Charles University in Prague

Faculty of Science

Ph.D. Program: Botany

Unveiling hidden species diversity in desmids (Desmidiales, Viridiplantae)

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Ph.D. Thesis

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LIST OF PAPERS

This thesis is based on the following eight papers, reffered to in the text as Papers I-VIII.

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

II. ĠŠastný, J. & Neustupa, J. (2008): *Cosmarium gauthierae* sp. nov. (Conjugatophyceae, Desmidiales) from an ephemeral pool in South-West Macedonia. *Cryptogamie Algologie* 29: 255-260

III. ĠŠastný, J. & Kouwets, F.A.C (2012): New and remarkable desmids (Zygnematophyceae, Streptophyta) from Europe: taxonomical notes based on LM and SEM observations. *Fottea* 12(2): 293-313

IV. ĠŠastný, J., Ġkaloud, P., Langenbach, D., Nemjová, K. & Neustupa, J. (2013): Polyphasic evaluation of *Xanthidium antilopaeum* and *Xanthidium cristatum* (Zygnematophyceae, Streptophyta) species complex. *Journal of Phycology* 49(2): 401-416

V. Ġkaloud, P., ĠŠastný, J., Nemjová, K., Mazalová, P. & Neustupa, J. (2012): Molecular phylogeny of baculiform desmid taxa (Zygnematophyceae). *Plant Systematics and Evolution* 298: 1281-1292

VI. Neustupa, J., ĠŠastný, J., Nemjová, K., Mazalová, P., Goodyer, E., Poulíļková, A. & Ġkaloud, 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(1): 223-239

VII. Nemjová, K., Neustupa, J., ĠŠastný, J., Ġkaloud, P. & Veselá, J. (2011): Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*. *Phycological Research* 59(3): 208-220

VIII. Neustupa, J., Ġkaloud, P. & ĠŠastný, J. (2010): The molecular phylogenetic and geometric morphometric evaluation of *Micrasterias crux-melitensis / M. radians* species complex. *Journal of Phycology* 46(4): 703-714

Author's contributions:

II. Me and Jiří Neustupa wrote the paper jointly, I made the figures and prepared the tables.

III. I wrote the major part of the text, performed the SEM work, made several line drawings and prepared the tables. Frans Kouwets participated on writing of the paper and made the majority of line drawings.

IV. I was responsible for the isolation of the *Xanthidium* strains for the study and their identification according to traditional morphology, performed the SEM works and wrote the major part of the text. Pavel Škaloud was responsible for molecular and Jiří Neustupa for geometric morphometric analyses, respectively, and they both wrote appropriate parts of the text. Dorothee Langenbach helped with molecular analyses and Katarína Nemjová with the isolation of strains.

V.-VII. I was in general responsible for isolation, cultivation and traditional morphological analyses of the strains, have performed the SEM works and participated on writing of the papers.

VIII. Jiří Neustupa wrote the major part of the paper and made the geometric morphometric analyses, Pavel Škaloud was responsible for molecular analyses and wrote appropriate parts of the text, I performed the SEM works.

Declaration: I hereby declare that I have written this thesis independently, using the listed references, or in cooperation with co-authors of the papers. I have submitted neither the thesis, nor any of its parts, to acquire any other academic degree.

Prague, 15th May 2013 ....

Jan Šťastný

In behalf of all co-authors, we declare the keynote participation of Jan Šťastný in this thesis, as described above.

....

Jiří Neustupa Pavel Škaloud

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ABSTRACT

The delineation of desmid species was traditionally based on purely morphological features. However, a frequent misinterpretation of morphological variability in desmids has led in the past to extensive taxonomical confusion within this important group of green algae which complicates the interpretation of their biodiversity in freshwater ecology, biogeography and biomonitoring. Consequently, I focused in this thesis predominantly on a previously neglected issue, the application of polyphasic approaches in the species-level taxonomy of desmids. In the most studies, a combination of both traditional morphological and modern molecular phylogenetic and geometric morphometric methods has been used to evaluate the taxonomy of selected desmid species, particularly representatives of the morphologically complex genera *Micrasterias* and *Xanthidium*. In two papers, I used the combination of traditional morphological and autecological data to clear up the taxonomy of several morphologically less prominent desmid taxa. Generally, the results of the thesis demonstrated that the way we recently see the diversity and distribution of desmids should be thoroughly changed. The real species diversity is mostly distinctly finer than that estimated by classical morphological taxonomy, often corresponds to varieties of the traditional morphologically defined species, and is usually well determinable using combination of molecular and morphological data. Consequently, true cryptic diversity appears to be a relatively rare phenomenon in desmids. Moreover, it is likely that the actual species diversity of desmids is for a much greater part than generally supposed related to the patterns of their geographic distribution or to the climatic factors. The biogeographical areas of these phylogenetic species are probably usually much smaller and the proportion of regionally restricted or even endemic species consequently much higher than recently assumed. Herewith, the results contradict the ñubiquity modelò as the possible distribution model of desmids, in favour of Foissnerôs ñmoderate endemicity modelò. The practical use of desmids in biomonitoring and other studies based on species composition data will need to be revised, but still seems to be much more promising than in the absolute majority of other microalgal groups, particularly due to the revealed monophyly of most of the traditional desmid morphospecies studied. Polyphasic approach, based on combination of several mehods, yields a new level of interpretations that could not be reached by the use of any of these methods alone. Nevertheless, it is obvious that the investigation of hidden species diversity in desmids is still at the beginning.

CONTENTS

1. INTRODUCTION

1.1. General introduction

Species are fundamental natural units and their proper circumscription is an essential requirement for both biodiversity assessments and correct understanding of their ecology, biogeography, evolutionary history, and speciation. However, microbial eukaryotes often reveal so few morphological characters that it is very difficult to delimit them corretly using morphological criteria alone. Contrary to macroorganisms, simple morphology often leads to the convergent morphological evolution across different genera or even classes. For example, species of the morphologically defined algal genus *Chlorella* were revealed to be dispersed over two classes of chlorophytes, the Chlorophyceae and the Trebouxiophyceae (Huss et al. 1999).

