

Genetic diversity and polyphyletic origin of the
Dictyosphaerium morphotype

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1 General introduction

Systematics of the Chlorophyta

Eukaryotic algae are one of the main primary producers in aquatic environments. The term Chlorophyta refers traditionally to a group of green algae which are characterized by chloroplasts with a double membrane which lack an endoplasmatic reticulum (ER), starch as a reserve polysaccharide, stacked thylacoids and chlorophyll a and b (Friedl 1997, Chapman et al. 1998). Nevertheless, colourless taxa like *Polytoma* Ehrenberg or *Prototheca* W. Krüger which lost their pigments in a secondary process and contain only leucoplasts are included as well (Pringsheim 1963). Chlorophyta comprise a huge diversity of morphological and ecological different algae, ranging from unicellular (e.g. *Chlorella* Beijerinck) to colonial (e.g. *Volvox* Linnaeus), from filamentous unbranched (e.g. *Oedogonium* Link ex Hirn) to branched forms (e.g. *Chaetomorpha* Kützing). They occur in a variety of different habitats, like freshwater or marine environments, can live as photobionts (e.g. *Trebouxia* Puymaly) or as terrestrial epiphytes (e.g. *Trentepohlia* Martius) and some occur in soil as well (e.g. *Bracteacoccus* Tereg).

The systematics of green algae went through major changes since the first approach for a natural system was done over 200 years ago which considered the organization level of the vegetative state as major feature (Blackman 1900, Blackman & Tansley 1902, Pascher 1918). The system was refined over the years resulting in a modified classification taking life cycles, ultrastructural data, shape and organization of vegetative cells, mitosis, cell wall composition and architectures of flagellated cells into account as well (e.g. Christensen 1962, Ettl & Komárek 1982, van den Hoek & Jahns 1978, van den Hoek et al. 1988, 1995, Mattox & Stewart 1984). Classes, families, genera and species were delineated by a combination of the different morphological criteria (Kornmann 1973, Ettl 1981, Komárek & Fott 1983, Mattox & Stewart 1984).

The introduction of molecular analyses gave further insight into the natural system of green algae (Melkonian & Surek 1995, Friedl 1997, Lewis & McCourt 2004). The common consensus is, that green algae evolved in two major lineages, called the Charophyte-clade and the Chlorophyte-clade *sensu* Lewis & McCourt (2004). The Charophyte-clade (or Streptophyta *sensu* Bremer 1985) comprises the land plants and a number of green algae groups such as the Mesostigmatophyceae, Chlorokybophyceae, Klebsormidiophyceae, Zygnemophyceae, Coleochaetophyceae and Charophyceae. The Chlorophyte-clade (or Chlorophyta) includes the majority of algae, which were traditionally referred to as green

algae. This clade contains the three monophyletic algae groups Chlorophyceae, Trebouxiophyceae and Ulvophyceae and the paraphyletic Prasinophyceae, consisting of at least six different clades (Lewis & McCourt 2004) (nine clades according to Pröschold & Leliaert 2007) at the basal part of the Chlorophyta (Fawley et al. 2000, Lewis & McCourt 2004, Marin et al. 2010).

By combining molecular and morphological data, the polyphyletic origin of several morphological defined families and genera was revealed. For example, Komárek & Fott (1983) included 28 genera within the Scenedesmaceae (Chlorophyceae). Molecular studies detected the phylogenetic relationship of genera like *Didymogenes* Schmidle, *Diclostera* Jao, Wei & Hu or *Makionella* Okada to the Trebouxiophyceae (Hegewald & Hanagata 2000, Hepperle et al. 2000, Krienitz et al. 2004). A similar pattern could be observed for the Chlorellaceae. SSU rRNA gene analyses showed, that the *Chlorella*-like morphology evolved independently within the Chlorellaceae and Trebouxiophyceae (Huss et al. 1999). *Chlorella vulgaris* Beijerinck, represented by the authentic strain SAG 211-11b, established a lineage within the Chlorellaceae (Trebouxiophyceae). Consequently, the generic name *Chlorella* Beijerinck is only valid for members of this group (Huss et al. 1999, Krienitz et al. 2004). As a result, several new genera were erected among the *Chlorella*-like algae (e.g. *Marinichlorella* Z.Aslam, W.Shin, M.K.Kim, W.-T.Im et S.-T.Lee, Aslam et al. 2007; *Kalinella* Neustupa, Němcová, Eliáš et Škaloud, Neustupa et al. 2009) and known species (e.g. *Chlorella saccharophila*, *C. ellipsoidea*) were new combined to different genera (e.g. *Chloroidium* Nadson) (Aslam et al. 2007, Neustupa et al. 2009, Darienko et al. 2010).

It was noted, that morphological characters are particularly subject to convergent or parallel evolution or may show phenoplasticity. Simple body forms can lead to an underestimation of genetic diversity as it was shown on the example of the “green ball” of *Chlorella* which was detected in several independent phylogenetic lineages, corresponding to different molecular defined genera and species (Huss et al. 1999, Aslam et al. 2007, Neustupa et al. 2009). The example of *Micractinium* Fresenius showed that a phenotype can be highly influenced by environmental conditions. Growing in dense cultures, *Micractinium* loses its bristles, becomes solitary and resembles the typical *Chlorella* phenotype. Under grazing pressure or when it is transferred to media from the rotifer *Brachionus*, a redevelopment of long bristles can be observed (Luo et al. 2005, 2006). Studies on different *Scenedesmus* species revealed a similar pattern. The colony size increased significantly when growing under influence of zooplankton such as *Daphnia* (Trainor 1998). Further on, single-celled isolates of *Scenedesmus* can display morphological features that span several morphological

groups and lead to an overestimation of species richness (Lüring 1998, 1999, Trainor 1998, Verschoor et al. 2004). Then again, morphological stasis (little or no morphological changes between/within species), as proven by the diatom *Skeletonema* Greville or in the colonial green algae *Pandorina morum* (O. Müller) Bory de Saint-Vincent can mask and underestimate genetic diversity (Gallagher 1982, Medlin et al. 1991). The question arose, how to distinguish species in the light of extremely high genetic variability compared with a limited amount of morphological characters on one hand, and phenotypic adaptation to environmental condition on the other hand.

Species concepts

The biological species concept (Mayr 1948), which regards the sexual compatibility of two taxa as criterion for species delineation is not applicable on groups where sexual reproduction is unknown or where mating experiments are not possible. A phylogenetic approach based on genetic markers is broadly applicable and more or less objective, but highly depends on the chosen genetic region. If a conserved region is analyzed, fewer species will be recognized and morphological characters may conflict with the position in the phylogenetic tree. If a highly variable region is chosen, the amount of species may be overestimated (Hoef-Emden 2007, Rindi et al. 2009b).

