Endophytic unicellular chlorophytes: a review of Chlorochytrium and Scotinosphaera

Daniel E. Wujek* and Rufus H. Thompson†

1Department of Biology, Central Michigan University, Mt Pleasant, Michigan 48859, USA
2Department of Botany, University of Kansas, Lawrence, Kansas 66045, USA

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The genera Nautococcopsis and Ectogeron are reduced to synonymy with Chlorochytrium. The inclusion of Scotinosphaera and Kentrosphaera within the genus Chlorochytrium is considered now untenable. Scotinosphaera and Kentrosphaera are considered synonymous, with the name Scotinosphaera having priority. Redefinitions are given for Chlorochytrium and Scotinosphaera based on seven opposed attributes. Chlorochytrium, in germination of the zoospore, secretes an attachment disc; has fundamentally a parietal chloroplast that develops many radiate lobes, few to many pyrenoids; and the vegetative cell contains few to many minute contractile vacuoles. Its first two to four divisions are vegetative. Further division of each protoplast results in biflagellate zoospores that are walled, compressed and have a flagellar papilla. Scotinosphaera lacks an attaching disc; is strictly unicellular with no vegetative divisions; contains an axile chloroplast with many radiate arms; has characteristically one large central pyrenoid; lacks contractile vacuoles; and division results in numerous biflagellate, terete, spine-shaped zoospores. Old cells may develop one or more localised lamellated, wart-like thickenings of the wall, externally or internally. Sexual fusion has been observed for Chlorochytrium but not for Scotinosphaera. The relationships of Chlorokoryne, Eremotyle, Nautococcus and Kentrosphaera are reviewed.

INTRODUCTION

Throughout the past half-century, the authors have had opportunities to study specimens of Chlorochytrium Cohn, Chlorokoryne Pascher, Nautococcus Korshikov, Ectogeron Dangeard, Scotinosphaera Klebs, Kentrosphaera Borzi and Excentrosphaera Moore. The taxa described in these genera are seldom reported in the literature, some only from their initial descriptions.

The genus Nautococcus was erected by Korshikov (1926). The fifth and last species he described at that time was N. constrictus Korshikov. It differed from the other four species in having a profusely radially lobed, parietal chloroplast that contained few to many pyrenoids and in having a central nucleus. The other four species were characterised by a massive axile chloroplast with a central pyrenoid and an acentric nucleus. Because of these differences, Geitler (1943) removed N. constrictus and erected the genus Nautococcopsis to hold it. Korshikov (1953), apparently unaware of Geitler’s treatment of N. constrictus, also erected the genus Nautococcopsis based on the same species. Müller, also in 1953, reported on N. constrictus from Hamburg, Germany, and redescribed the species. In 1947, Dangeard erected the genus Ectogeron with the type and only species E. elodeae Dangeard. In 1952, Geitler erected the genus Eremotyle on the type and only species, E. affixa Geitler. This species was placed in synonymy with E. elodeae by Bourrelly (1966).

Except for Korshikov’s description of the neustonic phase of N. constrictus, the descriptions of the species in these genera are identical. Furthermore, the alga Chlorochytrium kyanum Szymanski (1878), the life history was reported by Klebs (1881), is also considered identical with these. This report, however, identifies features distinctive for the genus Chlorochytrium, reviews its relationship to each genus and substantiates its recognition as a distinct genus. We also make comparisons with the closely allied genera Chlorokoryne, Nautococcus, Ectogeron, Scotinosphaera, Kentrosphaera and Excentrosphaera. No molecular data on this group of chlorophytes indicating their phylogenetic positions among the Chlorophyceae have yet been published.

MATERIAL AND METHODS

Phytoplankton samples and material isolated from floating leafy aquatic plants containing the ‘genera’ Chlorochytrium, Chlorokoryne, Nautococcus, Ectogeron, Scotinosphaera, Kentrosphaera and Excentrosphaera were collected from the following sites: Kansas – Potter Pond (March–June 1951–1978), railroad trestle pond north of Lawrence, late winter through early summers (1957–1978), and Nehrber’s farm pond 5 km east of Lawrence on Kansas Hwy. 10 (January–March 1957), Douglas County, prairie pond, Welda, Anderson County (March 1963); Michigan – Lake Geneserath and Millers Marsh, Charlevoix County (June–August 1990–1994), Coldwater Lake, Isabella County (spring and summer – sporadically 1970–1994); Minnesota – lakes and ponds in the Itasca State Park and adjoining waters, Becker, Clearwater, and Hubbard Counties (June–July 1964, 1965) (waters described in Meyer & Brook 1968).

