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PSEUDONEOCHLORIS MARINA (CHLOROPHYTA), A NEW COCCOID ULVOPHYCEAN ALGA, AND ITS PHYLOGENETIC POSITION INFERRED FROM MORPHOLOGICAL AND MOLECULAR DATA[1]

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Ultrastructural and molecular sequence data were used to assess the phylogenetic position of the coccoid green alga deposited in the culture collection of the University of Texas at Austin under the name of *Neochloris* sp. (1445). This alga has uninucleate vegetative cells and a parietal chloroplast with pyrenoids; it reproduces by forming naked biflagellate zoospores. Electron microscopy revealed that zoospores have basal bodies displaced in the counterclockwise absolute orientation and overlapped at their proximal ends. Four microtubular rootlets numbering 2 and 2/1 are alternatively arranged in a cruciate pattern. A system I fiber extends beneath each d rootlet and a system II fiber (rhizoplast) originates from each basal body and extends peripherally along each d rootlet. These features differ substantially from those of the three genera, *Ettlia* (Komárek) Deason et al., *Neochloris* (Starr) Deason et al., and *Parietochloris* Watanabe et Floyd, all of which were previously accommodated in the single genus *Neochloris* Starr. Sequence data from the nuclear small subunit ribosomal RNA gene were obtained and compared with published green algal sequences. Results from the ultrastructural and sequence data support the placement of *Neochloris* sp. (The Culture Collection of Algae at the University of Texas at Austin [UTEX] no. 1445) in the Ulvophyceae. This isolate is described as *Pseudoneochloris marina*, gen. et sp. nov. in the Ulotrichales, Ulvophyceae.

Key index words: **18S ribosomal RNA; flagellar apparatus; *Neochloris*; *Pseudoneochloris*; ultrastructure; Ulvophyceae**

Abbreviations: **b1, first basal body; b2, second basal body; DO, directly opposed; dr, d-rootlet; M, mitochondrial profile; N, nucleus; P, pyrenoid matrix; St, stigma; sr, s-rootlet; V, vacuole**

Unicellular green algae are one of the more difficult algal groups to resolve taxonomically because of the relatively small number of morphological traits present in their vegetative cells. However, intensive efforts to characterize flagellar apparatus architecture of coccoid green algae have aided in understanding their phylogenetic affinities (Watanabe and Floyd 1996). Also, studies using ultrastructural information in conjunction with sequence data have greatly clarified the systematics of selected coccoid green algal taxa (Lewis et al. 1992, Wilcox et al. 1992, Friedl and Zeltner 1994, Friedl 1996, Nakayama et al. 1996).

Neochloris Starr (1955) was established for coccoid green algae that possess a parietal chloroplast with pyrenoids and biflagellate zoospores that become spherical upon quiescence. Watanabe and Floyd (1989) divided the nine species of *Neochloris* into three phylogenetic lines based on the architecture of the flagellar apparatus, wall covering of the zoospores, nuclear condition of mature vegetative cells, and details of the pyrenoid matrix. Species of *Neochloris* with multinucleate vegetative cells and zoospores with directly opposed (DO) basal bodies that are naked or covered by fuzzy material were retained in *Neochloris* (Starr) Deason et al., but were transferred to the Sphaeropleales, Chlorophyceae (Deason et al. 1991). The pyrenoid matrix in this group is not penetrated by thylakoid membranes. A second set of species was transferred to *Ettlia* (Komárek) Deason et al. because of the characteristics of uninucleate vegetative cells and walled zoospores with clockwise orientation of basal bodies. Kouwets (1995) observed fine striations on the cell wall and an axial chloroplast in *Ettlia minuta*, one of

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two species of the genus *Ettlia*, and proposed to classify it in the genus *Nautococcus*. *Ettlia* and *Nautococcus* are both accommodated in the Chlorococcales, Chlorophyceae. *Parietochloris* (Bold) Watanabe et Floyd, a third genus, has uninucleate vegetative cells and zoospores that are naked with counterclockwise basal bodies. Motile cells of this genus also have a system II fiber that originates from each basal body and joins into a single strand, extending toward the nucleus. This genus was placed in the Pleurastrophyceae (Mattox and Stewart 1984), and later the Trebouxiophyceae (Friedl 1995).

The polyphyly of *Neochloris* found using ultrastructural data was tested through the use of sequence data from the nuclear small subunit (18S) ribosomal RNA gene (Lewis et al. 1992). Molecular data were in agreement with the ultrastructural data, demonstrating a problem associated with using vegetative cell morphology alone for classification of members of this genus. In addition to *Neochloris*, four other genera of coccoid green algae have been shown to be polyphyletic using combined ultrastructural and molecular data (*Characium*, Lewis et al. 1992, Floyd et al. 1993, *Trebouxia*, Friedl and Zeltner 1994, *Pleurastrum*, Friedl 1996, *Chlorococcum*, Nakayama et al. 1996).

In this article, the alga assigned to *Neochloris* sp. (The Culture Collection of Algae at the University of Texas at Austin [UTEX] no. 1445) is characterized using ultrastructural and molecular data. Both data types support the conclusion that this alga forms a lineage distinct from *Neochloris* and should be described as a new genus in the Ulvophyceae.

MATERIALS AND METHODS

An isolate of *Neochloris* sp. (UTEX no. 1445, hereafter referred to as *Pseudoneochloris marina*) was obtained from the culture collection of the University of Texas at Austin (Starr and Zeikus 1987). Cultures were maintained in f/2 medium with sea water (Guillard and Ryther 1962) and 9:1 medium, including nine parts of Bold's basal medium (Deason and Bold 1960). Cultural conditions and fixation methods used for TEM were those described elsewhere (Watanabe and Floyd 1989). The protocols for total DNA extraction, PCR amplification, and sequencing followed those described previously (Lewis 1997).