The Compensatory Base Change (CBC) approach of the internal transcribed spacer 2 (ITS-2) was often discussed as a compromise (Coleman 2000). The ITS-2 is a variable region, situated between the 5.8S and the LSU of the rRNA gene and shows a high degree of predictability among eukaryotes to fold up in a characteristic way (e.g. Coleman 2003, 2007, Schultz et al. 2005). By comparing the secondary structure of two related taxa, a CBC refers to a base change, 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 the second taxon (Gutell et al. 1994). In some microalgae, the occurrence of CBCs in conserved regions of the ITS coincidences with the sexual incompatibility between two species (Coleman & Mai 1997, Mai & Coleman 1997, Coleman 2000, 2003, 2009, Amato et al. 2007). Nowadays, the presence of CBCs or hemi-CBCs (only one-sided base changes) is also often used for species delineation in morphological difficult groups or when only asexual reproduction is known (Krienitz et al. 2004, Hoef-Emden et al. 2007).

DNA barcoding

In 2003, Hebert et al. proposed “DNA barcoding” as a way to identify species on the molecular level (Hebert et al. 2003, Tautz et al. 2003). The DNA barcode should be a short,

highly variable DNA sequence, unique for each species, easily amplifiable and allow to separate closely related taxa. For animals, part of the mitochondrial cytochrome oxidase c subunit I gene (COI, *cox1*) has been proposed and is now widely used in separation of species (Hajibabaei et al. 2007). However, this region seems to be too conserved for species delineation in land plants and green algae (Kress et al. 2005). Different markers were tested and proposed for diatoms. Evans et al. (2007) proposed the *cox1* as barcode and demonstrated the separation of species in *Sellaphora*. They stated that this region is short and variable and has already been used in combination with other genes for phylogenetic studies. However, Moniz & Kaczmarek (2010) suggested the usage of the 5.8S and part of the ITS-2 as genetic region for barcoding. They showed the applicability of this highly variable part in differentiating several diatom species. For green algae, the discussion is still ongoing. Whichever marker will be used in the future as barcode, the species identification can only be accurate in thoughtfully characterized groups (Meyer & Paulay 2005).

In the past, biodiversity studies of protists largely focused on morphological surveys (Beaver & Chrisman 1989, Gaedke & Wickham 2004). Due to the progress of high-throughput DNA sequence analyses, like metagenomics, biodiversity studies can now focus also on genetic differences in environmental samples (e.g. Moreira & Lopez-Garcia 2002, Richards et al. 2005, Slapeta et al. 2005). Huge discrepancies in species counts between morphological surveys and molecular approaches can be observed (Medinger et al. 2010). In addition, the high-throughput analyses often revealed new lineages without a close match to already known taxa. This indicates, that the genetic characterization of many protist groups is poorly studied so far (Rindi et al. 2009a, Medinger et al. 2010). Morphological convergence, which is often a response to habitat or herbivore pressure further add to the problem of assigning morphospecies to phylogenetic lineages. Special morphological features, such as mucilage envelopes or colony formation provide an ecological benefit for the algae and may have evolved independently in different evolutionary lineages.

First molecular analyses of members of the genus *Dictyosphaerium* Nägeli (Trebouxiophyceae) suggested that the genetic diversity of the genus is highly underestimated so far (Krienitz et al. 2004). The special colonial morphotype of this group with the surrounding mucilage may be the result of environmental influences, such as bottom-up or top-down regulations. It needs to be clarified, whether the morphotype evolved independently or not.

The genus *Dictyosphaerium*

General characteristics

Members of the genus *Dictyosphaerium* are colonial, coccoid photosynthetic green algae. The cells show the same properties as typical *Chlorella* cells with one parietal or cup-shaped chloroplast. The pyrenoid is covered by two starch grains. However, some taxa do not form a pyrenoid or it could not be observed (Komárek & Perman 1978). The cell walls of *Dictyosphaerium* species are smooth, with the exception of *Dictyosphaerium granulatum* where it is irregularly covered by granules of different size (Hindák 1977). The cell shapes range from spherical, to oval and almost ellipsoid with a size range from 1-15 µm (Komárek & Perman 1978). The cell form of some species can be age-dependent. In that case, young cells tend to be more elongate, getting more spherical during maturing (Hindák 1984, Komárek & Fott 1983).

The reproduction is typically via autospore formation with 2-4 autospores. The individual cells, resulting from one particular mother cell, are joined by a system of gelatinous stalks. These stalks are the remnants of the mother cell wall which ruptures in 2-4 flaps during the release of the autospores. In the most common type of release, the mother cell wall ruptures on the surface in four flaps and the autospores “slide” out of the cell-wall horizontally or slightly tilted and separate from each other without changing orientation. The flaps of the cell wall remnants become mucilaginous and thin out. In the second way of release, the autospores slant after the rupture of the mother cell wall in a 180° angle from the inside to the outside. The connection of the daughter cells to the stalks is at first at the broader side, but can later shift to the tips of the cells (Komárek & Perman 1978). These two release types lead to the characteristic colonies with 4-64 cells which are surrounded by a mucilage envelope. Sexual reproduction is rarely observed in *Dictyosphaerium* species. Only in *Dictyosphaerium indicum* Iyengar et Ramanathan, oogamy has been described (Iyengar & Ramanathan 1940).

The combination of cell size and shape, the connection to the gelatinous stalks, the process of the release of the autospores and special habitat preferences are to be considered as species specific (Komárek & Perman 1978). Problems in identification of culture strains are caused by loss of colonial life form and the disintegration into solitary cells (Komárek & Perman 1978).

Ecology and distribution of *Dictyosphaerium*

Members of *Dictyosphaerium* are mainly present in freshwater habitats with occasional reports from brackish waters as well (Komárek & Perman 1978, Feibicke 1994). Feibicke (1994) reported *D. tetrachotomum* from the Innere Schlei, a brackish water fjord of the Baltic Sea. Nevertheless, the main habitats are lakes, water reservoirs, rivers, swamps and peat bogs where they can reach high abundances (Komárek & Fott 1983). Irfanullah & Moss (2006) reported *D. pulchellum* as the dominating phytoplankton species from the small, shallow, acid Lake Delamere. There are also reports from eutrophic mixed lakes, where it can dominate during the summer period (Shubert 2003). A green water bloom caused by *Dictyosphaerium* was reported from a fish pond (John & Tsarenko 2002). Some taxa, like *D. chlorelloides* (Nauman) Komárek & Perman have also been repeatedly reported from soil (Komárek & Perman 1978, Zancan et al. 2005).