Observations were made both from freshly collected material and from short-term cultures grown in soil–water extract or Bold’s Basal Medium (Bold 1967), with additional soil water extract. Cultures no longer survive.

* Corresponding author (wujek1de@cmich.edu).
† Deceased 3 June 1980.
RESULTS AND DISCUSSION

The alga *Nautococcus constrictus* or *Nautococcopsis constricta* (Korshikov) Geitler has been collected frequently by R.H.T. and had been carried in culture. It exhibited all the characteristics of morphology and growth habit given by Korshikov (1953), Dangeard (1947) and Geitler (1943) for their genera. In the free-living condition, it develops two morphologically distinct forms depending on whether a zoospore germinates at the surface film or on a solid substratum.

At the surface film it develops the form typical of *Nautococcus* (Fig. 1). The compressed zoospore comes to rest with its broad side against the film. It secretes a heavier wall and produces a thin flange of wall material in contact with the surface film. This remains as a cap on the cell and functions as a flotation device as the cell body develops either above or beneath the surface. As the cell grows, it becomes pear-shaped to nearly spherical. The chloroplast in the zoospore is parietal with shallow, blunt, marginal lobes, but as it grows, it becomes thicker and begins to split into radial lobes. With enlargement of the cell, the chloroplast increases in size, the lobes elongate and their apices expand against the cell wall. This forces the inner surface of the plastid toward the centre of the cell. At the same time, the pyrenoid divides until there are few to many smaller pyrenoids scattered throughout, located mostly in the lobes. Between the lobes are few to many small, contractile vacuoles. During growth, there are vegetative divisions of the protoplast into 2–8 cells, each of which produces its own wall. Further divisions of each of these cells result in 16–32 zoosporocytes in each. At maturity, the outermost wall gelatinises and bursts to release the inner wall as a rapidly expanding vesicle containing the mass of metamorphosing zoosporocytes. These become very active within the vesicle and disperse when it bursts.

When a germinating zoospore comes to rest with its flat side against a submerged substratum, it secretes an additional wall and attaching flange. All growth of the cell is to one side. The original wall of the germling remains unchanged as a small knob at that point on the cell (Fig. 2). From this knob, the cell grows out in a narrow to broad fan-wise fashion and may be even or quite irregular with narrow marginal sinuses. As it grows, there is a spreading in the plane of the substratum, a growth that lifts the attaching flange of wall material. At the same time, a new, wide flange is also produced. Repetition of such growth results in the dorsal wall bearing the older flanges in a concentric contoured arrangement (Fig. 3). Vegetative division may occur during growth or it may not begin until the cell is near mature size. The surface of the chloroplast becomes rugose with closely compacted, radiate processes with numerous pyrenoids. In between the lobes, particularly visible at the margin, are few to many minute contractile vacuoles. By the time the zoosporocytes are fully delimited, the colour of the cell contents has changed from bright green to olive-brown to yellow-brown. The mass of zoosporocytes is extruded in a vesicle through a circular pore developed in the free, dorsal surface of the cell. The vesicle is usually lobulate and at first contains discrete masses separated into individual zoospores that soon are completely intermingled through their own activity (Fig. 4).

Zoospores are biflagellate, strongly compressed, flat on one side and convex on the opposite. They contain a parietal, en-
Figs 1–8. *Chlorochytrium lemnae*. Scale bars = 10 μm.

Fig. 1. Morphological 'Nautococcus constricta' form from surface film.

Fig. 2. Young germling with small attaching flange.

Fig. 3. Repeated growth showing concentric contoured arrangement.

Fig. 4. Zoospores in a vesicle after release from sporangium prior to discharge.

Fig. 5. Zoospore germination through opening in a dead *Sphagnum* cell.

Fig. 6. Cell enlargement away from point of attachment.

Fig. 7. Variety of vegetative cells in various stages of zoosporogenesis.

Fig. 8. Cell growth away from point of attachment.

Figs 9–14. *Scotinosphaera paradoxa*. Scale bars = 10 μm.

Fig. 9. Cell from recently germinated zoospore.

Figs 10, 11. Chloroplasts of vegetative cells with numerous radiate lobes containing one or two pyrenoids.

Fig. 12. Lamellated thickening of cell wall.

Figs 13, 14. Multinucleate cell with external knob(s) dividing into zoosporocytes.
receives some credence when one reads in his account that reproduction was not observed, that there were few parietal chromatophores without pyrenoids and that there were red oil globules. His illustrations are perfect for the depauperate phase of Chlorochytrium.

