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PHYTOPLANKTON Review Paper

Present state of the systematics of planktonic coccoid green algae of inland waters

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Abstract This review discusses the main developments in the systematics of coccoid green algae over the last three decades. The relationships of key groups of planktonic coccoid green algae are shown in the phylogenetic trees of Chlorophyceae and Trebouxiophyceae. The trees clearly show that the morphology of these algae do not adequately reflect their phylogenetic position. Different phylogenetic species can be hidden under one and the same morphotype. As most of the genera have a polyphyletic origin, they are in need of a systematic re-evaluation. Species classification using the phylogenetic species concept resulted in the establishment of new genera with smaller numbers of species and the description of new species that are not distinguishable by light microscopy. An overview of genera is given in tables and the revised designations of species as contained in the harmonized taxon list of the European Water Framework Directive lists is provided. In this transitional phase from an artificial to a more natural systematics of algae, field biologists and ecologists as well as molecular biologists must strengthen their interdisciplinary cooperation. The alignment of eco-functional groups of algae with true species identities using the barcoding conception will provide a better understanding of the interaction between organisms and their environment.

Keywords Coccoid green algae · Chlorophyceae · Trebouxiophyceae · Systematics · Genus and species concept · Barcodes · Functional groups

Introduction

The classification of algae is presently going through an extremely interesting stage. The old, artificial classification system is being replaced by a new, more natural, phylogenetic system. This is especially the case for the coccoid green algae, mainly because numerous organisms in this morphological group do not propagate sexually. Hence, phenotypic criteria have to a large extent been used in the establishment of taxonomic clades. Initially, the introduction of molecular phylogenetic methods into the systematics of green algae led to a fundamental revision of the concepts of higher taxonomic lineages such as divisions, classes and orders (Melkonian & Surek, 1995; Friedl, 1997; Chapman et al., 1998; Leliaert et al.,

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2012). Following these new phylogenetic conceptions the orders that contain coccoid green algae have been considerably changed. The systematics of lower taxonomic levels such as families and genera remain provisional because detailed taxon sampling for molecular sequence analyses have not been carried out for the taxa. Nevertheless, key groups of planktonic coccoid greens have in the recent past been studied by molecular methods, and as such a picture of their phylogenetic designation has started to take shape.

The central unit for biological classification is the species. The most established species concept is the biological species (Mayr, 1942), which refers to groups of interbreeding natural populations that are reproductively isolated. Because of its restriction to sexually reproducing organisms, this concept cannot be applied to most of the coccoid green algae. In an effort to deal with this limitation, limnologists adopted the morphological species concept using morphology-based diacritical characteristics. The climax of the morphological species concept for the coccoid green algae was reached following the publication of the famous handbook on "Chlorococcales" by Komárek & Fott (1983). Approximately 1,200 taxa from water, soil, and other habitats were compiled in this comprehensive work. The works of Hindák entitled "Studies on the Chlorococcal Algae (Chlorophyceae) I–V" (1977, 1980, 1984, 1988, 1990), which were published partly before and after the handbook of Komárek & Fott, have inspired many phytoplankton researchers. Combining a sharp eye for detail and experience, Hindák identified several new morphospecies. His observations triggered lively discussions on diacritic morphological characteristics such as presence or absence of pyrenoids, cell wall incrustations and mucilaginous envelopes.

Running parallel to these traditional approaches, an ultrastructural concept to classify green algae based on anatomy of flagellated cells and cytokinesis during mitosis was initiated by Melkonian (1982, 1984) and Mattox & Stewart (1984). Based on the orientation of the basal bodies of the flagellar apparatus, three main types have been suggested: *c*ounter *c*lock*w*ise orientation (CCW, 11–5 o'clock), *c*lock*w*ise orientation (CW, 1–7 o'clock), and *d*irectly *o*pposite orientation (DO, 12–6 o'clock). Later studies on molecular characteristics by Lewis et al. (1992) revealed that the relationships established on the basis of ultrastructural data were closely supported by molecular data.

As discussions on the relations between the various concepts of classifying green algae continued, the phylogenetic concept, which utilizes molecular markers to delineate taxa, steadily made its way into the daily routine of phycologists. The evidence that has so far accumulated clearly suggests that biological and morphological conceptions cannot solve the main questions on natural grouping of coccoid green algae. A polyphasic approach combining morphological, ecophysiological and molecular phylogenetic methods should contribute to modern systematics (Pröschold & Leliaert, 2007). In this review, we focus on the main developments in the systematics of coccoid green algae following the landmarks set by the works of Komárek & Fott, Hindák, and Mattox & Stewart in the middle of the 1980s.

Higher taxonomic lineages which contain coccoid green algae

The green algae evolved about 1,500 million years ago (Yoon et al., 2004) into two large lineages (divisions), the Chlorophyta and the Charophyta (Lewis & McCourt, 2004). In this review, we follow the work of Lewis & McCourt (2004) who suggested a "working classification of green algae and land plants" (Table 1).

The Charophyta has often been labeled the Streptophyta by Bremer et al. (1987). This division contains seven classes that include the Charophyceae (stoneworts), the Embryophyceae (higher land plants), the Zygnemophyceae (conjugates), and the Chlorokybophyceae (has only one species *Chlorokybus atmophyticus*, an aerophytic coccoid green alga). The Zygnemophyceae has within the order Desmidiales, a special type of coccoid green algae with a striking morphology characterized by two symmetrical halves (semicells). The majority of coccoid green algae considered here occur in several orders of Chlorophyceae, Trebouxiophyceae, and Prasinophyceae within the division Chlorophyta (Melkonian, 1990a; Fawley et al., 2000; Krienitz et al., 2003). Conventionally, these coccoid taxa were lumped in the order Chlorococcales *sensu lato* (*s.l.*) by Komárek & Fott (1983) and represented one of the most diverse groups of photoautotrophic cryptogams. However, this classical approach based on Pascher's (1918) idea of establishing orders according to life forms has not been supported by phylogenetic methods and is therefore not applicable in modern systematic considerations of green algae. Consequently, most of the taxa that do not belong to the Chlorococcales *s.l.* have been transferred to other orders.

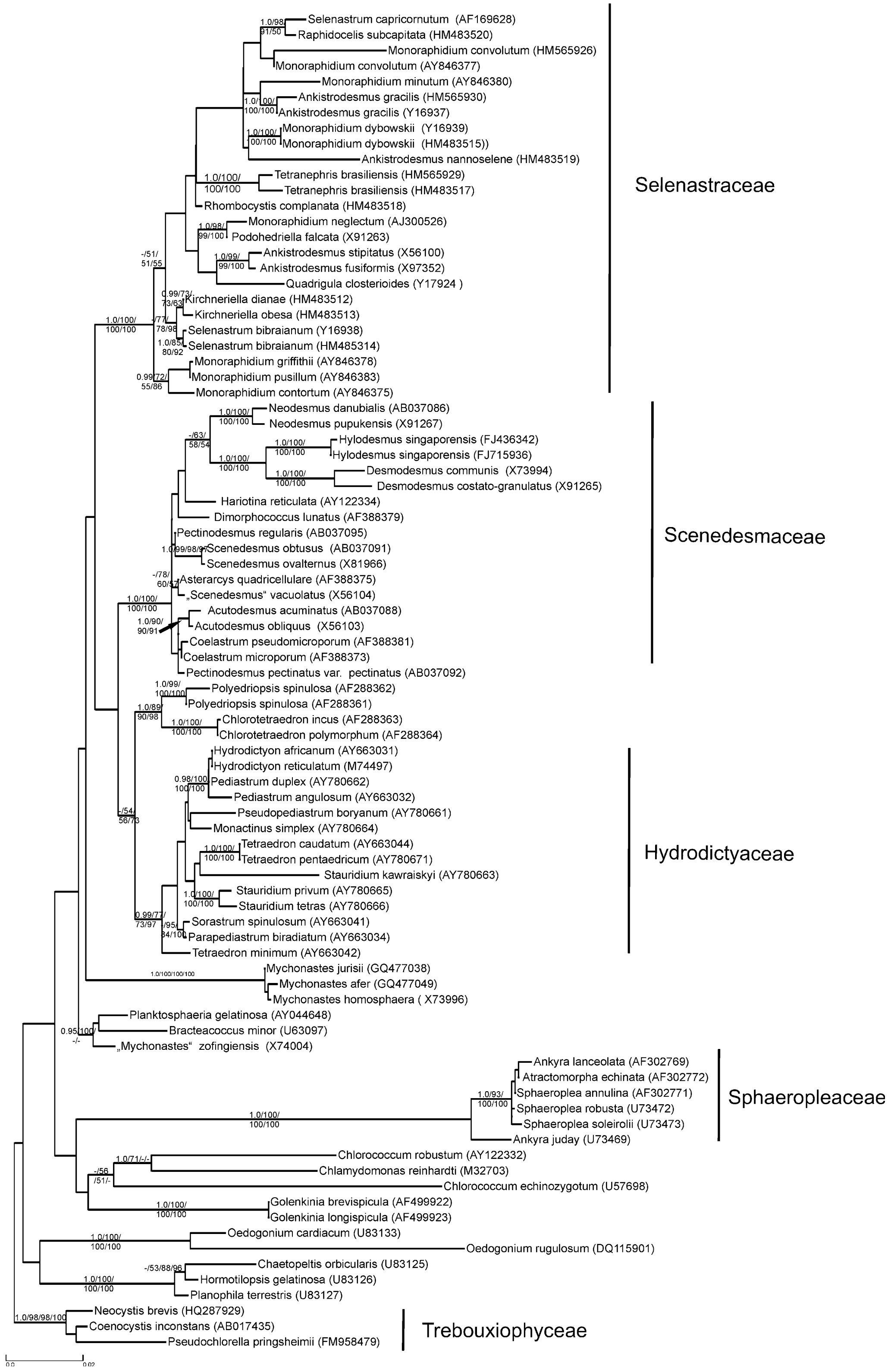
In the division Chlorophyta, coccoid taxa occur in three different classes; the Chlorophyceae, Trebouxiophyceae, and Prasinophyceae (Table 1). In the Chlorophyceae, the coccoid taxa occur in two orders; the Chlorococcales *sensu stricto* (*s.str.*) and Sphaeropleales. The only remaining taxa in the order Chlorococcales *s.str.* are those related to the polyphyletic genus *Chlorococcum* and some other genera that all are still under revision. It has also become evident that some taxa of Chlamydomonadales, Dunaliellales, and Volvocales occur in the order Chlorococcales *s.str.* (Nakayama et al., 1996; Booton et al., 1998; Chapman et al., 1998; Pröschold et al., 2001). The ultrastructures of all these taxa are characterized by a CW orientation of the basal apparatus of the flagella. Apart from the Chlorococcales *s.str.* and Sphaeropleales, several other lineages (incertae sedis) which do not correspond to the named clades have coccoid taxa. Future revisions of the CW-group of Chlorophyceae may probably lead to the establishment of several new orders. Furthermore, the content of ambiguous orders such as Actinochloridales, Chlorosarcinales, and Tetrasporales must be emendated.

