**Palacký University, Faculty of Science**

Department of Botany

# Biology and diversity of desmids

Petra Mazalová

**Ph.D. thesis**

Olomouc, 2013

Supervised by: **Prof. RNDr. Aloisie Poulíčková, CSc.**, Department of Botany, Palacký University

I hereby declare that this thesis is my independent work, using the listed references, or in co-operation with co-authors of the papers. I did not submit any of the theses or their any part to acquire any other academic title.

April 2013, Olomouc

................................

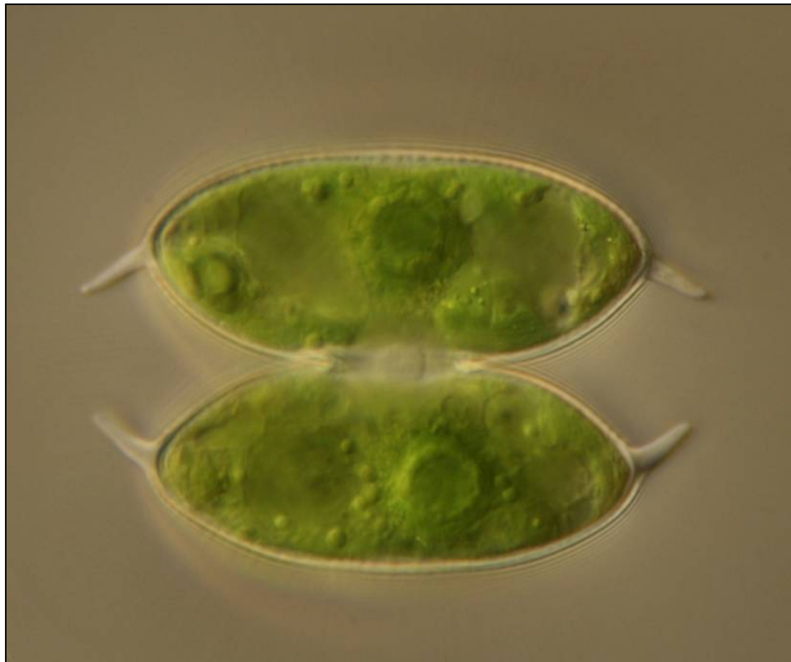
Mgr. Petra Mazalová

## Motto:

„Only nature knows what it wants. It never makes jokes and never makes mistakes. Those make only men."

„In nature we never see anything isolated, but everything in connection with something else which is before it, beside it, under it and over it."

(Johann Wolfgang von Goethe)



(*Staurodesmus convergens*; foto: P. Mazalová)

## Acknowledgements

I would like to thank my supervisor, Aloisie Poulíčková, for her systematic guidance, collaboration, support and inspiration. I also express my appreciation to colleagues from our algological laboratory for their contribution to a good working atmosphere and for helpful advice whenever I needed it. Finally I kindly thank my family for their unwavering support throughout my life.

## Abstract

Higher plants evolved from algae, and still share many common characteristics. However, studies on biology and diversity of algae lag behind those of higher plants. In this thesis, I have investigated the phylogenetic, morphological and geographical diversity of desmids and explored the DNA content variations for better understanding of the importance of polyploidy in their evolution. In addition, I propose there, a multi-step protocol that leads to the quantification of DNA content in desmids and some other microalgae using flow cytometry.

In the course of the study of nine mires in Central and Northern Moravia, altogether 109 desmid taxa were found. Five of those taxa are considered to be new records for the Czech Republic, 42 of them are new records for Moravia. The findings were discussed with previous records from Moravia and Czech Republic. Moreover, the character and origin of the unique locality Slavkov mire were discussed. The results indicate a lack of knowledge of desmid diversity in the Moravia region.

A pair of well-known desmid species, *Micrasterias rotata* and *M. fimbriata*, were tested for their phylogenetic and morphological homogeneity as well as their geographic distribution. The investigation was based on morphological and geometric morphometric studies, with genetic analyses of the $trnG^{ucc}$ sequences of strains, isolated mainly from Europe. *Micrasterias rotata* proved to be phylogenetically homogenous across Europe, while *M. fimbriata* turned out to be composed of two firmly delimited lineages. The lineages differ genetically as well as in their morphology. There are also differences in their geographic distribution patterns and likely in their ecological preferences. It has been suggested to describe one lineage as a new species of the genus *Micrasterias*.

Nuclear DNA content was measured in microalgae with a focus on desmids, using a new protocol based on flow cytometry measurements. To obtain the protoplasts, a mixture of enzymes (cellulase and macerozyme) was used. The nuclei suspension was prepared using LB01 nuclear isolation buffer, and nuclei were stained by propidium iodide. The leaves of four plant species were used as standards, and one new microalgal standard was suggested. The protocol proved to be useful for desmids and also worked for some Chlorophyta and Heterokontophyta. Optimization of enzyme mixture or nuclear isolation buffer for measuring other microalgae, as in those we did not succeed in measuring, deserves more attention.

The nuclear DNA content of 34 *Micrasterias* strains (25 *Micrasterias* species) was measured. Intraspecific and interspecific variation was observed and estimated DNA contents were mapped along the phylogenetic tree, based on concatenated SSU rDNA, psaA, and

coxIII alignment. The related species were mostly similar in their DNA content. Strong correlation between the absolute nuclear DNA content and chromosome numbers in species, for which the data were available, was found. Significant positive correlation between the DNA content and cell size of the investigated *Micrasterias rotata* strains was also found. In conclusion the evolutionary significance of genome size variation was confirmed in the genus *Micrasterias* and the importance of natural polyploidization in the evolution and speciation of it was verified.

# *Abstrakt*

*Vznik a vývoj vyšších rostlin úzce souvisí s řasami, vyšší rostliny s řasami doposud sdílejí mnoho společných znaků. Nicméně studie biologie a diverzity řas pokulhávají za rozsáhlostí studií vyšších rostlin. V této disertační práci byly zkoumány fylogenetická, morfologická a geografická diverzita krásivek a rozdíly v množství jejich jaderné DNA, za účelem lepšího pochopení významu polyploidie v jejich evoluci. Navíc zde navrhuji protokol pro měření množství jaderné DNA u krásivek a několika dalších mikrořas, a to pomocí průtokové cytometrie.*

*Během studia devíti mokřadních lokalit střední a severní Moravy bylo nalezeno celkem 109 taxonů krásivek. Pět z těchto taxonů je nových pro území ČR a 42 pro území Moravy. Byly diskutovány předchozí nálezy všech taxonů na území Moravy či celé České republiky. Zároveň byl zvážen charakter a původ unikátní lokality nacházející se poblíž vesnice Slavkov. Získaná data naznačila nízkou prozkoumanost krásivek na území Moravy.*

*Dva dobře známé druhy krásivek, Micrasterias rotata a M. fimbriata, byly zkoumány z pohledu jejich fylogenetické a morfologické homogenity a geografického rozšíření. Výzkum byl proveden pomocí morfologických a morfometrických analýz, spolu s fylogenetickou analýzou* $trnG^{ucc}$ *kmenů z různých lokalit Evropy. Micrasterias rotata se ukázala být fylogeneticky homogenní v rámci Evropy, zatímco M. fimbriata se rozdělila do dvou dobře podpořených linií. Populace těchto linií se lišily kromě fylogenetické analýzy také morfologickými a morfometrickými daty. Byly zjištěny rozdíly v jejich geografickém rozšíření a pravděpodobně rozdílné ekologické nároky. Na základě výsledků bylo navrženo popsat jednu z těchto linií jako nový druh rodu Micrasterias.*

*Pomocí průtokové cytometrie bylo měřeno množství jaderné DNA u krásivek a několika dalších mikrořas. Byl použit nový protokol vzniklý na základě poznatků z měření u vyšších rostlin. Pro získání protoplastů byla použita směs enzymů celulázy a mecerozymu. Suspenze jader byla získána použitím LB01 lyzačního pufru a následně byla jádra obarvena propidium jodidem. Jako standard byly použity listy čtyř různých rostlin a byl navržen nový standard z řad mikrořas. Protokol se ukázal vhodný pro použití u krásivek a fungoval i u několika zástupců z řad Chlorophyta a Heterokontophyta. Optimalizace enzymatické směsi či lyzačního pufru pro jiné skupiny mikrořas, a pro ty, u kterých nebyla metoda úspěšná, si zaslouží další pozornost.*

*U 34 kultur (25 druhů) rodu Micrasterias bylo změřeno množství jaderné DNA. Byla sledována mezidruhová a vnitrodruhová variabilita tohoto množství a zjištěné hodnoty DNA*

*byly namodelovány na fylogenetický strom založený na propojení analýz SSU rDNA, psaA a coxIII. Příbuzné druhy vykázaly podobná množství DNA. U druhů, pro které byla známa data o počtu chromozomů, byla zjištěna silná pozitivní korelace mezi množstvím DNA a počtem chromozomů. Pozitivní korelace se projevila i mezi množstvím DNA a rozměry buněk u sledovaného druhu Micrasterias rotata. Experimenty poskytly důkaz významu změny velikosti genomu v evoluci rodu Micrasterias a potvrdily důležitost přirozené polyploidizace v jeho evoluci a speciaci.*

# Contents

**1 Introduction** ... 10

**1.1 Systematic delimitation of desmids** ... 10

1.1.1 *Position of desmids within Plantae* ... 10

1.1.2 *Desmid classification* ... 11

**1.2 Basic characteristics of desmids: an outline** ... 12

1.2.1 *Main morphological features of desmids* ... 12

1.2.2 *Reproduction of desmids* ... 13

1.2.3 *Ecological preferences of desmids* ... 14

1.2.4 *Genus Micrasterias* ... 14

**1.3 Polyphyletism and cryptic diversity within desmids** ... 15

**2 Aims of the thesis** ... 17

**3 Conclusions and future prospects** ... 18

**3.1 Desmid flora of mires in Central and Northern Moravia** ... 18

**3.2 Delimitation of *Micrasterias fimbriata* and *M. rotata*** ... 19

**3.3 Quantification of DNA content in microalgae using flow cytometry** ... 20

**3.4 DNA content in evolution of the genus *Micrasterias*** ... 21

**4 Literature cited** ... 23

**5 List of papers included in the thesis** ... 30

**Curriculum vitae – Mgr. Petra Mazalová**

# 1 Introduction

The thesis is an investigation of different desmid species/genera (Zygnematophyceae, Streptophyta), their diversity, spatial distribution and taxonomy. Presented studies are based on a complex/polyphasic approach, combining classic morphological characters together with molecular data and cytology. Although application of sophisticated methods like flow cytometry (Papers III, IV) shifts desmid research significantly, classic floristic data are still valuable (Paper I). Special attention was paid to the genus *Micrasterias* (Papers II, IV), which is a well known model organism (Meindl 1993; Weiss et al. 1999; Neustupa & Šťastný 2006; Neustupa & Škaloud 2007; Neustupa et al. 2008, 2010; Nemjová et al. 2011; Škaloud et al. 2011).

## 1.1 Systematic delimitation of desmids

### 1.1.1 *Position of desmids within Plantae*

Desmids (Desmidiales) represent a group of green microalgae. Together with the order Zygnematales, they belong to the class Zygnematophyceae, phylum Charophyta, infrakingdom Streptophyta, kingdom Plantae (Lewis & McCourt 2004; Kalina & Váňa 2005). They have been always regarded as members of Zygnematophyceae (Conjugatophyceae, conjugating green algae) and Plantae, in classification schemes of algae. However, they were considered a distinct class within the phylum Chlorophyta until recently (Round 1963, 1971; Hindák 1978; Brook 1981; Kalina 1997; Cavalier-Smith 1998).

Recent ultrastructural research on the morphology and division, phylogenetic and biochemical studies has shown many different features within Chlorophyta (e.g. Fowke & Pickett-Heaps 1969; Pickett-Heaps 1979; Mattox & Stewart 1984; Bremer et al. 1987; Bhattacharya & Medlin 1998; Graham et al. 2000; Leliaert et al. 2012) which split them into two lineages: Chlorophyta (most “chlorophyte green algae”) and Charophyta/Streptophyta (few “charophyte green algae” together with species of land plants) (McCourt et al. 2004). The main characteristics along with the phylogenetic analyses which differentiate Streptophyta from Chlorophyta are: open mitosis and cell division with phragmoplast, characteristic arrangement of thylakoids into grana-like stacks, structure of the cellulose synthase complex, type of photorespiration and structure of flagellate reproductive cells, if they are present (Kalina & Váňa 2005; Wodniok et al. 2011). They are also different in their origins. According to Becker & Marin (2009) streptophyte algae have a primary freshwater

life style and were physiologically pre-adapted to terrestrial existence, whereas Chlorophyta evolved in marine habitats.

Nowadays is widely accepted the hypothesis that the land plants (embryophytes) evolved from streptophyte algae. Phylogenetic analyses indicate that the sister group of embryophytes (closest living relatives of land plants) are Zygnematales or Zygnematales together with Coleochaetales, instead of the Charales proposed for a long time (Wodniok et al. 2011; Becker 2012; Timme et al. 2012; Laurin-Lemay et al. 2012).

### 1.1.2 *Desmid classification*

Classification schemes of desmids and the whole class Zygnematophyceae underwent many changes in history. It was at first based on morphology with the stress on the cell wall features, cellular organization and chloroplast structure (Gontcharov 2008; Gontcharov & Melkonian 2010). These days, molecular data are widely used for their affirmation or reclassification (e.g. Denboh et al. 2001; Gontcharov & Melkonian 2005, 2008, 2010, 2011; Hall et al. 2008).

Desmids are traditionally classified into two distinct groups: saccoderm and placoderm desmids. Saccoderm desmids are characterized by compact cell wall, while placoderm desmids are characterised by a cell wall consisting of two or more parts (Smith 1950).

In 1972 Mix proposed division of Conjugatophyceae into two orders: Zygnematales (Saccodermae) and Desmidiales (Placodermae). This was suggested on the basis of electron microscopical investigation of the desmid cell walls. The cell wall of the first mentioned order is compact (consisting of one piece), and there are no pores present (it is smooth). The cell wall of the latter order consists of two or more parts and its cell wall is structured with pores, warts or spines. Mix (1972) further divided the order Zygnematales into two families: Mesotaeniaceae (unicellular organisms) and Zygnemataceae (filamentous organisms). The order Desmidiales was classified into two suborders: Archidesmidiinae and Desmidiinae. Suborder Archidesmidiinae with families Gonatozygaceae, Peniaceae and Closteriaceae was characterized by long, cylindrical or fusiform cells. The majority of species of the suborder Desmidiinae with only one family Desmidiaceae exhibited a more or less defined median constriction – isthmus (Mix 1972; Brook 1981; Denboh et al. 2001). This taxonomic system has been accepted and also supported by molecular methods, with the exception of the order Zygnematales which was found to be polyphyletic (McCourt et al. 2000; Kalina & Váňa 2005; Gontcharov 2008).

From the system of Mix (1972) Desmidiales can be defined as single-cell conjugates with a cell wall consisting of two or more parts. This conception corresponds to the perception of Desmidiales s. s. ("true desmids"). However within papers dealing with desmids, the conception of Desmidiales s. l. is rooted (except for taxonomical papers). They are defined as unicellular conjugates (true desmids + Mesotaeniaceae; e.g. Růžička 1977; Šťastný 2010). This broader understanding was also used in our study (Paper I).

The morphological diversity of desmids has led to the description of many species. The estimations of their total richness range from 1500 to 12000 (Gerrath 1993). More than 3000 species have been validly described and distributed over 35 genera (Gontcharov & Melkonian 2010). Many of these species contain additional subspecies, varieties and forms which are doubtful (Kouwets 2008), but some of these intraspecific taxa can represent new species. Phylogenetic analyses of desmids led to the conclusion that many traditional genera and also some species are polyphyletic (Gontcharov 2008; Gontcharov & Melkonian 2005, 2008, 2010, 2011; Hall et al. 2008). For this reason, revision of the system is needed. Our study on the genus *Micrasterias* (Paper II) also supported this notion.

## 1.2 Basic characteristics of desmids: an outline

Desmids are solely freshwater green algae. They have always been classified as members of kingdom Plantae principally because of their pigments and starch as a reserve product (Brook 1981). They comprise coccoid and colonial forms that are together with Zygnematales distinctive by their unique sexual reproduction (conjugation) and absence of flagella during their life cycle (Coesel & Meesters 2007; Gontcharov 2008; John & Williamson 2009). Desmidiales are peerless in morphology and inner-cell organization (Růžička 1977; Hindák 1978). The main component of their cell wall is cellulose, as in higher plants (Kalina & Váňa 2005; this was a very important feature for our study on the nuclear DNA content of microalgae – Paper III). However, the composition of desmid cell walls differs significantly (e.g. Lütz-Meindl & Brosch-Salomon 2000; Eder et al. 2008). Desmids have highly specific ecological demands and are sensitive to environmental changes, thus they are important indicators of wetland ecosystems (Coesel 1998; Hašler et al. 2008; Šťastný 2009, 2010).

### 1.2.1 *Main morphological features of desmids*

Desmids are distinguished by coccoid type of thallus but they can organize their cells into filamentous or other colonies too (Růžička 1977; Kalina & Váňa 2005). Filamentous colonies may be developed during reproduction, when the daughter cells after division remain attached

to each other (genera like *Hyalotheca*, *Desmidium*, *Sphaerozosma* and so on). Desmids exhibit great diversity in size. The length of the longest axis varied according to West & West (1904) from 8 to 1200 μm, according to Růžička (1977) from 10 to 1700 μm, Kouwets (2008) recorded a range from 10 to 1000 μm.

The shape of desmid vegetative cells in front view varies from spherical to cylindrical, elliptic, discoidal, fusiform or baculiform (Růžička 1977; Lenzenweger 1996; Coesel & Meesters 2007). A really noticeable feature which distinguishes them from other algae is their symmetry (Coesel & Meesters 2007). Longitudinal symmetry is distinct particularly in "true desmids" whose cells consists of two almost symmetrical halves, semicells, divided by a constriction called sinus (West & West 1904; Gontcharov 2008; John & Williamson 2009). The part which connects the semicells together is called the isthmus (West & West 1904). Longitudinal symmetry is also however present in other families of desmids but expressed there only in the internal arrangement of organelles (Růžička 1977; Kalina & Váňa 2005). Moreover, desmid cells from apical view show a radiate symmetry resembling ellipse (biradiate), triangle (triradiate), polygon (pluriradiate) or circle (omniradiate) (Coesel & Meesters 2007).

A conspicuous feature typical for some genera is the cell wall ornamentation. Parts of cell walls can be formed into rides, granules, tubercles or spines of various sizes and shapes. The cell wall can be provided with different large pits which usually join cell wall pores (Brook 1981; Coesel & Meesters 2007). Some granules visible under light microscope on the cell walls can originate from mucilage secreted by pores (Coesel & Meesters 2007).

In desmid cells, there is one nucleus located in the centre of isthmus (Kalina & Váňa 2005). The most visible organelles are chloroplasts. They are bright green and the content is predominantly chlorophyll a + b. Their shapes can vary from straight and rod-like to ridged, spirally twisted or may exhibit a radiating structure (West & West 1904). Usually there are two chloroplasts in each cell with two or more pyrenoids (Hindák 1978; Kalina & Váňa 2005; Coesel & Meesters 2007). The chloroplasts can be axial (situated in the centre of the cell) or parietal (situated round the periphery of the cell). Axial chloroplasts are more common, parietal are rather rare (Brook 1981).

1.2.2 *Reproduction of desmids*

Essentially, desmids are haploid organisms. The only diploid phase in their life cycle is the zygote (zygospore if mature) (Kalina & Váňa 2005).

The most frequent is asexual reproduction – cell division initiated typically by mitosis (Brook 1981; Kalina & Váňa 2005; Coesel & Meesters 2007).

Sexual reproduction in zygnematalean algae is relatively rare in nature and cultures and the main triggers are unknown (Coesel & Meesters 2007; Zwirn et al. 2013). Mating cells of desmids are not morphologically different from each other, or from vegetative cells. At first, mating cells draw closer. Then they are paired and their cell walls are broken in the isthmus part. Further, the protoplasts are fused and the zygote is formed. At the end the zygote creates a thick-walled zygospore which can withstand desiccation and it can remain in this phase for a long time before germinating (Coesel & Meesters 2007). The surface part of the cell walls of zygospores can be formed into various protuberances. The uniqueness of zygospores in zygnematalean algae can serve as characteristic for species identification (Hindák 1978; Poulíčková et al. 2007).

### 1.2.3 *Ecological preferences of desmids*

Desmids almost exclusively occur in freshwater habitats. However few species are also found in brackish waters. Although they can grow in different types of freshwater habitats and are present all over the world, they are not equally abundant in all of them. In general, the highest diversity of desmid flora is in mesotrophic to oligo-mesotrophic, slightly acidic clear waters. They occur mostly as metaphyton in shallow water bodies such as moorland pools or bogs with the occurrence of submerged macrophytes (e.g. *Utricularia*, *Potamogeton*, *Scorpidium*, *Myriophyllum*) and in acidic bogs with *Sphagnum*. They generally cannot tolerate permanently high pH values and high flow rates. In eutrophic waters they have to compete for life space with blue greens or chlorococcaleans, which have higher growth rates. For this reason, they are not abundant in limestone areas, running waters or waters rich in nutrients (West & West 1904; Coesel 1975; Brook 1981; Kalina & Váňa 2005; Coesel & Meesters 2007; Kouwets 2008). All of these preferences were taken into consideration when the localities in Moravia region were chosen and sampled (Paper I).

As already said, desmids are important indicators of wetland ecosystems. They are highly sensitive to environmental changes and have specific ecological demands (Coesel 1998; Hašler et al. 2008; Šťastný 2009, 2010).

### 1.2.4 *Genus Micrasterias*

The desmid genus *Micrasterias* was firstly characterized by Ralfs (1848) in his book "*The British Desmidieae*" – the first comprehensive monograph on desmids. The name of this

genus comes from Greek: mikros means “small” and aster means “star”, so we can say that *Micrasterias* is a name for a “little star”. The most of the taxa were described in the 19th century and only about one third of descriptions come from the 20th century (Škaloud et al. 2011). Nowadays, about 60 species of the genus are accepted. However AlgaeBase (Guiry & Guiry 2013) include 901 species and Index Nominum Algarum (2013) 936 records of this genus.

The genus is widely distributed and some species are considered cosmopolitan (Guiry & Guiry 2013). It has flattened and disc-like cells, consisting of two identical semicells. Each semicell is divided into three lobes – one polar lobe and two lateral lobes. These lateral lobes are further divided into lobules of the second, third, fourth or even fifth order (Krieger 1939; Prescott et al. 1977; Růžička 1977; Coesel & Meesters 2007). The cell wall may be smooth with pores or it is furnished with spines or granules (Coesel & Meesters 2007). Data from older molecular studies indicated that the genus may be paraphyletic (Gontcharov et al. 2003; Hall et al. 2008). According to the study of Škaloud et al. (2011) the genus is more likely monophyletic and includes species traditionally classified in different genera: *Cosmarium ralfsii*, *Triploceras gracile* and *Staurodesmus dickiei*. These species are morphologically different from *Micrasterias*. However according to Škaloud et al. (2011) this fact can be a result of their repeated morphological transformations in evolution. While certain morphological features correlate with the *Micrasterias* phylogeny, there are some characteristics which evolved independently. For example Černá & Neustupa (2010) found that the cell complexity reflects environmental factors (e.g. pH) and this does not correlate with phylogenetic data.

Various aspects (morphogenesis, plasticity, physiology or ultrastructure) have been studied on the genus *Micrasterias* during last decades (Meindl 1993; Weiss et al. 1999; Neustupa & Šťastný 2006; Neustupa & Škaloud 2007; Neustupa et al. 2008, 2010; Nemjová et al. 2011; Škaloud et al. 2011).

### 1.3 Polyphyletism and cryptic diversity within desmids

Phylogenetic analyses of the Zygnematophyceae started in 1985 (Hori et al. 1985). Since then, many papers on the molecular phylogeny of desmids has been written (Denboh et al. 2001; Gontcharov et al. 2003; Gontcharov & Melkonian 2005, 2008, 2010, 2011; Gontcharov 2008; Kouwets 2008). These studies show the monophyly of families Gonatozygaceae and Closteriaceae and doubtful concept of the families Peniaceae and Desmidiaceae (McCourt et

al. 2000; Denboh et al. 2001; Gontcharov et al. 2003; Hall et al. 2008). Recent research in the Desmidiaceae has confirmed substantial polyphyly on the level of traditional genera (Gontcharov et al. 2003; Gontcharov & Melkonian 2005, 2008; Hall et al. 2008). Species of most of the genera in the phylogenetic trees were spread out in several clades together with other genera (Gontcharov & Melkonian 2011). These results indicate the necessity for re-evaluation of the traditional desmid genera and family concept.

Re-evaluation is however needed not only on a genera or family level. More frequent use of molecular methods showed that cryptic and pseudocryptic species are a more commonly encountered phenomenon than previously thought (Evans et al. 2007). Cryptic species in general (not only in desmids) represent species which are morphologically identical, but genetically different. Thus, traditional morphospecies represent species complexes, consisting of genetically different, reproductively isolated entities – biological species. In the case of pseudocryptic species, morphological differences are inconspicuous, and sophisticated methods are necessary for their discovery (geometric morphometrics, interphase nuclei structure) (Bickford et al. 2007; Lundholm et al. 2012; Poulíčková et al. 2010). Intraspecific variation (varieties, forms) is well-known particularly in desmids (Růžička 1977; Coesel & Meesters 2007) and cryptic/pseudocryptic diversity has been illustrated in complexes *Micrasterias crux-melitensis*, *M. radians* (Neustupa et al. 2010), *M. truncata* (Nemjová et al. 2011). Combination of classic morphology and ecology together with molecular data and other techniques (cytology, DNA content – ploidy level) is known as the polyphasic/complex approach to diversity studies. This approach in general should lead to better understanding of diversity, dispersal and distribution of microalgae. Species concept and delimitation of desmid species certainly deserves further attention if only because of their use in many ecological applications (Coesel 1998, 2001, 2003; Neustupa et al. 2009).

# 2 Aims of the thesis

The principal goal of this thesis was to investigate desmid diversity using the polyphasic approach. The most important prerequisites include knowledge of desmid taxonomy, selection of localities with the occurrence of interesting species complexes and optimization of non-traditional methods potentially suitable for interpretation of desmid phylogeny. The genus *Micrasterias* was selected as a model genus.

In particular, the four principal aims of the thesis can be summarized as follows:

1) to create a current overview of desmid diversity from desmids inhabiting the mires of central and northern Moravia, which can help us to complete recent floristic studies of the Czech Republic and select model species complex and model localities
2) to revise a delimitation of the species *Micrasterias fimbriata* and *M. rotata* using the combination of different methods (polyphasic approach)
3) to develop a protocol for quantification of the nuclear DNA by flow cytometry which is suitable primarily for desmids
4) to test the evolutionary significance of polyploidy in the model genus *Micrasterias* (otherwise to test whether the phylogeny of the genus is associated with the DNA content variation).

# 3 Conclusions and future prospects

In this thesis, I have investigated the diversity of desmids using a polyphasic approach. Floristic data obtained from central and northern Moravia help to complete the knowledge on the desmid flora of the Czech Republic. Next, I applied flow cytometry to microalgae, particularly Streptophyta with the aim of using this method for testing of the nuclear DNA significance in desmid phylogeny. Further, I participated in the polyphasic characterization of two well-known *Micrasterias* species and applied DNA content measurements to interpretation of the phylogeny in the model genus *Micrasterias*.

## 3.1 Desmid flora of mires in Central and Northern Moravia

Knowledge of the distribution of microalgae in Moravia region (east part of the Czech Republic) is low in both scale, global (in contrast to higher plants) and regional (in contrast to other parts of the Czech Republic). In Paper I, I tried to fill the gap in this knowledge within desmids. Their diversity and distribution was studied there more than half a century ago and these studies focused mainly on habitats in the Jeseníky Mts (Fischer 1924, 1925; Lhotský 1949; Růžička 1954, 1956, 1957; Rybníček 1958). There were also some recent studies on desmid diversity in the Moravia region (Kitner et al. 2004; Šťastný 2010; Štěpánková et al. 2012), however our knowledge remained incomplete.

During the years 2008-2012, I collected samples from nine Moravian wetland localities. The localities ranged from diverse bogs, fens, and pools to ephemeral ditches. They were chosen with a consideration for the ecological preferences of desmids, including localities which have never been sampled before. The pH values of the studied sites ranged from 4.2 to 8.7, conductivity ranged from 22 to 360 $\mu S.cm^{-1}$ and trophic level ranged from oligo-mesotrophic to slightly eutrophic. Altogether, 109 taxa belonging to 14 genera were found, 42 of them newly described in Moravia, and five of them new for the Czech Republic. The distributional pattern of species corresponded with the main ecological gradients and species richness per locality ranged from 5 to 50 taxa. To date, it is accepted that desmids prefer mesotrophic to oligo-mesotrophic, slightly acidic shallow water bodies (Růžička 1977; Brook 1981; Coesel & Meesters 2007). In agreement, the locality with the greatest species richness was the mesotrophic transitional peat bog „Pstruží potok” with pH values between 6.3 and 6.4. On the other hand, low species richness was found at neutral to slightly alkaline localities „Filipovické louky” and „Panské louky”.

During our study, the following desmid taxa which are new and not recorded in the Czech Republic were found: *Closterium* cf. *costatum* var. *westii*, *Cosmarium asphaerosporum* var. *strigosum*, *C. exiguum* var. *pressum*, *C. incertum*, *C. transitorium*. However, the validity of the taxon *Closterium* cf. *costatum* var. *westii* is challenged (Růžička 1977; Šťastný & Kouwets 2012). The relatively large number of new taxa for Moravia illustrates the low exploration of this area. A unique locality near the village Slavkov, considered to be a possible Holocene relic (based on higher plants floristic data) was revealed during this study. Thus Paper I confirmed the importance of floristic and ecological research, broadened our knowledge of desmid diversity in the Czech Republic and revealed new sources for future taxonomic studies.

### 3.2 Delimitation of *Micrasterias fimbriata* and *M. rotata*

Desmid species *Micrasterias rotata* and *M. fimbriata* are known mostly from phytobenthos of peatlands. *M. rotata* is widely distributed and has been recorded from all continents excluding Antarctica (Tyler 1970). On the other hand, species *M. fimbriata* which is morphologically very similar to *M. rotata* has been recorded to be relatively rarer (Borge 1925; Krieger 1939; Kossinskaja 1960; Prescott et al. 1977; Růžička 1977; Medvedeva 2001; Coesel & Meesters 2007).

Samples of *M. fimbriata* were collected from different parts of Europe during the investigation of its morphology and inconspicuous morphological differences were found between their populations. Although the phylogenetic relation between *M. fimbriata* and *M. rotata* was illustrated by 18S rDNA analyses (Neustupa & Škaloud 2007), the monophyly and genetic structure of species remained unclear. Paper II is focused on their monophyly, further phylogenetic and morphological differentiation as well as geographic distribution. In this study a set of clonal strains and natural samples mostly from Europe, and published records from Europe and North America of these well known species were investigated. To analyze them we used phylogenetic analysis of trnG$^{ucc}$ (plastid gene that encodes transfer RNA-Gly and is appropriate for species delimitation; Shaw et al. 2005), geometric morphometrics, light microscopy, electron microscopy and the geographic distribution data.

Phylogenetic analysis of trnG$^{ucc}$ constructed using unrooted Bayesian inference confirmed the paraphyletic origin of *M. fimbriata*. Its strains formed two independent lineages, creating a clade together with *M. brachyptera*. We assigned these lineages A-lineage and B-lineage. They were also visibly different morphologically. Based on microscopic

observations and particularly on geometric morphometric analysis we found that both lineages differ in the shape of terminal lobules, in the depth of the incisions between polar lobe and lateral lobes, in length and shape of marginal spines of the polar lobes and in the presence of surface spine layers. The investigation of the geographical distribution of both lineages showed that B-lineage morphotype has never been reported from North America. On the other hand, A-lineage morphotype has never been found east of the Rhine River. The only parts of Europe, where both lineages co-occur are to our knowledge the Netherlands and Scotland. Both lineages seem to differ also in ecological preferences. While B-lineage specimens seem to prefer mesotrophic, slightly acidic wetland habitats, A-lineage representatives were mostly found in distinctly oligotrophic bogs at low pH. As the original description of *M. fimbriata* (Ralfs 1848) corresponds to the B-lineage, we proposed in Paper II a description of A-lineage as a new *Micrasterias* species. Members of the species *M. rotata* formed an independent lineage in the phylogenetic tree. They were also homogenous morphologically. Unfortunately, except for one strain we had strains of it only from Europe. So we can conclude that at least in Europe, populations of *M. rotata* represent a homogenous phylogenetic species lineage.

Paper II, in my opinion, supports the importance of the polyphasic approach in taxonomy.

### 3.3 Quantification of DNA content in microalgae using flow cytometry

Flow cytometry is routinely used for estimation of DNA amounts in higher plants. In comparison to other methods for DNA content measurement such as cytophotometry, flow cytometry is faster and large populations can be measured in a short time (Doležel & Bartoš 2005). Techniques, where the protoplasts for flow cytometry measuring are obtained by cell wall degrading enzymes, were developed in the 1960s (Cocking 1960). These techniques have been optimized for marine macroalgae only recently (Butler et al. 1990; Wakabayashi et al. 1999) and have never been used for microalgae before. Paper III pioneers the method of nuclear DNA quantification by flow cytometry within microalgae, using protoplasts after enzyme treatment.

Primarily cultures of Zygnematophyceae were tested. However representatives of other microalgal classes were added to find out the range of use of our protocol. Zygnematophyceae, closely related to higher plants (Wodniok et al. 2011; Becker 2012; Timme et al. 2012; Laurin-Lemay et al. 2012), share similar cell wall organization and main cell wall polymers (Eder et al. 2008). For this reason, we decided to obtain the protoplasts by

modifying the enzyme mixture of cellulase and macerozyme, which was used for higher plants (e.g. Gajdová et al. 2007) and marine green macroalgae (e.g. Chen & Shih 2000). As a nuclear isolation buffer, the LB01 lysis buffer (Doležel et al. 1989) was used and nuclear DNA was stained by propidium iodide (PI). As standards, young leaves of different plant species were used. They were prepared separately from algae by chopping with a razor blade and after adding the LB01 buffer they were mixed with algae, stained and analysed together. All samples were measured at least twice, usually three times to avoid possible differences between estimates produced on different days.

In Paper III we characterized a new possible standard for flow cytometry from the ranks of microalgae – *Micrasterias pinnatifida*. The suggested new standard is available in official culture collection, it is easy to cultivate, it shares with microalgae, identical sample preparation, it has a low coefficient of variation within our measurements (referring to the quality of measurements) and it seems to be free of compounds that interfere with PI staining.

Our protocol, mainly based on studies with higher plants and commercially available enzyme mixtures, proved to be useful for members of Zygnematophyceae, particularly desmids. Moreover we succeeded in some members of Chlorophyta (*Chloroidium ellipsoideum*, *Tetraselmis subcordiformis*) and Heterokontophyta (*Tribonema vulgare*). We had no success with microalgae with pellicle (*Euglena*), chlamys (*Chlamydomonas*), silica frustule (*Pinnularia*), alganan in the cell walls (*Chlorella*) or surrounded by a thick mucilaginous envelope (*Planktospheria*). The protocol also failed in some other microalgae (*Kentrosphaera*, *Pseudendoclonium*, *Stigeoclonium*, *Trentepohlia* and *Xanthidium*). Modification of the enzyme mixture or another nuclear isolation buffer could be helpful for these microalgae.

### 3.4 DNA content in evolution of the genus *Micrasterias*

Kew Plant DNA C-values database enters the data of only 253 algae, in contrast to over 7500 angiosperm C-value entries (Bennett & Leitch 2012). To increase the number of known C-values of algal species and to increase our knowledge of the role of nuclear DNA content in the evolution of algae, we surveyed the nuclear DNA content of *Micrasterias* species (Paper IV).

The nuclear DNA content of 34 *Micrasterias* strains (25 *Micrasterias* species) was investigated using the method described in Paper III. The absolute nuclear DNA level ranged from 2C = 2.1 to 39.2 pg; intraspecific variation being 16.0 – 31.1 pg in *M. rotata*, 15.4 – 21.7

pg in *M. truncata*, and 9.4 – 28.2 pg in *Triploceras gracile* (which genetically belong to the genus *Micrasterias*; Škaloud et al. 2011). These results were applied on the phylogenetic tree, created using Bayesian inference (BI) and based on concatenated SSU rDNA, psaA, and coxIII alignments and which were published before (see Methods in Paper IV). Most of the related species were significantly similar in their DNA content. However we also found some closely related strains differing greatly in their DNA content. We then tested the correlation between estimated DNA content values and the real number of chromosomes, previously published by Kasprik (1973). A strong correlation was found for all seven strains.
In addition, geometric morphometrics was used to test the correlations between DNA content and cell morphometric parameters. A significant correlation was found between the nuclear DNA content and average cell length in the genus *Micrasterias* and cell size in the investigated species *M. rotata*.

In fact, this is one of the first analyses of genomes in microalgae in the whole-genus-scale. It confirms the evolutionary significance of genome size variation in the desmid genus *Micrasterias* and highlights the resemblance to holocentric (holokinetic) higher plant genera (desmids have holocentric chromosomes; King 1960; Godward 1966; Guerra et al. 2010). The results confirm the importance of natural polyploidisation in the evolution and speciation of some microalgae.

# 4 Literature cited


BECKER, B. (2012): Snow ball earth and the split of Streptophyta and Chlorophyta. – Trends in Plant Science 20: 1-4.

BECKER, B. & MARIN, B. (2009): Streptophyte algae and the origin of embryophytes. – Annals of Botany 103: 999-1004.

BENNETT, M. D., LEITCH, I. J. (2012): Plant DNA C-values database (release 6.0, Dec. 2012) http://www.kew.org/cvalues/.

BHATTACHARYA, D. & MEDLIN, L. (1998): Algal phylogeny and the origin of land plants. – Plant Physiology 116: 9-15.

BICKFORD, D., LOHMAN, D. J., SODHI, N. S. et al. (2007): Cryptic species as a window on diversity and conservation. – Trends in Ecology and Evolution 22 (3): 148-155.

BORGE, O. (1925): Die von Dr. F. C. Hoehne während der Expedition Roosevelt-Rondon gesammelten Süsswasseralgen. – Arkiv für Botanik 19: 1-56.

BRANDHAM, P. E. & GODWARD, M. B. E. (1965): The inheritance of mating type in desmids. – New Phytologist 64 (3): 428-435.

BREMER, K., HUMPHRIES, C. J., MISHLER, B. D., CHURCHILL, S. P. (1987): On cladistic relationships in green plants. – Taxon 36: 339-349.

BROOK, A. J. (1981): The biology of desmids. University of California Press, Berkeley and Los Angeles.

BUTLER, D. M., EVANS, L. V. & KLOAREG, B. (1990): Isolation of protoplasts from marine macroalgae. – In: AKATSUKA, I. (ed.):Introduction to Applied Phycology. – 647-668 pp.,

SPB Academic Publication, The Hague, The Netherlands.

CAVALIER-SMITH, T. (1998): A revised six-kingdom system of life. – Biological Reviews 73: 203-266.

ČERNÁ, K. & NEUSTUPA, J. (2010): The pH-related morphological variations of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic. – Aquatic Ecology 44: 409-419.

CHAPMAN, D. J. (eds) Progress in phycological research 9. Biopress, Bristol.

CHEN, Y.-C. & SHIH, H.-C. (2000): Development of protoplasts of *Ulva fasciata* (Chlorophyta) for algal seed stock. – Journal of Phycology 36: 608-615.

COCKING, E. C. (1960): A method for the isolation of plant protoplasts and vacuoles. – Nature 187: 927-929.

COESEL, P. F. M. (1975): The relevance of desmids in the biological typology and evaluation of fresh waters. – Hydrobiological Bulletin 9: 93–101.

COESEL, P. F. M. (1998): Sieralgen en Natuurwaarden. Stichting Uitgeverij K. N. N. V., Utrecht.

COESEL, P. F. M. (2001): A method for quantifying conservation value in lentic freshwater habitats using desmids as indicator organisms. – Biodivers Conserv. 10 (2): 177-187.

COESEL, P. F. M. (2003): Desmid flora data as a tool in conservation management of Dutch freshwater wetlands. – Biologia 58 (4): 717-722.

COESEL, P. F. M. & MEESTERS K. J. (2007): Desmids of the lowlands. Mesotaeniaceae and Desmidiaceae of the European lowlands. KNNV Publishing, Zeist.

DENBOH, T., HENDRAYANTI, D. & ICHIMURA, T. (2001): Monophyly of the genus *Closterium* and the order Desmidiales (Charophyceae, Chlorophyta) inferred from nuclear small subunit rDNA data. – Journal of Phycology 37: 1063-1072.

DOLEŽEL, J. & BARTOŠ, J. (2005): Plant DNA flow cytometry and estimation of nuclear genome size. – Annals of Botany 95: 99-110.

DOLEŽEL, J., BINAROVÁ, P., LUCRETTI, S. (1989): Analysis of nuclear DNA content in plant cells ba flow cytometry. – Biologia Plantarum 31: 113-120.

EDER, M., TENHAKEN, R., DRIOUICH, A., LÜTZ-MEINDL, U. (2008): Occurrence and characterization of arabinogalactan-like proteins and hemicelluloses in *Micrasterias* (Streptophyta). – Journal of Phycology 44: 1221-1234.

EVANS, K. M., WORTLEY, A. H., MANN, D. G. (2007): An assessment of potential diatom “barcode” genes (*cox*1, *rbc*L, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). – Protist 158 (3): 349-364.

FISCHER, R. (1924): Ökologische Skizzen zur Algenflora des mährisch-schlesischen Gesenkes. – Schriften f. Süsswasser u. Meereskunde, Heft. 7: 1-20.

FISCHER, R. (1925): Ökologische Skizzen zur Algenflora des mährisch-schlesischen Gesenkes. – Verhandl. d. Naturf. in Brünn 59: 3-11.

FOWKE, L. C. & PICKETT-HEAPS, J. D. (1969): Cell division in *Spirogyra.* II. Cytokinesis. – Journal of Phycology 5: 273-281.

FRITSCH, F. E. (1930): Űber Entwicklungstendenzen bei Desmidiaceen. – Zeitschrift für Botanik 23: 402-418.

GAJDOVÁ, J., NAVRATILOVÁ, B., SMOLNA, J., LEBEDA, A. (2007): Effect of genotype, source tissue and media composition on *Cucumis* and *Cucurbita* protoplast isolation and regeneration. – Acta Horticulturae 731: 89-94.

GERRATH, J. F. (1993): The biology of desmids: a decade of progress. – In: ROUND, F. E.,

GODWARD, M. B. E. (1966): The chromosomes of the algae. Edward Arnold, London.

GONTCHAROV, A. (2008): Phylogeny and classification of Zygnematophyceae (Streptophyta): current state of affairs. – Fottea 8 (2): 87-104.

GONTCHAROV, A., MARIN, B. & MELKONIAN, M. (2003): Molecular phylogeny of conjugating green algae (Zygnematophyceae, Streptophyta) inferred from SSU rDNA sequence comparisons. – Journal of Molecular Evolution 56: 89-104.

GONTCHAROV, A. & MELKONIAN, M. (2005): Molecular phylogeny of *Staurastrum* Meyen ex Ralfs and related genera (Zygnematophyceae, Streptophyta) based on coding and noncoding rDNA sequence comparisons. – Journal of Phycology 41: 887-899.

GONTCHAROV, A. & MELKONIAN, M. (2008): In search of monophyletic taxa in the family Desmidiaceae (Zygnematophyceae, Viridiplantae): The genus *Cosmarium*. – American Journal of Botany 95 (9): 1079-1095.

GONTCHAROV, A. & MELKONIAN, M. (2010): Molecular phylogeny and revision of the genus *Netrium* (Zygnematophyceae, Streptophyta): *Nucleotaenium* gen. nov. – Journal of Phycology 46: 346-362.

GONTCHAROV, A. & MELKONIAN, M. (2011): A study of conflict between molecular phylogeny and taxonomy in the Desmidiaceae (Streptophyta, Viridiplantae): Analyses of 291 rbcL sequences. – Protist 162: 253-267.

GRAHAM, L. E., COOK, M. E., BUSSE, J. S. (2000): The origin of plants: Body plan changes contributing to a major evolutionary radiation. – Proc. Natl. Acad. Sci. USA 97: 4535-4540.

GUERRA, M., CABRAL, G., CUACOS, M. et al. (2010): Neocentrics and holokinetics (Holocentrics): Chromosomes out of the centromeric rules. – Cytogenetic and genome Research 129: 82-96.

GUIRY, M. D. & GUIRY, G. M. (2013): *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 22 March 2013.

HALL, J. D., KAROL, K. G., MCCOURT, R. M., DELWICHE, CH. F. (2008): Phylogeny of the conjugating green algae based on chloroplast and mitochondrial nucleotide sequence data. – Journal of Phycology 44: 467-477.

HAŠLER, P., ŠTĚPÁNKOVÁ, J., ŠPAČKOVÁ, J. et al. (2008): Epipelic cyanobacteria and algae: a case study from Czech ponds. – Fottea 8 (2): 133–146.

HINDÁK, F. (ed.) (1978): Sladkovodné riasy. – SPN, Bratislava.

