ASSESSING THE RELATIONSHIPS OF AUTOSPORIC AND ZOOSPORIC CHLOROCOCCALEAN GREEN ALGAE WITH 18S rDNA SEQUENCE DATA[1]

Lee W. Wilcox, Louise A. Lewis,[2] *Paul A. Fuerst,* and Gary L. Floyd*[3]

Departments of Plant Biology and *Molecular Genetics, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

We examined the relationships of autosporic and zoosporic taxa in the Chlorococcales by analyzing available complete nuclear-encoded small-subunit (18S) rRNA gene sequences along with two new sequences from Hydrodictyon reticulatum *(L.) Lagerh. and* Neochloris vigenis *Archibald. Some autosporic taxa grouped with the coenobial hydrodictyacean algae and related unicellular organisms having directly opposed basal bodies. These include* Scenedesmus obliquus *(Turp.) Kütz. and* Chlorella fusca *var.* vacuolata *Shihira et Krauss. Other autosporic organisms, including* Chlorella kessleri *Fott et Novakova, C.* minutissima *Fott et Novakova, C.* protothecoides *Krug., C.* vulgaris *Beij.,* Nanochlorum eucaryotum *Wilhelm, Eisenbeis, Wild, et Zahn, and* Prototheca wickerhamii *Soneda et Tubaki, form a separate group. Of the zoosporic taxa examined, this group would appear to have the greatest affinity to organisms having a counterclockwise displacement of basal bodies, the Pleurastrophyceae. Beyond the fact that* Ankistrodesmus stipitatus *(= A.* falcatus *var.* stipitatus *(Chodat) Lemm.) does not group with the latter organisms, its position remains in doubt. None of the autosporic taxa appear to be closely related to chlorophycean organisms possessing a clockwise basal body displacement.*

Key index words: Chlorococcales; Chlorophyceae; Chlorophyta; 18S rDNA; phylogeny; Pleurastrophyceae; sequence data

The Chlorococcales has long been thought to represent an unnatural assemblage of organisms (e.g. Fritsch 1935, Smith 1950). As noted by Fritsch (1935), virtually every attempt at classification (using vegetative morphological and life history characters) leads to unnatural groups. Phenotypic plasticity in some taxa (Trainor and Egan 1990, Egan and Trainor 1991) has further complicated the issue. As a result of such difficulties, the number of classification schemes proposed for this problematic order have nearly equaled the number of workers who have dealt with it.

The artificiality of the Chlorococcales has become even more apparent with recent compelling ultrastructural evidence, primarily coming from details of the flagellar apparatus (FA) of motile cells. Three distinct FA types are now known to occur among taxa once included in this order. The three each possess a cruciate system of microtubular rootlets and are distinguished primarily by the spatial relationship of the basal bodies, whether 1) displaced in a clockwise (CW) direction, 2) directly opposed (DO), or 3) displaced in a counterclockwise (CCW) direction when viewed from above (Watanabe and Floyd 1989b). Possessing the CW type are members of such traditional chlorococcalean genera as *Golenkinia* (Moestrup 1972), *Chlorococcum,* and *Protosiphon* (Watanabe and Floyd 1989a). The DO type is found in the hydrodictyacean taxa *Hydrodictyon* (Marchant and Pickett-Heaps 1972 a, b) and *Pediastrum* (Wilcox and Floyd 1988) and in the unicellular forms *Tetraedron* and *Chlorotetraedron* (Watanabe et al. 1988). Among organisms with the CCW type are *Friedmannia, Trebouxia,* and *Pseudotrebouxia* (Melkonian and Berns 1983). This group corresponds to the class Pleurastrophyceae (sensu Mattox and Stewart 1984). The CW, DO, and CCW FA types are each represented among the species of "*Neochloris*" (Watanabe and Floyd 1989b) and *Characium* (Floyd and Watanabe 1990).

Flagellar apparatus information is of course unavailable for chlorococcalean taxa that reproduce by way of non-motile spores. The paucity of taxonomically useful morphological characters has led workers to investigate a host of physiological and biochemical features (e.g. see Takeda 1991 for a brief overview of the approaches that have been taken in studying *Chlorella*). While providing a considerable amount of information, such studies have left many questions unanswered.