Also at species level, the taxonomy of various protists has been turned upside down over the past two decades with the increasing use of molecular methods, revealing an apparent rate of hidden (cryptic or pseudocryptic) diversity (e.g. Fawley et al. 2006, Hausmann et al. 2006, Slapeta et al. 2006, Evans et al. 2008, Lindstrom 2008, Kraft et al. 2010, Poulíļková et al. 2010, Ġkaloud & Peksa 2010, Fuļíková et al. 2011, Kucera & Saunders 2012, Moniz et al. 2012, Ġkaloud et al. 2012). Cryptic species are defined as morphologically identical but genetically distinct entities, while pseudocryptic (or semicryptic) ones present, besides genetic, also minor morphological differences (Mann & Evans 2007, Alverson 2008).

In spite of these increased efforts, the number of species investigated by molecular phylogenetics methods represent just the tip of the iceberg and the range and extent of the genotypic and phenotypic diversity are still unknown for the vast majority of extant, free-living protist species (Weisse 2008). Given that protists hold key roles in nearly all ecosystems (Cotterill et al. 2008) and are frequently used for the purposes of both basic and applied research, it is obvious that the matters of their diversity and species delineation still remains one of the central issues of contemporary protistan biology (Weisse 2008).

1.2. Desmids, introduction

Desmids are unicellular algae that belong to conjugating green algae (Zygnematophyceae, Viridiplantae). This group represents the largest and the most diverse lineage of the streptophyte green algae, from which the embryophyte land plants evolved

(Becker & Marin 2009). The feature which sets this class apart from other streptophytes and may have contributed to its successful diversification (Brook 1981), is the unique mode of sexual reproduction, the so called conjugation (fusion of amoeboid nonflagellate gametes emerging from the walls of adhering vegetative cells). Desmids *sensu lato* traditionally involve two groups of unicellular algae within Zygnematophyceae; the so called saccoderm (ñfalseò) desmids (members of family Mesotaeniaceae from order Zygnematales) and the placoderm (ñtrueò) desmids, members of the monophyletic order Desmidiales (McCourt et al. 2000, Denboh et al. 2001). In this thesis, I have focused on the representatives of the latter group, which is well characterized by a specific cell wall architecture and regarded as the most derived in the whole class Zygnematophyceae (Mix 1973, Brook 1981). Each cell consists of two (or seldom more) almost symmetrical and often profusely sculptured segments (see Fig. 1) with a system of complex pores penetrating the secondary cell wall, the primary cell wall having been shed after cell division (McCourt et al. 2000, Gontcharov et al. 2003, Hall et al. 2008, for details see also Coesel & Meesters 2007).

Desmids occurr exclusively in freshwaters, particularly in standing waters, such as ponds, lakes or shallow pools. The highest desmid diversity is found in mesotrophic, slightly acidic to slightly alkaline water bodies like moorland pools, peat pits or fen hollows (Coesel 1982, Coesel & Meesters 2007). They belong to the dominant phytobenthos groups in these habitats, both in terms of species richness and biomass (Watanabe et al. 2000, Coesel & Meesters 2007). Therefore, they have recently been several times used in various kinds of ecological studies (e.g. Pals et al. 2006, Krasznai et al. 2008, Neustupa et al. 2009, 2011, 2012). Moreover, due to their highly specific ecological demands, they are considered excellent indicator organisms and represent one of the most important groups in the ecological monitoring of freshwater habitats (Coesel 1998, 2001, 2003). Logically, as recently stressed by Coesel & Krienitz (2008), their use and reliability for the above mentioned purposes esentially require reliable species concepts and related knowledge on distributional patterns and extent of cryptic and pseudocryptic diversity in the particular taxa.