A majority of chlorophycean members of Chlorophyta are placed in the order Sphaeropleales (Fig. 1). Some members of these taxa such as Sphaeropleaceae, Hydrodictyaceae, and *Bracteacoccus* produce zoospores with DO-orientation of the flagellar apparatus (Lewis, 1997; Buchheim et al., 2001; Wolf et al., 2002a; Shoup & Lewis, 2003). However, many non motile unicellular or colonial taxa such as Selenastraceae and *Mychonastes* are also included in this order (Krienitz et al., 2001, 2003, 2011a, b, 2012). The Scenedesmaceae also belong to this order. Members of this family are commonly non motile. However, presence of zoospores in cultures of *Acutodesmus* has been reported (Trainor, 1963). Keller et al. (2008) confirmed the monophyly of Sphaeropleales; however, from several other studies the relations between the clade containing the type genus *Sphaeroplea* (Sphaeropleales *s.str.*) and the other clades

Table 1 Overview on main lineages of green algae

- Kingdom Chlorobionta
  - Division Chlorophyta
    - Class Chlorophyceae
      - Order Chlamydomonadales
      - Order Chlorococcales* (*Chlorococcum*)
      - Order Sphaeropleales* (*Ankyra*, *Pediastrum*, *Scenedesmus*, *Selenastrum*)
      - Order Oedogoniales
      - Order Chaetopeltidales
      - Order Chaetophorales
      - Incertae sedis* (*Actinochloris*, *Nautococcus*)
    - Class Ulvophyceae
      - Order Ulotrichales
    - Class Trebouxiophyceae
      - Order Trebouxiales* (*Trebouxia*, *Chloroidium*, *Myrmecia*)
      - Order Microthamniales
      - Order Prasiolales
      - Order Chlorellales* (*Chlorella*, *Dictyosphaerium*, *Micractinium*)
      - Incertae sedis* (*Choricystis*, *Coccomyxa*, *Botryococcus*)
    - Class Prasinophyceae
      - Order Pyramimonadales* (*Halosphaera*, *Pachysphaera*)
      - Order Mamiellales* (*Bathycoccus*, *Ostreococcus*)
      - Order Pseudoscourfieldiales* (*Pycnococcus*)
      - Order Prasinococcales* (*Prasinococcus*)
      - Order Chlorodendrales
      - Incertae sedis* (*Picocystis*)
  - Division Charophyta
    - Class Mesostigmatophyceae
    - Class Chlorokybophyceae
      - Order Chlorokybales* (*Chlorokybus*)
    - Class Klebsormidiophyceae
    - Class Zygnemophyceae
      - Order Zygnematales
      - Order Desmidiales* (*Cosmarium*)
    - Class Coleochaetophyceae
    - Subdivision Streptophytina
    - Class Charophyceae
    - Class Embryophyceae

The orders containing coccoid phenotypes are indicated by an asterisk, and some examples of genera belonging into these orders are given


Selenastrum capricornutum (AF169628)
Raphidocelis subcapitata (HM483520)
Monoraphidium convolutum (HM565926)
Monoraphidium convolutum (AY846377)
Monoraphidium minutum (AY846380)
Ankistrodesmus gracilis (HM565930)
Ankistrodesmus gracilis (Y16937)
Monoraphidium dybowskii (Y16939)
Monoraphidium dybowskii (HM483515))
Ankistrodesmus nannoselene (HM483519)
Tetranephris brasiliensis (HM565929)
Tetranephris brasiliensis (HM483517)
Rhombocystis complanata (HM483518)
Monoraphidium neglectum (AJ300526)
Podohedriella falcata (X91263)
Ankistrodesmus stipitatus (X56100)
Ankistrodesmus fusiformis (X97352)
Quadrigula closterioides (Y17924)
Kirchneriella dianae (HM483512)
Kirchneriella obesa (HM483513)
Selenastrum bibraianum (Y16938)
Selenastrum bibraianum (HM485314)
Monoraphidium griffithii (AY846378)
Monoraphidium pusillum (AY846383)
Monoraphidium contortum (AY846375)
Selenastraceae
1.0/98/ 91/50
1.0/100/ 100/100
1.0/100/ 100/100
1.0/100/ 100/100
1.0/98/ 99/100
1.0/99/ 99/100
-/51/ 51/55
0.99/73/ 73/63
-/77/ 78/98
1.0/85/ 80/92
0.99/72/ 55/86
1.0/100/ 100/100
Neodesmus danubialis (AB037086)
Neodesmus pupukensis (X91267)
Hylodesmus singaporensis (FJ436342)
Hylodesmus singaporensis (FJ715936)
Desmodesmus communis (X73994)
Desmodesmus costato-granulatus (X91265)
Hariotina reticulata (AY122334)
Dimorphococcus lunatus (AF388379)
Pectinodesmus regularis (AB037095)
Scenedesmus obtusus (AB037091)
Scenedesmus ovalternus (X81966)
Asterarcys quadricellulare (AF388375)
„Scenedesmus" vacuolatus (X56104)
Acutodesmus acuminatus (AB037088)
Acutodesmus obliquus (X56103)
Coelastrum pseudomicroporum (AF388381)
Coelastrum microporum (AF388373)
Pectinodesmus pectinatus var. pectinatus (AB037092)
Scenedesmaceae
1.0/100/ 100/100
-/63/ 58/54
1.0/100/ 100/100
1.0/100/ 100/100
1.0/100/ 100/100
1.0/99/98/97
-/78/ 60/57
1.0/100/ 100/100
1.0/90/ 90/91
Polyedriopsis spinulosa (AF288362)
Polyedriopsis spinulosa (AF288361)
Chlorotetraedron incus (AF288363)
Chlorotetraedron polymorphum (AF288364)
Hydrodictyon africanum (AY663031)
Hydrodictyon reticulatum (M74497)
Pediastrum duplex (AY780662)
Pediastrum angulosum (AY663032)
Pseudopediastrum boryanum (AY780661)
Monactinus simplex (AY780664)
Tetraedron caudatum (AY663044)
Tetraedron pentaedricum (AY780671)
Stauridium kawraiskyi (AY780663)
Stauridium privum (AY780665)
Stauridium tetras (AY780666)
Sorastrum spinulosum (AY663041)
Parapediastrum biradiatum (AY663034)
Tetraedron minimum (AY663042)
Hydrodictyaceae
1.0/99/ 100/100
1.0/89/ 90/98
1.0/100/ 100/100
0.98/100/ 100/100
1.0/100/ 100/100
1.0/100/ 100/100
-/54/ 56/73
0.99/77/ 73/97
-/95/ 84/100
Mychonastes jurisii (GQ477038)
Mychonastes afer (GQ477049)
Mychonastes homosphaera (X73996)
1.0/100/100/100
Planktosphaeria gelatinosa (AY044648)
Bracteacoccus minor (U63097)
„Mychonastes" zofingiensis (X74004)
0.95/100/ -/-
Ankyra lanceolata (AF302769)
Atractomorpha echinata (AF302772)
Sphaeroplea annulina (AF302771)
Sphaeroplea robusta (U73472)
Sphaeroplea soleirolii (U73473)
Ankyra juday (U73469)
Sphaeropleaceae
1.0/93/ 100/100
1.0/100/ 100/100
Chlorococcum robustum (AY122332)
Chlamydomonas reinhardti (M32703)
Chlorococcum echinozygotum (U57698)
1.0/71/-/-
-/56 /51/-
Golenkinia brevispicula (AF499922)
Golenkinia longispicula (AF499923)
1.0/100/ 100/100
Oedogonium cardiacum (U83133)
Oedogonium rugulosum (DQ115901)
1.0/100/ 100/100
Chaetopeltis orbicularis (U83125)
Hormotilopsis gelatinosa (U83126)
Planophila terrestris (U83127)
-/53/88/96
1.0/100/ 100/100
Neocystis brevis (HQ287929)
Coenocystis inconstans (AB017435)
Pseudochlorella pringsheimii (FM958479)
1.0/98/98/100
Trebouxiophyceae
0.0
0.02

◀ **Fig. 1** Molecular phylogeny of the Chlorophyceae based on SSU rRNA gene sequence comparisons. The phylogenetic tree shown was inferred using the maximum likelihood (ML) method (with substitution model: J1 [Optimum, Empirical]:G [Optimum]:5), based on 1558 aligned positions of 87 taxa using Treefinder (Jobb, 2008). Bayesian values (>0.95) (MB) were calculated by MrBayes 3.1 using GTR settings (Ronquist & Huelsenbeck, 2003; Posada & Buckley, 2004). The stationary distribution was assumed after 4 million generations when the average standard deviations of split frequencies between two runs was lower than 0.01. To test the tree confidence, bootstrap values (>50%) ML (1,000 replicates), neighbor-joining (NJ) (1,000 replicates; calculated using Paup 4.0), and maximum parsimony (MP) [1,000 replicates; calculated using Paup 4.0 (Swofford, 2002)] were determined. Support values are shown at the branches in the order: MB, ML, MP, NJ. *Scale bar* indicates substitutions per site. The sequences were obtained from Genbank [National Center for Biotechnology Information (NCBI)]. For each taxon, the NCBI accession number is given in brackets

(e.g., *Hydrodictyon*-clade, *Scenedesmus*-clade, *Ankistrodesmus*-clade, *Bracteacoccus*-clade) of the Sphaeropleales *s.l.* remain ambiguous (Fawley et al., 2005a; Pröschold & Leliaert, 2007; Krienitz et al., 2011a). Better-supported conclusions will require more data, based on sequences of a larger number of molecular markers.

Two major orders of coccoid taxa can be identified in the class Trebouxiophyceae; the Trebouxiales and Chlorellales (Fig. 2). Several other clades such as the *Choricystis*-clade and *Oocystis*-clade are distinctive and may probably be established as an independent order in future. Other clades (incertae sedis) hosting *Coccomyxa*, *Botryococcus*, *Coenocystis* and *Pseudochlorella* await delineation into higher taxonomic ranks. The Trebouxiales mainly comprises taxa from edaphic and aerophytic habitats as well as endosymbionts of lichens (Ettl & Gärtner, 1995; Friedl, 1995). Within Chlorellales (Fig. 3) are numerous new genera and species that were recently described from freshwaters (Fawley et al., 2005b; Bock et al., 2010, 2011a, b, c; Pröschold et al., 2010).

The class Prasinophyceae contains small flagellated and scaled green algae. A few of them have lost one or both the flagella and scales and now have a coccoid phenotype. Coccoid prasinophytes occur in different orders of the class Prasinophyceae (Table 1). It has been shown that this class is an artificial taxonomic lineage because these organisms have a paraphyletic origin; hence should better be named as prasinophytes (Steinkötter et al., 1994). The prasinophytes evolved in several independent lineages which probably represent independent classes. A report by Guillou et al. (2004) identifies seven different clades of the prasinophytes. Initial taxonomic revision of some of these clades has resulted in the description of the classes Nephroselmidophyceae (Cavalier-Smith, 1993), Chlorodendrophyceae (Masjuk, 2006), and Mamiellophyceae (Marin & Melkonian, 2010). The coccoid prasinophytes are restricted to marine habitats or saline inland waters (Guillou et al., 2004; Krienitz et al., 2012).

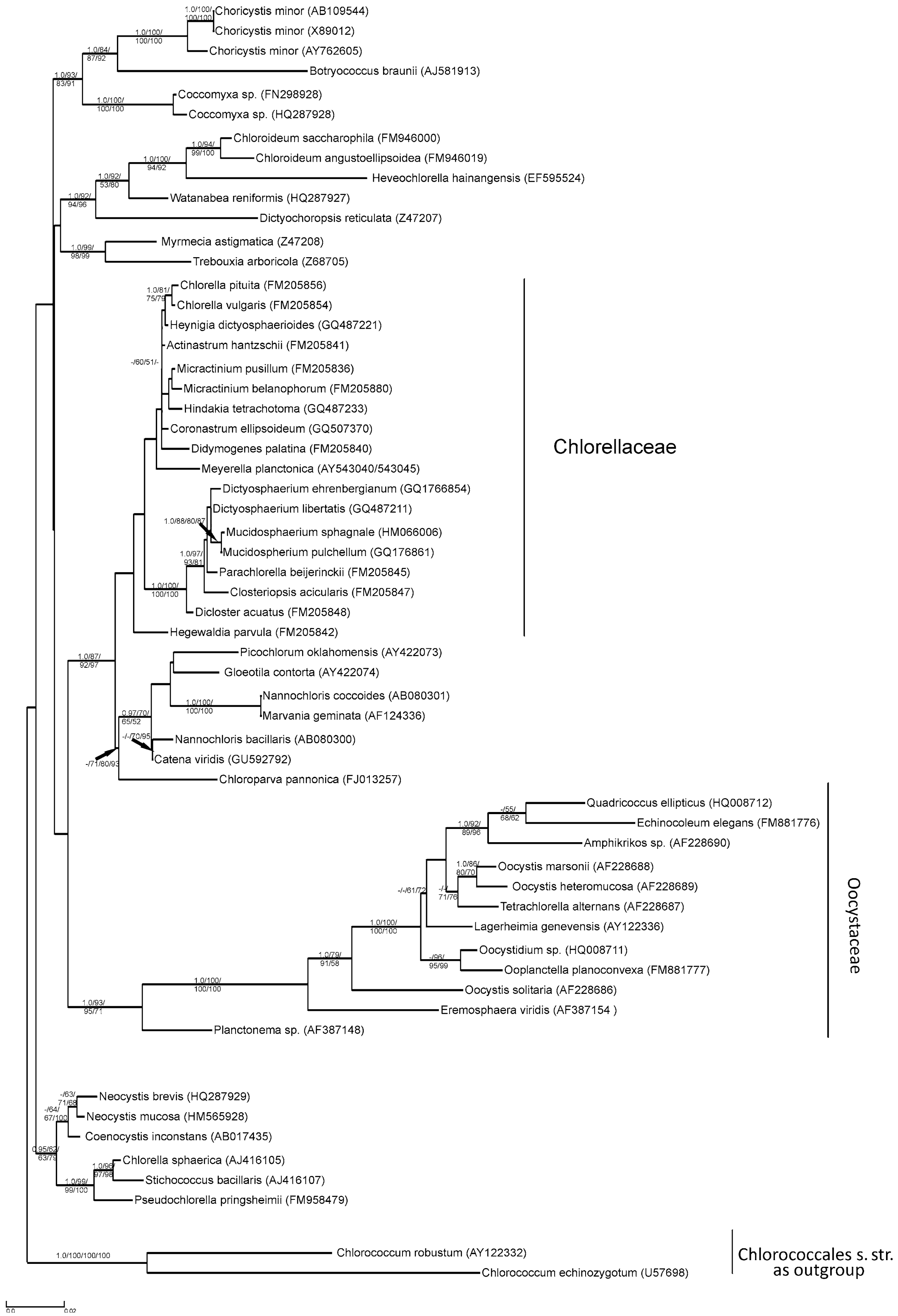
Recent systematics of key groups of coccoid green algae of inland waters

The ranking of the groups discussed in this section follow the topology in the phylogenetic trees (Figs. 1, 2, 3). These trees are based on a selection of sequences obtained from Genbank [National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/]. The accession numbers of these sequences are indicated in Figs. 1, 2, and 3).

