Pl. Syst. Evol. 177: 213–219 (1991)

Plant Systematics and Evolution

Taxonomic status of the species of the green algal genus *Neochloris*

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Received March 20, 1991

Key words: Algal taxonomy, *Chlorophyceae, Pleurastrophyceae, Chlorococcales, Sphaeropleales, Sphaeropleaceae, Hydrodictyaceae, Neochloridaceae, Chlorococcopsis, Ettlia, Neochloris, Parietochloris.*

Abstract: Komárek has recently reviewed the various species assigned to the green algal genus *Neochloris* Starr (*Chlorococcales, Chlorococcaceae*) and removed those with uninucleate vegetative cells to a new genus, *Ettlia*. Watanabe & Floyd, unaware of Komárek's work, also reviewed the species of *Neochloris* and distributed them among three genera – *Neochloris, Chlorococcopsis* gen. nov., and *Parietochloris* gen. nov. – on the basis of details of the covering of the zoospore and the arrangement of the basal bodies of the flagellar apparatus. This paper reconciles these two treatments and makes additional recommendations at the ranks of genus, family, order, and class.

Neochloris was established by Starr (1955) as a genus of *Chlorococcaceae* to accommodate a previously undescribed species, *N. aquatica*, which was isolated from the sides of an aquarium at Indiana University. It was distinguished from other genera in the family by the following suite of characters: (1) the production of zoospores without walls and with two equal flagella; (2) vegetative cells with a hollow spherical (parietal) chloroplast; and (3) the presence of at least one pyrenoid in the chloroplast of the vegetative cell. New species were added by Arce & Bold (1958), Bold (1958), Herndon (1958), Deason & Bold (1960), Chantanachat & Bold (1962), Bischoff & Bold (1963), Groover & Bold (1969), Archibald (1973), and Vinatzer (1975). To this group of new species was added *Neochloris wimmeri* (Rabenh.) Archibald & Bold (1971), based on *Chlorococcum wimmeri* Rabenhorst (1868: 30). Two of the total of 16 species were reduced to synonymy by Watanabe (1983). Recent studies of the various species of *Neochloris*, using light and electron microscopy, have revealed morphological polyphyleticism (Komárek 1989, Lewis 1990, Watanabe & Floyd 1990). Two attempts have been made to express this polyphyleticism taxonomically (Komárek 1989, Watanabe & Floyd 1990). Because the two treatments were developed and published independently, some overlapping and conflicting opinions resulted. In the present paper we reconcile these two treatments and make further proposals at the ranks of genus, family, order, and class.

Generic circumscriptions

The cells of *Neochloris aquatica*, the type species of its genus, were described by STARR (1955) as multinucleate. Species later described in the genus are either multinucleate or uninucleate. KOMÁREK (1989: 270) used nuclear number as a generic character, reserving *Neochloris* for multinucleate species and proposed a new genus *Ettlia* to accomodate the uninucleate species. *Ettlia* DESIKACHARY (in IYENGAR & DESIKACHARY 1981: 153), applied to a new genus in the *Volvocales*, is invalid because the protologue lacks a Latin description. KOMÁREK (1989) transferred six species of *Neochloris* into the new genus, but two of these (*N. terrestris* HERNDON and *N. texensis* ARCHIBALD) are in fact multinucleate and should be retained in *Neochloris*.