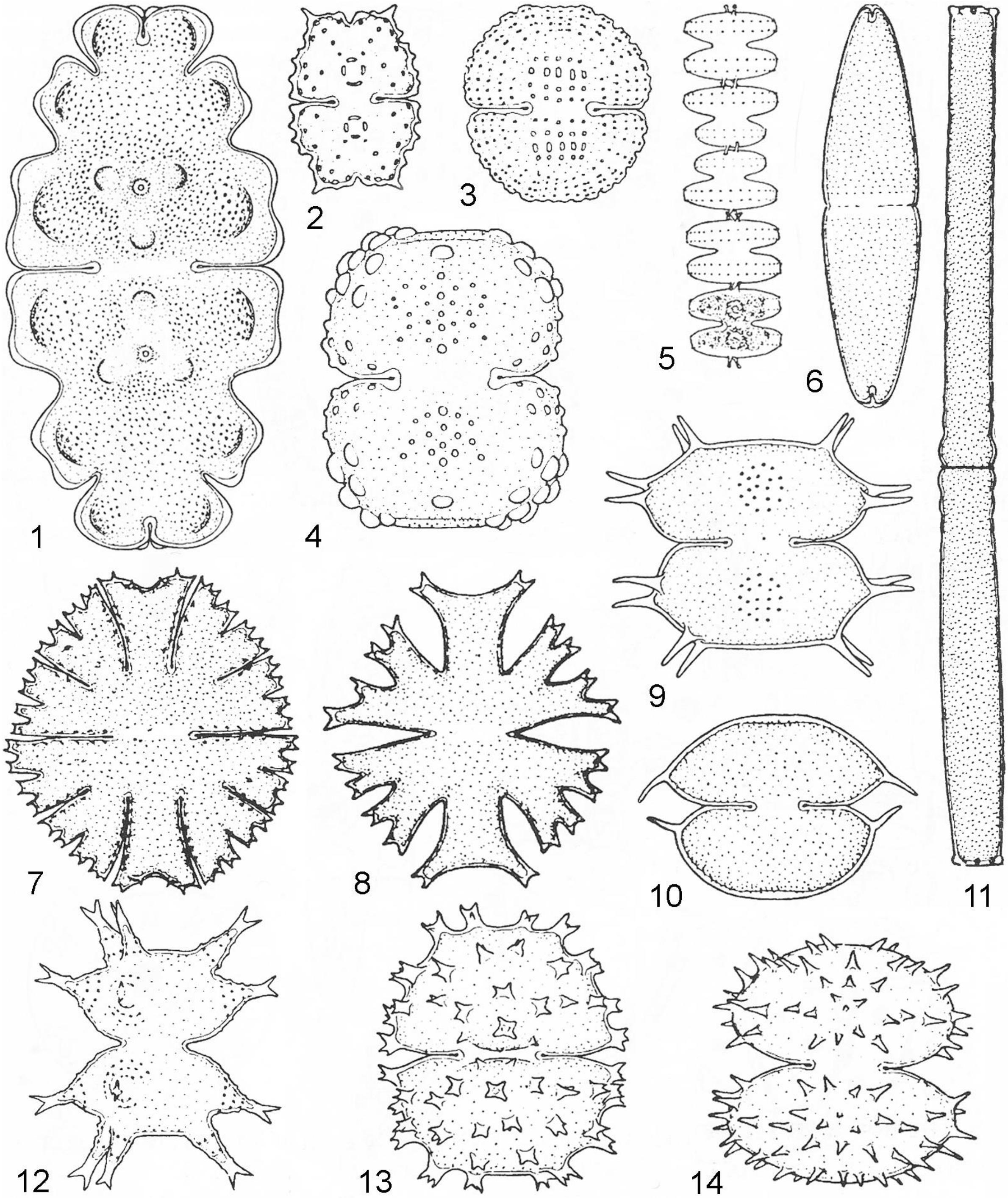

Fig. 1. Variability of cell shapes and ornamentation in various desmids. (1) *Euastrum oblongum* (2) *E. turneri* (3) *Cosmarium formosulum* (4) *C. ungerianum var. subtriplicatum* (5) *Sphaerozosma aubertianum* (6) *Tetmemorus granulatus* (7) *Micrasterias papillifera* (8) *M. crux-melitensis* (9) *Xanthidium antilopaeum* (10) *Staurodesmus convergens* (11) *Pleurotaenium ehrenbergii* (12) *Staurastrum furcigerum* (13) *S. spongiosum var. perbifidum* (14) *S. teliferum*. Modified after RŢģiļ ka, from Fott 1967.

1.3. Species concepts in desmids

As already mentioned above, problems concerning species definition in microalgae complicate interpretation of their biodiversity in ecological, biogeographical or biomonitoring studies. However, given two organisms, it is often very difficult to distinguish, whether they belong to the same species or not, particularly in closely related ones. That is the reason why many different species concepts are used by biologists. In this chapter, the most important and relevant ones are briefly characterized and discussed with respect to the desmids.

The morphological species concept

According to this species concept, species are groups of morphologically identical or similar organisms (Futuyma 1998). At the generic and species levels, desmids, similarly to other microalgae, are traditionally classified according to morphological characters of vegetative cells, such as shape, dimensions, number of symmetry planes, cell wall ornamentation or chloroplast configuration (Brook 1981). In some species, the shape of zygospores (products of the sexual reproduction) is used as additional discriminative feature (RŢğiļ ka 1977, Coesel & Meesters 2007).

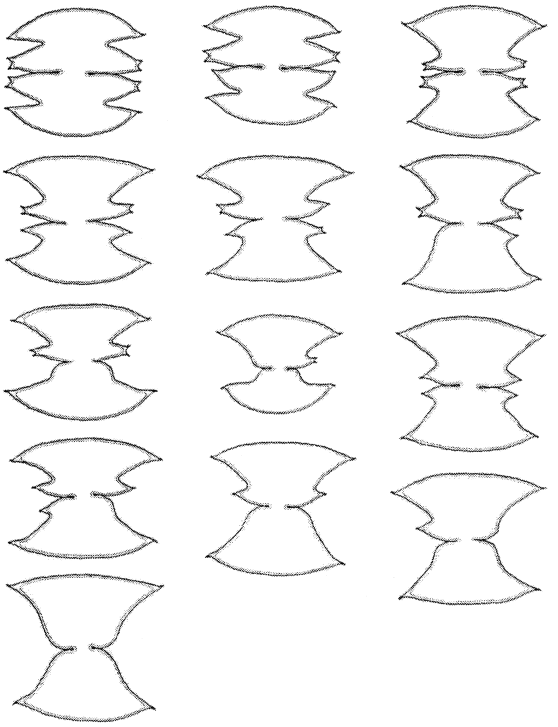

Fig. 2. Different morphological expressions seen in one population of *Micrasterias laticeps*. Modified after Bicudo & Sormus 1972.

However, most of these morphological discriminative characters of desmids may be extremely variable even within one natural population (e.g. Bicudo & Sormus 1972, Gerrath 1979, 1983, Kouwets 1984, see also Fig. 2) or depending on environmental conditions (e.g. RŢğiļ ka 1971, Neustupa et al. 2008).

Unfortunately, it was just this extensive morphological plasticity of complex desmid cells what built the basis for the existing huge confusion in their traditional morphological species-level taxonomy (Kouwets 2008). The ecomorphae were often so much different from the ñtypicalò form (contrary to other, usually morphologically much less prominent algal groups) that the desmid taxonomists were put up to describe them as separate taxa. Very often a new name and specific rank have been given to almost every variation encountered (Archer 1860), inducing De Wildeman (1894) to the lament that óDesmidiologists describe specimens not speciesô. Moreover, since it is easier to describe a new variety than a new species, these newly described and often ill-defined forms were frequently described as infraspecific taxa of apparently unrelated, only superficially similar species, in this way creating a large number of so-called collective species (Kouwets 2008) and further obscuring clear species definitions. Finally, in many cases no original figure or authentic material existed or the figures were very poor, what often led to the creation of a large series of taxonomic synonyms (e.g. Heimans 1969, p. 56., part about *Staurastrum echinatum*, see also Fig. 3).

