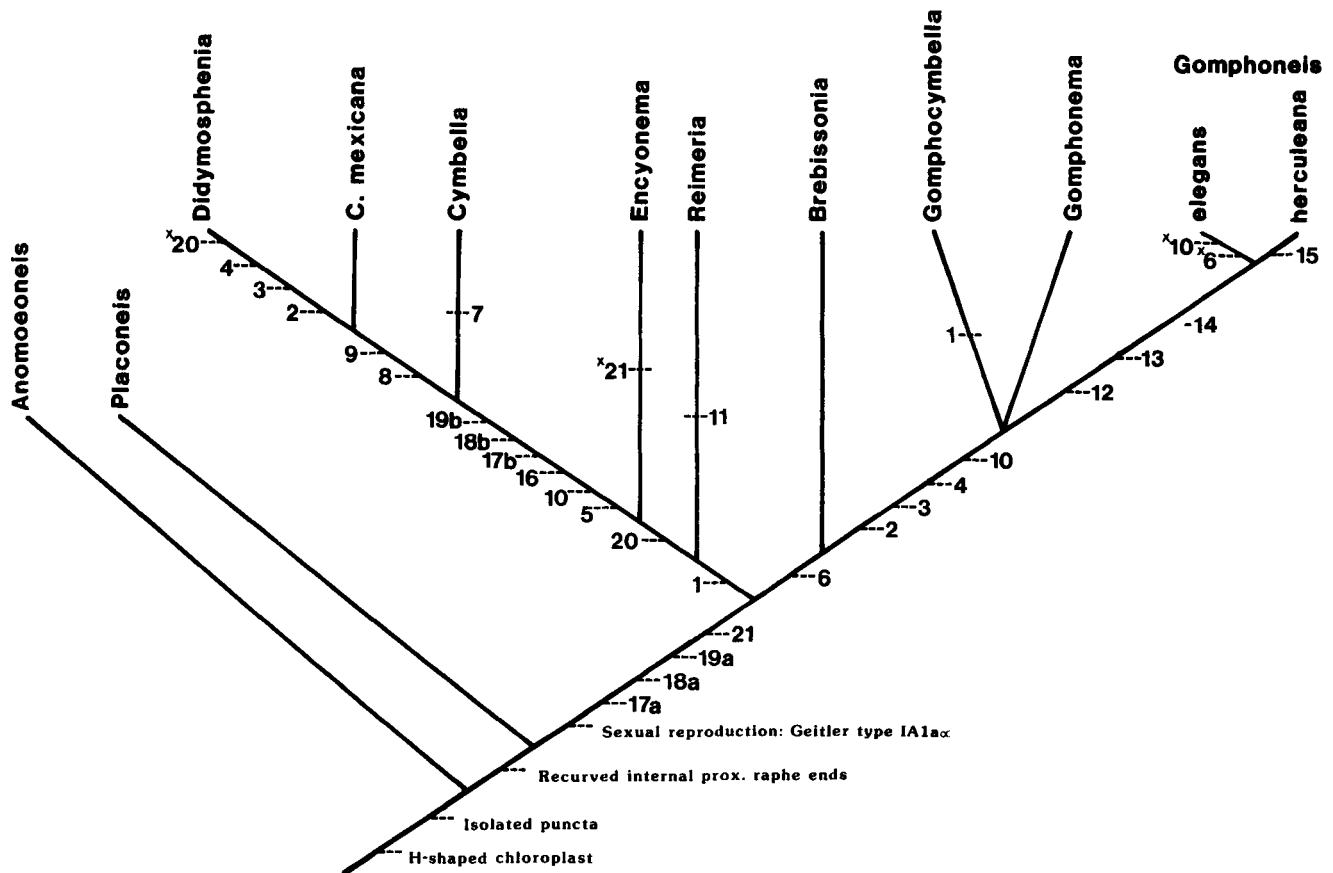
FIG. 2. Consensus cladogram of the three equally most parsimonious cladograms constructed by PAUP for the character state data presented in Table 1. Horizontal black bars represent shared, derived features. Numbers correspond to features described in the text and Table 1. "X" indicates evolutionary reversals.

and *Reimeria* (Geitler 1958). The dorsal stigma in *Gomphonema* species (see [18]) is positioned opposite the pyrenoid (Dawson 1972) and a similar condition is observed in *Gomphoneis herculeana* (Kociolek 1988). Thus, these two taxa have ventral chloroplasts. The position of the chloroplast in *Didymosphenia* apparently has not been reported. Our unpublished observations on *D. geminata* (Lyngb.) M. Schm. indicate the chloroplast is dorsal, and this orientation is indicated by Meyer's (1929) illustration of a dorsally-placed pyrenoid during reduction division in this species. The same rationale described for [17] and [18] above can be applied here to consider both ventrally rotated (a) and dorsally rotated (b) chloroplasts as derived and unordered.