With the advent of modern molecular methods, acquiring DNA sequence data has become nearly routine, thereby making a potentially powerful phylogenetic tool available to an increasing number of workers. In a recent study employing complete nuclear-encoded small-subunit (18S) rRNA gene sequences, Huss and Sogin (1990) have provided an additional line of evidence for the unnaturalness of the Chlorococcales. They found the autosporic genus *Chlorella* to be polyphyletic, with the species separating into two distinct groups. They also analyzed sequences from four other autosporic chlorococcalean genera, *Nanochlorum* (Sargent et al. 1988), *Ankistrodesmus, Prototheca,* and *Scenedesmus,* and found them to segregate along the same lines as the *Chlorella* species.

In another study employing complete 18S rDNA sequence data, we investigated species at one time placed in *Neochloris* and *Characium* representing the

---

CW, DO, and CCW FA groups (Lewis et al. 1992) and found the molecular data to support the earlier ultrastructural findings of Watanabe and Floyd (1989b).

In this report, we integrate much of the 18S rDNA sequence data currently available on the Chlorococcales, the majority coming from the two aforementioned studies, and report 18S sequences for two additional "directly opposed" taxa, *Hydrodictyon reticulatum* (L.) Lagerh. and *Neochloris vigenis* Archibald. The use of molecular data allows autosporic and zoosporic taxa to be placed in the same context and provides what may be the most useful means by which to assess their relationships.

MATERIALS AND METHODS

We present sequence data for *Hydrodictyon reticulatum* (Carolina Biological Supply) and *Neochloris vigenis* (UTEX #1981, Starr and Zeikus 1987). Both sequences have been deposited in GenBank (accession numbers are M74497 and M74496, respectively). We have previously described 18S rDNA sequences for *Dunaliella parva* Lerche, *Characium hindakii* Lee et Bold, *C. perforatum* Lee et Bold, *C. vacuolatum* Lee et Bold, *Friedmannia israelensis* Chantanachat et Bold, *Neochloris aquatica* Starr, *Parietochloris pseudoalveolaris* (Deason et Bold) Watanabe et Floyd, and *Pediastrum duplex* Meyen (Lewis et al. 1992). Sequences for *Ankistrodesmus falcatus* var. *stipitatus* (Chodat) Lemm., *Chlorella fusca* var. *vacuolata* Shihira et Krauss, *C. kessleri* Fott et Novakova, *C. minutissima* Fott et Novakova, *C. protothecoides* Krug., *Prototheca wickerhamii* Soneda et Tubaki, and *Scenedesmus obliquus* (Turp.) Kütz. are from Huss and Sogin (1990). "*A. stipitatus*" is used by Huss and Sogin for *A. falcatus* var. *stipitatus*. Those of *Chlorella vulgaris* Beij., *Nanochlorum eucaryotum* Wilhelm, Eisenbeis, Wild, et Zahn, and *Glycine max* (L.) Merr. come from Huss and Sogin (1989), Sargent et al. (1988), and Eckenrode et al. (1985), respectively.

Culture conditions/DNA extraction. Organisms were cultured at 20° C under a 16:8 h L:D cycle in soil water (*Hydrodictyon*) or 9:1 BBM: soil water extract (Starr and Zeikus 1987) (*Neochloris*). Vegetative *Hydrodictyon* cells were used for DNA extractions. For *N. vigenis*, DNA was extracted from zoospores, which were induced as described in Watanabe and Floyd (1989b). Cells were lysed in UNSET buffer (Garriga et al. 1984). *Hydrodictyon* cells were gently ground with a mortar and pestle. Following lysis, material was subjected to a standard phenol/chloroform extraction, ethanol-precipitated, and resuspended in TE buffer (Maniatis et al. 1982).

Polymerase chain reaction/cloning/sequencing. Total cellular DNA served as template for polymerase chain reaction (PCR) using primers complementary to the conserved 5' and 3' ends of the 18S rDNA molecule. Following an approach similar to that of Medlin et al. (1988), the amplification primers have built-in restriction sites allowing for efficient cloning into M13-mp18 and M13-mp19 sequencing vectors. Single-stranded DNA from approx. 10 M13 clones of each orientation was pooled for dideoxy chain-termination sequencing following the Sequenase ver. 2.0 protocol (USB).

