Süßwasserflora von Mitteleuropa
Freshwater Flora of Central Europe

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Chlorophyta: Ulvophyceae

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Chlorophyta

Ulvophyceae

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Editors' Preface

The precursor of the "Freshwater Flora of Central Europe" was founded by Alfred Pascher in 1913 under the title "Die Süßwasserflora Deutschlands, Österreichs und der Schweiz". The third edition of the "Freshwater Flora" was initiated in 1973 and currently includes 23 volumes. It is intended to serve as a reference work for the identification of lower and higher water plants under both field and culture conditions, with a special focus on algae and cyanobacteria. As the title implies, the series also considers bacteria, fungi and vascular macrophytes.

In recent years, molecular tools and studies on the subcellular level provided new insights into the true relationships of algae groups and underlined that the classification applied so far is artificial. Fundamental revisions of taxonomic groups were published in the last decades and these achievements need to be incorporated step by step into this compendium. Several practical reasons hampered this project. Within the 45 years of the current edition, a functional structure that turned out to be outdated was applied, especially for green algae. This necessitated a new outline of the volumes, which is presented below. This attempt considers both practical and phylogenetic aspects.

The current volume deals with a natural lineage of chlorophytes, the Ulvophyceae. This class has never before been treated in a taxonomic standard work in such a modern and comprehensive manner. We are very pleased that a team of enthusiastic authors – working actively on the frontline of phycology and representing the new generation of phycologists – spent their valuable time and knowledge to fill this major gap.

The Editors

Preface

This volume of the “Süßwasserflora von Mitteleuropa” (Freshwater Flora of Central Europe) covers the freshwater, aerophytic, and terrestrial species of Ulvophyceae. The class includes about 1700 species, and is one of the main classes of green algae (Chlorophyta). Although most of the species diversity of Ulvophyceae is found in the marine environment, a substantial number of species also occurs in brackish, freshwater, and aero-terrestrial habitats. These species are found in nine orders: Ulvales and Ulotrichales, which contain most freshwater species, the Trentepohliales which is exclusively aero-terrestrial, the smaller orders Chlorocystidales, Oltmannsiellopsidales, Scotinosphaerales, and Ignatiales, and the large but mainly marine orders Cladophorales and Bryopsidales. One other order, Dasycladales, only includes marine species and is thus not included in the present volume. Freshwater ulvophycean algae display a wide variety of thallus morphologies, ranging from microscopic unicellular organisms to larger, filamentous or parenchymatic algae. Species are also found in a wide diversity of habitats, generally attached, or sometimes free-floating. Several marine species occur over a broad salinity range, and are found in brackish to freshwater and semi-terrestrial habitats. Other species are restricted to freshwater or aero-terrestrial habitats. Several species are found in highly specialized habitats such as epizoic on freshwater snails or on carapaces of freshwater turtles, epiphytic on aquatic lichens or as lichen phycobionts, or endophytic in filamentous algae or mesophyll of vascular plants.

This volume serves as a reference work for identifying these ulvophyte green algae by providing keys, detailed descriptions, and illustrations of the more than 100 European species. As in other volumes of this series, this treatment is not restricted to European taxa, and includes descriptions and illustrations of more than other 100 taxa found on other continents.

The present study incorporates the latest findings in molecular phylogeny, ultrastructure and morphology for the classification, delimitation and identification of the species. In addition, it significantly revises the taxonomy of ulvophytes, in particular the orders Ulvales and Ulotrichales, based on new molecular phylogenetic data, combined with morphological data. We propose to resurrect one order and family (Chlorocystidales and Chlorocystidaceae), and describe five new families (Binucleariaceae, Planophilaceae, Hazeniaceae, Sarcinofilaceae, and Tupiellaceae). In addition, we formally describe the order Ignatiales and family Ignatiaceae based on published molecular and ultrastructural data.

This book is one of the first comprehensive treatments of species diversity of non-marine Ulvophyceae, and we hope that it will be a useful tool in ulvophyte research worldwide.

Acknowledgements

The authors would like to thank Michala Klimešová and Veronica Malavasi for their excellent and skillful sequencing work. We are in debt to Michael Guiry, Robert Lucking, Patrik Mráz and Karol Marhold for helpful discussion. We are very grateful to Maike Lorenz (SAG Culture Collection), David R. Nobles (UTEX Culture Collection), Barbara Melkonian (CCAC Culture Collection), Alena Lukešová and Josef Juráň (CCALA Culture Collection), who kindly provided cultured strains of ulvophyte algae.

Contents

Part I

Introduction to the freshwater Ulvophyceae

Circumscription of the class Ulvophyceae: ultrastructure and morphology

The Ulvophyceae K. R. Mattox & K. D. Stewart is one of the main classes of green algae, comprising about 1700 species according to AlgaeBase (Guiry 2012, Guiry & Guiry 2017). Most of this diversity is found in marine coastal waters, while a smaller number of species occurs in brackish, freshwater, and terrestrial habitats. The Ulvophyceae are best known for their macroscopic species (Brodie et al. 2007), but a large, less notable diversity comprise of microscopic species, which are found in marine, as well as in freshwater and terrestrial habitats.

The original circumscription of the class Ulvophyceae was based on a set of ultrastructural characteristics, including a counter-clockwise orientation of the flagellar root system (**Fig. 1**), cytokinesis by furrowing, a closed persistent mitotic spindle and the absence of a phycoplast (**Fig. 2**) (Floyd & O'Kelly 1990, Mattox & Stewart 1984, O'Kelly & Floyd 1984, Sluiman 1989). Some species have flagellate reproductive cells with cell walls covered by organic body-scales (Sluiman 1989), but in many other species zoids lack scales and even a cell wall. Cell wall composition of vegetative cells is variable, with several groups containing cellulose, mannans, xylans, glucans, and sulfated polysaccharides or sulfated rhamnogalacturonans, AGP and extensin (Carlberg & Percival 1977, Domozych et al. 2012). Ultrastructurally, the cell wall may consist of a structural fraction composed of irregularly arranged microfibrils, embedded in an amorphous matrix of complex heteropolysaccharides (e. g. Ulvales, Ulotrichales), a lamellated structure composed of parallel arranged crystalline cellulose I microfibrils with changing orientation in each lamella forming a crossed fibrillar arrangement (e. g. Cladophorales) (Kreger 1962, McCandless 1981, Mizuta 1987, Percival 1979, van den Hoek et al. 1995).

