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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

Authorôs contributions:

II. Me and JiŚí 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 Ġkaloud was responsible for molecular and JiŚí 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. JiŚí Neustupa wrote the major part of the paper and made the geometric morphometric analyses, Pavel Ġkaloud 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 ĠŠastný

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

....

JiŚí Neustupa Pavel Ġkaloud

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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, Poulíčková et al. 2010, Škaloud & Peksa 2010, Fučíková et al. 2011, Kucera & Saunders 2012, Moniz et al. 2012, Škaloud 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 ("false") desmids (members of family Mesotaeniaceae from order Zygnematales) and the placoderm ("true") 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.

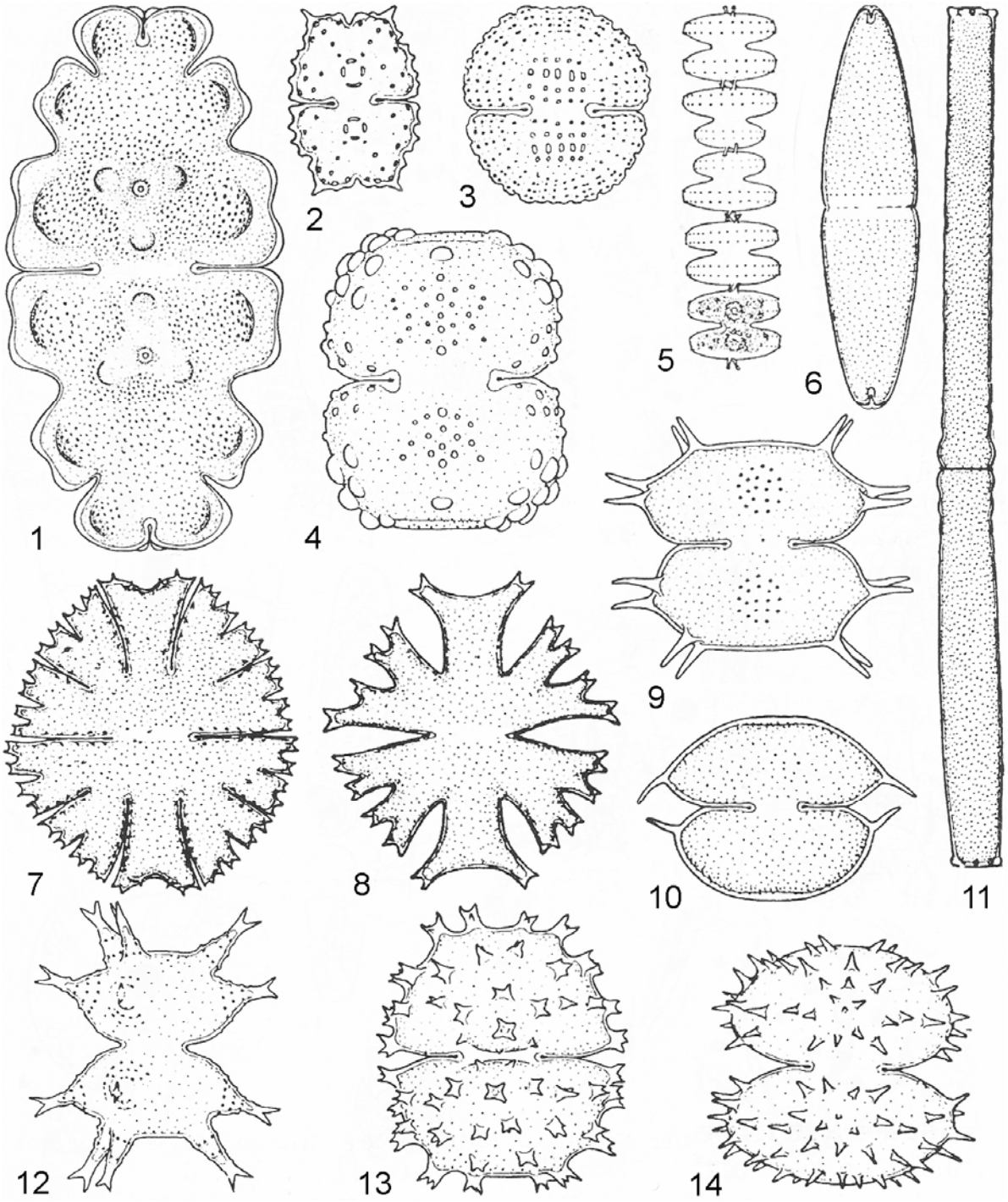

Fig. 1. Variability of cell shapes and ornamentation in various desmids. (1) *Euastrum oblongum* (2) *E. turneri* (3) *Cosmarium formosulum* (4) *C. ungerianum var. subtriplicatum* (5) *Sphaerozosma aubertianum* (6) *Tetmemorus granulatus* (7) *Micrasterias papillifera* (8) *M. crux-melitensis* (9) *Xanthidium antilopaeum* (10) *Staurodesmus convergens* (11) *Pleurotaenium ehrenbergii* (12) *Staurastrum furcigerum* (13) *S. spongiosum var. perbifidum* (14) *S. teliferum*. Modified after Růžička, 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 (Růžička 1977, Coesel & Meesters 2007).

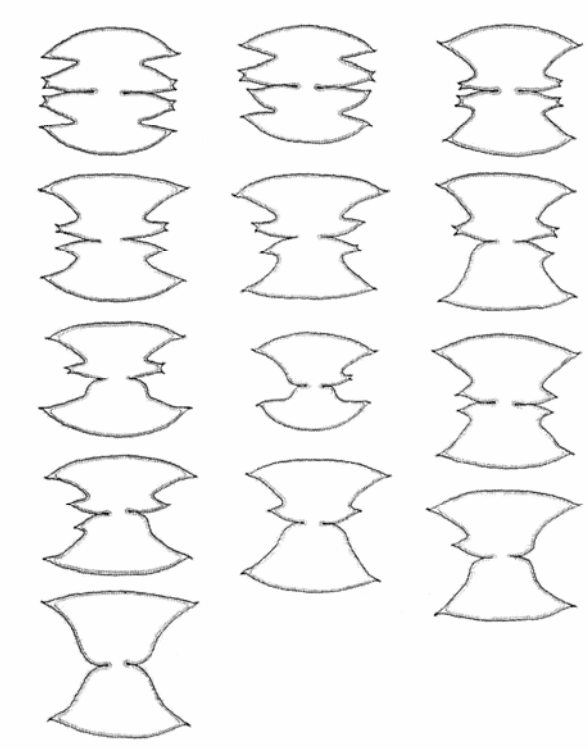

Fig. 2. Different morphological expressions seen in one population of *Micrasterias laticeps*. Modified after Bicudo & Sormus 1972.

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. Růžička 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 "typical" 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 'Desmidiologists describe specimens not species'. 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).

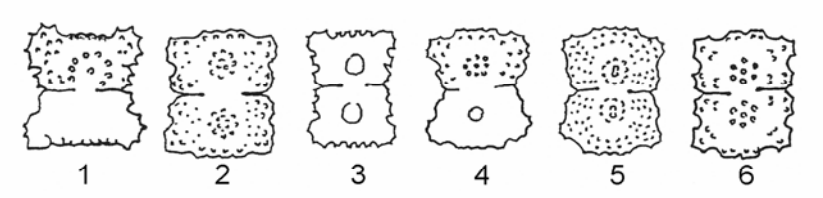

Fig. 3. Six Cosmarium species that are probably synonymous. (1) *Cosmarium wallichii* West & West (2) *C. seelyanum* Wolle (3) *C. nobile* (Turner) Krieg. (4) *C. naivashensis* Rich (5) *C. divergens* Krieg. (6) *C. subnobile* Hinode. After various authors, from Coesel & Krienitz 2008.

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, Škaloud 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. Fučíková et al. 2011, Škaloud et al. 2012), the nuclear ribosomal internal

transcribed spacer (ITS) regions (e.g. Brodie et al. 2007, Kynčlová et al. 2010) or the actin introns (e.g. Nelsen & Gargas 2006, Škaloud & 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 "byproduct", 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 "gold standard" 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, Škaloud 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 "good" 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 "ubiquity model", 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 "moderate endemicity model", 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).

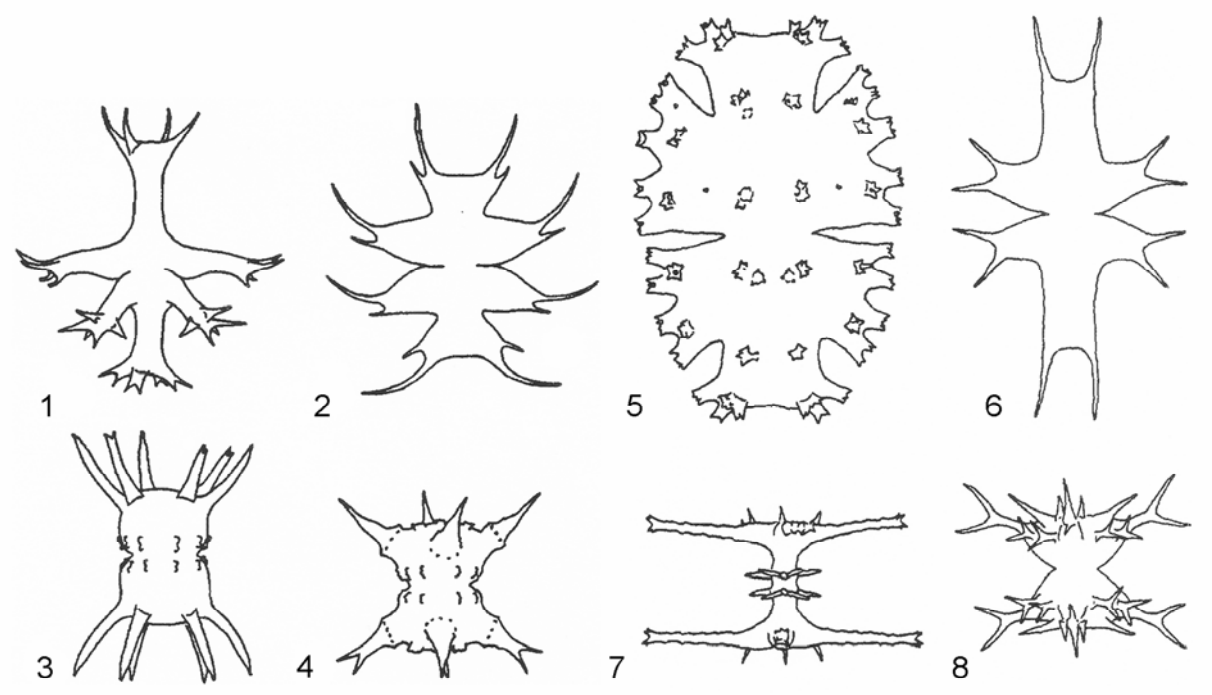

Fig. 4. Desmid taxa characteristic of the equatorial African (1-4) and the Indo-Malaysian/North Australian (5-8) region. (1) *Allorgeia incredibilis* (2) *Micrasterias sudanensis* (3) *Staurastrum rzoskae* (4) *S. fuellebornii* var. *evolutum* (5) *Micrasterias anomala* (6) *M. ceratofera* (7) *Staurastrum 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 "cosmopolitic" or "worldwide". 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 "core" *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 “good” 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 "ubiquity model" as the possible distribution model of desmids, in favour of Foissner's "moderate endemicity model". 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 "ecologically tolerant" 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^{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 – 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.
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Study abroad:

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Publications in SCI journals:

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ATTACHMENTS

Paper I

Desmids (Conjugatophyceae, Viridiplantae) from the Czech Republic; new and rare taxa, distribution, ecology

Jan Šťastný

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Desmids (Conjugatophyceae, Viridiplantae) from the Czech Republic; new and rare taxa, distribution, ecology

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Abstract: The present work summarizes the current diversity, distribution and autecology of desmids found within the Czech Republic; the focus is on the occurrence and autecology of rare taxa. Data are based on the author's extensive sampling from 2003–2009, during work for both his master's degree, and currently, his Ph.D. dissertation. Over 1 400 samples were collected, from various types of wetland habitats ranging from eutrophic fishponds, diverse bogs and fens, to ephemeral pools and various aerophytic habitats. Altogether, 526 taxa of desmids (401 species) belonging to 27 genera were found, 80 of them newly described in the Czech Republic. In the present work, 169 rare or otherwise noteworthy taxa, belonging to the following genera: *Mesotaenium* (1), *Netrium* (1), *Roya* (2), *Tortitaenia* (1), *Gonatozygon* (2), *Closterium* (14), *Haplotaenium* (2), *Pleurotaenium* (3), *Docidium* (1), *Actinotaenium* (6), *Euastrum* (9), *Micrasterias* (7), *Cosmarium* (78), *Xanthidium* (7), *Staurodesmus* (4), *Staurastrum* (25), *Cosmocladium* (2), *Sphaerozosma* (2), *Hyalotheca* (1) and *Desmidium* (1) are depicted by line drawings and briefly discussed with regard to their ecology, taxonomy or distribution within the Czech Republic or Central Europe. In addition, SEM images are provided for 45 taxa, and, finally, a comprehensive table is included with indicative notations concerning all taxa found.