Sequence analyses. Sequences were aligned manually using the sequence editor ESEE for the IBM-PC (Cabot and Beckenbach 1989). Regions corresponding to the PCR primers and those that were not clearly alignable for all taxa were excluded from the sequence analyses. These regions corresponding to positions 1–23, 133–136, 230–231, 491–493, 1350–1371, 1397, 1490–1491, 1684–1710, and 1770–1797 in the *Nanochlorum* sequence were excluded. The alignment is available from the authors upon request. The total number of positions remaining (including insertions/deletions) is 1728, which includes 193 phylogenetically informative sites. Both distance and parsimony methods were utilized to analyze the data. For the former, the DNADIST program of PHYLIP (Felsenstein 1990) was employed to generate a distance matrix (Table 1) with the Kimura two-parameter option, which was then input into FITCH to generate a distance tree. Parsimony analyses were performed using PAUP ver. 3.0L (Swofford 1990) with the following options: heuristic search, random stepwise addition (10 replicates), MULPARS, and TBR branch-swapping. A parsimony bootstrap analysis of 100 replications was done using the same options. Gaps were included in the parsimony analysis with those two or more nucleotides in length being reduced to a single nucleotide. The 18S rDNA sequence from soybean (Eckenrode et al. 1985) was used as an outgroup taxon (see Lewis et al. 1992). In the cases where informative sites were involved in base-pairing within stems of the secondary structure and where compensatory substitutions had occurred in all (or all but one) taxa, the weighting for each of the positions making up the pair was reduced by one-half. For positions where all but two taxa exhibited compensatory changes, weighting was reduced by one-quarter. Positions where three or more taxa did not have compensatory changes were given the same (full) weight as unpaired regions. Actual values used in the analysis were 4, 3, and 2.

RESULTS AND DISCUSSION

Polyphyly of autosporic taxa. In both distance (Fig. 1) and parsimony (Figs. 2, 3) trees, the autosporic taxa separate into two main lineages. Two taxa, *Scenedesmus* and *Chlorella fusca*, group with the "directly opposed" unicellular and coenobial taxa. The others, *Nanochlorum*, *Prototheca*, and the remaining *Chlorella* species, form a group that appears closest to the zoosporic taxa possessing the pleurastrophycean type of FA (Figs. 1–3). *Ankistrodesmus* was omitted from the analyses that generated the trees (see later). Huss and Sogin (1990) pointed out that the groups composed of 1) *Scenedesmus/C. fusca/[Ankistrodesmus]* and 2) *Prototheca/Nanochlorum/Chlorella* spp. might correspond to the Scenedesmaceae and Oocystaceae (sensu Bold and Wynne 1985), respectively. However, the latter group may not be chlorophycean but, rather, may be more closely allied with organisms placed in a separate class, the Pleurastrophyceae. None of the autosporic taxa exhibit a strong affinity to CW taxa, represented here by *Dunaliella parva* and *Characium vacuolatum*.

The position of *Ankistrodesmus* remains problematic. When included in the distance analysis, it branches from a point intermediate between the CW and DO taxa. However, parsimony analysis shifts it to a basal position on the clade containing the CW and DO groups. Furthermore, when *Ankistrodesmus* is included in a parsimony analysis that excludes the CCW taxa, it groups with *Scenedesmus* and *C. fusca*. In no instance was it found to group with the CCW zoosporic taxa and/or the assemblage of autosporic taxa, which includes *Nanochlorum*, *Prototheca*, and *Chlorella* species. Because of the instability in its position, we chose to omit *Ankistrodesmus* from the trees depicted here. Perhaps as data for additional zoosporic and autosporic taxa are included, the position of *Ankistrodesmus* will become clear.

The "classical" view of the evolution of autosporic organisms suggests that they arose from zoosporic