None of the abovementioned characters, however, are unique to the Ulvophyceae, and because of this lack of ultrastructural or biochemical synapomorphies, the monophyly of the Ulvophyceae has long been questioned (see section on phylogenetic relationships of the Ulvophyceae, below). For example, a counter-clockwise orientation of the flagellar root system is also found in species of Trebouxiophyceae, cytokinesis by furrowing occurs in most green algae, a closed mitosis also occurs in the Chlorophyceae, a persistent mitotic spindle is also characteristic for many charophyte green algae, and a phycoplast is absent in prasinophytes (Leliaert et al. 2012, Mattox & Stewart 1984, O'Kelly & Floyd 1984). Also organic body-scales covering the cell walls and flagella are generally regarded as an ancestral character of the green algae (Leliaert et al. 2011, Melkonian 1990).

The order Trentepohliales, which has been affiliated with the Ulvophyceae based on nuclear ribosomal DNA data (Zechman et al. 1990) forms an exception with atypical ultrastructural features, such as the presence of a phragmoplast and multilayered structures associated with flagellar bases in motile cells instead of a cruciate flagellar root system (Graham & McBride 1974).

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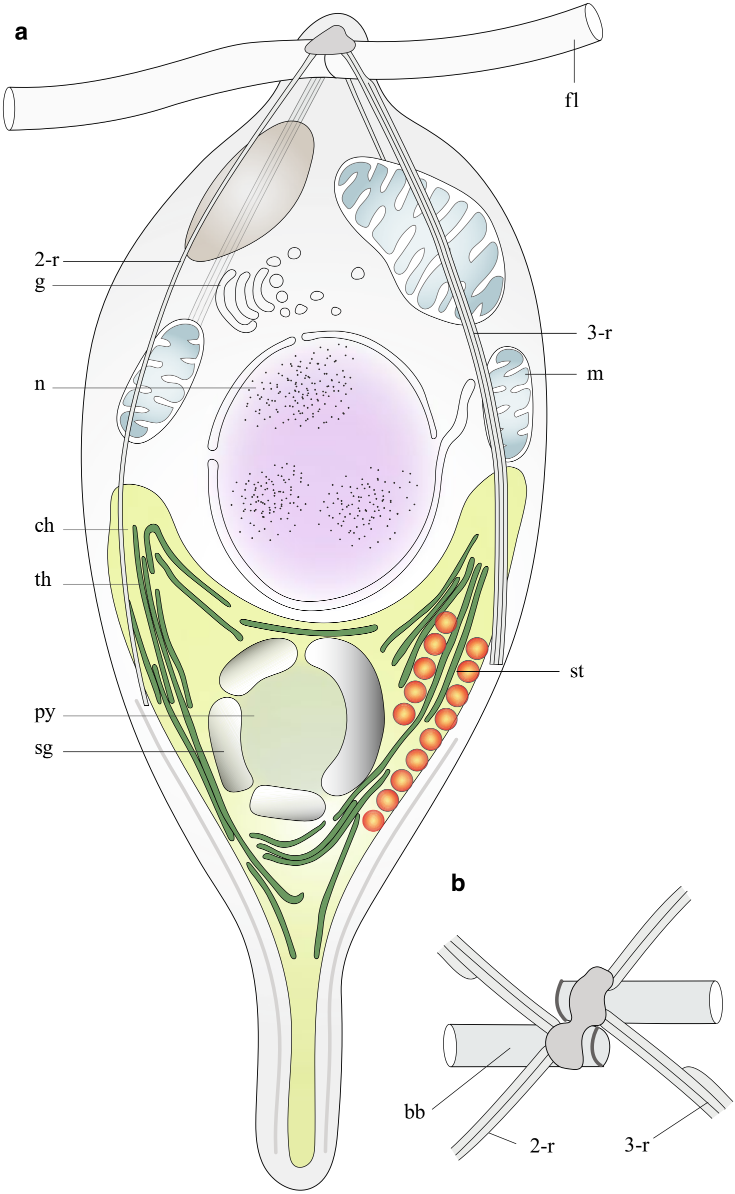
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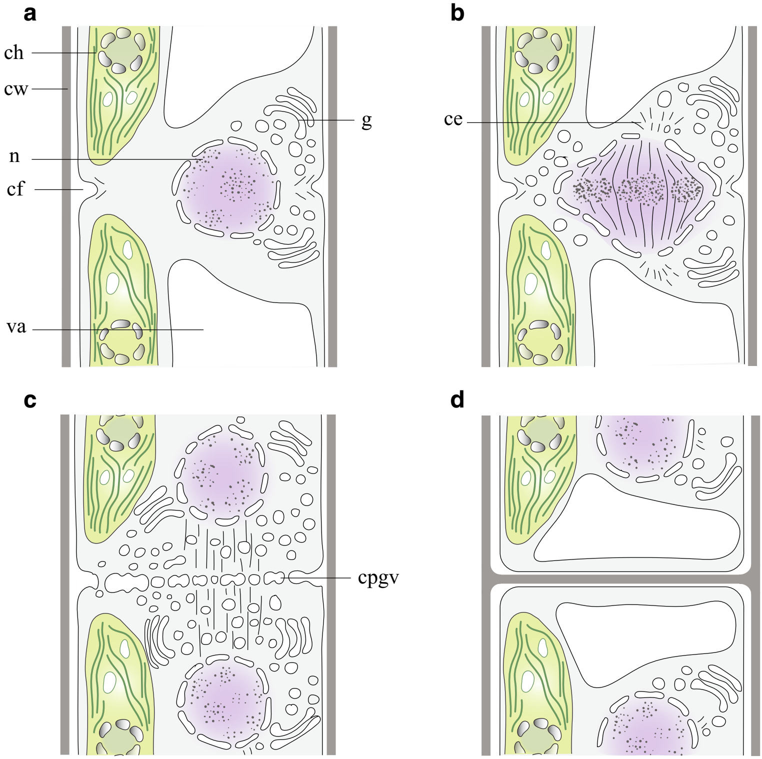
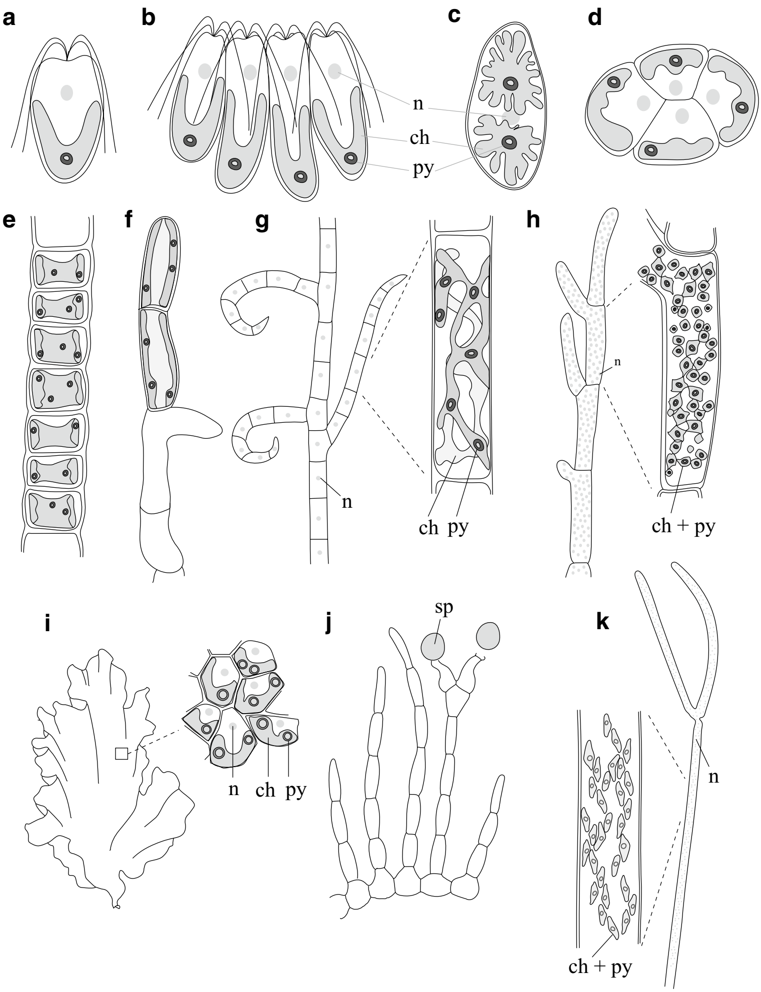