HORI, H., LIM, B.-L. & OSAWA, S. (1985): Evolution of green plants as deduced from 5S rDNA sequences. – Proc. Natl. Acad. Sci. USA 82: 820-823.

INDEX NOMINUM ALGARUM (2013): University Herbarium, University of California, Berkeley. Compiled by Paul Silva. Available online at http://ucjeps.berkeley.edu/CPD/; searched on 22 March 2013.

JOHN, D. M. & WILLIAMSON, D. B. (2009): A practical guide to the Desmids of the West of Ireland. – Martin Ryan Institute, National University of Ireland, Galway.

KALINA, T. (1997): Systém a vývoj sinic a řas. Karolinum, Praha.

KALINA, T. & VÁŇA, J. (2005): Sinice, řasy, houby, mechorosty a podobné organismy v současné biologii. Karolinum, Praha.

KASPRIK, W. (1973): Beiträge zur Karyologie der Desmidiaceen – Gattung *Micrasterias*. Beih – Nova Hedwigia 42: 115-137.

KING, G. C. (1960): The cytology of desmids: the chromosomes. – New Phytologist 59:65-72.

KITNER, M., POULÍČKOVÁ, A., NOVOTNÝ, R. & HÁJEK, M. (2004): Desmids (Zygnematophyceae) of the spring fens of a part of West Carpathians [Krásivky prameništ' části západních Karpat]. – Czech Phycology 4: 43-61.

KOSSINSKAJA, E. K. (1960): Flora sporovych rastenii SSSR. Tom V. Desmidievye vodorosli. Izdatelstvo Akademii Nauk SSSR, Moscow.

KOUWETS, F. A. C. (2008): The species concept in desmids: the problem of variability, infraspecific taxa and the monothetic species definition. – Biologia 63: 881-887.

KRIEGER, W. (1939): Die Desmidiaceen Europas mit Berücksichtigung der aussereuropäischen Arten. Band 13. Abteilung 1, Teil 2, Lieferung 1 of Dr. Rabenhorst's Kryptogamen-Flora von Deutschland. Ősterreich und der Schweiz. Akademische Verlagsgeselschaft M. B. H., Leipzig.

LAURIN-LEMAY, S., BRINKMANN, H. & PHILIPPE, H. (2012): Origin of land plants revisited in the light of sequence contamination and missing data. – Current Biology 22: 593-594.

LELIAERT, F., SMITH, D. R., MOREAU, H., HERRON, M. D., VERBRUGGEN, H., DELWICHE, CH. F. & DE CLERCK, O. (2012): Phylogeny and molecular evolution of the green algae. – Critical Reviews in Plant Sciences 31: 1-46.

LENZENWEGER, R. (1996): Desmidiaceenflora von Österreich, Teil 1. J. Crammer, Stuttgart.

LEWIS, L. A. & MCCOURT, R. M. (2004): Green algae and the origin of land plants. – Am. J. Bot. 91: 1535-1556.

LHOTSKÝ, O. (1949): Poznámka k flóře Desmidiaceí Hrubého Jeseníku. – Časopis Vlast. spolku mus. v Olomouci 58: 149-155.

LUNDHOLM, N., BATES, S. S., BAUGH, K. A., BILL, B. D., CONNELL, L. B., LÉGER, C., TRAINER, V. L. (2012): Cryptic and pseudo-cryptic diversity in diatoms – with description of *Pseudo-Nitzschia hasleana* sp. nov. and *P. fryxelliana* sp. nov. – Journal of Phycology 48: 436-454.

LÜTZ-MEINDL, U., BROSCH-SALOMON, S. (2000): Cell wall secretion in the green alga, Micrasterias. – J. Microsc. 198: 208-217.

MATTOX, K. R. & STEWART, K. D. (1984): Classification of the green algae: a concept based on comparative cytology. – In: IRVINE, D. E. G., JOHN, D. M. eds. (1984): The systematics of the green algae. Academic Press, London.

MCCOURT, R. M., DELWICHE, CH. F., KAROL, K. G. (2004): Charophyte algae and land plant origins. – Trends in ecology and evolution 19 (12): 661-666.

MCCOURT, R. M., KAROL, K. G., BELL, J., HELM-BYCHOWSKI, K. M., GRAJEWSKA, A., WOJCIECHOWSKI, M. F. & HOSHAW, R. W. (2000): Phylogeny of the conjugating green algae (Zygnemophyceae) based on rbcL sequences. – Journal of Phycology 36: 747-758.

MEDVEDEVA, L. A. (2001): Biodiversity of aquatic algal communities in the Sikhote-Alin biosphere reserve (Russia). – Cryptogamie Algologie 22: 65-100.

MEINDL, U. (1993): Micrasterias cells as a model system for research on morphogenesis. – Microbiological Reviews 57: 415-433.

MIX, M. (1972): Die Feinstruktur der Zellwände bei Mesotaeniaceae und Gonatozygaceae mit einer vergleichenden Betrachtung der verschiedenen Wandtypen der Conjugatophyceae und über deren systematischen Wert. – Archiv für Mikrobiologie 81: 197-220.

NEMJOVÁ, K., NEUSTUPA, J., ŠŤASTNÝ, J., ŠKALOUD, P. & VESELÁ, J. (2011): Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*. – Phycological Research 59: 208-220.

NEUSTUPA, J., ČERNÁ, K. & ŠŤASTNÝ, J. (2009): Diversity and morphological disparity of desmids assemblages in Central European peatlands. – Hydrobiologia 630: 243-256.

NEUSTUPA, J. & ŠKALOUD, P. (2007): Geometric morphometrics and qualitative patterns in the morphological variation of five species of *Micrasterias* (Zygnematophyceae, Viridiplantae). – Preslia 79: 401-417.

NEUSTUPA, J., ŠKALOUD, P. & ŠŤASTNÝ, J. (2010): The molecular phylogenetic and geometric morphometric evaluation of *Micrasterias crux-melitensis/M. radians* species complex. – Journal of Phycology 46: 703-714.

NEUSTUPA, J., ŠŤASTNÝ, J. (2006): The geometric morphometric study of Central European species of the genus Micrasterias (Zygnematophyceae, Viridiplantae). – Preslia 78 (2): 253-263.

NEUSTUPA, J., ŠŤASTNÝ, J., HODAČ, L. (2008): Temperature-related phenotypic plasticity in the green microalga *Micrasterias rotata*. – Aquatic Microbial Ecology 51 (1): 77-86.

PICKETT-HEAPS, J. D. (1979): Electron microscopy and the phylogeny of green algae and land plants. – Integrative and Comparative Biology 19 (2): 545-554.

POULÍČKOVÁ, A., VESELÁ, J., NEUSTUPA, J., ŠKALOUD, P. (2010): Pseudocryptic diversity versus cosmopolitanism in diatoms: A case study on *Navicula cryptocephala* Kütz. (Bacillariophyceae) and morphologically similar taxa. – Protist 161 (3): 353-369.

POULÍČKOVÁ, A., ŽIŽKA, Z., HAŠLER, P., BENADA, O. (2007): Zygnematalean zygospores: Morphological features and use in species identification. – Folia Microbiologica 52 (2): 135-145.

PRESCOTT, G. W., CROASDALE, H. T., VINYARD, W. C. (1977): A synopsis of North American desmids, part II. Desmidiaceae: Placodermae, section 2. University of Nebraska Press, Lincoln.

RALFS, J. (1848): The British Desmidieae. Reeve, Benham & Reeve, London.

ROUND, F. E. (1963): The taxonomy of the Chlorophyta. – British Phycological Bulletin 2 (4): 224-235.

ROUND, F. E. (1971): The taxonomy of the Chlorophyta. II. – British Phycological Journal 6 (2): 235-264.

RŮŽIČKA, J. (1954): Krásivky (Desmidiaceae) řeky Moravice a jejích přítoků. – Přírodov. sbor. Ostravského kraje 15: 290-303.

RŮŽIČKA, J. (1956): Krásivky pramenů Moravice (Velká Kotlina, Jeseníky). – Přírodov. sbor. Ostravského kraje 17: 38-58.

RŮŽIČKA, J. (1957): Krásivky z pramenišť na Malém Dědu (Hrubý Jeseník). – Časopis Slezského muzea 6: 108-121.

RŮŽIČKA, J. (1977): Die Desmidiaceen Mitteleuropas, Band 1, 2. Lieferung. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

RYBNÍČEK, K. (1958): Krásivky pramenné oblasti Branné v Hrubém Jeseníku. – Přírodov. sbor. Ostravského kraje 19: 97-122.

SHAW, J., LICKEY, E. B., BECK, J. T. et al. (2005): The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. – American Journal of Botany 92: 142-166.

ŠKALOUD, P., NEMJOVÁ, K., VESELÁ, J., ČERNÁ, K., NEUSTUPA, J. (2011): A multilocus phylogeny of the desmid genus *Micrasterias* (Streptophyta): Evidence for the accelerated

rate of morphological evolution in protists. – Molecular Phylogenetics and Evolution 61: 933-943.

SMITH, G. M. (1950): The fresh-water algae of the United States. McGraw-Hill, New York.

STARR, R. C. (1955): Isolation of sexual strains of Placoderm desmids. – Bulletin of the Torrey Botanical Club 82: 261-265.

ŠŤASTNÝ, J. (2009): The desmids of the Swamp Nature Reserve (North Bohemia, Czech Republic) and a small neighbouring bog: species composition and ecological condition of both sites. – Fottea 9 (1): 135–148.

ŠŤASTNÝ, J. (2010): Desmids (Conjugatophyceae, Viridiplantae) from the Czech Republic, new and rare taxa, distribution, ecology. – Fottea 10 (1): 1-74.

ŠŤASTNÝ, J. & KOUWETS, F. A. C. (2012): New and remarkable desmids (Zygnematophyceae, Streptophyta) from Europe: taxonomical notes based on LM and SEM observations. – Fottea 12: 293-313.

ŠTĚPÁNKOVÁ, J., HAŠLER, P., HLADKÁ, M. & POULÍČKOVÁ, A. (2012): Diversity and ecology of desmids of peat bogs in the Jeseníky Mts.: Spatial distribution, remarkable finds. – Fottea 12 (1): 111-126.

TIMME, R. E., BACHVAROFF, T. R., DELWICHE, CH. F. (2012): Broad phylogenetic sampling and the sister lineage of land plants. – Plos One 7 (1): e29696. doi: 10.1371/journal.pone.0029696

TYLER, P. A. (1970): Taxonomy of Australian freshwater algae I. The genus *Micrasterias* in south-eastern Australia. – British Phycological Journal 5: 211-234.

WAKABAYASHI, T., KUBOI, T., TUBOI, T., KAJI, M., HARA, M. (1999): Preparation of high yields of algal protoplasts using buccal juice of sea hare and commercial cellulase. – Marine Biotechnology 1: 407-410.

WEISS, D., LÜTZ, C., LÜTZ-MEINDL, U. (1999): Photosynthesis and heat response of the green alga Micrasterias denticulata (Desmidiaceae). Zeitschrift für Naturforschung 54c: 508-516.

WEST, W. & WEST, G. S. (1904): A monograph of the British Desmidiaceae. Volume I. The Ray Society, London.

WODNIOK, S., BRINKMANN, H., GLÖCKNER, G., HEIDEL, A. J., PHILIPPE, H., MELKONIAN, M., BECKER, B. (2011): Origin of land plants: Do conjugating green algae hold the key? – BMC Evolutionary Biology 11: 104.

ZWIRN, M., CHEN, CH., UHER, B. & SCHAGERL, M. (2013): Induction of sexual reproduction in *Spirogyra* clones – does an universal trigger exist? – Fottea 13 (1): 77-85.

## 5 List of papers included in the thesis

**Thesis is based on the following four papers, quoted in the text as Papers I-IV**:

**Paper I**

MAZALOVÁ, P., ŠTĚPÁNKOVÁ, J. & POULÍČKOVÁ, A. (2013): Desmid flora of mires in Central and Northern Moravia (Czech Republic). – Časopis Slezského zemského muzea, A – vědy přírodní (in press).

**Paper II**

NEUSTUPA, J., ŠŤASTNÝ, J., NEMJOVÁ, K., MAZALOVÁ, P., GOODYER, E., POULÍČKOVÁ, A. & ŠKALOUD, P. (2011): A novel, combined approach to assessing species delimitation and biogeography within the well-known desmid species *Micrasterias fimbriata* and *M. rotata* (Desmidiales, Streptophyta). – Hydrobiologia 667: 223-239.

**Paper III**

MAZALOVÁ, P., ŠARHANOVÁ, P., ONDŘEJ, V. & POULÍČKOVÁ, A. (2011): Quantification of DNA content in freshwater microalgae using flow cytometry: a modified protocol for selected green microalgae. – Fottea 11(2): 317-328.

**Paper IV**

POULÍČKOVÁ, A., MAZALOVÁ, P., VAŠUT, R. J., ŠARHANOVÁ, P., NEUSTUPA, J., ŠKALOUD, P. (2013): DNA content variation and its significance in the evolution of the genus Micrasterias (Desmidiales, Streptophyta) (in prep.).

# Paper I

ISSN 1211-3026 Čas. Slez. Muz. Opava (A), 62: 1-22, 2013
DOI: 10.2478/cszma-2013-0001

# Desmid flora of mires in Central and Northern Moravia (Czech Republic)

Petra Mazalová, Jana Štěpánková & Aloisie Poulíčková

Desmid flora of mires in Central and Northern Moravia (Czech Republic). – Čas. Slez. Muz. Opava (A), 62: 1-22, 2013.

Abstract: In contrast to higher plants, diversity and distribution of microalgae is not very well understood and floristic data is incomplete for many regions. This study focuses on filling this gap in case of desmids in the region, Moravia (Czech Republic). During the years 2008-2012, desmid flora of nine Moravian (Czech Republic) peat bogs and wetlands were studied. One hundred and nine taxa belonging to 14 genera have been found, 42 of them are new records for Moravia, and five of them are new for the Czech Republic (*Closterium* cf. *costatum* var. *westii*, *Cosmarium asphaerosporum* var. *strigosum*, *C. exiguum* var. *pressum*, *C. incertum*, *C. transitorium*). Species which have been found are briefly discussed with regard to their previous records for Moravia or for the whole Czech Republic. Line drawings of 66 taxa are included. Character and origin of the unique locality Slavkov mire is discussed.

Key words: Conjugatophyceae, Desmidiales, diversity, Moravia, mires

## Introduction

Several studies carried out in Moravia (east part of the Czech Republic) have been conducted with diversity of desmids. However, the majority of these studies are more than 50 years old and deal only with habitats in the Jeseníky Mts (Fischer 1924, 1925; Lhotský 1949; Růžička 1954, 1956, 1957; Rybníček 1958). It is a logical approach, because there are famous desmid-rich localities in this part of Moravia. Although desmids can be present in various types of freshwater habitats, including eutrophic waters or even ice, they prefer mesotrophic to oligo-mesotrophic acidic waters and *Sphagnum* bog pools (Coesel 1975; Brook 1981; Coesel and Meesters 2007). The Jeseníky Mts had been covered by glacier in the last ice age and there were good conditions for the formation of oligotrophic and oligo-mesotrophic raised bogs and transitional peat bogs (Reichholf 1998). In recent studies, special attention has been paid to diatom and desmid flora of ombrotrophic mires in the Jeseníky Mts (Štěpánková et al. 2012, Poulíčková et al. 2013), spring fens in the West Carpathians (Poulíčková 1987; Kitner et al. 2004) and epipelic assemblages of few fishponds (Hašler et al. 2008).

The most comprehensive study on desmids in the Czech Republic focused on Bohemia (western part of the Czech Republic) and included only six Moravian localities (Šťastný 2010), thus desmid floristic data is still incomplete. Particularly, in such areas like the Libavá military training area that have never been sampled before.

The main objective of this study is to deepen our knowledge of the occurrence and species composition of desmids in Moravia. Sampling sites were concentrated on wetland habitats such as transitional peat bogs and peat meadows, which represent the most desmid rich habitats of this region. Such habitats are situated mainly in Central and Northern Moravia, while they are lacking in the eastern and southern part. In accordance with this distribution pattern, we focused on the first mentioned area except for ombrotrophic peat bog localities in the Jeseníky Mts which were already well described by Štěpánková et al. (2012).

## Material and methods

In the period of four years (from 2008 to 2012), 67 samples from nine Moravian localities were taken. Various types of wetland habitats including diverse bogs and fens, wet peat meadows, marshes or ephemeral ditches were sampled. The geographic position of all sampling sites listed bellow is plotted on a map (Map 1).

Samples were collected by squeezing out the mosses (especially *Sphagnum* spp.), and submerged macrophytes. For collecting the algae from the sediment a plastic pipette was used. A part of each sample was left for LM observations of living material. The rest of samples was fixed by formaldehyde to a final concentration of 2 % on the day of collecting. Samples were examined under the light microscope Zeiss Primo Star. Desmids were identified using both living and fixed material according to: Coesel (1982, 1983), Coesel and Meesters (2007), Lenzenweger (1997, 1999), Růžička (1977, 1981) and West and West (1905, 1908, 1912). In the overview of taxa found during our study, some desmids are presented as rare and/or new for Moravia or even for the whole Czech Republic. Rareness of the taxa was evaluated according to Poulíčková et al. (2004), Šťastný (2010) and other literature cited.

Environmental variables (pH, conductivity) of water were measured using a pH/conductivity meter COMBO by Hanna (HI 98129).

**List of sampling sites, their geographic position and basic characteristic:**

**1.** Mesotrophic transitional peat bog „Pstruží potok” Nature Reserve (49°57'1"N, 17°13'1"E) – Jeseníky Mts Protected Landscape Area; pH = 6.3-6.4, cond. = 76-177 $\mu S.cm^{-1}$.

**2.** Mesotrophic transitional peat bog „Skalské rašeliniště” Nature Reserve (49°55'10"N, 17°12'23"E); pH = 5.9-6.4, cond. = 43-110 $\mu S.cm^{-1}$.

**3.** Oligo-mesotrophic transitional peat bog „Rašeliniště Skřítek” National Nature Reserve (49°59'33"N, 17°9'56"E) – Jeseníky Mts Protected Landscape Area; pH = 6.9-7.8, cond. = 22-106 $\mu S.cm^{-1}$.

**4.** Fen and waterlogged meadows „Filipovické louky” Nature Reserve (50°9'42"N, 17°9'59"E) – Jeseníky Mts Protected Landscape Area; pH = 7.9, cond. = 74 $\mu S.cm^{-1}$.

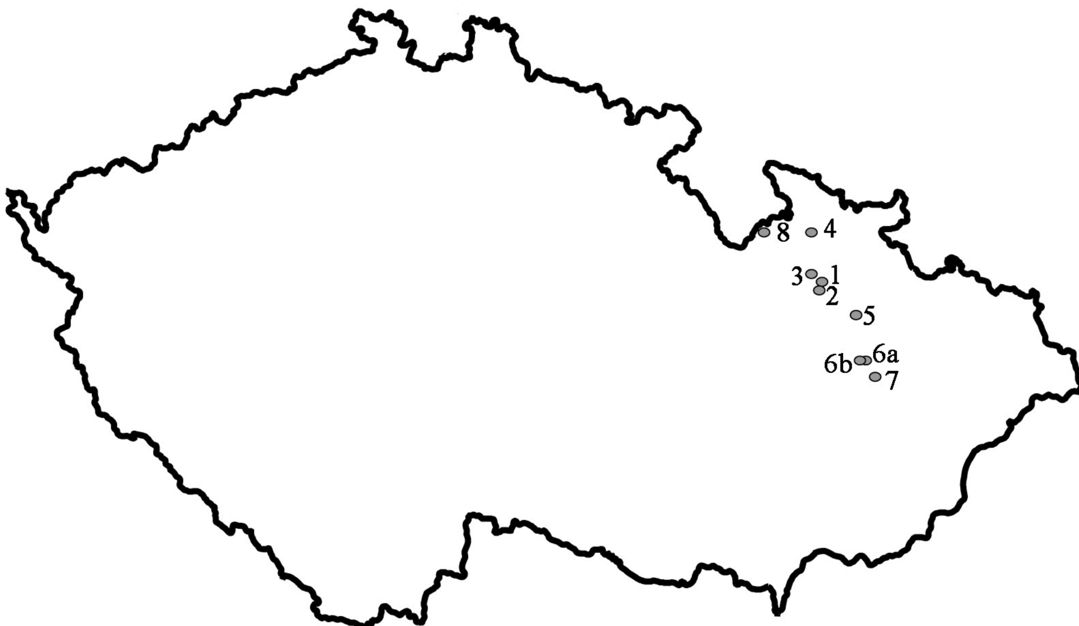
**5.** Bog spruce forest „Panské louky” Nature Reserve (49°49'56"N, 17°26'1"E); pH = 6.4-8.5, cond. = 62-260 $\mu S.cm^{-1}$.

**6.a** Bog between the hills „Radeška” and „Strážisko” (49°38'4"N, 17°29'57"E) – Libavá military training area; pH = 6.9-8.7.

**6.b** Mesotrophic ephemeral ditches nearby the hill „Strážisko” (49°38'19"N, 17°28'46"E) – Libavá military training area.

**7.** *Sphagnum* pools, pools with *Utricularia* spp. and fen hollows near the village Slavkov by Olomouc (49°34'16"N, 17°33'32"E) – Libavá military training area; pH 4.3-5.8.

**8.** *Sphagnum* pools in the area of the „Podbělka” and „Sušina” hills and the „Mokřiny” site (50°9'50"N, 16°51'19"E) – Králický Sněžník Mts Protected Landscape Area; pH = 4.2-5.13, cond. = 30-360 $\mu S.cm^{-1}$.




**Map 1:** Map of the sampling sites.

## Results and discussion

*Ecological part*

According to Šťastný (2005) there could occur around 500 desmid species in the Czech Republic. His estimates are based on complex study of desmid flora in Bohemia and data known from Central Europe, particularly the Netherlands and Austria (Coesel 1998 and Lenzenweger 2003). A total of 109 desmid taxa were identified at nine localities in Central and Northern Moravia, which represent at least one-fifth of the taxa estimated to be present in the Czech Republic. The species richness per locality ranged from 5 to 50 taxa and its distribution pattern corresponded with the main ecological gradients.

The most important ecological variable influencing microalgal distribution in mires seems to be pH and conductivity (Neustupa et al. 2009; Poulíčková et al. 2013). The high occurrence of desmid species appears to be correlated with low conductivity, calcium and alkalinity levels, pH values between 5.1-7.0, and the presence of free $CO_2$ (Woelkerling & Gough 1976; Růžička 1977, 1981; Brook 1981; Lenzenweger 1997). Desmids prefer mesotrophic to oligo-mesotrophic waters (Coesel 1975; Coesel and Meesters 2007). Their absence in eutrophic waters with nutrient-rich conditions is according to Coesel (2001) caused by the absence of competitiveness to taxa with higher growth rates. Moreover, pH in eutrophic waters is usually increasing above 8.3 (particularly with the peak of photosynthesis) and limitation by free $CO_2$ can be expected in such conditions (Poulíčková 2011).

The study sites represent wetland habitats, their pH ranged from 4.2 to 8.7, conductivity ranged from 22 to 360 $\mu S.cm^{-1}$ and expected trophic level ranged from oligo-mesotrophic to slightly eutrophic. Most desmid species were recorded from the mesotrophic transitional peat bog „Pstruží potok" with pH values between 6.3-6.4. Low species richness was found at neutral and slightly alkalic localities „Filipovické louky" and „Panské louky".

The most frequent taxa, occurring at five or more localities were *Cylindrocystis brebissonii*, *Netrium digitus*, *Closterium striolatum*, *Closterium rostratum* and *Closterium lunula*. Some of these species are traditionally defined as species typically inhabiting acidic peat bogs (*Cylindrocystis brebissonii*, *Netrium digitus*, *Closterium striolatum*), others are most common in slightly acidic to neutral waters (*Closterium rostratum* and *Closterium lunula*). However, they are also known for their relatively wide ecological valence in different pHs, e.g. *Closterium striolatum* - 4.0 to 7.0, *Closterium rostratum* - 3.9 to 7.0 or *Closterium lunula* - 4.5 to 8.0 (Růžička 1977; Coesel & Meesters 2007).

Among desmids found during our study, the following taxa are new, unrecorded in the Czech Republic: *Closterium* cf. *costatum* var. *westii*, *Cosmarium asphaerosporum* cf. var. *strigosum*, *C. exiguum* var. *pressum*, *C. incertum*, *C. transitorium* (they are designated with a triangle ▲ before the species name). Relatively high number of new taxa for Moravia (42 taxa – designated with an asterisk * before the species name) illustrate the lack of knowledge in this area.

Besides diatoms, desmids belong to the best bioindicators. Diversity and species composition of their assemblages correlates with character of a biotope quite well (Coesel 1998, 2001; Šťastný 2010). That is why we paid special attention to characterize the locality Slavkov mire (no. 7), which has been discovered quite recently by field botanists. Desmids probably represent the first group of organisms, which species list will be published for this locality.

The locality is particularly interesting because of the occurrence of higher plant taxa as *Eriophorum vaginatum* and *Vaccinium uliginosum* (leg. Zbyněk Hradílek). Their local populations are geographically isolated from others situated as far away as the mountain bogs in the Jeseníky Mts. (about 80 km far), suggesting a possible Holocene relic character of the Slavkov mire (Zbyněk Hradílek, personal communication). Regarding these finds, the locality could be considered to be valuable also because of its genesis. Its potential character of the

Holocene relic must be confirmed by further analyses. In such case, nomination of the Slavkov mire among protected areas would be desirable. Indeed, the character of this unique locality seems to be confirmed by desmid floristic data.

During our study, 22 desmid taxa were identified here. Among these, the following taxa are remarkable in relation to their distribution pattern. *Staurastrum senarium* and *S. gladiosum* are rare species, indicative for stable ecosystems and they appear to be bound to Holocene peat bogs (Coesel 1997, 1998; Coesel & Meesters 2007; Šťastný 2010). Regarding the origin of habitats, similar preferences have been reported for *Euastrum ansatum*, *E. elegans*, *Micrasterias thomasiana* var. *notata*. Besides the Holocene site, species were collected at the Pleistocene mires as well (Coesel 1985).

*Taxonomic part*

Systematical classification of species found during our study (bellow) corresponds to the conception of Desmidiales s.l., which contains the family Mesoteniaceae and the order of Desmidiales s.s. with families Gonatozygaceae, Peniaceae, Closteriaceae and Desmidiaceae (Mix 1972).

## Family Mesotaeniaceae

***Cylindrocystis brebissonii*** De Bary

Localities: 1, 3, 5, 6b, 8

In Moravia it is a very common species, particularly in *Sphagnum* vegetation. Previous records: Fischer (1924, 1925), Kitner et al. (2004), Lhotský (1949), Rosa (1957), Růžička (1956, 1957), Rybníček (1958), Štěpánková et al. (2012).

***Cylindrocystis crassa*** De Bary

Locality: 6b

This species was very rare, found only in one of the samples. In Moravia previously recorded by Lhotský (1949), who surprisingly refers to widespread occurrence in Moravia, and by Rybníček (1958). According to our experience it is rather rare nowadays. A possible explanation could be that it can be easily confused with *Cylindrocystis brebissonii*.

***Cylindrocystis gracilis*** I. Hirn

Synonym: *Cylindrocystis brebissonii* var. *minor* W. et G. S. West

Localities: 5, 6b

Up to date, this species has been reported only from Northern Moravia – localities Hrubý Jeseník (Růžička 1957; Štěpánková et al. 2012) and Velká Kotlina (Růžička 1956).

***Mesotaenium endlicherianum*** Nägeli

Locality: 1

Fig 1: 23

It is a rare species of benthic or subathmophytic habitats (Šťastný 2010). Previous records from Moravia come from Růžička (1957) and Štěpánková et al. (2012).

***Mesotaenium macrococcum*** (Kütz.) J. Roy et Bisset

Localities: 3, 6b

It was relatively rare. From Moravia previously reported only from the Hrubý Jeseník Mts (Lhotský 1949; Rosa 1957; Štěpánková et al. 2012).

***Netrium digitus*** Itzigs. et Rothe

Localities: 1, 2, 3, 6b, 7, 8

Fig 1: 15

It was common in acidic habitats, but present also in slightly acidic waters. Previous records from Moravia: Fischer (1924, 1925), Kitner et al. (2004), Lhotský (1949), Růžička (1956, 1957), Rybníček (1958), Štěpánková et al. (2012).

## Family Peniaceae

***Penium polymorphum*** Perty

Locality: 3

Together with our findings from Moravia, up to now it was reported only from the Jeseníky Mts (Fischer 1924, 1925; Rybníček 1958; Štěpánková et al. 2012).

****Penium spirostriolatum*** J. Barker

Localities: 6a, 6b, 7

Fig 1: 18

Although it is rather common in acidic places, this is the first record of this species from Moravia (except for the record of Lelková et al. 2004, which is doubtful – the authors themselves stated this record with an abbreviation cf.). However, it was previously reported from the Czech Republic – from Bohemia (Nováková 2002; Šťastný 2005, 2007, 2008a, 2009, 2010).

## Family Closteriaceae

****Closterium* cf. *baillyanum*** (Ralfs) Bréb.

Synonyms: *Closterium baileyanum* Bréb., *Cl. didymotocum* var. *baillyanum* Bréb. in Ralfs

Locality: 1

Fig 1: 7

According to Coesel and Meesters (2007) or Růžička (1977), the average value of cell length of this species is around 300-500 µm, cell breadth 30-50 µm. Dimensions of cells in our material were smaller (L: 230-250, B: 22.5-32 µm). It is widely known from Bohemia (western part of the Czech Republic; e.g. Šťastný 2005) but there was no record from Moravia until now.

****Closterium calosporum*** Wittr.

Synonym: *Closterium calosporum* f. *minus* Wille

Locality: 3

It had flattened apices with a clear end pore. In the Czech Republic it is quite common (e.g. Šťastný 2005). New taxon for Moravia.

****Closterium closterioides*** (Ralfs) A. Louis et Peeters

Synonyms: *Closterium libellula* Focke ex Nordst., *Penium closterioides* Ralfs, *P. libellula* (Focke) Nordst.

Locality: 5

Fig 1: 10

According to Růžička (1977), it prefers acidic to neutral habitats with pH around (4.8)-5.5-7.0. Our samples from locality no. 5 come however from habitat with higher pH – around 8.2. New taxon for Moravia.

***Closterium costatum*** Ralfs

Locality: 4

From Moravia previously recorded by Kitner et al. (2004) in the acidic spring fen Obidová (the Beskydy Mts – Protected Landscape Area).

▲***Closterium* cf. *costatum* var. *westii*** Cushman

Locality: 1

Fig 1: 4

However the nominate variety is quite common in the Czech Republic (e.g. Pascher 1906; Roubal 1958; Rosa 1969; Kitner et al. 2004; Šťastný 2007, 2008b, 2009, 2010), variety *westii* has not been recorded there until now. New taxon for the Czech Republic. According to Růžička (1977), this variety is rather rare in middle Europe. It should be pointed out, that the validity of the taxon is challenged (Růžička 1977; Šťastný and Kouwets 2012).

****Closterium dianae*** Ralfs

Synonym: *Closterium acuminatum* Kütz. ex Ralfs

Localities: 1, 3

It is a widely distributed species in the Czech Republic (e.g. Šťastný 2005). In spite of it, this is the first record for Moravia. Some specimens of var. *dianae* were hardly recognizable from var. *minus* – transitional forms.

*_**Closterium dianae**_ **var.** _**minus**_ Hieron.

Synonym: *Closterium dianae* f. *intermedium* Kossinskaja

Localities: 1, 2, 3

Rather rare in our samples and less common in Bohemia than the nominate variety (Šťastný 2010). New taxon for Moravia.

*_**Closterium directum**_ W. Archer

Synonyms: *Closterium ulna* Focke ex W.B. Turner, *Cl. ulna* var. *multinucleatum* Deflandre, *Cl. ulna* var. *striolato-punctatum* Elenkin

Locality: 2

The species was found in a slightly acidic *Sphagnum* locality. Many reports from Bohemia: from Břehyně and Pískovny Cep (Šťastný 2005), from the Jizerské hory Mts (Štěpánková et al. 2008), from the Krkonoše Mts (Nováková 2002). New taxon for Moravia.

_**Closterium ehrenbergii**_ Ralfs

Localities: 1, 4

Fig 1: 6

It was present in slightly acidic to slightly alkaline waters. Cells had a great range of dimensions. Previous records from Moravia: Dočkal and Sládeček (1974), Kočárková and Poulíčková (2001), Lelková et al. (2004), Sládečková et al. (1985). Losos and Marvan (1957) found *Closterium ehrenbergii* var. *malinvernianum* in Černý potok and Růžička (1954) in the river Moravice and its tributaries (Černý potok and Podolský potok).

*_**Closterium ehrenbergii**_ **var.** _**atumidum**_ Grönblad

Locality: 3

Cells were strongly arched, without swelling in the ventral side of the mid region (in contrast to the type variety). New taxon for Moravia.

*_**Closterium incurvum**_ Bréb.

Synonym: *Closterium venus* var. *incurvum* (Bréb.) Krieger

Localities: 2, 3, 7

Rather common in acidic, slightly acidic to slightly alkaline waters in Bohemia (Šťastný 2005). New taxon for Moravia.

*_**Closterium intermedium**_ Ralfs

Locality: 6a

The species occurred in samples with slightly acidic to neutral water reaction. It is already known from Bohemia (e.g. Šťastný 2010). New taxon for Moravia.

_**Closterium juncidum**_ Ralfs

Locality: 6a

Fig 1: 9

From Moravia known only from the river Moravice and its tributaries until now (Růžička 1954; Losos and Marvan 1957).

_**Closterium littorale**_ F. Gay

Locality: 5

Fig 1: 12

It was rare in slightly alkaline water bodies. From Moravia previously recorded from the Morava river (Poulíčková 1987). According to literature (e.g. Coesel and Meesters 2007) and also according to our experience, it prefers rather eutrophic habitats.

_**Closterium littorale**_ **var.** _**crassum**_ W. et G. S. West

Locality: 5

It was rare in slightly alkaline water bodies. Previous records from Moravia from the Moravice river (Losos and Marvan 1957; Růžička 1954).

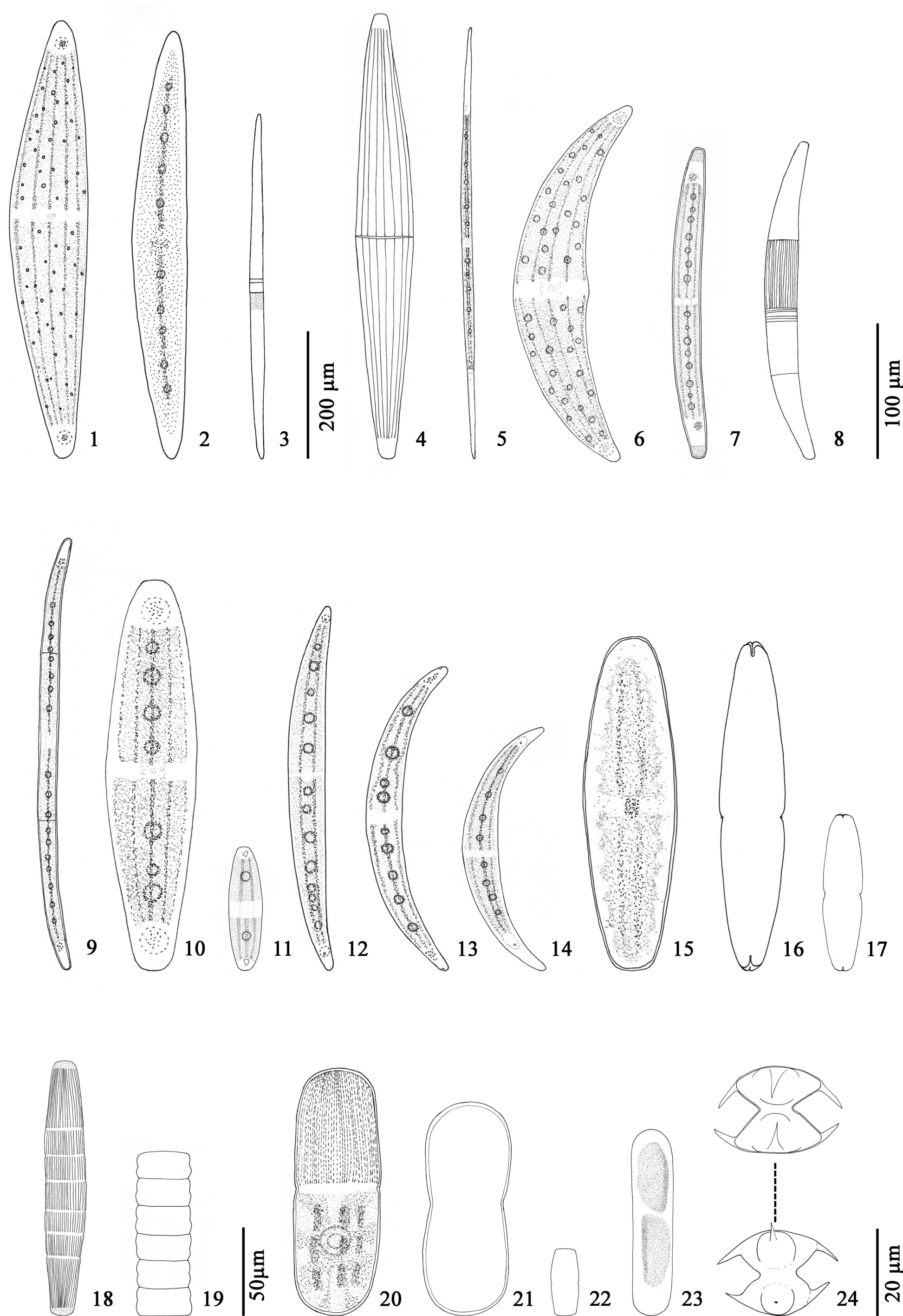



**Fig 1: 1** – *Closterium lunula*, **2** – *Cl.* cf. *pseudolunula*, **3** – *Cl.* cf. *pritchardianum* var. *angustum*, **4** – *Cl.* cf. *costatum* var. *westii*, **5** – *Cl. pronum*, **6** – *Cl. ehrenbergii*, **7** – *Cl.*cf. *baillyanum*, **8** – *Cl. striolatum*, **9** – *Cl. juncidum*, **10** – *Cl. closterioides*, **11** – *Cl. navicula*, **12** – *Cl. littorale*, **13** – *Cl. parvulum*, **14** – *Cl. tumidulum*, **15** – *Netrium digitus*, **16** – *Tetmemorus granulatus*, **17** – *T. laevis*, **18** – *Penium spirostriolatum*, **19** – *Hyalotheca dissiliens*, **20** – *Actinotaenium silvae-nigrae* var. *parallelum*, **21** – *A. diplosporum*, **22** – *A. pinicolum*, **23** – *Mesotaenium endlicherianum*, **24** – *Staurodesmus glaber*. Figs 1–3: scale 200 µm; 4–8: scale 100 µm; 9–19: scale 50 µm; 20–24: scale 20 µm.

## *Closterium lunula* Ralfs

Localities: 1, 5, 6a, 6b, 7

Fig 1: 1

It was found in acidic, slightly acidic to slightly alkaline water bodies. Previously reported from Obidová in the Beskydy Mts – Protected Landscape Area (Kitner et al. 2004) and from the Morava river (Hindák et al. 2006).

## **Closterium moniliferum* var. *concavum* Klebs

Locality: 3

This taxon belongs to desmids with a wide ecological valence, so it can occur in slightly acidic to alkaline, meso-eutrophic water bodies (Růžička 1977). In Moravia, *Closterium moniliferum* Ralfs was previously recorded by Dočkal and Sládeček (1974), Hindák et al. (2006), Kočárková and Poulíčková (2001), Lelková et al. (2004), Růžička (1954). Variety *concavum* is obviously less common than the nominate variety because this is the first record of it for Moravia.

## **Closterium navicula* (Bréb.) Lütkem.

Synonyms: *Closterium navicula* var. *granulatum* (W. et G. S. West) Krieger, *Penium fusiforme* F. Gay, *P. navicula* Bréb., *P. navicula* f. *willei* Schmidle

Locality: 6b

Fig 1: 11

According to Růžička (1977), it is a common species in middle Europe. It prefers slightly acidic water bodies but it can be present also in slightly alkaline waters. Even though it has been repeatedly recorded from localities in Bohemia (Šťastný 2005, 2007, 2008a, 2008b, 2009, 2010; Štěpánková et al. 2008 – var. *crassum*; Lukavský 2009) this is the first record for Moravia.

## *Closterium parvulum* Nägeli

Localities: 1, 6a

Fig 1: 13

In Moravia it was present in slightly acidic, neutral to slightly alkaline waters. It was previously recorded by Růžička (1956, 1957).

## **Closterium* cf. *pritchardianum* var. *angustum* Borzécki

Locality: 1

Fig 1: 3

The species occurred in slightly acidic water with *Sphagnum*. Cells were almost straight, thinner than 28 µm with truncately rounded apices. Cell wall was irregular punctate at the apices. New taxon for Moravia.

## *Closterium pronum* Bréb.

Locality: 7

Fig 1: 5

It was rather common in some of the acidic bog pools in the locality No. 7. From Moravia previously recorded by Poulíčková (1987) from the river Morava in Olomouc. In the book „Desmids of the Lowlands" (Coesel and Meesters 2007) the wide ecological range of this species is discussed.

## *Closterium* cf. *pseudolunula* Borge

Locality: 1

Fig 1: 2

This species was a rare element in the samples. It was found in slightly acidic water with *Sphagnum*. Cells were conspicuously larger (reaching up to 680 µm) than the average (Coesel 1983; Růžička 1977). Previous record from Moravia was made by Hašler et al. (2008).

## *Closterium rostratum* Ralfs

Localities: 1, 2, 3, 4, 5

This species is common in Moravia. We found it in slightly acidic to slightly alkaline habitats. In samples from the peat bog „Pstruží potok" (Locality 1), there were intermediate forms of *Closterium rostratum* and *Closterium kuetzingii* present. From Moravia previously recorded by Kitner et al. (2004), Losos and Marvan (1957) and Štěpánková et al. (2012).

******Closterium rostratum* **var.** ***brevirostratum*** W. West

Synonym: *Closterium rostratum* f. *brevirostratum* (W. West) Kossinskaja

Locality: 1

This taxon is probably often confused with the variety *rostratum*. It was very rare in one of the samples. New taxon for Moravia. However, according to Lenzenweger (1996) and Růžička (1977), the validity of this taxon is challenged.

***Closterium striolatum*** Ralfs

Localities: 1, 2, 3, 5, 6a, 6b, 7

Fig 1: 8

In Moravia, common and in some of our samples, relatively abundant. Our samples had wide range of dimensions: L: 237.5-330, B: 30-35 µm. Previous records: Kitner et al. (2004) and Marvan (1998).

***Closterium tumidulum*** F. Gay

Locality: 1

Fig 1: 14

This species is known for its wide ecological valence, present also in eutrophic waters (Růžička 1977). From Moravia previously recorded by Hašler et al. (2008), Losos and Marvan (1957), Růžička (1954, 1956, 1957) and Rybníček (1958) from rivers and from stagnant waters.

***Closterium tumidum*** Johns.

Locality: 6b

From Moravia more records are known: Růžička (1954, 1957) and Rybníček (1958). Růžička (1977) regarded this species as rare in Central Europe. But we do not share this opinion. According to us and with respect to the opinion of Šťastný (2010), it is probably rather overlooked.

## Family Desmidiaceae

****Actinotaenium colpopelta*** (Bréb.) Compère

Synonyms: *Actinotaenium colpopelta* f. *minus* (W. West) Compère, *A. viride* (Corda ex Josh.) Teiling, *Colpopelta viridis* Corda, *Cosmarium colpopelta* Bréb. ex W. Archer, *C. cordanum* Bréb. in Rabenhorst, *C. viride* (Corda) ex Josh., *Dysphinctium viride* (Corda) De Toni

Locality: 1

It was a very rare species, which we found in slightly acidic water. Also according to Coesel and Meesters (2007) and Růžička (1977), this species is rare. Dimensions of our specimens: L: 40-52.5, B: 21.5-25, I: 20-22.5 µm. New taxon for Moravia. Our specimens correspond to this depicted by Růžička (1981), Plate 55: Fig. 20.

***Actinotaenium cucurbita*** (Ralfs) Teiling

Synonym: *Cosmarium cucurbita* Ralfs

Localities: 1, 8

Although it prefers strictly acidic water bodies (Růžička 1981), we found it in an acidic and in slightly acidic waters. Previous records from Moravia: Marvan (1998) and Štěpánková et al. (2012).

***Actinotaenium cucurbitinum*** (Bisset) Teiling

Synonyms: *Cosmarium cucurbitinum* (Bisset) Lütkem., *Penium cucurbitinum* Bisset

Locality: 3

It was rather rare in the samples from a slightly acidic habitat. In Moravia previously found by Kitner et al. (2004).

***Actinotaenium diplosporum*** (P. Lundell) Teiling

Synonym: *Cosmarium diplosporum* (P. Lundell) Lütkem.

Localities: 1, 3

Fig 1: 21

Our specimens occurred in slightly acidic to neutral waters. From Bohemia it is known from the localities Břehyně, Krkonoše Mts (Šťastný 2005), Jizerské hory Mts (Štěpánková et al. 2008), Slavkovský les (Trojánková 2006) and from the fishpond Řežabinec (var. d*iplosporum*, var. *americanum* and var. *americanum* f. *minus*, Růžička 1973). From Moraviait was previously recorded from Malý Děd in the Hrubý Jeseník Mts (Růžička 1957) and source of the Moravice river (Růžička 1956).