As type species of *Ettlia*, KOMÁREK designated *E. carotinosa* KOMÁREK, newly described on the basis of the Prague subculture (Praha-Ac. 93) of a strain originally placed in the Culture Collection of Algae and Protozoa of Cambridge University (no. 213/4). This strain had been isolated and identified as *Chlorococcum wimmeri* RABENHORST (1868: 30) by F. MAINX and was subsequently established in the Culture Collection of Algae at Indiana University (no. 113). STARR (1954), while admitting that RABENHORST's description is hardly adequate for distinguishing a species of *Chlorococcum* from quiescent stages of other genera, nonetheless adopted the name, but redescribed the alga on the basis of observations on the MAINX strain. Neither STARR (1954) nor ARCHIBALD & BOLD (1971), who transferred the species into *Neochloris* on the basis of STARR's redescription, seemed to appreciate that RABENHORST did not take credit for *Chlorococcum wimmeri*, but in fact merely transferred into *Chlorococcum* a species previously published as *Protococcus wimmeri* HILSE (1861; in RABENHORST 1861: no. 1031). This species was based on a collection by HILSE from Peterwitz, near Strehlen (Strzelin), now in Poland, which was distributed by RABENHORST in his exsiccata, "Die Algen Europas." KOMÁREK (1989) chose not to follow STARR (1954) in accepting on faith the identification of the MAINX strain with *C. wimmeri*, claiming that the application of the epithet *wimmeri* is not determinable because of insufficient data in HILSE's diagnosis. Like STARR, however, he gave no indication that he had examined type material. We have made such an examination (specimen in FH) and agree with KOMÁREK (1990) that application of the epithet *wimmeri* is equivocal. It may be noted that DROUET & DAILY (1956: 164) concluded that HILSE's collection was representative of encysted flagellates.

WATANABE & FLOYD (1990) studied the ultrastructure of nine species of *Neochloris* and, unaware of KOMÁREK's (1989) treatment, emended the genus and established two new genera to reflect the polyphyleticism revealed by their study. To the generic characters previously used in this taxonomic group, they added structure of the flagellar apparatus of zoospores. Like KOMÁREK, WATANABE & FLOYD reserved *Neochloris* for multinucleate species. Zoospores of these multinucleate species were found to have the flagellar basal bodies directly opposed. In addition, they all are without cell walls. Three multinucleate species, *N. pseudostigmata, N. pyrenoidosa*, and *N. vigenis*, have zoospores with some unorganized fibrous material outside the cell membrane (the fuzzy material of WATANABE & FLOYD 1990), but this material does not constitute a cell wall.

Uninucleate species previously assigned to *Neochloris* were divided by WATANABE & FLOYD (1990) into two new genera. *Chlorococcopsis* was established for

Table 1. Taxonomy of the species formerly assigned to *Neochloris*

Chlorophyta
Chlorophyceae
Sphaeropleales
Neochloridaceae
* *Neochloris aquatica* STARR 1955 (type species)
N. conjuncta ARCHIBALD 1973
N. gelatinosa HERNDON 1958
* *N. pseudostigmatica* BISCHOFF & BOLD 1963
* *N. pyrenoidosa* ARCE & BOLD 1958
* *N. terrestris* HERNDON 1958
N. texensis ARCHIBALD 1973
* *N. vigenis* ARCHIBALD 1973

Chlorococcales
Chlorococcaceae
* *Ettlia carotinosa* KOMÁREK 1989 (type species)
* *E. minuta* (ARCE & BOLD) KOMÁREK 1989
E. oleoabundans (CHANTANACHAT & BOLD) KOMÁREK 1989

Pleurastrophyceae
Pleurastrales
Pleurastraceae
* ***Parietochloris alveolaris* (BOLD) WATANABE & FLOYD, comb. nov.** (type species)
Basionym: *Neochloris alveolaris* BOLD 1958: 737, figs. 1–16, 31–38.
* ***P. cohaerens* (GROOVER & BOLD) WATANABE & FLOYD, comb. nov.**
Basionym: *Neochloris cohaerens* GROOVER & BOLD 1969: 45, figs. 34, 134–42, 159: 29.
* ***P. pseudoalveolaris* (DEASON & BOLD) WATANABE & FLOYD, comb. nov.**
Basionym: *Neochloris pseudoalveolaris* DEASON & BOLD 1960: 28, figs. 33–37, 98–100.