With Chlorochytrium now involved, previous studies on this genus were reviewed. It was found that West (1904) reduced Stomatochytrium Cunningham (1887) and later (1916) reduced Scotinosphaera and Endosphera Klebs (1881) to synonymy with Chlorochytrium. In 1917, Bristol, West’s student, reduced the genus Kentrosphaera Borzi (1883) (= Centrosphaera) to synonymy with Chlorochytrium. Her reasons for the reduction developed out of a detailed study of Chlorochytrium grande Bristol. She showed that the chloroplast lobes widened toward the periphery. At the wall, each expanded into a thin disc, thus presenting the false impression of a parietal plastid with an inwardly directed and attenuated process. When the discoidal exsapid processes do not touch one another, there is the erroneous appearance of many discoidal, parietal plastids, each with an inward prolongation. This is the aspect that Klebs described for both Scotinosphaera and Endosphera and Borzi for Kentrosphaera. It is also the same aspect and misinterpretation that led Moore (1901) to erect the genus Excentrosphaera. Moore reported each ‘plastid’ to have ‘a minute pyrenoid’. When one reads that he based this on iodine-stained material, there is the suspicion that he mistook the scattered autochthonous starch grains for ‘minute’ pyrenoids.

In her next paper, Bristol (1920) reviewed the genus Chlorochytrium and tried to resolve the conflicting and inadequately described species into some order. In her summation, she listed six freshwater, four marine and three doubtful freshwater species. Except for two marine species, the descriptions afford no means of distinguishing one from another with certainty. The marine species, C. colnii Gardner and C. moorei Gardner (Gardner 1917), are described as having quadriflagellate zoospores of two sizes. Where it is known, the other species (five) have biflagellate zoospores or gametes of the same size. Zoospore dimensions are given for three species, but descriptions of their contents and shape are virtually useless. Cell dimensions overlap completely and cell shape among the species is too variable to be of use. Nevertheless, Bristol (1917) gave the following summation of Chlorochytrium species, while three species (C. laetum Schröter, C. viride Schröter and C. ruhrum Schröter) are species dubium et noncom post esse cognitum.

There are valid reasons for disagreeing with some of the generic reductions above. The reduction that initiated the point of view that culminated in Bristol’s summation was that of Scotinosphaera by West (1916). Bristol’s description of C. grande and her reduction of Kentrosphaera to synonymy with Chlorochytrium were natural extensions of West’s action. Her detailed study and interpretation of plastid morphology in C. grande and her interpretation of Borzi’s description of K. facciolaiae Borzi seem eminently reasonable. Smith (1933) rerecognized Kentrosphaera on the basis of its free-living habit and lack of sexual reproduction. In doing this, he used Bristol’s (1920) description and illustrations of material that he had identified as C. paradoxa (Klebs) G.S. West (= Scotinosphaera paradoxa) to erect the new species K. bristolae G.M. Smith. Kentrosphaera was described as free-living, but often associated with filamentous blue-green algae. One can collect this alga intermingled with many other algae in moist or seeping areas in the soil, and it may also be taken as surface plankton, where it may project above the surface or lie parallel to it. The cells are not always free, however, and they may be found frequently and in quantity within the tissues of dead Lemna, Potamogeton and Typha culms and the floating canes of Hibiscus americanus. The cells one finds here fulfill all the features of C. grande, C. paradoxa (sensu Bristol), S. paradoxa (sensu Klebs), K. facciolaiae (sensu Borzi) and Excentrosphaera (sensu Moore). They have the axile, multilobate chloroplast that, from the surface, suggests numerous parietal plastids in radial arrangement. They have one or two central pyrenoids and can exist endophytically or free.

West’s (1916) reduction of Scotinosphaera appears to have been based solely on its reported endophytic habit and he apparently attached no importance to Klebs’ description of spindleshaped, biflagellate zoospores. Though the germination-knob or germling ‘stopper’, as it was termed in Bristol’s papers, had been stressed as a character of Chlorochytrium, the lack of one by Scotinosphaera was not taken into account.