TABLE 1. *Pairwise distance values used to generate tree in Figure 1.*

|	Dp	Cv	Ch	Na	Hr	Nv	Pd	So	Cf	Ck	Cm	Cp	Pw	Ne	Cv	Pp	Cp	Fi
Dun. parva	—																	
Cha. vac.	0.032	—																
Cha. hind.	0.037	0.051	—															
Neo. aqua.	0.037	0.049	0.017	—														
Hyd. retic.	0.036	0.049	0.022	0.022	—													
Neo. vigen.	0.037	0.048	0.020	0.006	0.023	—												
Ped. duplex	0.040	0.052	0.027	0.027	0.007	0.028	—											
Scen. obl.	0.039	0.053	0.024	0.024	0.017	0.025	0.023	—										
Chlo. fus.	0.037	0.050	0.023	0.023	0.016	0.025	0.022	0.006	—									
Chlo. kess.	0.046	0.063	0.040	0.041	0.035	0.041	0.040	0.038	0.037	—								
Chlo. min.	0.043	0.055	0.040	0.042	0.038	0.043	0.043	0.037	0.036	0.020	—							
Chlo. prot.	0.080	0.092	0.080	0.080	0.075	0.080	0.078	0.077	0.075	0.058	0.058	—						
Prot. wick.	0.057	0.069	0.053	0.055	0.051	0.055	0.056	0.051	0.051	0.033	0.032	0.049	—					
Nano. euc.	0.046	0.062	0.045	0.044	0.040	0.045	0.045	0.041	0.041	0.024	0.017	0.058	0.032	—				
Chlo. vulga.	0.042	0.057	0.036	0.038	0.032	0.038	0.037	0.035	0.035	0.012	0.018	0.055	0.029	0.018	—			
Par. pseud.	0.045	0.061	0.047	0.051	0.045	0.051	0.051	0.046	0.046	0.036	0.033	0.066	0.043	0.036	0.031	—		
Cha. perf.	0.062	0.073	0.059	0.067	0.061	0.067	0.067	0.061	0.060	0.057	0.052	0.088	0.065	0.058	0.051	0.051	—	
Fr. israel.	0.047	0.061	0.046	0.049	0.041	0.050	0.046	0.044	0.043	0.038	0.034	0.070	0.050	0.037	0.035	0.038	0.050	—
Gly. max	0.111	0.126	0.110	0.111	0.109	0.110	0.111	0.112	0.110	0.108	0.110	0.137	0.123	0.112	0.108	0.118	0.125	0.109
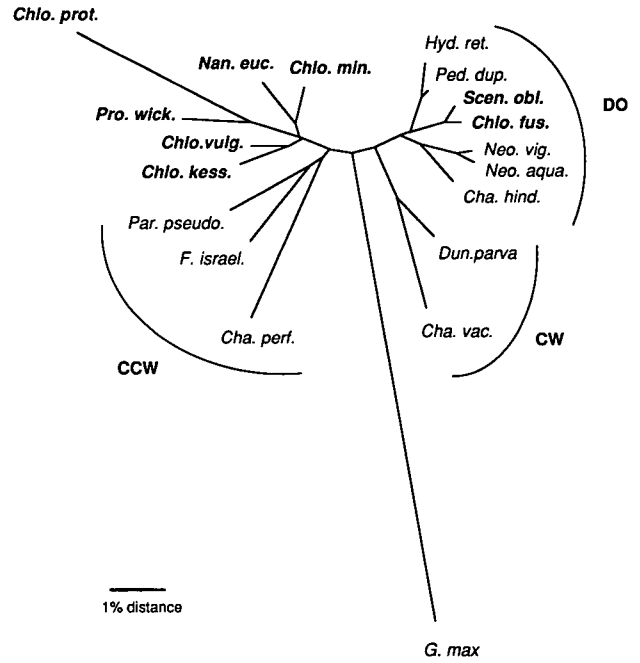
FIG. 1. Distance tree showing disposition of three flagellar apparatus groups of zoosporic taxa (CW, DO, CCW) in relation to autosporic taxa. Note that the autosporic taxa (in boldface) separate into two groups.

forms through the complete loss of motility (e.g. Fritsch 1935, Smith 1950). Fritsch (1935) stated that "suppression of motility occurred again and again along different evolutionary lines [in the Chlorococcales]." The presence of autosporic and zoosporic taxa within two of the major lineages in our trees (Figs. 1–3) provides strong evidence for this view. Schemes separating taxa on the basis of auto- vs. zoospore production clearly can lead to artificial groups. Likewise, because unicellular and coenobial forms (e.g. *Scenedesmus, C. fusca*) may group closely, this appears to be another unreliable distinction.

Relationships among zoosporic taxa with directly opposed basal bodies and their putative autosporic allies. As predicted from their FA ultrastructure, *Hydrodictyon reticulatum* and *Neochloris vigenis* group with other taxa having this type of flagellar apparatus. According to the taxonomic treatment of *Neochloris* by Watanabe and Floyd (1989b; see also Deason et al. 1991), *N. vigenis* should remain in the genus *Neochloris*, based on its possession of a FA nearly identical to that of the type species of the genus, *N. aquatica*. The molecular data support this view. The three unicellular DO zoosporic taxa group together (*N. vigenis, N. aquatica,* and *C. hindakii*), as do the two coenobial organisms (*H. reticulatum* and *P. duplex*).