A striking feature for all *Dictyosphaerium* species is the heavy production of colorless, diffluent mucilage. The excretion occurs already during the release of the autospores and continues during the growth phase of the individual cells (Komárek & Perman 1978). The envelopes of the different cells of the colonies merge later to one single mucilage sheath surrounding each colony which originates from one mother cell. The benefits of the mucilage production of algae were often discussed in the literature. One assumption is that it can reduce the density of the organism and therefore improve the buoyancy of the cells (Reynolds 2007). Moreover, a mucilaginous sheath can serve as a defense against grazing. The particle may be too large for microzooplankters to ingest, more difficult for mesoplankter raptors to grasp or less filterable for filter-feeding plankters (Reynolds 2007). And even if the cells are ingested, the mucilage protects the cells during digestion so that they can survive the passage through the consumers gut (Porter 1976). On the other side, the mucilaginous envelope could act as microhabitat for a bacterial flora. Heterotrophic bacteria can produce substances which provide the algae with additional nutrients or act as stimulatory effects (Cole 1982). Another point is that algae are able to uptake nutrients from the boundary layers of the cell. A mucilaginous coat might be an inexpensive way to increase the prospect of encounters with nutrients molecules in nutrient dilute environments (Reynolds 2007). Furthermore, the selective permeability of the mucilage can serve as an advantage against metal poisoning. Studies suggest that an interaction of the –OH groups in the polysaccharides with toxic cations (e.g. aluminum) could play an important role in reducing heavy metal poisoning (Freire-Nordi et al. 1998).

Short overview about the history of the systematics in *Dictyosphaerium*

The genus *Dictyosphaerium* was established in 1849 on the basis of *Dictyosphaerium ehrenbergianum* Nägeli with its oval cell form and the connection of the mucilaginous stalks at the broader side of the cells (Nägeli 1849). Nägeli's system of orders for single celled algae was based on four major criteria: the different modes of reproduction, form of growth, pigment content and morphological formation of the cell content (Nägeli 1849). Based on these criteria, he erected eight orders and placed the new genus *Dictyosphaerium* to the Palmellaceae. The production of mucilage and the connection of the cells via mucilaginous stalks gave the genus a special position in different systematic concepts during the last centuries. After the placement into the Palmellaceae (Nägeli 1849, Rabenhorst 1863) it was placed into several families such as the Tetrastromataceae (Wille 1897), Protococcaceae (West 1904), Oocystaceae (Allorge & Allorge 1930) or Scenedesmeceae (Collins 1909, Brunthaler 1915). However, several of the authors classified the genus *Dictyosphaerium* into the special subfamily Dictyosphaeriaceae, originally created by De-Toni (1889) as member of the Palmellaceae. West (1916) raised this subfamily to the status of an independent family which was later used by Komárek & Perman (1978) and Melkonian (1989), too. Unfortunately, the name has already been used for marine siphonous green algae ("Dictyosphaerieae Kütz. 1849") and is therefore invalid (Silva 1970). According to Silva (1970) the correct name of the family should be Botryococcaceae Wille 1909.

The combination of the different morphological characters led to the description of more than 20 species. In a first attempt to revise the genus, Komárek & Perman (1978) reduced the number to eleven species within the genus. Later, the description of new species from Cuba, Antarctica and former Czechoslovakia increased the number of species to 16 (Hindák 1980, Comas 1996, Ling & Seppelt 1998). Hindák (1988) questioned the placement of taxa with the *Dictyosphaerium* morphotype without pyrenoids as members of the genus. He considered the absence or presence of pyrenoids as genera specific characters and transferred the pyrenoid-less species to the genus *Pseudodictyosphaerium* Hindák. *Pseudodictyosphaerium* consists of picoplanktonic or nanoplanktonic species with a colonial or solitary life-form. The main differences to *Dictyosphaerium* are the absence of a pyrenoid and the irregularly branched gelatinous stalks.

Considerable doubts are over the significance of absence or presence of pyrenoids as generic criteria (John & Tsarenko 2002). The main morphological criterion to distinguish the uniflagellate genera *Chloromonas* Gobi from *Chlamydomonas* Ehrenberg was the absence of pyrenoids (Wille 1903). Recent molecular analyses showed that some strains of

Chlamydomonas and *Chloromonas* belong to the same clade, questioning the delineation based on pyrenoids (Pröschold et al. 2001). Nozaki et al. (1998) demonstrated on the example of *Chlorogonium* Ehrenberg that the occurrence of a pyrenoid can be depend on culture conditions. They could show that a pyrenoid can be observed if the strains were grown under photoautotrophic conditions in mineral media. The pyrenoid was absent under heterotrophic conditions and therefore without taxonomic relevance. Whether the pyrenoid has taxonomic significance in case of *Dictyosphaerium* needs to be clarified.

Aims and hypotheses of this study

The aim of this thesis was to reveal the phylogenetic relationship of green algae with the *Dictyosphaerium* morphotype. Comparative examinations of clonal strains of different *Dictyosphaerium* species from diverse habitats and biogeographical regions were necessary to cover as many inter- and intra-specific variations as possible.

This thesis addressed the following hypotheses:

- ***Dictyosphaerium* is a member of the Chlorellaceae**

In contrast to traditional morphological concepts which placed the colonial *Dictyosphaerium* to the Botryococcaceae, our molecular analyses showed a phylogenetic affinity to the Chlorellaceae formerly characterized exclusively as solitary. The polyphasic approach of morphological investigations, molecular analyses and secondary structure information proposed a first step towards a modern generic concept of chlorellacean algae (Chapters 2, 3).

- **The *Dictyosphaerium* morphotype is of polyphyletic origin**

Possession of mucilage results in different ecological advantages. Responding to these advantages, during the evolutionary process, mucilage bearing morphotypes evolved independently and repeatedly. The molecular analyses confirmed the polyphyletic origin of the *Dictyosphaerium* morphotype (Chapter 4). To outline the phylogeny of the genus *Dictyosphaerium* it was necessary to establish an epitype for the type species and to delineate other genera such as *Parachlorella*, *Closteriopsis* and *Dicloster*.

- **A high genetic diversity is hidden within morphological species**

Common species in inland waters like *D. ehrenbergianum*, *D. pulchellum* and *D. tetrachotomum* have a cosmopolitan distribution and occupy a huge range of different habitats. Since green microalgae are organisms whose genetic diversity is sometimes much higher than their simple morphology suggests, these species are supposedly complexes of genetic species of various genera, evolved through morphological convergence at different phylogenetic positions (Chapter 5, 6).

- **Some morphological criteria traditionally utilized in taxa distinction are phylogenetically irrelevant**

Morphological criteria like mucilage production and cell wall appendages are not suitable for distinguishing phylogenetic lineages. Molecular investigations of *Coronastrum ellipsoideum* proved this assumption, especially with regard to its relationship with the *Dictyosphaerium* morphotype (Chapter 7).

- **Some species of *Chlorella* are colonial with a mucilaginous envelope**

Chlorella is one of the most famous microalgal genera worldwide. Due to a scarcity of morphological features it is difficult to distinguish species. To test whether molecular signatures (barcodes) can lead to an easy identification of *Chlorella* species on the molecular level, the CBC approach was compared with Moniz & Kaczmarska`s (2010) barcode concept, revealing new species with mucilage envelopes (Chapter 8).