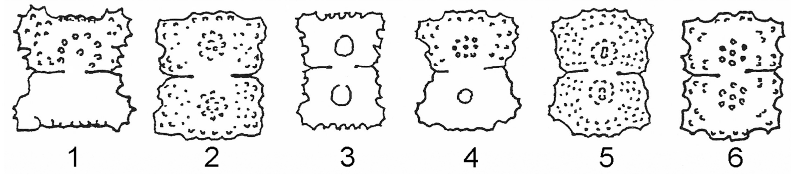

Fig. 3. Six Cosmarium species that are probably synonymous. (1) *Cosmarium wallichii* West & West (2) *C. seelyanum* Wolle (3) *C. nobile* (Turner) Krieg. (4) *C. naivashensis* Rich (5) *C. divergens* Krieg. (6) *C. subnobile* Hinode. After various authors, from Coesel & Krienitz 2008.

All the above mentioned facts clearly illustrate the problems of using the traditional morphological species concept in desmids (for details see Kouwets 2008). Its application for species delineations may further be complicated by the occasional occurrence of polyploid complexes (see Kapraun 2007) and by the fact that it does not recognize cryptic or sibling species (Behnke et al. 2004). However, previously published morphological investigations represent valuable information sources about morphological variability and distribution of particular desmid species, and the traditional morphology, if performed carefully and critically, may still provide a lot of useful information.

The biological species concept

This species concept is probably the most widely accepted among contemporary biologists. It defines species as groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1942, 1948). The life cycle of *Desmidiales* involves the sexual process and there are several examples of application of the biological species concept criteria based on reproductive isolation as a part of taxonomic evaluation of desmid species. For instance, Blackburn & Tyler (1987) tested the reproductive behaviour in clones of *Micrasterias thomasiana* and several similar *Micrasterias* species and revealed an unexpected hidden diversity of mating types [i.e. populations which mutually show (almost) complete sexual isolation; Ichimura 1981] within the traditional morphospecies. Such mating types can be considered syngens or biological species (Coesel & Krienitz 2008). Another well-known example is the *Closterium ehrenbergii* species complex where up to 18 mating types have been recognized (e.g. Ichimura 1981, Ichimura & Kasai 1989, Denboh et al. 2003). Often, the individual mating types slightly differ in morphology, ecology and/or geographical distribution (Ichimura & Kasai 1990; Ichimura et al. 1997), but they also may be morphologically identical, indicating true cryptic diversity in desmids.

It is more than likely that such sibling species will occur in many more desmid morphospecies, what would substantially increase the diversity of this algal group. Unfortunately, sexual reproduction, which is essential for applying the biologic species concept, is a relatively rare phenomenon in desmids (Coesel 1974, Coesel & Teixeira 1974, Coesel & Krienitz 2008) and of many species no sexual stages are known at all. Moreover, the breeding experiments are usually extremely time-consuming. Thus, a wide application of the biological species concept in desmid taxonomy remains highly problematic.

The phylogenetic species concept

The application of phylogenetic analyses based on specific molecular markers in the 1990s considerably extended the scope of algal systematics and taxonomy. Molecular data allowed to formulate specific phylogenetic hypotheses and to trace the phylogenetic relationships between individual taxa. Consequently, the phylogenetic species concept has been put into practice, in which a species is the smallest group of organisms that shares unique combination of character states (nucleotide states as well; Nixon & Wheeler 1990). Ideally, a phylogenetic species is also monophyletic, i.e. includes an ancestor and all its descendants. Hence, this concept defines a species as a group having a shared and unique evolutionary history. It can be applied practically for to organisms, but its strict application, regarding all evolutionary end-products (even a number of clonal asexual organisms) as unique species, could result in overestimating of the real species number (Wheeler & Platnick 2000, Ġkaloud 2008).

For investigation of recent speciation events or even for delimiting cryptic and phylogenetic algal species, the rapidly evolving and variable molecular markers are preferred, for instance the plastid *rbc*L gene (e.g. Lindstrom 2008, Moniz et al. 2012), mitochondrial *cox*1 gene (e.g. Fuļíková et al. 2011, Ġkaloud et al. 2012), the nuclear ribosomal internal transcribed spacer (ITS) regions (e.g. Brodie et al. 2007, Kynļlová et al. 2010) or the actin introns (e.g. Nelsen & Gargas 2006, Ġkaloud & Peksa 2010). However, in Streptophytes, an ideal molecular barcode (i.e. a marker that would allow reliable species-level identification and could be used universally) is still wanting (Hall et al. 2010).

The taxonomy of desmids has recently undergone major changes based on molecular phylogenetic analyses (e.g. McCourt et al. 2000, Gontcharov et al. 2003, Gontcharov 2008, Hall et al. 2008, Gontcharov & Melkonian 2004, 2005, 2008, 2010, 2011). However, most of these studies were concentrated on reconstruction of major lineages corresponding to families and orders or on the demonstration of the artificial nature of the traditional desmid genera (Gontcharov et al. 2003, Gontcharov & Melkonian 2005, 2008, Gontcharov 2008). As a ñbyproductò, the data of Gontcharov & Melkonian (2008) indicated possible pseudocryptic diversity in the traditional taxa *Staurodesmus extensus* and *Cosmarium punctulatum*. Yet, there was no study using molecular data that would specifically focus on the validity of traditional desmid species concepts.

From the above chapters it is clear that no from the mentioned approaches alone can be considered a ñgold standardò for species level taxonomy of algae. Therefore, the usage of ***polyphasic*** (multidisciplinary) ***approach*** (i.e. combining morphological, ultrastructural, molecular, ecological or biochemical data) has been recently recommended for species delimitation in various algal groups (e.g. Mann 1999, Pröschold & Leliaert 2007, Ġkaloud 2008), including desmids (Kouwets 2008).