Phylogenetic analysis. A total of 1631 nucleotides of the 18S rRNA gene sequence were determined for *P. marina*. The sequence of *P. marina* (DDBJ/EMBL/GenBank accession no. U41102) was incorporated into a data matrix with 38 published

TABLE 1. List of taxa and DDBJ/EMBL/GenBank accession numbers by class, showing sources of 18S rRNA sequence data.

Class	Taxon	DDBJ/EMBL/GenBank accession no.
Ulvophyceae	*Acrosiphonia* sp. Agardh	U03757
	Gloeotilopsis planctonica Iyengar et Philipose	Z28970
	Gloeotilopsis sarinoidea (as *Protoderma sarinoidea* [Groover and Bold] Tupa)	Z47998
	Pseudendoclonium basiliense Vischer	Z47996
	Pseudoneochloris marina (Neochloris sp. 1445)	U41102
	Ulothrix zonata (Weber et Mohr) Kützing	Z47999
Chlorophyceae	*Ankistrodesmus stipitatus* (= *A. falcatus* var. *stipitatus* [Chodat] Lemm.)	X56100
	Bracteacoccus minor (Chod.) Petrova	U63097
	Chaetophora incrassata (Huds.) Hazen	D86499
	Chlamydomonas moewusii Gerloff (Ettl et Schlösser)	U41174
	Chlamydomonas reinhardtii Dang	M32703
	Chlamydopodium vacuolatum (Lee et Bold) Ettl et Komárek	M63001
	Chlorococcum hypnosporum Starr	U41173
	Dunaliella parva Lerche	M62998
	Ettlia minuta (Arce et Bold) Komárek	M62996
	Hydrodictyon reticulatum (L.) Lagerh.	M74497
	Neochloris aquatica Starr	M62861
	Neochloris vigenis Archibald	M74496
	Pediastrum duplex Meyen	M62997
	Scenedesmus obliquus (Turp.) Kütz.	X56103
Trebouxiophyceae	*Chlorella ellipsoidea* Gerneck	X63520
	Chlorella kessleri Fott et Nováková	X56105
	Chlorella minutissima Fott et Nováková	X56102
	Chlorella saccharophila (Krüg.) Mig.	X63505
	Fusochloris perforatum (Lee et Bold) Floyd, Watanbe et Deason	M62999
	Leptosira obovata Vischer	Z68695
	Myrmecia biatorellae (Tschermak-Woess et Plessl) Petersen	Z28971
	Parietochloris pseudoalveolaris (Deason et Bold) Watanabe et Floyd	M63002
	Prototheca wickerhamii Soneda et Tubaki	X56099
	Trebouxia magna Archibald	Z21552
Prasinophyceae	*Halosphaera* sp. Schmitz	AB017125
	Mamiella sp. Moestrup	AB017129
	Mantoniella squamata (Manton et Parke) Desikachary	X73999
	Nephroselmis olivacea Stein	X74754
	Ostreococcus tauri Courties et Chrétiennot-Dinet	Y15814
	Nephroselmis pyriformis (Carter) Ettl (as *Pseudoscourfieldia marina* [Throndsen] Manton)	X75565
	Pyramimonas parkeae Norris et Pearson	AB017124
	Scherffelia dubia (Perty) Pascher	X68484
	Tetraselmis striata Butcher	X70802

18S rRNA sequences (Table 1). The alignment consisted of a total of 1777 aligned nucleotides. Although the alignment of most of the gene sequence was not problematic, 99 nucleotides were excluded from the analyses because sequence for these regions could not be unambiguously aligned across all taxa in the study. The number of excluded nucleotides and their locations correspond to the following secondary structure features proposed in the model by Neefs et al. (1993): 4 nucleotides in V1 (6); 5 in V2 (9); 7 in V3 (17); 5 in V4 (E23-1); 9 in (E23-2); 29 in V7(43); and 34 in V9 (49). This alignment is available from TreeBase (http://www.herbaria.harvard.edu/treebase/).

Maximum parsimony: Parsimony analyses were done in PAUP 4.0b (Swofford 1999) with all sites treated as unordered and with equal weight. Of the 1684 included sites, 1119 were constant and 375 were parsimony informative. A second parsimony analysis was done using a 1:2 transition to transversion step matrix, thereby assigning a greater cost to transversions over transitions ("weighted parsimony"). All tree searches for this and the following analyses were heuristic and used random step-wise addition of taxa, with tree-bisection-reconnection branch swapping holding all best-scoring trees at each step. For the equal-weight parsimony analysis, bootstrap values were obtained from 500 replicate data sets with heuristic searching.

Maximum likelihood: The HKY85 model (Hasegawa et al. 1985) was used, as implemented in PAUP 4.0 d65 (Swofford 1999), on a 667 MHz Microway Alpha Workstation (Microway, Inc., Kingston, MA) running Red Hat Linux 5.2 (Red Hat Linux, Durham, NC). Parameters for rate heterogeneity and the transition to transversion rate ratio were initially estimated under the likelihood criterion from the parsimony topology, then set to those values before the ML searches (see discussion in Swofford et al. 1996). In this case, the transition to transversion rate ratio was estimated to be 1.7. Site-to-site rate heterogeneity was modeled as a γ distribution (Yang 1994; with four discrete rate categories approximating the γ distribution having the shape parameter, α, set to 0.2). The bootstrap proportion of 100 replicate data sets (Felsenstein 1985) was determined using the same parameters used during the heuristic searches.

RESULTS

Morphological aspects. Cultures of *P. marina* in the f/2 medium showed vigorous growth, whereas those cultures in the 9:1 medium did not grow as well. Vegetative cells are spherical and approximately 3 μm in diameter when young and reached 17.5 μm in diameter at maturity in actively growing cultures and 20 μm in diameter in stationary cultures (Figs. 1 and 2). The cell wall is smooth, comprises several layers, and has an approximate 0.5 μm thickness in old cells. A large vacuole occupies nearly half of the volume of older cells, especially in liquid 9:1 medium (Fig. 2). A single nucleus is located peripherally (Figs. 1 and 2). The chloroplast in young cells lacking a vacuole assumes a hollow, spherical shape, although the chloroplast becomes cup-shaped or saucer-shaped when vacuoles are present (Fig. 2). A pyrenoid is located in the center of the chloroplast and the matrix is covered by starch segments and penetrated by one to two thylakoid membranes (Fig. 2).