In the experience of the writers, the endophytism of the forms under discussion is among the least useful of taxonomic characters. The idea that these algae, particularly Chlorochytrium, are characteristically if not wholly obligately endophytic is an accrued and erroneous belief stemming from Cohn’s original publication (1872) and perpetuated by the fact that this was the habitat that one looked for, found and identified Chlorochytrium. Their presence within a ‘host’ means no more than that a zoospore germinated there and grew into an opening in the surface, whether a stoma or an injury. The same development may occur on any dead, water-logged, floating plant tissue. In the same Lemna frond harbouring Chlorochytrium, there may be a wide variety of other algae inhabiting substomatal chambers and the intercellular spaces. In these sites, one can find such diatoms as Navicula, Rhopalodia, Nitzschia, Epithemia and Amphora in dense masses; various cocoid blue-greens; Euglena; Gymnodinium; Glenodinium; Ankistrodesmus; Scenedesmus and various other cocoid green algae; and filamentous forms such as Plectonema, Phormidium, Oscillatoria, Nostoc, Oedogonium, Stigeoclonium, Leptosiphon, Cylindrocapsa, Coleocladus, Chaetoportula and Aphanothece. Cohn’s fig. 5 (1872) depicts the tissues of a Lemna frond containing several cells of C. lemnæ, together with Calothrix, Nostoc, Oscillatoria and a cocoid form of some other alga. All of the above algae were described from their more prevalent free-living condition, so no one should attach any significance to their endophytism beyond their ability to get into and grow in such places.

Endophytic cells referable to the genus Chlorochytrium occur predominantly in dead tissues. One can find them in living Lemna and Potamogeton leaves, especially if through wind action the leaves have been submerged for a time. In this condition, it is only the substomatal chamber that is normally invaded because the alga cannot grow through the cuticle or force its way between the walls of healthy epidermal cells. In dead leaves of Lemna and Potamogeton, however, the zoospores not only enter the stoma but are able to grow between water-softened cells of the undersurface of the leaves. Zoospores that are released within such leaves are able to disperse widely throughout the water-filled spaces.
We conclude that, for *Chlorochytrium*, as for many other algae, aquatic plants are merely habitats, not obligate hosts. The very fact that *Chlorochytrium* can be carried for generations in inorganic media argues against the idea that it is an obligate endophyte. From field observations that *Chlorochytrium* occurs in or on substrata in the upper 30 cm of water, there is the further inference that its presence here is because of better aeration or the amount of light relative to oxygen and carbon dioxide tension.

With the above in mind and with the recognition that *Chlorochytrium lemmae* may be neustonic, epiphytic and endophytic and that *Scotinosphaera paradoxa* may be neustonic, endophytic or subaerial, the endophytic ability should no longer be considered a generic attribute.

We further believe that the separation of *Scotinosphaera*-like (or *Kentrosphaera*-like) forms from *Chlorochytrium* is warranted on the basis of seven distinctively contrasting features (Table 1).

### Separation of Chlorochytrium and Scotinosphaera

The two genera may be redefined as follows, with *Scotinosphaera* having priority over *Kentrosphaera*.

**CHLOROCHYTRIUM**: Unicellular to limittedly sarcinoid, with a parietal chloroplast that becomes perforate-vacuolate and splits into many radially oriented lobes with few to many pyrenoids. The protoplast contains few to many contractile vacuoles. Cells globular, pyriform to flattened and irregular. Zoospores compressed, walled and biflagellate. The flagella issue from opposite sides of a papilla. Plastid parietal with one pyrenoid and an anterio-lateral stigma. Zoospores released in a vesicle. On germination, they secrete an attachment disc and may or may not develop a tubular ‘neck’ before enlarging. While naked zoospores were noted by Watanabe & Floyd (1994), we can only surmise they may have been working with contaminated or misidentified cultures. Zoosporocytes may develop into aplanospores that can germinate into new thalli. Fusion of biflagellate isogametes both within the vesicle and after release has been reported (Klebs 1881). Species may be neustonic, epiphytic or endophytic on living or dead aquatic plants or other substrata.

**SCOTINOSPHAERA**: Unicellular, with a massive, axile chloroplast containing one or two large, central pyrenoids and divided into numerous radiate lobes that expand into thin discs at the periphery (Figs 9–11). Vegetative cells lack contractile vacuoles. Cells may become multinucleate, or nuclear divisions and cleavage into zoosporocytes may take place only upon reaching mature size. Cells linear, lanceolate or ovoid, globular or irregular. They characteristically develop localised, lamellated thickenings of the wall (Fig. 12) that may be external knobs or internal, veriform growths (Figs 13, 14). Cells may also develop into akinetes with thick, lamellated walls. Zoospores biflagellate, spindle-shaped or lanceolate and terete in section. One parietal chloroplast with stigma and pyrenoid. Daughter cells are either extruded in a vesicle or escape singly through a pore in the wall. Asexual reproduction may be by aplanospores. Sexual reproduction unknown. Species may be neustonic, endophytic in living or dead aquatic plants or subaerial in seepage areas in soil.