1.4. Diversity of desmids

The above-mentioned problems concerning the application of the morphological species concept in desmids, which has almost exclusively been used for species delineations, significantly hamper also the diversity estimates of the group. Differently broad species definitions by various authors resulted in rather dissimilar assessments of the desmid species richness, ranging from 1 500 to 12 000 spp (Cranwell et al. 1990, Hoshaw et al. 1990). The most recent and usually cited estimate of the total number of ñgoodò species known so far amounts to approximately 3 000 (Gerrath 1993). However, for instance Coesel (see Coesel & Krienitz 2008) when extrapolating the number of morphospecies distinguished in an ongoing inventory of European Staurastra, came to a comparable number. Thus, the real taxonomic diversity of the group is often considered very uncertain (e.g. Gontcharov 2008, Gontcharov & Melkonian 2011).

1.5. Biogeography of desmids

The research of hidden diversity in microalgae is obviously closely associated with the issue of the geographical distribution of the potentially recognized (pseudo)cryptic taxa. Two models concerning the distribution patterns of microorganisms have recently appeared in series of papers and became promptly one of the most contentious issues of microbial biogeography and ecology. The ñubiquity modelò, proposed by Finlay and Fenchel (Finlay 2002, Fenchel & Finlay 2004, Finlay et al. 2004, Fenchel 2005), says that all microorganisms occur everywhere the environment is suitable due to high dispersal ability and high individual numbers. On the other hand, the ñmoderate endemicity modelò, raised by Foissner (Foissner 2004, 2006), estimates that about one third of the taxa are due to various reasons endemic, in spite of suitable habitats in other regions. The main differences between both models are summarized in Table 1, for details see Foissner (2008).

Features	**Ubiquity model**	**Moderate endemicity model**
Absolute abundance of individuals within morphospecies	High	Low in the majority of species
Rates of migration species pool found locally	High	Low for most of the rare species
Proportion of global species pool found locally	High	Moderate; usually highly overestimated due to undersampling
Relative number of endemics	Low/None	Moderate (cca 30%)
Global number of morphospecies	Low	High due to long time to speciate
Conservation	Not needed	Needed
Human introductions	?	Likely high

Tab. 1. Comparison of the ubiquity and the moderate endemicity models. Modified after Foissner 2008.

In desmids, already West (1909) stated that no group of freshwater algae exhibits such marked geographical peculiarities as the desmids. He even suggested that these peculiarities would enable to recognize the rough geographical origin of any desmid collection. The main reason for such a geographical restriction might be that desmids only seldom form resistent, wind-transportable spores (Coesel 1974). Therefore, their dispersal is supposed to proceed mostly by vegetative cells carried by insects and birds (Brook 1981, Coesel at al. 1988, Kouwets 1998). These, however, may readily desiccate or be washed out in salty water so that the distances bridged in that way will generally be rather limited (Coesel 1996). Moreover, the geographical distribution of desmids may be significantly curtained by their highly specific ecological demands.

Indeed, there are several examples of peculiar, well-defined distribution patterns in desmids (Donat 1926, Heimans 1969) and Krieger (1933, 1937) and Coesel (1996) even distinguished ten desmid floral regions. However, since a reliable knowledge of geographical distribution patterns is confined only to taxa that can not be confused with any other ones (Heimans 1969, Coesel & Krienitz 2008), all these attempts were based only on a relatively small number of morphologically most conspicuous, clearcut taxa (flagship species) with a low confusion likelihood (see Fig. 4).