Zoospore production was promoted by transferring actively growing cells on agar media into newly prepared liquid culture. In mature vegetative cells, 4, 8, or 16 daughter cells are formed and are released as aplanospores (Bold and Wynne 1985, data not shown) or zoospores (Figs. 1, 3 and 4). Zoospores are active in the beginning of the light period for ~2 h. The zoospores assume a fusiform, pyriform, or raindrop shape, and measure 8–10 μm in length and 2–3 μm in width. No papilla were observed. At the end of the swimming period, the zoospores moved in a spiral manner and became spherical in shape when motility ceased. Neither cell wall nor scales are present on the surface of the cell body. Two flagella are inserted at the apex of the cell at an angle of 180°–230° (data not shown) between basal bodies. The nucleus is peripherally positioned in the anterior two-thirds of the cell, and the Golgi apparatus is anterior to the nucleus. The chloroplast obliquely covers the posterior end of the cell and contains one thylakoid-traversed pyrenoid in the posterior region and a stigma in the median-to-posterior position of the chloroplast. The stigma comprises carotenoid droplets arranged in a single layer (Fig. 4).

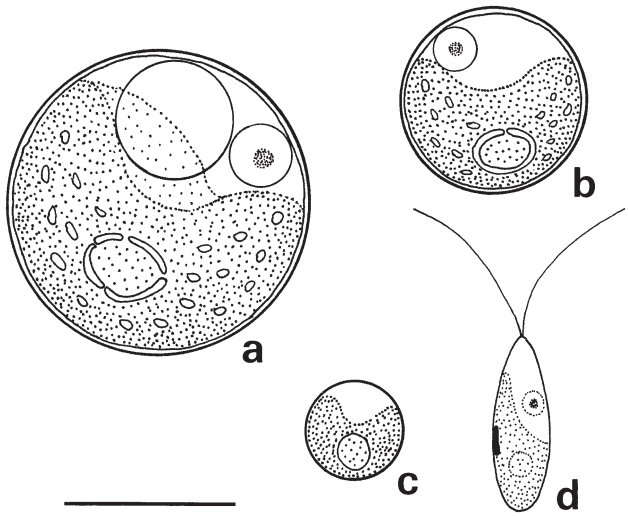


FIG. 1. Schematic light microscopic presentation of vegetative cells (a–c) and a zoospore (d). Scale bar = 10 mm.

In the zoospores, the two basal bodies are connected by a distal fiber with a central narrow electron-opaque band (Fig. 5). The basal bodies are displaced in the counterclockwise absolute orientation and overlap at their proximal ends (Figs. 6–11). When observed in cross-sectional view, the distal fiber is connected to two or three basal body triplets at their anterior flanks (Figs. 12 and 13). The two basal bodies are connected to one another at their lateral flanks (Figs. 7, 8, and 16). A thin, striated fiber is present between the proximal end of one basal body and an anterior triplet of the second basal body (Figs. 6, 15, and 17). Four rootlets comprising two types are arranged in a cruciate pattern (Figs. 7–11). The d (dexter)-rootlets (after Melkonian 1984) contain two microtubules (Fig. 23) with a system I fiber (striated microtubule-associated component, Floyd et al. 1980) beneath (Figs. 23–25). No striation was observed in the system I fiber. Terminal caps are not present on the anterior triplets where the d-rootlets insert; however, each basal body triplet is associated with electron-opaque material at its most proximal end (Fig. 6), which forms a proximal ring