Key words: bioindicators, Czech Republic, desmids, distribution, ecology, rare taxa, species diversity

Introduction

Although the study of desmids has a very long tradition in the Czech Republic (Šťastný 2005), and many studies carried out in this country have been considered, at least in part, with this group of algae, knowledge of the diversity and distribution of desmids in the Czech Republic remains scarce. This is largely due to the extremely small number of high–quality, specialized publications appearing over the past 40 years, and to the fact that many works, particularly older ones, lack algal illustrations, and therefore, their data must be regarded with much reserve. Moreover, due to the recent negative impact of human activity (acidification, eutrophication), which causes changes in the nature of wetland biotopes (Lederer 1998; Kouwets 1999), it is highly probable that the majority of previously acquired data is at present already invalid or obsolete. This, unfortunately, also applies to the localities that were once the most desmid–rich within the Czech Republic (see Pascher 1903, 1906; Lütkemüller 1910; Japp 1930b; Rosa 1951; Růžička 1973) that currently have either been destroyed, or their character has been completely altered.

The primary objective of the present work was to remedy the above–mentioned lack of reliable data on the occurrence and distribution of desmids in the Czech Republic, and the pragmatic reason for this undertaking is that the material is simply too voluminous to publish in the form of a journal article.

However, the present work was not designed to be a true flora, like e.g. Růžička (1977, 1981), Lenzenweger (1996, 1997, 1999, 2003b) or Coesel & Meesters (2007). These works are sufficient for a routine determination of desmids from the Czech Republic, because they are aimed at desmids from climatically similar regions, and, as they are adequate and quite satisfactory I thought it more effective to avoid overlapping descriptions or accounts of common taxa. Instead, it seemed much more consequential to focus in greater detail on rare, often poorly known, taxa, some of them even not mentioned in any of the floras mentioned above.

A fundamental problem in the traditional desmid taxonomy based upon morphology is the frequent descriptions of new taxa based

on unsubstantiated evidence of only slight morphological differences; this fails to take into account the natural morphological plasticity of the particular taxa (Růžička 1955b; Grönblad & Růžička 1959; Kouwets 1988, 2008). Therefore, where possible, all taxa discussed herein (especially little–known taxa), were illustrated by more than one drawing to demonstrate the extent of their morphological variability. Moreover, for every taxon some basal data [dimensions, reference to sampling sites, most frequently used synonym(s)] are listed, and each taxon is briefly discussed with respect to publications reporting it from the Czech Republic, and where available, some notes concerning its taxonomy or autecology are attached.

As an overview of all taxa found, and to allow a quick retrieval of basal information concerning a particular species, a comprehensive, notated table is included (Table 1). In this table, analogically to Coesel (1998a), the following aspects of the ecology of every taxon are evaluated:

Trophic state of the habitat. Three classes are distinguished: oligotrophic (low concentrations of nutrients, little aquatic biomass), mesotrophic (moderately rich in nutrients) and eutrophic (high concentrations of nutrients, large aquatic biomass).

Acidity. Three classes are distinguished: acidophilous (occurring at pH < 6.5), circumneutral (pH 6.5–7.5) and alkaliphilous (pH > 7.5).

Life form. Three types are distinguished: atmophytic (living in a thin water film of wet, periodically desiccating substrates), benthic (living on the under–water soil or associated with submerged aquatic plants) and planktonic (living in large water bodies, passively floating).

Rarity. Three categories are distinguished: (1) occasional occurrence; (2) rare; (3) very rare. Common and easily confusable species (in particular some small, smooth–walled *Cosmarium* taxa or some *Staurastrum* species with arm–like processes) are not labeled.

Ecological sensitivity. This parameter reflects the maturity and degree of inner complexity of the ecosystem that a particular species is indicative of, and, indirectly, also indicates its vulnerability, and the time necessary for its restoration. Clearly, the more complex (internally differentiated) an ecosystem, the more susceptible it becomes to being disturbed, and the more time is required for its restoration (Coesel 1998a, 2001). Marks range from 1 (moderately indicative, occurring also in earlier succession stages) to 3 (most indicative; the species in question seems to be characteristic of highly structured, finely balanced ecosystems). Taxa with a wide ecological amplitude, not being indicative of a vulnerable habitat type, and taxa easily confused with others were not labeled.

Contrary to Coesel (1998a), and to preclude inaccuracies, not only all species, but also all infraspecific taxa have been labeled in Table 1 as to their basic parameters, because they often observably represent separate species having ecological demands completely different from the nominate variety (compare e.g. *Xanthidium antilopaeum* Kütz. and its "var." *laeve* Schmidle). Moreover, some infraspecific taxa clearly representing only phenotypical forms of the nominate variety (e.g. *Micrasterias americana* Ralfs var. *boldtii* Gutw.) were not included in the table. All values for individual parameters listed (particularly "rarity" values) are based only on the author's personal opinion, records and experience; earlier records were not considered. Therefore, the data reflect only the current situation, but considering the large number of samples they are based upon, and the wide spectrum of habitats they represent, they can be presumed to be representative of the Czech Republic as a whole, although the true current biodiversity of desmids within the Czech Republic is undoubtedly somewhat greater. For instance, Štěpánková et al. (2008) reported some species from the Jizerské hory Mts not included in Table 1, and the author himself found several taxa that he was unable to identify reliably; some of these will be described as new species elsewhere.

The last–mentioned parameter (ecological sensitivity of the individual species), may provide additional (next to e.g. species diversity), interesting information about the particular habitat sampled. It has been put into practice by Coesel who used the fact that desmids are ecologically highly sensitive bioindicators and developed a complex method for evaluating and quantifying the nature conservation value of a given aquatic site based on the vulnerability of the ecosystem as indicated by the composition of its desmid flora (Coesel 1998a, 2001; see also Coesel 2003 and Coesel & Meesters 2007; CD attached). Principally, this method is based on three criteria (species diversity, the occurrence of rare species and the occurrence of ecologically sensitive species), and has been primarily tailored to Dutch

conditions, but, as mentioned by Coesel himself, it might also be applicable in geographically similar areas, which would include the Czech Republic. The very processes of evaluation and collection of the data necessary for the assessment of nature values (particularly the transformation schemes for individual water types) are suitable for Czech conditions, and may be found in the above–mentioned publications (Coesel 1998a, 2001; Coesel & Meesters 2007; CD attached) along with some examples of the method's application.

Nevertheless, there are some differences between these two countries, and due to considerable contrast particularly as concerns the rarity of some taxa in both countries, it seemed more accurate to calibrate the species–related data according to the Czech conditions, as summarized in Table 1.

Material and methods

The samples were taken from various types of wetland habitats ranging from eutrophic fishponds, diverse bogs and fens, to ephemeral pools and various aerophytic habitats. The sampling sites are designated on a map (Fig. 1).

The algal material was collected by squeezing out dominant aquatic plants and mosses and aspirating the algae from the sediment with a syringe. Plankton was sampled using a plankton net with 20 or 40 µm mesh. Environmental variables (pH, conductivity) were measured either with Combo HI 98129 (HANNA, Germany) portable instrument or with a pH–meter WTW 330 and conductometer WTW LF 315 (WTW, Germany). Preparations were made by mixing one drop of material with one drop of glycerin, and drawings were made with the aid of a drawing apparatus. The following abbreviations are used in the text: (Syn.) synonym(s); (Dim.) dimensions; (L) length; (B) breadth; (Ls) length without spines; (Lc) length with spines; (Bs) breadth without spines; (Bc) breadth with spines; (I) isthmus; (Occ.) occurrence; (rr) very rare; (r) occasional; (c) abundant; (cc) very abundant; (m) mass occurrence.

Records of the individual taxa within the Czech Republic were ascertained from the publications of Lhotský & Rosa (1955) and Poulíčková et al. (2004) and several other sources not included in those works, such as: Roubal (1939), Lhotský (1954), Šimek (1992, 1997), Lederer (1998), Lederer et al. (1998), Neustupa et al. (2002), Šejnohová et al. (2003), Nováková (2003, 2004), Kitner et al. (2004), Hašler et al. (2008) and Štěpánková et al. (2008). Taxa that are new for the Czech desmid flora, are designated with an asterisk (*) before the species name.

For scanning electron microscopy (SEM) glass coverslips (10 mm in diameter) were washed with acetone, placed on a heating block, and coated three times with a poly–L–lysine solution (1:10 in distilled water) to ensure better adhesion of the desmid cells. After cooling, a drop of the formaldehyde–fixed material was placed on the glass and when almost dry, it was transferred into 30% acetone and dehydrated by an acetone series (30, 50, 70, 90, 95, 99% and 2x in 100%, 10 minutes each). Finally, the cells were dried to critical–point with liquid CO_2, subsequently sputter coated with gold and examined using Phenom Desktop scanning electron microscope.

Results and discussion

***Mesotaenium caldariorum* (Lagerh.) Hansg. (Fig. 2)**
Dim.: L: 35–48 µm, B: 11.5–12 µm
Occ.: 36rr, 40rr

Aerophytic species readily distinguished by the conically attenuated and usually slightly asymmetrical (Coesel & Meesters 2007) cell poles. It is typically reported from artificial habitats such as rain drain pipes (see Coesel et al. 2006). Its two previous findings from within the territory of the Czech Republic (Czurda 1946; Komárek & Rosa 1957) were from this type of habitat, however, interestingly, I found it only in natural settings.

****Netrium pseudactinotaenium* Coesel (Figs 3–6)**
Dim.: L: 36–59 µm, B: 17.5–20 µm
Occ.: 1rr (at an oligotrophic, acidic site with pH = 4.4), 30r, 102rr

Strictly acidophilous, only very recently described species characterized in particular by plasmatic strings that create the impression of a grooved cell edge (Coesel 2002). To date it has been reported, to my knowledge, only from the type site in The Netherlands; thus, my case constitutes its second global published record. Judging from the nature of my sampling sites, its occurrence is limited to well–preserved biotopes, almost untouched by human activity.

****Roya cambrica* W. et G.S.West (Fig. 7)**
Dim.: L: 133–171 µm, B: 6.5–7 µm
Occ.: 17rr

Roya cambrica, most likely, is a very rare species,

reported within Europe only from Great Britain and Norway (Krieger 1937), and recently also from The Netherlands (Coesel & Meesters 2007).

***Roya closterioides* Coesel (Figs 8–12)**
Dim.: L: 50–125 µm, B: 2.5–3.3 µm
Occ.: 1rr, 6rr, 9rr, 13cc, 17m, 24rr, 25r, 47rr, 99rr

This species has only been described very recently by Coesel (2007). Its cells differ from those of the similar *Roya pseudoclosterium* (J.Roy) W. et G.S.West described by West & West (1896) by their fusiform shape, somewhat more irregular curving and, on average, a slightly shorter length. So far, *R. closterioides* is known only from the Dutch type locality, but the frequency of my findings indicates that it is probably not actually rare, but due to its extreme inconspicuousness and insufficient knowledge among researchers it often remains unnoticed or misidentified. From the Czech Republic only *R. pseudoclosterium* has been reported thus far (Lütkemüller 1910; Štěpánková et al. 2008), but the rather short cell length of Lütkemüller's specimens (34–118 µm) indicate that his finding may in fact represent the species in question, rather than *R. pseudoclosterium*.

***Tortitaenia bahusiensis* (Nordst. et Lütkem.) Coesel (Fig. 13)**
Syn.: *Spirotaenia bahusiensis* Nordst. et Lütkem.
Dim.: L: 25–45 µm, B: 10–10.5 µm;
Occ.: 91rr

Rare, aerophytic species that appears to be more common on artificial substrates (Rieth 1982; Tomaszewicz & Hindák 2008; see also Šťastný 2008) than in natural habitats.

***Gonatozygon aculeatum* Hastings (Figs 14–15, 340)**
Dim.: L: 87–283 µm, B: 7–11 µm
Occ.: 1r, 4r, 5r, 47rr

In Central Europe, according to Růžička (1977), a very rare species, from the Czech Republic previously reported only by Růžička (1973). The spines of my specimens were rather short, up to 2.5 µm, the apices typically club–shaped (Fig. 15), only in some specimens from sampling sites no. 1 and 47 similar to that of *G. monotaenium* De Bary, i.e. slightly dilated (Fig. 14).