The apparent affinity of *Scenedesmus* and *C. fusca* var. *vacuolata* with the DO zoosporic taxa appears genuine. Both distance and parsimony analyses generate the same topology, and bootstrap analysis using parsimony groups them with the DO taxa 92%
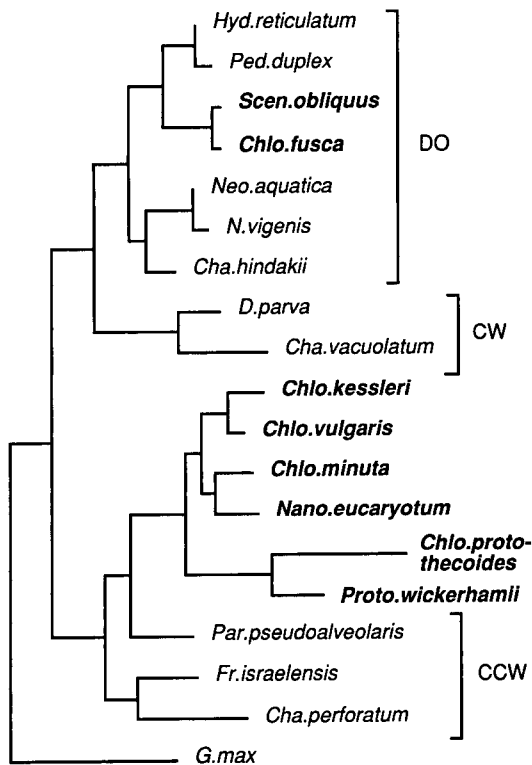
FIG. 2. Shortest parsimony tree found in an heuristic search. Note that autosporic taxa (in boldface) again fall into two groups, one included among the DO taxa and the other closest to the CCW forms. Tree length is 1649 steps (see Materials and Methods for details on character weighting). Consistency index (C.I.) = 0.553.
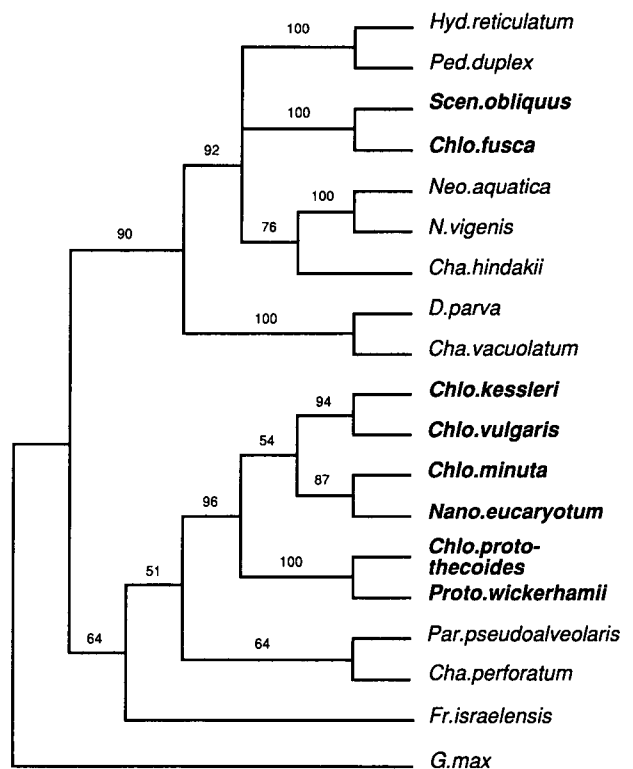

FIG. 3. Parsimony bootstrap tree. Numbers indicate the number of times in 100 replications that the groups (to the right of the number value) were represented. Note that *Scenedesmus* and *C. fusca* grouped with the DO taxa 92% of the time and that the remaining autosporic taxa grouped with the CCW taxa 64% of the time.

of the time (Fig. 3). That these organisms might be related is not unexpected because they have many similarities. The retention of at least occasional motility in *Scenedesmus obliquus* (Trainor 1963, Trainor and Burg 1965) along with its coenobial growth form suggests a possible affinity to the Hydrodictyaceae. The grouping of *C. fusca* var. *vacuolata* with these organisms is likewise not surprising, as it has been suggested to be closely related to *Scenedesmus* based on ultrastructural, biochemical, and DNA hybridization studies (see discussion in Huss and Sogin 1990).