***Actinotaenium pinicolum*** Rosa
Localities: 1, 3
Fig 1: 22
It seems to be a relatively common species in acidic to slightly acidic habitats of Moravia. In the past it was probably often overlooked. Štěpánková et al. (2012) found it in eight localities of the Jeseníky Mts, with a strong preference for subatmophytic sites.

***Actinotaenium silvae-nigrae* var. *parallelum*** (Krieger) Kouwets et Coesel
Synonym: *Penium silvae nigrae* var. *parallelum* Krieger
Localities: 1, 3, 8
Fig 1: 20
It occurred in acidic to slightly acidic waters with *Sphagnum*. Previously it was recorded by Štěpánková et al. (2012).

***Actinotaenium* cf. *spinospermum*** (Josh.) Kouwets et Coesel
Synonym: *Penium spinospermum* Josh.
Locality: 3
We are not sure with identification, because we did not found the zygospores of the species. Our specimens were present in slightly acidic waters with *Sphagnum*. They corresponded to these depicted by Růžička (1977) in Table 5: Figs. 12-14. The species was previously reported by Štěpánková et al. (2012).

**▲*Cosmarium asphaerosporum* cf. var. *strigosum*** Nordst.
Locality: 1
Fig 2: 20
Cells were a little bit narrower and had a broader isthmus than those mentioned in West and West (1905) – the dimensions of our specimens were: L: 10-10.5, B: 7.5-8 µm. We found it in slightly acidic *Sphagnum* water. This is new taxon for the Czech Republic.

***Cosmarium botrytis*** Ralfs
Localities: 4, 6a
Our specimens came from slightly alkaline waters. They were a little bit bigger than those described in Coesel and Meesters (2007). Dimensions of our specimens were: L: 85-97, B: 65-75 µm. It was previously reported by Kitner et al. (2004), Losos and Marvan (1957). Another variety – var. *tumidum* was reported by Hašler et al. (2008).

***Cosmarium caelatum*** Ralfs
Localities: 1, 3
Fig 2: 9
The species was found in slightly acidic habitats. According to Coesel and Meesters (2007), it is rare in the Netherlands. However, in the Czech Republic it is relatively common, from Moravia reported by Kitner et al. (2004), Růžička (1956), Rybníček (1958) and Štěpánková et al. (2012).

***Cosmarium crenatum*** Ralfs
Locality: 6b
There were no records from Moravia for a long time. The last time it was reported by Růžička (1957) and Rybníček (1958). According to our observations it is rather a rare species.

*****Cosmarium decedens*** (Reinsch) Racib.
Synonyms: *Cosmarium carpaticum* (Racib.) Migula, *C. plicatum* var. *decedens* Reinsch, *Euastrum anomalum* F. Gay, *E. decedens* (Reinsch) J. Roy
Locality: 3
Fig 2: 12
Our specimens come from an ephemeral puddle in the neighbourhood of the peat bog „Rašeliniště Skřítek". It is rare and this is the first record of the species for Moravia.

***Cosmarium difficile*** Lütkem.
Locality: 1
Fig 2: 13
It is a relatively common species in the Czech Republic. Previous reports from Moravia: Kitner et al. (2004), Růžička (1956, 1957) and Štěpánková et al. (2012).

▲***Cosmarium exiguum* var. *pressum*** W. et G. S. West
Locality: 1
Fig 2: 19
Our specimens corresponded to the figures from West and West (1908), Plate 70: Fig. 23-24. The taxon occurred in a large amount's in one of the samples. New taxon for the Czech Republic.

****Cosmarium galeritum*** Nordst.
Locality: 1
Fig 2: 6
The species occurred in a slightly acidic habitat and was rare in one of the samples. From the Czech Republic, it was previously recorded only twice, by Roubal (1938) and Šťastný (2010). However, Šťastný (2010) found a different morphospecies with just one central pyrenoid. Our finding can be regarded as the first for Moravia.

***Cosmarium homalodermum*** Nordst.
Synonym: *Cosmarium hammeri* var. *homalodermum* (Nordst.) W. et G. S. West
Locality: 1
Fig 2: 11
Rather a rare species with variable number of pyrenoids (one or two; Kouwets 1997). Chloroplasts of our specimens contained two pyrenoids in each semicell. In Moravia previously found by Růžička (1956, 1957).

***Cosmarium hornavanense*** Gutw.
Locality: 2
Fig 2: 7
Dimensions of our specimens: L: 95-97.5, B: 70-72.5 µm. Our specimens were a little bit narrower than those described by Lenzenweger (1999). According to Růžička (1957), it is a common species in mountains of the Czech Republic, however always scattered. From Moravia previously recorded by Růžička (1956, 1957) and Rybníček (1958).

▲***Cosmarium incertum*** Schmidle
Locality: 1
Fig 2: 14
It was very rare, with dimensions: L: 29-31.75, B: 17.75-19 µm. New taxon for the Czech Republic.

***Cosmarium laeve* var. *octangulare*** (Wille) W. et G. S. West
Synonyms: *Cosmarium laeve* var. *undulatum* Schmidle, *C. meneghinii* f. *octangulare* Wille
Locality: 1
The variety was found in a slightly acidic mesotrophic peat bog. *Cosmarium laeve* was previously reported from Moravia by Losos and Marvan (1957), Růžička (1954, 1956), Marvan (1998) and Kitner et al. (2004). Růžička (1954, 1956) reported a broad variability in this species and figure No. 19 in the Table 2 (Růžička 1956) seems to be more likely var. *octangulare* than var. *laeve*.

***Cosmarium nasutum* f. *granulatum*** Nordst.
Locality: 1
Fig 2: 10
According to Kouwets (1999), this forma should be united with the nominal variety. Previous record for Moravia is from the locality Obidová (Kitner et al. 2004).

***Cosmarium ochthodes*** Nordst.
Localities: 1, 2, 3
According to Lenzenweger (1999), it is an adaptable species. In our samples, it occurred in slightly acidic water bodies. Relatively wide range of dimensions: L: 75-97.5, B: 62.5-70 µm. The specimens corresponded to these

depicted by Lenzenweger (1999), Plate 55: Fig. 5-6. From Moravia it was previously recorded by Růžička (1956).

### *Cosmarium pachydermum* P. Lundell

Locality: 1
Fig 2: 2
Kitner et al. (2004) found it in three rather acidic Moravian localities: Adámky, Horní Lomná and Obidová. We detected it in the peat meadow „Pstruží potok" which is slightly acidic.

### **Cosmarium praemorsum* Bréb.

Locality: 1
This could be the first record of the species from Moravia, because the validity of the only previous record from Růžička (1954) is doubtful. Although the shape and dimensions of his specimens fitted, he was not able to see the ornamentation to be sure with determination.

### *Cosmarium pseudonitidulum* Nordst. **var.** *validum* W. et G. S. West

Localities: 1, 3
Fig 2: 3
Other findings from Moravia: Růžička (1956, 1957) and Rybníček (1958).

### **Cosmarium pseudoornatum* B. Eichl. et Gutw.

Locality: 6b
Fig 2: 15
The species found in a slightly acidic ephemeral pool was already reported from the Czech Republic (e.g. Šťastný 2005, Trojánková 2006). However, this is a new taxon for Moravia.

### **Cosmarium quadratum* **var.** *willei* (Schmidle) Krieger et Gerloff

Synonyms: *Cosmarium quadratum* f. *maius* Manguin, *C. quadratum* f. *willei* (Schmidle) W. et G. S. West, *Dysphinctium quadratum* var. *willei* Schmidle
Locality: 3
Fig 2: 5
We found this variety in a slightly acidic locality. Previous reports from Moravia belong to the nominate variety (Růžička 1956; Rybníček 1958; Losos and Heteša 1972; Kitner et al. 2004; Štěpánková et al. 2012) while variety *willei* was recorded only recently in Bohemia (Lukavský 2009). New taxon for Moravia.

### **Cosmarium ralfsii* Ralfs

Synonyms: *Cosmaridium ralfsii* (Bréb.) Hansg., *Pleurotaeniopsis ralfsii* (Bréb.) P. Lundell
Locality: 1
Fig 2: 1
The species found in a slightly acidic habitat is probably rare in Moravia. New taxon for Moravia.

### *Cosmarium regnellii* **var.** *minimum* B. Eichl. et Gutw.

Locality: 2
Fig 2: 17
It occurred in a slightly acidic habitat. Previous reports from Moravia are from Hašler et al. (2008) and Štěpánková et al. (2012).

### **Cosmarium subcostatum* **var.** *minus* (W. et G. S. West) Kurt Först.

Synonym: *Cosmarium subcostatum* f. *minus* W. et G. S. West
Locality: 7
Fig 2: 16
The variety was found in a slightly acidic bog pool. Cells were a little bit smaller than those described by West and West (1908). Dimensions of our specimens: L: 17.5-18.5, B: 15-17 µm. New taxon for Moravia.

### *Cosmarium subcucumis* Schmidle

Locality: 4
This species found in a slightly alkaline locality was relatively frequently reported from Moravia: Růžička (1954, 1956), Losos and Marvan (1957), Rybníček (1958), Kitner et al. (2004).

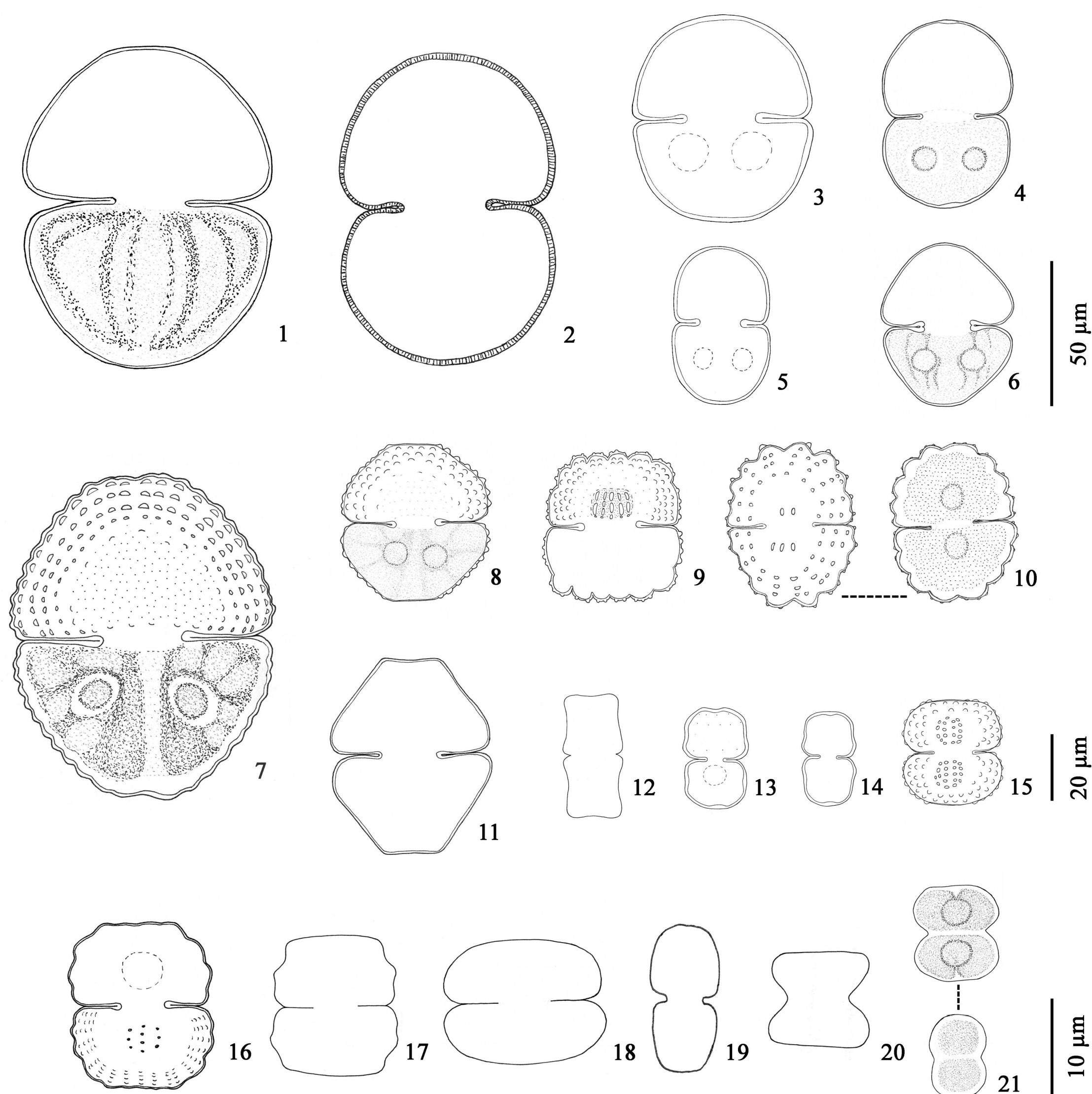



**Fig 2: 1** – *Cosmarium ralfsii*, **2** – *C. pachydermum*, **3** – *C. pseudonitidulum* var. *validum*, **4** – *C. transitorium*, **5** – *C. quadratum* var. *willei*, **6** – *C. galeritum*, **7** – *C. hornavanense*, **8** – *C. vexatum* var. *concavum*, **9** – *C. caelatum*, **10** – *C. nasutum* f. *granulatum*, **11** – *C. homalodermum*, **12** – *C. decedens*, **13** – *C. difficile*, **14** – *C. incertum*, **15** – *C. pseudoornatum*, **16** – *C. subcostatum* var. *minus*, **17** – *C. regnellii* var. *minimum*, **18** – *C. subquadrans* var. *minor*, **19** – *C. exiguum* var. *pressum*, **20** – *C. asphaerosporum* cf. var. *strigosum*, **21** – *C. tinctum* var. *intermedium*. Figs 1–6: scale 50 µm; 7–15: scale 20 µm; 16–21: scale 10 µm.

### ***Cosmarium subquadrans* var. *minor*** Symoens ex Coesel

Locality: 1
Fig 2: 18
This taxon is rare in the Czech Republic. Our specimens come from a slightly acidic *Sphagnum* locality. From Moravia previously only recently recorded by Štěpánková et al. (2012).

### **Cosmarium tinctum* var. *intermedium*** Nordst.

Locality: 7
Fig 2: 21

Šťastný (2010) recorded two known varieties of this species from the Czech Republic: nominate variety and var. *subretusum*. Variety *intermedium* was found many years ago by Roubal (1958). This is the first record of var. *intermedium* for Moravia.

### ▲*Cosmarium transitorium* (Heimerl) Ducellier

Synonym: *Cosmarium pachydermum* f. *transitoria* Heimerl
Locality: 1
Fig 2: 4
The species was rarely found on a *Sphagnum* habitat and this is the first record of it from the Czech Republic.

### *Cosmarium vexatum* W. West

Locality: 1
It occurred in slightly acidic waters. From Moravia previously reported by Štěpánková et al. (2012). In Moravia, Hašler et al. (2008) found another variety: var. *lacustre*, a rare taxon in central Europe (Lenzenweger 2003).

### **Cosmarium vexatum* **var.** *concavum* Schmidle

Localities: 1, 2
Fig 2: 8
It requires the same conditions as the nominate variety. The first record from the Czech Republic comes from Šťastný (2010). It is hard to distinguish it from *Cosmarium sportella* Bréb., which differs only with central ornamentation of semicells (Lenzenweger 1999). Its taxonomic position is challenged by some authors (Guiry & Guiry 2013). If the taxon is valid, our record is the first for Moravia.

### **Euastrum ansatum* Ralfs

Synonyms: *Cosmarium ansatum* (Ehr.) Corda, *C. pseudopyramidatum* var. *ansatum* Krieger et Gerloff, *Euastrum ansatum* var. *commune* Ducellier, *E. ansatum* var. *dideltiforme* Ducellier, *E. ansatum* var. *emarginatum* Hansg., *E. ansatum* var. *simplex* Ducellier, *E. rotundum* Playfair
Localities: 6a, 7
Fig 3: 8–9
According to Růžička (1981), it is a well adaptable species which prefers slightly acidic waters but can also occur in acid *Sphagnum* waters or in neutral sites. Although it has been frequently reported from Bohemia, records from Moravia do not exist. The only record from Moravia is from Kitner et al. (2004), who reported *Euastrum ansatum* var. *pyxidatum* from locality Obidová. New taxon for Moravia.

### *Euastrum bidentatum* Nägeli

Locality: 6b
Fig 3: 10
Růžička (1981) stated that the nominate variety of *Euastrum bidentatum* is common in Central Europe. It prefers meso-oligotrophic moorland pools and fen hollows (Coesel and Meesters 2007). Previous record from Moravia: Štěpánková et al. (2012).

### *Euastrum binale* Ralfs

Localities: 1, 7, 8
Rather rare in Central Europe (Růžička 1981), however Lhotský (1949) assumed it to be widely distributed across the whole Moravia. Nevertheless, this is after his record the only one from this region.

### *Euastrum binale* **var.** *gutwinskii* (Schmidle) Homfeld

Localities: 1, 6a, 6b
Fig 3: 13
The taxon is relatively common in the Czech Republic with preference to acidic or slightly acidic habitats. From Moravia it was previously recorded by Fischer (1924, 1925), Lhotský (1949), Rybníček (1958) and Štěpánková et al. (2012).

### *Euastrum dubium* Nägeli

Localities: 6a, 6b
It is rather rare in Moravia. We found it in slightly acidic to neutral habitats. Previous record: Kitner et al. (2004).

### ***Euastrum elegans*** Ralfs
Locality: 7
Fig 3: 11
The species was found in a mesotrophic acidic pool. From Moravia it was previously recorded by Růžička (1957) from the Hrubý Jeseník Mts.

### ****Euastrum gayanum*** De Toni
Synonym: *Euastrum formosum* F. Gay
Locality: 7
Fig 3: 14
It prefers mesotrophic acidic habitats (Coesel and Meesters 2007). New taxon for Moravia.

### ***Euastrum humerosum*** Ralfs
Locality: 6a
The species occurred in acidic to slightly acidic and neutral waters. From Moravia it is already known from the Jeseníky Mts, particularly in consequence with aerial liming (locality Trojmezí and Sedlo pod Vozkou; Štěpánková et al. 2012).

### ***Euastrum insulare*** (Wittr.) J. Roy
Locality: 6b
Fig 3: 12
The species was found in a mesotrophic slightly acidic ephemeral ditch. Kitner et al. (2004) recorded it from the West Carpathians.

### ***Euastrum oblongum*** Ralfs
Localities: 1, 2, 6a, 6b
Fig 3: 7
It occurred in slightly acidic mesotrophic habitats and was relatively abundant in the samples. From Moravia previously recorded by Kitner et al. (2004) and by Růžička (1957).

### ***Hyalotheca dissiliens*** Ralfs
Localities: 1, 2, 6b, 7
Fig 1: 19
The nominate variety is much more common than other varieties in the Czech Republic. Although it is not extremely rare in Moravia, it was previously recorded only once by Růžička (1957).

### ****Micrasterias americana*** Ralfs
Synonyms: *Euastrum americanum* Ehr., *Micrasterias americana* var. *taylori* Krieger, *M. morsa* Ralfs
Locality: 6b
Fig 3: 4
Rather a rare species in the Czech Republic (Šťastný 2010) occurred in our specimens in slightly acidic and neutral waters of ephemeral ditches. New taxon for Moravia.

### ****Micrasterias denticulata* var. *angulosa*** (Hantzsch) W. et G. S. West
Synonym: *Micrasterias angulosa* Hantzsch
Localities: 1, 6b
Fig 3: 3
It is common in acidic, slightly acidic and neutral habitats (Růžička 1981). We were able to find specimens with its typical form, however according to Coesel and Meesters (2007), transitional forms to *Micrasterias thomasiana* var. *notata* are more common. It was reported several times from localities in Bohemia (Šťastný 2005 – nominate variety, 2008b – var. *angulosa*, 2009 – var. *angulosa*), but it can be classified as a very rare taxon in the Czech Republic. New taxon for Moravia.

### ***Micrasterias papillifera*** Ralfs
Localities: 1, 2, 6a
Fig 3: 5
The species is relatively rare in the Czech Republic and occurs in slightly acidic habitats. Previous record from Moravia is from the locality Obidová (Kitner et al. 2004).

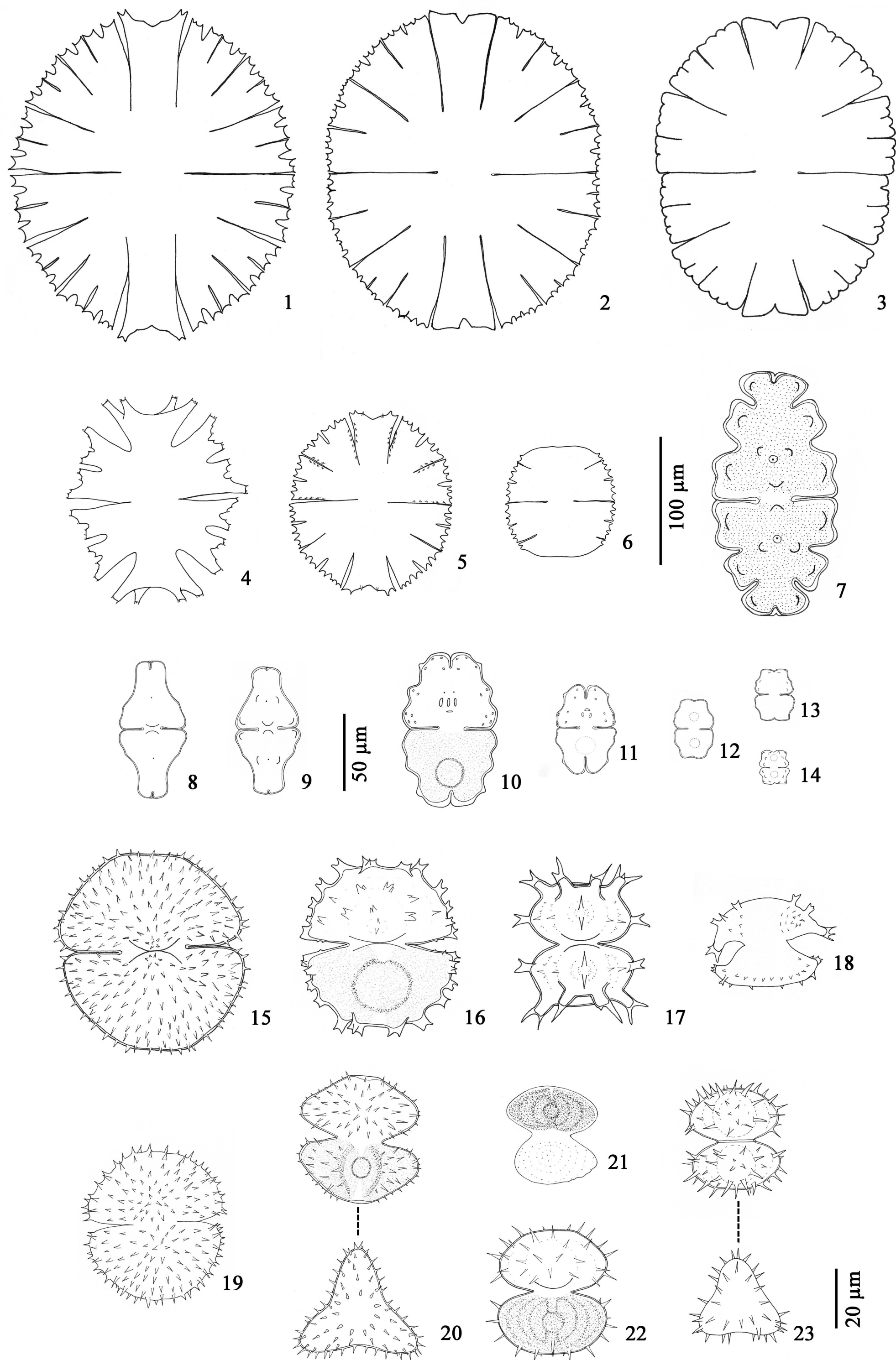



**Fig 3: 1** – *Micrasterias rotata*, **2** – *M. thomasiana* var. *notata*, **3** – *M. denticulata* var. *angulosa*, **4** – *M. americana*, **5** – *M. papillifera*, **6** – *M. truncata*, **7** – *Euastrum oblongum*, **8–9** *E. ansatum*, **10** – *E. bidentatum*, **11** – *E. elegans*, **12** – *E. insulare*, **13** – *E. binale var. gutwinskii*, **14** – *E. gayanum*, **15** – *Staurastrum pyramidatum*, **16** – *St. spongiosum* var. *perbifidum*, **17** – *St. senarium*, **18** – *St. controversum*, **19** – *St. hirsutum*, **20** – *St. kouwetsii*, **21** – *St. punctulatum*, **22–23** *St. teliferum* var. *ordinatum*. Figs 1–6: scale 100 µm; 7–9: scale 50 µm; 10–23: scale 20 µm.

***Micrasterias rotata*** Ralfs

Localities: 2, 6a

Fig 3: 1

Our populations were found in slightly acidic mesotrophic habitats. Although it is rather a rare species in the Czech Republic, over the years it was recorded from many places in Bohemia: Břehyně, Borkovická blata, Pískovny Cep, Rašeliniště Pele, Vizír (Šťastný 2005), Chvojnov (Šťastný 2008b), Slavkovský les (Trojánková 2006), localities Odměny and Staré jezero in the Třeboň basin (Chattová 2007); var. *evoluta* from Blatná and the Šumava Mts (Rosa 1951); var. *pulchra* from the Šumava Mts (Pascher 1906). From Moravia, it was known only from the locality Obidová (Kitner et al. 2004).

****Micrasterias thomasiana*** W. Archer

Synonym: *Micrasterias denticulata* var. *subnotata* W. West, *M. denticulata* f. *thomasiana* (W. Archer) Jacobsen, *Micrasterias thomasiana* f. *maior* W. West

Localities: 6a, 6b

It was found in slightly acidic habitats. Although it was over the years several times recorded from Bohemia, it is rare in the Czech Republic (Šťastný 2010). New taxon for Moravia. This species is listed in the Red Book of endangered and rare species of the Slovak and the Czech Republic (Kotlaba 1995).

****Micrasterias thomasiana* var. *notata*** (Nordst.) Grönblad

Synonym: *Micrasterias denticulata* var. *notata* Nordst.

Localities: 2, 6a, 7

Fig 3: 2

According to Coesel and Meesters (2007), it is more common than the nominal variety according to Růžička (1981) both varieties are quite frequent in the middle Europe. New taxon for Moravia.

***Micrasterias truncata*** Ralfs

Localities: 1, 2

Fig 3: 6

The species is relatively common in the Czech Republic. However, only two records are known from Moravia: the Jeseníky Mts (Růžička 1956) and the Beskydy Mts (Kitner et al. 2004).

***Pleurotaenium trabecula*** Nägeli

Locality: 2

The species was found in a slightly acidic bog with *Sphagnum*. According to Růžička (1977), it often occurs in Central Europe. From Moravia previously recorded by Lelková et al. (2004) and by Sládečková et al. (1985).

****Staurastrum controversum*** Ralfs

Synonym: *Staurastrum anatinum* var. *controversum* (Bréb. in Meneghini) Brook

Localities: 6a, 7

Fig 3: 18

It was found in mesotrophic, acidic and slightly acidic waters. It is rather rare in the Czech Republic (Šťastný 2010). New taxon for Moravia.

****Staurastrum dilatatum*** Ralfs

Locality: 2

The species was found in a slightly acidic habitat. It is already known from Bohemia (e.g. Šťastný 2010). New taxon for Moravia.

***Staurastrum furcatum* var. *aciculiferum*** (W. West) Coesel

Locality: 8

It occurred in an acidic habitat with *Sphagnum*. From Moravia it is already known from the Jeseníky Mts (Rybníček 1958, Štěpánková et al. 2012), particularly from ombrotrophic mires.

****Staurastrum gladiosum*** W. B. Turner

Locality: 7

The species prefers mesotrophic peat bog pools of Holocene character (Coesel 1997; Coesel & Meesters 2007). New taxon for Moravia.

***Staurastrum hirsutum*** Ralfs
Localities: 1, 3, 6b, 8
Fig 3: 19
The species inhabit acidic to slightly acidic waters. Although it used to be bound to oligotrophic habitats (Coesel and Meesters 2007), we found it in mesotrophic waters. From Moravia previously recorded by Štěpánková et al. (2012).

*****Staurastrum kouwetsii*** Coesel
Localities: 2, 3, 6b
Fig 3: 20
According to Coesel and Meesters (2007), it is often misidentified as *Staurastrum subbrebissonii* or *Staurastrum pilosum* in algological literature. It occurs in acidic to slightly acidic mesotrophic habitats (e.g. Coesel 1997, 1998; Šťastný 2010). New taxon for Moravia.

***Staurastrum margaritaceum*** Ralfs
Localities: 3, 6a, 6b, 8
The species inhabit acidic to slightly acidic waters. Coesel and Meesters (2007) characterized it as an oligotrophic species. However, we found it also in mesotrophic waters. It is not rare in the Czech Republic. Previous records from Moravia were made by Fischer (1924, 1925), Rybníček (1958) and recently by Štěpánková et al. (2012).

***Staurastrum orbiculare*** Ralfs
Locality: 7
The species was rarely recorded in one of our samples. It was previously found in Moravia e.g. by Rybníček (1958) in the Jeseníky Mountains. Růžička (1958) found another variety, *Staurastrum orbiculare* var. *extensum* Nordst. in the same mountains.

***Staurastrum punctulatum*** Ralfs
Locality: 7
Fig 3: 21
The species was found in an acidic bog pool. From Moravia previously recorded by Kitner et al. (2004) from the Beskydy Mts and by Štěpánková et al. (2012) from the Jeseníky Mts.

*****Staurastrum pyramidatum*** W. West
Synonyms: *Cosmoastrum pyramidatum* (W. West et G. S. West) Palamar-Mordvintseva, *Staurastrum muricatum* var. *acutum* W. West, *S. muricum* var. *acutum* W. West
Locality: 1
Fig 3: 15
The species was rare in the samples with dimensions: L: 67.5, B: 52.5-57.5 µm. Our specimens corresponded to those depicted by Lenzenweger (1997), Plate 28: Fig. 1-2. It is a rare species in the Czech Republic (Šťastný 2010). New taxon for Moravia.

***Staurastrum senarium*** Ralfs
Synonym: *Desmidium senarium* Ehr.
Locality: 7
Fig 3: 17
We found the species in a mesotrophic peat bog pool of potential Holocene character. In Moravia previously recorded by Kitner et al. (2004).

*****Staurastrum sexcostatum* cf. var. *productum*** W. West
Synonym: *Staurastrum sexcostatum* subsp. *productum* W. West
Locality: 6b
The taxon was found in a mesotrophic ephemeral ditch. It is rather rare in the Czech Republic (Šťastný 2010). New taxon for Moravia. However, the validity of this variety is challenged (Guiry and Guiry 2012).

***Staurastrum spongiosum*** Ralfs
Locality: 6b
The species was found in a slightly acidic, mesotrophic habitat. It is rare in the Czech Republic (Šťastný 2010). Dimensions: L: 50-55, B: 42.5-46, I: 17.5 μm. New taxon for Moravia.

***Staurastrum spongiosum* var. *perbifidum*** W.West
Locality: 6b
Fig 3: 16
We found this variety in slightly acidic, mesotrophic ephemeral ditches.
It is rare in the Czech Republic (Šťastný 2010). Our specimens corresponded to this depicted by Lenzenweger (1997), Plate 30: Fig. 12. New taxon for Moravia.

***Staurastrum subavicula*** (W. West) W. et G. S. West
Synonyms: *Staurastrum arcuatum* subsp. *subavicula* W. West, *S. arcuatum* var. *subavicula* W. West, *S. vastum* Schmidle
Locality: 7
The species was found in a mesotrophic acidic pool. It is a new record for Moravia. However, for Bohemia it was already recorded (e.g. Šťastný 2010).

***Staurastrum teliferum*** Ralfs
Locality: 7
The species occurred in acidic to slightly acidic bog pools. New taxon for Moravia.

***Staurastrum teliferum* var. *ordinatum*** F. C. E. Børgesen
Locality: 6a
Fig 3: 22–23
The variety was found in a mesotrophic slightly acidic bog. The nominate variety is already known from some Bohemian localities. Also variety *ordinatum* was previously reported by Růžička (1973) from a Bohemian locality. However, our finding can be regarded as new record for Moravia. It could be caused by an uncertainty related to validity of this taxon (Coesel 1997).

***Staurodesmus glaber*** (Ralfs) Teiling
Synonyms: *Arthrodesmus incus* f. *brebissonii* Racib., *A. ralfsii* var. *brebissonii* (Racib.) G.M. Smith, *Desmidium glabrum* Ehr., *Staurastrum glabrum* Ehr. ex Ralfs
Locality: 7
Fig 1: 24
The species occurred in acidic bog pools. New record for Moravia.

***Tetmemorus granulatus*** Ralfs
Localities: 1, 2, 3
Fig 1: 16
The species was found in acidic, slightly acidic and rarely in neutral habitats. It was previously recorded from Moravia from the Jeseníky Mts (Lhotský 1949) and from the Beskydy Mts (Kitner et al. 2004).

***Tetmemorus laevis*** Ralfs
Localities: 1, 2, 5, 6a
Fig 1: 17
The species was commonly found in acidic and slightly acidic habitats. Previous records from Moravia: Fischer (1924, 1925), Kitner et al. (2004), Poulíčková (1998), Růžička (1956, 1957) and Štěpánková et al. (2012).

**Acknowledgements:** We would like to thank to Dr. Zbyněk Hradílek from the Palacký University in Olomouc for his help in field sampling and to Mgr. Steven Morris for English language corrections. This research was supported by the projects: GACR 206/08/0389 of the Grant Agency of the Czech Republic; Přf_2012_001of the Internal Grant Agency of the Faculty of Science, Palacký University; PdF_2013_024 of the Internal Grant Agency of the Faculty of Education, Palacký University; CETPO CZ.1.07/2.3.00/20.0166.

## References


Brook A. J. (1981): The biology of desmids. University of California Press, Berkeley and Los Angeles.

Coesel P. F. M. (1975): The relevance of desmids in the biological typology and evaluation of fresh waters. – Hydrobiological Bulletin 9: 93–101.

- (1982): De Desmidiaceeën van Nederland – Sieralgen. Deel 1 Fam. Mesotaeniaceae, Gonatozygaceae, Peniaceae. Wetenschappelijke Mededelingen KNNV, Hoogwoud NH.

- (1983): De Desmidiaceeën van Nederland – Sieralgen. Deel 2 Fam. Closteriaceae. Wetenschappelijke Mededelingen KNNV, Hoogwoud NH.

- (1985): De Desmidiaceeën van Nederland – Sieralgen. Deel 3 Fam. Desmidiaceae 1. Wetenschappelijke Mededelingen KNNV, Hoogwoud NH.

- (1997): De Desmidiaceeën van Nederland – Sieralgen. Deel 6 Fam. Desmidiaceae 4. Wetenschappelijke Mededelingen KNNV, Hoogwoud NH.

- (1998): Sieralgen en Natuurwaarden. Stichting Uitgeverij K. N. N. V., Utrecht.

- (2001): Biology and diversity of desmids. Compendium from training course, September 14-17, 2001. Dept. of Environm. Assesm., Swedish Univ. of Agricul. Sci., Uppsala.

Coesel P. F. M. & Meesters K. J. (2007): Desmids of the Lowlands. Mesotaeniaceae and Desmidiaceae of the European Lowlands. KNNV Publishing, Zeist.

Chattová B. (2007): Algologická studie rašelinných okrajů rybníků Třeboňské pánve. (Bachelor thesis). Masaryk University in Brno.

Dočkal P. & Sládeček V. (1974): Znečištění řeky Jihlavy u Třebíče. – Bulletin metod. střed. vodoh. laboratoří 25: 111–125.

Fischer R. (1924): Ökologische Skizzen zur Algenflora des mährisch-schlesischen Gesenkes. – Schriften f. Süsswasser u. Meereskunde, Heft. 7: 1–20.

- (1925): Ökologische Skizzen zur Algenflora des mährisch-schlesischen Gesenkes. – Verhandl. d. Naturf. in Brünn 59: 3–11.

Guiry M. D. & Guiry G. M. (2012): *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 15 November 2012.

- (2013): *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 11 February 2013.

Hašler P., Štěpánková J., Špačková J., Neustupa J., Kitner M., Hekera P., Veselá J., Burian J. & Poulíčková A. (2008): Epipelic cyanobacteria and algae: a case study from Czech ponds. – Fottea 8 (2): 133–146.

Hindák F., Hindáková A., Marvan P., Heteša J. & Hašler P. (2006): Diversity, abundance and volume biomass of the phytoplankton of the Morava River (Czech Republic, Slovakia) and Dyje River (Czech Rapublic) in November 2005. Diverzita, abundancia a objemová biomasa fytoplanktónu rieky Moravy (Česká republika, Slovensko) a rieky Dyje (Česká republika) v novembri 2005. – Czech Phycology 6: 77–97.

Kitner M., Poulíčková A., Novotný R. & Hájek M. (2004): Desmids (Zygnematophyceae) of the spring fens of a part of West Carpathians [Krásivky pramenišť části západních Karpat]. – Czech Phycology 4: 43–61.

Kočárková A. & Poulíčková A. (2001): Algal species composition of pools in the Litovelské Pomoraví Protected Landscape Area [Druhové spektrum řas v planktonu tůní Litovelského Pomoraví]. – Czech Phycology 1: 37–44.

Kotlaba F. [ed.] (1995): Červená kniha ohrožených a vzácných druhů rostlin a živočichů SR a ČR, 4. díl (Sinice, řasy, houby, lišejníky, mechorosty). – Príroda, Bratislava.

Kouwets F. A. C. (1997): Contributions to the knowledge of the French desmid flora I. New and noteworthy taxa from the Central and Eastern Pyrenees. – Arch. Protistenk. 148: 33–51.

- (1999): A check-list of desmids (Chlorophyta, Zygnemaphyceae) of France. –Patrimoines Natureles 41: 1–148.

Lelková E., Kočárková A. & Poulíčková A. (2004): Phytoplankton ecology of two floodplain pools near Olomouc [Ekologie fytoplanktonu dvou tůní u Olomouce]. – Czech Phycology 4: 111–121.

Lenzenweger R. (1996): Desmidiaceenflora von Österreich. Teil 1. J. Crammer, Stuttgart.

- (1997): Desmidiaceenflora von Österreich. Teil 2. J. Crammer, Stuttgart.

- (1999): Desmidiaceenflora von Österreich. Teil 3. J. Crammer, Stuttgart.

- (2003): Desmidiaceenflora von Österreich. – In: Crammer, J. (ed.): Bibliotheca Phycologica 111/4: 1–87 pp. Gebrüder Borntraeger Verlagsbuchhandlung, Berlin-Stuttgart.

Lhotský O. (1949): Poznámka k flóře Desmidiaceí Hrubého Jeseníku. – Časopis Vlast. spolku mus. v Olomouci 58: 149–155.

Losos B. & Heteša J. (1972): Plankton plůdkových rybníků. UJEP, Brno.

Losos B. & Marvan P. (1957): Hydrobiologické poměry řeky Moravice a jejích přítoků Podolského a Černého potoka. – Sborník Vysoké školy zemědělské a lesnické v Brně, řada A: Spisy fakulty agronomické a zootechnické 1: 41–69.

Lukavský J. (2009): Algae, Cyanobacteria and Chytridiales of Černé Lake in the Bohemian Forest (Šumava, Czech Republic). – Silva Gabreta 15 (1): 1–48.

Marvan P. (1998): Řasová flóra stojatých vod, mokřadů a toků širší oblasti vlivu energetické soustavy Dukovany–Dalešice. – Přírod. Sbor. Západomorav. Muz. Třebíč 34: 1–136.

Mix M. (1972): Die Feinstruktur der Zellwände bei Mesotaeniaceae und Gonatozygaceae mit einer vergleichenden Betrachtung der verschiedenen Wandtypen der Conjugatophyceae und über deren systematischen Wert. – Archiv für Mikrobiologie 81: 197-220.

Neustupa J., Černá K. & Šťastný J. (2009): Diversity and morphological disparity of desmid assemblages in Central European peatlands. – Hydrobiologia 630 (1): 243-256.

Nováková S. (2002): Algal flora of subalpine peat bog pools the Krkonoše Mts. – Preslia 74: 45–56.

Pascher A. (1906): Neuer Beitrag zur Algenflora des südlichen Böhmerwaldes. – Lotos 54: 1–36.

Poulíčková A. (l987): Algae in ground water below the active stream of a river (Basin of the Morava River, Czechoslovakia). – Arch.Hydrobiol. / Suppl. 78, Algological Studies 46: 65-88.

- (l998): Významné algologické lokality po padesáti letech I. Prameny Střední Opavy [Important algological localities after the period of 50 years]. – In: Poulíčková, A. and Palochová, A. eds. (1998): Sborník referátů 38. prac. konf. AS ČBS.

- (2011): Základy ekologie sinic a řas. UP Olomouc, Olomouc.

Poulíčková A., Bergová K., Hnilica R. & Neustupa J. (2013): Epibryic diatoms from ombrotrophic mires: diversity, gradients and indicating options. – Nova Hedwigia (In press).

Poulíčková A., Lhotský O. & Dřímalová D. (2004): Prodromus sinic a řas České Republiky. – Czech Phycology 4: 19–33.

Reichholf J. (1998): Pevninské vody a mokřady. Ekologie evropských sladkých vod, luhů a bažin. Ikar, Praha.

Rosa K. (1951): Algenflora von Südböhmen I. Die Algen der Umgebung von Blatná. – Studia Botanica Cechoslovaca 12: 173–232.

- (1957): Výzkum mikroedafonu ve smrkovém porostu na Pradědu. – Přírodovědecký sborník Ostravského kraje 18 (1):17–73.

- (1969): Příspěvek k výzkumu řasové flory v okolí Vlašimi II. – Sborník vlastivědných prací z Podblanicka, Benešov 10: 32–75.

Roubal J. (1938): Flora desmidiaceí rašelinných jam u Příbraze. – Čas. Nár. Mus. 112: 83–95.

- (1958): Desmidiologické poznámky. – Sborník Vyšší pedagogické školy v Plzni, biologie-chemie 1: 71–124.

Růžička J. (1954): Krásivky (Desmidiaceae) řeky Moravice a jejích přítoků. – Přírodov. sbor. Ostravského kraje 15: 290–303.

- (1956): Krásivky pramenů Moravice (Velká Kotlina, Jeseníky). – Přírodov. sbor. Ostravského kraje 17: 38–58.

- (1957): Krásivky z pramenišť na Malém Dědu (Hrubý Jeseník). – Časopis Slezského muzea 6: 108–121.

- (1973): Die Zieralgen des Naturschutzgebietes „Řežabinec" (Südböhmen). – Preslia 45: 193–241.

- (1977): Die Desmidiaceen Mitteleuropas. Band 1, 1. Lieferung. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

- (1981): Die Desmidiaceen Mitteleuropas. Band 1, 2. Lieferung. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Rybníček K. (1958): Krásivky pramenné oblasti Branné v Hrubém Jeseníku. – Přírodov. sbor. Ostravského kraje 19: 97–122.

Sládečková A., Sládeček V. & Musilová J. (1985): Jakost vody ve vodárenských nádržích Fryšták, Ludkovice a Slušovice. – Vodní Hosp. B 35 (2): 39–42.

Šťastný J. (2005): Diverzita a ekologie krásivek ve vybraných oblastech České republiky. (Master's thesis). Charles University in Prague.

- (2007): Die Desmidiaceenflora von Pískovny Cep (Südböhmen). – Novit. Bot. Univ. Carol. 18: 53–62.

- (2008a): Desmids from ephemeral pools and aerophytic habitats from the Czech Republic. – Biologia 63 (6): 888–894.

- (2008b): Krásivky Přírodních rezervací „Chvojnov" a „Na Oklice". [Desmids of the Nature Reserves „Chvojnov" and „Na Oklice"]. – Acta rerum naturalium 4: 13–16.

- (2009): The desmids of the Swamp Nature Reserve (North Bohemia, Czech Republic) and a small neighbouring bog: species composition and ecological condition of both sites. – Fottea 9 (1): 135–148.

- (2010): Desmids (Conjugatophyceae, Viridiplantae) from the Czech Republic; new and rare taxa, distribution, ecology. – Fottea 10 (1): 1–74.

Šťastný J. & Kouwets F. A. C. (2012): New and remarkable desmids (Zygnematophyceae, Streptophyta) from Europe: taxonomical notes based on LM and SEM observations. Fottea 12: 293–313.

Štěpánková J., Vavrušková J., Hašler P., Mazalová P. & Poulíčková A. (2008): Diversity and ecology of desmids of peat bogs in the Jizerské hory Mts. – Biologia 63: 891–896.
Štěpánková J., Hašler P., Hladká M. & Poulíčková A. (2012): Diversity and ecology of desmids of peat bogs in the Jeseníky Mts.: Spatial distribution, remarkable finds. – Fottea 12 (1): 111–126.
Trojánková K. (2006): Krásivková flóra CHKO Slavkovský les. – Erica 13: 3–15.
West W. & West G. S. (1905): A monograph of the British Desmidiaceae, Vol. II. – The Ray Society, London.
- (1908): A monograph of the British Desmidiaceae, Vol. III. – The Ray Society, London.
- (1912): A monograph of the British Desmidiaceae, Vol. IV. – The Ray Society, London.
Woelkerling W. J. & Gough S. B.: (1976): Wisconsin desmids, III. Desmid community composition and distribution in relation to lake type and water chemistry. – Hydrobiologia 51 (1): 3-31.