20. *Difference between dorsal and ventral mantle heights.* Krammer (1981) indicates that species of *Cymbella s. l.* possess a difference in valve mantle heights. Of the terminal taxa considered here only *Cymbella, C. mexicana* and *Encyonema* possess this feature, which, owing to its absence in the outgroups, is considered derived.

21. *Apical pore fields present.* All terminal taxa, except *Encyonema*, possess apical pore fields. This feature is lacking in the outgroups, suggesting presence of APF's is derived.

MATERIALS AND METHODS

Coding of the character states for the ten terminal taxa is presented in Table 1. Three most parsimonious cladograms were identified by PAUP (Phylogenetic Analysis Using Parsimony, Version 2.4.0, Swofford 1986) using the Branch and Bound option. A consensus cladogram was constructed from the most parsimonious cladograms using the subroutine Contree. Putative synapomorphies (shared, derived characters) were mapped on the consensus cladogram and identified numerically.

RESULTS

The consensus cladogram (Fig. 2) indicates the lineage of freshwater APF-bearing cymbelloid and gomphonemoid diatoms and their close allies is defined by the presence of APF's, the IA1aα type of sexuality described by Geitler, as well as a dorsal stigma, distal raphe ends that are deflected ventrally, and a ventrally rotated chloroplast with ventral pyrenoid and dorsal nucleus. The lineage is divided into two clades; one comprised predominately of cymbelloid diatoms, the other comprised almost entirely of gomphonemoid diatoms. The cymbelloid clade is defined by asymmetry about the apical axis and contains the taxa *Reimeria, Encyonema, Cymbella, C. mexicana* and the gomphonemoid genus *Didymosphenia.* The gomphonemoid lineage is defined by

the presence of slit-like stigmata and contains *Brebissonia*, *Gomphocymbella*, *Gomphonema* and the two subgroups of *Gomphoneis*.

Within the cymbelloid lineage, *Reimeria* branches first and possesses the autapomorphic (derived, but unique) feature of unequal APF's. The rest of the lineage is defined by having dorsal and ventral mantles of different heights. *Encyonema* branches next and, according to the hypothesis, members lack APF's through secondary loss. The remaining members of the cymbelloid clade (*Cymbella*, *C. mexicana* and *Didymosphenia*) form a group which is defined by the features of differentiated APF's, lack of an intermissio, APF's not bisected by the raphe, ventrally placed stigmata, distal raphe ends deflected dorsally, and a dorsally rotated chloroplast with a dorsal pyrenoid and ventral nucleus. The genus *Cymbella* branches at this point and is defined by the presence of dendritic stigmata. *Didymosphenia* and *C. mexicana* are shown to share a more recent common ancestor based on the features of complex foraminae and conical, silica-covered stigmata. The genus *Didymosphenia* is shown to have the derived features of asymmetry about the transapical and pervalvar axes, an APF at one pole only, and to have secondarily lost differences in valve mantle height.

In the lineage predominated by gomphonemoid taxa, the genus *Brebissonia* branches first, and it is without any defining features. Asymmetry about the transapical and pervalvar axes, APF's restricted to one pole and differentiated APF's define the rest of the clade. Asymmetry features and APF's at one pole are considered convergences between this gomphonemoid lineage and *Didymosphenia*. At this level of hierarchy in the gomphonemoid line there is an unresolved trichotomy between *Gomphocymbella*, *Gomphonema* and *Gomphoneis*. Asymmetry about the apical axis (a convergence with the cymbelloid lineage) defines *Gomphocymbella* whereas presence of septa, pseudosepta and marginal laminae define the genus *Gomphoneis*. *Gomphonema* lacks defining features. Within *Gomphoneis*, *G. elegans* is defined by undifferentiated APF's and lack of stigmata (both features are considered evolutionary reversals) and the *G. herculeana* subgroup is defined by the presence of an axial plate.