Chlorophyceae

Selenastraceae

Morphologically, this group comprises of "needles and capricorns." Members of this family are very common in freshwaters and exhibit a high morphological diversity. They exclusively propagate by autospores. Discussions on the diacritical features of this group have been very intense. Marvan et al. (1984) investigated 18 genera of the Selenastraceae by means of numerical evaluation of morphological and ontogenetic characteristics. The shape of the cells or colonies, the arrangement of autospores inside the mother cell, the development of mucilage and incrustations on the cell wall, and the presence, number, and type of pyrenoids were used to differentiate the genera. According to this conception, several genera differ from each other only by one of the above characters. For example, *Selenastrum* differs from *Ankistrodesmus* by the curvature of the cells (Komárek & Comas, 1982), *Raphidocelis* differs from *Kirchneriella* by cell wall incrustations (Hindák, 1977), and *Chlorolobion* differs from *Monoraphidium* by the presence of a pyrenoid with starch envelope (Komárek, 1979; Heynig & Krienitz, 1982). Fawley et al. (2005a) correlated the morphological features of


1.0/100/
100/100
Choricystis minor (AB109544)
Choricystis minor (X89012)
1.0/100/
100/100
Choricystis minor (AY762605)
1.0/84/
87/92
Botryococcus braunii (AJ581913)
1.0/93/
83/91
Coccomyxa sp. (FN298928)
1.0/100/
100/100
Coccomyxa sp. (HQ287928)
Chloroideum saccharophila (FM946000)
1.0/94/
99/100
Chloroideum angustoellipsoidea (FM946019)
1.0/100/
94/92
Heveochlorella hainangensis (EF595524)
1.0/92/
53/80
Watanabea reniformis (HQ287927)
1.0/92/
94/96
Dictyochoropsis reticulata (Z47207)
Myrmecia astigmatica (Z47208)
1.0/99/
98/99
Trebouxia arboricola (Z68705)
1.0/81/
75/79
Chlorella pituita (FM205856)
Chlorella vulgaris (FM205854)
Heynigia dictyosphaerioides (GQ487221)
Actinastrum hantzschii (FM205841)
-/60/51/-
Micractinium pusillum (FM205836)
Micractinium belanophorum (FM205880)
Hindakia tetrachotoma (GQ487233)
Coronastrum ellipsoideum (GQ507370)
Didymogenes palatina (FM205840)
Meyerella planctonica (AY543040/543045)
Dictyosphaerium ehrenbergianum (GQ1766854)
Dictyosphaerium libertatis (GQ487211)
1.0/88/80/87
Mucidosphaerium sphagnale (HM066006)
1.0/97/
93/81
Mucidospherium pulchellum (GQ176861)
Parachlorella beijerinckii (FM205845)
1.0/100/
100/100
Closteriopsis acicularis (FM205847)
Dicloster acuatus (FM205848)
Hegewaldia parvula (FM205842)
Chlorellaceae
Picochlorum oklahomensis (AY422073)
1.0/87/
92/97
Gloeotila contorta (AY422074)
1.0/100/
100/100
Nannochloris coccoides (AB080301)
0.97/70/
65/52
Marvania geminata (AF124336)
-/-/70/95
Nannochloris bacillaris (AB080300)
-/71/80/93
Catena viridis (GU592792)
Chloroparva pannonica (FJ013257)
-/55/
68/62
Quadricoccus ellipticus (HQ008712)
1.0/92/
89/96
Echinocoleum elegans (FM881776)
Amphikrikos sp. (AF228690)
1.0/86/
80/70
Oocystis marsonii (AF228688)
-/-/61/72
-/-/
71/76
Oocystis heteromucosa (AF228689)
Tetrachlorella alternans (AF228687)
1.0/100/
100/100
Lagerheimia genevensis (AY122336)
-/96/
95/99
Oocystidium sp. (HQ008711)
1.0/79/
91/58
Ooplanctella planoconvexa (FM881777)
1.0/100/
100/100
Oocystis solitaria (AF228686)
Eremosphaera viridis (AF387154)
1.0/93/
95/71
Planctonema sp. (AF387148)
Oocystaceae
-/63/
71/68
Neocystis brevis (HQ287929)
-/64/
67/100
Neocystis mucosa (HM565928)
Coenocystis inconstans (AB017435)
0.95/62/
63/79
1.0/96/
97/98
Chlorella sphaerica (AJ416105)
Stichococcus bacillaris (AJ416107)
1.0/99/
99/100
Pseudochlorella pringsheimii (FM958479)
1.0/100/100/100
Chlorococcum robustum (AY122332)
Chlorococcum echinozygotum (U57698)
Chlorococcales s. str.
as outgroup
0.0
0.02

◀ **Fig. 2** Molecular phylogeny of the Trebouxiophyceae based on SSU rRNA gene sequence comparisons. The phylogenetic tree shown was inferred using the maximum likelihood (ML) method (with substitution model: J1 [Optimum, Empirical]:G [Optimum]:5), based on 1,429 aligned positions of 58 taxa using Treefinder (Jobb, 2008). Bayesian values (>0.95) (MB) were calculated by MrBayes 3.1 using GTR settings (Ronquist & Huelsenbeck, 2003; Posada & Buckley, 2004). The stationary distribution was assumed after 4 million generations when the average standard deviations of split frequencies between two runs was lower than 0.01. To test the tree confidence, bootstrap values (>50%) for ML (1,000 replicates), NJ (1,000 replicates; calculated using Paup 4.0), and MP [1,000 replicates; calculated using Paup 4.0 (Swofford, 2002)] were calculated. Support values are shown at the branches in the order: MB, ML, MP, and NJ. *Scale bar* indicates substitutions per site. The sequences were obtained from Genbank [National Center for Biotechnology Information (NCBI)]. For each taxon, the NCBI accession number is given in brackets

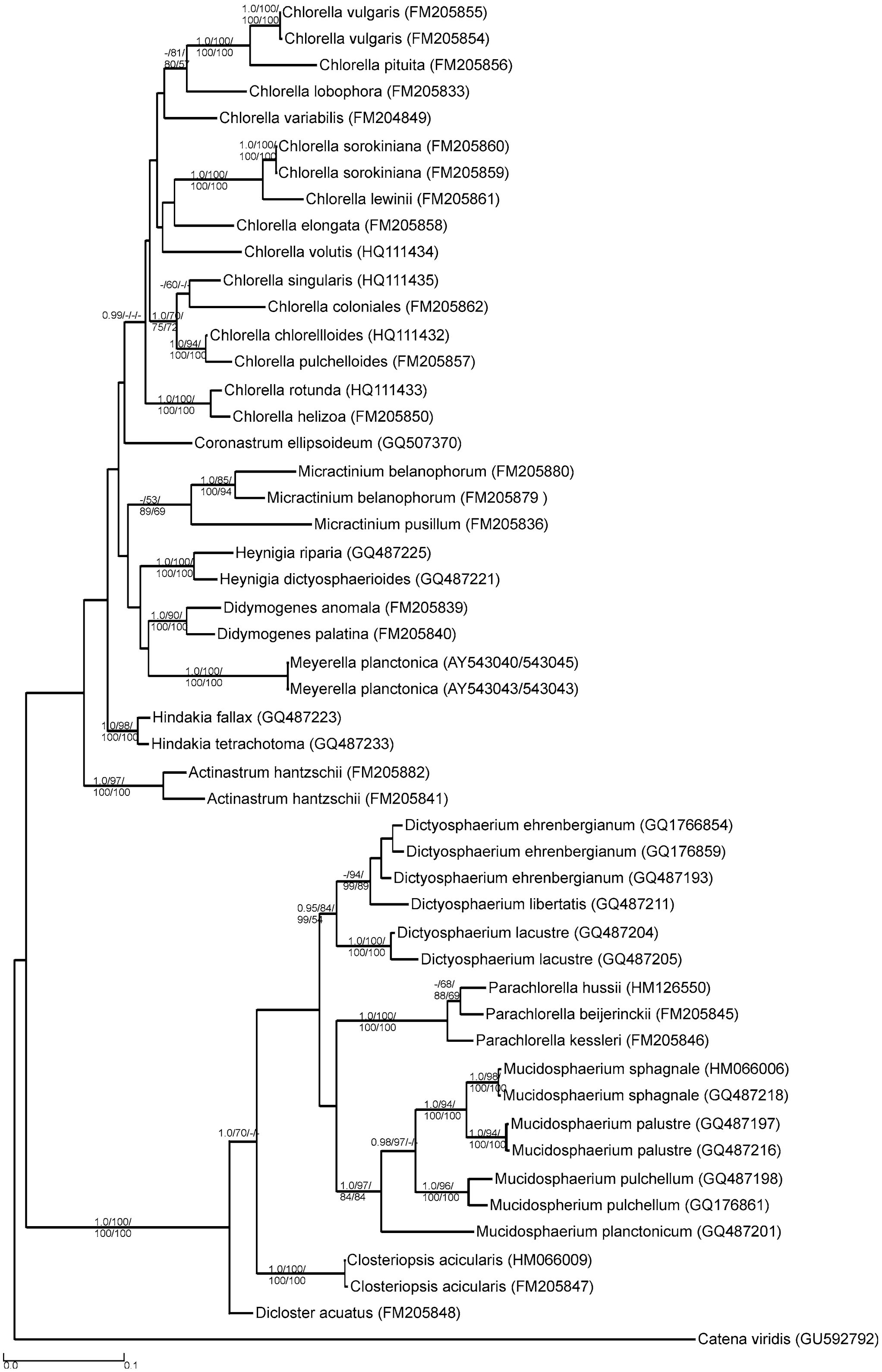
several morphospecies of Selenastraceae with their molecular characteristics and found that one morphotype can cover different phylotypes. The data suggested that a broad morphospecies concept would result in a substantial underestimation of species diversity. Subsequent molecular studies have confirmed the existence of "small" genera in Selenastraceae containing only a few species (Krienitz et al., 2001, 2011b). From the molecular studies, 10 different genera have been confirmed in this family (Table 2). Several other genera are yet to be subjected to detailed molecular investigation the outcome of which may be the description of new genera. This is especially the case for the relatives of the needle-shaped *Ankistrodesmus s.l.* and *Monoraphidium s.l.* which are found in nine different clades and probably represent different genera (Krienitz et al., 2011b). However, further taxon sampling is essential before conclusions on the taxonomic identities of these clades can be drawn.

The adjustment of the diacritical value of the main morphological characteristics in line with the molecular findings has revealed the following: the solitary versus colonial life form, the general shape of cells and colonies are relatively good criteria. Although changes in environmental conditions can result in some colonies disintegrating, the arrangement of autospores in the mother cells can be used for the differentiation of genera. The establishment of mucilage and incrustations are of limited taxonomic value because of the wide variability. Similarly, views on the value of starch formation and the presence of pyrenoids have changed over time. Traditionally, most of the genera were thought to be devoid of pyrenoids. However, this position was later, revised following the observation of pyrenoids in some genera. The first report on the presence of pyrenoids was made by Eloranta (1979), who used the TEM to observe pyrenoids in *Monoraphidium griffithii*. This finding was later confirmed for other species (Krienitz et al., 1985, 2001; Krienitz & Scheffler, 1994). In subsequent studies, pyrenoids were detected in all taxa studied; some exhibited a naked matrix, while others were equipped with starch grains covering the pyrenoid matrix (Krienitz et al., 2011b). In aerated cultures, it was observed that the concentration of CO_2 influenced pyrenoid formation (Miyachi et al., 1986). In *Monoraphidium terreste*, pyrenoids were well developed under normal air conditions. However, they were found to disappear under CO_2 enrichment (Krienitz & Klein, 1988). Hence the presence or absence of pyrenoids cannot be used as a basis for the differentiation of members of the Selenastraceae family.