**Authors' address:** Petra Mazalová & Aloisie Poulíčková, Department of Botany, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic, e-mail: petra.mazalova@upol.cz (corresponding author)
Jana Štěpánková, Department of Biology, Faculty of Education, Palacký University, Purkrabská 2, CZ-779 00 Olomouc, Czech Republic

# Paper II

Hydrobiologia (2011) 667:223–239
DOI 10.1007/s10750-011-0650-3

PRIMARY RESEARCH PAPER

# A novel, combined approach to assessing species delimitation and biogeography within the well-known desmid species *Micrasterias fimbriata* and *M. rotata* (Desmidiales, Steptophyta)

**Jiří Neustupa · Jan Šťastný · Katarína Nemjová · Petra Mazalová · Emma Goodyer · Aloisie Poulíčková · Pavel Škaloud**

Received: 28 December 2010 / Revised: 15 February 2011 / Accepted: 19 February 2011 / Published online: 4 March 2011
© Springer Science+Business Media B.V. 2011

**Abstract** Morphological species of freshwater microalgae often have broad geographic distribution. However, traditional species concepts have been challenged by the results of molecular phylogenetic analyses that mostly indicate higher diversity than was previously recognized by purely morphological approaches. A degree of phenotypic differentiation or different geographic distribution of species defined by molecular data remains largely unknown. In this study, we analyzed a pair of well-known and widely distributed desmid species (*Micrasterias fimbriata* and *M. rotata*) and tested for their phylogenetic and morphological homogeneity as well as their geographic distribution. Geometric morphometric and morphological attributes of cells were used in combination with genetic analysis of the *trnG*$^{ucc}$ sequences of 30 strains isolated from a variety of European locations and obtained from culture collections. *Micrasterias rotata* proved to be phylogenetically homogenous across Europe while *M. fimbriata* turned out to be composed of two firmly delimited lineages, differing by molecular as well as by morphometric and morphological data. Published records of traditional *M. fimbriata* were also included in the classification discrimination analysis and were placed into the newly identified lineages upon comparison to the morphometric data collected from living material. Largely disparate geographic patterns were revealed within traditional *M. fimbriata*. One phylogenetic lineage is frequent in central and eastern Europe, but occurs also in the British Isles. A second lineage has been recorded in North America and in Western Europe, where its distribution is possibly limited to the west of the Rhine River. Interestingly, the morphometric analyses of the published records illustrated that the geographic differences have remained largely unchanged since the 1850s indicating a previously unknown distributional stability among microalgal species groups such as the desmids.

**Keywords** Microalgae distribution · Desmidiales · Geometric morphometrics · Green algae · Molecular phylogenetics · *Micrasterias* · *trnG*$^{ucc}$

**Electronic supplementary material** The online version of this article (doi:10.1007/s10750-011-0650-3) contains supplementary material, which is available to authorized users.

Handling editor: Luigi Naselli-Flores

J. Neustupa (✉) · J. Šťastný · K. Nemjová · P. Škaloud
Department of Botany, Faculty of Science,
Charles University of Prague, Benátská 2,
128 01 Praha, Czech Republic
e-mail: neustupa@natur.cuni.cz

P. Mazalová · A. Poulíčková
Department of Botany, Faculty of Science,
Palacký University Olomouc, 17. listopadu 12,
771 46 Olomouc, Czech Republic

E. Goodyer
Royal Botanic Garden Edinburgh (RBGE),
20a Inverleith Row, Edinburgh EH3 5LR, UK

## Introduction

Although desmids have been recognized as important indicators of water quality, particularly eutrophication and acidification (Coesel, 1982), knowledge of their ecological and geographic distribution relies on purely morphological delimitations of individual taxa (Coesel, 1996). Species concepts of green microalgae have recently been undergoing major conceptual changes in the light of increasing evidence stemming from molecular phylogenetic studies. The artificial nature of traditional species and genera has been demonstrated in various taxonomic groups (Huss et al., 1999; Mikhailyuk et al., 2008; Gontcharov & Melkonian, 2010a, b). Repeated evolution of cryptic, morphologically unrecognizable species has been suggested in green microalgae that frequently occur in freshwater phytoplankton and phytobenthos (Müller et al., 2005; Luo et al., 2006). In the Desmidiales, about 2,500 traditional, morphologically defined species were described from freshwater habitats worldwide (Gontcharov & Melkonian, 2010b). However, the large number of traditionally defined, and—in many cases—doubtful infraspecific taxa considerably obscure the taxonomy of the group (Kouwets, 2008). The genus *Micrasterias* C.Agardh ex Ralfs represents some of the most conspicuous and well-known species of the group. It is also one of the "flagship" freshwater algae constantly attracting attention of amateur scientists and nature-lovers (e.g., Le Naturaliste, 2007; Brochard, 2008). Apparent morphological variability of large and richly ornamented *Micrasterias* cells led the early (e.g., Ralfs, 1848), as well as modern phycologists, to describe about 900 species and infraspecific taxa (Guiry & Guiry, 2010). The genus has been refined by Krieger (1939), and even then about 202 taxa were recognized. Růžička (1981) synonymized many infraspecific taxa, and included 51 species and varieties of *Micrasterias* in his critical revision of the European members of the genus. However, phylogenetic reliability of individual species, and especially of their subspecific taxa, remained questionable.

Members of the genus *Micrasterias* form a single lineage within Desmidiales supported by multigene phylogenetic analyses (Gontcharov & Melkonian, 2008; Hall et al., 2008). However, several morphologically different species, traditionally classified in different genera, such as *Cosmarium ralfsii*, *Staurodesmus dickiei* and *Triploceras gracile*, were found nested within the *Micrasterias*-lineage (Gontcharov & Melkonian, 2008; Hall et al., 2008). Recent species-level studies of *Micrasterias crux-melitensis*/*M. radians* (Neustupa et al., 2010) and *Micrasterias truncata* complexes (Nemjová et al., accepted), employing combined morphological and molecular approaches have revealed that these traditional taxa mostly represent taxonomically meaningful units, but some of the varieties are apparently independent species. Several morphologically defined infraspecific taxa were shown to be artificial, and probably lack taxonomic value (e.g., *M. crux-melitensis* var. *janeira* or *M. truncata* var. *neodamensis*). However, individual species-level phylogenetic lineages were always found to be morphologically identifiable, both by careful microscopic analysis, as well as by quantitative geometric morphometric methods. Consequently, cryptic species have not yet been detected within the genus *Micrasterias* (Neustupa et al., 2010).

In this study, we concentrated on what is probably the most conspicuous *Micrasterias* species—*M. rotata*, together with its close relative, *M. fimbriata*. Both of these traditional species occur mostly in the phytobenthos of peatlands. *Micrasterias rotata* has been collected on all continents, excluding Antarctica (Krieger, 1939; Tyler, 1970). On the other hand, collections of traditional *M. fimbriata* are rarer, with specimens being recorded from Europe (Růžička, 1981; Coesel & Meesters, 2007), North America (Prescott et al., 1977), and Northern Asia (Kossinskaja, 1960; Medvedeva, 2001). There is also a single report of *M. fimbriata* var. *brasiliensis* from South America (Borge, 1925; Krieger, 1939). The phylogenetic relation between *M. rotata* and *M. fimbriata* was illustrated by Neustupa & Škaloud (2007) on the basis of 18S rDNA sequence analysis. However, genetic structure and monophyly of these two conspicuous and well-known taxa remained unclear. For this study, we assembled a set of clonal strains, natural samples, and published records (the main focus for which being continental Europe) to test for the monophyly of species and their eventual further phylogenetic and morphological differentiation as well as for geographic distribution of individual taxa. In the past records (e.g., West & West, 1905), *M. fimbriata* has been considered a variety of the broadly defined *M. apiculata* (West & West, 1905). Therefore, we also included sequences of *M. apiculata* var. *apiculata* and the closely similar

*M. brachyptera* into the study. However, our main attention was paid to the illustration of contrasting species concepts and distribution of traditional *M. rotata* and *M. fimbriata*. Molecular analyses were based upon the group II intron sequences of the plastid gene that encodes transfer RNA-Gly ($trnG^{ucc}$). This plastid-encoded marker was found to be very efficient in species delimitation within the *Micrasterias* lineage of Desmidiales (Neustupa et al., 2010; Nemjová et al., accepted). Qualitative morphological data were obtained by a combination of light microscopy (LM) and scanning electron microscopy (SEM) of samples. Morphological differences in cell shape were quantified using geometric morphometrics to establish a morphospace which spanned the variation between specimens. This was conducted separately for each lineage so that differences between populations and individual taxa could be statistically evaluated (Neustupa et al., 2008, 2010). *Micrasterias* species, being one of the most conspicuous unicellular organisms visible in the light microscope, have been frequently reported and illustrated since the 1850s. In this study, we illustrate that these historical records from the literature may be useful for morphometric reconstruction of the geographic distribution of previously unrecognized taxa.

## Materials and methods

### Localities and sampling

Sampling locations were chosen to maximize the spread of sites across continental Europe. Three vast regions—Czech Republic, the French departments Landes, and Gironde in Aquitaine, and western regions of Ireland—were chosen for detailed screening. In total, over 1,000 samples from the Czech Republic were searched for *Micrasterias* (Neustupa et al., 2009; Šťastný, 2010). In addition, about 120 samples from Aquitaine and 100 samples from western Ireland were also investigated. Clonal strains were isolated from the natural populations using the single-cell isolating method. Additional strains of the investigated species available in culture collections were also obtained. In total, 30 strains of *M. fimbriata* and *M. rotata* were used in the molecular and morphometric analyses (Table 1). The strains were cultured in MES-buffered DY IV liquid medium at 20°C and illuminated at 40 µmol photons $m^{-2}$ $s^{-1}$ from 18 W cool fluorescent tubes (Philips TLD 18W/33, Royal Philips Electronics, Amsterdam, the Netherlands), at a light:dark (L:D) regime of 12:12 h. In addition to the cultured material, natural populations were also used for morphological and morphometric studies. Sampling of natural populations concentrated on reported European records of traditional *M. fimbriata.* These sampling localities were in Denmark, on the Baltic island of Bornholm (Nordstedt, 1888; Burchardt & Kowalski, 2009), the Lake District in northern England (Brodie et al., 2007), several localities in The Netherlands (Coesel & Meesters, 2007), Walchsee bog in Tyrol, Austria (Šťastný & Lenzenweger, 2008), Estonia and Belgium (Supplementary Table 2). The available published figures of *M. fimbriata* were also used for morphological and morphometric comparisons. First, microphotographs available from public domain websites were utilized (Sieralgen in Nederland, 2003; Webber, 2006; Le Naturaliste, 2007, 2010a, b; Brochard, 2008; Photomacrography, 2008; Encyclopedia of Life, 2010; Oyadomari, 2010). Second, specimens published in desmid monographs, and in numerous floristic and taxonomic articles from the U.S.—Alaska (Croasdale, 1956), Louisiana (Förster, 1972), and other different U.S. locations (Wolle, 1892; Prescott et al., 1977); France—Auvergne (Wurtz, 1945; Kouwets, 1987); Vosges Mts. (Comère, 1901); Austria (Lenzenweger, 1981); Belgium (Gysels, 2005); Britain (Ralfs, 1848; Cooke, 1887; West & West, 1905; Brook & Johnson, 2002); Canada, Ontario (Irenee-Marie, 1938); Finland (Kallio, 1953); Germany (Mix, 1970); Poland (Raciborski, 1885); Scotland (Roy & Bisset, 1893); and finally the former Soviet Union (Kossinskaja, 1960). We were also glad to obtain samples collected by Peter F.M. Coesel from De Weerribben, and microphotographs from Koos J. Meesters from Polder Westbroek, The Netherlands. Finally, Alasdair Joyce kindly provided us with the unpublished drawings made by his late father Alan Joyce, originating from different localities in the northwest of Scotland. All the figures used in our analyses are listed in the Supplementary Table 2.

### Molecular phylogenetics

For the phylogenetic analyses, the group II intron of the plastid encoded RNA-Gly transfer gene ($trnG^{ucc}$) was chosen. Shaw et al. (2005) illustrated that $trnG^{ucc}$

**Table 1** The list of strains used in morphological and molecular analyses

| Strain designation | Original identification | Locality | Geographic coordinates | Accession numbers |
|---|---|---|---|---|
| C1 | *Micrasterias fimbriata* | Chvojnov wetland, Czech Republic | 49°24′23.39″N 15°25′10.24″E | FR731997 |
| C5 | *Micrasterias fimbriata* | Chvojnov wetland, Czech Republic | 49°24′23.39″N 15°25′10.24″E | Identical with FR731997 |
| C11 | *Micrasterias fimbriata* | Marienteich, Czech Republic | 50°32′43.53″N 14°40′39.44″E | Identical with FR731997 |
| C14 | *Micrasterias fimbriata* | A bog near Rod pond, Czech Republic | 49°07′13.99″N 14°45′07.24″E | Identical with FR731997 |
| B1 | *Micrasterias fimbriata* | Bastemose, Bornholm, Denmark | 55°07′37.63″N 14°56′42.15″E | Identical with FR731997 |
| I5 | *Micrasterias fimbriata* | An unnamed pool near Lecknavarna, Ireland | 53°34′10.60″N 9°48′29.57″W | Identical with FR731997 |
| I7 | *Micrasterias fimbriata* | An unnamed pool near Lecknavarna, Ireland | 53°34′10.60″N 9°48′29.57″W | Identical with FR731997 |
| I10 | *Micrasterias fimbriata* | Eirk Lough, Ireland | 51°56′28.21″N 9°37′41.03″W | Identical with FR731997 |
| I11 | *Micrasterias fimbriata* | Eirk Lough, Ireland | 51°56′28.21″N 9°37′41.03″W | Identical with FR731997 |
| W1 | *Micrasterias fimbriata* | Schwemm near Walchsee, Tyrol, Austria | 47°39′34.52″N 12°17′50.51″E | Identical with FR731997 |
| C9 | *Micrasterias apiculata* | Břehyně wetland, Czech Republic | 50°34′58.21″N 14°42′11.54″E | FR731998 |
| SVCK 247 | *Micrasterias apiculata* | A bog near Zeller See, Austria | 47°18′15″N 12°48′33″E | Identical with FR731998 |
| SVCK 65 | *Micrasterias brachyptera* | Bogs close to Korvanen, Finland | 67°56′13″N 27°50′25″E | FR731996 |
| CAUP K608 | *Micrasterias fimbriata* | Pools near Hostens, Aquitaine, France | 44°29′54.83″N 00°38′19.06″W | FR691070 |
| Q2 | *Micrasterias fimbriata* | A bog near Étang Hardy, Aquitaine, France | 43°43′08.60″N 01°22′09.42″W | Identical with FR691070 |
| Q10 | *Micrasterias fimbriata* | A bog near Étang Hardy, Aquitaine, France | 43°43′08.60″N 01°22′09.42″W | Identical with FR691070 |
| Q14 | *Micrasterias fimbriata* | A bog near Étang Hardy, Aquitaine, France | 43°43′08.60″N 01°22′09.42″W | Identical with FR691070 |
| L1 | *Micrasterias fimbriata* | Torver Tarn, Lake District, United Kingdom | 54°19′29.57″N 03°06′23.70″W | Identical with FR691070 |
| SAG 162.80 | *Micrasterias fimbriata* | Texas, USA | – | Identical with FR691070 |
| CAUP K604 | *Micrasterias rotata* | Pools by Cep, Czech Republic | 48°55′23.65″N 14°50′23.96″E | FR691071 |
| SVCK 1 | *Micrasterias rotata* | An unknown locality near Potsdam, Germany | – | Identical with FR691071 |
| SVCK 26 | *Micrasterias rotata* | Wildes Moor bei Husum, Germany | 54°24′56.11″N 09°14′56.22″E | Identical with FR691071 |

**Table 1** continued

| Strain designation | Original identification | Locality | Geographic coordinates | Accession numbers |
|---|---|---|---|---|
| SVCK 78 | *Micrasterias rotata* | Bogs close to Korvanen, Finland | 67°56′13″N 27°50′25″E | Identical with FR691071 |
| SVCK 93 | *Micrasterias rotata* | Hammerfest, Norway | 70°39′33″N 23°41′07″E | Identical with FR691071 |
| SVCK 212 | *Micrasterias rotata* | Timmer Moor near Hamburg, Germany | 53°39′47.62″N 10°08′25.26″E | Identical with FR691071 |
| SVCK 243 | *Micrasterias rotata* | A bog near Sappel close to Millstatt, Kärnten, Austria | 46°47′52.60″N 13°37′47.46″E | Identical with FR691071 |
| SVCK 287 | *Micrasterias rotata* | Burnham's Swamp near Falmouth, Massachusetts, USA | – | Identical with FR691071 |
| Q1 | *Micrasterias rotata* | Pools near Hostens, Aquitaine, France | 44°29′54.83″N 00°38′19.06″W | Identical with FR691071 |
| Q6 | *Micrasterias rotata* | A bog near Étang Hardy, Aquitaine, France | 43°43′08.60″N 01°22′09.42″W | Identical with FR691071 |
| C8 | *Micrasterias rotata* | A mountain fen near Nové Hamry, Czech Republic | 50°21′50.46″N 12°39′21.90″E | Identical with FR691071 |
| C12 | *Micrasterias rotata* | Marienteich, Czech Republic | 50°32′43.53″N 14°40′39.44″E | Identical with FR691071 |
| C13 | *Micrasterias rotata* | A bog near Rod pond, Czech Republic | 49°07′13.99″N 14°45′07.24″E | Identical with FR691071 |
| I6 | *Micrasterias rotata* | Muckross Lake, Ireland | 52°00′41.33″N 09°31′45.64″W | Identical with FR691071 |

intron is one of the most variable plastid-encoded molecular phylogenetic markers suitable for species delimitation. Being a low-copy marker, *trnG^ucc^* overcomes drawbacks of utilizing multiple-copy genes and introns (Álvarez & Wendel, 2003). Recently, *trnG^ucc^* intron sequences were used in phylogenetic studies of different groups of Streptophytes (Pedersen & Hedenäs, 2003; Turmel et al., 2005; Bayer et al., 2009; Neustupa et al., 2010).

Genomic DNA was extracted from the strains (Table 1) according to the following method: After centrifugation, cells were disrupted by shaking for 10 min with glass beads at 1,800 rpm in Retch-MM200. Consequently, genomic DNA was extracted using Invisorb Spin Plant Mini Kit (Invitek) according to the manufacturer's protocol. The polymerase chain reaction was carried out in 20-µl volumes of 13.9 µl of sterile Mili-Q water, 2 µl of $MgCl_2$ (25 µM), 2 µl of PCR Buffer 10× (Applied Biosystems), 0.4 dNTP (10 µM), 0.25 µl of each trnG-ucc primers (Neustupa et al., 2010), 0.2 µl of AmpliTaq GOLD polymerase (5 U/µl), and 1 µl of DNA (not quantified). PCR amplification was set to an initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturing at 94°C for 1 min; annealing at 62°C for 1 min; elongation at 72°C for 1.5 min; and final extension at 72°C for 10 min. The PCR products were purified with JetQuick PCR Purification Kit (Genomed) according to manufacturer's protocol. Consequently, they were sequenced using the same primers by Macrogen Inc. on an automatic 3730XL DNA sequencer. Sequencing readings (encompassing 724–772 base pairs) were assembled and edited using the Seqassem software (Hepperle, 2004). The ClustalW algorithm, set to default parameters, was used for aligning sequences in Mega 4.0 (Tamura et al., 2007). Only unique sequences were left in the alignment, and the alignment stability was assessed in SOAP v1.2 alpha 4 (Löytynoja & Milinkovitch, 2001) comparing alignments produced under different gap-

opening and gap-extension penalties (7–20/2.5; 2–10/1.5). Only stable blocks of alignment were left in the final alignment (see in Supplementary Table 1). The substitution model was selected using the Akaike Information Criterion (AIC) estimated with PAUP/MrMtGui v1.0b (Nylander, 2004). The general reversible model with allowance for invariable sites (GTR+I) was selected as being the most suitable for the data set. The phylogenetic tree was inferred with Bayesian inference (BI) using MrBayes version 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Two parallel runs were carried out for 10,000,000 generations, each with three heated and one cold chain. Trees and parameters were sampled every 100 generations. Convergence of the two cold chains was checked, and burn-in was determined using the program's "sump" command.

Bootstrap analyses were performed with maximum likelihood (ML) and maximum parsimony (MP) analyses. The ML analyses were performed in GARLI v. 0.951 (Zwickl, 2009) and consisted of rapid heuristic searches (100 pseudo-replicates) using automatic termination (gentreshfortopoterm = 100,000). The MP bootstrap values were inferred from 10 000 replicates using the Close–Neighbor–Interchange algorithm with search level 3 in Mega 4.0 (Tamura et al., 2007). The obtained phylogenetic tree was displayed in FigTree (Rambaut, 2009) and Mega 4.0 (Tamura et al., 2007). Finally, the displayed phylogenetic tree was graphically adjusted in Adobe Illustrator CS3 v13.0.1.

### Light and electron microscopy

Microphotographs were taken on an Olympus BX51 light microscope with Olympus Z5060 digital photographic equipment (Olympus Corporation, Tokyo, Japan). The formaldehyde-fixed samples for SEM were pipetted on acetone-washed glass coverslips that had, subsequently, been coated three times with a poly-L-lysine solution (1:10 in deionized water) to ensure adhesion of cells, and dried on a heating block. Then, samples were transferred in 30% acetone, dehydrated by an acetone series (10 min successively in 30, 50, 70, 90, 95, 99%, and 2× in 100%), and critical point dried with liquid $CO_2$. Finally, they were sputter coated with gold and examined using the JEOL 6380 LV scanning electron microscope.

### Morphometric methods

For each strain, 20–25 adult semicells were randomly chosen for geometric morphometric analysis. The analysis was based on the position of 49 structurally defining cell perimeter landmarks (Supplementary Fig. 1). In addition, parallel morphometric analysis of terminal lobules closest to the lateral semicell incision was also conducted in *M. fimbriata* specimens. The lowest terminal lobule of the upper lateral lobule (i.e., the terminal lobule adjacent to the lateral incision) was chosen (Supplementary Fig. 2). In total, there were 11 landmarks depicted on these terminal lobules, including four sliding landmarks, which were used for capturing the lobule outline variation. The TPS-series software (publicly available at http://life.bio.sunysb.edu/morph/) was used (Rohlf, 2008). Positions of landmarks were digitized in TpsDig, ver. 2.12. The landmark configurations were superimposed by generalized Procrustes analysis (GPA) in TpsRelw, ver. 1.42. Correlation between Procrustes and the Kendall tangent space distances was assessed using TpsSmall, ver. 1.20, to ensure that the variation in shape was small enough to allow subsequent analyses (Zelditch et al., 2004). Indeed, this correlation was very high ($r = 0.999$), and so we proceeded with further statistical analyses. The landmark configurations of *Micrasterias* semicells were symmetrized using a standard method of Klingenberg et al. (2002). A principal component analysis (PCA) of geometric morphometric data was conducted on the entire set of 294 semicells acquired from strains subjected to molecular characterization. Scores of the objects on the non-zero principal component (PC) axes were used for two-group linear discrimination analysis (LDA), whose significance was assessed by the Hotelling's $T^2$ test in PAST, ver. 2.01 (Hammer et al., 2001). This analysis was designed for statistical evaluation of differences in shape of individual species. The additional semicells from natural samples, and from the published figures, were also landmark-registered for the geometric morphometric analysis. Then, the GPA-aligned configurations of these semicells were subjected to the classification discrimination analysis using the above-defined set based on an independent grouping criterion, i.e., molecular data. This analysis served as a parallel procedure to confirm morphological identification of the newly identified species based

on a qualitative, expert-based, and taxonomic assessment.

## Results

### Molecular phylogeny

The analyzed *trnG*$^{ucc}$ intron sequences data set consisted of 709 characters, of which 112 were parsimony informative. According to the unrooted Bayesian analysis (Fig. 1), all of the strains were clearly separated from all of the other *Micrasterias*-lineage members, whose *trnG*$^{ucc}$ intron sequences were available in the GenBank database. The *M. fimbriata* strains formed two independent lineages, constituting a moderately supported clade together with *M. brachyptera* (1.00/81/94, Bayesian posterior probability/ML/MP). These lineages of traditional *M. fimbriata* have been tentatively assigned as A-, and B-lineages ("A" for Aquitaine, and "B" for Bohemia as regions of first isolation). The A-lineage comprised all the *M. fimbriata* strains isolated from Aquitaine (France), Lake District (UK) and a single strain from Texas (USA). Together with *M. brachyptera*, the A-lineage formed a clade with moderate statistical support (1.00/72/86, BI/ML/MP). The B-lineage, composed of strains isolated from Bohemia, Western Ireland, Bornholm and Tyrol, was inferred in a sister position to this clade. The strains

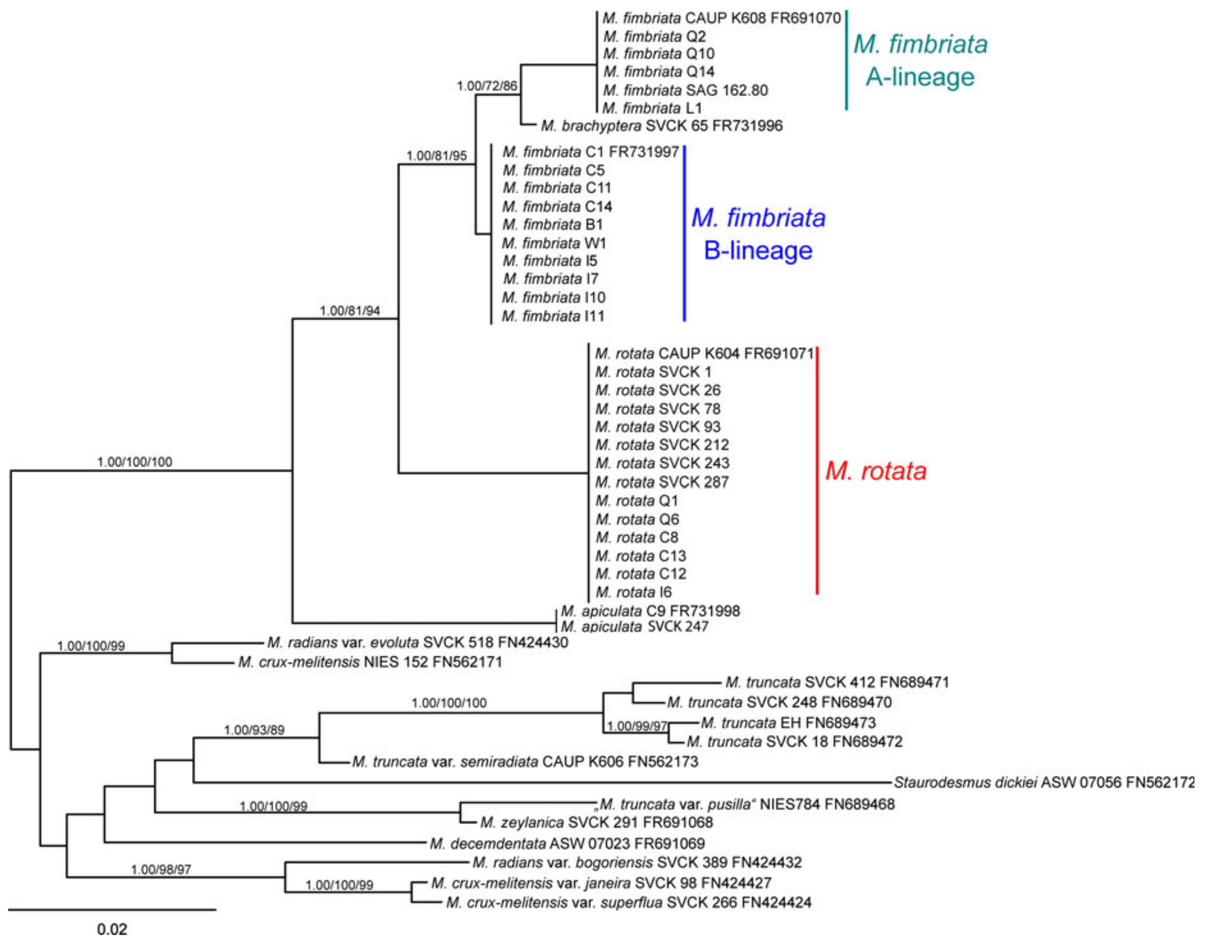



**Fig. 1** Unrooted Bayesian phylogenetic tree of *trnG*$^{ucc}$ sequences. The *scale bar* shows the estimated number of substitutions per nucleotide. The posterior probabilities lower then 0.70 and bootstrap support levels below 50% are omitted. The indicators of statistical significance are provided as follows: Bayesian posterior probability/ML bootstrap support/ MP bootstrap support

of *M. rotata* and *M. apiculata* formed independent lineages. Among themselves, all the *M. rotata* strains, as well as all the strains belonging to either the A- or B-lineage of *M. fimbriata*, had identical *trnG*$^{ucc}$ sequences. The strains of *M. apiculata* had the most divergent sequence, differing from the other strains by a unique insertion of 43 nucleotides in its *trnG*$^{ucc}$ intron sequence.

## Morphology and geometric morphometrics

The cells of *Micrasterias rotata* (Fig. 2i) had typical unequally divided lateral lobules, and the terminal lobules were usually shortly bidentate. The polar lobe was gradually broadening toward the apex, which always had two bidentate marginal outgrowths. Importantly subapical as well as surface spines were completely lacking. The cell size varied from 205 to 312 µm long (apex–apex) and from 200 to 252 µm broad. In contrast, the investigated strains identified as *M. fimbriata* according to traditional criteria were not homogenous, and formed two morphological groups corresponding to phylogenetic lineages illustrated by our molecular analysis. The members of the B-lineage had slightly unequal lateral lobes and rounded terminal lobules ending with abruptly protruding spines, i.e., so called *fimbriae* (Fig. 2a–d). Apart from two bidentate marginal apices, the polar lobe typically had two subapical spines (Fig. 2b). In some cells, several surface spines were also observed, especially along the major cell incisions (Fig. 2b). The A-lineage cells had unequally divided lateral lobes, but their terminal lobules were not rounded, but instead they gradually tapered toward the apex and did not possess any spines (Fig. 2e–h). However, similarly to B-lineage cells, and contrary to *M. rotata*, they always had two emergent subapical spines on the polar lobes (Fig. 2f). The surface spines along the major cell incisions were present on most cells of the A-lineage (Fig. 2f). The dimensions of cells from the A-lineage varied from 192 to 263 µm in length and from 181 to 228 µm in width. On the other hand, the cells of the B-lineage were slightly larger and varied from 201 to 276 µm in length and from 197 to 248 µm in width. Both the A- and B-lineages clearly differed from *M. apiculata* and *M. brachyptera*, both in cell size as well as in cell shape and lobulation pattern (Fig. 2j, k).

**Fig. 2** Light microscopy and SEM pictures of *Micrasterias* ▶ strains. *M. fimbriata*, B-lineage (strain C11), overall morphology (**a**), apical part of the cell (**b**), note two subapical spines (*asterisks*) and surface spines (*arrowheads*) on the polar lobe, details of the lateral lobe showing rounded terminal lobules ending with abruptly protruding spines (**c**, **d**). *M. fimbriata*, A-lineage (strain CAUP K608), overall morphology (**e**), apical part of the cell (**f**), note two subapical spines on the polar lobe (*asterisks*) and numerous surface spines along the major cell incisions (*arrowheads*), details of the lateral lobe showing terminal lobules gradually tapered toward the apex (**g**, **h**) *M. rotata* (strain C12) (**i**), *M. apiculata* (strain SVCK 247) (**j**), *M. brachyptera* (strain SVCK 65) (**k**). Scale bars: 20 µm (**a**, **e**, **i**–**k**), 50 µm (**b**–**d**, **f**–**h**)

The PCA of geometric morphometric data illustrated that cells belonging to three lineages established on the basis of *trnG*$^{ucc}$ sequence data differed in their overall shape characteristics (Fig. 3a, b). The first PC axis explained 42.9% of the morphometric variation and reflected differences between *Micrasterias rotata* (negative PC1 values) and two lineages of traditional *M. fimbriata*. The second and third PC axes accounted for 12.1 and 10.9% of the variation, respectively. They described shape variation within the phylogenetic groups and, especially in case of the third PC axis the difference between the A-lineage of *M. fimbriata* (positive PC3 values), and other two lineages. The canonical variate analysis (CVA) of scores on the non-zero PC axes illustrated highly significant shape discrimination among groups (Wilk's $\lambda = 0.022$, $F = 133.2$, $P < 0.00001$). The first CV axis (72.1% of the variance) spanned mostly the difference between *M. rotata* and both lineages traditionally assigned to *M. fimbriata*, whereas the second CV axis (27.9%) emphasized differences between both *M. fimbriata* lineages (Fig. 3c). The two-group discrimination analyses confirmed their highly significant shape differences (Hotelling's pairwise comparisons, Bonferroni corrected *P*-values $<0.00001$ in all the group pairs). The underlying Mahalanobis distances between individual group means indicated that the *M. rotata* cells were more similar to cells of the B-lineage ($D_M = 0.35$), than to cells of the A-lineage ($D_M = 0.51$). The pair of two traditional *M. fimbriata*-assigned lineages had $D_M = 0.38$.

The LDA of geometric morphometric data from *M. fimbriata* strains illustrated 100% correct classification of semicells into their a priori groups based on molecular data (Fig. 4a). Likewise, there was also

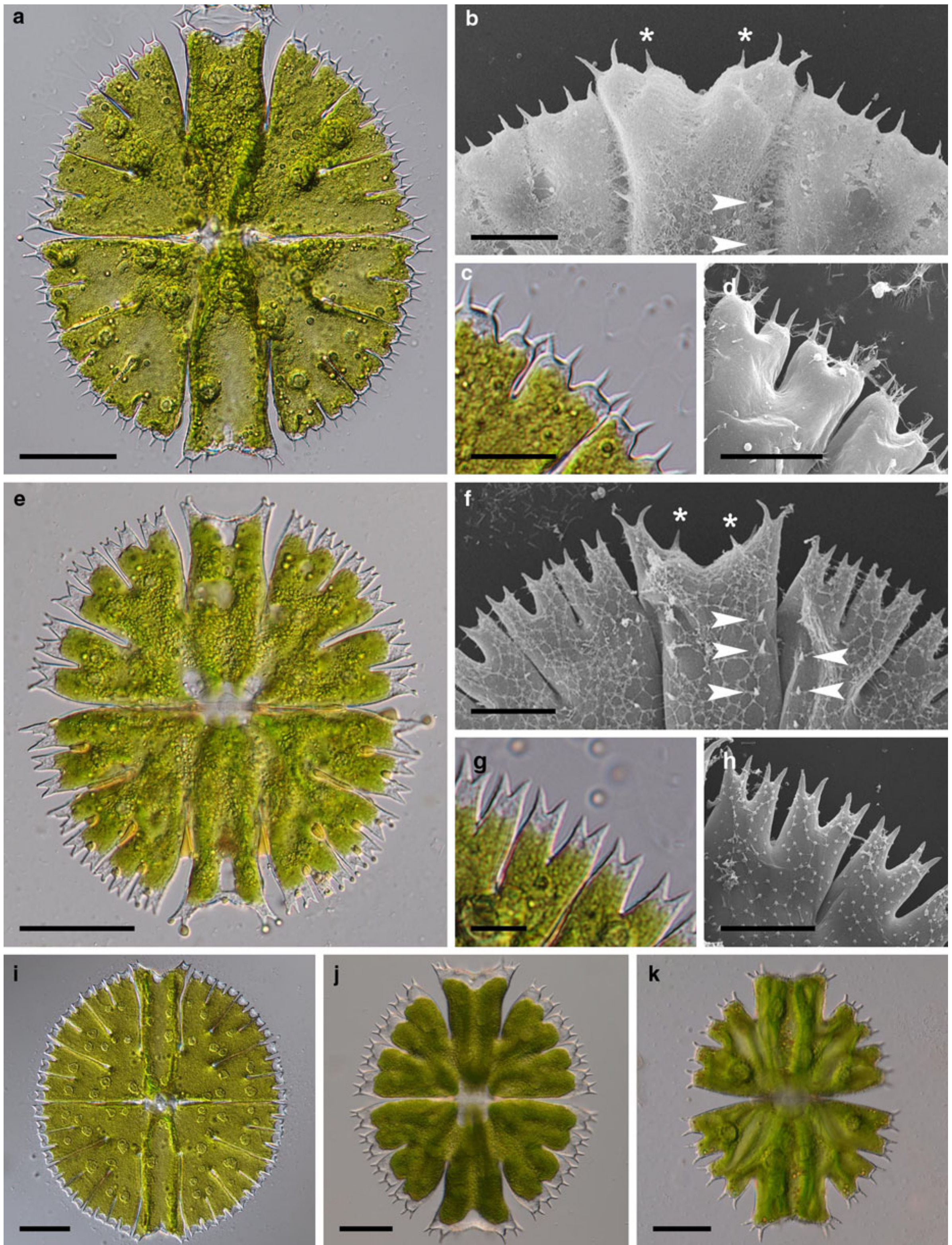
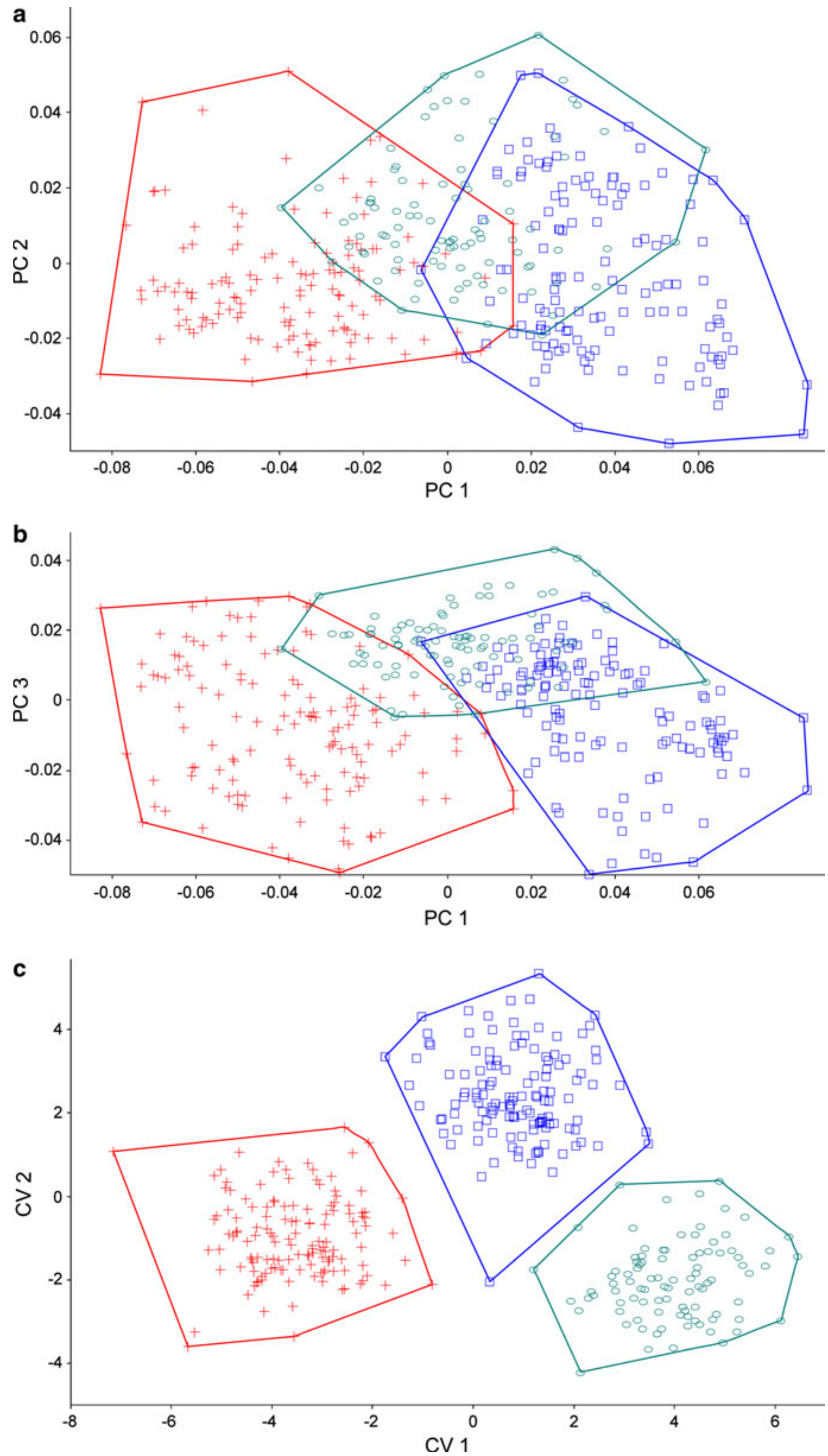

a
b
c
d
e
f
g
h
i
j
k

**Fig. 3** The PCA and CVA ordination plots of geometric morphometric data of *Micrasterias rotata*, and *M. fimbriata* (A- and B-lineages) semicells. The PC1 versus PC2 (**a**), PC1 versus PC3 (**b**), and CV1 versus CV2 plots (**c**) are depicted. *Crosses*: *M. rotata*, *ellipses*: A-lineage of *M. fimbriata*, *squares*: B-lineage of *M. fimbriata*



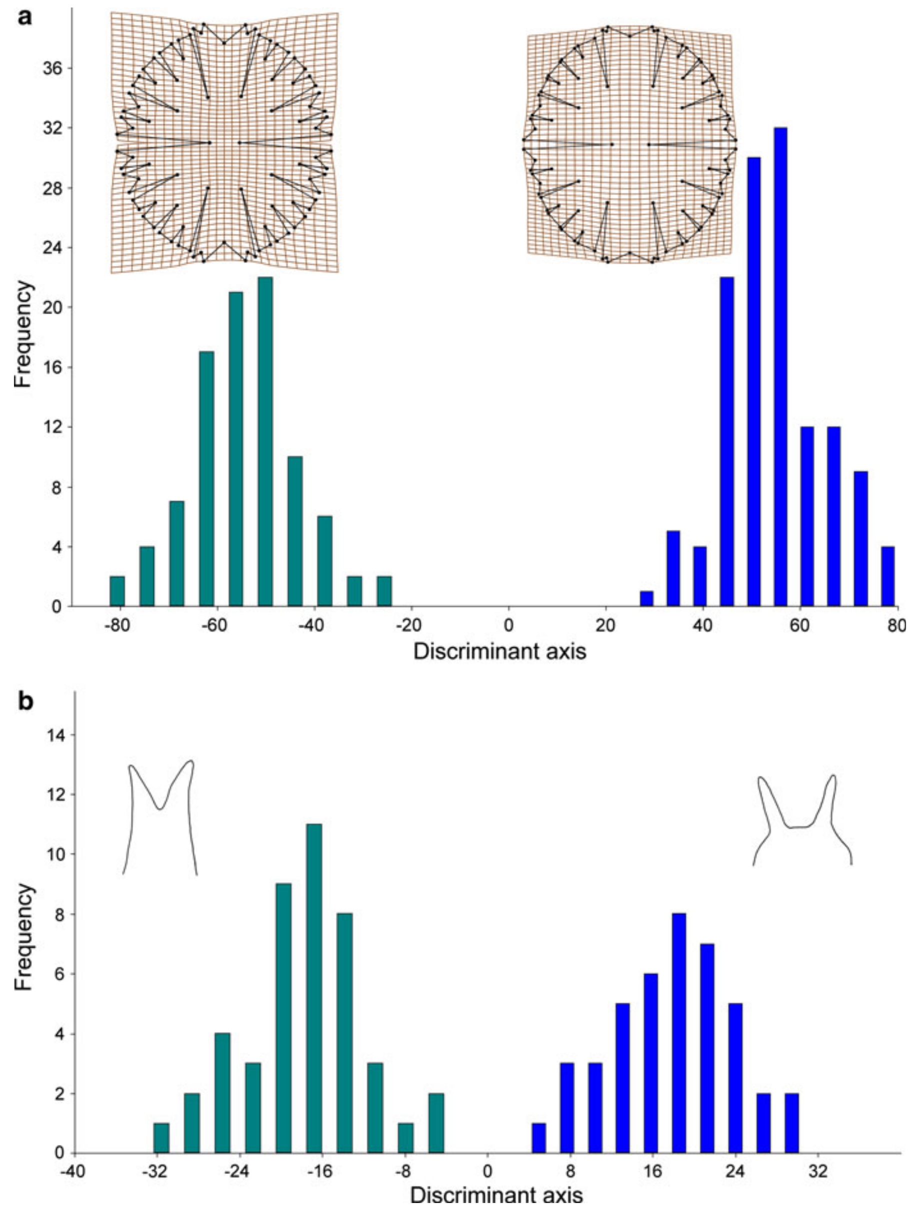



**Fig. 4** The linear discrimination analyses of geometric morphometric data of *M. fimbriata* strains. The landmarks analyses of semicells (**a**), and terminal lobules (**b**) are depicted. The A-lineage of *M. fimbriata* is depicted in *left bars*, the B-lineage in *right bars*

an unambiguous discrimination of *M. fimbriata* semicells on the basis of their terminal lobule shapes (Fig. 4b). At this stage, we included data from figures of natural *M. fimbriata* populations (Supplementary Figs. 3, 4) and from the literature records to the discrimination analysis of terminal lobule shapes. The classification discrimination analysis was conducted for each cell, and their values on the discriminant axis and subsequently their group assignment were ascertained (Supplementary Table 2). In fact, this analysis was largely confirmatory, as the morphological differences in the shape of terminal lobules were readily recognizable by qualitative judgment (see Supplementary Figs. 3, 4). The combined molecular, morphological. and morphometric analyses were used for reconstruction of geographic distribution of two *M. fimbriata* phylogenetic lineages. They illustrated their rather surprising and largely disparate distributional patterns (Fig. 5). In North America, we have not been able to confirm any report of the *M. fimbriata* B-lineage morphotype. On the contrary, all the North American literature comprising a span of more than

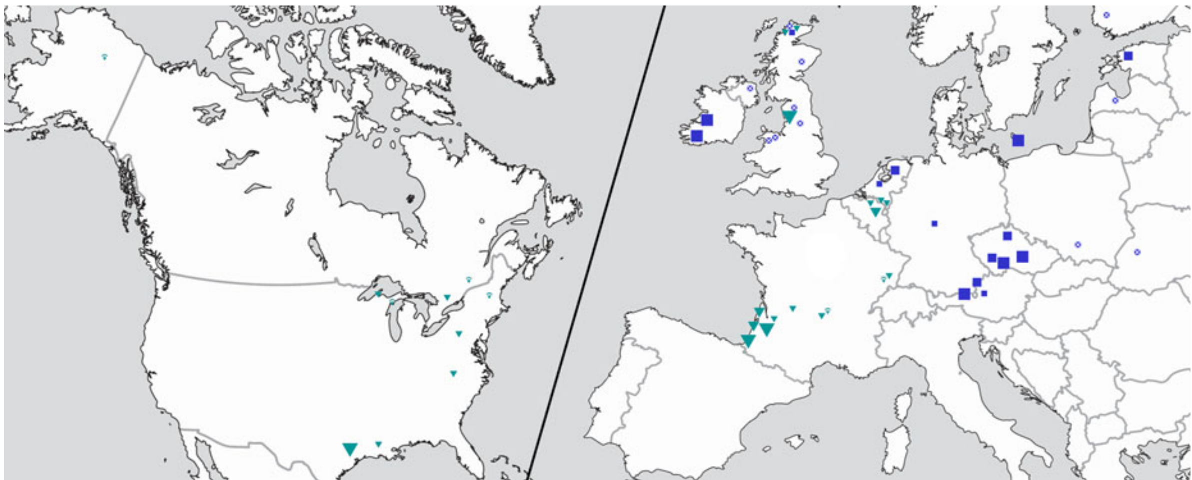


**Fig. 5** The *map* illustrating presumptive distribution of A- and B-lineages of *M. fimbriata* in Europe and North America. *Triangles* represent A-lineage and *squares* represent B-lineage findings. *Large symbols* indicate localities of clonal strains, *middle-sized symbols* indicate localities of investigated natural populations, and *small symbols* indicate other published records. The *crossed symbols* indicate records older than 50 years

100 years, and records from Alaska to New England, recently published microphotographs and available strains fitted well into the morphological characteristics of the A-lineage. On the other hand, in Europe, both lineages were encountered. The members of the A-lineage, or the records morphologically corresponding to this lineage, were found in the western parts of the continent, from England to Aquitaine, where they sometimes occurred in abundance. In fact, our broad sampling of desmid assemblages from wetlands and étangs of south-western France resulted in frequent findings of the A-lineage populations, whereas the B-lineage of *M. fimbriata* was not encountered in this region. Similarly, the A-lineage morphotypes were the only ones that were found in our samples from Belgium, and from the Lake District. Surprisingly, there have not been any reported findings of this lineage east of the Rhine River. The two *M. fimbriata* culture strains (SVCK 178 and UTEX LB 766) with an unknown origin also belonged to the A-lineage on the basis of morphological data.