Fig. 2 Schematic representation of ultrastructural details of mitosis and cytokinesis in *Ulothrix* (Ulotrichales), a uniseriate, filamentous multicellular alga with uninucleate cells, closed mitosis with a persistent telophase spindle, cytokinesis via a cleavage furrow to which golgi vesicles are added. Ultrastructural details are shown during early prophase (**a**), metaphase (**b**), late telophase (**c**), and early interphase (**d**). ce = centrioles; cf = cleavage furrow; ch = chloroplast with pyrenoid; cpgv = cell plate of golgi vesicles; cw = cell wall; g = golgi body; n = nucleus; va = vacuole. Based on van den Hoek et al. (1995) and Sluiman et al. (1983)

Fig. 1 Schematic representation of the ultrastructure of a gamete of *Acrosiphonia* (**a**), a member of the Ulotrichales, with a typical counter-clockwise orientation of the flagellar root system (shown in top view of the flagellar apparatus in figure **b**). bb = basal body; ch = chloroplast; fl = part of the flagellum; g = golgi body; m = mitochondrion; n = nucleus; py = pyrenoid; st = stigma; sg = starch grain; th = thylakoid; 2-r = two-stranded microtubular root; 3-r = three-stranded microtubular root. Based on van den Hoek et al. (1995) and Miyaji and Hori (1984)

Morphologically, the class is very diverse, and includes a wide range of thallus organizations and cytological types (**Fig. 3**). Thallus architectures include flagellate unicells (e. g. *Oltmannsiellopsis unicellularis*), non-flagellate unicells (e. g. *Pseudoneochloris marina*, *Scotinosphaera*, *Planophila*), flagellate colonies (e. g. *Oltmannsiellopsis viridis*), non-flagellate colonies (e. g. *Ignatius tetrasporus*, *Pleurastrum*, *Pseudendoclonium, Hazenia*), multicellular unbranched filaments (e. g. *Ulothrix* spp., *Binuclearia, Sarcinofilum*), branched filaments (e. g.



Cladophora, *Acrosiphonia*, *Cloniophora*, *Trentepohlia*), tubular and blade-like thalli (e. g. *Ulva* spp.), and siphonous thalli (e. g. Bryopsidales).

Four main cyto-morphological types can be distinguished in the Ulvophyceae (Cocquyt et al. 2010). Type 1 are flagellate or non-flagellate unicellular or colonial organisms with a single nucleus, which is found in some species of Ulotrichales (Friedl & O'Kelly 2002), Scotinosphaerales (Škaloud et al. 2013a), Oltmannsiellopsidales (Chihara et al. 1986), and *Ignatius* (Bold & MacEntee 1974, Watanabe & Nakayama 2007). Type 2 are multicellular thalli (filaments, discs or blades) composed of uninucleate cells. This type is found in Ulvales, Ulotrichales and Trentepohliales. Type 3 (siphonocladous type) include multicellular thalli composed of multinucleate cells with regularly spaced nuclei, and no cytoplasmic streaming (McNaughton & Goff 1990, Motomura 1996). This occurs in species of Cladophorales, some species of the Ulotrichales (e. g., *Urospora* and *Acrosiphonia*), and in *Blastophysa*. Type 4 (siphonous type) are thalli composed of a single giant tubular cell (siphon) that generally contain thousands of nuclei and chloroplasts that are transported throughout the siphon by cytoplasmic streaming (Menzel 1994). Siphonous thalli range from microscopic siphons to large and morphologically complex seaweeds with differentiated thallus structures (e. g., rhizoids, stolons, and fronds). The siphonous type is found in the orders Bryopsidales and Dasycladales, although in many species of Dasycladales, the thallus remains uninucleate throughout much of the life cycle with a giant diploid nucleus that only divides prior to reproduction (Berger & Kaever 1992). The giant-cells of siphonocladous and siphonous green algae are characterized by unique ultrastructural and physiological features, including unique mechanisms of wounding response (reviewed in Mine et al. 2008).