Based on molecular phylogeny, several taxa traditionally included in the Selenastraceae were removed from this family. *Choricystis minor* is member of a picoplanktonic lineage in the Trebouxiophyceae (Krienitz et al., 1996a; Darienko et al., 2010). *Keratococcus bicaudatus* and *Pseudococcomyxa simplex* are closely related to *Choricystis* (Friedl, 1996). The authentic strain of *P. simplex* was recovered in the *Elliptochloris*-clade and found to be closely related to other strains of *Coccomyxa* (Pröschold et al., 2011). Consequently, the taxon *Coccomyxa simplex* has been re-established. Hence, other members of *Pseudococcomyxa* need a taxonomic revision. *Dicloster acuatus* is a needle-shaped coenobial member of Chlorellaceae (Hegewald & Hanagata, 2000). *Closteriopsis acicularis* is a solitary, needle-shaped member of the Chlorellaceae and is closely related to *Dicloster* (Ustinova et al., 2001). The ultrastructure of its pyrenoid is comparable to that of *Chlorella* and other Chlorellaceae (Hegewald & Schnepf, 1986). The apochloric *Hyaloraphidium curvatum* is a member of the lower fungi (Ustinova et al., 2000).

Scenedesmaceae

This is the largest group of coccoid green algae in freshwater ecosystems. Among the genera, the genus *Scenedesmus s.l.* with its extremely wide morphological variability is a nightmare for field ecologists who


1.0/100/ 100/100
Chlorella vulgaris (FM205855)
Chlorella vulgaris (FM205854)
1.0/100/ 100/100
-/81/ 80/57
Chlorella pituita (FM205856)
Chlorella lobophora (FM205833)
Chlorella variabilis (FM204849)
1.0/100/ 100/100
Chlorella sorokiniana (FM205860)
Chlorella sorokiniana (FM205859)
1.0/100/ 100/100
Chlorella lewinii (FM205861)
Chlorella elongata (FM205858)
Chlorella volutis (HQ111434)
-/60/-/-
Chlorella singularis (HQ111435)
Chlorella coloniales (FM205862)
0.99/-/-/-
1.0/70/ 75/72
Chlorella chlorellloides (HQ111432)
1.0/94/ 100/100
Chlorella pulchelloides (FM205857)
Chlorella rotunda (HQ111433)
1.0/100/ 100/100
Chlorella helizoa (FM205850)
Coronastrum ellipsoideum (GQ507370)
Micractinium belanophorum (FM205880)
1.0/85/ 100/94
-/53/ 89/69
Micractinium belanophorum (FM205879)
Micractinium pusillum (FM205836)
Heynigia riparia (GQ487225)
1.0/100/ 100/100
Heynigia dictyosphaerioides (GQ487221)
Didymogenes anomala (FM205839)
1.0/90/ 100/100
Didymogenes palatina (FM205840)
Meyerella planctonica (AY543040/543045)
1.0/100/ 100/100
Meyerella planctonica (AY543043/543043)
Hindakia fallax (GQ487223)
1.0/98/ 100/100
Hindakia tetrachotoma (GQ487233)
Actinastrum hantzschii (FM205882)
1.0/97/ 100/100
Actinastrum hantzschii (FM205841)
Dictyosphaerium ehrenbergianum (GQ1766854)
Dictyosphaerium ehrenbergianum (GQ176859)
-/94/ 99/89
Dictyosphaerium ehrenbergianum (GQ487193)
0.95/84/ 99/54
Dictyosphaerium libertatis (GQ487211)
Dictyosphaerium lacustre (GQ487204)
1.0/100/ 100/100
Dictyosphaerium lacustre (GQ487205)
-/68/ 88/69
Parachlorella hussii (HM126550)
Parachlorella beijerinckii (FM205845)
1.0/100/ 100/100
Parachlorella kessleri (FM205846)
Mucidosphaerium sphagnale (HM066006)
1.0/98/ 100/100
Mucidosphaerium sphagnale (GQ487218)
1.0/94/ 100/100
Mucidosphaerium palustre (GQ487197)
1.0/70/-/-
0.98/97/-/-
1.0/94/ 100/100
Mucidosphaerium palustre (GQ487216)
Mucidosphaerium pulchellum (GQ487198)
1.0/96/ 100/100
1.0/97/ 84/84
Mucidospherium pulchellum (GQ176861)
1.0/100/ 100/100
Mucidosphaerium planctonicum (GQ487201)
Closteriopsis acicularis (HM066009)
1.0/100/ 100/100
Closteriopsis acicularis (FM205847)
Dicloster acuatus (FM205848)
Catena viridis (GU592792)
0.0
0.1

◀ **Fig. 3** Molecular phylogeny of the Chlorellaceae based on a partitioned dataset of SSU, ITS1, 5.8S, and ITS2 gene sequences. The phylogenetic tree shown is based on 2,536 manually aligned base positions of 50 taxa, calculated by Treefinder (Jobb, 2008) using the maximum likelihood (ML) method under different substitutional models for each partition. The substitution models were as follows: 18S (1,686 bases) J2 [Optimum, Empirical]:GI [Optimum]:5; ITS1 (388 bases) J1 [Optimum, Empirical]:G [Optimum]:5; 5.8S (137 bases) HKY [{3,1,1,1,1,3}, Empirical]:G [Optimum]:5; and ITS2 (325 bases) GTR [Optimum, Empirical]:G [Optimum]:5. The Bayesian values (>0.95) (MB) were calculated by MrBayes 3.1. A general time reversible model with gamma shape parameter and proportion of invariable sites (GTR+I+G) was applied to each partition. The parameters were unlinked and allowed to vary across the partitions (Ronquist & Huelsenbeck, 2003; Posada & Buckley, 2004). The stationary distribution was assumed after 4 million generations when the average standard deviations of split frequencies between two runs was lower than 0.01. To test the tree confidence, bootstrap values (>50%) for ML (1,000 replicates), NJ (1,000 replicates; using Paup 4.0), and MP (1,000 replicates; using Paup 4.0 (Swofford, 2002) were calculated. Support values are shown at the branches in the order: MB, ML, MP, and NJ. *Scale bar* indicates substitutions per site. The sequences were obtained from Genbank [National Center for Biotechnology Information (NCBI)]. For each taxon, the NCBI accession number is given in brackets

wish to determine a taxon in a fixed sample under the inverted microscope. The "Annotated catalogue of *Scenedesmus* and nomenclaturally related genera," published by Hegewald & Silva (1988) is a milestone in the history of conventional systematics of green algae. More than 800 taxa were included in details that provide a clear testimony of how the game of nature creates a multitude of morphospecies. Although this catalogue is a good guide for microscopists, at the end of the day it is difficult to decide on the species delineation in Scenedesmaceae. Several publications, each justifying the different views of the authors, have been written.

According to Hegewald (1997), the great morphological variability of Scenedesmaceae can be attributed to the strictly non-sexual propagation. This argument may also be valid for many other coccoid green algae such as the Selenastraceae. The simple reproduction by means of autospores results in a situation whereby all mutations occurring, which do not significantly influence growth and ability of the mutants to compete, remain and are not lost by genetic processes. This discussion leads to the old question on the possible occurrence of flagellated stages in *Scenedesmus*, first reported by a Ukrainian phycologist J. J. Valz more than 130 years ago (according to Hegewald, 1997). Trainor (1963) recorded the presence of flagellates in cultures of *Scenedesmus*, while Lukavský (1991) and Cepák (1993) found them in outdoor mass cultures. Trainor (1996) has described the production and germination of zygospores. However, the majority of these algae propagate asexually by autospores, and the reasons given by Hegewald to explain the wide morphological variability remain valid.

Revisions of the class followed the introduction of molecular methods. First, it became evident that the morphologically differentiated subgenera should be upgraded to generic status. Consequently, the genus *Desmodesmus* was separated from *Scenedesmus* (An et al., 1999). Whereas *Desmodesmus* comprises species characterized by many substructures on the cell wall and are equipped with teeth, rosettes, warts and spines, the species of *Scenedesmus* have a smooth, non-ornamented cell wall (Hegewald, 2000). The genus *Acutodesmus*, which comprises of more or less ellipsoidal, spindle-shaped taxa that show longitudinal ridges under the TEM, was established by Tsarenko & Petlevanny (2001). Later, *Pectinodesmus* was erected for taxa with similar morphology as *Acutodesmus* but differing in molecular phylogeny (Hegewald et al., 2010). Several genera which are presently monotypic such as *Hylodesmus* and *Comasiella* have recently been established (Eliáš et al., 2010; Hegewald et al., 2010).

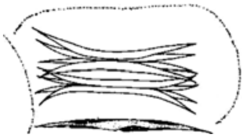
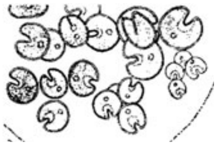
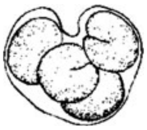
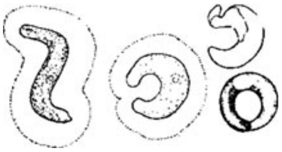
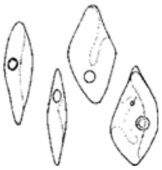
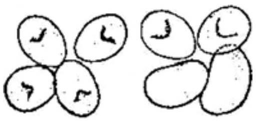
A major surprise resulting from the molecular studies is the clustering of *Coelastrum*-morphotypes with *Scenedesmus* suggesting that the flat coenobia with cells arranged in one or two rows of *Scenedesmus*-relatives are phylogenetically closely related to the spherical coenobia of *Coelastrum* and allied species (Fig. 1) (Hegewald et al., 2010). Following confirmation through molecular studies of the separate position of *Coelastrum reticulatum*, the old name *Hariotina* was reintroduced (Hegewald et al., 2002).

At present, 13 genera of Scenedesmaceae are phylogenetically and morphologically well defined (Table 3). Several other genera of this family, such as all the crucigenoid groups, are yet to be revised.

Hydrodictyaceae

The Hydrodictyaceae comprises microscopic colonies of *Pediastrum*, *Euastropsis*, and *Sorastrum* as well as macroscopic colonies of *Hydrodictyon*. The most

Table 2 Genera of Selenastraceae confirmed by 18S rRNA gene phylogeny and their main diacritic morphological characteristics

Genus	Drawing	Main diacritic morphology
Ankistrodesmus		Needle-shaped cells, in colonies, parallel arrangement of autospores
Kirchneriella		Semilunate- to crescent-shaped cells, in colonies, serial arrangement of autospores
Monoraphidium		Needle- to rod-shaped cells, solitary, serial arrangement of autospores
Nephrochlamys		Semilunate-shaped cells, in colonies, serial arrangement of autospores, widening mother cell wall
Podohedriella		Needle-shaped cells, solitary, heteropolar, serial arrangement of autospores
Quadrigula		Cylindrical cells with rounded ends, in quadricellular colonies, parallel arrangement of autospores
Raphidocelis		Capricorn-shaped cells, arcuated, solitary or in irregular colonies, serial arrangement of autospores
Rhombocystis		Cells rhomboidal with slightly thickened poles, solitary or colonial, parallel arrangement of autospores
Selenastrum		Semilunate-shaped cells in regular colonies, parallel arrangement of autospores
Tetranephris		Bean-shaped cells in quadricellular colonies, touched at the poles, serial arrangement of autospores

Drawings after Komárek & Fott (1983)

recent review reduced the number of *Pediastrum*-species to 24 (Komárek & Jankovská, 2001). Hydrodictyaceae exhibit distinct reproduction strategies: they produce asexually inside the parental cells through biflagellated zoospores that aggregate after swarming to daughter colonies which are released from the mother-sporangiae in a gelatinous bubble. Sexually, Hydrodictyaceae reproduce by isogametes. The ultrastructure of the flagellar apparatus is characterized by a directly opposite (DO) configuration in *Hydrodictyon* and *Pediastrum* (Wilcox & Floyd, 1988).

Phylogenetic analysis of *Hydrodictyon*, *Pediastrum*, and *Sorastrum* has revealed a pattern of colony-form evolution within the family from two-dimensionality to three-dimensionality (McManus & Lewis, 2005). Another molecular phylogenetic study of 28 hydrodictyacean strains revealed polyphyly in *Pediastrum* and resulted in taxonomic conclusions (Buchheim et al., 2005). Beside *Pediastrum*, the genera *Monactinus*, *Parapediastrum*, *Pseudopediastrum,* and *Stauridium* were delineated. It is interesting to note that *Pediastrum duplex* with a complex morphology of colonies evolved polyphyletically (McManus & Lewis, 2011). Consequently, a new genus *Lacunastrum* was erected (McManus et al., 2011). It has also been shown that members of the genus *Tetraedron* evolved as a sister clade to the Hydrodictyaceae. However, the evolutionary link between the tetraedric unicells of autosporic *Tetraedron*, zoosporic *Chlorotetraedron*, and the zoosporic colonial Hydrodictyaceae remains obscure (McEntee et al., 1977; Komárek & Kováčik, 1985; Hegewald et al., 2001; Buchheim et al., 2005). Presently, the Hydrodictyaceae has 10 genera as confirmed by morphological and molecular analyses (Table 4).