Recent reports of the B-lineage morphotype originate mostly from the Central and Eastern Europe. The B-lineage populations are very probably the only ones of the traditional *M. fimbriata* that occur in the Czech Republic (after examining in excess of 1,000 samples collected from this country). Similarly, the B-lineage is probably the exclusive inhabitant of traditional *M. fimbriata* on Bornholm, where most of the suitable *Micrasterias* habitats were sampled, and the encountered populations analyzed. In addition, all of our findings and the available literature records of *M. fimbriata* from Austria, Germany, and eastern European sites referred to the B-lineage morphotype. However, this lineage has also been encountered in the western parts of Europe. In Western Ireland, where about 100 samples had been collected, only specimens conforming to the B-lineage were found. The natural populations of the B-lineage were also ascertained after material from The Netherlands provided by the courtesy of Peter Coesel and Koos Meesters, and there are also several published reports of this morphotype from England. In addition, Alan Joyce's unpublished records from north-west Scotland sampled between 1985 and 2006 illustrate both A-, and B-lineages. Therefore, to our knowledge, The Netherlands and Scotland are the only parts of Europe where the populations of both lineages co-occur.

## Discussion

Remarkable phylogenetic homogeneity of morphologically defined *Micrasterias rotata* across different European region has illustrated its well-defined and robust species concept. The single North American strain of *M. rotata* included in our study was also identical to European populations. Based on these

data, we cannot reject—at least for Europe—the hypothesis that all the populations of *M. rotata* truly represent a homogenous phylogenetic species lineage. This pattern is similar to that illustrated in traditional *M. crux-melitensis* (Neustupa et al., 2010). In this morphospecies, all of the European strains were also found to be identical in their *trnG*$^{ucc}$ and ITS2 sequences. However, the single East-Asian strain of *M. crux-melitensis*—NIES 152—clustered independently, and likely represents a different species. Similarly, we cannot preclude that *M. rotata* may also be found phylogenetically heterogeneous on a global scale.

In *M. fimbriata*, the second traditional species investigated in this study, a more complicated taxonomic structure was revealed. In fact, our results illustrated a previously unrecognized, morphological and phylogenetic differentiation of this traditional species. During our morphological investigation of *M. fimbriata* samples from Aquitaine, we were captivated by their seemingly different morphology from the Central European populations. The molecular analyses confirmed that this traditional species is actually composed of two independent lineages that can also be well defined by careful microscopic observations, as well as by geometric morphometric analysis. The morphological differences between two lineages can be summarized as follows:

(a) Shape of terminal lobules (Fig. 2a–h). This unambiguous character was used in geometric morphometric analysis for discrimination of published figures and natural populations.
(b) The incisions between polar lobe and lateral lobes are shallower in the B-lineage cells. The marginal spines of the A-lineage polar lobes are often long and inwardly bow-shaped (Fig. 2f), whereas they are shorter and straight in the B-lineage.
(c) The surface spine layers (that were used for delimitation of *M. fimbriata* var. *spinosa* in the past) are always present on A-lineage cells (Fig. 2f). On the other hand, they are more rarely encountered on the B-lineage cells (Fig. 2b).

Unfortunately, we do not have abiotic data for all the collection sites available. However, next to the above mentioned morphological differences, there also seems to be a striking contrast in the ecological preferences of the representatives of both lineages. Although the B-lineage specimens in samples from The Czech Republic (Šťastný, 2010), Austria (Šťastný & Lenzenweger, 2008), Ireland, and Bornholm generally originate from mesotrophic, slightly acidic wetland habitats, the sites of the A-lineage were mostly distinctly oligotrophic bogs at low pH. The same tendency concerning autecology of both lineages was also observed in the Netherlands (Peter Coesel, pers. comm.). The well-supported morphological and morphometric differentiation of both phylogenetic lineages made it possible to analyze the previously published morphological data for *M. fimbriata*, and to correlate these figures with discrimination patterns based on morphological data of investigated strains. Obviously, we will never be able to acquire sequence data out of the past literature records. However, close and straightforward correlation of morphological and phylogenetic data enabled us to establish the morphology-based discriminative framework for the identification of a presumptive phylogenetic affiliation of published illustrations of morphotypes. Although microphotographs objectively reflect the morphology of cells, the accuracy of drawings can never be assured. Nevertheless, the robustness of our presumptive geographic pattern of distribution, constructed from correlations between the older published data, and more recent findings from the same regions generally confirmed very good reliability of traditional desmidiological drawings. Our geometric morphometric analysis of the published records produced a rather interesting pattern of possible geographic structure for both lineages. In North America, not a single analyzed report corresponded to the B-lineage morphotypes. Moreover, Prescott et al. (1977) illustrated solely the A-lineage specimens in their treatise of North American desmids, but they did not indicate their exact locations. Interestingly, the single South American record of *M. fimbriata* from Brazil (Borge, 1925; Krieger, 1939) also fitted into the A-lineage. As this study was primarily designed for investigation of European data, we did not obtain a more significant amount of North American samples. However, the available strains, recently published microphotographs, as well as all the older literature records support the hypothesis that the A-lineage may be the only American form of traditional *M. fimbriata*. In Europe, tentative distribution of the A-lineage seems to be limited to oceanic

parts of the continent, west of the Rhine. However, outright climatic control of the European A-lineage distribution was supported neither by the American data, where the A-lineage morphotypes were reported also from cold temperate and subarctic regions, such as Ontario or Alaska, nor by its occurrence under rather harsh conditions of the Vosges Mts., at an altitude of more than 900 m above sea level (Lac de Lipsach). Our data also did not support the hypothesis of a recent invasion from North America, as the morphologically well-fitting A-lineage specimens were reported from Western Europe at least twice in the last 100 years (Comère, 1901; Wurtz, 1945). Moreover, these records originated from regions where the A-lineage of *M. fimbriata* was also recently recorded: Vosges Mts. (Le Naturaliste, 2007) and Auvergne (Kouwets, 1987).

The B-lineage seems to be more frequent in central and eastern parts of the European continent, where the A-lineage has not been detected. Interestingly, the B-lineage has never been reported from regions south of the Alps, not even from countries with detailed local accounts and detailed recent checklists of desmids (e.g., Spain—Cambra Sánchez et al., 1998, Italy—Abdelahad et al., 2003 or Romania—Cărăuş, 2002). However, it also occurs in areas of Western Europe, such as Ireland, or the Netherlands. It seems to have also been relatively widely distributed in Britain at the end of the nineteenth century, as Ralfs (1848), Cooke (1887), Roy & Bisset (1893), and West & West (1905) unanimously recorded morphotypes corresponding to this lineage. In addition, the unpublished drawings by Alan Joyce also include two apparent findings of the B-lineage from Scotland. However, our investigation of the Lake District samples yielded only the A-lineage populations. In addition, solely the A-lineage morphotype of *M. fimbriata* was illustrated (with no collecting locality specified) by Brook & Johnson (2002) in their review of British desmids. Kossinskaja (1960), Gontcharov (1998), and Medvedeva (2001) reported *M. fimbriata* populations from the Far East regions of Russia. However, no original published figures specifically tied to Far Eastern localities are available. At the same time, there are many published records of *M. fimbriata* from different European countries with no original figures included. The species has apparently been considered so well known by traditional desmidiologists that they—unfortunately—did not deem it necessary to draw or photograph their findings anymore. That is why our notion on past distributional patterns of these two *Micrasterias* lineages in Europe will necessarily remain fragmentary, despite the fact that the traditional species was recorded on many occasions. For the analysis of recent data, we used, apart from our own findings, a number of microphotographs published on the internet pages of amateur microscopists who are nowadays often equipped with good light microscopes and produce excellent figures e.g., André Advocat (Le Naturaliste, 2007), Christophe Brochard (Brochard (2008), Wim van Egmond (Sieralgen in Nederland, 2003), and others. We believe that in this study we have illustrated that this relatively new phenomenon of increasing interest of amateur nature-lovers in freshwater algae, coupled with fast publication of their findings on the internet, makes a valuable and accessible contribution to scientific investigation. Therefore, we anticipate that publication of our data on two-fold structure of traditional *M. fimbriata* may soon result in many new findings and localities of both lineages, completing their detailed continentwide distribution. Relative importance of environmental (e.g., climatic) versus historical (e.g., spatial isolation) factors in geographic distribution of microalgae has recently been the subject of intense debates (for a review see e.g., Foissner, 2008). Coesel (1996) and Coesel & Krienitz (2008) suggested that some *Micrasterias* species (such as *M. hardyi* in Australia or *M. sudanensis* in tropical Africa) may represent fine examples of historically constrained geographic distribution areas in unicellular algae. Our data generally concur with these findings and the presently known distributional areas of both *M. fimbriata* lineages in Europe are strikingly similar to phytogeographic patterns of vascular plants taxa (see e.g., Cox & Moore, 2005). Therefore, we cannot exclude that some of the large *Micrasterias* species with low dispersal frequencies may also have largely vicariant and stable distributions, similar to different macroscopic groups.

Differentiation of two *M. fimbriata* lineages warrants their description as separate species. They formed clearly delimited lineages on the *trnG*$^{ucc}$ tree, even with the *Micrasterias brachyptera* strain nested within this clade. At the same time, morphological discriminative characters readily distinguish cells belonging to both lineages. At this point, we should note that the original drawing of *M. fimbriata*

(Ralfs, 1848, Table 8, Fig. 2) apparently corresponds to the B-lineage. At the same time, most of the A-lineage findings were referred to as *M. fimbriata* var. *spinosa*, because of more conspicuous surface spines on these cells (e.g., Wurtz, 1945; Croasdale, 1956; Kouwets, 1987; Engels, 2002). However, the type of this variety—originally described from Scotland—clearly belongs to the B-lineage (Roy & Bisset, 1893) and the presence of surface spine layers can by no means be taken as a discriminative character between the A-, and B-lineages. While these are usually more conspicuous on the cells of the A-lineage, they can also be found on B-lineage specimens. Other varieties (such as var. *obtusiloba*, var. *elefanta*, var. *caudata*, or var. *nuda*) were considered synonymous with the type by Růžička (1981). We certainly have no reason to doubt his taxonomic opinion on the basis of our observations. Our data suggest that the twofold phylogenetic division of *M. fimbriata* was not reflected by any of the traditional subspecific taxa. Therefore, description of the A-lineage as a new *Micrasterias* species would probably be necessary. However, the multigenic phylogenetic revision of the genus is ongoing, and may probably result in some quite far-reaching taxonomic conclusions. Therefore, we think that description of the A-lineage as a separate species—supported by multigenic phylogenies—should be undertaken together with other nomenclatoric changes. Even if the ongoing genuswide study will probably yield more complex insight into the interspecific phylogenetic structure, still, we can now conclude that our A-, and B-lineages are separate, paraphyletic species. *Micrasterias brachyptera* was recovered as a sister species to the A-lineage. However, both these closely related species differ by a number of conspicuous morphological features, such as cell size, degree of lobulation, or the overall cell shape (see e.g., Fig. 2). Based on these data, we can conclude that morphological features of individual *Micrasterias* species can evolve relatively rapidly and, therefore, phylogenetic inferences at the among-species level should be based on molecular data. The main focus of this study was the illustration of the concerted use of molecular and geometric morphometric analyses, as well as of detailed morphological observations, and a combination of these techniques may yield more complex results on the species structure of desmids than could possibly be achieved by applying any of these techniques in isolation.

**Acknowledgments** This study was supported by the following grants: 206/09/0906, Czech Science Foundation; and 21620828, Czech Ministry of Education. The authors thank Frans Kouwets for providing invaluable advice on the distribution of *Micrasterias* in Aquitaine localities. They are also deeply indebted to Peter F.M. Coesel and National Herbarium of the Netherlands in Leiden for their kind provision of a sample of *M. fimbriata* from De Weerribben, and to Koos J. Meesters for his kind provision of the microphotograph of the same species from Westbroek. They also thank Pieter Vanormelingen for providing some hardly accessible literature records. The recommendations of the anonymous reviewers led to improve the manuscript. Last but not the least, the authors thank Mr. Alasdair Joyce for his kind provision of unpublished drawings of his late father, Alan Joyce, including many *M. fimbriata* findings from Scotland.

## References

Abdelahad, N., G. Bazzichelli & R. D'Archino, 2003. Catalogo delle Desmidiaceae (Chlorophyta, Zygnematophyceae) segnalate in Italia. Academia Nazionale delle Science, Rome.

Álvarez, I. & J. F. Wendel, 2003. Ribosomal ITS sequences and plant phylogenetic inference. Molecular Pyhlogenetics and Evolution 29: 417–434.

Bayer, R. J., D. J. Mabberley, C. Morton, C. H. Miller, I. K. Sharma, B. E. Pfeil, S. Rich, R. Hitchcock & S. Sykes, 2009. A molecular phylogeny of the orange subfamily (Rutaceae: Aurantioideae) using nine cpDNA sequences. American Journal of Botany 96: 668–685.

Borge, O., 1925. Die von Dr. F. C. Hoehne während der Expedition Roosevelt-Rondon gesammelten Süsswasseralgen. Arkiv för Botanik 19: 1–56.

Brochard, C., 2008. Cristophe Brochard Nature Photography [available on Internet at http://www.cbrochard.com].

Brodie, J., D. M. John, I. Tittley, M. J. Holmes & D. B. Williamson, 2007. Important Plant Areas for Algae: A Provisional Review of Sites and Areas of Importance for Algae in the United Kingdom. Plantlife International, Salisbury.

Brook, A. J. & L. R. Johnson, 2002. Order Zygnematales. In John, D. M., B. A. Whitton & A. J. Brook (eds), The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae. Cambridge University Press, Cambridge: 479–593.

Burchardt, L. & W. W. A. Kowalski, 2009. Diversity of the algae, habitats, and aquatic ecosystems of Bornholm Island. Oceanological and Hydrobiological Studies 38(Supplement 2): 13–20.

Cambra Sánchez, J., M. Álvarez Cobelas & M. Aboal Sanjurjo, 1998. Lista florística y bibliográfica de los clorófitos (Chlorophyta) de la Península Ibérica, Islas Baleares e Islas Canarias. Asociación Española de Limnología, Burgos.

Cărăuş, I., 2002. The algae of Romania. Studii şi Cercetări, Biologie 7: 1–694.

Coesel, P. F. M., 1982. Structural characteristics and adaptations of desmid communities. Journal of Ecology 70: 163–177.

Coesel, P. F. M., 1996. Biogeography of desmids. Hydrobiologia 336: 41–53.
Coesel, P. F. M. & L. Krienitz, 2008. Diversity and geographic distribution of desmids and other coccoid green algae. Biodiversity and Conservation 17: 381–392.
Coesel, P. F. M. & J. Meesters, 2007. Desmids of the Lowlands. KNNV Publishing, Zeist.
Comère, J., 1901. Les Desmidiées de France. Paul Klincksieck, Paris.
Cooke, M. C., 1887. British Desmids. A Supplement to British Fresh-Water Algae. Williams & Norgate, London.
Cox, C. B. & P. D. Moore, 2005. Biogeography. An Ecological and Evolutionary Approach. John Willey & Sons, New York.
Croasdale, H., 1956. Freshwater algae of Alaska I. Some desmids from the interior. Part 2: *Actinotaenium*, *Micrasterias* and *Cosmarium*. Transactions of the American Microscopical Society 75: 1–70.
Encyclopedia of Life, 2010. *Micrasterias fimbriata* Ralfs [available on Internet at http://www.eol.org/pages/922464].
Engels, M., 2002. Liste der Sammlung von Conjugaten-Kulturen (SVCK) am Institut für Allgemeine Botanik der Universität Hamburg. Institut für Allgemeine Botanik der Universität Hamburg [available on Internet at http://www.biologie.uni-hamburg.de/b-online/d44_1/44_1.htm].
Foissner, W., 2008. Protist diversity and distribution: some basic considerations. Biodiversity and Conservation 17: 235–242.
Förster, K., 1972. Desmidieen aus dem Südosten der Vereinigten Staaten von America. Nova Hedwigia 23: 515–644.
Gontcharov, A. A., 1998. Contribution to the desmid flora of the Primorsky Territory, Russia. Bulletin of the National Science Museum, Tokyo, Series B 23: 59–80.
Gontcharov, A. A. & M. Melkonian, 2008. In search of monophyletic taxa in the family Desmidiaceae (Zygnematophyceae, Viridiplantae): the genus *Cosmarium*. American Journal of Botany 95: 1079–1095.
Gontcharov, A. A. & M. Melkonian, 2010a. Molecular phylogeny and revision of the genus *Netrium* (Zygnematophyceae, Streptophyta): *Nucleotaenium* gen. nov. Journal of Phycology 46: 346–362.
Gontcharov, A. A. & M. Melkonian, 2010b. A study of conflict between molecular phylogeny and taxonomy in the Desmidiaceae (Streptophyta, Viridiplantae): analyses of 291 *rbc*L sequences. Protist. doi:10.1016/j.protis.2010.08.003
Guiry, M. D. & G. M. Guiry, 2010. AlgaeBase. National University of Ireland, Galway [available on Internet at http://www.algaebase.org].
Gysels, J., 2005. Ridderkruisen (*Micrasterias* Ag. ex Ralfs): oude en nieuwe vindplaatsen in de provincie Antwerpen. Antwerpse Koepel voor Natuurstudie, Antwerp.
Hall, J. D., K. G. Karol, R. M. McCourt & C. F. Delwiche, 2008. Phylogeny of the conjugating green algae based on chloroplast and mitochondrial nucleotide sequence data. Journal of Phycology 44: 467–477.
Hammer, Ø., D. A. T. Harper & P. D. Ryan, 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9.
Hepperle, D., 2004. SeqAssem©. A Sequence Analysis Tool, Contig Assembler and Trace Data Visualisation Tool for Molecular Sequences [available on Internet at http://www.sequentix.de/software_seqassem.php].
Huelsenbeck, J. P. & F. Ronquist, 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.
Huss, V. A. R., C. Frank, R. C. Hartmann, M. Hirmer, A. Kloubоucek, B. M. Seidel, P. Wenzeler & E. Kessler, 1999. Biochemical taxonomy and molecular phylogeny of the genus *Chlorella* (Chlorophyta). Journal of Phycology 35: 587–598.
Irenee-Marie, F. I. C., 1938. Flore Desmidiale de la Region de Montreal. Laprairie, Montreal.
Kallio, P., 1953. The effect of continued illumination on the desmids. Annales Botanici Societatis Zoologicae Botanicae Fennicae Vanamo 8: 58–74.
Klingenberg, C. P., M. Barluenga & A. Meyer, 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56: 1909–1920.
Kossinskaja, E. K., 1960. Flora Sporovych Rastenii SSSR. Tom V. Desmidievye vodorosli. Izdatelstvo Akademii Nauk SSSR, Moscow.
Kouwets, F., 1987. Desmids from the Auvergne (France). Hydrobiologia 146: 193–263.
Kouwets, F., 2008. The species concept in desmids: the problem of variability, infraspecific taxa and the monothetic species definition. Biologia 63: 877–883.
Krieger, W., 1939. Die Desmidiaceen. Kryptogamen Flora Bd. XIII, Teil 2. Akademisches Verlag, Leipzig.
Le Naturaliste, 2007. *Micrasterias fimbriata* Ralfs. Posted by André [available on Internet at http://www.lenaturaliste.net/forum/viewtopic.php?f=91&t=68].
Le Naturaliste, 2010a. *Micrasterias* (identification). Posted by Bruno Dosso [available on Internet at http://www.lenaturaliste.net/forum/viewtopic.php?f=39&t=5125].
Le Naturaliste, 2010b. *Micrasterias*, j'en ai trouvé une! Posted by Kévin Pounot [available on Internet at http://www.lenaturaliste.net/forum/viewtopic.php?f=39&t=6446].
Lenzenweger, R., 1981. Heimische Zieralgen (*Desmidiaceen*)—gefährdete Mikroflora. Öko-L 3: 15–19.
Löytynoja, A. & M. C. Milinkovitch, 2001. SOAP, cleaning multiple alignments from unstable blocks. Bioinformatics 17: 573–574.
Luo, W., S. Pflugmacher, T. Pröschold, N. Walz & L. Krienitz, 2006. Genotype versus phenotype variability in *Chlorella* and *Micractinium* (Chlorophyta, Trebouxiophyceae). Protist 157: 315–333.
Medvedeva, L. A., 2001. Biodiversity of aquatic algal communities in the Sikhote-Alin biosphere reserve (Russia). Cryptogamie Algologie 22: 65–100.
Mikhailyuk, T. I., H. J. Sluiman, A. Massalski, O. Mudimu, E. M. Demchenko, S. Y. Kondratyuk & T. Friedl, 2008. New streptophyte green algae from terrestrial habitats and an assessment of the genus *Interfilum* (Klebsormidiophyceae, Streptophyta). Journal of Phycology 44: 1586–1603.
Mix, M., 1970. Die Desmidiaceen des Zeller Lochs bei Fulda. Mitteilungen aus dem Staatsinstitut für Allgemeine Botanik in Hamburg 13: 71–92.
Müller, J., T. Friedl, D. Hepperle & M. Lorenz, 2005. Distinction between multiple isolates of *Chlorella vulgaris* (Chlorophyta, Trebouxiophycee) and testing for conspecificity using amplified fragment length polymorphism and

ITS rDNA sequences. Journal of Phycology 41: 1236–1247.

Nemjová, K., J. Neustupa, J. Št'astný, P. Škaloud & J. Veselá, accepted. Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*. Phycological Research.

Neustupa, J. & P. Škaloud, 2007. Geometric morphometrics and qualitative patterns in the morphological variation of five species of *Micrasterias* (Zygnemophyceae, Viridiplantae). Preslia 79: 401–417.

Neustupa, J., J. Št'astný & L. Hodač, 2008. Temperature-related phenotypic plasticity in the green microalga *Micrasterias rotata*. Aquatic Microbial Ecology 51: 77–86.

Neustupa, J., K. Černá & J. Št'astný, 2009. Diversity and morphological disparity of desmid assemblages in Central European peatlands. Hydrobiologia 630: 243–256.

Neustupa, J., P. Škaloud & J. Št'astný, 2010. The molecular phylogenetic and geometric morphometric evaluation of *Micrasterias crux-melitensis/M. radians* species complex. Journal of Phycology 46: 703–714.

Nordstedt, C. F. O., 1888. Desmidieer från Bornholm, samlade och delvis destämda af R. T. Hoff. Videnskabelige meddelelser fra den Naturhistoriske forening i Kjöbenhavn 1888: 182–213.

Nylander, J. A. A., 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University [available on Internet at http://www.abc.se/~nylander].

Oyadomari, J. K., 2010. Freshwater Algae (and Protozoans) of the Keweenaw Peninsula, MI [available on Internet at http://www.keweenawalgae.mtu.edu/].

Pedersen, N. & L. Hedenäs, 2003. Phylogenetic investigations of a well supported clade within the acrocarpous moss family Bryaceae: evidence from seven chloroplast DNA sequences and morphology. Plant Systematics and Evolution 240: 115–132.

Photomacrography, 2008. Photography Through the Microscope [available on Internet at http://www.photomacrography.net/forum/viewtopic.php?t=5266&highlight=micrasterias].

Prescott, G. W., H. T. Croasdale & W. C. Vinyard, 1977. A Synopsis of North American Desmids, Part II. Desmidiaceae: Placodermae, Sect. 2. University of Nebraska Press, Lincoln.

Raciborski, M., 1885. De nonnulis Desmidiaceis novis vel minus cognitis, quae in Polonia inventae sunt. Pamiętnik wydziału matematyczno-przyrodniczego Akademii Umiejętności 10: 57–100.

Ralfs, J., 1848. The British Desmidieae. Reeve, Benham & Reeve, London.

Rambaut, A., 2009. FigTree. Tree Figure Drawing Tool. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Rohlf, F. J., 2008. Tps Series. Department of Ecology and Evolution, State University of NewYork at Stony Brook, New York.

Ronquist, F. & J. P. Huelsenbeck, 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Roy, J. & J. P. Bisset, 1893. On Scottish Desmidieae I. The Annals of Scottish Natural History 5: 106–111.

Růžička, J., 1981. Die Desmidiaceen Mitteleuropas, Band 1, 2. Lieferung. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling & R. L. Small, 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92: 142–166.

Sieralgen in Nederland, 2003. Desmid of the Month, July 2003. *Micrasterias fimbriata* [available on Internet at http://www.desmids.nl/maand/english/juleng03.html].

Št'astný, J., 2010. Desmids (Conjugatophyceae, Viridiplantae) from the Czech Republic; new and rare taxa, distribution, ecology. Fottea 10: 1–74.

Št'astný, J. & R. Lenzenweger, 2008. A supplement to the desmid flora (Desmidiales, Zygnematophyceae) of the "Schwemm" near Walchsee in Northern Tyrol (Austria). Phyton—Annales Rei Botanicae 48: 37–49.

Tamura, K., J. Dudley, M. Nei & S. Kumar, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Turmel, M., C. Otis & C. Lemieux, 2005. The complete chloroplast DNA sequences of the charophycean green algae *Staurastrum* and *Zygnema* reveal that the chloroplast genome underwent extensive changes during the evolution of the Zygnematales. BMC Biology 3: 22–34.

Tyler, P. A., 1970. Taxonomy of Australian freshwater algae I. The genus *Micrasterias* in south-eastern Australia. British Phycological Journal 5: 211–234.

Webber, M., 2006. Personal www pages of Mark Webber [available on Internet at http://www.markwebber.org/images/dcc0607/index.html].

West, W. & G. S. West, 1905. A Monograph of the British Desmidiaceae, Vol. 2. Ray Society, London.

Wolle, F., 1892. Desmids of the United States and List of American Pediastrums. Moravian Publication Office, Bethlehem.

Wurtz, A., 1945. Recherches ecologiques sur les lacs et tourbières et étangs d'Auvergne. Annales de la Station Centrale d'Hydrobiologie Appliquée 1: 89–199.

Zelditch, M. L., D. L. Swiderski, D. H. Sheets & W. L. Fink, 2004. Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, London.

Zwickl, D. J., 2009. Garli. Phylogenetic Analysis of Molecular Sequence Data Using the Maximum-Likelihood Criterion [available on Internet at http://code.google.com/p/garli/].

# Paper III

# Quantification of DNA content in freshwater microalgae using flow cytometry: a modified protocol for selected green microalgae

Petra Mazalová, Petra Šarhanová, Vladan Ondřej & Aloisie Poulíčková

*Department of Botany, Faculty of Science, Palacký University Olomouc, Šlechtitelů 11, CZ–783 71 Olomouc, Czech Republic; e–mail: petra.mazalova@upol.cz*

**Abstract:** The study of genome size variation in microalgae lags behind that of comparable research in higher plants and seaweeds. This situation is essentially caused by: (1) difficulties in obtaining sufficient biomass for experiments; (2) problems with protoplast isolation due to cell–wall heterogeneity and complexity; and (3) the absence of suitable standards for routine measurements. We propose a multi–step protocol that leads to the quantification of DNA content in desmids using flow cytometry. We present detailed culture conditions, the minimal biomass necessary for three repetitive measurements, a method to isolate protoplasts and selection of suitable standards. Our protocol, which is mainly based on studies with higher plants and commercially available enzyme mixtures, is useful in Streptophyta, especially members of the Zygnematophyceae, because of their close phylogenetic relationship to higher plants, in particular the similarity of their cell wall organization. Moreover, the suggested protocol also works for some Chlorophyta (*Chloroidium ellipsoideum*, *Tetraselmis subcordiformis*) and Heterokontophyta (*Tribonema vulgare*). We suggest and characterize a new standard for flow cytometry of microalgae (*Micrasterias pinnatifida*). Modification of the enzyme mixture is probably necessary for microalgae whose cell walls are surrounded by a mucilaginous envelope (*Planktosphaeria*), those that contain alganan (*Chlorella*), monads with a pellicle or chlamys (*Euglena*, *Chlamydomonas*). While we did not anticipate any success with diatoms (*Pinnularia*), because of their silica frustules, the enzyme mixture also failed for some other green microalgae (*Xanthidium, Kentrosphaera, Stigeoclonium, Trentepohlia* and *Pseudendoclonium*).

**Keywords:** DNA content, flow cytometry, desmids, microalgae, standards, *Micrasterias pinnatifida*

## Introduction

Although data relating to genome size are extremely important in the assessment of phylogenetic relationships in plants, there remain enormous gaps in the current genome size database. DNA content have so far been determined for 7058 species, comprising 6287 angiosperms, 204 gymnosperms, 82 pteridophytes, 232 bryophytes and 253 algae (Bennett & Leitch 2010). Genome size estimates have been studied by Kapraun (2005, 2007), who reported values for almost 400 species of red, green and brown macroscopic algae. Unicellular freshwater microalgae have rarely been studied (Kapraun 2007), but genome size data can be used for testing the role of polyploidy in algal evolution and speciation (Mann & Poulíčková 2010), particularly in microalgal groups with sexual reproduction (Zygnematophyceae, Bacillariophyceae). Our research interests include cryptic diversity and reproductive isolation in desmids and so we tried to apply existing genome size estimation methods to this group of microalgae in particular.

Several methods have been used to quantify nuclear DNA in algae, primarily Feulgen microdensitometry and similar microspectrophotometric methods (Hardie et al. 2002; Kapraun 2007). Although flow cytometry (FC) is routinely used in higher plants, it has rarely been used for microalgae (Le Gall et al. 1993; Simon et al. 1994; Veldhuis et al. 1997). Moreover these studies estimated nuclear DNA content from whole cells, but recently it turned out that only estimates based on isolated nuclei are accurate enough for general comparisons (Kapraun 2007).

In contrast to higher plants and seaweeds, studies on microalgae require isolation of single cells and maintainence of unialgal cultures to obtain a reasonable amount of biomass for experiments. As a result, research on microalgae is time consuming. In addition, we have found that methods for nuclei separation used in higher

plants (chopping tissue with a razor blade; Galbraith et al. 1983), do not work in our model microalgae, the desmids. Consequently, we tried grinding the material in a mortar, but this did not yield any positive results. Techniques for cell wall removal using cell wall–degrading enzymes have previously been used in higher plants for intact plant cells and in the preparation of somatic hybrids after protoplast fusion (Doležel et al. 2007). This technique has also been used for some seaweeds (Millner et al. 1979; Butler et al. 1990). However, algae are a heterogeneous group and exhibit a variety and complexity of cell wall organization which contrasts with the relative homogeneity characteristic of higher plants. As in higher plants, the algal cell wall can sometimes be interpreted as a two phase system: a crystalline phase (the skeleton) embedded in a more amorphous phase (the matrix). However, the algal cell wall differs from land plants in the abundance of matrix compounds compared to the skeletal components (Butler et al. 1990). In higher plants the three primary components of the cell wall are cellulose, hemicellulose and pectins, and protoplasts can be isolated from virtually any plant species using a combination of cellulases, hemicellulases and pectinases (Butler et al. 1990). Because of the phylogenetic position of desmids close to higher plants (Streptophyta), we decided to start with commercial preparations of enzymes available for the routine isolation of higher plant protoplasts. Propidium iodide was used as a fluorescent dye which intercalates quantitatively to the double–stranded DNA, therefore absolute DNA amount can be quantified.

Commonly used higher plant standards (Doležel et al. 1992), *Pisum sativum*, *Lycopersicon esculentum*, *Raphanus sativus* and *Zea mays* have been tested and used for characterization of new desmid standard.

In this study we aimed to develop a protocol for the quantification of nuclear DNA by flow cytometry suitable primarily for desmids. As the method consists of several steps, we aimed to find 1) an easy and fast culturing method to obtain a minimal biomass necessary for the experiments, 2) an easy way to isolate protoplasts and 3) a suitable standard for measurement of DNA content and/or define a new microalgal standard. We also tried to verify, by selective screening, whether our protocol is useful for other microalgae.

## Materials and methods

**Algal strains, media, culture conditions, cropping of cells and LM observations.** The algal material used in these experiments was obtained from clonal unialgal cultures. Algal strains were either our isolates or those obtained from culture collections (Table 1). Our strains (699, 723, 745, Pin1Cra) were isolated using standard methods (Andersen 2005) from Irish or Scottish lakes. They are held in the culture collection of the Department of Botany, Palacký University in Olomouc. Cultures were maintained at 16 °C with 12:12 light:dark cycle, under cool–white fluorescent lights at an irradiation of 20 $\mu mol.m^{-2}.s^{-1}$.

Two basic liquid culture media were used: an oligotrophic medium for desmids used in the CAUP collection (OGM; Černá & Neustupa 2010) and Bold‘s Basal medium for other microalgae (BBM; Bold 1949). *Euglena* was cultured in soil extract medium (Andersen 2005) diluted 1:1 with distilled water, *Tetraselmis* in ASW medium (McLachlan 1964) and *Pinnularia* in diatom medium WC with silicate (Guillard & Lorenzen 1972). Storage cultures were kept in 50 mm Petri dishes. Subsequently, 2 weeks before planned flow cytometric measurements, a rich inoculum of each strain (ca 1 ml) was transfered to fresh medium in 100 mm Petri dishes and kept at a higher irradiation (40 $\mu mol.m^{-2}.s^{-1}$) with 16:8 light:dark cycle. Approximately 10 days after subculturing, the bottom of the Petri dish was conspicuously green and such an amount of cells in exponential growth phase was used for cytometric measurements. For three replacated DNA measurements of each algal strain, we used three Petri dishes of material. Desmids were usually growing on the bottom of the Petri dish. Therefore superfluous medium was gently poured off and cells were carefully scraped from the bottom using a bacteriological squeegee and transfered into 15 ml centrifuge tubes and centrifuged (1500 rpm for 5 min). Superfluous medium was again gently removed and the sedimented cells were used for subsequent treatment. LM observations were done under a light Zeiss AxioImager microscope and Zeiss Axiovert inverted microscope.

**Enzyme treatment.** Conspicuously green cultures in the exponential phase of growth (10–15 days after inoculation) were concentrated by centrifugation so that the tip of a conical 15 ml tube was filled with biomass. Then, 2 ml of the enzymatic mixture of 2% Cellulase Onozuka R–10 (Duchefa Biochemie, Netherlands), 0.5% Macerozyme R–10 (Duchefa Biochemie, Netherlands) dissolved in modified rinsing solution PGly (Table 2) was added (Debeaujon & Branchard 1992). Suspensions were kept for 16 h in the dark at 27 °C. After 16 h, suspensions were centrifuged (700 rpm for 5 min), the supernatant was poured off and replaced by 1 ml of PGly to rinse the pellet. Suspensions were recentrifuged (700 rpm for 5 min) and PGly was

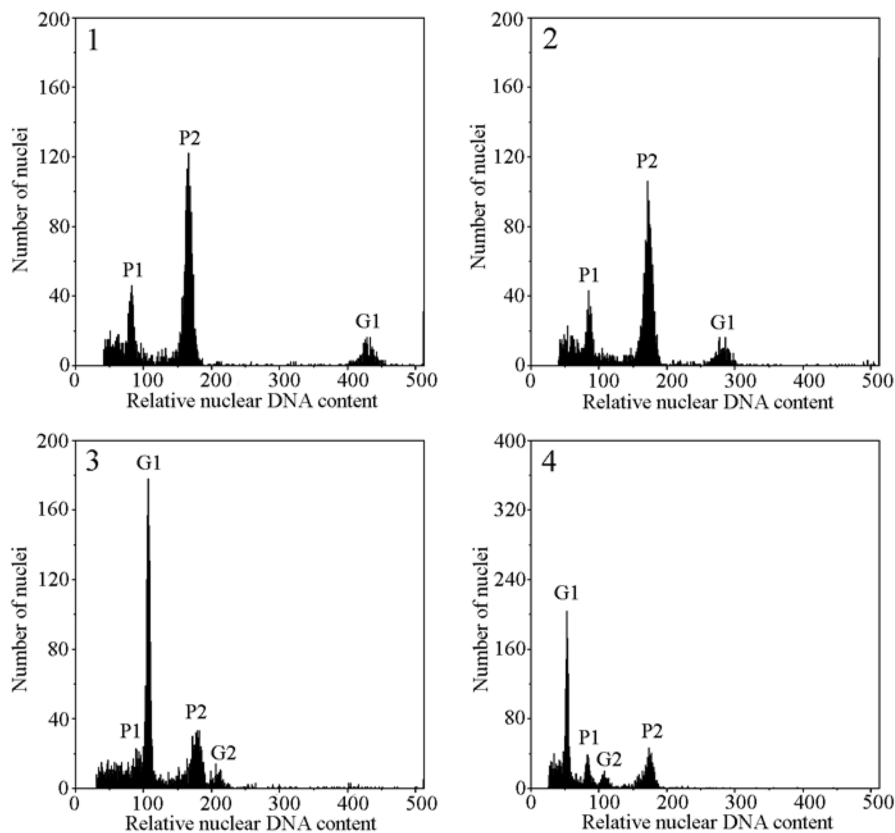



Fig. 1. Histograms of relative nuclear DNA content of *Micrasterias pinnatifida* (RALFS) and various standards: (1) *Pisum sativum*, (2) *Zea mays*, (3) *Lycopersicon esculentum*, (4) *Raphanus sativus*. (G1) G1 phase of the standards, (G2) G2 phase of the standards, (P1) haploid stage of the sample, (P2) diploid stage of the sample.

removed. The obtained biomass of cells was placed on ice before lysis buffer (flow cytometry) was added to protect the cells from regenerating cell walls and to enhance release of the nuclei from the treated cells.

**Flow cytometry and DNA amount estimation.** The absolute nuclear DNA amount of the studied algae was estimated by FC using a ML CyFlow instrument (Partec GmbH, Münster, Germany). Each sample was analyzed separately to choose a proper standard of different genome size, but still relatively close enough to the sample to avoid nonlinearity of the instrument. A second measurement of the studied samples was done together with the internal standard. *Raphanus sativus* cv. Saxa (2C = 1.11 pg, Doležel et al. 1992), *Lycopersicon esculentum* cv. Stupicke (2C = 1.96 pg, Doležel et al. 1992), *Zea mays*, CE–777, (2C = 5.46 pg, Suda personal communication) or *Pisum sativum* cv. Ctirad (2C = 8.76 pg, Suda personal communication) were used as standards. Leaf tissues of standards were chopped with a razor blade in 1 ml of LB01 lysis buffer (15 mM TRIS; 2 mM EDTA; 0.5 mM spermine–4HCl; 80 mM KCl; 20 mM NaCl; 0.1 % Triton X–100; 15mM 2–mercaptoethanol.; pH 8.0; Doležel et al. 1989). Then the algal solution was added to the chopped standard, mixed and the suspension of nuclei was filtered through nylon mesh (42 µm) into a tube containing another 300 µl of lysis buffer. After 1 hour incubation on ice, the suspension was decanted to a new tube to remove the sediment and 50 µl of propidium iodid (PI) was added. Measurements were done on the flow cytometer up to 3000 particles. The absolute nuclear DNA amount was calculated from a linear ratio between G1 peak position of the standard and the peak of the sample according to Doležel & Bartoš (2005): (sample G1 peak mean / standard G1 peak mean) * standard 2C DNA content.

All samples were measured at least twice, usually three times. Some authors had shown that greater ploidy levels in one algal culture can occur (Hamada 1987; Haig 2010). Although we did not study the number of chromosomes or ploidy level, in those cases where we obtained two peaks, we labelled the first lower ploidy peak as 1C and the second main peak as 2C in accordance with Greilhuber et al. (2005).

**New microalgal standard identification.** The vigorously growing desmid strain available in the Hamburg culture collection (Sammlung von Conjugaten–Kulturen), *Micrasterias pinnatifida* (Kützing) ex Ralfs (SVCK 411; http://www.biologie.uni–hamburg.de/b–online/d44_1/44_1.htm) has been characterized as a first microalgal standard.

*Micasterias pinnatifida* strain was measured seventeen times with different standards following the method described above. As internal standards, *Pisum sativum* was measured ten times, *Zea mays* four times, *Lycopersycon esculentum* twice and *Raphanus sativus* once. To cross–check genome sizes, standards were also analysed in combination: a) *P. sativum, Z. mays* and *R. sativus* and b) *P. sativum* and *L. esculentum*.