Fig. 3 Morphological variation found in the Ulvophyceae. Thallus architectures include: **a** – Flagellate unicells (*Oltmannsiellopsis unicellularis*), redrawn from Chihara et al. (1986). **b** – Flagellate colonies (*Oltmannsiellopsis viridis*) redrawn from Chihara et al. (1986). **c** – Non-motile unicells (*Scotinosphaera paradoxa*), redrawn from Wujek and Thompson (2005). **d** – Non-motile colonies (*Ignatius tetrasporus*), redrawn from Bold & MacEntee (1974). **e** – Unbranched filaments; cells uninucleate with parietal chloroplast (*Ulothrix* sp.). **f** – Branched filaments; cells uninucleate with parietal chloroplast (*Ulvella* sp.). **g** – Branched filaments composed of uninucleate cells (*Spongomorpha aeruginosa*); each cell with a single parietal, perforate (reticulate) chloroplast with several pyrenoids; redrawn from Jónsson (1962). **h** – Branched filaments composed of multinucleate cells (*Cladophora* sp.), multiple chloroplast with pyrenoid forming a parietal network. **i** – Multicellular blades composed of uninucleate cells, each with a single chloroplast with one or more pyrenoids (*Ulva* sp.). **j** – Branched filaments composed of prostrate and upright filaments (*Trentepohlia aurea*), redrawn from Fott (1971). **k** – Siphonous filament (*Dichotomosiphon tuberosus*); redrawn from Takagi (2003). ch = chloroplast; n = nucleus; py = pyrenoid; sp = sporangium

Life cycle

Ulvophyceae exhibit a wide diversity of life cycles. Species may reproduce asexually, sexually, or exhibit a combination of sexual and asexual reproduction. Sexual reproduction involves a heteromorphic or isomorphic diplohaplontic life cycle, or a haplontic life cycle (**Fig. 4**). Gametes are either isogamous or anisogamous. Diplohaplontic life cycles are almost exclusively found in marine Ulvophyceae, while haplontic life cycles occur both in marine and freshwater taxa. An example of an isomorphic, diplohaplontic life cycle, as found in *Ulva* sp. (Ulvales) is illustrated in **Fig. 4**a, and an example of a haplontic life cycle, as found in *Ulothrix* (Ulotrichales), is illustrated in **Fig. 4**b.

In several taxa of Ulotrichales (including *Acrosiphonia*, *Chlorothrix*, *Eugomontia*, *Gomontia*, *Monostroma*, *Protomonostroma*, *Spongomorpha*, and *Ulothrix*), the haplontic life cycle includes a dormant, microscopic, mostly attached zygote (sometimes interpreted as a unicellular sporophyte) as the diploid stage, known as *Codiolum* phase (Brodie et al. 2007, Gabrielson et al. 2006, Hanic & Lindstrom 2008, O'Kelly et al. 2004b).

Several sexually reproducing species, may also reproduce asexually during certain parts of the life cycle by biflagellate or quadriflagellate zoospores, aplanospores and akinetes (Bliding 1963, 1968, Hiraoka et al. 2003). Asexual reproduction may also be through vegetative fragmentation, whereby detached parts of the thallus have the ability to re-attach to the substrate and continue growth, e. g. several species of Cladophorales.

In many freshwater ulvophytes, sexual reproduction has never been observed. These include *Oltmannsiellopsis* (Oltmannsiellopsidales), and several genera of Ulvales, Ulotrichales, Cladophorales.

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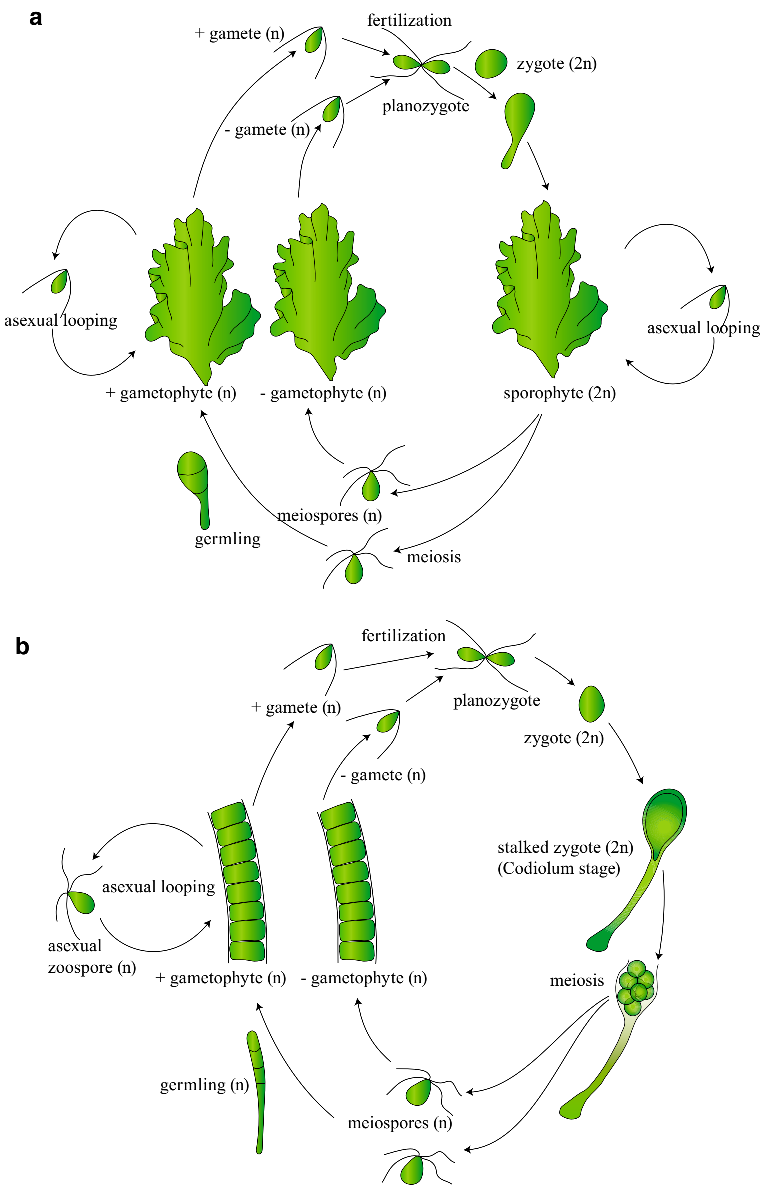
Fig. 4 Two main types of life cycles in the Ulvophyceae. **a** – Isomorphic diplohaplontic life cycle as found in *Ulva* (Ulvales) with alternation of two similar multicellular generations, based on Koeman and Van den Hoek (1981). **b** – Haplontic life cycle as found for *Ulothrix,* based on Lokhorst and Vroman (1972) and van den Hoek et al. (1995)