Sphaeropleaceae

This group provides a good example for demonstrating that life form sensu Pascher (1918) is not suitable for the natural grouping of algae. Two different life forms in this family, the filamentous (*Sphaeroplea*) and the coccoid (*Ankyra*) green algae cluster together (Fig. 1). *Sphaeroplea* is a filamentous green algal genus with multinucleate (coenocytic) cells (Buchheim et al., 2001). Its propagation is by asexual division of the cells in the unbranched filaments and oogamous sexual reproduction. *Actractomorpha* produces extremely long needle-shaped solitary cells that are coenocytic in character and can propagate asexually by zoospores and sexually by anisogamy or seldom by oogamy (Hoffman, 1983). Normally, this alga occurs mostly in soil. However, it has also been observed in freshwaters (Schmidt & Fehér, 1999–2000). Freshwater forms are not always correctly designated (e.g., as *Closteriopsis longissima* f. *gigantea* Heynig, 1980). Such planktonic cells can grow to lengths of 1,000–1,900 µm. The most frequently observed members of Sphaeropleaceae in the plankton are species of *Ankyra*, which produce spindle-shaped heteropolar cells with an anchor (Reymond & Hegewald, 1988). Species of *Ankyra* dominate in the clear water stages of stagnant waters (Barone & Naselli Flores, 1994). Although *Ankyra* mainly propagate asexually by zoospores, propagation in the genus needs more detailed investigation. Several types of unidentified aplanospores or resting stages have been observed in *Ankyra* cultures and field samples (Fott, 1971; Krienitz & Heynig, 1982).

Other clades

The *Mychonastes*-clade contains tiny, mostly spherical or oval cells of small size that occur as solitary cells or in colonies previously considered as two separate genera, *Mychonastes* and *Pseudodictyosphaerium*. Members of these genera belong to the most common pico- or small nanoplankton green algae in fresh or brackish waters. Molecular phylogenetic analyses have shown that both genera are mixed in the same clade (Krienitz et al., 1999, 2011a). Most species were previously described under the generic name of *Pseudodictyosphaerium* (Hindák, 1978a, b, 1988). However, since the genus *Mychonastes* was described four month earlier in 1978 (Simpson & Van Valkenburg, 1978), it therefore has nomenclatural priority. Consequently, the species of *Pseudodictyosphaerium* have been transferred to *Mychonastes*.

The *Bracteacoccus*-clade comprises mainly of soil algae. However, *Planktosphaeria gelatinosa*, which is a freshwater plankton commonly present, has been found to be a close relative of *Bracteacoccus* and *Radiococcus* (Wolf et al., 2003b). Unfortunately, this morphotype has been under-represented in recent taxon samplings for molecular considerations and needs further investigation. The edaphic "*Mychonastes*" *zofingiensis* does not belong to the true *Mychonastes* genus, and it is very likely that a new generic

Table 3 Genera of Scenedesmaceae confirmed by 18S rRNA or ITS gene phylogeny and their main diacritic morphological characteristics under LM and TEM

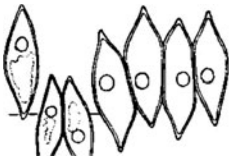
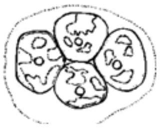
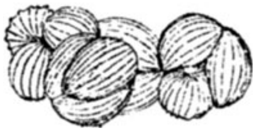
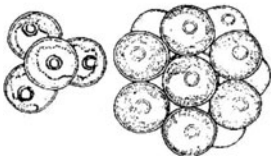
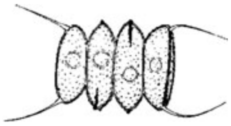
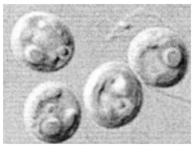
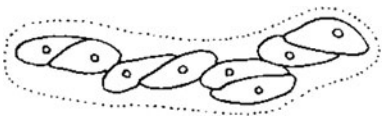
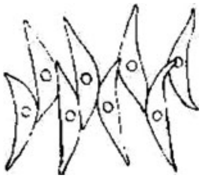
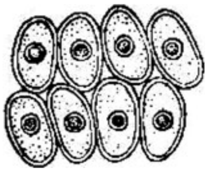
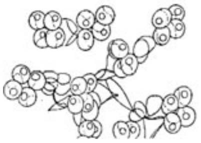
Genus	Drawing	Main diacritic morphology
Acutodesmus		Cells spindle-shaped with acute poles, without spines, in flat or curved coenobia or solitary, TEM: cell wall without or with few weak ridges
Asterarcys		Cells irregular ovoid, in 4- or 8-celled coenobia or solitary, chloroplast net-shaped
Coelastrella		Cells spherical or ellipsoid, in 2- or 4-celled groups, cell wall with meridional ribs
Coelastrum		Cells spherical or broad ellipsoidal, in spherical coenobia, cell wall smooth or rugose, no mucilage
Comasiella		Cells bean-shaped in flat, slightly curved coenobia, cell wall smooth
Desmodesmus		Cells cylindric, in flat coenobia, often with special cell wall ornamentations on an fourth outer layer, rosettes, tubes, warts, teeth, ribs, and spines
Dimorphococcus		Bean- or semilunate-shaped cells connected in syncoenobia by mucilaginous strands
Hariotina		Cells spherical, in large spherical coenobia connected by long cell wall extensions, mucilage
Hylodesmus		Cells spherical or oval, solitary, TEM: few delicate ribs
Neodesmus		Cells spindle- to drop-shaped, in 2-celled coenobia, which are connected in string-like of syncoenobia

Table 3 continued

Genus	Drawing	Main diacritic morphology
Pectinodesmus		Cells spindle-shaped, in flat or curved coenobia, TEM: cell wall with strong longitudinal ridges
Scenedesmus		Cells oval or cylindrical with obtuse or truncate poles, without spines, in flat or slightly curved coenobia, cell wall smooth
Westella		Cells spherical to ovoid, in 4-celled square-shaped coenobia, which are connected to syncoenobia

Drawings after Komárek & Fott (1983) and Krienitz (1990). Micrograph of *Hylodesmus* after Eliáš et al. (2010)

designation will be necessary for this taxon (Krienitz et al., 2011a).

The *Golenkinia*-clade is a member of the Chlorococcales *s.str.* (Fig. 1). The morphological and ontogenetic peculiarities, especially the CW basal body orientation shown by Hegewald & Schnepf (1984), have been supported by molecular analyses (Wolf et al., 2003a). Members of the polyphyletic genus *Chlorococcum* mostly occur in soils. However, after a heavy downpour, they can be washed out and transported into water bodies where they are able to propagate as observed in the case of *Chlorococcum robustum* (Krienitz et al., 1997).

Trebouxiophyceae

Chlorellaceae

This clade contains the classical "green balls." Following the description of the archetypical form of coccoid green algae, *Chlorella vulgaris* by Beijerinck (1890), more than 100 species of this genus have been described from freshwater, marine, and soil habitats. However, most of them need to be revised and transferred to other genera and other families. It has been shown that *Chlorella*-like green spheres evolved independently in different evolutionary lineages of Chlorophyceae, Trebouxiophyceae, and prasinophytes (Friedl, 1997; Chapman et al., 1998; Pröschold & Leliaert, 2007; Darienko et al., 2010). Based on the examination of biochemical and molecular data, Huss et al. (1999) have reduced the *Chlorella* genus to four species.

In the year 2000, a new development followed the work of Hegewald & Hanagata (2000) who found out that the coenobial ellipsoid *D. acuatus*, formerly classified in Scenedesmaceae, was closely related to *Chlorella kessleri*. The needle-shaped *C. acicularis*, traditionally considered as member of Selenastraceae, was also found to cluster in this lineage (Ustinova et al., 2001). *Actinastrum hantzschii*, a coenobial taxon formerly in the Coelastraceae, has also been transferred to Chlorellaceae (Wolf et al., 2002b). Krienitz et al. (2004) have identified two separate lineages within Chlorellaceae, designated as a *Chlorella*-clade and a *Parachlorella*-clade. Presently, these two clades have both old and new genera (Table 5; Figs. 2, 3).

The *Chlorella*-clade has eight different lineages that form clusters designated as genera. The scope of this genus has been extended to include 14 species. Among these species are solitary cells with or without mucilage and colonial forms that exhibit a morphology resembling *Dictyosphaerium* (Bock et al., 2011a). *Chlorella*-species occur in freshwater, soil and as endosymbionts (Pröschold et al., 2011). Fawley et al. (2005b) found *Meyerella*, a tiny sphere without pyrenoid, within the *Chlorella*-clade. According to

Table 4 Genera of Hydrodictyaceae confirmed by 18S or 26S rRNA gene phylogeny and their main diacritic morphological characteristics

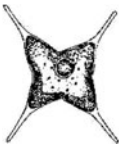
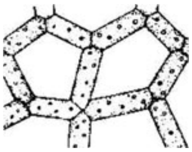
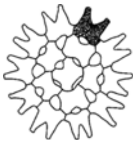
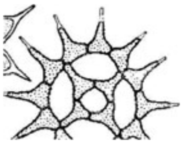
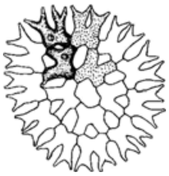
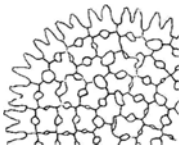
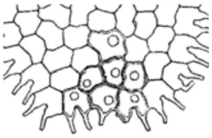
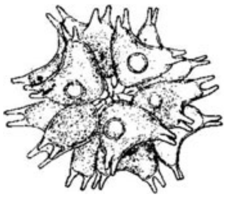
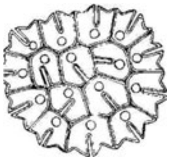
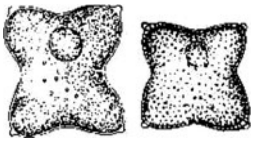
Genus	Drawing	Main diacritic morphology
Chlorotetraedron		Cells tetrahedral or polyhedral, with elongated cell wall protuberances at the corners, solitary
Hydrodictyon		Cells cylindrical in macroscopic net-like, one-layered coenobia
Lacunastrum		Flat coenobia with large intercellular spaces, marginal cells with two lobes
Monactinus		Flat coenobia with large intercellular spaces, marginal cells with one tapering lobe
Parapediastrum		Flat coenobia with intercellular spaces, marginal cells with 2 lobes each divided into 2 projections
Pediastrum		Flat coenobia with large intercellular spaces, marginal cells with 2 projections
Pseudopediastrum		Flat coenobia without intercellular spaces, marginal cells with two tapering lobes in one plane
Sorastrum		Three-dimensional coenobia, cells with 2 or 4 projections
Stauridium		Flat coenobia without intercellular spaces, marginal cells incised trapezoid or with projections in 2 planes

Table 4 continued

Genus	Drawing	Main diacritic morphology
Tetraedron		Cells flat or twisted, 3-, 4- or 5-sided, with rounded or elongated or spined corners, solitary

Drawings after Komárek & Fott (1983) and McManus et al. (2011)

Luo et al. (2010), the genera *Actinastrum*, *Didymogenes*, *Hegewaldia* and *Micractinium* also belong to this clade. *Hegewaldia* comprises taxa with facultative bristle production and oogamy (Pröschold et al., 2010). *Micractinium* usually occur in colonies and produce bristles. However, the colonies can disintegrate to form single cells without bristles. The formation of bristles can be triggered by substances produced by grazers such as the rotifer *Brachionus* (Luo et al., 2006). In addition to the several species of *Chlorella*, the *Chlorella*-clade has two other lineages with a colonial morphology similar to that of *Dictyosphaerium*. The two lineages belong to the genera *Heynigia* and *Hindakia* (Bock et al., 2010).

Six genera occur in the *Parachlorella*-clade. The genus *Parachlorella* has three species that are solitary or colonial, and covered by a mucilaginous envelope (Krienitz et al., 2004; Bock et al., 2011b). According to Krienitz et al. (2010), the *Dictyosphaerium*-morphotype evolved independently in different lineages of Chlorellaceae. The scope of the genus *Dictyosphaerium* has been reduced to three species, the type species *D. ehrenbergianum* (Bock et al., 2011c) and two new taxa. The genus *Mucidosphaerium* was established based on differences in its molecular phylogeny from that of *Dictyosphaerium*. *Mucidosphaerium* contains two former *Dictyosphaerium*-species, *D. pulchellum*, and *D. sphagnale*, as well as two new species. The taxa of the genera *Dictyosphaerium* and *Mucidosphaerium* are of great importance as they play an important role of establishing plankton communities. The spindle- to-needle-shaped genera *Closteriopsis* and *Dicloster*, as well as the marine, spherical *Marinichlorella* also belong to the *Parachlorella*-clade (Aslam et al., 2007).