Table 1. Basic characteristics of the strains under study. Sources of the strains: (ASW) Algensammlung Wien, University of Viena, nowadays deposit in the Culture Collection of Algae at the University of Cologne (CCAC); (CAUP) Culture Collection of Algae, Charles University in Prague, Czech Republic; (M) Research Culture Collection Melkonian, University of Cologne, Germany; (NIES) Microbial Culture Collection, National Institute for Environmental Studies, Japan; (SAG) Sammlung von Algenkulturen Göttingen, University of Göttingen, Germany; (SVCK) Sammlung von Conjugaten–Kulturen (http://www.biologie.uni-hamburg.de/b-online/d44_1/44_1.htm). The strains not held by above mentioned collections are keeping in culture collection of the Department of Botany, Palacký University in Olomouc (Czech Republic).

| | Species | Strain | Origin/locality | Medium |
|---|---|---|---|---|
| Zygnematophyceae | *Cosmarium contractum* Kirchner | M 3039 | Doksy (CZ) | OGM |
| | *Euastrum verrucosum* Ralfs | 699 | Lough an Fhraoigh (Connemara, IRL) | OGM |
| | *Micrasterias pinnatifida* Ralfs | SVCK 411 | Laguna de Mucubaji, Merida (YV) | OGM |
| | *Micrasterias rotata* Ralfs | SVCK 212 | near Potsdam (D) | OGM |
| | *Micrasterias truncata* var. *pusilla* G.S. West | NIES 783 | Centenial Park (Sydney, AUS) | OGM |
| | *Pleurotaenium ehrenbergii* (Ralfs) De Bary | 745 | vicinity of Upper Lake (Kerry, IRL) | OGM |
| | *Staurastrum arctiscon* (Ralfs) Lundell | 723 | Lough Eirk (Kerry, IRL) | OGM |
| | *Staurastrum orbiculare* Ralfs | M 2217 | vicinity of Vladivostok (RU) | OGM |
| | *Staurodesmus dickiei* (Ralfs) Lillieroth | ASW 07056 | fen Rotmoos near Hornspitz (AUS) | OGM |
| | *Triploceras gracile* Bailey | SAG 24.82 | Rotary Pond at Falmouth (MA, USA) | OGM |
| | *Triploceras gracile* Bailey | SVCK 366 | Sumatra | OGM |
| | *Xanthidium octocorne* Ralfs | M3057 | Pískovny Cep (CZ) | OGM |
| | *Zygnema circumcarinatum* Czurda | CAUP K 402a | meadow ditch near Doksy (CZ) | BBM |
| | *Zygnema cylindricum* Transeau | CAUP K 403 | Doksy (CZ) | BBM |
| Klebsormidiophyceae | *Klebsormidium flaccidum* (Kützing) Silva, Mattox & Blackwell | CAUP J 302 | Adršpach (CZ) | BBM |
| Trebouxiophyceae | *Chloroidium ellipsoideum* (Gerneck) Darienko et al. | CAUP H 1949 | on a tree bark, Adršpach (CZ) | BBM |
| | *Chlorella vulgaris* Beyerinck | CAUP H 1993 | Žebrákovský creek, Czech-Moravian Highlands (CZ) | BBM |
| Chlorophyceae | *Stigeoclonium* sp. | CAUP J 603 | Žebrákovský creek, river basin of Sázava, Czech-Moravian Highlands (CZ) | BBM |
| | *Chlamydomonas geitleri* Ettl | CAUP G 224 | pond near Opatov (CZ) | BBM |
| | *Desmodesmus communis* (E. Hegewald) E. Hegewald | CAUP H 522 | unknown | BBM |
| | *Planktosphaeria gelatinosa* G.M.Smith | CAUP H 1401 | soil from garden, Woods Hole (MA, USA) | BBM |

*Table 1 Cont.*

| | | | | |
|---|---|---|---|---|
| Ulvophyceae | *Kentrosphaera* sp. | CAUP H 5308 | soil, top of the Boreč Hill (České Středohoří Mts, CZ) | BBM |
| | *Pseudendoclonium basiliense* VISCHER | SAG 466-2 | bog water, Bot. Gard. Univ. Basel (CH) | BBM |
| | *Trentepohlia* sp. | CAUP J 1601 | bark, Singapore | BBM |
| Prasinophyceae | *Tetraselmis subcordiformis* (WILLE) BUTCHER | CAUP M 201 | Sandy Hook (NJ, USA) | ASW |
| Bacillariophyceae | *Pinnularia* cf. *gibba* EHRENBERG | Pin1Cra | Loch of Craiglush (UK) | WC |
| Euglenophyceae | *Euglena gracilis* KLEBS | CAUP E 201 | peaty pool nearby Třtice (CZ) | soil extract medium |
| Xanthophyceae | *Tribonema vulgare* PASCHER | CAUP D 501 | Palach Pond near Lednice (CZ) | BBM |

## Results and discussion

### Range of tested microalgae

This paper proposes an easy protocol for the quantification of nuclear DNA by flow cytometry, suitable primarily for Zygnematophyceae, but also useful for some other microalgae, depending in particular on cell wall organization. We tested 28 microalgal cultures and succeeded with Streptophyta representatives (Desmidiales, Zygnematales and Klebsormidiales; Table 3). In addition, 39 desmid cultures of the genus *Micrasterias* sensu lato have been successfully analyzed in another study (ŠKALOUD unpublished). Although the protocol (particularly protoplast isolation) failed in Bacillariophyceae, Euglenophyta and most of the tested Chlorophyta, it was successful for *Chloroidium ellipsoideum* (Oocystaceae), *Tetraselmis subcordiformis* (Prasinophyceae) and *Tribonema vulgare* (Xanthophyceae, Heterokontophyta).

### Minimum biomass requirement and culture conditions

In contrast to higher plants and seaweeds, biomass for microalgal experiments originates from clonal unialgal cultures. From our experience, most of the tested species grow well in liquid media. We mostly used culture media for green microalgae and desmids (Bold‘s Basal medium–BBM; BOLD 1949 and oligotrophic medium for desmids as used in the CAUP collection–OGM; ČERNÁ & NEUSTUPA 2010) and an elevated irradiance level compared to maintenance cultures (40 μmol.m$^{-2}$.s$^{-1}$). Under such conditions we obtained from each Petri dish sufficient cell suspension for at least one measurement. In the case of *Micrasterias rotata,* a greater degree of biomass cover of the Petri dish was necessary to yield a sufficient amount of cell suspension. In contrast, the suspensions of small genome size species including *Tetraselmis subcordiformis* and *Tribonema vulgare* were highly concentrated and one microliter of the suspension was sufficient for the flow cytometric analysis. The species with larger genome sizes tend to have bigger cells (GREGORY 2001; LEITCH & BENNETT 2007) and therefore the cover of the Petri dish seems to be more intense. Consequently the real amount of cells rather than the intensity of green colour is crucial for sufficient concentration of cells in the FC–solution. Culturing in Petri dishes is cheap, does not require a lot of space and the health and density of the culture can easily be checked under an inverted microscope (POULÍČKOVÁ & MANN 2006).

### Protoplast isolation

Isolated protoplasts are living plant cells from which the walls have been removed. They provide a large, relatively homogeneous suspension of wall–less, single plant cells for physiological and biochemical research. Protoplasts surrounded only by a plasma membrane are a convenient starting material for obtaining high yields of delicate cell organelles such as nuclei or microbodies. Techniques for the removal of the cell wall using cell wall–degrading enzymes were developed in the 1960s and are routinely used in

Table 2. Composition of the modified solution PGly (original: DEBEAUJON & BRANCHARD 1992). With continuous stirring dissolve all components in distilled water. Bring the total volume to 1000 ml. Adjust to pH 5.8 with 1M HCl and sterilize by filtration. *MES 2–(*N*-morpholino)ethanesulfonic acid

| | Component | g.l$^{-1}$ $H_2O$ |
|---|---|---|
| PGly | $KH_2PO_4$ | 0.0272 |
| | $KNO_3$ | 0.101 |
| | $CaCl_2$ (anhydrous) | 1.1176 |
| | $MgSO_4.7H_2O$ | 0.246 |
| | glycine | 11.15 |
| | glucose | 18.016 |
| | MES* | 0.5857 |
| | manitol | 65.58 |

higher plants (TAKEBE & OTSUKI 1969; COCKING 1960; DOLEŽEL et al. 2007) for biotechnological purposes. In principle, there is no reason why such techniques cannot be successfully applied to algae. Methods for preparation of protoplasts of marine macroalgae have been developed only recently (BUTLER et al. 1990; INOUE et al. 2010; WAKABAYASHI et al. 1999) and have never been used for microalgae. The algal cell wall differs from land plants in its heterogeneity and complexity. Commercial mixtures of enzymes routinely used in higher plants mostly consist of cellulases, hemicellulases and pectinases. Cellulose is very abundant in green algae, in which it accounts for up to 70 % of the cell wall (PRESTON 1974). On the other hand, the cellulose content of most brown and red algae is low, being less than 10 % and some algae do not contain any cellulose (BUTLER et al. 1990). Our recent research focuses primarily on desmids, which belong to Streptophyta, thus a high similarity of their cell wall organization with higher plants can be expected. There exist actually differences in cell wall composition within different algal taxa (STACE 1991), but the concentrations of the main cell wall polymers of land plants are similar to that of Streptophyta (EDER et al. 2008, ANDERSON & KING 1961).

We modified the enzyme mixture used for the genera *Cucumis* and *Cucurbita* (GAJDOVÁ et al. 2007; ONDŘEJ et al. 2009) and marine green macroalgae (REDDY et al. 2006; UPPALAPATI & FUJITA 2002; CHEN & SHIH 2000). After this treatment we were able to measure the amount of DNA in desmids (*Cosmarium contractum*, *Euastrum verrucosum*, *Micrasterias pinnatifida*, *Micrasterias rotata*, *Micrasterias truncata Pleurotaenium ehrenbergii*, *Staurastrum arctiscon*, *Staurastrum orbiculare*, *Staurodesmus dickiei*, *Triploceras gracile*), some other microalgae within Streptophyta (*Klebsormidium flaccidum*, *Zygnema circumcarinatum* and *Zygnema cylindricum*), a few Chlorophyta (*Chloroidium ellipsoideum*, *Tetraselmis subcordiformis*) and Heterocontophyta (*Tribonema vulgare*). However, in contrast to higher plant samples, the algal cell walls have not been digested completely by the enzymes in some cases. Sometimes the desmid cell wall was eliminated only from one (younger) semicell, or protoplast left the cell wall by the split in the isthmus area. However, even these samples were often useful for flow cytometric measurements. This is the reason why young cultures (in the exponential growth phase) are recommended, because the cell wall is not fully developed. The method failed in the cases of *Chlorella vulgaris* and *Desmodesmus communis.* Their cell walls contain alganan and *Planktosphaeria gelatinosa* has a robust mucilaginous envelope. As we expected, we did not successed in the diatom *Pinnularia* cf. *gibba* on account of its silica frustule, nor monads with protein–containing periplast [*Euglena gracilis, Chlamydomonas geitleri*] (GRAHAM et al. 2008). The enzyme mixture also failed for *Xanthidium octocorne, Kentrosphaera* sp., *Stigeoclonium* sp., *Trentepohlia* sp. and *Pseudendoclonium basiliense*. Specific enzyme mixtures will be necessary for these microalgae.

**Flow cytometry and DNA amount estimation**

FC is the optimal method for nuclear genome size estimation. Compared to traditional methods like Feulgen microdensitometry, scanning microspectrophotometry or DNA image cytometry which were used in the past, the FC enables the measurement of large amounts of cells in a short period of time (DOLEŽEL & BARTOŠ 2005). Usability of FC for estimation of DNA amount from whole cells or protoplasts refers to cytoplasmatic autofluorescens and nonspecific background which lead to overestimation of results (KAPRAUN 2007; VELDHUIS et al. 1997). Therefore release of the nuclei from the protoplast by lysis buffer enables precise estimation of the nuclear genome size. One of the most important steps in genome size estimation is the selection of an appropriate standard. It should have a genome size close to the peaks of the target material, but not overlap with

Table 3. Absolute nuclear DNA amount in species. **Raphanus sativus* cv. saxa (2C = 1.11 pg, DOLEŽEL et al. 1992), *Lycopersicon esculentum* cv. stupicke (2C = 1.96 pg, DOLEŽEL et al. 1992), *Zea mays* CE–777 (2C = 5.46 pg, Suda personal communication) or *Pisum sativum* cv. ctirad (2C = 8.76, Suda personal communication); (x) measurement failed, (AVG) average, (STD) standard deviation.

| | Species | Strain | DNA amount (pg) AVG ± STD | Standard species* |
|---|---|---|---|---|
| Zygnematophyceae | *Cosmarium contractum* KIRCHNER | M 3039 | 5.72 ± 0.11 | *Pisum a Zea* |
| | *Euastrum verrucosum* RALFS | 699 | 14.84 ± 0.12 | *Pisum* |
| | *M. pinnatifida* RALFS | SVCK 411 | 3.40 ± 0.07 | *Lycopersicon, Pisum, Raphanus, Zea* |
| | *M. rotata* RALFS | SVCK 212 | 26.35 ± 0.12 | *Zea* |
| | *M. truncata* var. *pusilla* G.S. WEST | NIES–783 | 5.75 ± 0.10 | *Pisum* |
| | *Pleurotaenium ehrenbergii* (RALFS) DE BARY | 745 | 8.97 ± 0.17 | *Zea* |
| | *Staurastrum arctiscon* (RALFS) LUNDELL | 723 | 7.20 ± 0.01 | *Zea* |
| | *Staurastrum orbiculare* RALFS | M 2217 | 2.81 ± 0.01 | *Pisum* |
| | *Staurodesmus dickiei* (RALFS) LILLIEROTH | ASW 07056 | 2.05 ± 0.00 | *Zea* |
| | *Triploceras gracile* BAILEY | SAG 24.82 | 9.39 ± 0.07 | *Zea* |
| | *Triploceras gracile* BAILEY | SVCK 366 | 28.20 ± 0.14 | *Pisum* |
| | *Xanthidium octocorne* RALFS | M 3057 | x | x |
| | *Zygnema circumcarinatum* CZURDA | CAUP K 402a | 3.07 ± 0.06 | *Zea* |
| | *Zygnema cylindricum* TRANSEAU | CAUP K 403 | 2.51 ± 0.00 | *Zea* |
| Klebsormidiophyceae | *Klebsormidium flaccidum* (KÜTZING) SILVA, MATTOX & BLACKWELL | CAUP J 302 | 0.28 ± 0.01 | *Lycopersicon* |
| Trebouxiophyceae | *Chloroidium ellipsoideum* (GERNECK) DARIENKO et al. | CAUP H 1949 | 1.21 ± 0.03 | *Zea* |
| | *Chlorella vulgaris* BEYERINCK | CAUP H 1993 | x | x |
| Chlorophyceae | *Stigeoclonium* sp. | CAUP J 603 | x | x |
| | *Chlamydomonas geitleri* ETTL | CAUP G 224 | x | x |
| | *Desmodesmus communis* (E. HEGEWALD) E. HEGEWALD | CAUP H 522 | x | x |
| | *Planktosphaeria gelatinosa* G.M.SMITH | CAUP H 1401 | x | x |
| Ulvophyceae | *Kentrosphaera* sp. | CAUP H 5308 | x | x |
| | *Pseudendoclonium basiliense* VISCHER | SAG 466–2 | x | x |
| | *Trentepohlia* sp. | CAUP J 1601 | x | x |
| Prasinophyceae | *Tetraselmis subcordiformis* (WILLE) BUTCHER | CAUP M 201 | 1.20 ± 0.03 | *Lycopersicon, Zea* |
| Bacillariophyceae | *Pinnularia* cf. *gibba* EHRENBERG | Pin1Cra | x | x |
| Euglenophyceae | *Euglena gracilis* KLEBS | CAUP E 201 | x | x |
| Xanthophyceae | *Tribonema vulgare* PASCHER | CAUP D 501 | 0.41 ± 0.00 | Raphanus |

the cells of interest (PRICE & JOHNSTON 1996). If the genome sizes are too dissimilar, non–linearity of the instrument can arise. We chose different plant standards for different samples to avoid overlapping or nonlinearity of the instrument (Table 3). In three cases (*Micrasterias pinnatifida*, *Cosmarium contractum* and *Tetraselmis subcordiformis*) more standards were used to choose the optimal one. Results did not lead to significant differences in estimated genome size.

At present two standard fluorescent dyes are routinely used for DNA staining for FC measurements: 4',6–diamidino–2–phenylindole (DAPI) binding to AT–rich regions (BARCELLONA et al. 1986) and propidium iodide (PI) intercalating to the whole DNA (CRISSMAN & STEINKAMP 1973). The applicability of DAPI staining in absolute DNA amount measurements clashes

with unbalanced proportion of different bases in the genome. Barow & Meister (2002) have shown that the proportion of AT:GC–rich regions in higher plants is not equal between families nor species. The intraspecific differences were also documented by Šmarda et al. (2008) on fescues which range from 42.53% to 46.41%. Similar variation with a range from 42.9% to 46.7% was detected for several algae species by Le Gall et al. (1993). The most marked discrepancy caused by the use of specifically binding dyes was shown by Doležel et al. (1992). They found that the difference was caused not only by the differences in overall AT/GC ratios for studied material and standard, but also by the species–specific differences in binding of these fluorochromes to DNA. Hence, use of PI for absolute genome size estimation is unambiguous.

Our protocol succeeded in 17 cases from 28 cultures used in this study. The estimated absolute nuclear DNA amount in these 17 microalgal cultures varied from 0.28 pg to 28.2 pg (Table 3). The two smallest genomes belong to the families Klebsormidophyceae (*Klebsormidium flaccidum* – 0.28 pg) and Xanthophyceae (*Tribonema vulgare* – 0.41 pg). Variability in the family Zygnematophyceae ranged from 2.05 to 28.2 pg, which probably reflects an increase in ploidy level during diversification and evolution of the species. This phenomenon was previously described by Wang et al. (1986) for polyploid series of *Spirogyra maxima* (Zygnemataceae) in both culture and wild populations. The original culture developed into three ploidy levels: 2x, 3x and 4x in accordance to findings in natural populations. In contrast, Hoshaw et al. (1985) observed a spontaneous decrease in ploidy level in clonal cultures of *Spyrogira singularis*. Kapraun (2007) recorded a range of 2C nuclear DNA content for Chlorophyta from 0.01 to 5.8 pg and for Streptophyta from 0.2 to 6.4 pg excluding the highly polyploid Desmidiales which have geonome sizes of up to 46.8 pg. Our results are broadly comparable with recorded ranges, however as we did not determine ploidy level of the studied cultures, we used terminology of 2C value only in cases with two peaks (desmids, Zygnematophyceae). In the case of primitive unicellular algae (*Chloroidium ellipsoideum*) the DNA amount (Table 3) should represent the haploid vegetative stage, while the situation in algae with sexual reproduction (desmids, diatoms) can be more complicated.

We detected in 5 of 17 algal cultures (*Cosmarium contractum*, *Micrasterias pinnatifida*, *M. truncata* var. *pusilla*, *Staurastrum arctiscon* and *Zygnema circumcarinatum*) two peaks = ploidy levels where the first peak (most likely 1C level) was notably smaller than the second (2C). This phenomenon was reviewed for several algae species by Haig (2010). They explain that occurence of both haploid and diploid cells is caused by the meiosis and syngamy both followed by mitosis leading to vegetative growth of the two ploidy levels in culture.

Interesting differences were found in the case of two strains of *Triploceras gracile*. The strain isolated from Sumatra with 28.2 pg has a DNA content three times higher than the strain isolated from USA (9.39 pg). The same phenomenon was detected by Kasprik (1973), who counted numbers of chromosomes of several *Micrasterias* species. He detected different numbers of chromosomes in each population of *M. americana* corresponding to three ploidy levels: 2x, 3x and 4x.

The coefficient of variation (CV) refers to the quality of the relative fluorescent peaks. In most cases CVs below 3% are acceptable as CVs below 5% are considered to be sufficient for "problematic" species (Doležel & Bartoš 2005). DAPI staining shows lower CVs than those for PI as the binding of DNA is not influenced by chromatine structure (Santisteban et al. 1992). Coefficients of variation in our study varied from 2.35% (*Micrasterias pinnatifida,* selected as a

Table 4. Values of the absolute nuclear DNA amount (pg) of standards used in our study, from different sources.

| | Doležel et al. (1992) | Doležel et al. (1998) | Lysák & Doležel (1998) | Johnston et al. (1999) | Suda personal communication |
|---|---|---|---|---|---|
| *Raphanus sativus* | 1.11 | 1.41 | | | 1.12 |
| *Lycopersicon esculentum* | 1.96 | | | | 1.96 |
| *Zea mays* | 5.72 | 5.82 | 5.43 | 5.73 | 5.46 |
| *Pisum sativum* | 9.07 | 9.09 | | 9.39 | 8.76 |

new standard for microalgae) to 5.38%, with only one exception (*Tetraselmis subcordiformis*; 14.76 %), which was excluded from the analysis. Gating of the peaks (understand as trimming of the peak with high background) was necessary in the case of *Micrasterias rotata* and *Chloroidium ellipsoideum*, to obtain explicit results. Modification of the protoplast isolation enzymatic mixture for these species could lead to better results.

In the case of the filamentous species *Zygnema circumcarinatum* and *Z. cylindricum,* chopping of the sample with a razor blade after enzyme treatment was necessary for nuclei isolation. We tested the hypothesis that chopping without enzymatic treatment is sufficient for cytometric analyses for *Z. cylindricum* as it is possible for most of the higher plants. Coefficient of variation in that case was 21.65% in comparison with the chopped sample treated with enzymes – CV = 5.15%. Nevertheless, analysis of unchopped and enzymatically treated sample did not give any results.

### New microalgal standard *M. pinnatifida*

As we have shown in our study, the genome size of microalgae may differ largely from one to another species or population. Selection of a suitable standard is necessary for precise analyses. In the last few decades, different standards for FC analyses were used e.g. human leucocytes (Lysák et al. 2000), chicken red blood cells (CRBCs, Galbraith et al. 1983; Veldhuis et al. 1997), salmon erythrocytes (Iversen & Laerum 1987) or different plant species (various authors). As was proven by many authors (Doležel et al. 1992; Johnston et al. 1999; Galbraith et al. 1983; Tiersch et al. 1989), human and chicken cells do not seem to be a convenient standard for genome size estimation due to intraspecific and sex–related variation (De Vita et al. 1994; Mefford et al. 1997). For absolute genome size estimation, use of biologically similar material with identical sample preparation is recommended (Doležel & Bartoš 2005). Therefore we suggest a new algal standard, *Micrasterias pinnatifida* for FC estimations, which can be the starting point for FC–standards development suitable for material of isolated protoplasts. The species match most of the requirements for such standards: unproblematic preparation leading to narrow peaks with low CV, simultaneous preparation of standard and protoplast samples prior to cytometric analyses, available in sufficient quantities, easy to cultivate and distribute to other laboratories and stable genome size due to vegetative propagation. In addition it seems to be free of compounds interfering with PI staining.

In the majority of the 17 measurements of *Micrasterias pinnatifida,* two peaks of nuclear DNA were detected. In four measurements the first peak was not detected, although CV for the second peak was low, indicating that the absence was not caused by debris in the sample. To identify the genome size of *M. pinnatifida,* all four plant standards were used as the primary reference standards (Fig. 1). However, the use of *Lycopersicon* and *Raphanus* as internal standards refers to alternating of 1C and 2C peaks (Fig. 1) and increase the CV and inaccuracy of the measurement. Therefore we decided to exclude these standards from absolute genome size estimation. The genome size of new algal standard was counted from 14 analyses with *Pisum* and *Zea*, whose means of CVs were 3.63% for alga, 2.36% for *Pisum* and 3.59% for *Zea*. In all cases peak of 2C–value of alga was narrow and with maximum CV = 4.61%. The determined size of *Micrasterias pinnatifida* from Hamburg culture collection is 3.4 pg ± 0.07.

To validate the DNA amount of internal standards (Table 4) we measured them in combination: a) *Pisum sativum, Zea mays* and *Raphanus sativus* or b) *Pisum sativum* and *Lycopersicon esculentum*. Cross calculation gave means of genome sizes: 8.77 pg for *P. sativum*, 5.48 pg for *Z. mays,* 1.95 pg for *L. esculentum* and 1.09 pg for *R. sativus*. The standard deviation of known and counted genome sizes did not exceed 0.01 and we consider use of known genome sizes as legitimate.

**Acknowledgements**

The authors are grateful to Kath Evans (University of Edinburgh) for English corrections. The research was supported by projects GACR 206/09/0906, 206/08/0389 and IGA PrF_2011_003. We are grateful to P. Škaloud (Charles University of Prague) for inspirative comments and for providing us with cultures.

### References

Andersen, R. A. (ed.) (2005): Algal culturing technique. – 596 pp., Academic Press, London.

Anderson, D.M.W. & King, N. J. (1961): Polysaccharides of the Characeae III. The carbohydrate content of *Chara australis*. – Biochimica et Biophysica Acta 52: 449–454.

Barcellona, M.L., Favilla, R., von Berger, J., Avitabile, M., Ragusa, N. & Masotti, L. (1986): DNA–4‘–6–diamidine–2–phenylindoilnete ractions: A comparative study employing fluorescence and ultraviolet spectroscopy. – Arch Biochem Biophys 250: 48–53.

Barow, M. & Meister, A. (2002): Lack of Correlation Between AT Frequency and Genome Size in Higher Plants and the Effect of Nonrandomness of Base Sequences on Dye Binding. – Cytometry 47: 1–7.

Bennett, M.D. & Leitch, I.J. (2010): Plant DNA C–values database. http://data.kew.org/cvalues/updates.html

Bold, H. C. (1949): The morphology of *Chlamydomonas chlamydogama* sp. nov. – Bulletin of the Torrey Botanical Club 76: 101–108.

Butler, D.M., Evans, L.V. & Kloareg, B. (1990): Isolation of protoplasts from marine macroalgae. – In: Akatsuka, I. (ed.): Introduction to Applied Phycology. – pp. 647–668, SPB Academic Publication, The Hague, The Netherlands.

Černá, K. & Neustupa, J. (2010): The pH–related morphological variations of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic. – Aquatic Ecology 44: 409–419.

Chen, Y.–C. & Shih, H.–C. (2000): Development of protoplasts of *Ulva fasciata* (Chlorophyta) for algal seed stock. – Journal of Phycology 36: 608–615.

Cocking, E.C. (1960): A method for the isolation of plant protoplasts and vacuoles. – Nature 187: 927–929.

Crissman, H.A. & Steinkamp, J.A. (1973): Rapid simultaneous measurement of DNA, protein and cell volume in single cells from large mammalian cell populations. – Journal of Cell Biology 59: 766–771.

Debeaujon, I. & Branchard, M. (1992): Induction of somatic embryogenesis and callogenesis from cotyledons and leaf protoplasts–derived colonies of melon (*Cucumis melo* L.). – Plant Cell Reports 12: 37–40.

De Vita, R., Cavallo, D., Eleuteri, P. & Dellomo, G. (1994): Evaluation of interspecific DNA content variation and sex identification in falconiformes and strigiformes by flow cytometric analysis. – Cytometry 16: 346–350.

Doležel J. & Bartoš J. (2005): Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size. – Annals of Botany 95: 99–110.

Doležel, J., Binarová, P. & Lucretti, S. (1989): Analysis of nuclear DNA content in plant cells by flow cytometry. – Biologia Plantarum 31: 113–120.

Doležel, J., Greilhuber, J., Lucretti, S., Meister, A., Lysák, M.A., Nardi, L. & Obermayer, R. (1998): Plant genome size estimation by flow cytometry: inter–laboratory comparison. – Annals of Botany 82 (Supplement A): 17–26.

Doležel, J., Greilhuber, J. & Suda, J. (2007): Flow cytometry with plants: an overview. – In: Doležel, J., Greilhuber, J. & Suda, J. (eds): Flow cytometry with plant cells. – pp. 41–65, Wiley–VCH Verlag Gmbh & Co. KGaA, Weinheim.

Doležel, J., Sgorbati, S. & Lucretti, S. (1992): Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. – Physiologia Plantarum 85: 625–631.

Eder, M., Tenhaken, R., Driouich, A. & Lütz–Meindl, U. (2008): Occurence and characterization of arabinogalactan–like proteins and hemicelluloses in *Micrasterias* (Streptophyta). – Journal of Phycology 44: 1221–1234.

Gajdová, J., Navratilová, B., Smolna, J. & Lebeda, A. (2007): Effect of genotype, source tissue and media composition on Cucumis and Cucurbita protoplast isolation and regeneration. – Acta Horticulturae 731: 89–94.

Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P. & Firoozabady, E. (1983): Rapid flow cytometric analysis of the cell cycle in intact plant tissues. – Science 220: 1049–1051.

Graham, J.E.; Wilcox, L.W., Graham, L.E. (2008): Algae (2nd Edition). – 720 pp., Benjamin Cummings, San Francisco, CA.

Gregory, T.R. (2001): Coincidence, coevolution, or causation? DNA content, cell size, and the C–value enigma. – Biological reviews 76: 65–101.

Greilhuber, J., Doležel, J., Lysák, M.A. & Bennett, M.D. (2005): The origin, evolution and proposed stabilization of the terms ‘Genome Size’ and ‘C–Value’ to describe nuclear DNA contents. – Annals of Botany 95: 255–260.

Guillard, R.R.L. & Lorenzen, C.J. (1972): Yellow–green algae with chlorophyllide C. – Journal of Phycology 8: 10–14.

Haig, D. (2010): What do we know about Charophyte (Streptophyta) life cycles? – Journal of Phycology 46: 860–867.

Hamada, J. (1987): Diploidy in DNA content in vegetative cells of Closterium ehrenbergii (Chlorophyta). – Journal of Phycology 23: 541–546.

Hardie, D.C., Gregory, T.R. & Herbert, P.D.N. (2002): From pixels to picograms: A beginners‘ guide to genome quantification by Feulgen image analysis densitometry. – The Journal of Histochemistry & Cytochemistry 50: 735–749.

Hoshaw, R.W., Wang, J.–C., McCourt, R.M. & Hull, H. M. (1985): Ploidal changes in clonal cultures

of *Spirogyra communis* and implications for species definition. American Journal of Botany 72:1005–11.

Iversen, O.E. & Laerum, O.D. (1987): Trout and salmon erythrocytes and human leukocytes as internal standards for ploidy control in flow cytometry. – Cytometry 8: 190–196.

Inoue, A., Mashino, C., Kodama, T. & Ojima, T. (2010): Protoplast preparatoin from *Laminaria japonica* with recombinant alginate lyase and cellulase. – Marine Biotechnology 13: 256–263.

Johnston, J.S., Bennett, M.D., Rayburn, A.L., Galbraith, D.V. & Price, H.J. (1999): Reference standards for determination od DNA content of plant nuclei. – American Journal of Botany 86: 609–613.

Kapraun, D.F. (2005): Nuclear DNA content estimates in multicellular green, red and brown algae: Phylogenetic considerations. – Annals of Botany 95: 7–44.

Kapraun, D.F. (2007): Nuclear DNA content estimates in green algal lineages: Chlorophyta and Streptophyta. – Annals of Botany 99: 677–701.

Kasprik, W. (1973): Beiträge zur Karyologie der Desmidiaceen.Gattung Micrasterias AG. – Beih. Nova Hedwigia 42: 115–137.

Le Gall, Y.,Brown, S., Marie, D., Mejjad, M. & Kloareg, B. (1993): Quantification of nuclear DNA and G–C content in marine macroalgae by flow cytometry of isolated nuclei. – Protoplasma 173: 123–132.

Leitch, I.J. & Bennett, M.D. (2007): Genome size and its uses: the impact of flow cytometry. – In: Doležel, J., Greilhuber, J. & Suda, J. (eds): Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes and Genomes. – pp. 153–176, Wiley–VCH, Weinheim, Germany.

Lysák, M.A. & Doležel, J. (1998): Estimation of nuclear DNA content in *Sesleria* (*Poaceae*). – Caryologia 52: 123–132.

Lysák, M.A., Rostková, A., Dixon, J.M., Rossi, G. & Doležel, J. (2000): Limited genome size variation in *Sesleria albicans*. – Annals of Botany 86: 399–403.

Mann, D.G. & Poulíčková, A. (2010): Mating system, auxosporulation, species taxonomy and evidence for homoploid evolution in *Amphora* (Bacillariophyta). – Phycologia 49: 183–201.

McLachlan, J. (1964): Some considerations of the growth of marine algae in artificial media. – Canadian Journal of Microbiology 10: 769–782.

Mefford, H., van den Engh, G., Friedman, C. & Trask, B.J. (1997): Analysis of variation in chromosome size among diverse human populations by bivariate flow karyotyping. – Human Genetics 100: 138–144.

Millner, P.A., Callow, M.E. & Evans, L. V. (1979): Preparation of protoplasts from the green alga *Enteromorpha intestinalis* (L.) Link. – Planta 147: 174–177.

Ondřej, V., Kitner, M., Doležalová, I., Nádvorník, P., Navrátilová, B. & Lebeda, A. (2009): Chromatin structural rearrangement during dedifferentiation of protoplasts of Cucumis sativus L. – Molecules and Cells 27: 443–447.

Preston, R.D. (1974): The physical biology of plant cell walls. – 491 pp., Chapman & Hall, London.

Price, H.J. & Johnston, J.S. (1996): Analysis of plant DNA content by Fuelgen micro–spectrophotometry and flow cytometry. – In: Jauhur, P. (ed.): Methods of Genome Analysis in Plants: Their merits and Pitfalls. Chpt. 7. – pp. 115–132, CRC Press Inc., Boca Raton, FL.

Poulíčková, A. & Mann, D.G. (2006): Sexual reproduction in *Navicula cryptocephala* (Bacillariophyceae). – Journal of Phycology 42: 872–886.

Reddy, C.R.K., Dipakkore, S., Kumar, G.R., Jha, B., Cheney, D.P. & Fujita, Y. (2006): An improved enzyme preparation for rapid mass production of protoplasts as seed stock for aquaculture of macrophytic marine green algae. – Aquaculture 260: 290–297.

Santisteban, M.S., Montmasson, M.P., Giroud, F., Ronot, X. & Brugal, G (1992): Fluorescence image cytometry of nuclear DNA content versus chromatin pattern: a comparative study of ten fluorochromes – J. Histochem. Cytochem. 40: 1789–97.

Simon, N., Barlow, R.G., Marie, D., Partensky, F. & Vaulot, D. (1994): Characterization of oceanic photosynthetic picoeucaryotes by flow cytometry. – Journal of Phycology 30: 922–935.

Stace, C. A. (1991): Plant taxonomy and biosystematics (2nd Edition). – 272 pp., University Press, Cambridge.

Šmarda, P., Bureš, P., Horová, L., Foggi, B. & Rossi, G. (2008): Genome Size and GC Content Evolution of Festuca: Ancestral Expansion and Subsequent Reduction. – Annals of Botany 101: 421–433.

Takebe, I. & Otsuki, Y. (1969): Infection of tobacco mesophyll protoplasts by tobacco mosaic virus. – Proceedings of the National Academy of Sciences USA 64: 843–848.

Tiersch, T.R., Chandler, R.W., Wachtel, S.S. & Elias, S. (1989): Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. – Cytometry 10: 706–710.

Uppalapati, S.R. & Fujita, Y. (2002): A simple method for mass isolation of protoplasts from species of *Monostroma*, *Enteromorpha* and *Ulva* (Chlorophyta, Ulvales). – Journal of Applied

Phycology 14: 165–168.
Veldhuis, M.J., Cucci, T.L., Sieracki, M.E. (1997): Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. – Journal of Phycology 33: 527–541.
Wakabayashi, T., Kuboi, T., Tuboi, T., Kaji, M. & Hara, M. (1999): Preparation of high yields of algal protoplasts using buccal juice of sea hare and commercial cellulase. – Marine Biotechnology 1: 407–410.
Wang, J.C., Hoshaw, R.W. & McCourt, R.M. (1986): A polyploid species complex of *Spirogyra communis* (Chlorophyta) occuring in nature. – Journal of Phycology 22: 102–107.

*© Czech Phycological Society (2011)*
*Received December 17, 2010*
*Accepted May 1, 2011*

# Paper IV

1 **DNA content variation and its significance in the evolution of the genus *Micrasterias***
2 **(Desmidiales, Streptophyta)**
3

4 **Aloisie Poulíčková[1,*], Petra Mazalová[1], Radim J. Vašut[1], Petra Šarhanová[1], Jiří**
5 **Neustupa[2] and Pavel Škaloud[2]**
6

7 *[1]Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 11,*
8 *CZ-783 71 Olomouc, Czech Republic, [2]Department of Botany, Charles University in Prague,*
9 *Benátská 2, CZ -128 01 Prague, Czech Republic*
10 **For correspondence. E-mail aloisie.poulickova@upol.cz*
11

12 - *Background and Aims* It is now clear that whole genome duplications have
13 occurred in all eukaryotic evolutionary lineages, and that the vast majority of
14 flowering plants have experienced polyploidisation in their evolutionary history.
15 However, study of genome size variation in microalgae lags behind that of
16 higher plants and seaweeds. In this study, we have addressed the question
17 whether microalgal phylogeny is associated with DNA content variation in order
18 to evaluate the evolutionary significance of polyploidy in the model genus
19 *Micrasterias*.
20 - *Methods* We applied flow-cytometric techniques of DNA quantification to
21 microalgae and mapped the estimated DNA content along the phylogenetic tree.
22 Correlations between DNA content and cell morphometric parameters were also
23 tested using geometric morphometrics.
24 - *Key Results* In total, DNA content was successfully determined for 34 strains of
25 the genus *Micrasterias*. The estimated absolute 2C nuclear DNA amount ranged
26 from 2.1 to 39.2 pg; intraspecific variation being 15.4 – 21.7 pg in *M. truncata*
27 and 16.0 – 31.1 pg in *M. rotata*. We found strong correlation between the
28 absolute nuclear DNA content and chromosome number in all seven strains for
29 which the chromosome counts were available. The related species were similar
30 in their DNA content, indicating the evolutionary significance of genome size
31 variation. We also found significant positive correlation between the DNA
32 content and cell size of the investigated *Micrasterias rotata* strains.

- *Conclusions* This study confirms the evolutionary significance of genome size variation in the desmid genus *Micrasterias* and highlights resemblance to holocentric higher plant genera. Overall, the results verify the importance of natural polyploidisation in the evolution and speciation of some microalgae.


**Key words**: microalgae, DNA content, flow-cytometry, polyploidy, phylogeny, *Micrasterias*

## Introduction

Streptophycean green algae are a sister group to land plants (McCourt et al., 2004; Becker and Marin, 2009; Wodniok et al., 2011). Desmids are the unicellular representatives of Zygnematophyceae (Streptophyta), and characterized by thousands of morphotypes. They are a logical target group for investigating nuclear DNA content variation (Kapraun, 2005). They possess both asexual and sexual reproduction within their life cycle. In common with other members of the Zygnematophyceae, desmids are haploid in the vegetative stage, only the zygospore being diploid (Brook, 1981).

Cytogenetic research on Desmids is intriguing due to the presence of holocentric (holokinetic) chromosomes (Guerra et al., 2010) which have a kinetochore located along the whole chromosome. In general, this type of chromosome is uncommon though described in some higher plant families (*Cyperaceae* and *Juncaceae*) and in the genera *Drosera*, *Myristica*, *Cuscuta* and *Chionographis* (Rotreklová et al., 2011). It is also described in some algae, arthropods and nematodes, including the model *Ceanorhabditis elegans*. By their nature, holocentric chromosomes have high potential for rapid chromosome rearrangements as chromosome fragments can undergo both normal meiosis and mitosis. For this reason, holocentric organisms are characterised by enormous diversity in both number and size of chromosomes. Chromosome number in desmids ranges from 14 to 592 (King, 1953) and numbers characteristic of an aneuploid series have also been found (King, 1960; Brandham, 1964). These phenomena suggest that symploidy or agmatoploidy prevails over polyploidy in these taxonomic groups.

Polyploidization, i.e. whole genome duplication (WGD), is considered one of a major evolutionary process in higher plants (Wendel, 2000; Soltis et al., 2009). Since early reviews of polyploidy in the late 1930’s, the number of known polyploid events in the evolution of higher plants has been continuously growing along with the development of new research methods (Soltis et al., 2009). Recently, with an advent of new sequence techniques it has become evident that by far the vast majority of flowering plants have experienced (ancient)

polyploidisation in their evolutionary history (Bowers et al., 2003; Paterson et al., 2004; Buggs et al., 2012; Schnable et al., 2012). Polyploid series have their origin in reproductive isolation at different ploidy levels due to unbalanced meiosis. These are often treated as different taxa in higher plants at ranks of varieties to species. Several processes at the genome level, such as the reciprocal loss of duplicated genes (fractionation), gene silencing, chromosome rearrangements and others, might lead to cytotype differentiation at different levels, i.e. morphologically, ecologically. Desmidiales has been characterized by extensive polyploidy, with both inter- and intraspecific variation in chromosome complements reported (Hoshaw and McCourt, 1988). Although there is practically no information about natural populations, changes in the chromosome complements of desmid cells have been artificially induced in cultures. Stable polyploid forms (triploid, tetraploid) of numerous *Micrasterias* species have been produced by Waris and Kallio (1957). Diploid cells were always larger than haploid in all *Micrasterias* species in which these conditions were induced (Brook, 1981).

In higher plants, detection of polyploid taxa within polyploid series became a routine procedure with the development of DNA flow-cytometry (FC) using several different protocols (Doležel et al., 2007; Duchoslav et al., 2010; Sonnleitner et al., 2010; Herben et al., 2012; Kolář et al., 2012). However, the microscopic size and highly variable cell wall composition of microalgae has limited its use for microalgae (Le Gall et al., 1993; Simon et al., 1994; Veldhuis et al., 1997). Thus, study of genome size variation in microalgae lags behind that of higher plants and seaweeds (Kapraun, 2005, 2007). The multi-step protocol proposed by Mazalová et al. (2011) has been found useful for quantification of DNA content in Streptophyta, particularly desmids and a microalgal standard for FC measurement has been suggested.