- **The absence of pyrenoids is of generic significance in distinguishing *Dictyosphaerium* from *Pseudodictyosphaerium***

The inclusion of *Dictyosphaerium*-like algae into the genus *Pseudodictyosphaerium* based on the absence of pyrenoids as proposed by Hindák (1988) was often questioned, since the development of pyrenoids can be culture dependent. To corroborate this hypothesis, *Pseudodictyosphaerium* strains were tested with molecular tools to recover their peculiar phylogenetic position (Chapter 9).

- **Solitary versus colonial life form have no diacritic relevance**

Morphological features like a colonial versus a solitary life form were used to separate the picoplanktonic genera *Mychonastes* and *Pseudodictyosphaerium*. Since environmental conditions influence the morphology of green algae, it was assumed that the colonial life form is a phenotypic adaptation and of no systematic relevance (Chapter 9).

Chapter 2

Generic concept in *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae)

S. 11-20

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Chapter 3

**Polyphyletic origin of bristle formation in Chlorellaceae:
Micractinium, *Didymogenes* and *Hegewaldia* gen. nov. (Trebouxiophyceae,
Chlorophyta)**

S. 21-29

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Chapter 4

Polyphyletic origin of the *Dictyosphaerium*-morphotype within Chlorellaceae (Trebouxiophyceae)

S. 30-35

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Chapter 5

Two new *Dictyosphaerium*-morphotype lineages of the Chlorellaceae (Trebouxiophyceae): *Heynigia* gen. nov. and *Hindakia* gen. nov.

S. 36-47

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<http://www.informaworld.com/smpp/content~db=all~content=a926374460~frm=titlelink>

Chapter 6

Updating the genus *Dictyosphaerium* and description of *Mucidosphaerium* gen. nov. (Trebouxiophyceae) based on morphological and molecular data

S. 48-74

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Chapter 7

Phylogenetic relationship of *Coronastrum ellipsoideum* and allied species

S. 75-90

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Chapter 8

Taxonomic reassessment of the genus *Chlorella* (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species

S. 91-111

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Chapter 9

**Taxonomic reassessment of the genus *Mychonastes* (Chlorophyceae, Chlorophyta)
including the description of eight new species**

S. 112-130

This Chapter is published as an original article in *Phycologia*.

The original article is online available at <http://www.phycologia.org/loi/phy>

10 General discussion

The systematics of green algae have undergone major rearrangements due to an increase in available tools for investigations over time. One of the newest developments, the molecular phylogenetic analyses based on different gene markers, resulted in an immense change in the understanding of the evolutionary history of green algae (Chapman et al. 1998, Lewis & McCourt 2004, Pröschold & Leliaert 2007).

Apart from the higher-level classification of algae, great discrepancies are observed by comparing molecular results with morphological features in families, genera and species (Nozaki & Krienitz 2001, Buchheim et al. 2005, McManus & Lewis 2005). DNA sequence data show that morphological characters are poor indicators of phylogenetic relationships in many cases (Huss et al. 1999, Rindi et al. 2009b). This thesis demonstrates how molecular results can reshape the classification and understanding of genera and species delineation of mucilaginous spheres.

Dictyosphaerium in the system of Chlorellaceae

The family Chlorellaceae is morphologically defined as comprising solitary or seldom in colonies living green algae which reproduce mainly by autospores. Their cells are in rare cases covered with a very fine gelatinous envelope and are always without bristles (Komárek & Fott 1983). Molecular phylogenetic studies on SSU and ITS rRNA gene sequences demonstrate that the traditional classification of the Chlorellaceae based on this morphological criteria is artificial and genera and species need to be revised (Huss et al. 1999, Wolf et al. 2002, Neustupa et al. 2009, Darienko et al. 2010). Based on molecular phylogeny, a partition of the Chlorellaceae into two sister clades is proposed by Krienitz et al. (2004): the *Chlorella* clade and the *Parachlorella* clade. In addition to molecular investigations, biochemical analysis on sterol composition as well as differences in the ultrastructures of the cell wall support this separation in many parts (Yamamoto et al. 2005, Görs et al. in press). Comparative analyses on morphology and molecular phylogeny reveal that former members of different families and with a high range of morphological characters cluster in both clades (Chapter 2).

The *Parachlorella* clade comprises solitary taxa like *Parachlorella* (with or without mucilage) and *Marinichlorella* (Krienitz et al. 2004, Aslam et al. 2007), needle shaped taxa like *Closteriopsis* (formerly Selenastraceae) and coenobial ellipsoid like *Dicloster* (formerly Scenedesmaceae) (Hegewald & Hanagata 2000, Ustinova et al. 2001, Krienitz et al. 2004). Within the *Chlorella* clade, simple organisation types like the single celled *Chlorella* are

closely related to the colonial, bristle exhibiting *Micractinium* (formerly Micractiniaceae), the coenobial *Didymogenes* (with or without bristles; formerly Scenedesmaceae), *Actinastrum* (ellipsoidal, coenobial; formerly Coelastraceae), *Meyerella* (single celled, without pyrenoids) and *Hegewaldia* (facultative oogamic) (Wolf et al. 2002, Krienitz et al. 2004, Fawley et al. 2005, Luo et al. 2010, Pröschold et al. 2010). Also one strain with the *Dictyosphaerium* morphology was detected within the *Chlorella* clade (Krienitz et al. 2004). The genus *Dictyosphaerium* was morphological classified into the same family than colonial and mucilaginous oil-algae of the genus *Botryococcus* Kützing (Botryococcaceae; Komárek & Fott 1983). This study tries to reveal the phylogenetic position of *Dictyosphaerium* and its close relatives.

Genera and species delineation of *Dictyosphaerium* morphotypes

The phylogenetic position of a genus is determined by the lineage containing the type species. Since no authentic strain from any described *Dictyosphaerium* species is available, it was necessary to designate epitypes for the analysed species. The newly designated epitype of the type species *Dictyosphaerium ehrenbergianum* Nägeli forms an independent lineage within the *Parachlorella* clade (Chapter 4; Krienitz et al. 2010). This assigns the genus *Dictyosphaerium* to the *Parachlorella* clade of the Chlorellaceae (Chapter 6; Bock et al. 2010). Molecular analyses confirmed further, that the *Dictyosphaerium* morphotype is of polyphyletic origin within the Chlorellaceae. Figure 1 shows an overview of the Chlorellaceae (all strains sequenced in this study are marked in bold). The mucilaginous morphotype evolves in new lineages within the *Chlorella* and *Parachlorella* clades and is also present in established genera like *Chlorella* and *Coronastrum* (clades with the *Dictyosphaerium* morphotype are coloured) (Chapter 7). Surprisingly, this high genetic range does not correspond to a high morphological diversity. For instance, two new lineages of *Dictyosphaerium* morphotypes are closely related to the epitype of *D. ehrenbergianum* with its oval cells and the attachment of the stalks at the broader side of the cells. These taxa show only small morphological differences compared to *D. ehrenbergianum*, but considerable changes in the ITS secondary structure. According to Coleman's CBC approach (Coleman 2007), they should be addressed as different species (Chapter 6). Convergent evolution is also observed for the spherical morphotype of *Dictyosphaerium*. Two typical species with spherical cells are *D. pulchellum* and *D. sphagnale*. Phylogenetic studies on new assigned epitypes establish *D. pulchellum* and *D. sphagnale* as members of the new genus *Mucidosphaerium* in the *Parachlorella* clade (Chapter 6).