Contrary to the claim presented by Puncíchaňová (1992) in recognising *Kentrosphaera* and not accepting *Scotinosphaera* as a valid genus, we believe that Klebs (1881), and later Bristol (1917), do present data relating to cell cycle, cell morphology and cytomorphic variability as sufficient in recognising *Scotinosphaera*. Care must be taken to interpret Klebs’, and later Bristol’s, words precisely, recognising the context in which the original work was done and the type of data that were available at the time. Even the application of the Botanical Code of Nomenclature can create problems, and commonsense may be as important as scientific strictness.

### Species accounts of Chlorochytrium and Scotinosphaera

The following revision is based on the work of Bristol (1917, 1920) and the writers’ observations.

**Chlorochytrium Cohn 1872** [including *Stomatochytrium* Cunningham (1887); *Endosphaera* Klebs (1881); *Nautococcopsis* Geitler (1943; Korshikov 1953); *Ectogeron* Dangeard (1947), and *Eremotyle* Geitler (1952)]

*C. lemmae* Cohn, type species

= *C. limnanthemum* (Cunningham) G.S. West 1904
= *Stomatochytrium limnanthemum* Cunningham 1887
= *C. biene* (Klebs) G.S. West 1916
= *Endosphaera biennis* Klebs 1881
= *C. knyanum* Szymanski 1878
= *C. pallidum* Klebs 1881
= *C. archerianum* Hieronymous 1888
= *Nautococcopsis constricta* (Korshikov) Geitler 1943
= *Nautococcus constrictus* Korshikov 1926
= *Nautococcopsis constricta* (Korshikov) Korshikov 1953
= *Ectogeron elodeae* Dangeard 1947
= *Eremotyle affixa* Geitler 1952
= *Chlorochytrium willei* Printz 1926
= *Chlororokoryne petrovae* Pascher 1938
= *Chlorosphaeropsis lemmae* Moewus 1950

*Scotinosphaera* Klebs 1881 [including *Kentrosphaera* Borzi (1883) and *Excentrosphaera* G.T. Moore (1901)]

*S. paradoxa* Klebs, type species

= *Chlorochytrium paradoxa* (Klebs) G.S. West 1916
= *Chlorochytrium faccioideae* (Borzi) Bristol 1917
= *Kentrosphaera faccioideae* Borzi 1883
= *C. gloeosiphium* Bohlin 1897
= *K. bristolae* G.M. Smith 1933
= *Excentrosphaera viridis* G.T. Moore 1901

*S. grande* (Bristol) nov. comb.
= Kentrosphaera grande (Bristol) G.M. Smith 1933
= Chlorochytrium grande Bristol 1917, pp. 107–126, plates 5, 6.
S. gibberosa var. gibberosa (Vodeničarov & Bendereiv) nov. comb.
= Kentrosphaera gibberosa Vodeničarov & Bendereiv 1971, p. 228, fig. 2.
S. gibberosa var. polymorpha (Punččoňáková) nov. comb.
= Kentrosphaera gibberosa var. polymorpha Punččoňáková 1992, p. 234, fig. 4.
S. lennae (Punččoňáková) nov. comb.
= Kentrosphaera lennae Punččoňáková 1992, p. 234, figs. 5, 6
S. austriaca (Punččoňáková) nov. comb.
= Kentrosphaera austriaca Punččoňáková 1992, p. 237, figs. 7, 8.

The marine species have not been included in the above summation because the writers have no knowledge of them; indeed Kornmann and Sahling (1983) have redescribed them as a new genus, Chlorocystis, within the Ulvophyceae. Lewin (1984) indicated the marine species may be unicellular diploid stages of certain filamentous algae such as Spongomonapha and henceforth should be referred to as ‘chlorochytrium stages’ in the same way we now use the term ‘conchocelis stage’ for certain red algae. The species C. colnii and C. moorei Gardner (1917), reported to have quadrigellate zoospores of two sizes, need restudy because they may not belong to this freshwater group. Conceivably, each could be a depauparate form of Enteromorpha.