Recent taxonomic research on microalgae suggests that traditional species/genera boundaries based largely on cell morphology have underestimated the real species diversity (Bickford et al., 2007; Poulíčková et al., 2010). In addition, numerous traditional genera and higher taxa proved to be polyphyletic so that they have to be revised using molecular phylogenetic methods (Denboh et al., 2001; Gontcharov et al., 2003; Gontcharov and Melkonian, 2008). *Micrasterias*, a model genus of desmids (Desmidiales, Streptophyta), includes about 60 morphospecies and its cryptic diversity, phylogeny and biogeography have recently been reported (Neustupa et al., 2010, 2011; Nemjová et al., 2011; Škaloud et al., 2011; Neustupa, 2013). The results of the recent phylogenetic studies indicate monophyletic origin of the genus followed by substantial morphological transformation of individual

1 infrageneric lineages (Gontcharov and Melkonian, 2008, 2011; Hall et al., 2008; Neustupa et
2 al., 2010; Škaloud et al., 2011). According to a multigenic phylogeny of 41 *Micrasterias* taxa,
3 the genus comprises at least eight lineages (Škaloud et al., 2011). Mapping morphological
4 diversification of the genus, on the phylogenetic tree has revealed profound differences in the
5 phylogenetic signal of selected phenotypic features. Whereas the branching pattern of the
6 cells clearly correlates with the phylogeny, the morphological complexity possibly reflects
7 their adaptive morphological response to environmental conditions (Škaloud et al., 2011).
8 Kasprik (1973) recognized four groups within the *Micrasterias* species based on
9 chromosome morphology. The first group have small chromosomes with a tendency to
10 aggregation and includes mostly representatives of clade A (see Škaloud et al., 2011, Fig. 1),
11 with the exception of *M. americana* from clade H. The second group possessing well-
12 separated chromosomes, includes representatives of clade G (Škaloud et al., 2011, Fig. 1),
13 with the exception of *M. rotata* from clade C. The third group with short, thick, relatively
14 compact chromosomes belongs mostly to clades C and D except for *M. muricata* from clade
15 H (Škaloud et al., 2011, Fig. 1). The fourth group, characterized by long, compact
16 chromosomes which appear to be joined together, includes *M. thomasiana*.
17 In this study, we asked whether the phylogeny of the genus *Micrasterias* is associated
18 with DNA content variation. To answer this question, we focussed on: 1) assessment of
19 overall DNA content variation; 2) recognition of the significance of DNA content in the
20 evolution at generic and species levels, and 3) the correlations between DNA content and
21 selected cell morphometric parameters.
22
23 **Material and methods**
24 ***Origin and cultivation of strains***
25 The strains we used were obtained from five public culture collections: Sammlung von
26 Conjugaten-Kulturen, University of Hamburg (SVCK); Culture Collection of Algae, Charles
27 University in Prague (CAUP); Culture Collection of Algae, University of Vienna (ASW),
28 currently deposited in the Culture Collection of Algae at the University of Cologne (CCAC);
29 Culture Collection of Algae, University of Göttingen (SAG); and Microbial Culture
30 collection, National Institute for Environmental Studies, Tsukuba (NIES). Some strains come
31 from the personal collection of Jiří Neustupa (Nemjová et al., 2011) (Table 1). They were
32 grown in 50 mm plastic Petri dishes in a liquid oligotrophic medium used in the CAUP
33 culture collection (OGM; Černá and Neustupa, 2010). Storage cultures were kept at a
34 temperature of 16 ºC, under an illumination of 20 µmol.m$^{-2}$.s$^{-1}$ with 12:12 light:dark cycle

1 (cooling box Helkama C5G). Subsequently, two weeks before planned flow cytometric
2 measurements, a rich inoculum of each strain (ca 1 ml) was transferred to fresh medium in a
3 100 mm Petri dishes and kept at a higher irradiation (40 μmol.m$^{-2}$.s$^{-1}$) with 16:8 light:dark
4 cycle. All cultures were regularly examined under inverted microscope (Zeiss Axiovert), to
5 check their fitness, cell density and estimation of cell division intensity. Well grown cultures
6 were used for cytometric measurements in exponential phase of growth (14-18 days after
7 subculturing), few slow growing cultures were maintained longer to obtain sufficient cell
8 density. Although the cell density was priority, intensively dividing cultures were preferred
9 because their cell walls were more sensitive to enzymatic disintegration (Mazalová et al.,
10 2011). For three repetitions of DNA measurements of each algal strain, we used three Petri
11 dishes of material.
12
13 ***Flow cytometry***
14 Protoplasts were isolated using an enzymatic mixture of 2% Cellulase Onozuka R–10
15 (Duchefa Biochemie, Netherlands), 0.5% Macerozyme R–10 (Duchefa Biochemie,
16 Netherlands) dissolved in modified rinsing solution PGly (Mazalová et al., 2011). The
17 absolute quantity of nuclear DNA of the algae was estimated by FC using a ML CyFlow
18 instrument (Partec GmbH, Münster, Germany). *Raphanus sativus* cv. Saxa (2C = 1.11 pg,
19 Doležel et al., 1992), *Lycopersicon esculentum* cv. Stupicke (2C = 1.96 pg, Doležel et al.,
20 1992), *Zea mays*, CE–777 (2C = 5.47 pg, Doležel et al., 2007) or *Pisum sativum* cv. Ctirad
21 (2C = 8.76 pg, Doležel et al., 1998) were used as standards. Young leaves of standard plants
22 were grown separately from algae. They were not affected by enzyme mixture, but chopped
23 with a razor blade in lysis buffer. Then the algal suspension was added to the chopped
24 standard, filtered, incubated and stained. As a fluorescent dye for DNA staining for FC
25 measurements, propidium iodide was used. The whole multi-step protocol has been published
26 in detail elsewhere (Mazalová et al., 2011).
27 All samples were measured at least twice, usually three times, always 3000 particles were
28 measured. Each of the repetitive measurements of the same clone was independent (desmid
29 suspension originated from different culture vessels), flow-cytometric measurements were
30 done other day after new calibration of cytometer. Thus the possibilities of errors in DNA
31 measurements were minimized. As the majority of cultures represented proliferating cultures
32 with sufficient amount of dividing cells (synchronized by photoperiod), the histogram of
33 relative nuclear DNA content usually shows 2 peaks – first peak represent vegetative haploid
34 cells (1C), second peak represent cells in G2 mitosis phase (2C) (Greilhuber and Doležel,

1 2009; Greilhuber et al., 2005). All clonal cultures used for measurement were strictly
2 vegetative, neither sexual reproduction nor zygotes were observed, thus none of the peak
3 represent generative polyploidy caused by hybridization. Although we cannot exclude
4 autopolyploidization known from literature (Kasprik, 1973).
5
6 ***Phylogenetic analysis, character evolution***
7 The phylogenetic tree used to map the character of the evolution was created using Bayesian
8 inference (BI), MrBayes, version 3.1 (Ronquist and Huelsenbeck, 2003). The analysis was
9 conducted on the concatenated SSU rDNA, psaA, and coxIII alignment published by Škaloud
10 et al. (2011), reduced to 26 taxa for which the cytometric data were obtained. The genetic
11 identity of *M. 'pusilla'* (NIES 783) and *M. radians* var. *evoluta* (SVCK 519) strains with those
12 molecularly characterized by Škaloud et al. (2011) has been described by Neustupa et al.
13 (2010) and Nemjová et al. (2011). Subsequently, eight *M. rotata* and *M. truncata* strains
14 having identical SSU rDNA sequences with those included in the alignment (Nemjová et al.,
15 2011; Neustupa et al., 2011) were added to the dataset. The final alignment included 34 taxa.
16 The existence of a phylogenetic signal in DNA content variation was tested by
17 calculating Pagel's lambda (Pagel, 1999). The maximum likelihood optimization of Lambda
18 value was performed using the 'fitContinuous' function of the Geiger package (Harmon et al.,
19 2008). To test for the existence of phylogenetic signal in the dataset, we compared the
20 negative log likelihoods obtained from a tree without phylogenetic signal ($\lambda = 0$) and the
21 original topology, using likelihood ratio test. The ancestral states of DNA content data were
22 calculated using the Ape and Geiger packages (Paradis et al., 2004; Harmon et al., 2008). The
23 ancestral states were reconstructed by the function "ace", using the maximum likelihood
24 optimization. All calculations were done in the program R, ver. 2.9.2 (The R Foundation for
25 Statistical Computing 2009, http://www.r-project.org/). The output from R was mapped onto
26 the Bayesian phylogenetic tree with TreeExtender v1.03 (Verbruggen, 2009), using a simple
27 list parser (option – p list). Finally, the evolution of DNA content was traced on a Bayesian
28 phylogenetic tree as colours along a gradient with TreeGradients v1.03 (Verbruggen, 2009).
29
30 ***Correlation analyses, geometric morphometrics***
31 Correlation analyses were conducted separately on 1) a dataset of all taxa for which the
32 cytometric data were obtained, and 2) a subset of five *M. rotata* strains. The relationship
33 between DNA content and selected cell morphometric parameters (cell length, cell
34 complexity and number of terminal lobes) was evaluated by linear correlation analyses in

PAST, ver. 2.01 (Hammer et al., 2001). The morphometric parameters tested were obtained from Škaloud et al. (2011). To analyze the correlation between the DNA content and cell size in the *M. rotata* subset precisely, we determined the centroid size (CS) of the cells per each strain by a landmark-based shape analysis. Centroid size, a widely used dimension-free size measure, which is linearly correlated with traditional univariate cell size measures of desmids, such as cell length or width (Neustupa et al., 2008), is defined as the square root of the sum of squared distances from all the analyzed landmarks to their centroid (Zelditch et al., 2004). For each *M. rotata* strain, 46-51 randomly chosen cells were photographed. In total, we defined 40 landmarks spanning the outline shape of the cells (see Neustupa et al., 2008, 2011) in TpsDig, ver. 2.16, and the centroid size values were acquired from the general Procrustes analysis of the entire dataset in TpsRelw, ver. 1.49 (software available at http://life.bio.sunysb.edu/morph/).

## Results

### *Overall DNA content variation*

In total, DNA content was successfully determined for 34 strains of *Micrasterias* species, including the phylogenetically nested species *Triploceras gracile* (Table 2). To analyse both interspecific and intraspecific DNA content variation, several different strains belonging to the species *M. rotata* and *M. truncata* were analysed, as well. The estimated absolute 2C nuclear DNA quantity varied from 2.1 to 39.2 pg. The smallest genomes belonged to morphologically distinct species *M. dickiei*, *M. novae-terrae*, *M. papillifera*, *M. pinnatifida* and *M. swainei*. In contrast, the biggest genomes were detected in *M. ceratofera* and several strains of *M. rotata* (Table 2). Generally, the majority of investigated strains possessed rather small DNA content. In fact, the estimated 2C DNA content was in the lower fifth of the measured values (2 – 10 pg) in more than half of the strains. Interestingly, high variability in DNA content was also detected among different strains belonging to a single species. In *M. truncata*, the estimated absolute 2C nuclear DNA quantity varied from 15.4 to 21.7 pg. The variability in 2C DNA content among the *M. rotata* strains was even bigger (16.0 – 31.1 pg).

To test the correlation between the estimated DNA content values and real number of chromosomes, we compared our measured values with the chromosome data published by Kasprik (1973). For the purpose of this test, we used seven *Micrasterias* SVCK strains used in both studies (Table 2). As can be seen from the regression analysis (Fig. 2), a logarithmic regression fits the data very well ($r = 0.991$, $R^2 = 0.983$, P-value $< 0.001$), suggesting a strong correlation between absolute nuclear DNA content and chromosome number.

***Evolution of DNA content***

To better understand the evolutionary history of DNA content changeover during the diversification of the genus *Micrasterias*, we mapped the estimated DNA content values along the phylogenetic tree. A phylogram was constructed by the Bayesian inference method, based on the concatenated SSU rDNA, psaA, and coxIII alignment published by Škaloud et al. (2011). Since the protoplast isolation and subsequent DNA content determination was not successful in all strains (Mazalová et al., 2011), our phylogram contains only 26 of 36 *Micrasterias* species with known sequence data. The topology of the resulting phylogenetic tree was highly congruent with the phylogram constructed by Škaloud et al. (2011), with strains inferred as members of 6 main clades (A, C, D, E, G, and H).

The existence of a phylogenetic signal in nuclear DNA content was tested using Pagel's lambda calculations. The related species were significantly similar in their DNA content ($\lambda = 0.836$, p-value = 0.002), indicating the evolutionary significance of genome size variation. The existence of a phylogenetic signal was also revealed by the maximum likelihood reconstruction of the ancestral states (Fig. 1). Whereas all species of clade G showed a clear tendency to rather low DNA content, all six *M. rotata* strains forming the clade C tended to have much higher amounts of DNA. On the other hand, clear differences were found in some closely related strains, indicating potential rapid evolutionary dynamics in the genome size. For example, two *Triploceras gracile* strains differed three times in their 2C nuclear DNA content (9.4 and 28.2 pg, respectively). Similarly, *M. crux-melitensis* (7.4 pg) and *M. radians* var. *bogoriensis* (6.7 pg) had 2C DNA content more than five times lower than the closely related *M. ceratofera* (39.2 pg).

***Correlations between DNA content and cell morphometric parameters***

In addition to analysis of the evolutionary significance of the DNA content variation, correlations between the genome size and selected morphometric characteristics were determined. Statistical relationships, described by correlation coefficients between the estimated DNA content and cell length, cell complexity and number of terminal lobes, are presented in Table 2 and illustrated in Fig. 3A-C. The only significant correlation was a positive relationship between the DNA content and average cell length (Fig. 3A). In fact, 27.2 % of the overall variability in cell lengths could be explained by the genome size data.

As mentioned above, significant differences in DNA content were also found between different strains of a single species. To test whether these differences could also affect the cell size, we analyzed the correlation between DNA content and centroid size in five *M. rotata*

strains having identical SSU rDNA sequences. Strong positive correlation was found (Table 3; Fig. 3D) and altogether 82.8 % of cell size variability detected could be explained by the different nuclear DNA contents of analyzed strains.

## Discussion

### *Overall DNA content variation and correlations with chromosome numbers*

Genome size measurement in algae is presently at the beginning and, frequently methodologically more demanding than in embryophytes. This is also reflected in the amount of the Kew Plant DNA C-values database algae entries of only 253, in contrast to the presently over 7500 angiosperm C-value entries (Bennett and Leitch, 2012).

Measurements of DNA content in 34 strains of the genus *Micrasterias*, ranging from 2C = 2.1 to 39.2 pg, are in congruence with previously published data.

For Streptophyta, 2C nuclear DNA content range from 0.2 to 6.4 pg, excluding the highly polyploid Charales and Desmidiales, which have 2C nuclear DNA content of up to 14.8 and 46.8 pg, respectively. In general, nuclear DNA content is smaller in Zygnematales than in Desmidiales (Kapraun, 2007). Algae are a highly diverse organism group and phylogenetically at the basis of land plants. Therefore, a broader knowledge of genome size would be enormously valuable for the evaluation of the role of nuclear DNA amount in evolution. Nuclear DNA content data for Streptophyta superimposed on a contemporary molecular phylogeny indicated that early diverging lineages, including some members of Chlorokybales, Coleochaetales and Klebsormidiales, have genomes as small as 2C = 0.1 – 2.7 pg (Kapraun, 2007; Mazalová et al., 2011). It has been proposed that the Streptophyte ancestral nuclear genome, common to both the charophyte and the embryophyte lineages, can be characterized as 1C = 0.2 pg and 1n = 6. Moreover, the DNA contents of the freshwater charophyceans and zygnemataleans are significantly larger than that of Rhodophyta (2C = 0.2 – 2.8 pg) and Phaeophyta (2C = 0.2 – 1.8 pg) (Kapraun, 2005). Although greater values for DNA content exist in polyploid bryophytes, more than 80% of the nuclear DNA 1C-values in mosses have been reported to occur in a narrow peak between 0.25 and 0.6 pg (Voglmayr, 2000). The size of algal genomes is best appreciated when compared with the minimum amount of DNA estimated in angiosperms. The smallest angiosperm genome is known in the carnivorous plant species *Genlisea margaretae* Hutch. (Lentibulariacea) with 1C = 0.065 pg equalling ca. 63.4 Mbp (Greilhuber et al., 2006). The genome of the genetic model species, *Arabidopsis thaliana* (L.) Heynhold with five chromosomes only, is approximately only 1C = 157 bp large (0.16 pg) (Bennett et al., 2003).

Many of the Zygnemataceae appear to be characterized by polyploid „species complexes" (Hoshaw and McCourt, 1988) and the large cell sizes reported for many Desmidiales, suggest that polyploidy in these uninucleate, unicellular organisms has produced some of the largest nuclear genome sizes known in plants. These suggestions are in congruence with our measurements. Broad variation has been found in different clones of the same species: *Micrasterias rotata* (2C = 16.0 – 31.1pg) almost 2-fold; *M. truncata* (2C = 15.4 – 21.7 pg) 1.4-fold; and *Triploceras gracile* (2C = 9.4 – 28.2 pg) 3-fold variation. Angiosperms with holocentric chromosomes have in general, large genome size variation. Individual species of the genus *Carex* have almost 8-fold variation in their 4C nuclear DNA content (Nishikawa et al., 1984), genus *Luzula* up to 6-fold variation in their 2C nuclear DNA content (Halkka, 1964; Bačič et al., 2007). Likewise, in the genus *Schoenus* up to 14.8-fold variation in 2C DNA content (Kaur et al., 2012) and in *Eleocharis* even up to 22.1-fold (0.25 pg in *E. acicularis* and 5.53 pg in *E. palustris*; Bennett and Leitch, 2010) were reported. In *Luzula*, the DNA content has only low variation among diploid species (from 0.83 to 0.97 pg), but roughly doubled in tetraploids and tripled in hexaploid (Bačič et al., 2007). The low variation of DNA content within a single species was confirmed for *L. nivea*, *L. luzuloides* and *L. multiflora*, although their chromosome numbers vary considerably (Kuta et al., 2004). The hexaploid *L. multiflora* ranged in chromosome number between 2n = 12 – 2n = 84, but the DNA content only varied from 3.125 to 3.271 pg.

Although the strains were maintained as long as 40 years and changes in chromosome numbers should be expected in cultures, our results support the earlier findings within Desmidiales (Kapraun, 2005) that chromosome complements and nuclear DNA contents are highly correlated, providing circumstantial evidence for the pervasive role of polyploidy in the evolution of this group of algae. In contrast, in the marine species of Ulvophyceae there is low correlation, consistent with a high occurrence of aneuploidy, i.e. chromosomal fusion and/or fission events. On the larger scale, genome variation in holocentric genera does not always correlate with chromosome numbers. Although the chromosome number in *Carex* varies greatly, between 6 and 62, genome size evolution exhibits a nearly constant rate (Rotreklová et al., 2011; Chung et al., 2012). Similar dissonance between the chromosome number and DNA content is found in another holocentric organisms, such as *Schoenus* (Kaur et al., 2012) and *Juncus* (Grif, 2000).

The dynamic nature of holocentric chromosomes can be demonstrated by the nuclear DNA variation within a single variety of sedge species *Carex scoparia* var. *scoparia*, in

which the chromosome counts varies from 2n = 62 to 2n = 68 and the 1C DNA content varies from 0.342 pg to 0.361 pg (Chung et al., 2011).

***Significance of DNA content in evolution***

Genome size is an important species-specific characteristic in organisms with centromeric chromosomes. Although limited data are available for microalgae, genome size was extensively studied in past decade in order to describe its changes during the evolution of angiosperms (Greilhuber et al., 2010). The correlation between the C-value and other traits was extensively investigated, not only from the taxonomic point-of-view, but also from a broader biological perspective. In general, the DNA amount serves as a reliable taxonomic indicator which can help to understand the taxonomic problems in angiosperms (Bennett and Leitch, 2011).

Although there are more than 7500 records of angiosperms in the Bennett's & Leitch's Kew Plant DNA C-value Database (Bennett and Leitch, 2012), only a limited number of papers have dealt with the relationship between the phylogeny and DNA content in holocentric angiosperms. To date, the published results indicate variation in DNA content within phylogenetic clades. In *Schoenus*, two out of three clades had generally small genomes (2C < 4 pg), however, in the third clade, small genomes are mixed with large ones (Kaur et al., 2012). In *Schoenus pauciflorus*, there was an increase in 2C DNA content which correlated with the increase in chromosome number. In contrast, *S. maschalinus* and *S. fluitans* differed 2.27-fold in their 2C DNA values, although they had the same chromosome number. Comparison of 45 diploid *Carex* species revealed that the DNA content inversely correlates with chromosome number (Nishikawa et al., 1984). However, Chung et al. (2012) demonstrated that chromosome number in *Carex* evolved independently of genome size when they studied the whole-genus scale. They also showed that only the *Ovales* section shows limited variation in 1C DNA content, whereas other sections across continents show large genome variation. These studies suggest that some clades demonstrate a narrow range of genome size variability, whereas others exhibit great variation. Although, interspecific C-value variation can be found in taxa with monocentric chromosomes too (e.g. Morgan-Richards et al., 2004) it seems to be more typical/frequent in holocentric genera. Indeed, similar results were obtained in *Micrasterias.* For example, clade G consists of strains with a narrow range of genome size variability. However, within other clades, clear differences were found in some closely related strains, indicating the potentially rapid evolutionary dynamics

in the genome size. Even more, great variability in genome size was detected in different strains of a single species (*Micrasterias rotata*, *M. truncata* and *Triploceras gracile*).

***Influence of DNA content on cell size and morphology***

Small genome size in angiosperms appears to be correlated with phenotypic characteristics such as rapid seedling establishment, short minimum generation times, reduced cost of reproduction and increased reproductive rate (Bennett, 1987; Midgley and Bond, 1991; Bennett and Leitch, 2005*a*, *b*). It has been recognized that although nuclear genome size is highly correlated with many cellular and ecological parameters, „correlation“ and „causation“ are far from interchangeable (Gregory, 2005*a*, *b*; Mann and Poulíčková, 2010).

Ploidy level in conjugating green algae may be of taxonomic significance as cell dimensions are considered to be diagnostic (Hoshaw and McCourt, 1988) and highly correlated with genome size (Wang et al., 2005). Diploid cells in *Micrasterias* were found to be usually larger than that of haploids. Waris and Kallio (1957) and Brandham (1965) observed that larger cells of *Closterium, Cosmarium* and *Staurastrum* were polyploid.

Kasprik (1973) reported an interesting case of an aneuploid series correlated with morphological differences in *Micrasterias thomasiana*. The basic chromosome number of this desmid is n=39, but morphologically irregular variants contained n=40, n=46 or even n=70 and 75. Some of the cells of this latter clone were inclined to develop more or less typical morphologies, except that they were significantly larger (Kasprik, 1973). Correlations between the DNA content and cell length, cell complexity and number of terminal lobes were tested in this study. The only significant correlation found was the positive relationship between the DNA content and average cell length. Changes in the degree of radiation may in some desmids, result from an increase in the level of ploidy, as concluded by Starr (1958) in his study of a heterothallic strain of *Cosmarium turpinii*. Starr considered the production of large forms to be a response to increase in nuclear quantity; whereas the change in shape (bi-, tri-, quadriradiate cells) he explained as a response to the increase in cell volume.

We cannot support this hypothesis by our data, as the cell complexity and number of terminal lobes variation were not significantly correlated with DNA content.

**Funding**

This work was supported by the Czech science foundation [grant number 206/09/0906] and the Internal Grant Agency of the Palacký University [grant number Prf-2013-003].

1 **Literature cited**


2 **Bačič T, Jogan N, Koce JD. 2007**. *Luzula* sect. *Luzula* in the south-eastern Alps - karyology
3 and genome size. *Taxon* **56**: 129–136.

4 **Becker B, Marin B. 2009.** Streptophyte algae and the origin of embryophytes. *Annals of*
5 *Botany* **103**: 999–1004.

6 **Bell G. 1994.** The comparative biology of the alternation of generations. *Lectures on*
7 *Mathematics in the Life Sciences* **25**: 1–26.

8 **Bennett MD. 1987.** Variation in genomic form in plants and its ecological implications. *New*
9 *Phytologist* **106** (Supplement): 177–200.

10 **Bennett MD, Leitch IJ. 2005*a*.** Nuclear DNA amounts in angiosperms: progress, problems
11 and prospects. *Annals of Botany* **95**: 45–90.

12 **Bennett MD, Leitch IJ. 2005*b*.** Plant genome size research: a field in focus. *Annals of*
13 *Botany* **95**: 1-6.

14 **Bennett MD, Leitch IJ. 2010.** Plant DNA C-values database (release 5.0). Available at:
15 http://data.kew.org/cvalues/. Accessed 19 December 2012.

16 **Bennett MD, Leitch IJ. 2011.** Nuclear DNA amounts in angiosperms: targets, trends and
17 tomorrow. *Annals of Botany* **107**: 467–590.

18 **Bennett MD, Leitch IJ. 2012.** Plant DNA C-values database (release 6.0, Dec. 2012)
19 http://www.kew.org/cvalues/.

20 **Bennett MD, Leitch IJ, Price HJ, Johnston JS. 2003.** Comparisons with Caenorhabditis
21 (similar to 100 Mb) and Drosophila (similar to 175 Mb) using flow cytometry show genome
22 size in Arabidopsis to be similar to 157 Mb and thus similar to 25 % larger than the
23 Arabidopsis genome initiative estimate of similar to 125 Mb. *Annals of Botany* **91**: 547–557.

24 **Bickford D, Lohman DJ, Sodhi NS et al. 2007.** Cryptic species as a window on diversity
25 and conservation. *Trends in Ecology and Evolution* **22**: 148–155.

26 **Bowers JE, Chapman BA, Rong JK, Paterson AH. 2003**. Unravelling angiosperm genome
27 evolution by phylogenetic analysis of chromosomal duplication events. *Nature* **422**: 433–438.

28 **Brandham PE. 1964.** *Cytology, sexuality and mating type in culture of certain desmids*. PhD
29 Thesis, University of London, United Kingdom.

30 **Brandham PE. 1965.** The effect of temperature on the radial symetry of *Staurastrum*
31 *polymorphum*. *J. Phycol.* **1**: 55–57.

32 **Brandham PE,Godward MBE. 1964.** The production and inheritance of the haploid form in
33 *Cosmarium botrytis*. *Phycologia* **4**: 75–83.

**Brook AJ. 1981.** *The biology of desmids.Botanical monographs,*vol. 16. Berkeley and Los Angeles: University of California Press.

**Buggs RJA, Renny-Byfield S, Chester M et al. 2012**. Next-generation sequencing and genome evolution in allopolyploids. *American Journal of Botany* **99**: 372–382.

**Chung KS, Hipp AL, Roalson EH. 2012**. Chromosome number evolves independently of genome size in a clade with nonlocalized centromeres (*Carex*: Cyperaceae). *Evolution* **66**: 2708–2722.

**Chung KS, Weber JA, Hipp AL. 2011**. Dynamics of chromosome number and genome size variation in a cytogenetically variable sedge (*Carex scoparia* var. *scoparia*, Cyperaceae). *American Journal of Botany* **98**: 122–129.

**Černá K, Neustupa J. 2010.** The pH-related morphological variations of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic. *Aquatic Ecology* **44**: 409–419.

**Denboh T, Hendrayanti D, Ichimura T. 2001.** Monophyly of the genus *Closterium* and the order desmidiales (Charophyceae, Chlorophyta) inferred from nuclear small subunit rDNA data. *Journal of Phycology* **37**: 1063–1072.

**Doležel J, Greilhuber J, Lucretti S, Meister A, Lysák MA, Nardi L, Obermayer R. 1998.** Plant genome size estimation by flow cytometry: inter-laboratory comparison. *Annals of Botany* **82**: 17–26.

**Doležel J, Greilhuber J, Suda J. 2007**. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* **2**: 2233–2244.

**Doležel J, Sgorbati S, Lucretti S. 1992.** Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* **85**: 625–631.

**Duchoslav M, Šafářová L, Krahulec F. 2010.** Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of Botany* **105**: 719–735.

**Gontcharov AA, Marin B, Melkonian M. 2003.** Molecular phylogeny of conjugating green algae (Zygnematophyceae, Streptophyta) inferred from SSU rDNA sequence comparisons. *Journal of Molecular Evolution* **56**: 89–104.

**Gontcharov AA, Melkonian M. 2008.** In search of monophyletic taxa in the family Desmidiaceae (Zygnematophyceae, Viridiplantae): The genus *Cosmarium*. *American Journal of Botany* **95 (9)**: 1079–1095.

**Gontcharov AA, Melkonian M. 2011.** A study of conflict between molecular phylogeny and taxonomy in the Desmidiaceae (Streptophyta, Viridiplantae): Analyses of 291 rbcL sequences. *Protist* **162 (2)**: 253–267.

**Gregory TR. 2005*a*.** The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. *Annals of Botany* **95**: 133–146.

**Gregory TR. 2005*b*.** Synergy between sequence and size in large-scale genomics. *Nature Reviews* **6**: 699–708.

**Greilhuber J, Borsch T, Muller K, Worberg A, Porembski S, Barthlott W. 2006**. Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size. *Plant Biology* **8**: 770–777.

**Greilhuber J, Doležel J. 2009.** 2C or not 2C: a closer look at cell nuclei and their DNA content. *Chromosoma* **118**: 391–400.

**Greilhuber J, Doležel J, Leitch IJ, Loureiro J, Suda J. 2010**. Genome size. *Journal of Botany* **2010**: Article ID 946138, 4 pp. doi: 10.1155/2010/946138.

**Greilhuber J, Doležel J, Lysák MA, Bennett MD. 2005.** The origin, evolution and proposed stabilization of the terms “Genome Size” and “C-Value” to describe nuclear DNA contents. *Annals of Botany* **95**: 255–260.

**Grif VG. 2000**. Some aspects of plant karyology and karyosystematics. *International Review of Cytology - a Survey of Cell Biology* **196**: 131–175.

**Guerra M, Cabral G, Cuacos M et al. 2010**. Neocentrics and holokinetics (Holocentrics): Chromosomes out of the centromeric rules. *Cytogenetic and Genome Research* **129**: 82–96.

**Halkka O. 1964**. A photometric study of *Luzula* problem. *Hereditas-Genetiskt Arkiv* **52**: 81–88.

**Hall JD, Karol KG, McCourt RM, Delwiche CF. 2008.** Phylogeny of the conjugating green algae based on chloroplast and mitochondrial nucleotide sequence data. *Journal of Phycology* **44**: 467–477.

**Hammer Ø, Harper DAT, Ryan PD. 2001.** PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 1–9.

**Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008.** GEIGER: Investigating evolutionary radiations. *Bioinformatics* **24**: 129–131.

**Herben T, Suda J, Klimešová J, Mihulka S, Říha P, Šímová I. 2012**. Ecological effects of cell-level processes: genome size, functional traits and regional abundance of herbaceous plant species. *Annals of Botany* **110**: 1357–1367.

**Hoshaw RW, McCourt RM. 1988.** The Zygnemataceae (Chlorophyta): a twenty-year update of research. *Phycologia* **27**: 511–548.

**Hoshaw RW, McCourt RM, Wang J-C. 1990.** Conjugatophyta. In: Margulis L, Corliss CO, Melkonian M, Chapman DL, eds. *Handbook of Protoctista*. Boston: Barlett & Jones Publishers, 119–131.

**Kapraun DF. 2005.** Nuclear DNA content estimates in multicellular green, red and brown algae: Phylogenetic considerations. *Annals of Botany* **95**: 7–44.

**Kapraun DF. 2007.** Nuclear DNA content estimates in green algal lineages: Chlorophyta and Streptophyta. *Annals of Botany* **99**: 677–701.

**Kasprik W. 1973.** Beiträge zur Karyologie der Desmidiaceen-Gattung *Micrasterias* AG. *Beih. Nova Hedwigia* **42**: 115–137.

**Kaur N, Datson PM, Murray BG. 2012**. Genome size and chromosome number in the New Zealand species of *Schoenus* (Cyperaceae). *Botanical Journal of the Linnean Society* **169**: 555–564.

**King GC. 1953.** Chromosome numbers in the desmids. *Nature* **172**: 592.

**King GC. 1960.** The cytology of the desmids: the chromosomes. *New Phytologist* **59**: 65–72.

**Kolář F, Fér T, Štech M et al. 2012**. Bringing together evolution on serpentine and polyploidy: Spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (*Dipsacaceae*). *Plos One* **7**: e39988. doi:10.1371/journal.pone.0039988.

**Kuta E, Bohanec B, Dubas E, Vižintin L, Przywara L. 2004.** Chromosome and nuclear DNA study on *Luzula* – A genus with holokinetic chromosomes. *Genome* **47**: 246–256.

**Le Gall Y, Brown S, Marie D, Mejjad M, Kloareg B. 1993.** Quantification of nuclear DNA and G-C content in marine macroalgae by flow cytometry of isolated nuclei. *Protoplasma* **173**: 123–132.

**Leitch IJ, Chase MW, Bennett MD. 1998.** Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Annals of Botany* **82** (Supplement A): 85–94.

**Leitch IJ, Soltis DE, Bennett MD. 2005.** Evolution of DNA amounts across land plants (Embryophyta). *Annals of Botany* **95**: 207–217.

**Mable BK, Otto SP. 1998.** The evolution of life cycles with haploid and diploid phases. *BioEssays* **20**: 453–462.

**Mann DG, Poulíčková A. 2010.** Mating system, auxosporulation, species taxonomy and homoploid evolution in *Amphora* (Bacillariophyta). *Phycologia* **49**: 183–201.

**Mazalová P, Šarhanová P, Ondřej V, Poulíčková A. 2011.** Quantification of DNA content in freshwater microalgae using flow cytometry: a modified protocol for selected green microalgae. *Fottea* **11 (2)**: 317–328.

**McCourt RM, Delwiche CHF, Karol KG. 2004.** Charophyte algae and land plant origins. *Trends in Ecology and Evolution* **19 (12)**: 661–666.

**Midgley JJ, Bond WJ. 1991.** Ecological aspects of the rise of angiosperms: a challenge to the reproductive superiority hypotheses. *Biological Journal of the Linnean Society* **44**: 81–92.

**Morgan-Richards M, Trewick SA, Chapman HM, Krahulcová A. 2004.** Interspecific hybridization among Hieracium species in New Zealand: Evidence from flow cytometry. *Heredity* **93 (1)**: 34–42.

**Nemjová K, Neustupa J, Šťastný J, Škaloud P, Veselá J. 2011.** Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*. *Phycological Research* **59**: 208–220.

**Neustupa J. 2013.** Patterns of symmetric and asymmetric morphological variation in unicellular green microalgae of the genus Micrasterias (Desmidiales, Viridiplantae). *Fottea* **13**: 53–63.

**Neustupa J, Škaloud P, Šťastný J. 2010.** The molecular phylogenetic and geometric morphometric evaluation of *Micrasterias crux-melitensis* / *M. radians* species complex. *Journal of Phycology* **46**: 703–714.

**Neustupa J, Šťastný J, Hodač L. 2008.** Temperature-related phenotypic plasticity in the green microalga *Micrasterias rotata*. *Aquatic Microbial Ecology* **51**: 77–86.

**Neustupa J, Šťastný J, Nemjová K, et al. 2011.** A novel, combined approach to assessing species delimitation and biogeography within the well-known desmid species *Micrasterias fimbriata* and *M. rotata* (Desmidiales, Steptophyta). *Hydrobiologia* **667**: 223–239.

**Nishikawa K, Furuta Y, Ishitobi K. 1984**. Chromosomal evolution in genus *Carex* as viewed from nuclear-DNA content, with special reference to its aneuploidy. *Japanese Journal of Genetics* **59**: 465–472.

**Pagel M. 1999.** Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

**Paradis E, Claude J, Strimmer K. 2004.** APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

**Paterson AH, Bowers JE, Chapman BA. 2004**. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 9903–9908.

1 **Poulíčková A, Veselá J, Neustupa J, Škaloud P. 2010.** Pseudocryptic diversity versus
2 cosmopolitanism in diatoms: A case study on *Navicula cryptocephala* Kütz.
3 (Bacillariophyceae) and morphologically similar taxa. *Protist* **161**: 353–369.
4 **Ronquist F, Huelsenbeck JP. 2003.** MrBayes 3: Bayesian phylogenetic inference under
5 mixed models. *Bioinformatics* **19**: 1572–1574.
6 **Rotreklová O, Bureš P, Řepka R et al. 2011**. Chromosome numbers of *Carex*. *Preslia* **83**:
7 25–58.
8 **Schnable JC, Freeling M, Lyons E. 2012**. Genome-wide analysis of syntenic gene deletion
9 in the grasses. *Genome Biology and Evolution* **4**: 265–277.
10 **Simon N, Barlow RG, Marie D, Partensky F, Vaulot D. 1994.** Characterization of oceanic
11 photosynthetic picoeucaryotes by flow cytometry. *Journal of Phycology* **30**: 922–935.
12 **Soltis DE, Albert VA, Leebens-Mack J et al. 2009**. Polyploidy and angiosperm
13 diversification. *American Journal of Botany* **96**: 336–348.
14 **Sonnleitner M, Flatscher R, García PE et al. 2010**. Distribution and habitat segregation on
15 different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio*
16 *carniolicus* (Asteraceae) in the Eastern Alps. *Annals of Botany* **106**: 967–977.
17 **Starr RC. 1958.** The production and inheritance of the triradiate form in *Cosmarium turpinii*.
18 *Amer. J. Bot.* **45**: 243–248.
19 **Škaloud P, Nemjová K, Veselá J, Černá K, Neustupa J. 2011.** A multilocus phylogeny of
20 the desmid genus *Micrasterias* (Streptophyta): Evidence for the accelerated rate of
21 morphological evolution in protists. *Molecular Phylogenetics and Evolution* **61**: 933–943.
22 **Veldhuis MJ, Cucci TL, Sieracki ME. 1997.** Cellular DNA content of marine
23 phytoplankton using two new fluorochromes: taxonomic and ecological implications. *Journal*
24 *of Phycology* **33**: 527–541.
25 **Verbruggen H. 2009.** TreeGradients v 1.02. Available at: http://www.phycoweb.net.
26 Accesed January 2012.
27 **Voglmayr H. 2000.** Nuclear DNA amounts in mosses (Musci). *Annals of Botany* **85**: 531–
28 546.
29 **Wang W, Tanurdzic M, Luo M, et al. 2005.** Construction of a bacterial artificial
30 chromosome library from the spikemoss *Selaginella moellendorffii*: a new resource for plant
31 comparative genomics. *BioMed Central Plant Biology* **5**: 10.
32 **Waris H, Kallio P. 1957.** Morphogenesis effects of chemical agents and micro-cytoplasmic
33 relations in *Micrasterias. Ann. Acad. Sci. Fenn. A* **37**: 1–20.
34 **Wendel JF. 2000**. Genome evolution in polyploids. *Plant Molecular Biology* **42**: 225–249.

1 **Wodniok S, Brinkmann H, Glöckner G, et al. 2011.** Origin of land plants: Do conjugating
2 green algae hold the key? *BMC Evolutionary Biology* **11**: 104.
3 **Zelditch ML, Swiderski DL, Sheets DH, Fink WL. 2004.** *Geometric Morphometrics for*
4 *Biologists: A Primer*. Elsevier Academic Press, London.

**Table 1.** List of the strains used in this study.

| Species | Strain number | Location | GenBank accession numbers | | |
|---|---|---|---|---|---|
| | | | SSU rDNA | psaA | coxIII |
| *Micrasterias americana* | SVCK 290 | Burnham's Swamp at Falmouth, Massachusetts, USA | FR852595 | FR852626 | FR852666 |
| *M. ceratofera* | SAG 21.97 | Sumatra, Indonesia | FR852598 | FR852630 | FR852670 |
| *M. conferta* | SVCK 110 | Moore bei Korvanen, Finland | FR852600 | FR852632 | FR852672 |
| *M. crux-melitensis* | SVCK 128 | A pond in Rheinland, Germany | =AM419206 | = FR852633 | = FR852673 |
| *M. decemdentata* | ASW 07023 | n.a. | FR852602 | FR852635 | FR852675 |
| *M. dickiei* | ASW 07056 | n.a. | FR852623 | FR852661 | FR852701 |
| *M. furcata* | CAUP K609 | Oorid Lough, Connemara, Ireland | FR852605 | FR852639 | FR852679 |
| *M. hardyi* | SVCK 249 | Lake Sorell, Tasmania, Australia | FR852606 | FR852640 | FR852680 |
| *M. jenneri* | SVCK 298 | Mt.Wellington, Tasmania, Australia | FR852607 | FR852641 | FR852681 |
| *M. laticeps* | SVCK 430 | N. Deming Pond, Itasca State Park, Minnesota, USA | FR852608 | FR852642 | FR852682 |
| *M. mahabuleshwarensis* | SVCK 324 | Nacogdoches, Texas, USA | FR852609 | FR852643 | FR852683 |
| *M. muricata* | SAG 157.80 | Texas, USA | FR852610 | FR852644 | FR852684 |
| *M. novae-terrae* | SAG 158.80 | n.a. | FR852611 | FR852645 | FR852685 |
| *M. papillifera* | CAUP K603 | Borkovická Blata, Czech Republic | AM419208 | FR852646 | FR852686 |
| *M. pinnatifida* | SVCK 411 | Laguna de Mucubaj¡Paramo de Mucubaj¡, Merida, Venezuela | FR852612 | FR852647 | FR852687 |
| *M. 'pusilla'* | NIES 783 | Sydney, Centenial Park Australia | = FR852621 | = FR852658 | = FR852698 |
| *M. radians* var. *bogoriensis* | SVCK 389 | Kuching, Borneo, Malaysia | FR852613 | FR852648 | FR852688 |
| *M. radians* var. *evoluta* | SVCK 519 | Lake Ol Bolossat, Kenya | = FR852614 | = FR852649 | = FR852689 |
| *M. radiosa* | SVCK 303 | Lake along the road east of Clifden, Ireland | FR852615 | FR852650 | FR852690 |
| *M. rotata* | SVCK 287 | Burnham's Swamp at Falmouth, Massachusetts, USA | =AM419209 | =FR852651 | =FR852691 |
| *M. rotata* | SVCK 26 | Wildes moor Schwabstedt by Husum, Germany | =AM419209 | =FR852651 | =FR852691 |
| *M. rotata* | SVCK 212 | Timmer Moor near Hamburg, Germany | =AM419209 | =FR852651 | =FR852691 |
| *M. rotata* | SVCK 1 | An unknown locality near Potsdam, Germany | =AM419209 | =FR852651 | =FR852691 |
| *M. rotata* | C8 | A mountain fen near Nové Hamry, Czech Republic | =AM419209 | =FR852651 | =FR852691 |
| *M. rotata* | CAUP K604 | Benthos of flooded quarry pools near Cep village, Czech Republic | AM419209 | FR852651 | FR852691 |

| | | | | | |
|---|---|---|---|---|---|
| *M. semiradiata* | CAUP K606 | Peat bog pool in “Borkovická Blata” Nature Reserve, Czech Republic | AM419211 | FR852659 | FR852699 |
| *M. swainei* | SVCK 138 | Summerfield Pond north of Woods Hole, Massachusetts, USA | FR852616 | FR852652 | FR852692 |
| *M. tetraptera* | SVCK 195 | Hickson's Bog I north of Woods Hole, Massachusetts, USA | FR852617 | FR852653 | FR852693 |
| *M. truncata* | HS2 | Pools by Hostens, Aquitaine, France | =FR852620 | =FR852657 | =FR852697 |
| *M. truncata* | SVCK 51 | Zeller Loch by Fulda, Germany | =FR852620 | =FR852657 | =FR852697 |
| *M. truncata* var. *neodamensis* | SVCK 412 | Laguna de Mucubaj¡Paramo de Mucubaj¡, Merida, Venezuela | FR852620 | FR852657 | FR852697 |
| *M. zeylanica* | SVCK 291 | Victoria or New South Wales (Australia) | FR852599 | FR852631 | FR852671 |
| *Triploceras gracile* | SAG 24.82 | Rotary Pond at Falmouth, Massachusetts, USA | AJ428089 | EF371259 | EF371151 |
| *Triploceras gracile* | SVCK 366 | Sumatra, Indonesia | FR852624 | FR852662 | FR852702 |

Sources of the strains: (ASW) Algensammlung Wien, University of Vienna, nowadays deposit in the Culture Collection of Algae at the University of Cologne (CCAC); (CAUP) Culture Collection of Algae of Charles University in Prague; (NIES) Microbial Culture Collection, National Institute for Environmental Studies, Japan; (SAG) Sammlung von Algenkulturen Göttingen, Germany; (SVCK) Sammlung von Conjugaten-Kulturen (http://www.biologie.uni-hamburg.de/b-online/d44_1/44_1.htm). The strains not held by above mentioned collections are keeping in personal culture collection of Jiří Neustupa, Charles University in Prague (Czech Republic).

= identical access numbers with Nemjová et al. (2011) and Neustupa et al. (2011) - see methods.