***Gonatozygon brebissonii* De Bary var. *alpestre* Růžička (Figs 16–18)**
Dim.: L: 25–65 µm, B: 5.5–7.5 µm
Occ.: 36cc, 40rr, 43rr

A rare taxon with a probably arctic–alpine distribution (Kouwets 1997; Šťastný 2008). To date it has been reported only from the type locality in the High Tatra Mountains (Růžička 1967) and from the Eastern Pyrenees (Kouwets 1997).

***Closterium archerianum* Cleve var. *pseudocynthia* Růžička (Figs 19–20)**
Dim.: L: 88–118 µm, B: 11.5–13 µm
Occ.: 8rr, 21r, 25r, 29rr, 47r, 48r, 49r, 86rr, 98r, 123r

Published findings thus far only from the type locality (Růžička 1973), from several localities in France (Kouwets 1997), from Austria (Šťastný & Lenzenweger 2008) and from the West of Ireland (John & Williamson 2009). The taxon under discussion, however, is most probably often confused with the frequently co–occurring (Kouwets 1997; personal observation) and generally much more common *Cl. cynthia* De Not., as mentioned by Růžička (1977).

***Closterium braunii* Reinsch (Figs 21–22)**
Dim.: L: 560–634 µm, B: 41–45 µm
Occ.: 47rr

This species is distinguished by its very remarkable cell wall sculpture in combination with the brownish color of the cell wall. In Central Europe is it very rare (Růžička 1973), my finding is the first from the Czech Republic. For discussion concerning the characteristic wall–sculpture see Kouwets (1991).

***Closterium calosporum* Wittr. var. *brasiliense* Børges. (Fig. 23)**
Dim.: L: 150–205 µm, B: 9–10 µm
Occ.: 1r, 4r, 17rr

According to Růžička (1977) in Central Europe a rather common taxon that, however, seems to be rare in the Czech Republic, being formerly reported only by Růžička (1973).

***Closterium cornu* Ralfs var. *upsaliense* Nordst. (Figs 24–25)**
Dim.: L: 42–67 µm, B: 4–5.5 µm
Occ.: 1rr, 4r, 36r, 37r, 43r, 50rr, 54rr, 100r, 118rr

According to Růžička (1977), this taxon is rare in Central Europe, but it is possible that it often remains unnoticed because of its small dimensions. So far only one finding has been reported from the Czech Republic (Roubal 1958). Interestingly, in my samples the alga in question always grew sub–aerophytically. The specimens depicted by Williamson (1997, pl. 1, fig. 5) and designated as *Cl. pygmaeum* Gutw. also clearly represent the taxon in question.

***Closterium delpontei* (G.A.Klebs) Wolle (Figs 26–27)**
Dim.: L: 530–813 µm, B: 28–38 µm
Occ.: 1r, 4r, 9r, 25r, 47rr, 48 rr

Rather rare species of well–preserved mesotrophic habitats. Previous findings from the Czech Republic only from Southern Bohemia (Rosa 1951, 1969; Roubal 1959; Růžička 1973). Note, it was only recently described from Austria (Šťastný & Lenzenweger 2008), which is one of the best explored European countries in regards to desmids. Possibly, this might be due to the fact that it is similar to the closely related *Cl. lineatum* Ralfs and the occasionally occurring intermediate forms are very hard to identify (Růžička 1977).

***Closterium exile* W. et G.S.West (Fig. 28)**
Dim.: L: 80–100 µm, B: 7.5–8 µm
Occ.: 61rr, 113rr, 119rr

This species is readily distinguishable from similar curved *Closterium* species predominantly by a rounded apex that lacks any light–microscopically visible pore (Růžička 1977). It's ecology is also very characteristic; it is usually detected at higher altitudes and typically grows sub–aerophytically. Previous reports of this species within the Czech Republic (Růžička 1956, 1957a, 1957b), as well as my present findings, have been from this type of habitat.

****Closterium nematodes* Joshua var. *proboscideum* W.B. Turner (Figs 29–31)**
Dim.: L: 213–325 µm, B: 22.5–30 µm
Occ.: 4rr, 26rr, 99r

According to Krieger (1937) and Růžička (1977) a species found mostly ordinarily in the tropics. Occurrence of the type variety (*Cl. nematodes* Joshua var. *nematodes*) is not convincingly documented from Central Europe; var. *proboscideum* is rare in the region (Růžička 1977). The characteristic thickening of the cell wall below the apex was well developed in the population from sampling site no. 26 (Fig. 29), on the contrary, in populations from sampling sites no. 4 and 99 it was rather inconspicuous (Figs 30–31).

****Closterium pseudopygmaeum* Kouwets (Figs 32–33)**
Dim.: L: 28–63 µm, B: 5.8–7 µm
Occ.: 36rr, 43r, 87rr

This species is characterized by its small dimensions, a slanted apex with an apical pore (Kouwets 2001), and occurrence predominantly in ephemeral pools and on wet substrates. However, the pore is often quite inconspicuous and may be easily overlooked, especially in fixed material. Thus far this alga has only been reported from several sites in France (Kouwets 2001).

***Closterium pusillum* Hantzsch (Figs 34–36)**
Dim.: L: 28–59 µm, B: 7.5–12 µm
Occ.: 7r, 38rr, 104c

Rather rare species of ephemeral habitats. Previous records from the Czech Republic include: Lütkemüller (1910, *Cl. pusillum* var. *monolithum* Wittr.), Roubal (1959), Rosa (1969) and Šejnohová et al. (2003).

***Closterium subulatum* (Kütz.) Bréb. (Figs 37–38)**
Dim.: L: 149–232 µm, B: 6–9 µm
Occ.: 15r (in the pools), 28rr, 59rr, 121rr

Apparently a quite adaptable species. It is reported most frequently from mesotrophic, slightly acidic to neutral waters (Růžička 1977; Coesel 1979a; Lenzenweger 1996; Coesel & Meesters 2007), alternatively, Förster (1965) found it in a peat–bog, most likely a markedly more acidic habitat, and moreover, all of my findings came from more or less eutrophic and alkaline habitats. So far only three findings have been reported from the Czech Republic (Rosa 1951, 1968, 1969).

***Closterium tortitaenoides* Coesel (Figs 39–41)**
Dim.: L: 51–68 µm, B: 8–8.5 µm
Occ.: 24c (in a strongly acidic, oligotrophic pool)

This species was previously only reliably known from several sites in The Netherlands, but it is possible that it has been confused with *Cl. navicula* (Bréb.) Lütkem. For notes concerning its taxonomy see Coesel (2002, 2007).

***Closterium tumidum* Johns. (Figs 42–43)**
Dim.: L: 92–145 µm, B: 14–18 µm
Occ.: 35cc, 52rr, 54rr, 73r, 110rr, 112cc, 113c

This species is considered to be rare in Central Europe (Růžička 1977), and is seldom reported even from neighboring countries (Gutowski & Mollenhauer 1996; Marhold & Hindák 1998; Lenzenweger 2003b). However, it is probably quite abundant in suitable biotopes (oligotrophic, slightly acidic to neutral waters, e.g. mountain brooks or spring areas), where it usually grows sub–aerophytically. This assumption is supported by the relatively numerous findings of this species at such sites in the Czech Republic (Dvořák 1920, 1920/21; Fischer 1924; Rosa 1939, 1951, 1969; Růžička 1954, 1957a) as well as by the relative high number of my own records. At site no. 35, I found a very rare form with clearly recognizable apical pores (Fig. 42), the occurrence of which was only mentioned previously by Růžička (1954, 1957a) .

***Closterium tumidum* Johns. var. *nylandicum* Grönblad (Fig. 44)**
Dim.: L: 125–157 µm, B: 10–11.5 µm
Occ.: 23r (growing sub–aerophytically)

A rare alga (Růžička 1977) that might be easily confused with the much more common *Cl. cornu* Ralfs, whose cells, however, are more slender and lack a distinctly inflated ventral side. To date there have been four documented findings from the Czech Republic (Rosa 1939, 1951, 1969; Rybníček 1958; as *Cl. tumidum*).

***Closterium turgidum* Ralfs var. *giganteum* (Nordst.) De Toni (Fig. 45)**
Dim.: L: 690–812 µm, B: 74–85 µm
Occ.: 9rr, 14rr, 25rr

This species is described as having a cell wall delicately striate, and pyrenoids scattered throughout the chloroplast. In my opinion, it is debatable whether the taxon in question is related to the "real" *Cl. turgidum* Ralfs, because there are considerable differences between both taxa as regards their morphology (see Růžička 1977) as well as their ecology; *Cl. turgidum* is a benthic species of slightly acidic, mesotrophic bogs and fens, while, my findings, by contrast, originate from the tychoplankton of greater water bodies.

***Haplotaenium indentatum* Kouwets var. *latius* Kouwets, morpha (Figs 46–53, 341–342)**
Dim.: L: 66–155 µm, B: 14–18 µm
Occ.: 1rr, 3cc, 4c

The taxon in question has only been described rather recently (Kouwets 1991) from France, but is probably often confused with the similar *Haplotaenium minutum* (Ralfs) Bando that, however, lacks the apical indentation (e.g. pl. 32, fig. 8 in Coesel & Meesters 2007 most likely represents *H. indentatum* var. *latius*). My material differs from that of Kouwets (1991) by a shorter cell length resulting in a lower length/breadth ratio (L/B = 5.5–10, Kouwets reports L/B = 11–15); however, as these characters were often extremely variable, even within populations from the same sampling site, the description of the taxon in question as a new variety of *H. indentatum* may not be justified. Judging from the nature of my sampling sites, *H. indentatum* var. *latius* seems to prefer a distinctly acidic and oligotrophic environment, which is in accordance with the data of Kouwets (1991). I recently also found it in the desmid–rich "Schwemm bei Walchsee" bog in Austria in a similar habitat type (Šťastný, unpublished; see also Lenzenweger 2000b; Šťastný & Lenzenweger 2008).

***Haplotaenium rectum* (Delponte) Bando (Figs 54–55)**
Syn.: *Pleurotaenium rectum* Delponte
Dim.: L: 230–310 µm, B: 18.5–21.5 µm
Occ.: 1r, 4r, 5r, 8rr, 19rr, 25r

This species was considered to be quite rare in Central Europe (Růžička 1977), and the only previous report of it in the Czech Republic was mentioned by Roubal (1939).

***Pleurotaenium eugeneum* (W.B.Turner) W. et G.S.West (Figs 56–58)**
Dim.: L: 520–828 µm, B: 37–47 µm
Occ.: 1r, 9r, 13rr

The only previous finding of this species in the Czech Republic was stated by Rosa (1951), in Central Europe it is considered to be rare (Růžička 1977). The specimen from site no. 1 (Fig. 58) differed from "typical" *Pl. eugeneum* (see Růžička 1977) as found at sites no. 9 and 13 (Figs 56–57) by, on average, more robust cells slightly tapering toward the apices, and by semicells scarcely having detectable swellings beyond the basal inflation. Nevertheless, their determination as *Pl. eugeneum* appears to be justified enough, particularly due to the presence of the characteristic whorl of spherical granules at the apices, 9–12 granules being visible in face view (Růžička 1977).

***Pleurotaenium simplicissimum* Grönblad (Figs 59–61, 343)**
Dim.: L: 573–740 µm, B: 32.5–40 µm
Occ.: 1rr, 4rr, 5rr

A species easily distinguished by rod like cells, a very slight basal inflation of the semicells, and by truncate apices provided with a whorl of tiny, longish, often inconspicuous granules (Figs 60, 343), 8–12 being visible in face view (Růžička 1977). Interestingly, at sampling site no. 4, I usually found cells with slightly inflated semicells (Fig. 61) that might be considered an anomaly. According to Růžička (1977), *Pleurotaenium simplicissimum* is extremely rare in Central Europe.

***Pleurotaenium tridentulum* (Wolle) W.West (Figs 62–64)**
Dim. (dimensions apply to half–cells only): L: 95–131 µm, B: 13.5–15 µm
Occ.: 30r

A strictly acidophilous, in Central Europe very rare species (Růžička 1977), with only one previous finding from the Czech Republic (Roubal 1939). I repeatedly found the alga at this site, always in the form of empty half–cells only, but quite abundant in one of the pools; the occurrence of living specimens at this particular site, or at some other high moors location within the "Modravské slatě" complex, is nonetheless likely.