The branching order of these two autosporic taxa and the DO organisms found in the parsimony (and distance) tree was not supported more than 50% of the time by bootstrapping (Fig. 3). This may be due to the fact that, because relatively few characters define this branching pattern, there is a low probability that any one bootstrap replication would include these characters. The examination of additional, more-variable, sequences might provide additional characters useful in evaluating this branching pattern. Nevertheless, if we assume the topology of the tree in Figure 2 to be correct, the autosporic taxa *S. obliquus* and *C. fusca* share a more recent common ancestor with the hydrodictyacean algae than with the unicellular motile forms. This

could suggest that the coenobial habit was established when these two groups diverged and that *Chlorella fusca* represents a secondarily derived unicellular growth form. Alternatively, the coenobial habit may have evolved independently in the Hydrodictyaceae and Scenedesmaceae. The distance tree (Fig. 1) suggests that the unicellular zoosporic taxa, *Neochloris vigenis*, *N. aquatica*, and *Characium hindakii* may have diverged just prior to the coenobial DO forms. This is not surprising, as it is difficult to imagine a scenario where the coenobial taxa would not have originated from a unicellular ancestor.

A number of additional organisms need to be investigated in order to provide a more complete picture of the relationships of DO organisms and their allies. Especially important in this regard are sphaeroplealean taxa, which have flagellar apparatuses similar to, yet distinct from, those of hydrodictyacean forms (Cácares and Robinson 1980, Hoffman 1984, Buchheim and Hoffman 1986). It will also be interesting to determine the affinities of *Tetraedron* and *Chlorotetraedron*, which can produce both auto- and zoospores (see Watanabe et al. 1988). More of the (apparently) strictly autosporic organisms also need to be examined. It is very likely that additional unnatural genera and families will be exposed. It

has been suggested, for example, that *Ankistrodesmus* may not be monophyletic (Kessler 1980, Huss and Sogin 1990). Also, *Dactylococcus*-like forms in *S. obliquus* cultures (see Smith 1950), *Tetraedron*-like forms in *Hydrodictyon* and *Oocystis,* and *Chlorella*-like forms in cultures of a number of chlorococcalean genera (see Fritsch 1935) suggest that finding additional instances where very morphologically distinct organisms possess very similar 18S sequences, such as *S. obliquus* and *C. fusca* (Huss and Sogin 1990), is likely.

Relationships among zoosporic organisms with counterclockwise-displaced basal bodies and the Nanochlorum/Prototheca/Chlorella *assemblage.* The relationships of zoosporic and autosporic organisms is less clear here than in the case of the DO organisms and *Scenedesmus/C. fusca.* Parsimony bootstrap analyses support the grouping of the CCW taxa with this second group of autosporic taxa between 60 and 70% of the time, with the finer-level relationships even less certain (Fig. 3). The shortest parsimony tree (Fig. 2) pairs *Friedmannia* and *Characium perforatum* and places *Parietochloris* at the base of the autosporic group, while the bootstrap tree (Fig. 3) pairs *C. perforatum* and *Parietochloris* and shows *Friedmannia* branching off prior to this pair. The distance tree (Fig. 1) shows the CCW and autosporic organisms to comprise two separate lineages. By themselves, these autosporic taxa appear to make up a coherent group. It appears that the closest zoosporic relatives of these autosporic taxa have not yet been examined. Of the organisms constituting the three FA groups, however, these autosporic taxa show the greatest affinity to those in the Pleurastrophyceae. Clearly, it will be necessary to examine additional organisms, pleurastrophycean, chlorococcalean, and ulvophycean, to better understand the relationships of these taxa.

Summary. The autosporic chlorococcalean taxa for which 18S rDNA sequence data are available do not form a monophyletic group. Two taxa, *Scenedesmus obliquus* and *Chlorella fusca* var. *vacuolata,* group with zoosporic forms having directly opposed basal bodies. The remaining *Chlorella* species, *Nanochlorum eucaryotum* and *Prototheca wickerhamii,* comprise a second group that appears distinct from other traditional chlorococcalean taxa, both autosporic and zoosporic. Of the latter, this group seems to have the greatest affinity to organisms possessing a CCW displacement of basal bodies (the Pleurastrophyceae). As additional taxa are examined, it should become clearer as to whether or not this group may better belong in a class other than the Chlorophyceae. The position of *Ankistrodesmus stipitatus* remains somewhat enigmatic. It clearly does not group with this second assemblage of autosporic organisms. New 18S rDNA sequences for *Hydrodictyon reticulatum* and *Neochloris vigenis* show these organisms to group with other taxa having directly opposed basal bodies, as predicted from FA studies. *Hydrod-*

ictyon pairs with another coenobial organism, *Pediastrum duplex,* while *Neochloris* groups with the other unicellular DO forms, *N. aquatica* and *Characium hindakii.*

Bold, H. C. & Wynne, M. J. 1985. *Introduction to the Algae. Structure and Reproduction,* 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey, 720 pp.