DISCUSSION

Previously published interpretations of interrelationships of the taxa included among the freshwater APF-bearing cymbelloid and gomphonemoid diatoms and their close allies, which envision cymbelloid diatoms as primitive, gomphonemoid diatoms as derived, and these two groups joined by intermediates such as gomphocymbelloid species (Cleve 1894) or *Reimeria sinuata* (Krammer and Lange-Bertalot 1985), are not supported by the present results. Results of the cladistic analysis suggest that a *Placoneis*-like taxon, having undifferentiated APF's at both poles, a process of reduction

division and gamete pairing as well as an orientation of cytological and valve features similar to *Encyonema*, *Reimeria* and *Gomphonema*, was ancestral to the entire lineage. That this ancestral form resembled a member of *Placoneis* is strengthened by the observation of Cox (1987) which shows recurved internal proximal raphe ends, a feature also observed in many gomphonemoid and cymbelloid species (Dawson 1973c, Krammer 1982b, Kociolek and Stoermer 1986b, c). The cymbelloid diatoms and gomphonemoid diatoms then each diverged independently from the naviculoid archetype.

Within the cymbelloid line, *Reimeria*, then *Encyonema*, branched, and the eventual evolution of *Cymbella s. s.* and its allies was accomplished through a polarity reversal of organellar and valve features, as hypothesized by Mann (1983b). It seems likely that members of *Cymbopleura*, a heterogenous group of cymbelloid diatoms, may have branched independently at various points along the cymbelloid clade. Additional observations on the ultrastructure and cytology of these enigmatic taxa will be necessary to assess their systematic positions.

The analytical result which shows *Didymosphenia* and *Cymbella mexicana* to share a more recent common ancestor than either does with *Cymbella s. s.* or other gomphonemoid diatoms suggests that the feature of valve symmetry, which has been given so much weight in the construction of classification schemes for diatoms, may not be a good indicator of genealogical relationships, particularly in this group. Cox (1979) has previously made this point with regard to *Gyrosigma* Hass. and *Pleurosigma* W. Sm. A consequence of the specific result here is that for the genus *Cymbella s. s.* to be a monophyletic group (*sensu* Hennig 1966) either *Didymosphenia* must be included in the genus or be joined by *C. mexicana* outside *Cymbella*. In either case the results clearly indicate *Didymosphenia* to be more closely related to *Cymbella* species than to other gomphonemoid diatoms.

Within the gomphonemoid line, the two subgroups of *Gomphoneis* are shown to be joined by the features of marginal laminae, septa and pseudosepta. The features of marginal laminae and multi-punctate striae were identified by Krammer and Lange-Bertalot (1985) to distinguish *Gomphoneis*. Expression of doubly-punctate striae in species of cymbelloid diatoms (Hustedt 1942, 1944, Okuno 1974, Germain 1981), *Reimeria* (Krammer 1982a, Kociolek and Stoermer 1987), *Gomphonema* (Hustedt 1942, 1945, Krammer and Lange-Bertalot 1985) and *Brebissonia* (Mahoney and Reimer 1986) suggest this feature may actually be derived for the whole group and thus not a defining feature of *Gomphoneis*. By the criterion of presence of marginal laminae, other gomphonemoid diatoms which possess this structure (e.g. *Gomphopleura* Reich., R. Mahoney, pers. comm.) would have to be included in the genus. The diatom *Gomphonema ventricosum* Greg. has been reported to

possess a septum (Dawson 1973c) and may represent a form intermediate between *Gomphonema* and *Gomphoneis*.

Although not included as terminal taxa in the present investigation, comment here on the taxonomic position of some doubly-punctate species of *Gomphonema* (*G. olivaceum* (Lyngb.) Kütz., *G. quadripunctatum* Østr.) seems appropriate. Dawson (1974) included these species in *Gomphoneis* on the basis of their dissimilarity to most other *Gomphonema* species, including the presence of doubly-punctate striae. Kociolek and Rosen (1984) argued for their retention in *Gomphonema* due to a lack of an axial plate. Lack of marginal laminae in these species was used as evidence to retain them in *Gomphonema* (Krammer and Lange-Bertalot 1985, 1986). Undifferentiated APF's and septa have been observed in these species (Dawson 1974, Okuno 1974, see also Skabitschevsky 1984). Additionally, because these species may be astigmate (like *Gomphoneis transsilvanica* (Pant.) Krammer *in* Krammer and Lange-Bertalot) or have several stigmoids about the central area (as in *G. elegans*), an argument can be made for their affinity with the *G. elegans* subgroup of *Gomphoneis*. The condition of marginal laminae would have to have been secondarily lost. Following the generic definition proposed by Krammer and Lange-Bertalot (1985), the taxonomic implication would be that the *G. olivaceum/G. quadripunctatum* group best belongs in *Gomphoneis*. Thus, *Gomphoneis* is a polythetic taxon, that is not all taxa exhibit the features which define it (Wiley 1981). Similarly, the entire ingroup considered in the present report is polythetic, since although presence of APF's help to define the lineage, *Encyonema* species lack them yet are still included in the clade.