a
+ gamete (n)
fertilization
zygote (2n)
planozygote
- gamete (n)
asexual looping
asexual looping
+ gametophyte (n)
- gametophyte (n)
sporophyte (2n)
germling
meiospores (n)
meiosis
b
fertilization
+ gamete (n)
planozygote
zygote (2n)
- gamete (n)
stalked zygote (2n)
(Codiolum stage)
asexual looping
asexual
zoospore (n)
+ gametophyte (n)
- gametophyte (n)
meiosis
germling (n)
meiospores (n)

Ecology of freshwater and terrestrial ulvophytes

Although the largest diversity of Ulvophyceae is found in marine habitats, a substantial number of species may be found over a broad salinity range, and occur in brackish to freshwater and semi-terrestrial habitats. For example, some marine *Ulva* and *Blidingia* species are able to penetrate into freshwater habitats (Messyasz & Rybak 2011). Other ulvophyte species or genera are entirely confined to brackish (marshes and estuaries), freshwater, damp terrestrial, or aeroterrestrial habitats. Species that are found in freshwater and terrestrial species are treated in this book, including marine/brackish species extending into freshwater habitats. Freshwater habitats range from still (ponds and lakes) to flowing waters (small streams to rivers), and include temporary pools of water. Freshwater ulvophytes are generally found attached, epilithic on rocky substrate, epipelic on sediment surface, epiphytic on aquatic leaf surfaces or on other algae, epizoic on freshwater snails, carapaces of freshwater turtles or on feathers of aquatic birds (e. g. *Ulvella* spp., *Aegagropilopsis* spp.), or attached to other substrates such as wood or artificial substrates such as glass and plastic. Some species are shell-boring, growing into calcified substrates (e. g. *Ulvella* spp.). Some species are known as epiphytes on aquatic lichens, or as lichen phycobionts (e. g. *Pseudendoclonium* spp.). Other species (e. g. *Ulvella* spp.) grow endophytic in various freshwater filamentous algae, including *Pithophora*, *Cladophora* and *Rhizoclonium*. Species may also grow as unattached, free-floating thalli (e. g. *Rhizoclonium* spp., *Pithophora* spp., *Ulva* spp.), and some of these are known to form typical spherical thalli, known as "lake balls" (e. g. *Aegagropila*).

Terrestrial habitats range from damp soil habitats, to subaerial habitats, including rocks, artificial substrates (concrete, carved stone, plastics, metal), tree bark, leaves, liverworts, mosses and lichens. Terrestrial species of ulvophytes may grow epilithic, epiphytic or endophytic within tissues of vascular plants. A few examples of terrestrial epizoic Ulvophyceae have also been reported (e. g. *Trentepohlia* growing on spiders in Queensland, Australia; Cribb 1964).

In Europe (and temperate regions, in general), freshwater ulvophytes are most abundant during spring and summer, when light and temperature are optimal for growth.

A number of freshwater ulvophytes are notorious for forming blooms as a result of anthropogenic eutrophication. For example, species of *Cladophora* and *Pithophora* have been found to form nuisance-level populations in the Laurentian Great Lakes in response to phosphorus enrichment (Auer et al. 2010, Dodds & Gudder 1992, Higgins et al. 2008, O'Neal et al. 1985). Species of *Ulva* may also form blooms in freshwater environments. Several *Ulva* species penetrate in salt marshes, enriched ponds, and other continental waters with increased salinity (Mares et al. 2011). In highly eutrophic conditions (e. g. waters influenced by wastewater or agricultural

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runoff), blooms of *Ulva* populations have been observed in North American and European waters (Kaštovský et al. 2010, Lougheed & Stevenson 2004, Messyasz 2006).

On a more positive note, many ulvophytes play an important and beneficial ecological role. For example, *Cladophora* has been described as an ecosystem engineer, providing an ecological niche for a diverse range of other aquatic organisms, ranging from microbial epiphytes to associated benthic and pelagic animals (Ward & Ricciardi 2010, Zulkifly et al. 2012, 2013).

The Trentepohliales are atypical ulvophytes from an ecological point of view because they are entirely restricted to terrestrial habitats. Species are found epiphytic or endophytic on or in leaves of land plants, or form red-orange crusts on the surface of trees, rocks and artificial substrata (e. g. *Trentepohlia*) (Rindi & Guiry 2002). Several species of Trentepohliales engage in symbiosis with fungi, forming lichens (Nelsen et al. 2011). Some species of Trentepohliales cause diseases in economic plants in tropical regions. For example, *Cephaleuros* (Trentepohliales) forms rusty spots on leaves of tea and other plants (Brooks et al. 2015).

Phylogenetic position of the Ulvophyceae and evolutionary relationships among its main lineages

The Ulvophyceae are currently regarded as one of the main classes of the Chlorophyta, but the relationship of the class with other classes of Chlorophyta is still a matter of debate. Also the relationship among ulvophyte lineages have been difficult to resolve, and in addition, monophyly of the class has been questioned.

Current view of phylogenetic relationships and uncertainties are summarized in **Fig. 5**. The Chlorophyta are one of the two main clades of green plants (also known as Viridiplantae or Chloroplastida), the other clade being the Streptophyta, which includes several freshwater green algae and the land plants (Embryopsida). The Chlorophyta includes several early diverging, species-poor clades of mostly unicellular marine and freshwater green algae (Prasinophytes), and the morphologically and ecologically diverse core Chlorophyta. The core Chlorophyta includes two smaller classes (Chlorodendrophyceae, comprising scaly quadriflagellates from freshwater, brackish water, marine and hypersaline habitats (Arora et al. 2013, Norris et al. 1980), and the Pedinophyceae, including asymmetric, uniflagellate, mostly naked green algae from marine, freshwater or soil habitats (Marin 2012)), and three large classes Ulvophyceae, Trebouxiophyceae and Chlorophyceae.