Buchheim, M. A. & Hoffman, L. R. 1986. Ultrastructure of male gametes of *Sphaeroplea robusta. J. Phycol.* 22:176–85.

Cabot, E. L. & Beckenbach, A. T. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Comput. Appl. Biosci.* 5:233–4.

Cácares, E. J. & Robinson, D. G. 1980. Ultrastructural studies on *Sphaeroplea annulina* (Chlorophyceae). 2. Spermatogenesis and male gamete structure. *J. Phycol.* 17:173–80.

Deason, T. R., Silva, P. C., Watanabe, S. & Floyd, G. L. 1991. Taxonomic status of the species of the green algal genus *Neochloris. Pl. Syst. Evol.* 177:213–9.

Eckenrode, V. K., Arnold, J. & Meagher, R. B. 1985. Comparison of the nucleotide sequence of soybean 18S rRNA with the sequences of other small-subunit rRNAs. *J. Mol. Evol.* 21: 259–69.

Egan, P. F. & Trainor, F. R. 1991. Phenotypic plasticity in *Scenedesmus communis* Hegew. (Chlorophyceae). II. Examples of algal cyclo- and noncyclomorphosis. *Am. J. Bot.* 78:37–52.

Felsenstein, J. 1990. PHYLIP manual version 3.3. University Herbarium, University of California, Berkeley.

Floyd, G. L. & Watanabe, S. 1990. Comparative ultrastructure of the zoospores of eight species of *Characium* (Chlorophyceae). *J. Phycol.* 26(Suppl.):11.

Fritsch, F. E. 1935. *The Structure and Reproduction of the Algae,* Vol. I. Cambridge University Press, London, 791 pp.

Garriga, G. H., Bertrandt, H. & Lambowitz, A. 1984. RNA splicing in *Neurospora* mitochondria: nuclear mutants defective in both splicing and 3' end synthesis of the large rRNA. *Cell* 36:623–34.

Hoffman, L. R. 1984. Male gametes of *Atractomorpha echinata* Hoffman (Chlorophyceae). *J. Phycol.* 20:573–4.

Huss, V. A. R. & Sogin, M. L. 1989. Primary structure of the *Chlorella vulgaris* small subunit RNA coding region. *Nucleic Acids Res.* 17:1255.

—— 1990. Phylogenetic position of some *Chlorella* species within the Chlorococcales based upon complete small-subunit ribosomal RNA sequences. *J. Mol. Evol.* 31:432–42.

Kessler, E. 1980. Physiological and biochemical contributions to the taxonomy of the genera *Ankistrodesmus* and *Scenedesmus.* V. Starch hydrolysis and new assignment of strains. *Arch. Microbiol.* 126:11–4.

Lewis, L. A., Wilcox, L. W., Fuerst, P. A. & Floyd, G. L. 1992. Concordance of molecular and ultrastructural data in the study of zoosporic chlorococcalean green algae. *J. Phycol.* 28: 375–80.

Maniatis, T., Fritsch, E. F. & Sambrook, J. 1982. *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, New York, 545 pp.

Marchant, H. J. & Pickett-Heaps, J. D. 1972a. Ultrastructure and differentiation of *Hydrodictyon reticulatum.* 3. Formation of the vegetative daughter net. *Aust. J. Biol. Sci.* 25:265–78.

—— 1972b. Ultrastructure and differentiation of *Hydrodictyon reticulatum.* 4. Conjugation of gametes and the development of zygospores and azygospores. *Aust. J. Biol. Sci.* 25:279–91.

Mattox, K. R. & Stewart, K. D. 1984. Classification of the green algae: a concept based on comparative cytology. In Irvine, D. E. G. & John, D. M. [Eds.] *The Systematics of the Green Algae.* Academic Press, London, pp. 29–72.

Medlin, L., Hille, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 71:491–9.

Melkonian, M. & Berns, B. 1983. Zoospore ultrastructure in the green alga *Friedmannia israelensis*: an absolute configuration analysis. *Protoplasma* 114:67–84.

Moestrup, Ø. 1972. Observations of the fine structure of spermatozoids and vegetative cells of the green alga *Golenkinia*. *Br. Phycol. J.* 7:169–83.

Sargent, M., Zahn, R., Walters, B., Gupta, R. & Kaine, B. 1988. Nucleotide sequence for the 18S rDNA from the microalga *Nanochlorum eucaryotum*. *Nucleic Acids Res.* 16:4156.