The genus *Gomphonema*, as shown in the cladistic analysis, lacks any defining characters, i.e. it is a "Not-A" group (Eldredge and Cracraft 1980). In many cases "Not-A" groups represent heterogenous assemblages of taxa, sometimes sharing only the commonality of lacking derived features (Wiley 1981). The myriad of forms assigned to *Gomphonema* (Dawson 1973c, Krammer and Lange-Bertalot 1986, Kociolek et al. 1988) suggest this possibility. Additional studies on the ultrastructure of *Gomphonema* species are warranted to identify whether they comprise a monophyletic taxon.

With regard to character evolution, Mann (1983b) suggested the features of ventrally-placed stigmata, external distal raphe ends that are deflected dorsally, and a dorsally-rotated chloroplast possibly arose together via polarity reversal from the opposite, ancestral condition, and his hypothesis is supported by our analysis. That these features were derived from a single process is suggested by their 100% correlation (see Mayr 1969). Similarly, the three features of valve asymmetry in gomphonemoid diatoms, namely asymmetry with regard to the transapical axis, asymmetry with regard to the pervalvar axis,

and APF's restricted to the footpole may be the result of a single process. That these three features also appear to be 100% correlated, even in apparently unrelated groups such as the araphid genus *Licmophora* C. A. Ag. (Hendey 1964, Wahrer et al. 1985), suggests that a single ontogenetic pathway might produce gomphonemoid heteropolarity.

The cladistic analysis of phylogenetic relationships among the freshwater APF-bearing cymbelloid and gomphonemoid diatoms and their close allies represents a testable hypothesis concerning interrelationships of the terminal taxa and the evolution of characters. As our understanding of the pool of taxa presently classified in *Navicula* becomes better known and as the ultrastructure of other cymbelloid and gomphonemoid species is described, the validity of this hypothesis as an estimator of the groups' genealogy may be evaluated.

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ULTRASTRUCTURE OF FERTILIZATION IN A CRYPTOMONAD[1]

Paul Kugrens[2]

Department of Biology, Colorado State University, Fort Collins, Colorado 80523

and

Robert E. Lee

Department of Anatomy, Colorado State University, Fort Collins, Colorado 80523

ABSTRACT

By means of various electron microscopic techniques, the ultrastructure of fusing gametes in a cryptomonad is described for the first time. The isolate used in this study is bisexual, and vegetative cells may act as isogametes. Plasmogamy usually is initiated at the posterior end of one gamete and the mid-ventral region of the other gamete. A posterior, pointed protuberance may be a specialized mating structure which initiates the fusion process. Fusion proceeds toward the anterior end, forming a quadriflagellate cell which becomes spherical and settles to the bottom of the culture flask. The quadriflagellate, spherical cell contains two nuclear-nucleomorph-chloroplast complexes which remain intact throughout karyogamy. During karyogamy the nuclei are positioned close to each other and become lobed on the sides where fusion takes place. At the points where the lobes touch, the nuclear membranes break down and direct karyogamy is initiated. Nuclear fusion continues and eventually a single zygotic nucleus is formed. The zygote nucleus and the two nucleomorphs and chloroplasts become enclosed in a common periplastidial compartment. The nucleomorphs, however, remain apart and do not fuse. Meiosis presumably is zygotic, but the stages of post-karyogamy remain to be elucidated.

Key index words: cryptomonads; fertilization; ultrastructure

The most common means of reproduction in cryptomonads is by mitotic cell division (Oakley and Dodge 1976, Oakley and Bisalputra 1977, McKerracher and Gibbs 1982, Oakley and Santore 1982, Meyer and Pienaar 1984a, b). Sexual reproduction apparently is rare and generally is perceived to be absent because direct evidence for a sexual process is lacking. For instance, Wawrik provided brief descriptions and diagrams of gamete fusion in field collections of *Cryptomonas* (Wawrik 1969) and *Chroomonas* (Wawrik 1971), but unfortunately these are not documented with photomicrographic evidence. Furthermore, the diagrams do not depict karyogamy. Hill and Wetherbee (1986), by utilizing fluorescence microscopic techniques on the marine cryptomonoad *Proteomonas* Hill and Wetherbee, detected haploid and diploid cells in a clonal culture. In addition, electron microscopy of *Proteomonas* cells provided structural differences between the two cell types, suggesting a diplomorphic life history. The