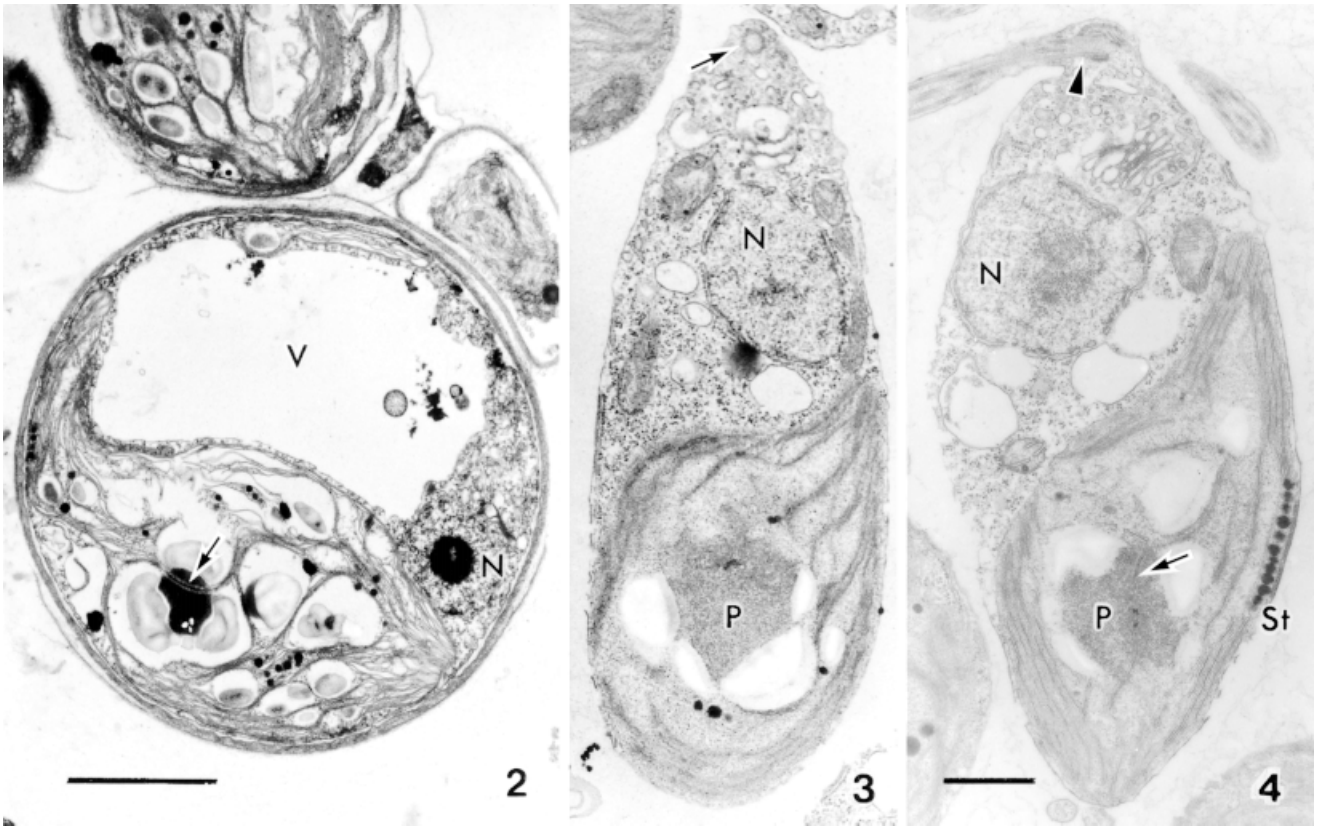


FIG. 2. Ultrastructural cross-section of a mature vegetative cell cultured in 9:1 medium, containing a large vacuole (V). The pyrenoid matrix is traversed by thylakoid membranes (arrow), and the nucleus (N) is peripherally located. Scale bar = 5 mm.

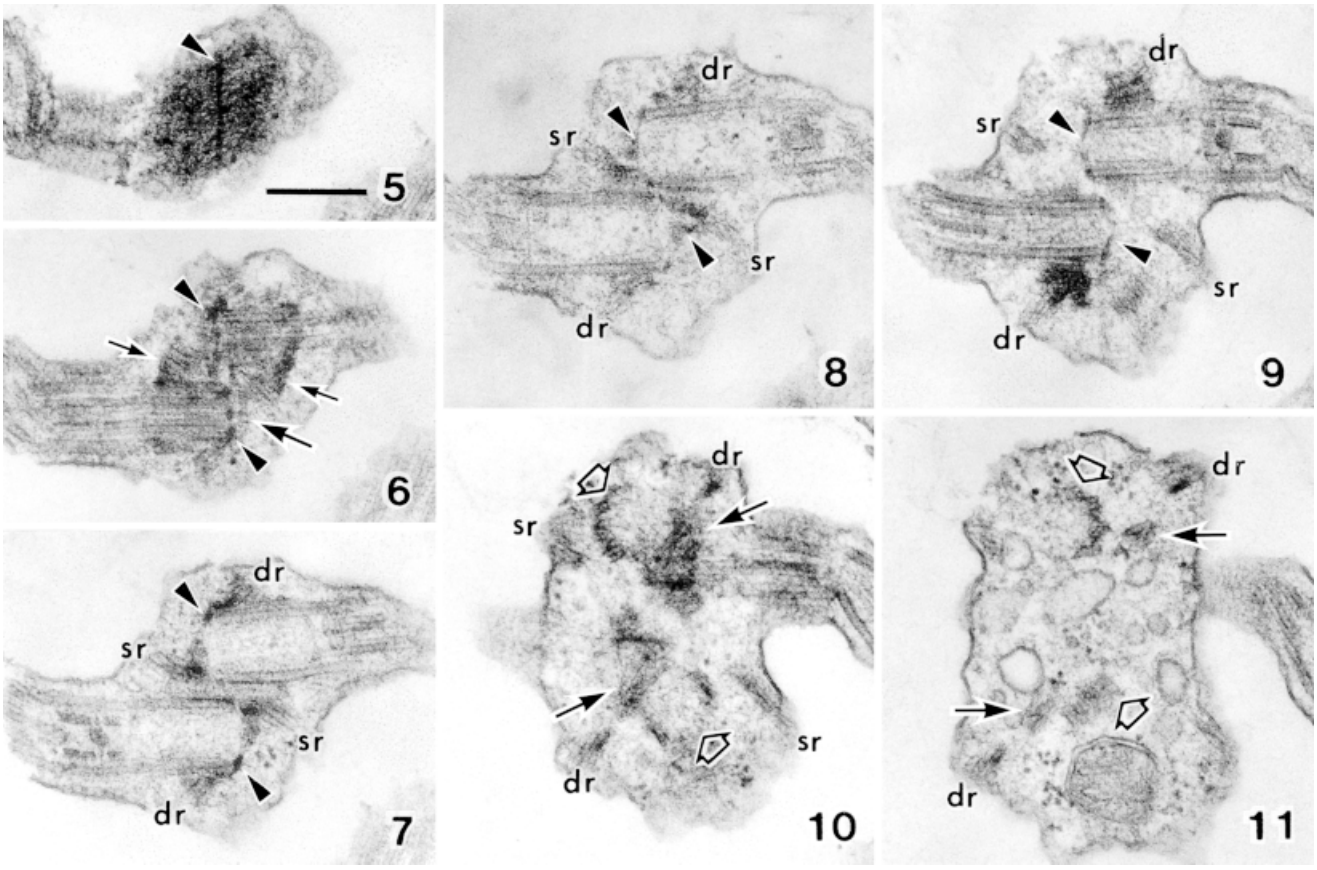
FIGS. 3–4. Longitudinal sections of zoospore. FIG. 3 includes a cross-section of an anterior basal body (arrow). FIG. 4 includes a longitudinal section of a basal body (arrowhead) and a thylakoid membrane (arrow) penetrating the pyrenoid matrix (P). The stigma (St) is visible as a dense layer of droplets in the chloroplast. Scale bar = 1 mm for FIGS. 3 and 4.