Smith, G. M. 1950. *The Fresh-Water Algae of the United States.* McGraw-Hill, New York, 719 pp.

Starr, R. C. & Zeikus, J. A. 1987. UTEX—the culture collection of algae at the University of Texas at Austin. *J. Phycol.* 23(Suppl.):1–47.

Swofford, D. L. 1990. PAUP—phylogenetic analysis using parsimony version 3.0L. Illinois Natural History Survey, University of Illinois, Champaign.

Takeda, H. 1991. Sugar composition of the cell wall and the taxonomy of *Chlorella* (Chlorophyceae). *J. Phycol.* 27:224–32.

Trainor, F. R. 1963. Zoospores in *Scenedesmus obliquus*. *Science (Wash. D.C.)* 142:1673–4.

Trainor, F. R. & Burg, C. A. 1965. *Scenedesmus obliquus* sexuality. *Science (Wash. D.C.)* 148:1094–5.

Trainor, F. R. & Egan, P. F. 1990. *Lagerheimia hindakii* is the unicellular stage of a *Scenedesmus*. *J. Phycol.* 26:535–9.

Watanabe, S. & Floyd, G. L. 1989a. Variation in the ultrastructure of the biflagellate motile cells of six unicellular genera of the Chlamydomonadales and Chlorococcales (Chlorophyceae), with emphasis on the flagellar apparatus. *Am. J. Bot.* 76:307–17.

—— 1989b. Comparative ultrastructure of the zoospores of nine species of *Neochloris* (Chlorophyta). *Pl. Syst. Evol.* 168:195–219.

Watanabe, S., Floyd, G. L. & Wilcox, L. W. 1988. Ultrastructure of the zoospores and vegetative cells of *Tetraedron* and *Chlorotetraedron* (Chlorophyceae). *J. Phycol.* 24:490–5.

Wilcox, L. W. & Floyd, G. L. 1988. Ultrastructure of the gamete of *Pediastrum duplex* (Chlorophyceae). *J. Phycol.* 24:140–6.

SEPARATING *TABELLARIA* (BACILLARIOPHYCEAE) SHAPE GROUPS BASED ON FOURIER DESCRIPTORS[1]

Daiqing Mou and Eugene F. Stoermer[2]

Center for Great Lakes and Aquatic Sciences, University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT

A random sample of 490 Tabellaria *specimens was analyzed using the harmonic amplitudes of the Fourier transformations of their valve outlines as shape descriptors. Principal component analysis (PCA) was applied to the sample to reduce dimensionality. The problem of non-normal distribution of these descriptors due to cell division was solved by sub-sectioning the entire data set based on its distribution on the first three components (PC1, PC2, and PC3) of the overall PCA. Each of the subsets was then analyzed by PCA. Shape groups from subset clusters were compared with one another and then similar groups were congregated into one growth series. Eight distinct shape groups were found. The results agree with some previous classical observations on the genus and at the same time reveal many new morphological characteristics related to valve shape. These new characteristics are impossible to obtain without appropriate specimen sampling, quantitative shape description, and data analysis techniques.*

Key index words: Bacillariophyceae; Fourier descriptors; morphometric; shape analysis; Tabellaria

Taxonomy of the diatom genus *Tabellaria* is very difficult, and there is poor agreement concerning the morphological limits of specific and sub-specific taxa. Almost all morphological characters observed in the genus have been reported to be highly variable (Knudson 1952, 1953a, b, Stoermer and Yang 1969, Koppen 1973, 1975) although the range and form of variation have not been established. Traditional diatom classification has become increasingly suspect for two primary reasons. Nearly all modern studies of diatom genera have revealed greater diversity than that expressed in traditional classifications. There is some recent evidence for this in *Tabellaria* (Lange-Bertalot 1988). At the same time, modern ecological and paleoecological investigations have revealed apparently consistent morphotypes associated with particular geographical regions or ecological conditions, and investigators have increasingly resorted to arbitrary designations to convey information hidden by current formal classifications (Koppen 1978, Kingston et al. 1986). Our operational hypothesis is that unresolved order exists within morphological characteristics of *Tabellaria* and that resolution of this order will result in a more natural and informative systematics.

Valve outline shape is considered important in *Tabellaria* taxonomy for two reasons. 1) *Tabellaria* species, as currently understood, are relatively character-poor. The first and most easily available taxonomic character of this genus is the valve outline