**Table 2.** Cytogenetic and morphometric characteristics of the analyzed strains. Species names are given in Table 1.

| Strain number | Chromosome number[1] (n) | 2C DNA amount (pg) AVG ± STD | Standard species | Centroid size | Number of terminal lobes per semicell[2] | Cell complexity[2] | Cell length[2] (µm) |
|---|---|---|---|---|---|---|---|
| SVCK 290 | - | 18.05 ± 0.33 | *Z. m.* | - | 4 | 1.59064 | 140 |
| SAG 21.97 | - | 39.23 ± 0.13 | *P. s.* | - | 2 | 1.448164 | 131 |
| SVCK110 | 39 | 4.12 ± 0.02 | *Z. m.* | - | 8 | 1.623739 | 90 |
| SVCK 128 | ± 100 | 7.39 ± 0.41 | *L. e., Z. m.* | - | 4 | 1.845687 | 107 |
| ASW 07023 | - | 4.11 ± 0.03 | *P. s., Z. m.* | - | 2 | 1.334061 | 50 |
| ASW 07056 | - | 2.05 ± 0.00 | *P. s.* | - | 0 | 0.75505 | 39 |
| CAUP K609 | - | 9.12 ± 0.38 | *L. e., Z. m.* | - | 4 | 2.545707 | 153 |
| SVCK 249 | - | 4.52 ± 0.05 | *P. s.* | - | 2 | 2.143069 | 216 |
| SVCK 298 | - | 8.50 ± 0.15 | *Z. m.* | - | 8 | 1.341453 | 157 |
| SVCK 430 | - | 9.59 ± 0.19 | *L. e., P. s.* | - | 1 | 1.185784 | 150 |
| SVCK 324 | - | 20.43 ± 0.12 | *Z. m.* | - | 2 | 1.802018 | 160 |
| SAG 157.80 | - | 19.19 ± 0.14 | *Z. m.* | - | 2 | 1.58329 | 175 |
| SAG 158.80 | - | 3.26 ± 0.11 | *P. s., Z. m.* | - | 6 | 2.242455 | 124 |
| CAUP K603 | - | 3.12 ± 0.00 | *P. s., Z. m.* | - | 8 | 2.299155 | 130 |
| SVCK 411 | - | 3.40 ± 0.07* | *L. e., P. s., R. s., Z. m.* | - | 1 | 1.220962 | 61 |
| NIES 783 | - | 5.75 ± 0.10* | *Z. m.* | - | 4 | 1.348246 | 60 |
| SVCK 389 | - | 6.69 ± 0.09 | *Z. m.* | - | 6 | 2.355276 | 102 |
| SVCK 519 | - | 4.56 ± 0.20 | *P. s.* | - | 4 | 1.999237 | 107 |
| SVCK 303 | - | 12.13 ± 0.07 | *Z. m.* | - | 14 | 3.135475 | 175 |
| SVCK 287 | - | 16.02 ± 0.06 | *Z. m.* | 3369.2 | 12 | 2.164567 | 260 |
| SVCK 26 | 226 | 22.24 ± 0.00 | *Z. m.* | 3417.4 | 12 | 2.164567 | 260 |
| SVCK 212 | - | 26.35 ± 0.12* | *Z. m.* | - | 12 | 2.164567 | 260 |
| SVCK 1 | 229 | 27.32 ± 0.48 | *Z. m.* | 3763.7 | 12 | 2.164567 | 260 |
| C8 | - | 30.53 ± 0.12 | *Z. m.* | 4341.8 | 12 | 2.164567 | 260 |
| CAUP K604 | - | 31.09 ± 0.00 | *P. s.* | 4421.9 | 12 | 2.164567 | 260 |
| CAUP K606 | - | 14.17 ± 0.34 | *P. s.* | - | 4 | 1.482767 | 96 |
| SVCK 138 | 26 | 3.14 ± 0.14 | *Z. m.* | - | 10 | 3.142072 | 155 |
| SVCK 195 | 48 | 5.42 ± 0.05 | *P. s.* | - | 8 | 2.056462 | 116 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HS2 | - | 15.35 ± 0.02 | *Z. m.* | - | 2 | 1.278057 | 100 |
| SVCK 51 | - | 17.00 ± 0.25 | *Z. m.* | - | 2 | 1.278057 | 100 |
| SVCK 412 | - | 21.69 ± 0.13 | *P. s.* | - | 2 | 1.278057 | 100 |
| SVCK 291 | - | 4.44 ± 0.17 | *P. s.*, *Z. m.* | - | 2 | 1.169259 | 57 |
| SAG 24.82 | - | 9.39 ± 0.07* | *P. s.* | - | 0 | 0.6973971 | 400 |
| SVCK 366 | - | 28.20 ± 0.14* | *P. s.* | - | 0 | 0.6973971 | 400 |

[1] according to Kasprik (1973); [2] according to Škaloud et al. (2011); (AVG) average, (STD) standard deviation

* data already published by Mazalová

Standards: *L. e. = Lycopersicon esculentum, P. s. = Pisum sativum, R. s. = Raphanus sativus, Z. m. = Zea mays*

1 **Table 3.** Correlation between the 2C DNA content and selected cell morphometric
2 parameters. Correlation analyses were performed for the whole dataset as well as for the
3 *Micrasterias rotata* subset (N = 46-51 specimens).

| Trait | r | $R^2$ | p-value |
|---|---|---|---|
| ***Micrasterias* dataset** | | | |
| Cell length | 0.522 | 0.272 | **0.001** |
| Cell complexity | -0.027 | 0.001 | 0.878 |
| Number of terminal lobes | 0.231 | 0.053 | 0.182 |
| ***Micrasterias rotata* dataset** | | | |
| Centroid size | 0.91 | 0.828 | **0.032** |

4

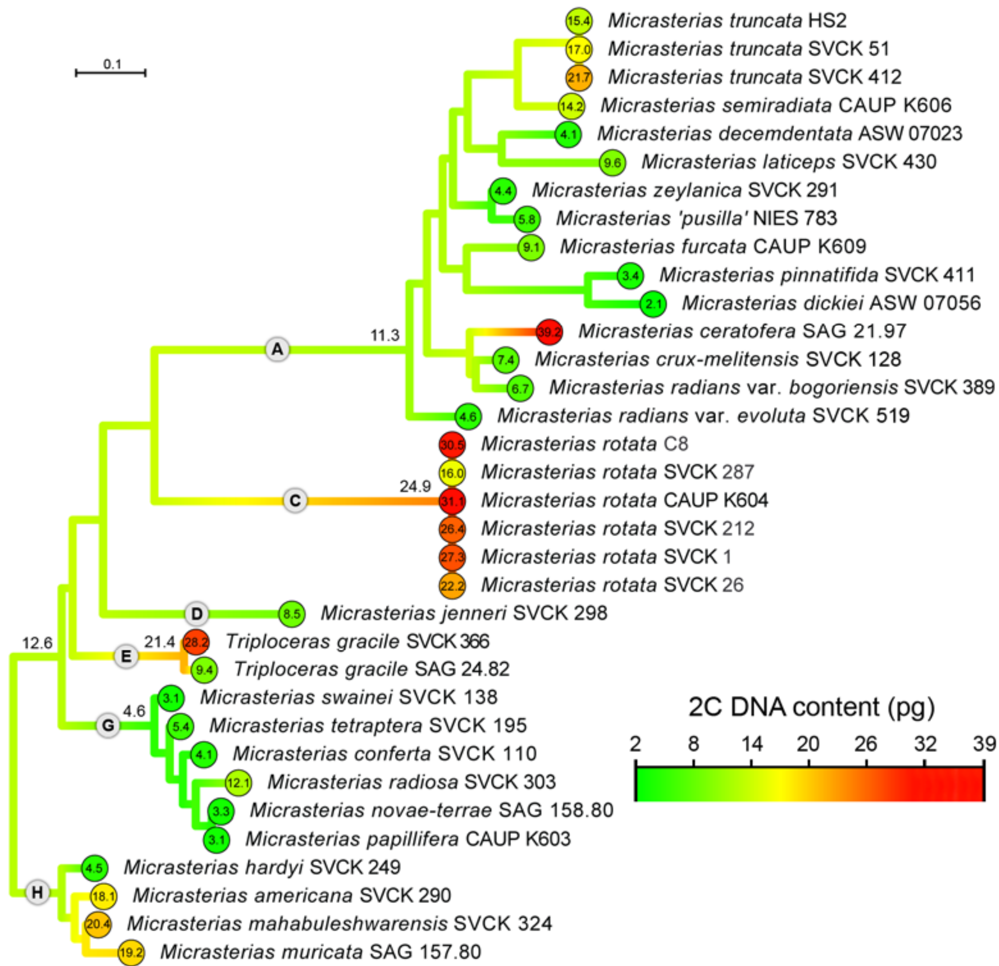
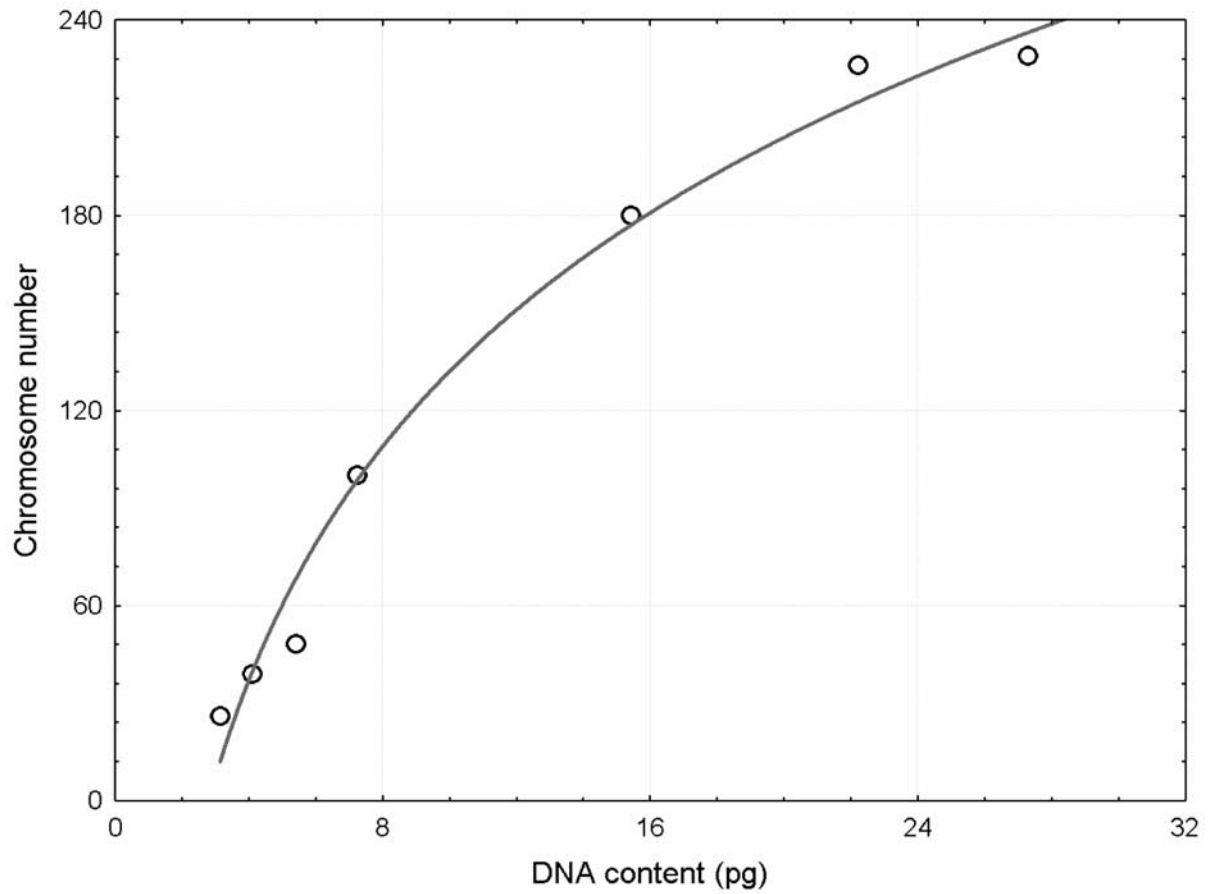


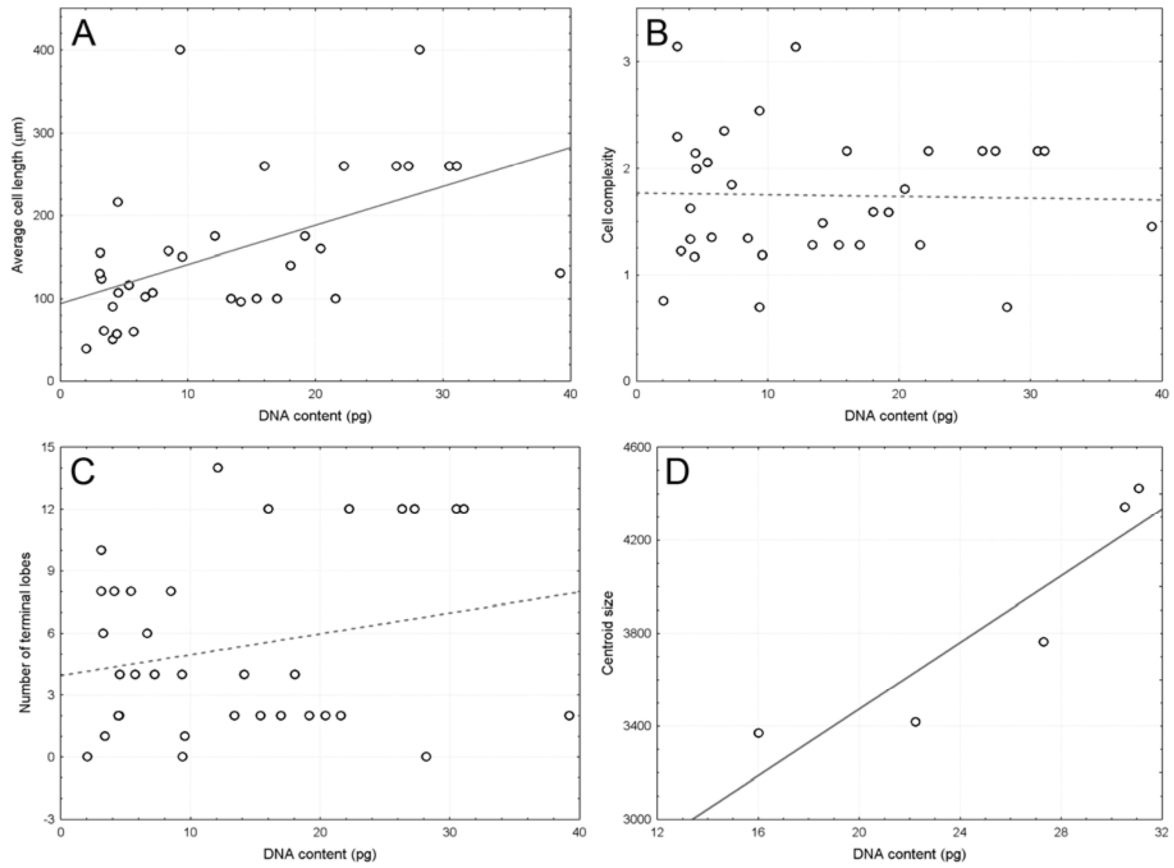

**Fig. 1.** Estimated evolution of DNA content mapped onto the phylogenetic tree of *Micrasterias* (maximum likelihood method). The phylogenetic analysis was conducted on the alignment published by Škaloud et al. (2011). Species affiliation to eight clades (A–H) is indicated. Estimated 2C DNA content is shown at the base of each clade. Scale bar – estimated number of substitutions per site.

1




2 **Fig. 2.** Logarithmic regression of measured DNA content on the chromosome data published

3 by Kasprik (1973) in seven *Micrasterias* strains (r = 0.991, $R^2$ = 0.983, P-value < 0.001).




1

2 **Fig. 3.** Correlation of 2C DNA content and (A) average cell length, (B) cell complexity, (C)
3 number of terminal lobes, (D) centroid size in *Micrasterias* strains. Significant correlations
4 are given by solid lines.

5

## CURRICULUM VITAE - Mgr. Petra MAZALOVÁ

Palacký University, Faculty of Science

Department of Botany

Šlechtitelů 11

Olomouc-Holice

CZ-783 71

e-mail: petra.mazalova@upol.cz

Study and practice:

**since 2009**: PhD. study in botany, Palacký University, Faculty of Science, Olomouc

**1. 6. - 30. 11. 2009** Research position at the Department of Botany, Faculty of Science, Palacký University, Olomouc

**2007- 2009**: Graduate study in biology and geography, Faculty of Science, Palacký University, Olomouc

**2004- 2007**: Undergraduate study in biology and geography, Faculty of Science, Palacký University, Olomouc

Stay abroad:

**1. 9.-30. 11. 2010** Cologne University, Germany, the laboratory of Prof. M. Melkonian

Publications impacted:

- ŠTĚPÁNKOVÁ, J., VAVRUŠKOVÁ, J., HAŠLER, P., **MAZALOVÁ, P.** & POULÍČKOVÁ, A. (2008): Diversity and ecology of desmids of peat bogs in the Jizerské hory Mts. - Biológia 63 (6): 891-896.
- NEUSTUPA, J., ŠŤASTNÝ, J., NEMJOVÁ, K., **MAZALOVÁ, P.**, GOODYER, E., POULÍČKOVÁ, A. & ŠKALOUD, P. (2011): A novel, combined approach to assessing species delimitation and biogeography within the well-known desmid species *Micrasterias fimbriata* and *M. rotata* (Desmidiales, Streptophyta). - Hydrobiologia 667: 223-239.
- **MAZALOVÁ, P.**, ŠARHANOVÁ, P., ONDŘEJ, V. & POULÍČKOVÁ, A. (2011): Quantification of DNA content in freshwater microalgae using flow cytometry: a modified protocol for selected green microalgae. - Fottea 11(2): 317-328.

- ŠKALOUD, P., ŠŤASTNÝ, J., NEMJOVÁ, K., **MAZALOVÁ, P.**, POULÍČKOVÁ, A. & NEUSTUPA, J. (2012): Molecular phylogeny of baculiform desmid taxa (Zygnematophyceae). - Plant Systematics and Evolution 298 (7): 1281-1292.

Popularizational papers:

- DVOŘÁK, P. & **MAZALOVÁ, P.** (2010): Úžasný svět sinic a řas. - Naše příroda (1): 8-13.
- **MAZALOVÁ, P.** (2012): Vývoj jizerskohorských rašelinišť. - Naše příroda (5): 52-54.

Abstracts:

- ŠTĚPÁNKOVÁ, J., VAVRUŠKOVÁ, J., HAŠLER, P., **MAZALOVÁ, P.** & POULÍČKOVÁ, A. (2007): Diversity and ecology of desmids of peat bogs in the Jizerské hory Mts. - In: HINDÁK, F. (ed.): Biology and Taxonomy of Green Algae International Symposium, Programme & Abstracts, 25-29 June 2007, Smolenice, Slovakia, p. 76.
- POULÍČKOVÁ, A., HAŠLER, P., ŠTĚPÁNKOVÁ, J., **MAZALOVÁ, P.** (2010): Overlooked diversity: ombrotrophic mires in Czech borderland. – In: KOMÁREK, J., HAUER, T. & KAŠTOVSKÝ, J. (ed.): Book of abstracts, 18th Symposium of the International Association for Cyanophyte Research, 15-20 August 2010, České Budějovice, Czech Republic, p. 77.
- **MAZALOVÁ, P.**, ŠTĚPÁNKOVÁ, J. & POULÍČKOVÁ, A. (2011): Desmidiological comparison of peat bogs in two mountain regions with different environmental burden. - In: Fifth European Phycological Congress, Rhodes, Greece, 4-9 September 2011, Programme and Book of Abstracts. Taylor & Francis, pp. 170-171.

Reports at conferences:

- ŠTĚPÁNKOVÁ, J., VAVRUŠKOVÁ, J., HAŠLER, P., **MAZALOVÁ, P.** & POULÍČKOVÁ, A. (2007): Diversity and ecology of desmids of peat bogs in the Jizerské hory Mts. - Biology and Taxonomy of Green Algae V, International Symposium, 25-29 June 2007, Smolenice, Slovakia (poster).
- POULÍČKOVÁ, A., HAŠLER, P., ŠTĚPÁNKOVÁ, J. & **MAZALOVÁ, P.** (2010): Overlooked diversity: ombrotrophic mires in Czech borderland. - 18th Symposium of the International Association for Cyanophyte Research, 16-20 August 2010, České Budějovice, Czech Republic (poster).

- **MAZALOVÁ, P.**, ŠTĚPÁNKOVÁ, J. & POULÍČKOVÁ, A. (2011): Desmidiological comparison of peat bogs in two mountain regions with different environmental burden. - Fifth European Phycological Congress, 4-9 September 2011, Rhodes, Greece (poster).
- **MAZALOVÁ, P.**, ŠARHANOVÁ, P., ONDŘEJ, V. & POULÍČKOVÁ, A. (2011): Kvantifikace množství jaderné DNA u vybraných mikrořas pomocí průtokové cytometrie. – 52nd Conference of the Czech Phycological Society 13. 9.-16. 9. 2011, Praha, Czech Republic (talk).
- **MAZALOVÁ, P.**, MELKONIAN, M., GONTCHAROV, A., HAŠLER, P., SAUSEN, N. & POULÍČKOVÁ, A. (2012): Význam morfologických charakteristik v systému desmidií. – 53rd Conference of the Czech Phycological Society 10. 9.-13. 9. 2012, Ostrava, Czech Republic (talk).

**Palacký University Olomouc**

**Faculty of Science**

**Department of Botany**

# Biology and diversity of desmids

Petra Mazalová

**P1527 Biology – Botany**

**Summary of Ph.D. Thesis**

**Olomouc 2013**

**Palacký University Olomouc**

**Faculty of Science**

**Department of Botany**

# Biology and diversity of desmids

Petra Mazalová

**P1527 Biology – Botany**

**Summary of Ph.D. Thesis**

**Olomouc 2013**

This Ph.D. thesis was carried out within the framework of internal Ph.D. studies at the Department of Botany, Faculty of Science, Palacký University Olomouc during the years 2009-2013.

Aspirant: **Mgr. Petra Mazalová**
Department of Botany, Faculty of Science, Palacký University Olomouc, Czech Republic

Supervisor: **Prof. RNDr. Aloisie Poulíčková, CSc.**
Department of Botany, Faculty of Science, Palacký University Olomouc, Czech Republic

Opponents: **Prof. RNDr. František Hindák, PhD. DSc.**
Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia

**Univ. Prof. Mag. Dr. Michael Schagerl**
Department of Limnology, Faculty of Life Sciences, University of Vienna, Austria

**Doc. RNDr. Yvonne Němcová, Ph.D.**
Department of Botany, Faculty of Science, Charles University in Prague

The review of the Ph.D. thesis has been written by Department of Botany, Faculty of Science, Palacký University Olomouc.

The summary of Ph.D. thesis was sent out on...............................

The oral defence will take place in front of the Commission for Ph.D. Theses for Study subject Botany in the conference room at the Department of Botany, Faculty of Science, Palacký University Olomouc, Olomouc Holice, Šlechtitelů 11 on.............................................

The Ph.D. thesis is available in the Library of Biological Department of Faculty of Science at the Palacký University Olomouc, Olomouc Holice, Šlechtitelů 11.

**Prof. Ing. Aleš Lebeda, DrSc.**
Chairman of the Commission for Ph.D.
Theses for Study Subject Botany
Faculty of Science, Palacký University Olomouc

## Contents

1. Introduction ... - 4 -
2. Aims ... - 7 -
3. Results ... - 8 -
3. 1 Desmid flora of mires in Central and Northern Moravia ... - 8 -
3. 2 Delimitation of *Micrasterias fimbriata* and *M. rotata* ... - 8 -
3. 3 Quantification of DNA content in microalgae using flow cytometry ... - 8 -
3. 4 DNA content in evolution of the genus *Micrasterias* ... - 9 -
4. Conclusions ... - 10 -
5. References ... - 12 -
6. List of author's papers ... - 15 -
7. Presentations at meetings ... - 16 -
8. Souhrn (Summary, in Czech) ... - 17 -

# 1 Introduction

Desmids (Desmidiales) represent a group of green microalgae. Together with the order Zygnematales, they belong to the class Zygnematophyceae, phylum Charophyta, infrakingdom Streptophyta, kingdom Plantae (Lewis & McCourt 2004; Kalina & Váňa 2005).

They have always been classified as members of kingdom Plantae principally because of their pigments and starch as a reserve product (Brook 1981). They comprise coccoid and colonial forms that are together with Zygnematales distinctive by their unique sexual reproduction (conjugation) and absence of flagella during their life cycle (Coesel & Meesters 2007; Gontcharov 2008; John & Williamson 2009). Desmidiales are peerless in morphology and inner-cell organization (Růžička 1977; Hindák 1978). The main component of their cell wall is cellulose, as in higher plants (Kalina & Váňa 2005). Essentially, desmids are haploid organisms. The only diploid phase in their life cycle is the zygote (zygospore if mature) (Kalina & Váňa 2005).

Desmids exhibit great diversity in size. The length of the longest axis varied according to West & West (1904) from 8 to 1200 µm, according to Růžička (1977) from 10 to 1700 µm, Kouwets (2008) recorded a range from 10 to 1000 µm. The shape of desmid vegetative cells in front view varies from spherical to cylindrical, elliptic, discoidal, fusiform or baculiform (Růžička 1977; Lenzenweger 1996; Coesel & Meesters 2007). A really noticeable feature which distinguishes them from other algae is their symmetry (Coesel & Meesters 2007). Longitudinal symmetry is distinct particularly in ”true desmids” (Desmidiales s. s.) whose cells consists of two almost symmetrical halves, semicells, divided by a constriction called sinus (West & West 1904; Gontcharov 2008; John & Williamson 2009). The part which connects the semicells together is called the isthmus (West & West 1904). Longitudinal symmetry is also however present in other families of desmids (Desmidiales s. l. = true desmids + Mesotaeniaceae) but expressed there only in the internal arrangement of organelles (Růžička 1977; Kalina & Váňa 2005). Moreover, desmid cells from apical view show a radiate symmetry resembling ellipse (biradiate), triangle (triradiate), polygon (pluriradiate) or circle (omniradiate) (Coesel & Meesters 2007).

A conspicuous feature typical for some genera is the cell wall ornamentation. Parts of cell walls can be formed into rides, granules, tubercles or spines of various sizes and shapes. The cell wall can be provided with different large pits which usually join cell wall pores (Brook 1981; Coesel & Meesters 2007). Some granules visible under light microscope on the cell walls can originate from mucilage secreted by pores (Coesel & Meesters 2007).

In desmid cells, there is one nucleus located in the centre of isthmus (Kalina & Váňa 2005). The most visible organelles are chloroplasts. They are bright green and the content is predominantly chlorophyll a + b. Their shapes can vary from straight and rod-like to ridged, spirally twisted or may exhibit a radiating structure (West & West 1904). Usually there are two chloroplasts in each cell with two or more pyrenoids (Hindák 1978; Kalina & Váňa 2005; Coesel & Meesters 2007). The chloroplasts can be axial (situated in the centre of the cell) or parietal (situated round the periphery of the cell). Axial chloroplasts are more common, parietal are rather rare (Brook 1981).

Desmids almost exclusively occur in freshwater habitats. However few species are also found in brackish waters. In general, the highest diversity of desmid flora is in mesotrophic to oligo-mesotrophic, slightly acidic clear waters. They occur mostly as metaphyton in shallow water bodies such as moorland pools or bogs with the occurrence of submerged macrophytes (e.g. *Utricularia*, *Potamogeton*, *Scorpidium*, *Myriophyllum*) and in acidic bogs with *Sphagnum* (West & West 1904; Coesel 1975; Brook 1981; Kalina & Váňa 2005; Coesel & Meesters 2007; Kouwets 2008). Desmids have highly specific ecological demands and are sensitive to environmental changes, thus they are important indicators of wetland ecosystems (Coesel 1998; Hašler et al. 2008; Šťastný 2009, 2010).

Classification schemes of desmids and the whole class Zygnematophyceae underwent many changes in history. It was at first based on morphology with the stress on the cell wall features, cellular organization and chloroplast structure (Gontcharov 2008; Gontcharov & Melkonian 2010). These days, molecular data are widely used, and phylogenetic analyses of desmids led to the conclusion that many traditional genera and also some species are polyphyletic (Gontcharov 2008; Gontcharov & Melkonian 2005, 2008, 2010, 2011; Hall et al. 2008). For this reason, revision of the traditional desmid genera and family concept is needed.

Re-evaluation is however needed not only on a genera or family level. More frequent use of molecular methods showed that cryptic and pseudocryptic species are a more commonly encountered phenomenon than previously thought (Evans et al. 2007). Cryptic/pseudocryptic diversity has been illustrated in complexes *Micrasterias crux-melitensis*, *M. radians* (Neustupa et al. 2010), *M. truncata* (Nemjová et al. 2011). Combination of classic morphology and ecology together with molecular data and other techniques (e.g. cytology, DNA content – ploidy level) is known as the polyphasic/complex approach to diversity studies. This approach in general should lead to better understanding of diversity, dispersal and distribution of microalgae.

Various aspects (morphogenesis, plasticity, physiology or ultrastructure) have been studied on the genus *Micrasterias* C. Agardh ex Ralfs during last decades (Meindl 1993;

Weiss et al. 1999; Neustupa & Šťastný 2006; Neustupa & Škaloud 2007; Neustupa et al. 2008, 2010; Nemjová et al. 2011; Škaloud et al. 2011). The genus is widely distributed and some species are considered cosmopolitan (Guiry & Guiry 2013). According to the study of Škaloud et al. (2011) the genus is more likely monophyletic and includes species traditionally classified in different genera: *Cosmarium ralfsii*, *Triploceras gracile* and *Staurodesmus dickiei*. While certain morphological features correlate with the *Micrasterias* phylogeny, there are some characteristics which evolved independently. For example Černá & Neustupa (2010) found that the cell complexity reflects environmental factors (e.g. pH) and this does not correlate with phylogenetic data.

# 2 Aims of the thesis

The principal goal of this thesis was to investigate desmid diversity using the polyphasic approach. The most important prerequisites include knowledge of desmid taxonomy, selection of localities with the occurrence of interesting species complexes and optimization of non-traditional methods potentially suitable for interpretation of desmid phylogeny. The genus *Micrasterias* was selected as a model genus.

In particular, the four principal aims of the thesis can be summarized as follows:

1) to create a current overview of desmid diversity from desmids inhabiting the mires of central and northern Moravia, which can help us to complete recent floristic studies of the Czech Republic and select model species complex and model localities
2) to revise a delimitation of the species *Micrasterias fimbriata* and *M. rotata* using the combination of different methods (polyphasic approach)
3) to develop a protocol for quantification of the nuclear DNA by flow cytometry which is suitable primarily for desmids
4) to test the evolutionary significance of polyploidy in the model genus *Micrasterias* (otherwise to test whether the phylogeny of the genus is associated with the DNA content variation).

# 3 Results

## 3. 1 Desmid flora of mires in Central and Northern Moravia

During the years 2008-2012, samples from nine Moravian wetland localities were collected. The localities ranged from diverse bogs, fens, and pools to ephemeral ditches. Altogether, 109 taxa belonging to 14 genera were found, 42 of them newly described in Moravia, and five of them new for the Czech Republic. The pH values of the studied sites ranged from 4.2 to 8.7, conductivity ranged from 22 to 360 $\mu S.cm^{-1}$ and trophic level ranged from oligo-mesotrophic to slightly eutrophic. The distributional pattern of species corresponded with the main ecological gradients and species richness per locality ranged from 5 to 50 taxa. A unique locality near the village Slavkov, considered to be a possible Holocene relic (based on higher plants floristic data) was revealed during this study.

## 3. 2 Delimitation of *Micrasterias fimbriata* and *M. rotata*

Set of clonal strains and natural samples mostly from Europe, and published records from Europe and North America of species *Micrasterias fimbriata* and *M. rotata* were investigated. Phylogenetic analysis of $trnG^{ucc}$ constructed using unrooted Bayesian inference confirmed the paraphyletic origin of *M. fimbriata*. Its strains formed two independent lineages, creating a clade together with *M. brachyptera*. They are also visibly different morphologically: both lineages differ in the shape of terminal lobules, in the depth of the incisions between polar lobe and lateral lobes, in length and shape of marginal spines of the polar lobes and in the presence of surface spine layers (based on microscopic observations and particularly on geometric morphometric analysis). Both lineages differ also in geographical distribution and seem to differ in ecological preferences.

Members of the species *M. rotata* formed an independent lineage in the phylogenetic tree. They were also homogenous morphologically.

## 3. 3 Quantification of DNA content in microalgae using flow cytometry

The protoplasts were obtained by modifying the enzyme mixture of cellulase and macerozyme. As a nuclear isolation buffer, the LB01 lysis buffer (Doležel et al. 1989) was used and nuclear DNA was stained by propidium iodide (PI). As standards, young leaves of different plant species were used.

Our protocol, mainly based on studies with higher plants and commercially available enzyme mixtures, proved to be useful for members of Zygnematophyceae, particularly desmids.

Moreover we succeeded in some members of Chlorophyta (*Chloroidium ellipsoideum*, *Tetraselmis subcordiformis*) and Heterokontophyta (*Tribonema vulgare*). We had no success with microalgae with pellicle (*Euglena*), chlamys (*Chlamydomonas*), silica frustule (*Pinnularia*), alganan in the cell walls (*Chlorella*) or surrounded by a thick mucilaginous envelope (*Planktospheria*). The protocol also failed in some other microalgae (*Kentrosphaera*, *Pseudendoclonium*, *Stigeoclonium*, *Trentepohlia* and *Xanthidium*). New possible standard for flow cytometry from the ranks of microalgae – *Micrasterias pinnatifida*, was characterized.

### 3. 4 DNA content in evolution of the genus *Micrasterias*

The absolute nuclear DNA level of 34 *Micrasterias* strains (25 *Micrasterias* species) ranged from 2C = 2.1 to 39.2 pg; intraspecific variation being 16.0 – 31.1 pg in *M. rotata*, 15.4 – 21.7 pg in *M. truncata*, and 9.4 – 28.2 pg in *Triploceras gracile* (which genetically belong to the genus *Micrasterias*; Škaloud et al. 2011). When the results were applied on the phylogenetic tree, created using Bayesian inference (BI) and based on concatenated SSU rDNA, psaA, and coxIII alignments, most of the related species were significantly similar in their DNA content. However some closely related strains differing greatly in their DNA content were also found. When the correlation between estimated DNA content values and the real number of chromosomes (previously published by Kasprik [1973]) were tested, a strong correlation was found for all seven strains. In addition, geometric morphometrics was used to test the correlations between DNA content and cell morphometric parameters. A significant correlation was found between the nuclear DNA content and average cell length in the genus *Micrasterias* and cell size in the investigated species *M. rotata*.

# 4 Conclusions

In the presented thesis, I have investigated the diversity of desmids using a polyphasic approach. Floristic data obtained from central and northern Moravia help to complete the knowledge on the desmid flora of the Czech Republic. Next, I applied flow cytometry to microalgae, particularly Streptophyta with the aim of using this method for testing of the nuclear DNA significance in desmid phylogeny. Further, I participated in the polyphasic characterization of two well-known *Micrasterias* species and applied DNA content measurements to interpretation of the phylogeny in the model genus *Micrasterias*.

1. The relatively large number of new desmid taxa for Moravia was found. It illustrates the low exploration of Moravian region. It was confirmed the importance of floristic and ecological research, broadened our knowledge of desmid diversity in the Czech Republic and revealed new sources for future taxonomic studies.

2. Two different lineages of *Micrasterias fimbriata* have been separated based on molecular data, different morphology, geographical distribution and probable different ecology. It has been suggested to describe one lineage as a new species of the genus *Micrasterias*.

*Micrasterias rotata* proved to be phylogenetically homogenous. They were also homogenous morphologically. Unfortunately, except for one strain we had strains of it only from Europe. So we can conclude that at least in Europe, populations of *M. rotata* represent a homogenous phylogenetic species lineage. It was also shown that polyphasic approach may be advantageous in taxonomy of algae.

3. Our protocol proved to be useful for members of Zygnematophyceae, particularly desmids. Moreover we succeeded in some members of Chlorophyta and Heterokontophyta. The protocol failed in some microalgae, mainly characterized by cell wall organization and components which contrasts with the organization and components of it in higher plants. Modification of the enzyme mixture (not modified for cell walls similar to higher plants) or another nuclear isolation buffer could be helpful for these microalgae.

4. Our results confirms the evolutionary significance of genome size variation in the desmid genus *Micrasterias* and highlights the resemblance to holocentric (holokinetic) higher plant genera (desmids have holocentric chromosomes; King 1960; Godward 1966; Guerra et al. 2010). The results confirm the importance of natural polyploidisation in the evolution and speciation of some microalgae.

# 5 References

BROOK, A. J. (1981): The biology of desmids. University of California Press, Berkeley and Los Angeles.

ČERNÁ, K. & NEUSTUPA, J. (2010): The pH-related morphological variations of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic. – Aquatic Ecology 44: 409-419.

COESEL, P. F. M. (1975): The relevance of desmids in the biological typology and evaluation of fresh waters. – Hydrobiological Bulletin 9: 93–101.

COESEL, P. F. M. (1998): Sieralgen en Natuurwaarden. Stichting Uitgeverij K. N. N. V., Utrecht.

COESEL, P. F. M. & MEESTERS K. J. (2007): Desmids of the lowlands. Mesotaeniaceae and Desmidiaceae of the European lowlands. KNNV Publishing, Zeist.

EVANS, K. M., WORTLEY, A. H., MANN, D. G. (2007): An assessment of potential diatom “barcode” genes (*cox*1, *rbc*L, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). – Protist 158 (3): 349-364.

DOLEŽEL, J., BINAROVÁ, P., LUCRETTI, S. (1989): Analysis of nuclear DNA content in plant cells ba flow cytometry. – Biologia Plantarum 31: 113-120.

GODWARD, M. B. E. (1966): The chromosomes of the algae. Edward Arnold, London.

GONTCHAROV, A. (2008): Phylogeny and classification of Zygnematophyceae (Streptophyta): current state of affairs. – Fottea 8 (2): 87-104.

GONTCHAROV, A. & MELKONIAN, M. (2005): Molecular phylogeny of *Staurastrum* Meyen ex Ralfs and related genera (Zygnematophyceae, Streptophyta) based on coding and noncoding rDNA sequence comparisons. – Journal of Phycology 41: 887-899.

GONTCHAROV, A. & MELKONIAN, M. (2008): In search of monophyletic taxa in the family Desmidiaceae (Zygnematophyceae, Viridiplantae): The genus *Cosmarium*. – American Journal of Botany 95 (9): 1079-1095.

GONTCHAROV, A. & MELKONIAN, M. (2010): Molecular phylogeny and revision of the genus *Netrium* (Zygnematophyceae, Streptophyta): *Nucleotaenium* gen. nov. – Journal of Phycology 46: 346-362.

GONTCHAROV, A. & MELKONIAN, M. (2011): A study of conflict between molecular phylogeny and taxonomy in the Desmidiaceae (Streptophyta, Viridiplantae): Analyses of 291 rbcL sequences. – Protist 162: 253-267.

GUERRA, M., CABRAL, G., CUACOS, M. et al. (2010): Neocentrics and holokinetics (Holocentrics): Chromosomes out of the centromeric rules. – Cytogenetic and genome Research 129: 82-96.

GUIRY, M. D. & GUIRY, G. M. (2013): *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 22 March 2013.

HALL, J. D., KAROL, K. G., MCCOURT, R. M., DELWICHE, CH. F. (2008): Phylogeny of the conjugating green algae based on chloroplast and mitochondrial nucleotide sequence data. – Journal of Phycology 44: 467-477.

HAŠLER, P., ŠTĚPÁNKOVÁ, J., ŠPAČKOVÁ, J. et al. (2008): Epipelic cyanobacteria and algae: a case study from Czech ponds. – Fottea 8 (2): 133–146.

HINDÁK, F. (ed.) (1978): Sladkovodné riasy. – SPN, Bratislava.

JOHN, D. M. & WILLIAMSON, D. B. (2009): A practical guide to the Desmids of the West of Ireland. – Martin Ryan Institute, National University of Ireland, Galway.

KALINA, T. & VÁŇA, J. (2005): Sinice, řasy, houby, mechorosty a podobné organismy v současné biologii. Karolinum, Praha.

KASPRIK, W. (1973): Beiträge zur Karyologie der Desmidiaceen – Gattung *Micrasterias*. Beih – Nova Hedwigia 42: 115-137.

KING, G. C. (1960): The cytology of desmids: the chromosomes. – New Phytologist 59: 65-72.

KOUWETS, F. A. C. (2008): The species concept in desmids: the problem of variability, infraspecific taxa and the monothetic species definition. – Biologia 63: 881-887.

LENZENWEGER, R. (1996): Desmidiaceenflora von Österreich, Teil 1. J. Crammer, Stuttgart.

LEWIS, L. A. & MCCOURT, R. M. (2004): Green algae and the origin of land plants. – Am. J. Bot. 91: 1535-1556.

MEINDL, U. (1993): Micrasterias cells as a model system for research on morphogenesis. – Microbiological Reviews 57: 415-433.

NEMJOVÁ, K., NEUSTUPA, J., ŠŤASTNÝ, J., ŠKALOUD, P. & VESELÁ, J. (2011): Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*. – Phycological Research 59: 208-220.

NEUSTUPA, J. & ŠKALOUD, P. (2007): Geometric morphometrics and qualitative patterns in the morphological variation of five species of *Micrasterias* (Zygnematophyceae, Viridiplantae). – Preslia 79: 401-417.

NEUSTUPA, J., ŠKALOUD, P. & ŠŤASTNÝ, J. (2010): The molecular phylogenetic and geometric morphometric evaluation of *Micrasterias crux-melitensis/M. radians* species complex. – Journal of Phycology 46: 703-714.

NEUSTUPA, J., ŠŤASTNÝ, J. (2006): The geometric morphometric study of Central European species of the genus Micrasterias (Zygnematophyceae, Viridiplantae). – Preslia 78 (2): 253-263.

NEUSTUPA, J., ŠŤASTNÝ, J., HODAČ, L. (2008): Temperature-related phenotypic plasticity in the green microalga *Micrasterias rotata*. – Aquatic Microbial Ecology 51 (1): 77-86.

PICKETT-HEAPS, J. D. (1979): Electron microscopy and the phylogeny of green algae and land plants. – Integrative and Comparative Biology 19 (2): 545-554.

RŮŽIČKA, J. (1977): Die Desmidiaceen Mitteleuropas, Band 1, 2. Lieferung. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

ŠKALOUD, P., NEMJOVÁ, K., VESELÁ, J., ČERNÁ, K., NEUSTUPA, J. (2011): A multilocus phylogeny of the desmid genus *Micrasterias* (Streptophyta): Evidence for the accelerated rate of morphological evolution in protists. – Molecular Phylogenetics and Evolution 61: 933-943.

ŠŤASTNÝ, J. (2009): The desmids of the Swamp Nature Reserve (North Bohemia, Czech Republic) and a small neighbouring bog: species composition and ecological condition of both sites. – Fottea 9 (1): 135–148.

ŠŤASTNÝ, J. (2010): Desmids (Conjugatophyceae, Viridiplantae) from the Czech Republic, new and rare taxa, distribution, ecology. – Fottea 10 (1): 1-74.

WEISS, D., LÜTZ, C., LÜTZ-MEINDL, U. (1999): Photosynthesis and heat response of the green alga Micrasterias denticulata (Desmidiaceae). Zeitschrift für Naturforschung 54c: 508-516.

WEST, W. & WEST, G. S. (1904): A monograph of the British Desmidiaceae. Volume I. The Ray Society, London.

# 6 List of author's papers

(* papers included in the thesis)

**Scientific papers:**

- Štěpánková, J., Vavrušková, J., Hašler, P., **Mazalová, P.** & Poulíčková, A. (2008): Diversity and ecology of desmids of peat bogs in the Jizerské hory Mts. – Biológia 63 (6): 891-896.
- *Neustupa, J., Šťastný, J., Nemjová, K., **Mazalová, P.**, Goodyer, E., Poulíčková, A. & Škaloud, P. (2011): A novel, combined approach to assessing species delimitation and biogeography within the well-known desmid species *Micrasterias fimbriata* and *M. rotata* (Desmidiales, Streptophyta). – Hydrobiologia 667: 223-239.
- ***Mazalová, P.**, Šarhanová, P., Ondřej, V. & Poulíčková, A. (2011): Quantification of DNA content in freshwater microalgae using flow cytometry: a modified protocol for selected green microalgae. – Fottea 11(2): 317-328.
- Škaloud, P., Šťastný, J., Nemjová, K., **Mazalová, P.**, Poulíčková, A. & Neustupa, J. (2012): Molecular phylogeny of baculiform desmid taxa (Zygnematophyceae). – Plant Systematics and Evolution 298 (7): 1281-1292.
- ***Mazalová, P.**, Štěpánková, J. & Poulíčková, A. (2013): Desmid flora in/of mires of Central and Northern Moravia (Czech Republic). – Časopis Slezského zemského muzea, A – vědy přírodní (in press).
- Poulíčková, A., Dvořák, P., **Mazalová, P**. & Hašler, P. (2013): Epipelic microphototrophs – an overlooked assemblage of lake ecosystems. – Freshwater Science (submitted).
- *Poulíčková, A., **Mazalová, P.**, Vašut, R. J., Šarhanová, P., Neustupa, J. & Škaloud, P. (2013): DNA content variation and its significance in evolution of the genus *Micrasterias* (Desmidiales, Streptophyta) (in prep.).

**Popular papers:**

- Dvořák, P. & **Mazalová, P.** (2010): Úžasný svět sinic a řas. Naše příroda (1): 8-13.
- **Mazalová, P.** (2012): Vývoj jizerskohorských rašelinišť. Naše příroda (5): 52-54.

# 7 Presentations at meetings

- Mazalová, P., Štěpánková, J. & Poulíčková, A. (2011): Desmidiological comparison of peat bogs in two mountain regions with different environmental burden. - Fifth European Phycological Congress, 4 - 9 September 2011, Rhodes, Greece (poster).
- Mazalová, P., Šarhanová, P., Ondřej, V. & Poulíčková, A. (2011): Kvantifikace množství jaderné DNA u vybraných mikrořas pomocí průtokové cytometrie. - 52$^{nd}$ Conference of the Czech Phycological Society 13. 9. - 16. 9. 2011, Praha, Czech Republic (talk).
- Mazalová, P., Melkonian, M., Gontcharov, A., Hašler, P., Sausen, N. & Poulíčková, A. (2012): Význam morfologických charakteristik v systému desmidií. - 53$^{rd}$ Conference of the Czech Phycological Society 10. 9. - 13. 9. 2012, Ostrava, Czech Republic (talk).

# 8 Souhrn (Summary, in Czech)


Vznik a vývoj vyšších rostlin úzce souvisí s řasami, vyšší rostliny s řasami doposud sdílejí mnoho společných znaků. Nicméně studie biologie a diverzity řas pokulhávají za rozsáhlostí studií vyšších rostlin. V této disertační práci byly zkoumány fylogenetická, morfologická a geografická diverzita krásivek a rozdíly v množství jejich jaderné DNA, za účelem lepšího pochopení významu polyploidie v jejich evoluci. Navíc zde navrhuji protokol pro měření množství jaderné DNA u krásivek a několika dalších mikrořas, a to pomocí průtokové cytometrie.

Během studia devíti mokřadních lokalit střední a severní Moravy bylo nalezeno celkem 109 taxonů krásivek. Pět z těchto taxonů je nových pro území ČR a 42 pro území Moravy. Byly diskutovány předchozí nálezy všech taxonů na území Moravy či celé České republiky. Zároveň byl zvážen charakter a původ unikátní lokality nacházející se poblíž vesnice Slavkov. Získaná data naznačila nízkou prozkoumanost krásivek na území Moravy.

Dva dobře známé druhy krásivek, *Micrasterias rotata* a *M. fimbriata*, byly zkoumány z pohledu jejich fylogenetické a morfologické homogenity a geografického rozšíření. Výzkum byl proveden pomocí morfologických a morfometrických analýz, spolu s fylogenetickou analýzou trnG$^{ucc}$ kmenů z různých lokalit Evropy. *Micrasterias rotata* se ukázala být fylogeneticky homogenní v rámci Evropy, zatímco *M. fimbriata* se rozdělila do dvou dobře podpořených linií. Populace těchto linií se lišily kromě fylogenetické analýzy také morfologickými a morfometrickými daty. Byly zjištěny rozdíly v jejich geografickém rozšíření a pravděpodobně rozdílné ekologické nároky. Na základě výsledků bylo navrženo popsat jednu z těchto linií jako nový druh rodu *Micrasterias*.

Pomocí průtokové cytometrie bylo měřeno množství jaderné DNA u krásivek a několika dalších mikrořas. Byl použit nový protokol vzniklý na základě poznatků z měření u vyšších rostlin. Pro získání protoplastů byla použita směs enzymů celulázy a mecerozymu. Suspenze jader byla získána použitím LB01 lyzačního pufru a následně byla jádra obarvena propidium jodidem. Jako standard byly použity listy čtyř různých rostlin a byl navržen nový standard z řad mikrořas. Protokol se ukázal vhodný pro použití u krásivek a fungoval i u několika zástupců z řad Chlorophyta a Heterokontophyta. Optimalizace enzymatické směsi či lyzačního pufru pro jiné skupiny mikrořas, a pro ty, u kterých nebyla metoda úspěšná, si zaslouží další pozornost.

U 34 kultur (25 druhů) rodu *Micrasterias* bylo změřeno množství jaderné DNA. Byla sledována mezidruhová a vnitrodruhová variabilita tohoto množství a zjištěné hodnoty DNA byly namodelovány na fylogenetický strom založený na propojení analýz SSU rDNA, psaA

a coxIII. Příbuzné druhy vykázaly podobná množství DNA. U druhů, pro které byla známa data o počtu chromozomů, byla zjištěna silná pozitivní korelace mezi množstvím DNA a počtem chromozomů. Pozitivní korelace se projevila i mezi množstvím DNA a rozměry buněk u sledovaného druhu *Micrasterias rotata*. Experimenty poskytly důkaz významu změny velikosti genomu v evoluci rodu *Micrasterias* a potvrdily důležitost přirozené polyploidizace v jeho evoluci a speciaci.