(Figs. 16–21). A d-rootlet is connected to the lateral flank of each basal body by a relatively thick, non-striated band (Figs. 9 and 14). The two s (sinister)-rootlets (after Melkonian 1984) are directly opposed to one another (Figs. 7 and 8). These rootlets originate at the central region of the flagellar apparatus as two microtubules (Fig. 24), but become three-membered (in a 2/1 configuration) away from the apex (Fig. 25). The s-rootlet of one basal body is connected to the second basal body by a fiber extending from the proximal ring (Figs. 8, 20, and 21).

A striated system II fiber extends in parallel with each d-rootlet from the posterior triplets of each basal body near the region where the d-rootlet begins (Figs. 10–17, 24, and 25), to a mitochondrion (Fig. 22). Its ending point was not observed. An accessory basal body is attached to each system II fiber between the d- and s-rootlets (Figs. 10 and 11). The ratio of length of proximal and distal cylinders in the transitional regions is ~2:1 (Fig. 20).

Sequence analysis. Phylogenetic analysis using the maximum likelihood criterion produced a tree of score lnL = −10584.43677 (Fig. 26). Maximum parsimony (MP) searches with equal weighting found six trees (length, L = 1522 steps; confidence interval [CI] = 0.4267), and weighted parsimony heuristic searches found one tree (L = 2124, CI = 0.4365) which was different from the six trees found using equal-weight parsimony (see below). For clarity, only the maximum likelihood (ML) tree is shown because any differences between this tree and the remaining trees can easily be described. The sequences of *Halosphaera* and *Pyramimonas* were used as outgroups and followed the results of Nakayama et al. (1998), which showed the basal position of these taxa in the green algae.

In all analyses, phylogenetic trees included the *Pseudoneochloris* sequence in a well-supported clade with the five ulotrichalean algal sequences. The ML and MP trees differed in the relative position of *Pseudoneochloris* within the ulotrichalean clade, and in minor rearrangements among the species in the DO clade. Specifically, *Pseudoneochloris* was either joined to *Acrosiphonia* sp. (in the ML analysis) or was attached at the base of the Ulotrichales (in the MP analysis). Both of these alternatives had low boostrap support. The single tree obtained from the weighted analysis differed from the equal weight parsimony analysis in that the weighted analysis (like the ML analysis) caused the Trebouxiophyceae to



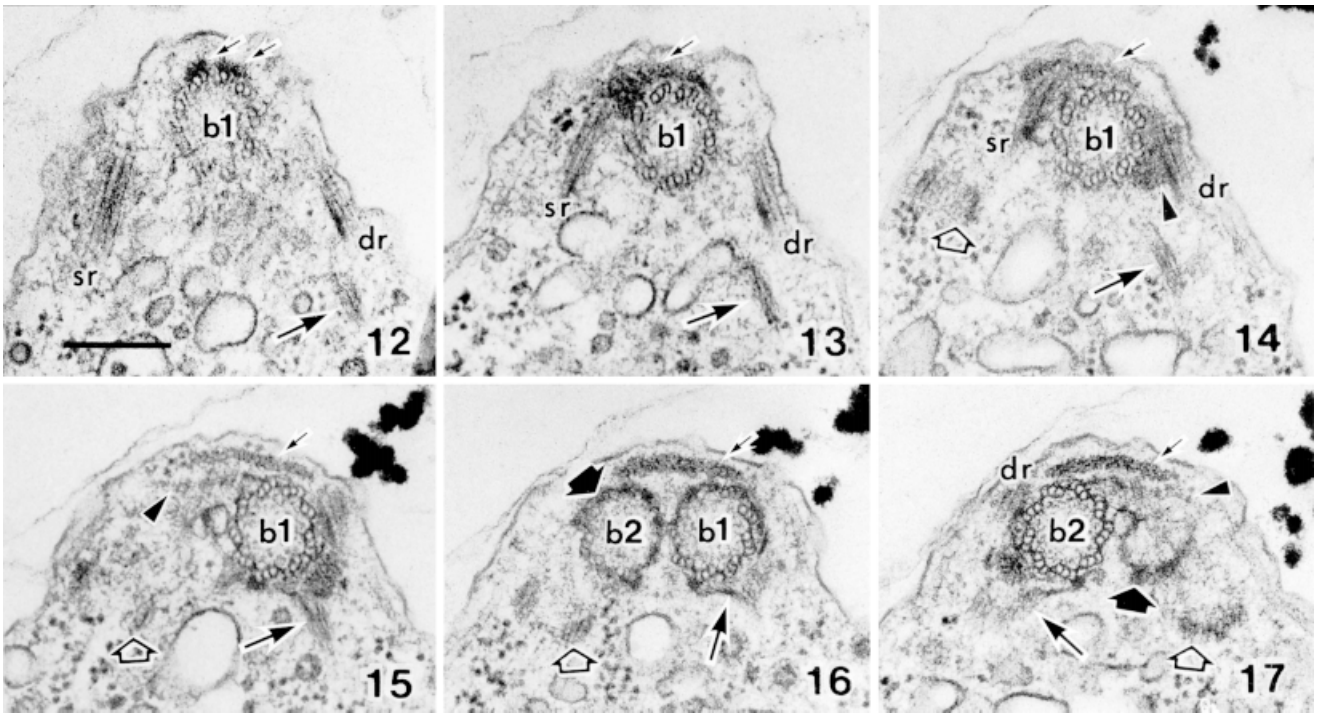
Figs. 5–11. Consecutive cross-sections of the anterior portion of a zoospore. Fig. 5. Horizontal section of the distal fiber with a central band (arrowhead). Scale bar = 200 nm for Figs. 5–11. Figs. 6–9. Basal bodies are displaced in counterclockwise absolute orientation, and two sets of d-rootlets (dr) and s-rootlets (sr) are arranged in a cruciate pattern. Small arrows indicate edges of the distal fiber, and arrowheads show electron-opaque material at the proximal end of microtubule triplets. A thin, striated fiber (large arrow) is associated with the proximal end of the basal body. Figs. 10 and 11. Two sections beneath the basal bodies include a system II fiber (arrows) running under d-rootlet (dr) and an attached accessory basal body (open arrows).

be monophyletic. Both ML and MP produced trees with the Chlorophyceae and Trebouxiophyceae as sister groups, although this node had low bootstrap support.