***Docidium baculum* Ralfs (Figs 65–66)**
Dim.: L: 238–295 µm, B: 13–14.5 µm
Occ.: 1r

In Central Europe this taxon occurs scattered (Růžička 1977), in the Czech Republic it is very rare. Past findings are recorded by Pascher (1903, 1906), Roubal (1939) and, more recently, Lederer at al. (1998); however, the latter, at site no. 99, was not confirmed by my findings.

***Actinotaenium cruciferum* (De Bary) Teiling (Figs 67–68)**
Syn.: *Cosmarium cruciferum* De Bary
Penium cruciferum (De Bary) Wittr.
Dim.: L: 17.5–20 µm, B: 10–11.5 µm
Occ.: 3rr, 8r

There are only three earlier findings within the territory of the Czech Republic (Rosa 1951; Růžička 1957a; Štěpánková et al. 2008). *A. cruciferum* might be easily confused with the much more common *Cosmarium goniodes* W. et G.S.West var. *subturgidum* W. et G.S.West that varies particularly by a somewhat different frontal and lateral view (see Fig. 152).

***Actinotaenium inconspicuum* (W. et G.S.West) Teiling (Figs 69–70, 344–345)**
Syn.: *Cosmarium bacillare* Lütkem.
Penium inconspicuum W. et G.S.West
Dim.: L: 12.5–19 µm, B: 5–6.5 µm
Occ.: 1r, 4rr, 5rr, 14rr, 24rr, 43cc

To date only two documented findings from the Czech Republic (Rosa 1941; Růžička 1973); however, due to its diminutive size it probably often goes unnoticed.

***Actinotaenium inconspicuum* (W. et G.S.West) Teiling var. *curvatum* Kouwets (Figs 71–72)**
Dim.: L: 13–17 µm, B: 4.5–5 µm
Occ.: 1r

This taxon is as yet known only from several sites in France (Kouwets 1991) and Austria (Šťastný & Lenzenweger 2008), however, due to similarity to the nominate variety, it might be easily overlooked. Gontcharov (1998) described *A. inconspicuum* var. *curvatum* f. *majus* that, however, is evidently identical to *A. infractum* (Messik.) Williamson (see Williamson 2007).

***Actinotaenium kriegeri* (Messik.) Kouwets (Figs 73–74)**
Syn.: *A. adelochondrum* (Elfving) Teiling var. *kriegeri* (Messik.) Růžička
Dim.: L: 24–30 µm, B: 11.5–13.5 µm
Occ.: 38rr, 43r, 45r, 50rr, 54cc

Rather rare species of oligomesotrophic, ephemeral and hemi–atmophytic habitats (KOUWETS 1997). Its characteristic feature are pores with pore–apparatuses increasingly protruding toward the apices (Fig. 73) rendering them seemingly scrobiculate. However, generally only some apical pores are visible (Fig. 74).

***Actinotaenium perminutum* (G.S.WEST) TEILING (Figs 75–77)**
Syn.: *Cosmarium perminutum* G.S.WEST
Cylindrocystis minutissima W.B.TURNER
Dim.: L: 10.5–14 µm, B: 6.5–7.5 µm
Occ.: 1r, 3rr, 4rr, 5rr

Apparently a rather rare taxon (RŮŽIČKA 1981) that, however, can easily be unnoticed because of its small size.

***Actinotaenium subsparsepunctatum* (GRÖNBLAD) COESEL (Figs 78–81)**
Syn.: *Cosmarium subtile* (W. et G.S.WEST) LÜTKEM. var. *subsparsepunctatum* GRÖNBLAD
Dim.: L: 14–16 µm, B: 9–10 µm
Occ.: 37cc

Although I did not find the characteristic zygospores (see COESEL 2002; figs 31–32), there is no doubt that the species in question has been identified correctly, because the cell shape, and the structure of chloroplasts (lobostelloid), as well as the ecology of my find site (in an ephemeral puddle) accurately correspond to Coesel's data.

***Euastrum biscrobiculatum* (WOLOSZ.) COESEL (Figs 82–84)**
Dim.: L: 23–33 µm, B: 17–24 µm, I: 5.5–7 µm
Occ.: 1r, 4rr, 5rr

This species is marked by its striking cell wall sculpture consisting of a combination of tubercles, scrobiculae and grooves (COESEL & MEESTERS 2007). Apparently it is very rare, and judging from the nature of the sampling sites where I found it, its occurrence seems to be limited to well–preserved biotopes with a high desmid diversity. However, it has a complicated taxonomy and might be readily confused with similar taxa (see COESEL 1984a, 2007). Interestingly, at site no. 1 in general I found exclusively larger cells possessing two central scrobiculae (Figs 82–83), on the other hand, at my other sites I found specimens with distinctly smaller cells having only one central scrobicula (Fig. 84).

***Euastrum brevisinuosum* (NORDST.) KOUWETS var. *dissimile* (NORDST.) KOUWETS (Figs 85–90)**
Dim.: L: 21–27 µm, B: 16–18.5 µm, I: 9–10 µm
Occ.: 84c
This rather rarely found taxon (KOUWETS 1984) was from the same sampling site reported already by ŠKALOUD (2004; pl. 11: p–q; pl. 27: f), but wrongly labeled as *E. crassangulatum* BØRGES. For details regarding its taxonomy see KOUWETS (1984).

***Euastrum crassicolle* P.LUNDELL (Figs 91–92, 346)**
Dim.: L: 23.5–27 µm, B: 12.5–14.5 µm, I: 5–6 µm
Occ.: 43r, 50rr

From the Czech Republic only *E. crassicolle* var. *dentiferum* NORDST. has been reported thus far (LÜTKEMÜLLER 1910; RŮŽIČKA 1973); most likely, however, these two varieties are only deviations caused by atypical ecology, e.g. aerophytic growth, which is quite common in this species (RŮŽIČKA 1981). This hypothesis is also supported by my findings at site no. 50, where transitions between the two varieties could be observed even within a single, not very abundant population with an aerophytic growth habit; similar observations of Grönblad have also been mentioned by RŮŽIČKA (1981).

***Euastrum crassum* RALFS (Fig. 93)**
Dim.: L: 135–147 µm, B: 68–75 µm, I: 22–25 µm
Occ.: 19rr, 23rr (at site no. 23 only some empty semicells were found)

In Central Europe this species is considered to be rare (RŮŽIČKA 1981), and from the Czech Republic was it recorded only two times at the beginning of the last century (PASCHER 1903, 1906).

***Euastrum germanicum* (SCHMIDLE) WILLI KRIEG. (Figs 94–95, 347–349)**
Dim.: L: 52–58 µm, B: 41–48 µm, I: 11–12.5 µm
Occ.: 1r, 2rr, 9c, 10rr, 11r, 12rr, 25rr (at all sampling sites in the littoral zone among submerged macrophytes, particularly *Myriophyllum spicatum* L.)

The only previous finding from the Czech Republic was mentioned by ROSA (1951). In Central Europe it is considered a very rare species (RŮŽIČKA 1981; LENZENWEGER 2000a); my findings, together with other recent ones (LENZENWEGER 2000b; LENZENWEGER & WERTL 2001; FEHÉR 2003) indicate,

however, that it is somewhat more common than supposed. It evidently prefers naturally slightly eutrophic habitats with luxurious macrophyte vegetation (see also Coesel 1978, 1998a; Kouwets 1998; Lenzenweger 2000a; Fehér 2003).

***Euastrum luetkemuelleri* F.Ducell. var. *carniolicum* (Lütkem.) Willi Krieg. (Figs 96–97, 350–352)**
Dim.: L: 22.5–29 µm, B: 16–20 µm, I: 6–7.5 µm
Occ.: 1r, 3rr, 4r, 5rr, 6rr

New for the Czech flora, according to Růžička (1981) and Krieger (1937) relatively rare in Central Europe. Not even the nominate variety of this species has been reported from anywhere in the Czech Republic.

***Euastrum montanum* W. et G.S.West (Fig. 98)**
Dim.: L: 27–30 µm, B: 18–20 µm, I: 5–6 µm
Occ.: 31rr, 32rr

There are only two previous reports of this species from the Czech Republic; Lütkemüller (1910) and Nováková (2004).

***Euastrum pinnatum* Ralfs (Figs 99–102)**
Dim.: L: 127–144 µm, B: 71–78 µm, I: 21–23 µm
Occ.: 4rr

I consistently found cells with an open sinus, which Růžička (1981, pl. 64, 4–5) regards as an anomaly. However, Scottish (Williamson 1997) and Dutch (Coesel & Meesters 2007) populations of this species were reported only having cells with an open sinus, therefore, it should not be regarded as anomalous, but as a variable character. *E. pinnatum* is very rare in Central Europe (Růžička 1981), and in the Czech Republic it has been described only twice (Pascher 1903, 1906).

***Euastrum turneri* W.West (Figs 103, 353)**
Dim.: L: 33.5–38.5 µm, B: 26–28.5 µm, I: 8.5–9 µm
Occ.: 1rr, 4rr, 8rr, 114rr

A species with scattered distribution in Central Europe (Růžička 1981), in our territory it is now clearly extremely rare. Previous findings: Lütkemüller (1910), Japp (1930b), Rosa (1951) and Růžička (1973). At site no. 114, most probably, the alga in question is not autochtonous but scoured from adjacent bogs situated in Zone I of Šumava National Park, as also indicated by its finding in a minimal abundance (one cell).

***Micrasterias apiculata* Ralfs (Fig. 104)**
Dim.: L: 192–260 µm, B: 180–220 µm, I: 33–39 µm
Occ.: 1r, 4r, 5rr, 8rr

Earlier findings from the Czech Republic were reported especially from Southern Bohemia (Pascher 1903, 1906; Dechant 1914; Cejp 1929; Roubal 1938, 1958; Rosa 1951; Růžička 1973), and from certain Moravian sites (Fischer 1920; Japp 1930b; Dvořák 1932); Sládeček mentions its presence in Padrťské rybníky (Sládeček 1950–51). At present, however, this species must be considered very rare on our territory. Růžička (1981) describes its distribution within Central Europe as scattered.

***Micrasterias brachyptera* P.Lundell (Fig. 105)**
Dim.: L: 185–237 µm, B: 144–177 µm, I: 30–37 µm
Occ.: 1r, 5rr, 20rr, 103rr

The only findings of this rare species (Růžička 1981) from within the Czech Republic were recorded by Roubal (1939) and Lhotský (1954). The latter also mentions another, unpublished finding by K. Rosa from Southern Bohemia. There are only two localities known from Austria (Lenzenweger 1996), in Germany it is considered to be an endangered species (Gutowski & Mollenhauer 1996).

***Micrasterias fimbriata* Ralfs (Figs 106, 354)**
Dim.: L: 222–273 µm, B: 197–248 µm, I: 30–34 µm
Occ.: 1r, 4r, 5r, 8r, 17rr, 19r, 20rr, 103r

Findings from the Czech Republic were reported from Southern (Pascher 1903; Kotek 1950; Růžička 1973) and Northern Bohemia (Lhotský 1954), and from some sites in Moravia [Fischer 1920, 1924 – as *M. apiculata* Ralfs var. *fimbriata* (Ralfs) Nordst., Čerňajev 1931]; recently it appears to be a rare species of slightly acidic, mesotrophic water bodies. In central Europe scattered (Růžička 1981).

***Micrasterias furcata* Ralfs (Figs 107–108)**
Syn.: *M. radiata* (Nägeli) W. et G.S.West
Dim.: L: 144–150 µm, B: 123–128 µm, I: 22–23 µm
Occ.: 5rr

Within Central Europe a very rare species (Růžička 1981), from the Czech Republic reported only from three sites (Pascher 1903; Cejp 1929; Japp 1930b) so far.

***Micrasterias jenneri* Ralfs (Figs 109, 355)**
Dim.: L: 122–145 µm, B: 90–102 µm, I: 20–23 µm
Occ.: 3c, 4c, 69rr

This rare (Růžička 1981), strictly acidophilous species has been reported from the Czech Republic only three times to date; from the Šumava Mts. (Pascher 1906), from site no. 3 (Mattauch 1936), and recently also from site no. 69 (Štěpánková et al. 2008)

***Micrasterias oscitans* Ralfs (Figs 110–111, 356)**
Dim.: L: 125–145 µm, B: 103–138 µm, I: 23–27 µm
Occ.: 4rr

A strictly acidophilous species, extremely rare in Central Europe (Růžička 1981). In the Czech Republic it has been found only in the Šumava Mts (Pascher 1903, 1906). Moreover, Mattauch (1936) mentions, from sampling site no. 3, *M. oscitans* var. *mucronata* (Dix.) Wille, which, however, falls within the variability of the nominal variety (see also Růžička 1981, p. 571) and should not be described as a separate taxon.