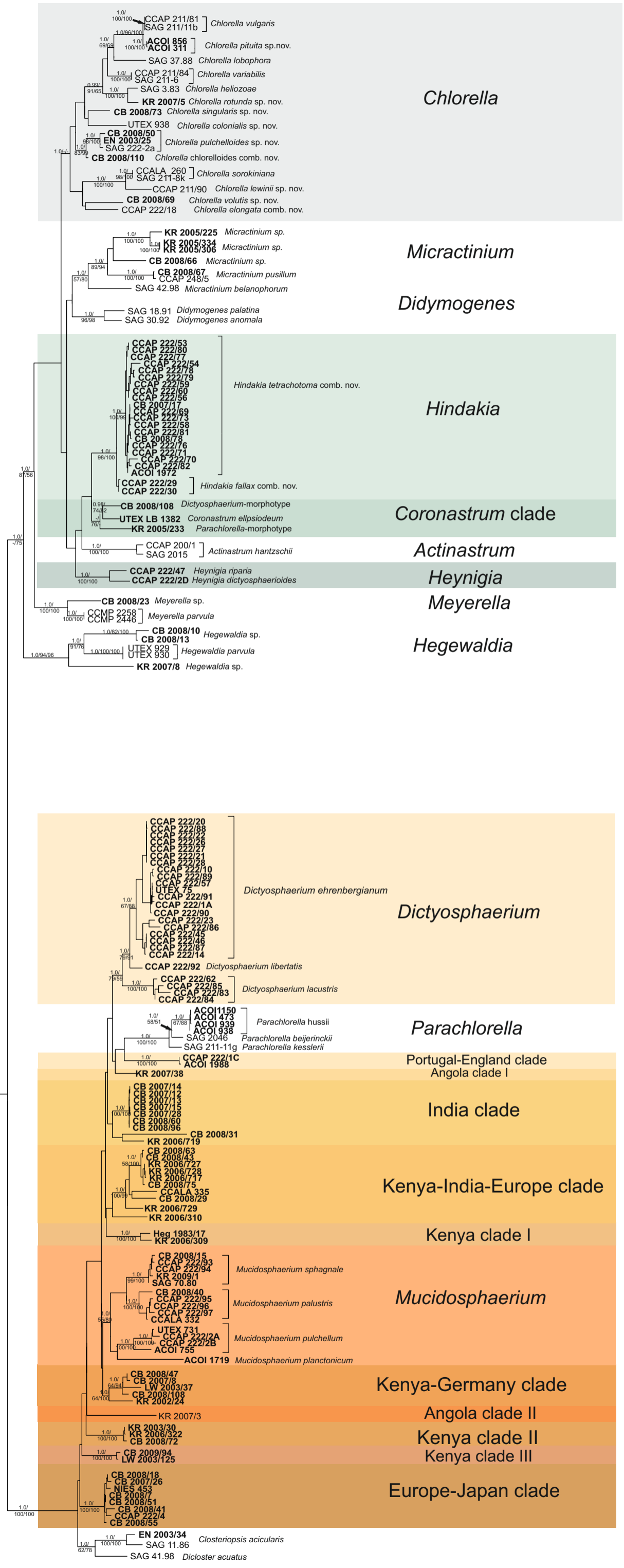


Figure 1. Rooted maximum likelihood tree of the Chlorellaceae inferred from a concatenated set of SSU and ITS rRNA gene sequences of 159 taxa and 2525 aligned base positions. Support values: posterior probabilities (MB; >0.95; from a partitioned dataset using MrBayes), maximum likelihood analyses (ML; from a partitioned dataset using Treefinder, 100 replicates) and maximum parsimony analyses (MP, 1000 replicates). Strains sequenced in this study are bold, clades where the *Dictyosphaerium* morphotype evolved are coloured.



Chlorella clade

Parachlorella clade

0.1 substitutions/site

MB ML/MP

In addition, the spherical morphotype clusters in the *Chlorella* clade within the new genus *Heyniga* (Chapter 8) and, more surprisingly, as sister to *Chlorella vulgaris* (*C. pituita* sp. nov. and *C. pulchelloides* sp. nov.). These examples confirm that several morphological criteria may be the result of convergent evolution and phenotypic adaptations to ecosystem conditions which do not reflect phylogenetic relationships. Several molecular studies on different green algae observed the similar pattern that the genetic diversity can be much higher than simple morphology suggested (Potter et al. 1997). Common genera like *Chlorococcum* Meneghini (Buchheim et al. 2002), *Klebsormidium* P.C. Silva, K. Mattox & W. Blackwell (Mikhailyuk et al. 2008) or *Trentepohlia* Martius (Rindi et al. 2009b) are polyphyletic.

Whether the natural system is more precisely circumscribed by small or large genera in coccoid green algae is still under discussion and should be considered for each group independently (An et al. 1999, Fawley et al. 2005). Most views on this topic are in agreement that one and the same system should be applied to closely related lineages (Rindi et al. 2009b). In case of the Chlorellaceae, the concept of genera in the *Parachlorella* clade should be congruent with the concept in the *Chlorella* clade.