DISCUSSION

Ultrastructural aspects. The present study determined that *P. marina* has coccoid, non-motile vegetative cells that remained uninucleate throughout vegetative growth. The chloroplast is cup- or saucer-shaped with a pyrenoid and its zoospores are biflagellate and become promptly spherical upon quiescence. Basal bodies of the zoospores are displaced in the counterclockwise orientation with imbrication at their proximal ends. Archibald (1973) reported that this isolate exhibited the plant mass characteristics and cellular morphology typical of *Neochloris aquatica* Starr when it was cultured in 3N Bold's basal agar medium. However, *Pseudoneochloris* is clearly different from this species in that mature vegetative cells of *N. aquatica* are multinuclear and its zoospores have directly opposed basal bodies. *P. marina* also differs in orientation of the flagellar apparatus and characteristics of the vegetative cells from members of the genus *Ettlia* (Watanabe and Floyd 1989).

Among the three genera into which the genus *Neochloris* Starr was divided (Watanabe and Floyd 1989), *P. marina* shares the most characters with the genus *Parietochloris* of the Trebouxiophyceae. Cytological features distinguishing the trebouxiophycean algae include the absence of scales on the cell surface, absence of system I fibers, and the presence of system II fibers that join together into a single strand (see Introduction) (Watson and Arnott 1973, Watson 1975, Melkonian and Berns 1983, Deason and Floyd 1987, Melkonian and Peveling 1988, Watanabe and Floyd 1989, Friedl 1995). Although *P. marina* shares some of these features, it does have a system I fiber beneath each d-rootlet and the system II fiber is of a different morphology. Another distinction is that all taxa of the Trebouxiophyceae known to date are exclusively found in freshwater habitats, such as soil or subarial environments, or found as phycobionts of lichens. Marine organisms like *P. marina* are not known to occur in this class of green algae. In addition, *P. marina* lacks the char-

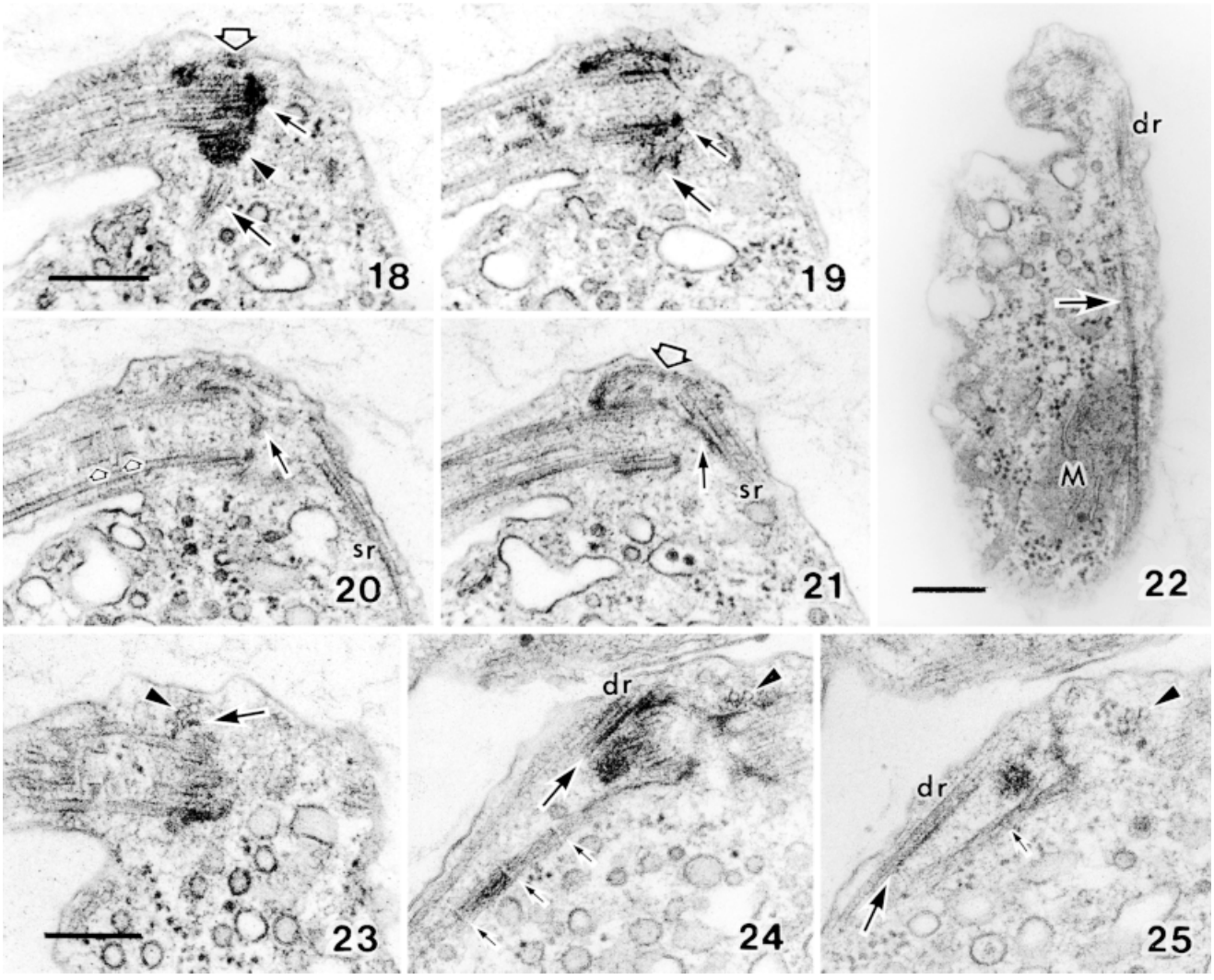


FIGS. 12–17. Consecutive cross-sections, including the first basal body (b1) (FIGS. 12–15), both basal bodies (FIG. 16), and the second basal body (b2) (FIG. 17). The distal fiber (small arrows) is connected to a basal body (FIGS. 12 and 13) and covers both basal bodies (FIG. 16). A system II fiber (large arrows) extends beneath each d-rootlet (FIGS. 12–14) and is connected to posterior triplets of basal body (FIGS. 15–17). A thin-striated fiber (arrowhead) is associated with the basal body (FIGS. 15 and 17). Electron-opaque material (short arrows) on the proximal end of basal body triplets are arranged to form a proximal ring (FIGS.16 and 17). Open short arrows indicate accessory basal bodies. Scale bar = 200 nm in FIG. 12 applies to FIGS. 12–17.