Oocystaceae

The family of Oocystaceae is a natural lineage in Trebouxiophyceae confirmed by ultrastructural and molecular criteria. Many members of this group are very common in the plankton of stagnant and flowing waters. The extended cell wall is multilayered and constructed from crystalline cellulose fibers. Molecular phylogenetic data have revealed the monophyly of Oocystaceae. However, the species concept in this group has not been confirmed (Hepperle et al., 2000; Pažoutová et al., 2010; Krienitz & Bock, 2011). Because of the reduced taxon availability in strain collections and remarkable uncertainties on the concept of the type genus *Oocystis*, the circumscription of the genera of Oocystaceae has remained obscure. Hindák (1988) re-applied Lemmermanns (1903) criteria for distinguishing between *Oocystis* (without pyrenoids) and *Oocystella* (with pyrenoids). Hence, the taxonomic relevance of pyrenoids in Oocystaceae must be resolved. Several genera are characterized by incrustations (*Amphikrikos*, *Granulocystis*, *Granulocystopsis*, and *Siderocelis*); however, their origins and taxonomical values are still the subject of ongoing discussions.

Other clades

Choricystis and *Botryococcus*. *Choricystis*-species exhibit tiny bean-shaped, solitary cells and were traditionally assigned to the Chlorophyceae family. However molecular data have revealed that they have a close affiliation to the Trebouxiophyceae (Krienitz et al., 1996a, 1999). Surprisingly, the colonial and oil-producing alga *Botryococcus braunii* clusters close to *Choricystis* (Senousy et al., 2004). Komárek & Marvan (1992) established a multitude of *Botryococcus* species based on morphology. However, according to Plain et al. (1993) the morphological features of *B. braunii* vary depending on growth conditions hence it is not reliable to define more species. The figures provided by Plain et al. (1993) were exclusively those of *B. braunii*. This study did not include any of the new

Table 5 Genera of Chlorellaceae confirmed by combined 18S rRNA and ITS gene phylogeny and their main diacritic morphological characteristics

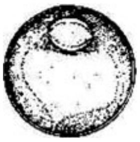
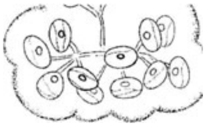
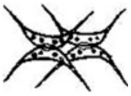
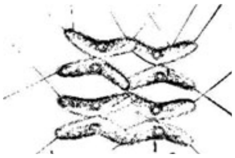
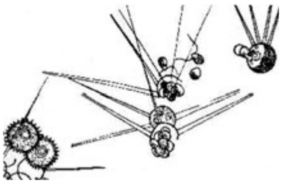
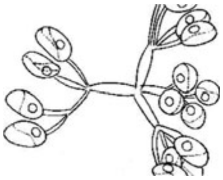
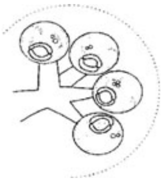
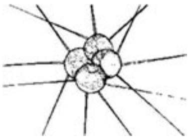
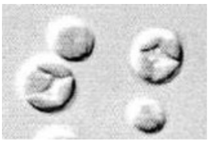
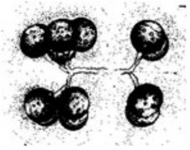
Genus	Drawing	Main diacritic morphology
Actinastrum		Cells rod-shaped, elongated, radially arranged in coenobia
Chlorella		Spherical or broad oval cells, with one pyrenoid, solitary or in mucilage covered colonies
Closteriopsis		Needle-shaped cells with several pyrenoids in the lateral chloroplast, solitary
Dictyosphaerium		Broad oval cells interconnected by strands attaching the elongated cell side, in mucilaginous colonies
Dicloster		Cells ellipsoidal arcuated with convex sides attached in 2- or 4-celled coenobia, 1 or 2 pyrenoids
Didymogenes		Cells cylindrical curved with convex side attached in 2-, 4- or 16-celled coenobia, one pyrenoid, spines
Hegewaldia		Spherical cells with or without bristles, solitary or in colonies, 1 pyrenoid, facultative oogamy
Hindakia		Broad oval cells interconnected by strands attaching the apical pole, in mucilaginous colonies, 1 pyrenoid
Heynigia		Spherical cells interconnected by mucilaginous strands in mucilaginous colonies, 1 pyrenoid

Table 5 continued

Genus	Drawing	Main diacritic morphology
Micractinium		Spherical cells with long bristles, in colonies, 1 pyrenoid
Meyerella		Cells short cylindrical, small, without mucilage, solitary, pyrenoid missing
Mucidosphaerium		Spherical cells interconnected by mucilaginous strands in mucilaginous colonies, 1 pyrenoid
Parachlorella		Spherical cells covered by mucilage, solitary or in groups, 1 pyrenoid

Drawings after Komárek & Fott (1983), Krienitz, et al. (2004), and Bock et al. (2010). Micrograph of *Meyerella* after Fawley et al. (2005b)

species described by Komárek & Marvan (1992) such as *B. terribilis* that has been shown to be very common in freshwater phytoplankton of diverse climatic regions (Krienitz et al., 1996b; Ballot et al., 2009; Fanés Treviño et al., 2009). The conversion of the *braunii*-morphotype into the *terribilis*-morphotype and vice versa was never demonstrated. It is therefore, important to as a matter of urgency include more *Botryococcus*-taxa into molecular studies. From an ecological point of view, the alliance of the picoplanktonic, invasive *Choricystis*, and the microplanktonic, attuning *Botryococcus* shown in Fig. 2, is not explainable. Padisák et al. (2009, 2010) grouped *Choricystis* into the functional group X1 and *Botryococcus* in group F. Later considerations will probably reveal the existence of some undiscovered lineages that have evolved between these two clades. Henley et al. (2004) documented a close relationship between *C. minor* and *Paradoxia multiseta* based on 18S rRNA phylogeny. However, according to the micrograph given (showing globular cells) in the catalogue of the UTEX strain collection, the identity of the *Paradoxia* strain investigated is doubtful.

Between the *Choricystis*-clade and the Chlorellaceae, several clades of green coccoid soil and aerophytic algae, such as *Chloroidium*, *Watanabea*, *Dictyochloris*, and the lichen symbionts of Trebouxiales have evolved (Friedl, 1995; Darienko et al., 2010; Rindi et al., 2010). These algae occasionally occur in the plankton of freshwaters. However, if a sufficient starting inoculum of these air- and soil-born algae is available and if the algae are able to propagate under the new conditions, then colonization of standing water bodies can occur rapidly (Happy-Wood, 1988).

Evolution of morphological peculiarities of coccoid green algae and their possible ecological functions

One of the most striking peculiarities of numerous coccoid green algae is the development of a uniform morphology hidden in the picoplankton size group which is nearly unidentifiable by LM. Convergent evolution resulted in a tiny, more or less spherical morphotype which covers an extremely high diversity with regard to phylogeny and physiology (Potter et al., 1997; Hepperle & Krienitz, 2001; Krienitz et al. 2011a). Based on its fast growth and high rates of reproduction and primary production, picophytoplankton can play a key role in food webs of freshwater, marine, and saline habitats (Stockner & Antia, 1986; Raven, 1999). Picoplankton may establish large populations under nearly all levels of trophy (Stockner, 1991; Weisse, 1993; Padisák et al., 1997; Hehmann et al., 2001; Hepperle & Krienitz, 2001). Even under very saline conditions they can dominate all the succession stages of eukaryotic primary producers (Henley et al., 2004; Somogyi et al., 2011; Krienitz et al., 2012).

Picoplanktons evolved as adaptive strategies in all the classes considered here. In the Chlorophyceae, the *Mychonastes*-clade contains several species of picoplankton (Krienitz et al., 2011a). In the Trebouxiophyceae, a number of lineages, which include *Picochloron* and *Chloroparva* from salt pans (Henley et al., 2004; Somogyi et al., 2011), *Choricystis*, and *Nannochloris* from freshwaters (Krienitz et al., 1996a; Yamamoto et al., 2003), have picoplankton species. *Marvania* is a picoplankton species from different inland waters characterized by vegetative propagation through budding (Hindák, 1976; Reymond et al., 1986). Among the prasinophytes, there are many picoplankton lineages which are usually abundant in the oceans (Guillou et al., 2004; Leliaert et al. 2012). Extreme saline inland waters have one lineage with *Picocystis* (Lewin et al., 2000; Hollibaugh et al., 2001; Roesler et al., 2002). *Picocystis salinarum* from saline inland waters represents a link between picoplankton from marine and freshwater habitats and is therefore of great ecological and phylogenetic interest (Krienitz & Kotut, 2010; Krienitz et al., 2012).

Comparison of the fatty acid's contents of green (*Choricystis*, *Mychonastes*) and eustigmatophycean (*Nannochloropsis*) freshwater picoplankton revealed surprising differences in the composition of polyunsaturated fatty acids. The sums of n-6 and n-3 fatty acids are ten times higher in *Nannochloropsis limnetica* than in the green picoplankton (Krienitz et al., 2000; Krienitz & Wirth, 2006). Hence, the eustigmatophycean picoplankton has a higher nutritional value for grazers than green algal picoplankton. This raises the question on how the grazers can differentiate between the nutritious eustigmatophycean and the less nutritious green algae. As a follow-up to Hartmann & Kunkel's (1991) statement "The paradigm of invariate, nonselective feeding by zooplankton is rejected," limnologists have come up with possible mechanism of food selection that include: chemosensory, electrical charge, surface hydrophobicity, and chemical cues (Weisse, 2004). All these interactions between green algae and possible grazers need to be further elucidated.

Incrustations on the surface of coccoid green algae are common across the whole range of systematic groups of green algae. For example, they have been observed in Selenastraceae (*Raphidocelis*), Scenedesmaceae (*Scenedesmus*), Oocystaceae (*Amphikrikos*, *Siderocelis*), and Radiococcaceae (*Coenochloris*) (Hindák, 1977; Crawford, 1978; Krienitz, 1986; Vanormelingen et al., 2007). These incrustations are precipitates of ferric and manganic hydroxides and are of crystalline or amorphous nature (Crawford & Heap, 1978). The genesis and structure of this cell wall deposits varies with taxon suggesting a genetic influence and give the impression that this process is under the control of the cell (Crawford & Heap, 1978). So far, no vesicle driven expression through pores from inner to outer cell wall has been observed. The ecological function of these incrustations remains a subject of discussion. From our field observation, it was apparent that numerous algae tend to develop incrustations under highly disturbed conditions in rivers (Krienitz, 1990, 1998). It can therefore be hypothesized that the incrustations increase the weight of the cells, and this allows the cells to sink to the lower less disturbed habitats, which are conducive for propagation.

Spines and bristles of the plankton algae are adaptive features that promote buoyancy and reduce grazing pressure (Van den Hoek et al., 1995). A large number of publications have documented the interaction of kairomon-producing grazers and spine- and bristle-formations by coccoid green algae (summarized by Van Donk, 2005). Spines and bristles have different origins and compositions. Scenedesmaceae produce rigid, tube-like spines as elaborated parts of the outer sporopollenin-like cell wall layer (Schnepf et al., 1980). In contrast to spines, bristles develop after cell wall formation and lack cellulosic fibers and algaenan substances (Schnepf et al., 1980; Hegewald & Schnepf, 1984). *Didymogenes* and *Micractinium* produce bristles of the same type (Schnepf & Hegewald, 1993). The spines of *Golenkinia* contain cellulosic fibers and are produced after cell wall formation (Hegewald & Schnepf, 1984). The morphological and biochemical differentiation of spines and bristles in the various groups of coccoid green algae closely agree with their differences based on molecular data. The spine producing Scenedesmaceae belong to the

Chlorophyceae (Hegewald, 1997). The close relationship between *Didymogenes* and *Micractinium* as members of Trebouxiophyceae was demonstrated by Luo et al. (2006). The polyphyletic origin of bristles within the Trebouxiophyceae was confirmed by Pröschold et al. (2010). The separate position of *Golenkinia* as a member of Chlorococcales *s.l.* was revealed by Wolf et al. (2003a).