As Figure 1 shows, the *Dictyosphaerium* morphotype clusters between morphological well-delineated genera like *Actinastrum* or *Didymogenes*, but also intermixing with members of traditional genera like *Chlorella* or *Coronastrum* (Chapters 1, 7; Krienitz et al. 2004). To delineate closely related genera, differences in the ITS-2 are taken into account as well (Marin et al. 2010). Analyses of the ITS-2 helices between the type species of the genera *Chlorella* (*C. vulgaris*) and *Micractinium* (*M. pusillum*) revealed 14 CBCs; between *Didymogenes* and *Actinastrum* only seven. By comparing the type species of the genera *Parachlorella* (*P. beijerinckii*) and *Dictyosphaerium* (*D. ehrenbergianum*), already 18 CBCs are observed. If this system is applied to the new lineages, several new genera and species evolve, delineated mainly by generic characteristics. It further implies, that the generic description of *Dictyosphaerium* is probably too broadly circumscribed. Only strains with oval cell form and the attachment to the stalks at their longer side remain within the genus at the moment (Chapter 6). This would suggest, that the remaining species would form new lineages as independent genera with a high probability. This is proven for *D. tetrachotomum*, which belongs to the new genus *Hindakia* within the *Chlorella* clade (Chapter 5). Unfortunately, the correlation of a morphological defined species to one clade can not be applied to all species. The spherical morphotype appears in several new lineages within the *Chlorella* and *Parachlorella* clades and only small morphological changes, like cell size and marginal

differences in cell shape are present (Chapters 5, 6, 8). Previously to our molecular analyses, these strains would probably be identified as one and the same species (Chapter 5; Komárek & Fott 1983). Due to the CBC approach of Coleman (2007), species are delineated within the different clades, even if morphological criteria are scarce. The Canadian Barcode Initiative tries to accomplish a similar goal (Hajibabaei et al. 2007). They propose to use a short highly variable DNA sequence to distinguish between closely related taxa. This might be especially helpful for environmental surveys and high throughput sequencing, where the diversity of a sample is assessed by sequencing clones without collecting the corresponding morphological data. Moniz and Kaczmarska (2010) introduced a barcoding system for diatoms based on 5.8S and parts of the ITS-2. This concept can be applied to the genus *Chlorella*, revealing the occurrence of colonial strains with mucilage envelopes within this genus. The delineation of species by barcoding matched the outline according to the CBC concept. Our results stress further the importance of thorough fully sampled and well characterized groups. Otherwise, a clear statement about the morphology and ecological condition of a certain lineage is not possible.

Pyrenoids as generic characters

In molecular phylogenies inferred from SSU rRNA gene sequences, one clade contains *Dictyosphaerium* strains without pyrenoids (Chapter 9). The discussion about the generic value of pyrenoids is still going on (John & Tsarenko 2002). The presence or absence of pyrenoids as generic criterion was not only used in distinguishing *Dictyosphaerium* from *Pseudodictyosphaerium*, but also in the generic concept of the Selenastraceae (Komárek & Fott 1983, Hindák 1988). Comparative examinations of clonal strains in combination with molecular analyses in Selenastracean algae show the high variability of this criterion and it can not be further used as taxonomical marker in this relationship (Krienitz et al. 2001, Fawley et al. 2005). Investigations on *Chlorogonium* (Haematococcaceae) reveal that the occurrence of pyrenoids depends on culture conditions (Nozaki et al. 1998). It is observed, that one strain of the same species can contain a pyrenoid and the next one not, like in *Chloromonas reticulata* (Pröschold et al. 2001). In 1988, Hindák transferred all *Dictyosphaerium* species without pyrenoids (*D. botryella* Komárek & Perman, *D. densum* Hindák, *D. anomalum* Koršikov, *D. elegans* Bachmann) to the genus *Pseudodictyosphaerium*. During my study, I analysed around 200 *Dictyosphaerium* strains, whereof 20 lacked pyrenoids. All strains without pyrenoid cluster together with members of the solitary *Mychonastes* in one clade within the Chlorophyceae. The analyses of the ITS-2 secondary

structure reveal two branched helices in the ITS-2, clearly different from the unbranched ones of the Chlorellaceae (Chapters 4, 9). The phylogenetic tree, inferred from combined 5.8S and ITS-2 data show no clear separation between solitary *Mychonastes* and colonial *Pseudodictyosphaerium* strains. This indicates that the absence of a pyrenoid is of greater generic value than the colonial life form.

Biogeography

Literature reports and our own sampling show that the *Dictyosphaerium* morphotype can be found in freshwater habitats on all major landmasses, e.g. Europe, North America, South America, Africa, Australia and Asia.

The biogeography of microorganism has become a subject of controversial discussions. One main view is based on the tenet introduced by Baas-Becking (1934): “Everything is everywhere, but the environment selects”. It assumes, that free-living eukaryotes (smaller than 1 mm) have a cosmopolitan distribution, occurring wherever their habitat requirements are satisfied. The argument behind this hypothesis is that small size and huge populations allows the production of large amounts of propagules which are capable for long distance transports (Fenchel & Finlay 2003, Finlay & Fenchel 2004). The opposite view believes in a more restricted distribution of microorganisms (Foissner 2006, Coesel & Krienitz 2008). They argue that the cosmopolitan distribution of many species relies on morphological species concepts and misidentifications. The analyses of *Dictyosphaerium* show that some species have a broad distribution, like *D. ehrenbergianum* which was isolated from Germany, UK, Mexico, Kenya, Tunisia and Zambia. More sampling on different continents would probably increase the knowledge of geographical distribution of this species. On the other hand, we see clades with restricted geographical distribution, like the Indian clade (Figure 1). One strain, CCAP 222/3 originally isolated from the South Orkney Islands, Antarctica differs in phylogeny and morphology from all other strains analysed so far. This strain shows the typical colonial life-form of *Dictyosphaerium* but lack a mucilaginous envelope. Phylogenetic analyses placed the strain near *Myrmecia* Printz within the Trebouxiophyceae, outside of the Chlorellaceae. CCAP 222/3 differs morphological from the one species which was described from the polar region, *D. dichotomum* which contains a thin mucilaginous envelope and reproduce only by 2 autospores (Ling & Seppelt 1998).

The large amount of sampled *Dictyosphaerium* strains in combination with morphological examinations, molecular phylogenetic analyses and secondary structure comparison presented in this work, provide new insights into the systematics of green algae. It

is shown, that the genetic diversity of this morphotype is much higher than previously assumed. Some taxa, delineated on morphological basis appear to be polyphyletic, comprising an assemblage of different species which can be clearly distinguished by molecular analyses. Open questions remain in several cases about the branching order of clades. Phylogenetic analyses based on a larger dataset with additional markers, like LSU or rbcL may give a better resolution. Moreover, it would be desirable to find morphological differences between the lineages. A focus on ultrastructural differences or chemical composition may give more attributes in distinguishing between phylogenetic lineages.

11 Summary

Colonial green algae exhibiting the *Dictyosphaerium* morphology are common members in freshwater habitats worldwide. They can be dominating the plankton of lakes and even form blooms eventually. The *Dictyosphaerium* morphotype is characterized by spherical or oval cells which are connected via gelatinous stalks and surrounded by mucilaginous envelopes.

In this thesis, the diversity and phylogenetic relationships of members of the *Dictyosphaerium* morphotype was evaluated by combining molecular phylogenetic analyses based on SSU, ITS-1, 5.8S and ITS-2 rRNA gene sequences, with secondary structure comparisons and morphological examinations.