Note: these three combinations were published earlier by WATANABE & FLOYD (1990), but they were invalid because the basionyms were not cited fully as required by Art. 33.2 of the International Code of Botanical Nomenclature. * Ultrastructure studied.

those species having zoospores with clockwise absolute orientation of the flagellar apparatus and thin cell walls. These walls are so thin that they have been observed only with the electron microscope. Zoospores with thin walls round up as if they were naked. Included in this genus were the MAINX strain of *N. wimmeri* and *N. minuta* ARCE & BOLD (1958). Both of these species fall within the circumscription of *Ettlia* so that *Chlorococcopsis* must be abandoned. Because KOMÁREK (1989) did not apply electron microscopy, he assumed that the zoospores of *Ettlia* are naked. His use of the words "zoosporae sine membrana firma" in the Latin diagnoses for both the genus and the type species is misleading, since it implies the presence of an infirm wall. In the English diagnosis of the type species, however, the wording is "zoospores naked."

Parietochloris, the second genus established by WATANABE & FLOYD (1990), includes those uninucleate species that have naked zoospores with counterclockwise

absolute orientation of the flagellar apparatus. To this genus were assigned three species originally described in *Neochloris*, namely *N. alveolaris* BOLD, *N. cohaerens* GROOVER & BOLD, and *N. pseudoalveolaris* DEASON & BOLD. The remaining species of *Ettlia* to be mentioned is *E. bilobata* (VINATZER) KOMÁREK. *Neochloris bilobata* VINATZER was shown to be a synonym of *N. alveolaris* BOLD by WATANABE (1983).

The disposition of the species formerly assigned to *Neochloris* is summarized in Table 1.

Suprageneric placement

The usefulness of the flagellar apparatus in proposing phylogenetic relationships among green algae has been demonstrated (MATTOX & STEWART 1984, O'KELLY & FLOYD 1984). Members of the *Chlorophyceae* have basal bodies which do not overlap and which have clockwise absolute orientation. These characters are correlated with the presence of a phycoplast. HOFFMAN (1984) argued that the *Sphaeropleaceae*, all members of which have motile cells with basal bodies directly opposed, should be included in the *Chlorophyceae* because of the presence of a phycoplast and the lack of basal body overlap. Members of the *Pleurastrophyceae* and the *Ulvophyceae* share the attribute of counterclockwise absolute orientation of motile cell basal bodies, but differ in the mechanism of cytokinesis. SLUIMAN (1989) included the *Pleurastrophyceae* as an order within the *Ulvophyceae*, but the present authors prefer to follow MATTOX & STEWART (1984). This decision is supported by the rRNA sequence data of KANTZ & al. (1990) showing that the *Pleurastrophyceae* is a sister group to the *Chlorophyceae* and not to the *Ulvophyceae*. The existence of three types of basal flagellar structures in *Neochloris* as previously circumscribed indicates polyphyleticism that is confirmed by molecular analysis (LEWIS 1990). As circumscribed by WATANABE & FLOYD (1990), *Neochloris* is restricted to species that have multinucleate cells in the vegetative state and zoospores that are either naked or covered with fuzzy material. A correlated character is that the basal bodies of zoospores are directly opposed. This arrangement is also found in the zoospores of *Hydrodictyon, Pediastrum*, and *Tetraedron* in the *Hydrodictyaceae* (HAWKINS & LEEDALE 1971, MARCHANT & PICKETT-HEAPS 1972, WILCOX & FLOYD 1988, WATANABE & al. 1988). *Sphaeroplea* C. AGARDH and *Atractomorpha* HOFFMAN in the *Sphaeropleaceae* (CÁCERES & ROBINSON 1981, HOFFMAN 1984, BUCHHEIM & HOFFMAN 1986), and *Chlorotetraedron* MCENTEE, BOLD & ARCHIBALD in the *Neochloridaceae* (WATANABE & al. 1988). Since the unicellular forms lack the microtubular bands involved in colony formation, they probably do not belong in the *Hydrodictyaceae* (WATANABE & al. 1988). We propose that *Tetraedron* be united with *Neochloris* and *Chlorotetraedron* in the *Neochloridaceae* ETTI & KOMÁREK (1982). Some species currently assigned to *Characium* may also belong to this family (FLOYD & WATANABE 1990). Motile cells of *Sphaeroplea* and *Atractomorpha* differ from those of *Hydrodictyon, Tetraedron*, and *Chlorotetraedron* in lacking a continuous striated microtubule-associated component (SMAC) and a partial cap, so that these genera stand apart in their own family, *Sphaeropleaceae*.