***Micrasterias pinnatifida* Ralfs (Figs 112, 357)**
Dim.: L: 60–64 µm, B: 62–74 µm, I: 11.5–14 µm
Occ.: 1r, 5r

Previous findings from the Czech Republic come from Southern Bohemia (Pascher 1903, 1906; Cejp 1929; Roubal 1939; Kotek 1950; Rosa 1969); in the 19th century the species was also found in Moravia (Nave 1863). In Central Europe it occurs only scattered (Růžička 1981), in the Czech Republic it is at present very rare.

****Cosmarium angulare* Johns. (Figs 113–115)**
Dim.: L: 30.5–35 µm, B: 29–32 µm, I: 9–10 µm
Occ.: 9rr, 59r

A rarely reported species of naturally eutrophic habitats with a well–developed macrophyte vegetation (Kouwets 1991; Lenzenweger & Wertl 2001) that agrees with the conditions of my finds (samples were collected by squeezing out submerged *Myriophyllum spicatum* and *Ceratophyllum demersum* L., respectively).

****Cosmarium basiornatum* (Grönblad) Coesel (Figs 116–117, 358–360)**
Dim.: L: 38–41.5 µm, B: 28–31 µm, I: 15–16.5 µm
Occ.: 7r, 38r, 43r, 45rr, 57rr

Thus far only scarcely reported, but most likely not a really rare alga of ephemeral habitats. Coesel & Meesters (2007) consider *C. basiornatum* identical to *C. cinctutum* Nordst., as described by Nordstedt (1875).

****Cosmarium berryense* Kouwets (Figs 118–119)**
Dim.: L: 20–22 µm, B: 18–20 µm, I: 6–6.5 µm
Occ.: 2rr, 121r

Very rare species of meso–eutrophic habitats, from the much more common species *C. humile* (F.Gay) Nordst. to be distinguished by differences in general cell shape and cell wall ornamentation. For a detailed discussion concerning its taxonomy see Kouwets (1998). Most likely, the figure of *C. humile* in Lenzenweger & Wertl (2001, pl. 2, fig. 14) actually represents *C. berryense*.

***Cosmarium bireme* Nordst. (Figs 120–121)**
Dim.: L: 14–16 µm, B: 13–15.5 µm, I: 3.5–4 µm
Occ.: 47r

Probably rare, but easily confused with some similar taxa (see discussion concerning *C. cyclops* Kouwets in Kouwets 2001). Earlier findings from the Czech Republic mentioned by Fischer (1924) and Růžička (1949, 1973).

****Cosmarium boitierense* Kouwets (Figs 122–124)**
Dim.: L: 16–19 µm, B: 14–16.5 µm, I: 4.5–5 µm
Occ.: 9rr, 10rr

C. boitierense and its var. *inambitiosum* have been described relatively recently from France (Kouwets 1998). According to the original diagnosis, the latter taxon differs from the nominal variety only by its smaller dimensions, and by having a more or less distinct median protuberance on either side of the semicells instead of a small papilla. However, this character can be very similar in both varieties (compare figs 55 and 61 in Kouwets 1998), moreover, the dimensions of both varieties can also vary quite considerably (see also Coesel & Meesters 2007). Therefore, taking into account the very similar ecological preferences of these varieties (Kouwets 1998), it would be better to consider *C. boitierense* only a single, albeit morphologically quite variable taxon, probably with a wide distribution in meso–eutrophic habitats, as predicted by Kouwets (1998).

***Cosmarium brebissonii* Ralfs (Figs 125–126)**
Dim.: L: 82–93 µm, B: 66–75 µm, I: 22–26 µm
Occ.: 8c, 29rr

Recently very rare, previous reports from the Czech Republic dating mostly from the first half of the last century (Pascher 1903, 1906; Japp 1930b; Dvořák 1932; Maloch 1937; Roubal 1939; Rosa 1969).

***Cosmarium carinthiacum* Lütkem. (Figs 127–128)**
Syn.: *C. cymatonotophorum* W.West forma *ornata* Messik.
C. messikommeri Coesel
Dim.: L: 7.5–8.8 µm, B: 8.8–10.2 µm, I: 3–4 µm
Occ.: 1r, 4r

An inconspicuous, very rare taxon of well preserved habitats with a high desmid diversity (see also Šťastný & Lenzenweger 2008).

***Cosmarium ceratophorum* Lütkem. (Figs 129, 361–362)**
Dim.: L: 31 µm, B: 25 µm, I: 7.5 µm
Occ.: 99rr

An extremely rare species, known from the type site in the Šumava Mts (Lütkemüller 1910), from eastern Finland (Grönblad 1921), and from a bog in Austria (Lenzenweger 2003b) that is a remnant of a glacial lake (Lenzenweger 2000b). Therefore, it can be generally considered arctic–alpine and a glacial relict at site no. 99, because the latter also arose during the Quarternary (Hájek & Vízdal 1998).

***Cosmarium commissurale* Ralfs var. *acutum* Bréb. (Fig. 130)**
Dim.: L: 30–33 µm, B: 39–42 µm, I: 11–12 µm
Occ.: 53rr

The taxon in question differs from the nominal variety by the acutely attenuated lateral lobes of the semicells, and a differently shaped apex (Coesel & Meesters 2007), but as shown by Williamson (1997; see also the discussion regarding *C. pseudocommisurale* Kouwets in Kouwets 1991), *C. commisurale* is a highly variable taxon and intermediate forms may occur. However, all cells I found represented *C. commisurale* var. *acutum,* which is considered to be rare in Williamson (1997) and is new for the Czech flora.

***Cosmarium contractum* Kirchn. var. *retusum* (W. et G.S.West) Willi Krieg. et Gerloff (Fig. 131)**
Dim.: L: 31–35 µm, B: 30–34 µm, I: 10–11 µm
Occ.: 1rr, 4rr, 5rr, 8rr, 25r

As indicated already by Coesel & Meesters (2007), this taxon is better kept separate from *C. contractum*, as there are considerable differences in the morphology, as well as in the ecology of these two taxa. Whereas *C. contractum* prefers oligo–mesotrophic milieu, the majority of my findings of var. *retusum* were made in mesotrophic habitats, and at site no. 25, as well as in The Netherlands (Coesel & Meesters 2007), it was even found in a slightly eutrophic and alkaline habitat .

***Cosmarium davidsonii* J.Roy et Bisset (Figs 132–133)**
Dim.: L: 32.5–37 µm, B: 22–24 µm, I: 13–14 µm
Occ.: 50rr, 61rr, 116r, 118r

Rather rare aerophytic species; mentioned in records from the Czech Republic by Lütkemüller (1910), Rosa (1951) and Růžička (1956, 1957a).

***Cosmarium decedens* (Reinsch) Racib. var. *minutum* (Gutw.) Willi Krieg. et Gerloff (Fig. 134)**
Dim.: L: 15.5–18.5 µm, B: 7–9 µm, I: 5.5–7 µm
Occ.: 43rr, 50rr, 54rr

Like the other varieties of *C. decedens*, this alga, too, occurs rather scarcely in ephemeral puddles and on wet soil (Lenzenweger 1999).

***Cosmarium dentiferum* Nordst. var. *alpinum* Messik. (Fig. 135)**
Dim.: L: 55–65 µm, B: 44–53 µm, I: 19–21 µm
Occ.: 61r, 63rr, 73rr, 117rr, 119rr

C. dentiferum var. *alpinum* is generally reported from oligotrophic, mountainous biotopes (Messikommer 1942; Lenzenweger 1999), which agrees well with my findings and, most likely, the alga discussed is relatively common in such habitats in our territory. No findings of this taxon have been reported from the Czech Republic, but considering the drawing of Rybníček [1958; pl. 3, fig. 39, as *C. margaritatum* (P.Lundell) J.Roy et Bisset], his findings from the Jeseníky Mts probably concern this very alga, a supposition supported by his data on the ecological parame-

ters. Possibly Růžička's record from a very similar habitat (Růžička 1956; pl. 3, fig. 29, as *C. margaritatum* forma) also represents *C. dentiferum* var. *alpinum*., in spite of its somewhat greater dimensions.

***Cosmarium didymoprotupsum* W. et G.S.West (Fig. 136)**
Dim.: L: 60–70 µm, B: 52–55 µm, I: 16–17.5 µm
Occ.: 1rr, 2r (at both sites in the littoral zone among *Myriophyllum spicatum*)

Apparently a rather rare tychoplanktonic alga of meso–eutrophic water bodies that, however, might be easily confused by non–specialists with other species exhibiting similar ecological parameters (e.g. *C. botrytis* Ralfs).

***Cosmarium dilatatum* Järnefelt et Grönblad (Figs 137–138)**
Dim.: L: 10.5–11 µm, B: 12–12.5 µm, I: 3.7–4 µm
Occ.: 22c (in plankton)

Apparently a very rare taxon of meso–eutrophic, larger water bodies, until present known only from several sites within Europe (Beijerinck 1926; Järnefelt & Grönblad 1960; Coesel 1989; Kouwets 1998), and from Japan and the Russian Far East (Gontcharov & Watanabe 1999). However, it might be easily overlooked.

***Cosmarium eichlerianum* (Grönblad) Messik. (Figs 139–140)**
Syn.: *C. rectangulare* Grunow var. *subrectangulare* (Lütkem. et Grönblad) Willi Krieg. et Gerloff
Dim.: L: 37–41 µm, B: 30–33 µm, I: 11–12.5 µm
Occ.: 1r, 5r

Rarely reported taxon of mesotrophic, slightly acidic habitats, new for the Czech Republic. However, the figure of Rosa (1951; pl. 13, fig. 5, as *C. sexangulare* P. Lundell) may represent this taxon. The cell wall is often somewhat thickened at the apical and lateral angles, which is not depicted in my drawings.

***Cosmarium fastidiosum* W. et G.S.West (Figs 141–143)**
Dim.: L: 40–53 µm, B: 35–50 µm, I: 14–19 µm
Occ.: 1c

The occurrence of this species is only rarely mentioned in literature (West & West 1908; Skuja 1929; Rosa 1951; Coesel 1979b; Coesel & Meesters 2007; Šťastný & Lenzenweger 2008), and most authors point to its rather great variability both in size and the development of the cell wall structure in the central part of the cell. These results are confirmed by my findings that showed extreme variability of the central ornamentation, even when comparing two semi–cells of the same cell. It is comprised of at least four, but sometimes many, irregularly placed flat granules, and the only stable feature is the presence of one supraisthmal granula. *C. fastidiosum* apparently is a very rare species, which seems to prefer mature ecosystems with a great diversity of desmids (see also Coesel 1979b; Šťastný & Lenzenweger 2008). From the Czech Republic it has only been reported once to date (Rosa 1951).

***Cosmarium fontigenum* Nordst. (Fig. 144)**
Dim.: L: 23–27 µm, B: 23–27 µm, I: 7–8 µm
Occ.: 9rr, 11rr, 25rr, 82c, 86cc

A rather rare species of mesotrophic to slightly eutrophic habitats (see e.g. Coesel 1998a; Lenzenweger 2000b; Fehér 2003). Previous reports from the Czech Republic: Lütkemüller (1910), Japp (1930b), Dvořák (1932), Růžička (1957a, 1973).

***Cosmarium galeritum* Nordst. (Figs 145–146)**
Dim.: L: 55–57 µm, B: 43–45 µm, I: 15–16 µm
Occ.: 50rr

My specimens differ from forms depicted by Coesel (1991) and Lenzenweger (1999), in having only one central pyrenoid, not two; however, this characteristic can be quite variable, sometimes even within a single population (see e.g. Kouwets 1997; discussion in section dedicated to *Cosmarium homalodermum*; Růžička 1973; note at *C. praemorsum*), and therefore, cannot be considered as taxonomically relevant. Also remarkable, and according to the literature, quite rare, was the aerophytic growth habit of my specimens. *C. galeritum*, so far, has been reported from the Czech Republic only by Roubal (1938).

***Cosmarium garrolense* J.Roy et Bisset (Figs 147–149)**
Dim.: L: 26.5–32.5 µm, B: 20–25 µm, I: 7.5–10 µm
Occ.: 72rr, 100cc

Rather rare aerophytic species, from the Czech Republic previously reported only by Růžička

(1956) and Rosa (1969).

***Cosmarium gibberulum* Lütkem. (Figs 150–151)**
Dim.: L: 30.5–34 µm, B: 26.5–29 µm, I: 8.5–9 µm
Occ.: 1r, 9rr, 21rr, 25rr, 55r, 82c, 121r

The morphology of my specimens corresponds well with the original description by Lütkemüller (1910; pl. 2., figs 17–20) from the Šumava Mts. It appears that *C. gibberulum* is a rare tychoplanktonic species of meso–eutrophic, well–preserved habitats (see also Lenzenweger 2000b, 2003b).