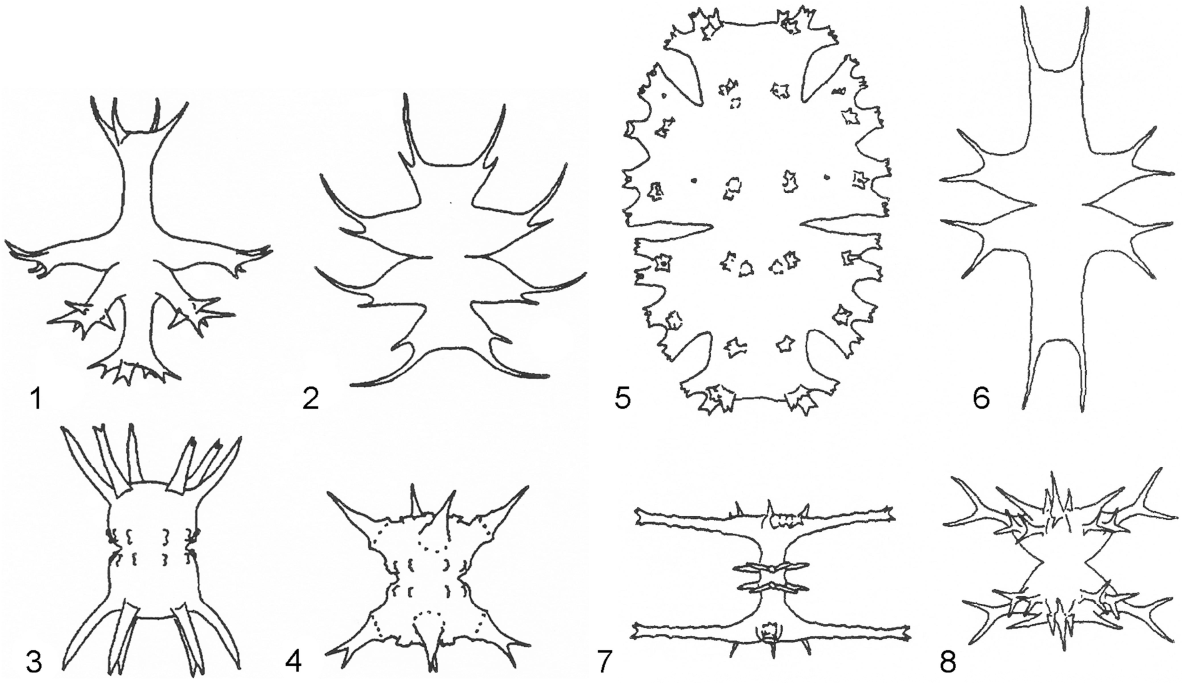

Fig. 4. Desmid taxa characteristic of the equatorial African (1-4) and the Indo-Malaysian/North Australian (5-8) region. (1) *Allorgeia incredibilis* (2) *Micrasterias sudanensis* (3) *Staurastrum rzoskae* (4) *S. fuellebornii* var. *evolutum* (5) *Micrasterias anomala* (6) *M. ceratofera* (7) *Staurastrum tauphorum* (8) *S. freemanii*. After Grönblad et al. 1958 and Scott & Prescott 1961, from Coesel 1996.

On the other hand, the majority of desmids is still said to show cosmopolitic distribution patterns (Coesel 1996) and there is a high number of taxa whose distribution is in reputable desmid monographs (e.g. Prescott et al. 1981, 1982) marked as ñcosmopoliticò or ñworldwideò. Yet, most interestingly, a closer look reveals that most of these taxa belong to rather vaguely and broadly defined species (e.g. *Cosmarium regnellii*, *C. laeve*, *Actinotaenium cucurbita*, *Xanthidium antilopaeum*). This indicates that a so far unrecognized hidden diversity may be responsible for the putative cosmopolitism of these traditional morphospecies and that they in fact may be a complex of several taxa with resctricted distribution.

Thus, it is obvious that for proper species delimitation allowing subsequent correct assessment of both the distribution patterns and diversity of desmids it is indeed crucial to use a polyphasic approach reconciling morphologic, genetic, and ecological features. This was also the main aim of this thesis.

2. RESEARCH OBJECTIVES OF THE THESIS

The general objective of this thesis was the taxonomical investigation and revision of several desmid species complexes using polyphasic approach. For this purpose, I focused particularly on the representatives of the conspicuous and morphologically complex genera *Micrasterias* and *Xanthidium*. Moreover, in two papers, I used the combination of traditional morphological and autecological data co clear up the taxonomy of several morphologically less prominent desmid taxa.

The particular aims can be summarized as follows:

i) to evaluate the diversity and distribution of desmids within the Czech Republic using the traditional morphological approach, and to select desmid species complexes with a potential hidden diversity suitable for further research (paper I)
ii) to evaluate the taxonomy of several problematic desmid taxa using the combination of traditional morphological and ecological data (papers II and III)
iii) to evaluate the taxonomy of selected desmid species using both traditional morphological and modern molecular phylogenetic and geometric morphometric approaches (papers IV-VIII)
iv) to investigate the distribution patterns of the recognized (pseudo)cryptic taxa using the combination of field and literature data (paper VI)

3. THESIS OUTLINE

Diversity and distribution od desmids within the Czech Republic

In **paper I**, the morphological species concept was adopted to assess the diversity, distribution and autecology of desmids within the Czech Republic. The examination of more than 1400 samples from about 150 various wetland habitats revealed altogether 526 desmid taxa, 80 of them being new for the Czech Republic. The paper focused particularly on the occurrence and autecology of rare taxa, however, as an overview of all taxa found, and to allow for a quick retrieval of basal information concerning a particular species, a comprehensive, notated table was included where several aspects of the ecology of every taxon are evaluated.

The extensive sampling also revealed a number of problematic taxa that apparently needed a more detailed study to clear up their taxonomy. Therefore, this paper served as a basis for further studies focused on the taxonomy of particular species (complexes) and has also revealed a number of desmid-rich localities (particularly minerotrophic fens in lowland areas) that served as a source of material for the isolation of numerous desmid strains within these studies. Finally, the data concerning the occurrence of rare taxa in the particular Czech wetland habitats may also be used in the future as a basis for comparative ecological studies tracing the development of these sites.

Taxonomy of selected desmid taxa using the combination of morphological and autecological data

In **paper II**, careful morphological analysis of a population of an unknown desmid from an ephemeral pool in Macedonia led to its description as a new species, *Cosmarium gauthierae*. Another finding of this species has been previously attributed to *C. onychonema*, from which, however, *C. gauthierae* clearly differs by a unique combination of typical morphological features as well as by its unusual ecology, so that our results illustrated a previously unrecognized, pseudocryptic diversity in *C. onychonema*.

In **paper III**, several potentially (pseudo)cryptic or otherwise taxonomically problematic desmid species have been studied using the combination of critical morphological observations and autecological data. In this study, we also used a scanning electron microscope to clearly illustrate the discriminative morphological characters of the individual taxa. We found pseudocryptic diversity in four desmid taxa (*Closterium costatum*, *Cosmarium punctulatum* var. *subpunctulatum*, *C. variolatum*, *Actinotaenium curtum*) what resulted in the description of four species new to science (*Cl. pseudocostatum*, *Cosmarium discrepans*, *C. hostensiense*, *Act. riethii*). In addition, our results confirmed the status of *Cosmarium cataractarum* and *C. cinctutum* as independent species and stressed the need for the raise of *C. subbroomei* var. *taylorii* to the rank of a separate species.