The *Neochloridaceae, Hydrodictyaceae*, and *Sphaeropleaceae*, all having motile cells with directly opposed basal bodies, should be grouped in a newly circumscribed order for which the oldest available name is *Sphaeropleales* LUERSSEN (1877). The change in circumscription is drastic and warrants an emended diagnosis.

Ettlia was assigned by KOMÁREK (1989) to the family *Neochloridaceae*, which in turn was assigned to the order *Chlorellales* sensu ETTL & KOMÁREK (1982). That order which was proposed by BOLD & WYNNE (1978) to include those chlorococcoid algae that lack the capacity for zoospore formation, was emended by ETTL & KOMÁREK (1982) to include all chlorococcoid algae except those that form walled zoospores. The latter algae are interpreted by ETTL & KOMÁREK (1982) as representing a cystoid evolutionary trend among flagellates of the *Chlamydomonas*-type and were retained in the order *Chlorococcales*, which was placed in the class *Chlamydophyceae*. We prefer to recognize the *Chlorococcales* in its original broad sense (cf. BOLD & WYNNE 1985), and to include in it as well as the *Sphaeropleales* in the class *Chlorophyceae* sensu MATTOX & STEWART (1984).

The counterclockwise absolute orientation of the flagellar apparatus of *Parietochloris* relates this genus to *Pleurastrum* (*Pleurastraceae, Pleurastrales, Pleurastrophyceae*).

Diagnoses

***Sphaeropleales* LUERSSEN 1877 emend.** Unicellular, filamentous, or coenobic nonmotile green algae producing motile cells with directly opposed basal bodies. Sexual reproduction oogamous, anisogamous, or isogamous.

***Sphaeropleaceae* KÜTZING 1849 emend.** Unicellular or filamentous coenocytic algae with incomplete septae and naked motile cells. Basal bodies of flagellar apparatus directly opposed but lacking a striated microtubule associated component (SMAC) and partial cap. Sexual reproduction oogamous or anisogamous.

***Hydrodictyaceae* DUMORTIER 1829 emend.** Coenobia producing zoospores that are naked or covered with fuzzy material. Basal bodies of flagellar apparatus directly opposed with SMAC and partial cap. Sexual reproduction isogamous. Coenobia developing from juxtaposed settled zoospores.

***Neochloridaceae* ETTL & KOMÁREK 1982 emend.** Spherical, subspherical, or angular unicells with smooth walls or walls with spines. Zoospores naked or covered with fuzzy material. Basal bodies of flagellar apparatus directly opposed with SMAC and partial cap.

***Neochloris* STARR 1955 emend.** Cells spherical or subspherical, multinucleate, chloroplast parietal with pyrenoids. Zoospores naked or covered with fuzzy material. Basal bodies of flagellar apparatus directly opposed.

***Ettlia* KOMÁREK 1989 emend.** Cells spherical or subspherical, uninucleate, chloroplast parietal with pyrenoids. Zoospores with thin wall. Basal bodies of flagellar apparatus having clockwise orientation.

We wish to express appreciation to Prof. WALTER HERNDON of The University of Tennessee, Knoxville for his help in obtaining references. The loan of the type specimen of *Protococcus wimmeri* HILSE by the Farlow Herbarium of Harvard University also is gratefully acknowledged.

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Accepted May 14, 1991 by D. J. CRAWFORD