***Cosmarium goniodes* W. et G.S.West var. *subturgidum* W. et G.S.West (Figs 152–154, 363)**
Syn.: *C. goniodes* W. et G.S.West var. *variolatum* W. et G.S.West
Dim.: L: 13–20 µm, B: 7–11 µm, I: 6–10 µm
Occ.: 1r, 4rr, 5r, 6r, 8rr, 13rr, 21r, 24rr, 25rr, 36rr, 47rr, 48rr, 55r, 85r, 99rr, 103rr

Curiously, in spite of being recently rather common, this taxon has been reported only twice in the Czech Republic (Lütkemüller 1910; Růžička 1973). A possible explanation appears to be that it is easily confused with some *Actinotaenium* species, particularly *A. cruciferum* and *A. perminutum*, which, however, have omniradiate cells whereas, in frontal view, *C. goniodes* var. *subturgidum* is different from its lateral view (compare Fig. 152).

***Cosmarium homalodermum* Nordst. (Figs 155–156)**
Syn.: *C. hammeri* Reinsch var. *homalodermum* (Nordst.) W. et G.S.West
Dim.: L: 59–65 µm, B: 52.5–58 µm, I: 17–18 µm
Occ.: 54r, 61rr

Rather rare aerophytic species with a variable number of pyrenoids (one or two, see Kouwets 1997), an observation confirmed by my finding from site no. 54, where cells with either number of pyrenoids were found. Previous reports from the Czech Republic: Fischer (1920, 1922–24), Růžička (1956, 1957b).

***Cosmarium jaoi* Kouwets (Figs 157–158)**
Syn.: *C. garrolense* J.Roy et Bisset var. *crassum* C.C.Jao
Dim.: L: 39–45 µm, B: 32–35 µm, I: 9.5–11.5 µm
Occ.: 1rr, 2r, 58r, 59r, 120rr, 122r

Kouwets (1998) considers this species very rare, but as indicated by my, and other recent findings (e.g. Lenzenweger & Wertl 2001; Fehér 2003), it is probably quite abundantly distributed in suitable biotopes (particularly in slightly eutrophic habitats with a copious macrophyte vegetation).

***Cosmarium kirchneri* Børges. (Figs 159, 364)**
Dim.: L: 57–63 µm, B: 50–55 µm, I: 15–17 µm
Occ.: 47r

At the sampling site I found this alga together with *C. margaritiferum* Ralfs; in the past *C. kirchneri* had been classified as one of the forms of the latter species [*C. margaritiferum* forma *kirchneri* (Børges.) W. et G.S.West]. However, my observation, similarly to that made by Coesel (1979b), showed two clearly distinguishable taxa, especially as regards the cell wall structure. This corroborates that distinguishing them as two separate species was well justified (for a detailed discussion see also Coesel 1991; Coesel & Meesters 2007). *C. kirchneri* is a rare species (contrary to *C. margaritiferum*, see also Lenzeweger 1999; p. 137), reported only by Růžička (1973; as *C. margaritiferum* f. *kirchneri*) from the Czech Republic.

***Cosmarium klebsii* Gutw. (Figs 160–161)**
Syn.: *C. subtumidum* Nordst. var. *klebsii* (Gutw.) W. et G.S.West
Dim.: L: 27–32 µm, B: 26–30 µm, I: 8–9 µm
Occ.: 1r, 9r, 11r

Rather rare species of meso–eutrophic habitats with well–developed macrophyte vegetation (see also e.g. Lenzeweger & Wertl 2001). From the morphologically similar *C. subtumidum*, as a variety of which it has been classified in the past, is *C. klebsii* distinguished by its entirely different ecological demands, and also by greater separation between cell wall pores, often marked by short filaments of produced cell material (Coesel & Meesters 2007).

***Cosmarium lagerheimii* Gutw. (Figs 162–163)**
Dim.: L: 16–18 µm, B: 13.5–16 µm, I: 4–4.5 µm
Occ.: 1rr, 9r, 11rr

Very rare species of meso–eutrophic, slightly acidic to alkaline water bodies, new for the Czech flora.

***Cosmarium limnophilum* Schmidle (Figs 164–165)**
Dim.: L: 32–37 µm, B: 26–30 µm, I: 8.5–9.5 µm
Occ.: 1r, 5r

A rare (Kouwets 1998) and apparently also morphologically rather variable (see e.g. Růžička 1949) species of meso–eutrophic habitats, all of its previous findings within the Czech Republic originate from site no. 15 (Růžička 1949, 1973; Šimek 1992, 1997). Intriguingly, the characteristic central ornamentation consisting of three granules arranged in a triangle that is generally considered to be hardly visible in frontal view (see e.g. Kouwets 1991; as *C. boeckii* Wille var. *isthmolaeve* Skuja; Kouwets 1998), was in my specimens comparatively well recognizable, at least when observing empty cells.

***Cosmarium medioretusum* Coesel (Figs 166–169)**
Dim.: L: 17–20 µm, B: 14–17 µm, I: 4.5–6 µm
Occ.: 1r

Very rare taxon, previously found only by Růžička (1973; as *C. subtransiens* Croasdale) in the Czech Republic. Interestingly, Lenzenweger (1986; as *C. umbilicatum* Lütkem. var. *borgei* Willi Krieg. et Gerloff) and Coesel (2007) report it from localities with a slight environmental disturbance, alternatively, both Czech reports, as well as a very recent finding from Austria (Šťastný & Lenzenweger 2008), originate from well preserved sites almost untouched by human activity.

***Cosmarium microsphinctum* Nordst. var. *crispulum* Nordst. (Figs 170, 365)**
Dim.: L: 37–41 µm, B: 25–27.5 µm, I: 14.5–16 µm
Occ.: 54rr, 66rr

A taxon well distinguished by a thick cell wall, slightly undulate margins and occurrence in hemi–atmophytic habitats, especially at higher altitudes. Only the nominate variety of *C. microsphinctum* has been reported in the Czech Republic (Rybníček 1958).

***Cosmarium netzerianum* Schmidle (Fig. 171)**
Syn.: *C. reniforme* (Ralfs) W.Archer var. *apertum* W. et G.S.West
Dim.: L: 49–56 µm, B: 47.5–50 µm, I: 20–21 µm
Occ.: 61rr
West & West (1908) consider *C. netzerianum* synonymous with their newly described *C. reniforme* var. *apertum,* and, since that publication, *C. netzerianum* was usually reported in the literature as *C. reniforme* var. *apertum*. However, in spite of a rough resemblance, *C. netzerianum* should not be considered a variety of *C. reniforme,* due to considerable differences between these taxa in the granulation of the cell wall (see Messikommer 1942; p.156) and significant differences in their ecology. Whereas *C. reniforme* is a typical tychoplanktonic species of meso–eutrophic water–bodies, *C. netzerianum* seems to have a clearly arctic–alpine distribution and often grows sub–aerophytically. Schmidle (1895) described it from a site situated 2 200 m a.s.l., furthermore, other findings (e.g. Insam & Krieger 1936; Messikommer 1942; Lenzenweger 1987, 1994, 2002, 2003a) are from sites at altitudes between 1 225 and 2 530 m a.s.l. As for sites in the Czech Republic *C. netzerianum* is only known from the Krkonoše Mts (Beck–Mannageta 1926).

****Cosmarium norimbergense* Reinsch var. *depressum* (W. et G.S.West) Willi Krieg. et Gerloff (Fig. 172)**
Dim.: L: 13–16.5 µm, B: 11.5–15 µm, I: 4–5 µm
Occ.: 63r

New for the Czech Republic. To date only *C. norimbergense* var. *boldtii* (Messik.) Růžička has been reported (Růžička 1973; pl. 12, figs 6–8), although, when examining Růžička's figures, it appears that a completely different, unrelated taxon is depicted.

***Cosmarium notabile* Bréb. (Figs 173–174)**
Dim.: L: 25–30 µm, B: 18–21 µm, I: 12.5–14 µm
Occ.: 36rr, 39rr, 41r, 45rr, 50rr, 61r, 64rr, 73r, 74r, 76r, 77r, 78r, 80rr

Rarely reported, but, judging from the number of my findings, probably a common species of ephemeral, oligo–mesotrophic habitats that, however, usually occurs in small numbers. Earlier reports from the Czech Republic: Wünsch (1939), Nováček (1941).

****Cosmarium notatum* (Grönblad) Coesel (Figs 175–176)**
Syn.: *C. jenisejense* Boldt var. *notatum* (Grönblad) Kurt Först.
Dim.: L: 21.5–25.5 µm, B: 21.5–26.5 µm, I: 8.5–9 µm
Occ.: 1r, 5rr

This alga, in all likelihood very rare in Central Europe, prefers colder areas (see e.g. Förster 1965; Lenzenweger 1999); it was, therefore, very surprising that it was found at sites only 275 m a.s.l. These sites, however, are portions of ponds that were established in the 14th century on the remnants of a glacial lake (Nováková & Popovský 1972) and, perhaps, *C. notatum* might represent a glacial relict.

***Cosmarium novae – semliae* Wille var. *granulatum* (Schmidle) Schmidle (Fig. 177)**
Dim.: L: 14–15 µm, B: 14.5–15.5 µm, I: 7 µm
Occ.: 24rr

Previously reported only by Lütkemüller (1910) in the Czech Republic, the occurrence of the nominate variety is mentioned by Fischer (1922–24) and Japp (1930a).

****Cosmarium obsoletum* (Hantzsch) Reinsch (Figs 178–179, 366–367)**
Dim.: L: 40–45 µm, B: 47.5–52.5 µm, I: 22.5–24 µm
Occ.: 1r, 4c, 5r

A rare taxon of oligo–mesotrophic, slightly acidic habitats, the occurrence of which seems to be confined to well–preserved biotopes. New for the Czech Republic.

***Cosmarium ocellatum* B.Eichler et Gutw. (Fig. 180)**
Dim.: L: 28.5–30 µm, B: 25–26 µm, I: 6–6.5 µm
Occ.: 25rr

Previous records from the Czech Republic: Lütkemüller (1910), Mattauch (1936).

***Cosmarium ocellatum* B.Eichler et Gutw. var. *notatum* (Nordst.) Willi Krieg. et Gerloff (Figs 181–183)**
Dim.: L: 25–28 µm, B: 23–26.5 µm, I: 6–7 µm
Occ.: 1r, 4rr, 8r

A rare taxon of undisturbed habitats, prior report from the Czech Republic only by Růžička (1973) .

***Cosmarium ordinatum* (Børges.) W. et G.S.West (Figs 184–185, 368)**
Dim.: L: 19–22.5 µm, B: 18–22.5 µm, I: 6.5–7.5 µm
Occ.: 1r, 4r, 8r

A quite rare species, in the Czech Republic, to date, reported only by Lütkemüller (1910). However, it has become evident that the findings of Rosa (1939; pl. 3, figs. 56–57) and Růžička (1973; pl. 10, fig. 12), both published as *C. orthostichum* P. Lundell refer in fact to *C. ordinatum*; *C. orthostichum* has, indeed, a similar cell wall sculpture, but differs in dimension by nearly twofold (see e.g. Lenzenweger 1999; Coesel & Meesters 2007).

****Cosmarium ornatulum* Coesel (Figs 186–188)**
Dim.: L: 20–22.5 µm, B: 20–22.5 µm, I: 6.5–7.5 µm
Occ.: 96c, 97r, 106c

Euplanktonic, recently described species (Coesel 2002) of eutrophic, alkaline habitats. In suitable habitats most likely quite common, as indicated by the information received from the hydrobiologist R. Geriš (Geriš, pers.com.), who regularly finds it in several eutrophic water reservoirs (Brno, Luhačovice, Mostiště, Plumlov).

****Cosmarium ornatulum* Coesel var. *depressum* Coesel (Fig. 189)**
Dim.: L: 22–23 µm, B: 25–26 µm, I: 8 µm
Occ.: 15rr

This variety differs from the type variety only by the greater breadth of its cells; its ecological requirements are similar (Coesel 2002).