acteristically flattened zoospores of motile cell-producing members of the Trebouxiophyceae (Tupa 1974, Melkonian and Peveling 1988). These differences clearly exclude *P. marina* from the Trebouxiophyceae.

Floyd and O'Kelly (1990) subdivided the class Ulvophyceae into five orders. Among them, *Pseudoneochloris* is most similar to the Ulotrichales in terms of the life cycle, morphology of the vegetative cells, and many ultrastructural features. Members of the order Ulotrichales include scale-bearing and scaleless types (O'Kelly and Floyd 1984); *P. marina* motile cells lack surface scales. A system I fiber has been demonstrated in gametes of *Ulothrix flacca* var. Roscoffensis (Berger-Perrot et al. 1986). The system II fiber extending along the d-rootlet of *P. marina* is also found in biflagellate swarmers of the Ulotrichales. Diagnostic features of the Ulotrichales include the terminal cap, a more or less prominent electron-opaque flap on the anterior flank of the proximal end of the basal body, and a wedge-shaped proximal sheath located at the posterior flank of the basal body (O'Kelly and Floyd, 1984). In *Pseudoneochloris,* neither the terminal cap nor the proximal sheath is present. Instead, a proximal ring exists on every basal body triplet. This structure may be homologous to the terminal cap.

Circumscription of the order Ulotrichales is still incomplete but includes the three informal groups "*Eugomontia*-group," "*Gayralia*-group," and "*Chlorocystis*-group," in addition to the Monostromataceae, Ulotrichaceae, and Acrosiphoniaceae (Floyd and O'Kelly 1990). The life cycle of the unicellular *Pseudoneochloris* contains a phase that includes the formation of zoospores or aplanospores but no sexual reproduction was observed. Among families and informal groups, *Pseudoneochloris* most resembles algae in the *Chlorocystis*-group, *Chlorocystis* and *Halochlorococcum.* According to O'Kelly and Floyd (1984), this group is characterized by coccoid, unicellular gametophytes. The quadriflagellate motile cells of *Chlorocystis cohnii* possess the ulotrichalean terminal cap and proximal sheath (O'Kelly and Floyd, unpublished observations). If *C. cohnii* produces biflagellate gametes, it is expected that they also have terminal caps and proximal sheaths, which are similar to the gametes of *Ulothrix flacca* var *roscoffensis* that possess these flagellar apparatus components (Berger-Perrot et al. 1986). The life cycle of *Pseudoneochloris* and *Chlorocystis* are also distinct. *Chlorocystis* forms a free-living gametophyte and an endophytic sporophyte and reproduces by quadriflagellate zoospores and biflagellate anisogametes (Kornmann and Sahling 1983), whereas only biflagellate zoospores have been observed for *Pseudoneochloris.* The second genus, *Halochlorococcum*, has spherical, uninucleate cells with a net-like or reticulate chloroplast containing a single pyrenoid. This alga produces both bi- and quadriflagellate zoospores according to species and also depending on cultural conditions (Korn-



FIGS. 18–25. FIGS. 18–21. Consecutive series including longitudinal sections of basal body. Electron-opaque material (small arrows) on proximal end of one basal body extends toward the s-rootlet of the second basal body. System II fiber (large arrows) originates from posterior region of basal body. Large short arrow shows distal fiber and small short arrows indicate the distal and proximal cylinders of transition region. Scale bar = 200 mm in FIG. 18 applies to FIGS. 18–21. FIG. 22. System II fiber (arrow) runs in parallel with d-rootlet, extending along the mitochondrial profile (M). Scale bar = 200 nm. FIG. 23. Cross-section of two microtubules of the d-rootlet (arrowhead) associated with the system I fiber (arrow). Scale bar = 200 nm for FIGS. 23–25. FIGS. 24 and 25. Consecutive sections including cross-section of most proximal part of s-rootlet (arrowheads) comprised of two microtubules (FIG. 24) and three microtubules (FIG. 25). System I fiber (large arrows) beneath d-rootlet and system II fiber (small arrows) with striations (FIG. 24).

mann and Sahling 1983, Guillard et al. 1975). *Pseudoneochloris* can be distinguished from *Halochlorococcum* in chloroplast morphology and in number of flagella per cell.

18S rRNA sequence data analyses. The 18S rRNA sequence data analyses results were in agreement with other recently published trees (Friedl 1996, Lewis 1997) in that the taxa currently or formerly classified as *Neochloris* are divided into three separate green algal lineages. These lineages correspond to data on the orientation of the flagellar apparatus. The taxon currently under study, *Pseudoneochloris,* did not group with any of these, but was in a clade with members of the Ulvophyceae. In all cases, a clade containing members of the Ulotrichales (including *Pseudoneochloris*) was supported by a bootstrap value of 100%. Within this clade, *Pseudoneochloris* grouped with *Acrosiphonia* at the base of the clade or nested between *Acrosiphonia* and the others, depending on the analysis. Interestingly, *Pseudoneochloris* was not grouped with *Ulothrix* or *Gloeotilopsis,* but was closest to *Acrosiphonia,* a marine alga that forms branched filaments and has multinucleate vegetative cells. Bold and Wynne (1985) classified this genus with *Spongomorpha* and *Urospora* in the Acrosiphoniaceae of the Acrosiphoniales, although Floyd and O'Kelly (1990) proposed to classify the family in the Ulotrichales. As already mentioned, the bootstrap value for the branch uniting *Acrosiphonia*