The production of mucilage by the cells is one of the most readily observed phenomena in coccoid green algae. It is commonly observed in all groups under discussion and has a polyphyletic origin. The production of mucilage largely depends on environmental influences and interaction with other species such as grazing pressure and resource competition (Reynolds, 2007). It may protect the algae from ingestion or, even if ingested, against digestion while passing through the intestinal tract of zooplankton (Porter, 1973). On the other side, the mucilaginous envelope can act as microhabitat for bacterial flora that produces substances with a nutritional value or have stimulatory effects (Cole, 1982). The mucilage can also act as a depository of nutrients (Decho, 1990). Furthermore, mucilage affects the buoyancy of the phytoplankton (Boney, 1981). The multitude of ecological functions makes it understandable that morphotypes characterized by mucilage evolved in different lineages and at different times as a response to the diverse interactions. This observation has clearly been demonstrated in the *Dictyosphaerium*-morphotype (Bock et al., 2010, 2011c; Krienitz et al., 2010). Even in one and the same genus such as *Chlorella* or *Mychonastes* mucilage possession has appeared and disappeared several times (Bock et al., 2011a; Krienitz et al., 2011a). Hence, the question on which of the contrasting features, “with mucilage” or “without mucilage,” is more ancestral remains open.

A very complicated case is the elucidation of the origin of the radiococcacean morphotype. Kostikov et al. (2002) revised this “family” based on a detailed examination of morphology and ontogeny. However, a few molecular phylogenetic studies have indicated a polyphyletic evolution of members of this family (Wolf et al., 2003b; Bock et al., 2011b). The study of this group has been hampered by scarcity of cultures, because of their poor growth performance in culture. Future studies subjecting single colonies to PCR can help to shed some light on the phylogeny of this “slimy colonial green spheres.”

Coccoid green algae represent the most diverse group among plankton algae. It is one of the groups well suitable for cultivation and therefore provides a wide range of experimental opportunities to study the genesis and ecological advantages of these organisms. Based on autecological features and functional characteristics, Reynolds et al. (2002) and Padisák et al. (2009) have placed most of the coccoid green algae in codon **X1** (shallow mixed layers in enriched conditions), and some of them (*Botryococcus*, *Dictyosphaerium*, and *Oocystis*) in codon **F** (clear epilimnia), and others (*Coelastrum*, *Pediastrum*, and *Scenedesmus*) in codon **J** (shallow enriched lakes, ponds, and rivers). Recent observations on species characterisics that include drastic changes in size through colony disintegration, and the periodic appearance and disappearance of features, such as mucilage, incrustations, and spines depending on interactions with abiotic and biotic environments suggest that a species can possibly deviate from this ecofunctional classification.

The phylogenetic species concept

The debate on the “right” species concept is certainly old and ongoing. In the post-Darwinian time, numerous concepts were proposed with different criteria for species delineation (e.g., Mayr, 1942; Henning, 1966; de Queiroz, 1998). As the biological (or reproductive) species concept of reproductive isolation (Mayr, 1942) is not applicable to asexually reproducing taxa (which is the case for many protists lineages, and especially the coccoid green algae), and the morphological species concept is very subjective, the phylogenetic species concept (or *diagnostic* concept sensu Mallet (2006)) has gained a lot of ground (Cracraft, 1989). This concept is based on genetic markers and recognizes the smallest monophyletic clusters of taxa worthy of taxonomic recognition as individual species. The populations/strains must share the same ancestor and all its descendants to be considered as individual species (Johansen & Casamatta, 2005; Mallet, 2006). This concept allows the delimitation of species by specific data like base changes in gene sequences. Hence, the number of recognized species largely depends on the chosen marker; the more conserved the analyzed region, the fewer the number of species recognized and vice versa (Hoef-Emden, 2007; Rindi et al., 2009). The same applies to the chosen threshold of genetic divergence between sequences. A higher threshold for the congruence of sequences (e.g., 98% congruence) results in more species recognized than a lower threshold (e.g., 94%). The small subunit (SSU) of the ribosomal rRNA (18S) gene sequence is conventionally used as marker in phylogenetic studies (Chapman et al., 1998; Huss et al., 1999). The 18S rRNA is a universal gene and plays a major role in protein translation. It contains highly conserved regions which favors the development of universal primers and simplifies sequence alignment of distant taxa (Long & David, 1980; Sogin et al., 1986). As it is present in numerous copies within the genome, it is easy to amplify during a PCR. These criteria makes the 18S rDNA a useful tool for the phylogenetic resolution of higher algal ranks such as classes and orders (Friedl, 1995; Krienitz et al., 2003, 2011a). Exhaustive studies combining molecular and morphological data have shown that the 18S is too conserved to separate closely related species in coccoid green algal lineages that are clearly distinguishable by morphological and/or ecological factors (e.g., Krienitz et al., 2004; Bock et al., 2010; Darienko et al., 2010).

New insights and methodological opportunities in the molecular techniques have shifted the focus to the internal transcribed spacer 2 (ITS2). The ITS2 is a fast evolving marker region, situated between the 5.8S and the 28S rRNA on the ribosomal gene. To obtain a mature rRNA, the ITS2 needs to be excised during the maturing stage so that it folds up into a characteristic secondary structure. The primary sequence of the ITS2 is highly variable, even between closely related taxa. However, the ITS2 secondary structure motive seems to be extremely conserved in all eukaryotes (Mai & Coleman, 1997; Coleman, 2003, 2007; Schultz et al., 2005). Indeed the ITS2 folds normally in four helices, with helix III being the longest and the most conserved (Coleman, 2007). Due to this specific folding pattern, it is possible to align closely related sequences unambiguously.

The Compensatory Base pair Changes (CBC) concept refers to base changes within the secondary structure where a matching pair of bases in a double stranded section of the structure in one taxon is exchanged by a different matching pair in a second taxon (Gutell, 1994). Mating experiments in sexual protist lineages in combination with CBCs within the ITS2 have pushed the discussion toward concepts merging Mayr's biological species concept with molecular data (Coleman, 2000; Denboh et al., 2003; Behnke et al., 2004; Hoef-Emden, 2007). In protist lineages with at least occasional sexual reproduction, the occurrence of CBCs in conserved regions of the ITS coincides with the sexual incompatibility between species (Mai & Coleman, 1997; Müller et al., 2007; Coleman, 2009). As a consequence, the presence of CBCs or hemi-CBCs (only one-sided base changes) is often used for species delineation in morphological difficult groups or when only asexual reproduction is known (Krienitz et al., 2004; Hoef-Emden, 2007). Additionally, the CBC concept includes the step of calculating the secondary structure. Software for secondary structure prediction can help to facilitate the procedure but are not reliable if the entire ITS2 sequence is directly submitted to a RNA folding server. The sequences have to be submitted in small pieces which correspond to the different expected helices to get consistent results (Zuker, 2003; Hoef-Emden, 2007; Schultz & Wolf, 2009).

Apart from the nuclear regions (18S, ITS, 28S), a variety of markers have been used for species delineation. Among these markers is the chloroplast encoded large subunit of the ribulose-bisphosphate carboxylase (*rbcL*) gene. This protein coding region results in a straightforward alignment and tends to be more variable than the 18S but not as variable as the ITS regions. Another advantage of this marker over the nuclear markers is that since it is a plastid gene, the risk of amplifying contaminants like fungi is reduced. As a protein-coding gene, the *rbcL* sequence can be partitioned into codons with first, the second, and the third base positions. The third base (wobble) position has a higher evolution rate than the first or the second base positions because of its substitutional saturation (Rindi et al., 2009). However, the usually applied *rbcL* sequence has been criticized because it is not variable enough for the different substitution rates for the wobble position may cause problems within the phylogeny. Several other markers have been tested on closely related algae in an effort to separate species but none has proved to be exhaustive for a large number of groups. Examples are the trnG^{ucc} intron sequences for Desmidiales (Neustupa et al., 2010; Nemjova et al., 2011), actin I locus sequences for *Asterochloris* (Škaloud & Peksa, 2010); the *psbA*/*rbcL* spacer in combination with the *rbcL* gene for the *Tribonemataceae* (Rybalka et al., 2009) and many more.

The recent discussion proposing the use of a “barcode” to facilitate the identification of taxa has resulted in the recognition of the need to identify a specific DNA region for species delineation again (Hebert et al., 2003). The ideal barcode should be a short sequence, easy to amplify by universal primers and with the power to resolve organisms at species level (e.g., Hebert et al., 2003; Zimmermann et al., 2011). Under this context, various barcodes have been proposed for different organisms. For animals, part of the mitochondrial cytochrome oxidase c subunit I gene (COI, cox1) has been proposed and is now widely used (Hajibabaei et al., 2007). Evans et al. (2007) have proposed the cox1 as barcode for diatoms. The V4 subregion on the 18S rRNA gene was proposed by Zimmermann et al. (2011) for diatoms. Moniz & Kaczmarska (2010) have suggested use of the 5.8S and part of the ITS2 for diatoms, and the same was also successfully tested on *Chlorella*-related strains (Bock et al., 2011a). The ITS2 is favored by many scientists because of its variability combined with the conserved folding pattern (Buchheim et al., 2011). As the ribosomal operon may contain several versions of ITS2, indels of numerous nucleotides may be present in the less-conserved parts of the different ITS2 versions, which prevents direct sequencing and requires cloning or the use of specific primers (Pröschold et al., 2005). Hence, the establishment of a barcode conception for algae is of great practical importance. Unambiguous designation of species is essential for water quality studies. Barcodes can be used for the identification of standard organisms because morphological concepts often fail to provide accurate identification of algae (Zimmermann et al., 2011). It is especially important to identify barcodes for indicator species similar to the functional group conception established by Reynolds et al. (2002). There are many other indices in different parts of the world. However, it is important that whichever index is used, it should give a correct species identification.

The role of the field workers and experts of phenotypic and ecological characterization of algae

In the assessment of the quality of inland waters, phytoplankton community structure provides a useful indicator tool (Salmaso et al., 2006; Pearl et al., 2007). This is due to the following important attributes:

- being the main pelagic primary producers the phytoplankton play a key role in the functioning of standing water bodies,
- as a consequence of its high reproduction rates, phytoplankton gives a rapid response to changes in environmental conditions,
- phytoplankton communities are generally more diverse than other eukaryotic populations within aquatic food webs,
- species composition of the phytoplankton community, i.e., its biodiversity, has a critical influence on many kinds of water-utilization by man.

Hence phytoplanktologists worldwide contribute to the establishment of water quality assessment methods. In Europe, the Water Framework Directive is the key approach to the evaluation and protection of the inland surface waters (Padisák et al., 2006; Anneville et al., 2008). To support the practical field work, harmonized taxon lists have been established. For the phytoplankton, such a list was developed by Mischke (2006) and Mischke & Nixdorf (2008). About 300 taxa of coccoid green algae are included in this list. However, the list is under continuous improvement, and there are initiatives to assist the field-workers with information systems on taxonomy of algae such as AlgaTerra (Jahn & Kusber, 2006), and AlgaeBase (Guiry & Guiry, 2011). The value of ecological data is to a large extent dependent on the correct identification of organisms as any incorrectly identified samples cannot be improved by any statistical treatment or other sophisticated methods (Kotut & Krienitz, 2011). In this paper (Table 6), we have included 84 coccoid green algae from the harmonized taxon list, which have already been subjected to molecular phylogenetic examinations, and provided their old and new taxonomic designations. It has become evident that many taxa are still missing, and joint research activities are necessary to facilitate the acquisition of more cultures from field samples that can be included in the morphological, ecophysiological, and molecular analyses.