***Cosmarium orthopunctulatum* Schmidle (Figs 190–191)**
Dim.: L: 26–30.5 µm, B: 25–28.5 µm, I: 9–11 µm
Occ.: 34c, 56r, 61rr, 62c, 67cc, 75rr, 81rr, 117r

A species with a predominantly arctic–alpine distribution (Coesel 1992), as is corroborated by previous reports of it in the Czech Republic (Fischer 1924; Lhotský 1949; Rybníček 1958), and by the majority of my findings originating from mountainous regions. My other samplings, from ravines in sandstone rock massifs (sites no. 75 and 81), are interesting from an ecological point of view. These ravines are situated only 450–500 m above sea level, but due to the inverse character of their climate, the temperatures at their bottom are often very low, comparable to that of much higher altitudes. The cell wall ornamentation can be variable in this alga, as shown by Messikommer (1942), on the other hand, a characteristic and stable feature is the rounded rhomboid shape of the cells in apical view (Fig. 190).

***Cosmarium ovale* Ralfs (Figs 192, 369–371)**
Dim.: L: 177–195 µm, B: 102–113 µm, I: 34–38 µm
Occ.: 1cc, 5r

Very rare taxon of well–preserved habitats, in the Czech Republic previously recorded only by Pascher (1903, as *Cosmaridium ovale* Hansg.) and Holzer [1931, as *Pleurotaeniopsis ovalis* (Ralfs) De Toni].

****Cosmarium paragranatoides* Skuja (Figs 193–197, 372–373)**
Dim.: L: 24–27.5 µm, B: 15–17.5 µm, I: 5–5.5 µm
Occ.: 1c, 4r, 5r, 25rr, 29rr

Relatively rare species of mesotrophic, slightly acidic habitats, new for the Czech Republic.

****Cosmarium parvulum* Bréb. var. *undulatum* Schmidle (Figs 198–200)**
Dim.: L: 24–30 µm, B: 12.5–13.5 µm, I: 10–11 µm
Occ.: 7r

Rare aerophytic taxon, new for the Czech Republic. The occurrence of the nominal variety of *C. parvulum* in the Czech Republic was mentioned by Fischer (1922–24, 1924), Rybníček (1958) and Roubal (1959), however, at least Rybníček's finding, from examination of his figure (Rybníček 1958; pl. 2, figs. 33–34), represents a different species, i.e. *Actinotaenium obcuneatum* (W.West) Teiling. The taxon in question might be, if observed in the lateral view, easily confused with *Actinotaenium kriegeri*, that also has a similar ecology (see above).

****Cosmarium paucigranulatum* Borge (Figs 201–203)**
Dim.: L: 10–11 µm, B: 9–10 µm, I: 3–3.5 µm
Occ.: 51cc, 118cc (in one of the mesotrophic pools)

Initially, my findings were determined to be *Xanthidium robinsonianum* W. Archer var. *alpinum* Bourrelly, described by Bourrelly (1987) from Austria (see also Lenzenweger 1997; pl. 18, fig. 10). However, the publication is invalid (Kouwets 2001) and, moreover, Bourrelly's material differs from *C. paucigranulatum*, as described by Borge (1923) in that the sides of semicells are angular instead of rounded, and have a slightly differently developed ornamentation (spinules instead of granules). These characters were already considered almost certainly variable by Kouwets (2001), who regarded both taxa as synonymous, and his conclusion is confirmed by my findings (Figs 201–203). *C. paucigranulatum*, most likely, is a very rare, but inconspicuous species preferring predominantly mesotrophic, slightly acidic habitats (see Bourrelly 1987).

****Cosmarium pericymatium* Nordst. (Figs 204–206, 374–376)**
Dim.: L: 41–51 µm, B: 26.5–31.5 µm, I: 21–24 µm
Occ.: 7r, 90r, 91m, 92r, 94r, 107r, 111rr, 115rr

A species typical for ephemeral, periodically desiccating habitats (see e.g. Williamson 2000; Brook 2001; Coesel et al. 2006; Šťastný 2008) that, however, seems to be much more common on artificial substrates like concrete than on "natural" substrates, such as wet rocks (see Šťastný 2008). If observed in the lateral view, the taxon in question might be easily confused with certain *Actinotaenium* species.

****Cosmarium pericymatium* Nordst. var. *corrugatum* Brook (Figs 207–208, 377–379)**
Dim.: L: 60–65 µm, B: 34–40 µm, I: 23–26 µm
Occ.: 91c, 111c

The ecological demands of this taxon are very similar to those of the nominal variety (see Šťastný 2008), thus far it has been exclusively reported from artificial, periodically desiccating substrates (Brook 2001; Williamson 2002; Šťastný 2008; Šťastný, unpublished record from The Netherlands). The series of corrugations, being present on each side of the isthmus and giving the taxon in question its name (Brook 2001) were in my specimen hardly ever visible under the light microscope (Figs 207–208), however, they are clearly distinct if the cells are observed with the aid of a SEM (Figs 377–378). A very similar corrugation along the basis of the semicells, but much more weakly developed than in the var. *corrugatum,* and visible exclusively under SEM, are sometimes present also in the nominate variety of *C. pericymatium* (Šťastný, personal observation).

****Cosmarium pericymatium* Nordst. var. *notabiliforme* Insam et Krieger (Figs 209–213)**
Dim.: L: 26–30 µm, B: 15–18 µm, I: 11–12.5 µm
Occ.: 52cc

Concerning their general morphology, it is very similar to the nominal variety, however, the cells

are much smaller and relatively slightly longer. Very rare, and only known from two sites in Austria (Insam & Krieger 1936). Notably, the material did not originate directly from the rocks in the "Pohořský" stream, but was collected by squeezing out mosses growing on a short artificial concrete portion of the bed of the stream close to its outflow from the pond, i.e. in a habitat similar to that, where the nominate and the var. *corrugatum* predominantly occur (see above).

***Cosmarium pokornyanum* (Grunow) W. et G.S.West (Figs 214–215)**
Dim.: L: 30–31 µm, B: 18–19 µm, I: 10 µm
Occ.: 29rr

A rather rare aerophytic taxon that within the Czech Republic was found only by Pascher (1903) and Roubal (1938) (both findings as *Euastrum pokornyanum* Grunow).

***Cosmarium prominulum* Racib. var. *subundulatum* W. et G.S.West (Figs 216–217, 380)**
Dim.: L: 14.5–17.5 µm, B: 14–18 µm, I: 6.5–7,5 µm
Occ.: 1rr, 3rr, 4rr

Very rare, from the Czech Republic previously reported only by Mattauch (1936) from site no. 3.

***Cosmarium pseudoexiguum* Racib. (Fig. 218)**
Dim.: L: 17–18 µm, B: 8–8.5 µm, I: 2.5 µm
Occ.: 9rr

Previous findings from the Czech Republic: Lütkemüller (1910), Růžička (1973).

***Cosmarium pseudoinsigne* Prescott (Figs 219–220, 381)**
Syn.: *C. insigne* Schmidle
Dim.: L: 48.5–52 µm, B: 38.5–40.5 µm, I: 14–16 µm
Occ.: 1rr (in the slightly eutrophic littoral zone), 58c

This rather rare tychoplanktonic species of mesotrophic–eutrophic, found in neutral to slightly alkaline habitats (see e.g. Coesel 1974, 1991; Lenzenweger & Wertl 2001; Fehér 2003) has been reported from the Czech Republic only by Roubal (1938) so far. Often, the cells of the species in question are covered with bacteria, probably feeding on some products secreted by the cell wall pores (Fig. 381).

***Cosmarium pseudoprotuberans* Kirchn. (Figs 221, 382–383)**
Dim.: L: 31–32 µm, B: 23–25 µm, I: 7.5–8 µm
Occ.: 1rr

Very rare species, reported only twice from the Czech Republic thus far (Rosa 1951; Růžička 1957a). However, judging from his figures (pl. 2, figs 41–42), Růžička's finding observably represents a completely different species. In addition, Lütkemüller (1910) mentions *C. pseudoprotuberans* var. *angustius* Nordst from the Šumava Mts.

****Cosmarium pseudoprotuberans* Kirchn. var. *sulcatum* (Nordst.) Coesel (Fig. 222)**
Dim.: L: 36.5–40 µm, B: 29–31 µm, I: 8–9 µm
Occ.: 9rr

This variety differs from the nominate only in having the median part of the semicell in apical view triundulate on either long side (Coesel & Meesters 2007).

****Cosmarium pseudoretusum* F.Ducell. (Figs 223–225, 384–385)**
Dim.: L: 26–30 µm, B: 20–23 µm, I: 6.5–7.5 µm
Occ.: 5c

A rare species, from similar taxa distinguished particularly by the papillate outgrowths at the basal angles being commonly present in well developed cells (Figs 223–225).

***Cosmarium pseudowembaerense* Kouwets (Figs 226–227)**
Syn.: *C. laeve* Rabenh. var. *pseudoctangulare* F.E.Fritsch et M.F.Rich
Dim.: L: 14–17 µm, B: 13–16 µm, I: 4–5 µm
Occ.: 1rr, 2rr, 12rr, 15c, 16rr, 120rr

Relatively rare species of larger, eutrophic and alkaline water bodies (Coesel 1998a; Kouwets 1998; Lenzenweger & Wertl 2001). In the Czech Republic previously found by Šimek (1992; as *C.* cf. *meneghinii* Ralfs) at site no. 15, and by Hašler et al. (2008).

***Cosmarium retusum* (Perty) Rabenh. (Figs 228–230)**
Dim.: L: 35.5–42.5 µm, B: 28–34 µm, I: 8.5–10 µm
Occ.: 1r, 4rr

A very rare species (e.g., only two records are

known from Austria, Šťastný & Lenzenweger 2008). Findings in the Czech Republic are only by Růžička (1949, 1973) from one site in southern Bohemia (forms with greatly reduced ornamentation).

***Cosmarium sexnotatum* Gutw. var. *bipunctatum* (Wolosz.) Coesel (Fig. 231)**
Dim.: L: 32–35.5 µm, B: 27–29 µm, I: 8.5–9 µm
Occ.: 1rr

Concerning the general cell shape and ecology, similar to *C. limnophilum* (see above), but clearly distinguished by the entirely different central ornamentation of the semicells that was usually, contrary to the observations of Coesel (1989) and Kouwets (1998), quite visible, even in frontal view, if empty cells were examined.

***Cosmarium sexnotatum* Gutw. var. *tristriatum* (Lütkem.) Schmidle (Figs 232–233, 386)**
Dim.: L: 18.5–22 µm, B: 16–19 µm, I: 6–7.5 µm
Occ.: 1rr, 4rr

This taxon is, along with the above–mentioned *C. sexnotatum* var. *bipunctatum*, new for the Czech Republic and apparently rather rare; however, it might be confused with, for instance, *C. blytii* Wille var. *novae–sylvae* W. et G.S.West.

***Cosmarium simplicius* (W. et G.S.West) Grönblad (Figs 234, 387)**
Syn.: *C. elegantissimum* P.Lundell var. *simplicius* W. et G.S.West

Dim.: L: 44–55 µm, B: 21–24 µm, I: 19–21 µm
Occ.: 1r, 4rr, 61rr
Previous findings from the Czech Republic reported only by Růžička (1949, 1956, 1973) and, evidently, Růžička's findings of *C. elegantissimum* f. *intermedium* Kaiser (Růžička 1956, 1957b) also correspond to the species in question.

***Cosmarium sphyrelatum* Coesel (Figs 235–240)**
Dim.: L: 15–18 µm, B: 11.5–14 µm, I: 4–5 µm
Occ.: 1cc, 5rr

Relatively recently described species (Coesel 1989), so far known only from The Netherlands, Austria (Šťastný & Lenzenweger 2008) and from the Orkneys (Williamson 2003); the latter finding, however, appears to represent a different species. According to the findings of *C. sphyrelatum* thus far, this species seems to prefer mature ecosystems with a high desmid diversity. For SEM images see Coesel (1984b, 1989).

***Cosmarium striolatum* (Nägeli) W. Archer (Fig. 241)**
Syn.: *C. tesselatum* (Delponte) Nordst.
Dim.: L: 125–150 µm, B: 67–76 µm, I: 50–60 µm
Occ.: 1r, 5rr

A very rare species with characteristic cell wall ornamentation that was previously reported from the Czech Republic only by Rosa (1951) and Růžička (1973).

***Cosmarium subadoxum* Grönblad (Fig. 242)**
Dim.: L: 9–10 µm, B: 9–10 µm, I: 2–2.5 µm
Occ.: 1rr

The morphology of my findings corresponds well to the species in question as illustrated by Kouwets (1987; pl. 13, figs 23–25), as well as Coesel & Meesters (2007; pl. 61, figs 35–36). Characteristic is the presence of a small central papilla on either side of the semicells that is fairly distinct, particularly in the apical view.