Taxonomy of selected desmid taxa using polyphasic approach

In **paper IV**, we evaluated the patterns of the phylogenetic and morphological differentiation in two of the taxonomically most problematic traditional species *Xanthidium antilopaeum* and *X. cristatum*. Altogether twenty six strains of *Xanthidium antilopaeum* and seven strains of *X. cristatum* were investigated. The molecular data based on *trn*G^{ucc} and ITS rDNA sequences illustrated the monophyly of both the complexes, with a single exception of *X. antilopaeum* var. *basiornatum*, which probably represents a separate species. Within *X. cristatum* complex, the traditional varieties *X. cristatum* var. *cristatum*, *X. cristatum* var. *uncinatum*, and *X. cristatum* var. *scrobiculatum* turned out to be separate taxa. Conversely, *X. cristatum* var. *bituberculatum* lacked any taxonomical value. Our data on *X. antilopaeum* illustrated extensive phylogenetic as well as phenotypic variability within this species complex. Although they did not result in any unambiguous pattern that would allow sound taxonomic classification, it is obvious that the real species diversity within this complex, as estimated by classical morphological taxonomy, has been largely underestimated and may actually be one or even two orders of magnitude higher. Interestingly, the phylogenetic tree based on the *trn*G^{ucc} data set also indicated several examples of possible geographical restriction among *Xanthidium* phylogenetic taxa.

Paper V focused on baculiform desmids, i.e. those with a rod-like morphology. Our phylogenetical data illustrated that this morphotype has evolved independently at least four times within Desmidiaceae. However, the most interesting result of this paper with respect to the subject of this thesis was the ascertained polyphyly of the traditional, very common taxa *Pleurotaenium ehrenbergii* and *P. trabecula*. There was clearly higher diversity within these taxa indicated by the *trn*G^{ucc} based phylogenetic tree than it was apparent on the basis of morphological data. This confirmed the generally poor morphological concept of some species of this genus, based on relatively few and often rather vague discriminating features. Moreover, our results confirmed the monophyly of *Pleurotaenium*, including the morphologically peculiar taxa *P. nodosum* and *P. ovatum*, and illustrated the phylogenetical position of the rare genus *Triplastrum*, which proved to be completely unrelated to other baculiform taxa.

In **paper VI**, we proved the homogeneity of 14 strains of the well-known desmid species *Micrasterias rotata* across Europe, based on *trn*G^{ucc} phylogeny. On the other hand, 16 strains of the other species studied, *M. fimbriata*, turned out to be composed of two clearly delimited lineages, differing by molecular as well as by morphometric and morphological data. This detected pseudocryptic diversity within *M. fimbriata* was all the more surprising in the light of the fact, that it belongs to the most conspicuous desmids at all and possesses quite a lot of morphological markers. Despite this, its traditional species concept has not been questioned by any desmid expert so far.

In **paper VII,** we investigated the morphological and molecular differentiation of the broadly perceived traditional species *Micrasterias truncata* which includes several infraspecific taxa with an unclear taxonomical value. In addition, we also studied strains of the morphologically similar species *M. decemdentata* and *M. zeylanica*. Molecular phylogenetic analysis based on *trn*G^{ucc} intron sequences revealed five well supported clades. Two Australian isolates of *M. truncata* var. *pusilla* turned to be closely related to *M. zeylanica* on the basis of molecular as well as geometric morphometric data and probably represent a separate species with a presumably tropical distribution. Similarly, two European strains of *M. truncata* var. *semiradiata* were phylogenetically as well as morphologically separated from all other strains. Thus, we proposed that this taxon should again be considered a separate species *M. semiradiata* (as it was originally described by Kützing) rather than a variety of *M. truncata*. All the other strains (including those attributable to traditional variety *M. truncata* var. *neodamensis*) formed a firmly supported group of the ñcoreò *M. truncata* which was subdivided into three clades. However, we were not able to find any morphological or biogeographical pattern that could be used for the taxonomic delimitation of these lineages. This may be caused by their relatively recent origin and they possibly represent a sympatric, truly cryptic species. This, however, will need to be confirmed by other features such as reproductive isolation or analyses of additional fast evolving molecular markers.

In **paper VIII**, we examined another representatives of the genus *Micrasterias*, *M. crux-melitensis* and *M. radians*. The traditional species boundaries in these morphologically closely similar taxa are rather indistinct. Consequently, in some studies, *M. radians* has been considered variety of *M. crux-melitensis* or both taxa have even been regarded as one morphologically variable species. However, our molecular data clearly rejected the hypothesis of possible conspecifity of these taxa as they revealed three distinct phylogenetical lineages. One of them comprised the European and North American strains that were morphologically identified as *M. crux-melitensis*. Within these lineage, the traditional varieties *M. crux-melitensis* var. *janeira* and *M. crux-melitensis* var. *superflua* turned out to have no taxonomical value. The strains of *M. radians* formed two separate phylogenetic lineages corresponding to traditional varieties *M. radians* var. *evoluta* and *M. radians* var. *bogoriensis*. The morphotypes corresponding to the former variety have, so far, only been reported from Africa, on the other hand, the single strain of *M. radians* var. *bogoriensis* originated from Southeast Asia. This apparent pattern of biogeographical resctriction among our three phylogenetical lineages indicated that geographic isolation may play an important role in species differentiation of relatively large freshwater protists, such as *Micrasterias*.

Investigation of distribution patterns of the recognized pseudocryptic taxa using the combination of field and literature data

In **paper VI**, we used a unique approach that allowed to include many published records into the analysis of the distribution patterns of the two recognized pseudocryptic taxa in traditional *M. fimbriata*. The published drawings and microphotographs of this species were included in a classification discrimination analysis and placed into the newly identified lineages upon comparison to the morphometric data collected from living material. This revealed largely disparate geographic patterns within traditional *M. fimbriata*. One phylogenetic species is frequent in central and eastern Europe, but occurs also in the British Isles. The second species 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.

4. CONCLUSIONS

The frequent misinterpretation of morphological variability in desmids has led in the past to a flood of ill-defined infraspecific taxa and to extensive taxonomical confusion within this important group of green algae. The traditional species concepts are often obscure, consequently hampering the interpretations of desmid biodiversity in the context of freshwater ecology, biogeography and biomonitoring.