The molecular analyses showed a polyphyletic origin of the *Dictyosphaerium* morphotype within the Chlorellaceae (Trebouxiophyceae). The molecular studies demonstrated the incongruence between phylogenetic relationships and traditional morphological classifications within the Chlorellaceae. Several ellipsoidal, needle-shaped, coenobial, spined or mucilage-covered taxa formerly grouped in different algal families clustered within the Chlorellaceae. The newly designated epitype-strain of the type species *D. ehrenbergianum* was member of a new phylogenetic subclade within the *Parachlorella* clade of the Chlorellaceae, assigning the genus *Dictyosphaerium* to this clade. Consequently, the generic name *Dictyosphaerium* is only valid for members of this subclade. Next to the epitype, two new lineages evolved which resembled *D. ehrenbergianum* (oval cell form, attached to the gelatinous stalks at their broader side), but differed considerably in their genetic signature. According to the Compensatory Base Change (CBC) approach for delineating taxa, these two new lineages are separate species, even if morphological criteria distinguishing them from *D. ehrenbergianum* are scarce.

Moreover, the mucilaginous morphotype evolved in new lineages within the *Chlorella* and *Parachlorella* clades. *Dictyosphaerium tetrachotomum* with its oval cells is one of the most frequent *Dictyosphaerium* species in European inland waters. The phylogenetic tree revealed a new subclade consisting of this morphotype within the *Chlorella* clade, referring to an independent genus. In addition, several lineages with the spherical morphotype were detected within the *Chlorella* and *Parachlorella* clades. This high genetic range did not correspond to a high morphological distinctness, since only small morphological changes, like cell size and marginal differences in cell shape, were observed between the lineages. This study further revealed that the *Dictyosphaerium* morphotype clustered closely to *Chlorella vulgaris*. By applying Moniz and Kaczmarek's barcoding system on the genus *Chlorella* we

were able to define nine new lineages within the genus, including seven species with mucilage and colonial life form. The delineation of *Chlorella* species by this barcoding system was congruent with the CBC approach.

The phylogenetic study further showed that the *Dictyosphaerium* morphotype evolved not only in the class Trebouxiophyceae but also within the Chlorophyceae. One clade containing *Dictyosphaerium* strains without pyrenoids clustered between members of the solitary genus *Mychonastes*. The phylogenetic tree revealed no clear distinction between solitary and colonial strains. Consequently, all strains were newly combined to *Mychonastes*.

This thesis demonstrated how molecular results can reshape the classification and understanding of conceptions of genera and species in colonial and mucilaginous green algae. Similar morphological traits may be the result of convergent evolution and phenotypic adaptations to ecosystem conditions and do not have to reflect phylogenetic relationships. In addition, our results stressed the importance of thoroughly sampled and well characterized groups to serve as model systems for the construction of the green algae tree of life.

12 Zusammenfassung

Koloniebildende Grünalgen des *Dictyosphaerium*-Phänotyps gehören weltweit zu den typischen Vertretern des Planktons in Süßwasser-Habitaten. In eutrophen Seen können sie die Zusammensetzung des Planktons dominieren und in Extremfällen Massenentwicklungen hervorrufen. Dieser Phänotyp ist geprägt durch runde oder ovale Zellen, die durch gallertartige Stränge miteinander verbunden sind. Die so entstandenen Kolonien sind von einer deutlichen Schleimhülle umgeben.

In dieser Dissertation wurden die Diversität und die Verwandtschaftsbeziehungen des *Dictyosphaerium*-Phänotyps mit Hilfe eines kombinierten Ansatzes aus molekular-phylogenetischen Untersuchungen, Sekundärstrukturanalysen und morphologischen Beobachtungen evaluiert.

Molekulare Untersuchungen haben gezeigt, dass der *Dictyosphaerium*-Phänotyp polyphyletisch innerhalb der Chlorellaceae (Trebouxiophyceae) entstanden ist. Phylogenetische Analysen ergaben dabei große Diskrepanzen zwischen den molekularen Ergebnissen und der traditionellen morphologischen Klassifizierungen innerhalb der Chlorellaceae. Neueste Resultate ergaben, dass Taxa aus verschiedenen Algenfamilien mit ganz unterschiedlicher Morphologie innerhalb der Chlorellaceae evolvierten. So können Vertreter dieser Familie sowohl runde als auch elliptische oder nadelförmige Zellen aufweisen; manche sind bestachelt, kolonial oder von einer Schleimhülle umgeben.

Durch die Designierung eines Stammes als Epitypus für die Typusart *Dictyosphaerium ehrenbergianum* konnte die Gattung *Dictyosphaerium* einer neuen phylogenetischen Linie im *Parachlorella*-Clade der Chlorellaceae zugeordnet werden. Folglich ist der Gattungsname *Dictyosphaerium* nur für Arten gültig, die mit dieser eng verwandt sind. Zwei weitere phylogenetische Linien entwickelten sich neben der des Epityps, deren Stämme *D. ehrenbergianum* morphologisch stark ähneln (ovale Zellen, Verbindung mit den Gallertsträngen an der breiten Seite der Zellen). Allerdings wies die genetische Signatur komplementäre Basenaustausche (CBCs) innerhalb der konservierten Regionen der ITS-2 auf. Demzufolge entsprachen diese phylogenetischen Linien nach Colemans CBC Konzept zur Artdifferenzierung unabhängigen Arten, auch wenn die morphologischen Unterschiede nur gering waren.

Eine der häufigsten phänotypischen *Dictyosphaerium*-Arten in europäischen Binnengewässern ist *D. tetrachotomum* mit ovalen Zellen, die an den Spitzen mit Gallertsträngen verbunden sind. Molekulare Untersuchungen zeigten, dass dieser Phänotyp

innerhalb einer neuen Linie im *Chlorella*-Clade der Chlorellaceae evolvierte und damit wurde er der neuen Gattung *Hindakia* zugeordnet. Des Weiteren konnten mehrere unabhängige phylogenetische Linien mit *Dictyosphaerium*-artigen Phänotypen und runden Zellen innerhalb der *Chlorella*- und *Parachlorella*-Clades entdeckt werden. Basierend auf ihrer phylogenetischen Position und molekularen Charakteristiken wurden neue Gattungen und Arten beschrieben. Dieses hohe genetische Spektrum spiegelte sich hingegen nicht in einer hohen morphologischen Vielfalt wieder. Nur geringe Unterschiede in Zellgröße und Zellform konnten zwischen den einzelnen Linien aufgezeigt werden, wodurch eine morphologische Identifikation erschwert wurde.

Überraschenderweise wurde eine nahe Verwandtschaft des *Dictyosphaerium*-Phänotyps zur solitären und schleimlosen Art *Chlorella vulgaris* nachgewiesen. Durch die Anwendung des Barcoding-Systems nach Moniz und Kaczmarek wurden innerhalb der Gattung *Chlorella* neun neue Linien aufgezeigt, wobei sieben davon koloniale Arten mit Schleimhülle darstellen. Die Abgrenzung sämtlicher *Chlorella*-Arten mit Hilfe des Barcoding-Systems erfolgte übereinstimmend mit dem CBC Artdifferenzierungskonzept. Basierend auf diesen Erkenntnissen musste das Gattungskonzept von *Chlorella* emendiert werden, um koloniale und schleimbildende Arten mit einzubeziehen.