The classes Ulvophyceae, Trebouxiophyceae and Chlorophyceae are species-rich and morphologically and ecologically diverse. Resolving the phylogenetic relationships among the main ulvophycean clades has been a difficult task, with nuclear ribosomal, chloroplast and mitochondrial multigene data yielding ambivalent and often contradicting results (Leliaert et al. 2012). Adding to the complexity, the monophyly of the Ulvophyceae and the Trebouxiophyceae has been questioned based on ultrastructural as well as molecular data.

Nuclear ribosomal DNA-based phylogenies (some of which supplemented with one or more chloroplast genes) have generally recovered the Ulvophyceae as a monophyletic group, although never with strong phylogenetic support (e. g., López-Bautista & Chapman 2003, Watanabe & Nakayama 2007). These studies recovered two distinct clades of Ulvophyceae: the Ulvales–Ulotrichales clade and a clade consisting of Trentepohliales, Cladophorales, Dasycladales and Bryopsidales, along with some lineages of uncertain affinity, including Oltmannsiellopsidales, Scotinosphaerales and the *Ignatius*-clade (Cocquyt et al. 2009, Škaloud et al. 2013a, Watanabe et al. 2001, Zechman et al. 1990). A phylogenetic study based on 10 genes (eight nuclear and two plastid genes) recovered the class as a well-supported monophyletic group, and confirmed the divergence of two main ulvophycean clades (Cocquyt et al. 2010). On the other hand, chloroplast phylogenomic analyses either indicated a polyphyletic Ulvophyceae, consisting of two or more separate lineages, or a monophyletic Ulvophyceae, but with low support (Fučíková et al. 2014, Leliaert & Lopez-Bautista 2015, Turmel et al. 2017).

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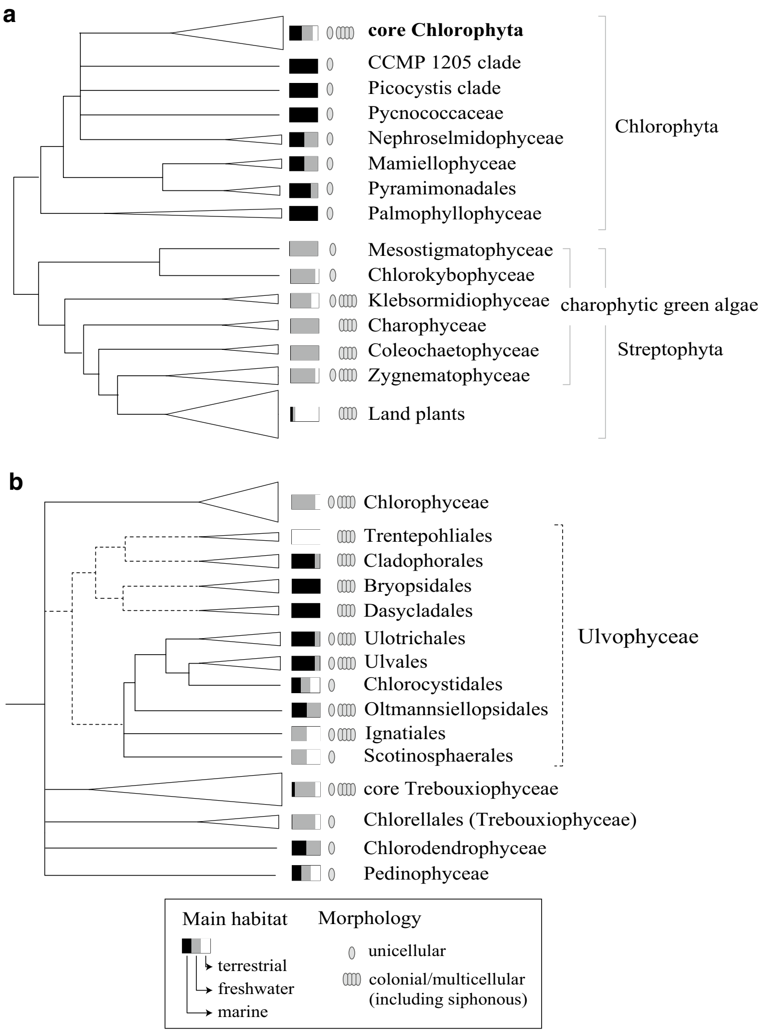

Fig. 5 Current view of phylogenetic relationships of the green plants (Viridiplantae). **a** – Position of the core Chlorophyta. **b** – Relationships and phylogenetic uncertainties in the core Chlorophyta. Dashed lines indicate relationships among ulvophyte clades that are supported in the phylogenetic analyses based on 8 nuclear and 2 plastid genes (Cocquyt et al., 2010; Škaloud et al., 2013a), but which are not supported in chloroplast phylogenomic analyses (Leliaert and Lopez-Bautista, 2015; Turmel et al., 2016)

Classification: orders and families

Current classification of the Ulvophyceae includes 32 families in 8 orders (Leliaert et al. 2015). The present study significantly revises the classification of ulvophytes, in particular the Ulvales and Ulotrichales. One order (Chlorocystidales) is resurrected, and seven families are newly described or resurrected. The *Ignatius*-clade, which has been recognized as a distinct clade of Ulvophyceae based on ultrastructural and molecular data, is here formally described as a new order and family (Ignatiales, Ignatiaceae). An overview of the order and family level classification, as presented in the current treatment, with indication of habitat type (up to the family level) is given in **Table 1**.

Order-level classification has been relatively stable over the past decades, with the notable exceptions of the Ulotrichales, which is likely non-monophyletic as traditionally circumscribed (Leliaert et al. 2012, O'Kelly et al. 2004b, Škaloud et al. 2013b). Also the Siphonocladales/Cladophorales complex has a confusing taxonomic history, but the current consensus has settled on the recognition of a single order Cladophorales (Boedeker et al. 2016, Leliaert et al. 2003).