Therefore, I focused in this thesis predominantly on a previously completely neglected issue, the application of polyphasic approaches, based on combination of various methods, in the species-level taxonomy of desmids. Though, as demonstrated in Papers II and III, also the traditional morphology, if performed carefully and critically and supported for instance by ecological data, may still provide valuable taxonomical data. Generally, however, for resolving the taxonomy of closely related species, approaches involving molecular phylogenetic analyses seem to be essential.

The results of the core part of thesis (papers IV-VIII), where polyphasic approaches were used to reveal the hidden diversity within several model desmid species complexes, can be summarized as follows:

As supposed on the basis of similar studies on various protistan species, the use of polyphasic approach mostly revealed a considerable rate of hidden diversity. Among our target taxa, given that numerous strains were studied, only *Micrasterias rotata* and *M. crux-melitensis* turned out to be phylogenetically homogenous. On the other hand, the number of traditional morphospecies that were found to be composed of several phylogenetic species (e.g. *Micrasterias fimbriata*, *M. truncata*, *M. radians*, *Xanthidium antilopaeum*, *X. cristatum*, *Pleurotaenium ehrenbergii*, *Pl. trabecula*) was much higher. Within *X. antilopaeum* species complex, the real species diversity appears to be even one or two orders of magnitude higher than that estimated by classical morphological taxonomy. Moreover, the number of traditional infraspecific taxa that turned out to have no taxonomical value (e.g. *Micrasterias crux-melitensis* var. *janeira*, *M. crux-melitensis* var. *superflua* or *Xanthidium cristatum* var. *bituberculatum*) was relatively low.

Therefore, it can be assumed that the real species diversity of Desmidiales which has sometimes been (e.g. Coesel & Krienitz 2008) considered rather lower than the recently accepted estimates (ca 3 000 spp.) due to the high number of current synonyms, will actually be considerably higher. Since the potential rate of hidden diversity within morphospecies with simple morphology and less discriminative characters would likely be even higher than that of morphologically complex taxa that we mostly studied so far, I believe that a future wide employment of molecular methods in the species-level taxonomy of Desmidiales would probably lead to revealing several times higher diversity than those about 3 000 recently accepted ñgoodò species.

The individual species-level phylogenetic lineages were mostly found to be morphologically identifiable, both by careful microscopic analysis, as well as by quantitative geometric morphometric methods. This indicates that a true cryptic diversity is probably a relatively rare phenomenon in desmids.

The results of the thesis (particularly those on the distribution of the *Micrasterias fimbriata* and *M. radians* lineages, on *M. truncata* var. *pusilla* and on several representatives of *Xanthidium antilopaeum* and *X. cristatum* species complexes) also indicate that the species differentiation and actual species diversity of desmids may for a much greater part than generally supposed be related to the patterns of their geographic distribution or to the climatic factors.

Herewith, they contradict the ñubiquity modelò as the possible distribution model of desmids, in favour of Foissnerôs ñmoderate endemicity modelò. However, it is questionable whether there are any cosmopolitan species at all among desmids. Many supposedly cosmopolitic taxa are reported from various parts of the world to occur in ecologically profoundly different habitats. But, in the light of our data and considering the generally very narrow ecological amplitude of desmids, it appears possible that these putative ñecologically tolerantò taxa may in fact be a complex of several species with restricted distribution and specific ecological demands. In this context, future wide employment of the unique approach based on geometric morphometric data, that helped in Paper VI to define the distribution patterns of two pseudocryptic taxa in *Micrasterias fimbriata*, could be very useful concerning the delimitation of distribution patterns of other desmid taxa.

As for the issue of the practical use of desmids in biomonitoring and ecological studies based on species composition data; it is obvious that the methods of their use in such a way will need to be revised and should be based on the newly encountered phylogenetic taxa since their both ecological demands and distribution may (such as in the two *Micrasterias fimbriata* lineages) differ profoundly. On the other hand, these attempts still seem to be much more promising than in the most other groups of microscopic algae. Firstly due to the supposedly low rate of true cryptic diversity in desmids and secondly since their traditional species concepts are, in spite of frequent inner differentiation, still much more robust than in the absolute majority of other microalgal groups, because of the revealed monophyly of most species studied.

Last but not least, the results of this thesis provided data about the phylogenetic and taxonomic identity of several very rare and morphologically peculiar taxa (e.g. *Triplastrum simplex*, *Pleurotaenium nodosum* or *Staurastrum tumidum*). They also demonstrated the usefulness of geometric morphometric methods for taxonomic revisions of desmid species as the majority of their descriptions are based on the iconotypes. Finally, the *trn*G$^{\mathrm{ucc}}$ marker turned out to be very useful for resolving intraspecific diversity within various desmid species complexes and is therefore considered a good candidate for a green algal barcode, in particular for Streptophytes.

To sum up: Obviously, the way we recently see the diversity and distribution of desmids should be thoroughly changed. The real phylogenetic species diversity is mostly finer, often corresponds to varieties of the traditional morphologically defined species and is usually well determinable using combination of molecular and morphological data. The biogeographical areas of these phylogenetic species are probably usually much narrower and the proportion of regionally restricted or even endemic species consequently much higher than generally supposed. This makes desmids, that are already well known for their vulnerability to all kinds of pollution and disturbance, even more susceptible to losses in biodiversity and further stresses the need for conservational strategies of their habitats. Polyphasic approach, based on combination of several mehods, yields a new level of interpretations that could not be reached by the use of any of these methods alone. Nevertheless, it is obvious that the investigation of hidden species diversity in desmids is still at the beginning.

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6. CURRICULUM VITAE

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Study and practice:

- since October 2005 ï position of a research worker at the Department of Botany, Faculty of Science, Charles University in Prague
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- 1999 - 2005 - M.Sc. study of Biology, Faculty of Science, Charles University in Prague; Title of the M.Sc. thesis: Diversity and ecology of desmids in selected areas of the Czech Republic.
- since 2005 ï PhD. study in Botany, Faculty of Science, Charles University in Prague

Study abroad:

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Publications in SCI journals:

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