As a final note on Chlorochytrium, it should be pointed out that, if the reported observations of meiosis (Kurssanov & Schemahanova 1927), gamete fusion and the subsequent germination of the motile zygote are correct, as well as the setchell & gardner. Where to intercalate this family is a new problem. As suggested earlier, it conceivably is a form derived by reduction from an attached, parenchymatous ancestor. As such, it might be placed among the Ulvales or Ulotrichales. On the other hand, when one examines the nature of the vegetative Chlorocystis, one finds that its unique characteristics of a gelatinous wall (instead of a rigid, cellulosic one) and incised or fissured chloroplast with numerous small contractile vacuoles along the fissures compare favourably with the similar characteristics of Characioclytris and Characiosispi. The zoospore of Chlorochytrium are markedly different, however. They are rigid walled, flattened, and bear a fairly large stigma. Those of Paraclytris and Characiosispi are obconic, biflagellate, with a distinct or a minute flagellar papilla and a conspicuous elongate-oval stigma; their wall is plastic. The zoospore plastid in all three genera is parietal, shallowly lobed, and contains a single pyrenoid.

The vegetative plant body of all three becomes multicellular and, as each grows in cellularity, the original wall inherited from the zoospore is added to and expanded. The individual cells have either a plasmalemma only or also a delicate external sheath. In Chlorochytrium, the sheaths of the individual cells are thicker but gelatinous in texture. This genus likewise differs in that the outer zoosporic wall is not added to and expanded but functions either in attachment or as a flotation cap when neustonic. At germination, the zoospore secretes an additional sheath that expands as the cell grows beneath or away from the zoosporic sheath. With further growth, this second sheath is stretched and ruptured as a new one is secreted within it. Thus, Chlorochytrium, if it does have a relative affinity with Characiosispi and Characiosispi, exhibits greater reduction in size and cellularity but has, to a greater degree, a residual retention of an ancestral wall formation.

If we accept these similarities as significant, then the Chlorochytriaeae may well be placed with the Characiosisphaeaeaeceae in the Characioclytrales, an order of unknown affinity within the Chlorophyta. However, Buchheim et al. (2002), using molecular techniques, suggest that the Characioclytriales form ‘a clade near the base of the “Dunaliella” group almost every conceivable kind of diversity in cell morphology and colonial agglomeration.

Chlorochytrium, with its limited sarcinoid development (in that each cell secretes its own wall and functions independently in production of zoospores) has no place in the Chlorophyceae. It likewise has no place in the Chlorosarcinaceae (= Chlorosphaeraceae), a family of packet-forming green algae that has lost nearly all plant body organisation. Each cell divides in three planes to produce a cubical or an irregular packet of cells. With repetition of this growth, a larger mass is produced that then fragments into the smaller packets. There is zoosporic reproduction by biflagellate or quadrigellate zoospores, depending on the genus. In all cases known, these are naked, and when biflagellate, of the Protostichon type. None are flattened and none are walled. Furthermore the vegetative cells of algae in this family lack contractile vacuoles.

It is thus clear that Chlorochytrium has none of the attributes of the Chlorococcaceae or of the Chlorosarcinaceae, and we therefore propose that it be placed in a family of its own, the Chlorochytriaeaeae (G.S. West) Setchell & Gardner.

The systematic placement of Scotinosphaera remains within the Chlorococcaceae. The family placement of Chlorochytrium, however, presents some difficulty. Fritsch (1935) placed it in a tribe or section, Chlorochytria, in the Chlorococcaceae of the Chlorococcales, while Smith (1950) placed it in the Endosphaeraceae within the Chlorococcales. Bourrelly (1966) placed it in the Chlorococcaceae, into which he merged the Endosphaeraceae. Lewin (1984) transferred it to the order Chaetophorales, family Chlorosarcinaceae. Melkonian (1990) suggested placing it in a new family, the Chlorochytriaeaeae within the Chlorococcales, but did not formally describe it. However, the family had already been recognised (see Komárek & Fott 1983). More recently, Watanabe & Floyd (1994) suggest that Chlorochytrium and Scotinosphaera (as Kentrosphaera) should be classified in the Chlorophyceae and Pleu- rastrophycaceae, respectively. We, however, believe both are best retained within the Chlorophyceae and not split between the two classes, as also proposed in the classification by Mattox & Stewart (1984), until molecular and further ultrastructural studies their resolve taxonomic placement at the class level (also see arguments below).

The order Chlorococcales is an unwieldy, artificial conglomerate of unicellular and colonial forms that exhibit nearly every conceivable kind of diversity in cell morphology and colonial agglomeration.
within the chlamydomonad lineage”. Further molecular and ultrastructural studies may resolve this taxonomic problem.

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