There is no doubt that there are difficulties in the microscopic identification of many species of coccoid green algae. Different phylogenetic species can be hidden under one and the same morphotype (Potter et al., 1997; Šlapeta et al., 2005). On the other hand,

Table 6 Coccoid green algae from the harmonized taxon list of the European Water Framework Directive, subjected to molecular phylogenetic examination, and their old, revised, or confirmed designations

Old designation	Revised or confirmed designation	Reference
*Actinastrum hantzschii**	*Actinastrum hantzschii** Lagerheim	Wolf et al. (2002b)
Amphikrikos sp.	*Amphikrikos* sp.	Hepperle et al. (2000)
Ankistrodesmus bibraianus	*Selenastrum bibraianum** Reinsch	Krienitz et al. (2011b)
Ankistrodesmus fusiformis	*Ankistrodesmus fusiformis* Corda	Krienitz et al. (2001)
Ankistrodesmus gracilis	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Ankistrodesmus nannoselene	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Ankistrodesmus stipitatus	*Ankistrodesmus stipitatus* (Chodat) Komárková-Legnerová	Krienitz et al. (2001)
Ankyra judayi	*Ankyra judayi* (G.M. Smith) Fott	Wolf et al. (2002a)
Ankyra lanceolata	*Ankyra lanceolata* (Korshikov) Fott	Wolf et al. (2002a)
*Botryococcus braunii**	*Botryococcus braunii** Kützing	Senousy et al. (2004)
Chlorella ellipsoidea	*Chloroidium ellipsoideum* (Gerneck) Darienko et al.	Darienko et al. (2010)
Chlorella minutissima	*Mychonastes homosphaera* (Skuja) Kalina & Punčochářová	Krienitz et al. (2011a)
Chlorella pyrenoidosa	*Pseudochlorella pyrenoidosa* (Zeitler) Lund	Darienko et al. (2010)
*Chlorella vulgaris**	*Chlorella vulgaris** Beijerinck	Huss et al. (1999)
Chlorotetraedron incus	*Chlorotetraedron incus* (Teiling) Komárek & Kováčik	Hegewald et al. (2001)
*Choricystis minor**	*Choricystis minor** (Skuja) Fott	Krienitz et al. (1996a, b)
Closteriopsis acicularis	*Closteriopsis acicularis* (G.M. Smith) Belcher & Swale	Ustinova et al. (2001)
Coelastrum astroideum	*Coelastrum astroideum* De Notaris	Hegewald et al. (2010)
Coelastrum microporum	*Coelastrum microporum* Nägeli	Hegewald et al. (2010)
Coelastrum morum	*Coelastrum morum* W. et G.S. West	Hegewald et al. (2010)
Coelastrum pseudomicroporum	*Coelastrum pseudomicroporum* Korshikov	Hegewald et al. (2010)
Coelastrum reticulatum	*Hariotina reticulata** Dangeard	Hegewald et al. (2010)
*Coelastrum sphaericum**	*Coelastrum sphaericum** Nägeli	Hegewald et al. (2010)
Coenochloris hindakii	*Parachlorella hussii* C. Bock, Pažoutová & Krienitz	Bock et al. (2011c)
*Coenochloris polycocca**	*Radiococcus polycoccus* (Korshikov) Kostikov et al.	Wolf et al. (2003b)
Coronastrum ellipsoideum	To be included in a new genus not yet designated	Bock et al. (2011c)
Crucigeniella rectangularis	To be included in a new genus not yet designated	Krienitz et al. (2003)
Dictyosphaerium chlorelloides	*Chlorella chlorelloides* (Naumann) C. Bock, Krienitz & Pröschold	Bock et al. (2011a)
Dictyosphaerium ehrenbergianum	*Dictyosphaerium ehrenbergianum* Nägeli	Krienitz et al. (2010)
Dictyosphaerium pulchellum	*Mucidosphaerium pulchellum* (Wood) C. Bock, Pröschold & Krienitz	Bock et al. (2011b)
Dictyosphaerium tetrachotomum	*Hindakia tetrachotoma* (Printz) C. Bock, Pröschold & Krienitz	Bock et al. (2010)
Didymocystis inermis	*Didymocystis inermis* (Fott) Fott	An et al. (1999)
*Didymogenes palatina**	*Didymogenes palatina** Schmidle	Luo et al. (2010)
*Golenkinia radiata**	*Golenkinia radiata** Chodat	Wolf et al. (2003a)
Kirchneriella aperta	*Kirchneriella aperta* Teiling	Krienitz et al. (2001)
*Kirchneriella obesa**	*Kirchneriella obesa** (W. West) Schmidle	Krienitz et al. (2011b)
Kirchneriella dianae	*Kirchneriella dianae* (Bohlin) Comas	Krienitz et al. (2011b)
Kirchneriella subcapitata	*Raphidocelis subcapitata* (Korshikov) Nygaard et al.	Krienitz et al. (2011b)
*Lagerheimia genevensis**	*Lagerheimia genevensis** (Chodat) Chodat	Krienitz et al. (2003)
*Micractinium pusillum**	*Micractinium pusillum** Fresenius	Luo et al. (2006)
Monoraphidium contortum	*Monoraphidium contortum* (Thuret) Komárková-Legnerová	Krienitz et al. (2011b)

Table 6 continued

Old designation	Revised or confirmed designation	Reference
Monoraphidium convolutum	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Monoraphidium dybowskii	To be included in a new genus not yet designated	Krienitz et al. (2011b)
*Monoraphidium griffithii**	*Monoraphidium griffithii** (Berkeley) Komárková-Legnerová	Krienitz et al. (2001)
Monoraphidium minutum	*Nephrochlamys subsolitaria* (G.S. West) Korshikov	Krienitz et al. (2011b)
Monoraphidium pusillum	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Neodesmus danubialis	*Neodesmus danubialis* Hindák	Hegewald & Hanagata (2000)
Nephrochlamys subsolitaria	*Nephrochlamys subsolitaria* G.S. West	Krienitz et al. (2011b)
Oocystis marssonii	*Oocystis marssonii* Lemmermann	Hepperle et al. (2000)
Oocystis solitaria	To be included in a new genus not yet designated	Hepperle et al. (2000)
*Paradoxia multiseta**	*Paradoxia multiseta** Svirenko	Henley et al. (2004)
Pediastrum biradiatum	*Parapediastrum biradiatum* (Meyen) E. Hegewald	Buchheim et al. (2005)
Pediastrum boryanum	*Pseudopediastrum boryanum* (Turpin) E. Hegewald	Buchheim et al. (2005)
*Pediastrum duplex**	*Pediastrum duplex** Meyen	Buchheim et al. (2005)
Pediastrum kawraiskyi	*Pseudopediastrum kawraiskyi* (Schmidle) E. Hegewald	Buchheim et al. (2005)
Pediastrum simplex	*Monactinus simplex* (Meyen) Corda	Buchheim et al. (2005)
Pediastrum tetras	*Stauridium tetras* (Ehrenberg) E. Hegewald	Buchheim et al. (2005)
*Planktosphaeria gelatinosa**	*Planktosphaeria gelatinosa** G.M. Smith	Wolf et al. (2003b)
*Polyedriopsis spinulosa**	*Polyedriopsis spinulosa** (Schmidle) Schmidle	Hegewald et al. (2001)
Pseudococcomyxa simplex	*Coccomyxa* sp.	Pröschold et al. (2011)
Pseudodictyosphaerium jurisii	*Mychonastes jurisii* (Hindák) Krienitz et al.	Krienitz et al. (2011a)
Quadricoccus ellipticus	*Quadricoccus ellipticus* Hortobágyi	Krienitz & Bock (2011)
*Quadrigula closterioides**	*Quadrigula closterioides** (Bohlin) Printz	Krienitz et al. (2001)
Scenedesmus arcuatus	*Comasiella arcuata** (Lemmermann) E. Hegewald et al.	Hegewald et al. (2010)
Scenedesmus acuminatus	*Acutodesmus acuminatus* (Lagerheim) Tsarenko	Hegewald & Wolf (2003)
Scenedesmus armatus	*Desmodesmus armatus* (Chodat) E. Hegewald	Hegewald et al. (2010)
Scenedesmus arthrodesmiformis	*Desmodesmus arthrodesmiformis* (Schröder) An, Friedl & E. Hegewald	An et al. (1999)
Scenedesmus costato-granulatus	*Desmodesmus costato-granulatus* (Skuja) E. Hegewald	Vanormelingen et al. (2007)
Scenedesmus denticulatus	*Desmodesmus denticulatus* (Lagerheim) An, Friedl & E. Hegewald	An et al. (1999)
Scenedesmus falcatus	*Pectinodesmus pectinatus** (Meyen) E. Hegewald et al.	Hegewald et al. (2010)
Scenedesmus obliquus	*Acutodesmus obliquus* (Turpin) Tsarenko	Hegewald & Wolf (2003)
Scenedesmus obtusus	*Scenedesmus obtusus* Meyen	Hegewald & Wolf (2003)
Scenedesmus opoliensis	*Desmodesmus opoliensis* (P. Richter) E. Hegewald	Hegewald et al. (2010)
Scenedesmus ovalternus	*Scenedesmus ovalternus* Chodat	Kessler et al. (1997)
Scenedesmus quadricauda	*Desmodesmus communis* (E. Hegewald) E. Hegewald	Kessler et al. (1997)
Scenedesmus serratus	*Desmodesmus serratus* (Corda) An, Friedl & E. Hegewald	An et al. (1999)
Scenedesmus subspicatus	*Desmodesmus subspicatus* (Chodat) E. Hegewald & Ant. Schmidt	Hegewald et al. (2005)
*Schroederia setigera**	*Schroederia setigera** (Schröder) Lemmermann	Buchheim et al. (2001)
*Tetrachlorella alternans**	*Tetrachlorella alternans** (G.M. Smith) Korshikov	Hepperle et al. (2000)
Tetraedron caudatum	*Tetraedron caudatum* (Corda) Hansgirg	Buchheim et al. (2005)
Tetraedron minimum	*Tetraedron minimum* (A. Braun) Hansgirg	Buchheim et al. (2005)

Table 6 continued

Old designation	Revised or confirmed designation	Reference
Treubaria setigera	*Treubaria setigera* (Archer) G.M. Smith	Buchheim et al. (2001)
Treubaria schmidlei	*Treubaria schmidlei* (Schröder) Fott & Kováčik	Buchheim et al. (2001)
*Westella botryoides**	*Westella botryoides** (W. West) De-Wildeman	Hegewald et al. (2010)

Species indicated by an asterisk are the type species of the genus. Species studied by molecular phylogeny however not designated to a genus are commented in column 2

one and the same genotype can exhibit different morphological peculiarities during its ontogeny or as result of interactions with the environment (Lürling & Beekman, 1999; Verschoor et al., 2004). Currently, molecular biologists are screening the contents of public strain collections (Day et al., 2004). However, these collections are not representative of the diversity of taxa in the field. Similarly, the designations of many taxa in culture collections are doubtful (Hegewald, 1989). Many algae are difficult to maintain in cultures and are not available for the sequencing work.

In the present situation, workers of both the types, the traditional phenotypic ones and those with a modern genotypic approach, should come together and forge interdisciplinary collaborations. Field limnologists have the privilege of working directly in ecosystems where evolution takes place. Organisms in their natural habitats are constantly interacting with each other and with their environment, while the natural selection process is acting on them. It is therefore so difficult to interpret the organismic structure of the ecosystem using the old phenotypic taxonomic units created by man using an artificial system. They do not adequately reflect how nature works. For ecologists, the genetic diversity within taxa is of major interest because of possible genetic shifts in populations as an adaptive response to a changing environment (Lynch et al., 1991; Wood & Leatham, 1992). Discussions on species concepts should in addition to the phenotype take into consideration intraspecific genetic variation. For species with well-studied phenotypic population characteristics from field observations, the description should be completed by genetic characteristics, especially for those cultured strains that conform to the original type description (Wood & Leatham, 1992). Limnologist should therefore support the efforts of molecular biologists to circumscribe phylogenetic species. Alongside this, large-scale ecophysiological experiments focusing on autecological features, physiological, and biochemical characteristics should be correlated to morphological variability of cultures of these phylospecies. Limnologists can also support an extensive taxon sampling. Through this exercise, they can advise on interesting algae, which need taxonomic revision and provide fresh samples, and isolate interesting strains for the public strain collections. On the other hand, molecular biologists have the basic tools for supporting the natural system of organisms. Their work should include developing methods for evaluating species composition in field (environmental) samples and comparing them with microscopic findings (Fawley et al., 2004; Richards et al., 2005; Medinger et al., 2010; Luo et al., 2011). A good example is the multiphased study of freshwater samples from México by Tavera & Diéz (2009).

In the near future, molecular tools may be used to identify individual filaments, colonies or cells picked from samples. It may also be possible to identify individuals directly within the samples. Promising results have already been published on cyanobacteria (Hayes et al., 2002), large sulfur bacteria (Salman et al., 2011), dinoflagellates (Ki & Han, 2005) and chrysophytes (Jost et al., 2010). Once the tools are fully developed, specialists with a good knowledge on morphospecies will have a good opportunity to align these individual phenotypes with the molecular findings (Auinger et al., 2008). However, presently we have to content with the use of two different and parallel systems. Although field biologists have no other option than to apply the traditional approach, they should be careful to provide very detailed documentation of their findings. The handbook by Komárek & Fott (1983) and the five volumes of Hindák's studies (1977, 1980, 1984, 1988, 1990) can be of great help in the determination of morphospecies of coccoid green algae. In the near future, the establishment of molecular signatures, the barcoding conception, will allow an unambiguous designation of organisms. The alignment of eco-functional groups of algae with true species identities using the barcoding conception will provide a better understanding of the interaction between organisms and their environment. The reasons why and how an individual species adapts to different environmental conditions by producing spines, mucilage or incrustations, or losing them, establishing colonies or disintegrating them, exhibiting striking colonial appearances or returning to the simple green ball habit as a survival tactic will become evident.

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