Conversely, family circumscriptions have changed substantially over the years. For example, the family Cladophoraceae originally included a wide range of freshwater and marine algae, characterized by branched or unbranched, uniseriate filaments (including *Aegagropila*, *Chaetomorpha*, *Chaetonella*, *Cladophora*, *Cladophoropsis*, *Pithophora*, *Rhizoclonium*, *Spongocladia*, *Acrosiphonia* and *Urospora*) (Wille 1890). A first important change of the family circumscription was the transfer of *Spongocladia* and *Acrosiphonia* to the Ulotrichales based on differences in cell wall composition, chloroplast structure and life cycle (Jónsson 1962, 1999). More recently, the family circumscription was altered by molecular phylogenetic evidence, and involved the transfer of several genera (*Aegagropila*, *Basicladia/Arnoldiella*, *Pithophora*, and *Wittrockiella*) to the resurrected family Pithophoraceae, and some species of *Cladophora* to the new family Pseudocladophoraceae (Boedeker et al. 2016, Boedeker et al. 2012). Similarly, family circumscriptions with the Ulvales-Ulotrichales were drastically reformed by molecular phylogenetic data (Carlile et al. 2011, Hayden and Waaland 2002, O'Kelly et al. 2004a, b, c, Škaloud et al. 2013b), and several new taxonomic changes at the family level are proposed in the current treatment.

Although the orders and families represent the main branches in the phylogeny of ulvophytes, some distinct clades are not recognized as higher-rank taxa. These include the marine genus *Blastophysa*, which forms a clade possibly related to the Cladophorales (Cocquyt et al. 2010), and a distinct clade of uncertain affinity, including the genera *Ignatius* (unicellular or irregular tetrad aggregations composed of 2 to 8 daughter cells within a mother cell wall, found in damp terrestrial habitats and lakes) and *Pseudocharacium* (unicellular alga growing epiphytically on fresh-

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water algae and plants) (Bold & MacEntee 1974, Cocquyt et al. 2010, Škaloud et al. 2013a, Watanabe & Nakayama 2007).

Table 1 Order and family level classification of the Ulvophyceae, with indication of habitat type. Only genera occurring in freshwater or (aero)terrestrial habitats are listed. Taxonomic changes, including resurrected and newly described orders and families, proposed in this work are indicated with an asterisk (*).

Order/Family	**Habitat and notes on freshwater and brackish genera**
Order Ulvales Blackman & Tansley	
Bolbocoleonaceae O'Kelly & Rinkel	Marine
Cloniophoraceae A. L.Carlile, C. J. O'Kelly & A. R. Sherwood	Brackish and freshwater *Cloniophora*: brackish and freshwater, globally distributed in (sub)tropics
Kornmanniaceae Golden & Cole	Marine, freshwater and terrestrial; artic to temperate *Blidingia*: marine to freshwater, temperate regions *Pseudendoclonium*: marine, freshwater, terrestrial and aerophytic, also as lichen phycobiont, globally distributed *Paulbroadya*: freshwater and marine, terrestrial and phycobiont of marine lichens, temperate and arctic *Lithotrichon*: phycobiont of freshwater lichens; Europe
Phaeophilaceae D. F. Chappell, C. J. O'Kelly, L. W. Wilcox & al.	Marine; temperate to tropical
Ulvellaceae Schmidle	Marine, brackish or freshwater *Ulvella*: marine, brackish or freshwater, worldwide distribution
Ulvaceae J. V. Lamouroux ex Dumortier	Marine to brackish and freshwater *Ulva* Linnaeus: brackish and freshwater, worldwide *Percursaria*: marine to brackish and freshwater, worldwide *Ochlochaete*: marine, brackish or freshwater, worldwide
Ctenocladiaceae Borzì	Marine, freshwater and aerophytic *Ctenocladus:* marine, freshwater in inland saline lakes, aerophytical; worldwide distribution *Pseudopleurococcus*: freshwater or aerophytic, scattered worldwide distribution *Spongioplastidium*: aerophytic, scattered worldwide distribution

Order/Family	**Habitat and notes on freshwater and brackish genera**
Order Ulotrichales Borzì	
Ulotrichaceae Kützing	Marine, brackish and freshwater habitats *Ulothrix*: marine, brackish and freshwater habitats; temperate. *Pearsoniella*: freshwater
Binucleariaceae Škaloud & Leliaert, fam. nov. *	Freshwater *Binuclearia*: freshwater; worldwide distribution
Planophilaceae Škaloud & Leliaert, fam. nov. *	Freshwater and terrestrial *Planophila*: terrestrial, on damp soil; temperate *Fernandinella*: terrestrial; temperate *Tetraciella*: freshwater; Europe *Chloroplana*: terrestrial; temperate to arctic *Pseudendocloniopsis*: freshwater; Europe
Hazeniaceae Škaloud & Leliaert, fam. nov. *	Freshwater to terrestrial *Hazenia*: freshwater or terrestrial; temperate to arctic
Helicodictyaceae Whitford & G. J. Schumacher	Freshwater and terrestrial *Helicodictyon*: freshwater; temperate *Protoderma*: freshwater; temperate *Rhexinema*: terrestrial, on damp soil; temperate *Pleurastrum*: freshwater; worldwide distribution
Sarcinofilaceae Škaloud & Leliaert, fam. nov. *	Freshwater and terrestrial *Sarcinofilum*: freshwater and terrestrial; mainly temperate to arctic *Filoprotococcus*: freshwater; Europe
Tupiellaceae Škaloud & Leliaert, fam. nov. *	Freshwater *Tupiella:* freshwater; temperate *Vischerioclonium*: freshwater; North America
Gomontiaceae De Toni	Marine, brackish or freshwater *Gomontia*: Marine and freshwater; worldwide distribution
Monostromataceae Kunieda	Marine, brackish and freshwater *Monostroma*: Marine, brackish and freshwater; worldwide distribution *Gayralia*: Marine to brackish; temperate
Kraftionemaceae Wetherbee & Verbruggen	Marine
Acrosiphoniaceae Jónsson	Marine to brackish; temperate to tropical