Weiterhin zeigten die phylogenetischen Studien, dass der *Dictyosphaerium*-Phänotyp auch innerhalb der Klasse der Chlorophyceae evolvierte. Sämtliche pyrenoidfreien *Dictyosphaerium*-Stämme (von Hindák zu *Pseudodictyosphaerium* gestellt) formten ein gemeinsames Cluster mit solitären Vertretern der Gattung *Mychonastes*. Diese phylogenetischen Untersuchungen zeigten keine klare Gruppierung zwischen den solitären und kolonialen Stämmen, aufgrund dessen wurden die *Pseudodictyosphaerium*-Arten in die Gattung *Mychonastes* überführt.

Vorliegende Ergebnisse zeigen, in welchem Maße molekulare Resultate unser Verständnis über die Klassifizierung und die Konzepte zur Gattungs- und Artabgrenzung von kolonialen und schleimbildenden Grünalgen verändern können. Viele morphologische Merkmale sind das Resultat konvergenter Evolution und phänotypischer Anpassung an die Bedingungen im Ökosystem und stellen keine direkte phylogenetische Verwandtschaft dar. Diese Erkenntnisse verdeutlichten weiterhin die Bedeutung von gut untersuchten und genau charakterisierten Algengruppen als Modelle für die Konstruktion des Stammbaumes der Grünalgen.

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List of relevant publications this work is based upon with declaration

This thesis is based on the following original articles and manuscripts.

- I** Luo, W., Pröschold, T., Bock, C. & Krienitz, L. 2010. Generic concept in *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). *Plant Biology* 12: 545-553.
- II** Pröschold, T., Bock, C., Luo, W. & Krienitz, L. 2010. Polyphyletic origin of bristle formation in Chlorellaceae: *Micractinium*, *Didymogenes* and *Hegewaldia* gen. nov. (Trebouxiophyceae, Chlorophyta). *Phycological Research* 58: 1-8.
- III** Krienitz, L., Bock, C., Luo, W. & Pröschold, T. 2010. Polyphyletic origin of the *Dictyosphaerium* morphotype within Chlorellaceae (Trebouxiophyceae). *Journal of Phycology* 46: 559-563.
- IV** Bock, C., Pröschold, T. & Krienitz, L. (2010). Two new *Dictyosphaerium*-morphotype lineages of the Chlorellaceae (Trebouxiophyceae): *Heynigia* gen. nov. and *Hindakia* gen. nov. *European Journal of Phycology* 45: 267-277.
- V** Bock, C., Pröschold, T. & Krienitz, L. (under review). Updating of the genus *Dictyosphaerium* and description of *Mucidosphaera* gen. nov. (Trebouxiophyceae) based on morphological and molecular data. Submitted to *Journal of Phycology*.
- VI** Bock, C., Pažoutová, M. & Krienitz, L. (under review). Phylogenetic relationship of *Coronastrum ellipsoideum* and allied species. Submitted to *Biologia*.
- VII** Bock, C., Krienitz, L. & Pröschold, T. (under review). Taxonomic reassessment of the genus *Chlorella* (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. Submitted to *Plant Biology*.
- VIII** Krienitz, L., Bock, C., Dadheech, P.K. & Pröschold, T. (in press). Taxonomic reassessment of the genus *Mychonastes* (Chlorophyceae, Chlorophyta) including the description of eight new species. *Phycologia*.

List of relevant publications

All articles are written by the first author. The contributions of the co-authors are as follows:

- I** Luo Wei and Lothar Krienitz initiated and coordinated the study. Sequencing, alignment, phylogenetic analyses and secondary structure comparison was contributed by Christina Bock, Thomas Pröschold and Luo Wei. Results were interpreted and discussed by all authors. Christina Bock contributed 20% to this paper.
- II** Thomas Pröschold and Lothar Krienitz initiated the study. Sequencing, alignment, phylogenetic analyses and secondary structure comparison was contributed by Christina Bock, Thomas Pröschold and Luo Wei. Results were interpreted and discussed by all authors. Christina Bock contributed 20% to the paper.
- III** Lothar Krienitz initiated the study and contributed morphology and taxonomy. Sequencing, alignment and phylogenetic analyses was contributed by Christina Bock under guidance of Thomas Pröschold. Secondary structure comparison was contributed by Thomas Pröschold and Christina Bock. Luo Wei provided three sequences and strains. Results were interpreted and discussed by all authors. Christina Bock contributed 30% of the work to the paper.
- IV** Christina Bock and Lothar Krienitz initiated the study. Sequencing, alignment, phylogenetic analyses and secondary structure comparison were carried out by Christina Bock under guidance of Thomas Pröschold. Morphology and taxonomy was contributed by Christina Bock. Results were interpreted and discussed by all authors. Christina Bock contributed 90% of the work to the paper.
- V** Christina Bock and Lothar Krienitz initiated the study. Sequencing, alignment, phylogenetic analyses and secondary structure comparison was done by Christina Bock under guidance of Thomas Pröschold. Isolation of strains, morphology and taxonomy was contributed by Christina Bock. Results were interpreted and discussed by all authors. Christina Bock contributed 90% of the work to the paper.
- VI** Christina Bock and Lothar Krienitz initiated the study. Sequencing, alignment, phylogenetic analyses and secondary structure comparison were carried out by Christina Bock. Marie Pažoutová provided three sequences. Morphology and taxonomy was contributed by Christina Bock and Marie Pažoutová. Results were interpreted and discussed by all authors. Christina Bock contributed 85% of the work to this manuscript.
- VII** Christina Bock and Lothar Krienitz initiated the study. Sequencing, alignment, phylogenetic analyses, secondary structure comparison morphology and taxonomy was contributed by Christina Bock. Results were interpreted and discussed by all authors. Christina Bock contributed 90% of the work to this manuscript.
- VIII** Lothar Krienitz initiated the study and contributed morphology and taxonomy. Sequencing, alignment, phylogenetic analyses and secondary structure comparison was contributed by Christina Bock and Thomas Pröschold. Pawan Dadheech provided five strains and guided the field work in India. Results were interpreted and discussed by all authors. Christina Bock contributed 35% of the work to this paper.

The work was conducted at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Stechlin-Neugolobsow) in the frame of the project: “The *Dictyosphaerium*-morphotype of green algae – its paraphyletic origin and variability in response to ecosystem conditions”, granted by the Deutsche Forschungsgemeinschaft (DFG) KR1262/11-1, 2.

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Curriculum Vitae

Der Lebenslauf ist in der Online-Version
aus Gründen des Datenschutzes nicht enthalten