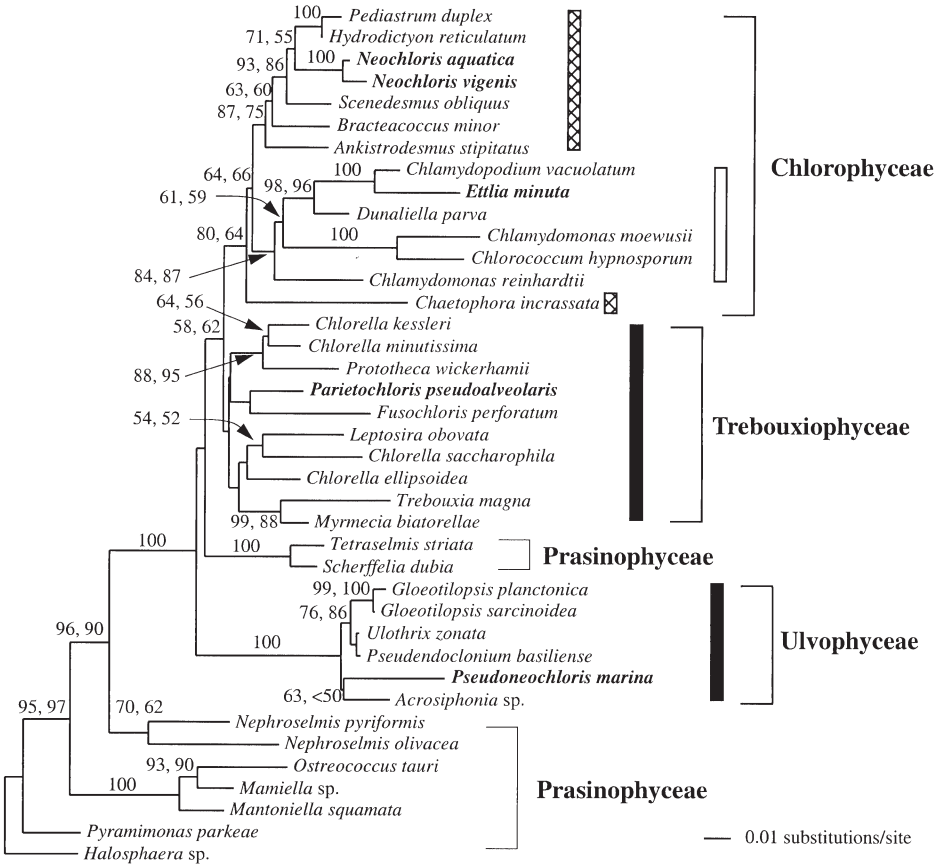


FIG. 26. Phylogenetic tree inferred using maximum likelihood from 18S ribosomal RNA gene sequence data showing the position of *Pseudoneochloris marina* in the Ulvophyceae, distinct from other taxa currently or formerly classified in the genus *Neochloris* (taxon names are in boldface text). Horizontal branch lengths are proportional to the mean number of nucleotide substitutions per site. Numbers associated with nodes indicate bootstrap values obtained using maximum likelihood (100 replicate samples) followed by values for equal weighted maximum parsimony (500 replicate samples). Nodes with bootstrap values of less than 50% are not labeled. Filled vertical bars indicate the taxa that produce motile cell with counterclockwise absolute orientation. Hatched vertical bars indicate algae with the directly opposed orientation; open bars indicate algae with the clockwise orientation.

and *Pseudoneochloris* is very low and additional taxon sampling is necessary to better resolve the relationship among these taxa.

Taxonomic revision. We propose a new genus for *Neochloris* sp. (UTEX no. 1445) in the Ulotrichales, Ulvophyceae. The generic and specific diagnoses follow:

Pseudoneochloris Watanabe, Himizu, Lewis, Floyd, et Fuerst, gen. nov.

Cellulae vegetativae uninucleatae, singulares, sphaericae, pariete laevi. Chloroplastus parietalis, cupulatus, acetabuliformis, pyrenoidibus praeditus. Reproduction asexualis per aplanosporis et zoosporis effectis. Zoosporae sine parietibus cellularum, duobus flagellis aequalibus. Corpora basalia zoosporarum imbricata, in absoluta dispositione antihelicte, et fibra systematis II oriunda unumquidque corporio basali, descendens sub unaquaeque dextera radicella.

Vegetative cells are uninucleate, solitary, spherical with smooth cell wall. Chloroplast parietal, cup-shaped, saucer-shaped with pyrenoids. Asexual reproduction by aplanospores and zoospores. Zoospores without cell wall, with two flagella of equal length. Basal bodies of zoospores overlapped and arranged in counterclockwise absolute orientation, and system II fiber originates from each basal body, descending under each dexter rootlet.

Type species: *Pseudoneochloris marina* Watanabe, Himizu, Lewis, Floyd, et Fuerst.

Pseudoneochloris marina Watanabe, Himizu, Lewis, Floyd, et Fuerst, sp. nov.

Cellulae vegetativae sphaericae, 3–20 μm diametro, interdum cum vacuolis. Chloroplastus cupulatus, acetabuliformis, pyrenoidibus praeditus. Zoosporae sine parietibus cellularum, pyriformes, obovoideae, 8–10 × 2–3 μm amplitudine.

Vegetative cells spherical, 3–20 μm in diameter, sometimes containing vacuoles. Chloroplast cup-shaped, saucer-shaped, with pyrenoids. Zoospores without cell wall, pyriform, obovoid, 8–10 × 2–3 μm in size.

Holotype: Figs. 1–25.

Isotype: Embedded material deposited at University of Connecticut.

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