Order/Family	**Habitat and notes on freshwater and brackish genera**
Order Chlorocystidales Kornmann & Sahling *	
Chlorocystidaceae Kornmann & Sahling *	Marine and terrestrial *Desmochloris*: marine and terrestrial
Order Oltmannsiellopsidales T. Nakayama, S. Watanabe & I. Inouye	
Oltmannsiellopsidaceae T. Nakayama, S. Watanabe & I. Inouye	Marine, brackish and freshwater *Oltmannsiellopsis*: marine to brackish habitats, temperate *Dangemannia*: freshwater or marine waters, temperate regions, including Europe
Order Scotinosphaerales Škaloud et al.	
Scotinosphaeraceae Škaloud et al.	Freshwater or terrestrial habitats *Scotinosphaera*: freshwater and terrestrial habitats, temperate regions
Order Ignatiales Leliaert & Škaloud, ord. nov. *	
Ignatiaceae Leliaert & Škaloud, fam. nov. *	Freshwater and terrestrial *Ignatius*: terrestrial *Pseudocharacium*: freshwater
Order Cladophorales Haeckel	
Anadyomenaceae Kützing	Marine, mostly tropical
Cladophoraceae Wille	Marine, brackish and freshwater; arctic and temperate to tropical *Cladophora*: Marine, brackish and freshwater species. Freshwater species with global distribution. *Rhizoclonium*: Marine, brackish and freshwater species. Freshwater species ("*R. hieroglyphicum*") globally distributed.
Okellyaceae Leliaert & Rueness	Marine, temperate
Pithophoraceae Wittrock	Marine, brackish and freshwater. Several freshwater genera:, *Aegagropila*: freshwater, N Hemisphere *Aegagropilopsis*: freshwater, SE Asia *Arnoldiella*: freshwater, global distribution *Pithophora*: freshwater, global distribution *Wittrockiella*: brackish water, global distribution
Pseudocladophoraceae Boedeker & Leliaert	Marine, temperate to subtropical
Siphonocladaceae F. Schmitz	Marine, mostly tropical
Valoniaceae Kützing	Marine, mostly tropical

Order/Family	**Habitat and notes on freshwater and brackish genera**
Order Bryopsidales J. H. Schaffner	
Bryopsidaceae Bory de Saint-Vincent	Marine
Codiaceae Kützing	Marine
Derbesiaceae Hauck	Marine
Caulerpaceae Kützing	Marine
Dichotomosiphonaceae Chadefaud ex G. M. Smith	Marine and freshwater. *Dichotomosiphon*: Marine and freshwater species. Freshwater species reported from Europe, North America and Asia.
Halimedaceae Link	Marine
Pseudocodiaceae L. Hillis-Colinvaux	Marine
Rhipiliaceae O. Dragastan et al.	Marine
Udoteaceae J. Agardh	Marine
Ostreobiaceae P. C. Silva	Marine
Order Dasycladales Pascher	
Dasycladaceae Kützing	Marine, mostly tropical
Polyphysaceae Kützing	Marine, mostly tropical
Order Trentepohliales Chadefaud ex R. H. Thompson & D. E. Wujek	
Trentepohliaceae Hansgirg	Various subaerial habitats, including epiphytic and endophytic species, and lichen phycobionts; temperate to tropical regions *Cephaleuros*: subcuticular or endophyllous, (sub) tropics *Phycopeltis*: epiphyllous, mainly tropical, but also recorded from W Europe *Printzina*: subaerial habitats, global distribution (polyphyletic genus) *Stomatochroon*: Plant parasitic, (sub)tropics *Trentepohlia*: subaerial habitats and lichen phycobionts, global distribution (polyphyletic genus).

Genus classification

Circca 110 genera of ulvophytes are currently circumscribed, most of which are restricted to the marine environment. Although most of the traditionally circumscribed genera are easily distinguishable based on gross morphological features, DNA sequence data have revealed that many genera are not monophyletic. For example, *Pseudendoclonium*, a genus occurring in marine, freshwater, and damp terrestrial habitats, has been shown as polyphyletic in several molecular phylogenetic studies (Carlile et al. 2011, Škaloud et al. 2013b, Darienko & Pröschold 2017, this study), and only for a few species the placement in the genus has been confirmed, while other species were transferred to genera *Paulbroadya*, *Tupiella*, *Rhexinema*, and *Hazenia*. Other genera that are non-monophyletic as traditionally circumscribed are *Cladophora* (Boedeker et al. 2016), *Phycopeltis* (Zhu et al. 2014), *Printzina* (Rindi et al. 2009), *Trentepohlia* (Rindi et al. 2009), and *Ulva* (Hayden et al. 2003). Our phylogenetic investigations further reveal the paraphyly of two other genera, *Planophila* and *Rhexinema*.

Species concept and identification

About 1700 species of Ulvophyceae are currently recognized according to AlgaeBase (Guiry & Guiry 2017). Like in other algal groups, traditional species boundaries in the Ulvophyceae have been largely based on morphological data (John & Maggs 1997). There is, however, a growing consensus that accurate species delimitation and identification in green algae should also include evidence from molecular data (Leliaert et al. 2014).

The morphological characters traditionally used to distinguish species vary amongst the different orders of ulvophytes, but generally include thallus shape, cell dimensions of vegetative and reproductive cells, and chloroplast morphology. These characters are often variable or plastic, which may impede accurate species delimitation and identification. In addition, cryptic species diversity is prevalent in green algae, making species delimitation using morphological data problematic (John & Maggs 1997, Leliaert et al. 2014). Because of these difficulties, the use of DNA-sequence data for species delimitation and identification in algae is becoming standard practice.

For ulvophytes, the most commonly used markers for species delimitation (DNA barcode markers) include the plastid encoded *tufA* and *rbcL* genes, and the nuclear small subunit ribosomal DNA (SSU rDNA), large subunit (LSU) rDNA and the rDNA internal transcribed spacer (ITS) (Fučíková et al. 2011, Leliaert et al. 2009, Luo et al. 2010, Rindi et al. 2011, Saunders & Kucera 2010, Škaloud & Peksa 2010,

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Škaloud & Rindi 2013, Subirana et al. 2013, Verbruggen et al. 2007). Different molecular markers have been assessed for species-level taxonomy in freshwater green algae by Hall et al. (2010), who concluded that of the markers tested, *rbcL*, ITS rDNA and *tufA* are the most promising for use as DNA barcodes (although it should be noted that ulvophytes were not included as test group in that study).