***Cosmarium subbroomei* Schmidle (Figs 243–244)**
Dim.: L: 35–42 µm, B: 32–37 µm, I: 13–14 µm
Occ.: 15r (in the pools)

In the Czech Republic this species was only found by Šimek (1992, 1997), however, from examination of his figures (Šimek 1997, Fig. 18), it appears that his material is highly consistent with *C. subbroomei* f. *isthmochondrum* Coesel (see below). It differs from this taxon only by the absence of the prominent supraisthmal granula on each semicell. However, this character is likely quite variable, as also indicated by my findings (Figs 245–246). Therefore, I believe Šimek's findings most likely represent *C. subbroomei* f. *isthmochondrum*. The nominate variety of *C. subbroomei*, on the other hand, is characterized by cells with an almost quadrate outline and a somewhat distinct granulation pattern (see e.g. Růžička 1972; Šťastný & Lenzenweger 2008); see my findings (Figs 243–244).

***Cosmarium subbroomei* Schmidle. f. *isthmochondrum* Coesel (Figs 245–246)**
Dim.: L: 35–40 µm, B: 33.5–36 µm, I: 12–13.5 µm
Occ.: 1r

New for the Czech Republic, however, most likely identical to *C. subbroomei* found by Šimek (1997, Fig. 18) at site no. 15 (see above). Within The Netherlands the taxon in question is predominantly reported from mesotrophic, slightly acidic to neutral habitats (Coesel 1975, as *C. subbroomei*, 1989), and this is consistent with the conditions of my findings. As indicated by Coesel & Meesters (2007), the taxon discussed is probably not related to the "real" *C. subbroomei* and is preferably designated as a separate species.

***Cosmarium subprotumidum* Nordst. var. *pyramidale* Coesel (Figs 247–248)**
Dim.: L: 21–23.5 µm, B: 19–21.5 µm, I: 6–6.5 µm
Occ.: 9r, 11r

This taxon is principally distinguished from the somewhat more common, but often co–occurring nominal variety by cells that are pyramidal in outline (in stead of trapeziform, Coesel & Meesters 2007) and have smaller dimensions.

***Cosmarium subquadrans* W. et G.S.West var. *minor* Symoens (Fig. 249)**
Dim.: L: 12.5–14 µm, B: 16–18 µm, I: 4.5–5 µm
Occ.: 1rr, 3rr, 4rr, 18cc

The taxon in question is distinguished from morphologically similar taxa by its rhomboid shape in apical view, and by its ecology; it prefers strongly acidic, oligotrophic waters. From the Czech Republic, thus far, only the nominal variety of *C. subquadrans* has been reported (Roubal 1958).

***Cosmarium subspeciosum* Nordst. (Fig. 250)**
Dim.: L: 52.5–57.5 µm, B: 43–46 µm, I: 15–16 µm
Occ.: 42r

From the Czech Republic only *C. subspeciosum* var. *transiens* has been reported; this, however, represents a completely different, unrelated species. Coesel (1991) labeled a forma that morphologically fully corresponds with my findings *C. subspeciosum* var. *simplicius* Jao, but this variety probably is of little taxonomic significance (see Coesel & Meesters 2007).

***Cosmarium subtumidum* Nordst. var. *groenbladii* Croasdale (Figs 251, 388)**
Dim.: L: 35–39 µm, B: 29–32 µm, I: 10–11.5 µm
Occ.: 1r, 8rr

To date, only the nominal variety of *C. subtumidum* is known from the Czech Republic (Beck–Mannageta 1926; Japp 1930b; Dvořák 1932; Rosa 1933, 1941, 1951, 1969). This differs from the taxon discussed in that the semicells in outline are rounded trapeziform instead of rounded rectangular to hexagonal (Coesel & Meesters 2007). In addition, the type variety of *C. subtumidum* lacks the locally thickened cell wall that is usually present and distinct in var. *groenbladii* (Fig. 251).

***Cosmarium taxichondriforme* B.Eichler et Gutw. (Figs 252–253, 389)**
Dim.: L: 40–43 µm, B: 40–43 µm, I: 14–16 µm
Occ.: 1c, 5rr

Rare species (Růžička 1955a; Coesel 1974), in the Czech Republic found only by Lütkemüller (1910) and Růžička (1973).

***Cosmarium tetrachondrum* P.Lundell forma (Figs 254–255)**
Dim.: L: 20.5–22.5 µm, B: 23.5–26.5 µm, I: 6.5–7.5 µm
Occ.: 1c, 4rr

Very rare, mentioned in the Czech Republic only by Růžička (1949, 1973) from one site in southern Bohemia.

***Cosmarium truncatellum* Perty (Figs 256–261)**
Dim.: L: 9–11 µm, B: 10–12.5 µm, I: 6.5–7.3 µm
Occ.: 30r, 69cc, 70r, 71rr

The morphology and ecology (occurrence in oligotrophic, strongly acidic milieu) of my findings agree very well with the data of Kouwets (1987) and Coesel & Meesters (2007). The only difference is the shape of the apex that is generally considered to be flat or slightly concave (Kouwets 1987), but in my material, particularly that from site no. 30, a slight inflation at the top of the apex was often visible.

***Cosmarium ungerianum* (Nägeli) De Bary var. *subtriplicatum* W. et G.S.West (Figs 262–263)**
Syn.: *C. ungerianum* (Nägeli) De Bary var. *nodosum* (Andersson) Lütkem.
Dim.: L: 57–65 µm, B: 51–59 µm, I: 18–22 µm
Occ.: 1c

A very rare alga, within the Czech Republic found

only by RŮŽIČKA (1949, 1973) and ROSA (1951).

***Cosmarium variolatum* P.LUNDELL (Figs 264–265, 390–392)**
Dim.: L: 32–35 µm, B: 20–21 µm, I: 7–8 µm
Occ.: 1r

A rare taxon, well characterized by a coarsely scrobiculated cell wall (each scrobiculation bears a distinct pore; Figs 390–392).

***Cosmarium variolatum* P.LUNDELL var. *cataractarum* RACIB. (Figs 266, 393–395)**
Dim.: L: 37–41.5 µm, B: 26.5–29 µm, I: 7.5–9 µm
Occ.: 1rr, 9c, 10rr, 11r, 58r

Although (if observed under light microscopy), the cell wall sculpture of this taxon roughly resembles *C. variolatum*, in the SEM (Figs 393–395) it appears completely different. Therefore, and additionally because of the essentially dissimilar ecological demands of these taxa, it appears desirable to distinguish *C. variolatum* var. *cataractarum* as a separate species. This revision, however, will be published elsewhere. The only report of the taxon in question within the Czech Republic was by HAŠLER et al. (2008).

***Cosmarium varsoviense* RACIB. (Figs 267, 396–397)**
Dim.: L: 38–45 µm, B: 34–38 µm, I: 16.5–18 µm
Occ.: 1c, 5r, 6rr, 9rr, 25rr, 26rr, 47rr

From the Czech Republic, only *C. varsoviense* RACIB. var. *tumidum* RŮŽIČKA has been reported (ŠIMEK 1992, 1997), but when studying that author's depiction (ŠIMEK 1997, Fig. 19), his finding has no resemblance to *C. varsoviense,* and might represent *C. rectangulare* GRUNOW. However, LÜTKEMÜLLER (1910) described *C. lomnicense* LÜTKEM. from the Šumava Mts which, most likely, represents the same taxon (see e.g. SKUJA 1934; KRIEGER & GERLOFF 1965). One of the typical features of the nominate variety of *C. varsoviense* is the presence (in the centre of the semi–cell) of a characteristic structure consisting of a rosette of clearly visible scrobiculae (RACIBORSKI 1889). This structure was always clearly recognizable in my material, at least in cases where I could observe empty cells (see Figs 267, 396–397); however, according to LENZENWEGER (1999) and KOUWETS (2001) this feature is often greatly reduced, or even be missing entirely. In the Czech Republic, *C. varsoviense* seems to be a quite rare species of mesotrophic, slightly acidic habitats.

***Cosmarium vogesiacum* LEMAIRE (Figs 268–269)**
Syn.: *C. bipunctatum* BÖRGES.
C. polonicum RACIB.
Dim.: L: 22.5–25 µm, B: 20–22.5 µm, I: 6–8 µm
Occ.: 4rr, 24r, 31cc, 36r

C. vogesiacum is a morphologically variable species (especially the central ornamentation, KOUWETS 1987) that is reported particularly from mountainous regions (COESEL 1998b). In the Czech Republic it has been reported only by LÜTKEMÜLLER (1910) and ROSA (1951) under the synonym *C. bipunctatum*. Another of Rosa's findings of *C. bipunctatum* most probably represents, judging from his figure (ROSA 1939; pl. 3, fig. 52), *C. ordinatum* (see above).

***Xanthidium aculeatum* EHRENB. (Figs 270–271, 398)**
Dim.: Ls: 67–77 µm, Lc: 78–95 µm, Bs: 63–73 µm, Bc: 77–93 µm, I: 18–22 µm
Occ.: 101cc

An extremely rare alga, to date only reported by NAVE (1863) and PASCHER (1903, 1906) from the Czech Republic. *X. aculeatum* differs from the somewhat similar *X. brebissonii* RALFS by having a relatively greater number of spines not arranged in definite pairs, and by the regular presence of an additional, rather variably developed ornamentation between the central ornamentation and the apex. Therefore, I think it likely that the figure of *X. brebissonii* forma in COESEL & MEESTERS (2007; pl. 81; fig. 2), showing a developed subapical ornamentation, actually represents *X. aculeatum.*

***Xanthidium basidentatum* (BØRGES.) COESEL (Fig. 272)**
Syn.: *X. aculeatum* EHRENB. var. *basidentatum* (BØRGES.) W. et G.S.WEST
Dim.: Ls: 70–72 µm, Lc: 85–88 µm, Bs: 61–63 µm, Bc: 78–82 µm, I: 24–25 µm
Occ.: 1rr

Very rare, within the Czech Republic reported only twice, by LÜTKEMÜLLER (1910, as *X. brebissonii* RALFS var. *basidentatum* BØRGES.), and by ROSA 1969 [as *X. fasciculatum* var. *basidentatum* (BØRGES.) RŮŽIČKA]. For a detailed discussion of

the taxonomy of this species see Coesel (1993).

***Xanthidium bifidum* (Bréb.) Deflandre (Figs 273–275)**
Syn.: *Arthrodesmus bifidus* Bréb.
Dim.: Ls: 12.5–17 µm, Bs: 12.5–17 µm, I: 5–6 µm
Occ.: 1r, 5r, 8rr, 24r

Very rare, but rather inconspicuous alga, reported from the Czech Republic only by Pascher (1906), Rosa (1941) and Růžička (1973).

***Xanthidium concinnum* W.Archer (Figs 276–277)**
Dim.: Ls: 10.5–12.5 µm, Bs: 11.5–13.5 µm, I: 3–3.5 µm
Occ.: 47c

An inconspicuous, but probably very rare species, the occurrence of which in the Czech Republic is mentioned by Lütkemüller (1910), Fischer (1924) and Lederer & Soukupová (2002). When observed in frontal view, some specimens seem to have several warts directly under the apex; however, these are probably just rough pores (see also Kouwets 1987).

***Xanthidium cristatum* Ralfs (Figs 278–279, 399)**
Dim.: Ls: 45–50 µm, Lc: 59–66 µm, Bs: 36–42 µm, Bc: 49–57 µm, I: 12–13.5 µm
Occ.: 1rr, 4rr, 5rr, 8r, 47c, 114rr

Older data on the occurrence of this species in the territory of the Czech Republic are relatively frequent [in Bohemia it was found by Pascher (1903), Roubal (1939), Rosa (1939, 1951), Růžička (1973) and Lederer et al. (1998), in Moravia by Dvořák (1910, 1934), Fischer (1920), Japp (1930a, 1930b) and (Holzer 1931)], recently it has become rare.

****Xanthidium cristatum* Ralfs var. *uncinatum* Ralfs forma *polonicum* Gutw. (Figs 280–281)**
Dim.: Ls: 59–64 µm, Lc: 76–84 µm, Bs: 50–58 µm, Bc: 72–77 µm, I: 17 – 18 µm
Occ.: 1r

In the Czech Republic only the nominate forma of *X. cristatum* var. *uncinatum* has been reported (Pascher 1903; Fischer 1920; Japp 1930b; Rosa 1939, 1951, 1969; Růžička 1973), which lacks, contrary to the forma *polonicum*, the granulation of the basal angles.

****Xanthidium fasciculatum* Ralfs var. *oronense* W. et G.S.West (Figs 282–283)**
Dim.: Ls: 50–55 µm, Lc: 65–75 µm, Bs: 45–53 µm, Bc: 62–70 µm, I: 15–16.5 µm
Occ.: 1r

The alga in question is new for the Czech Republic; however, it differs from the nominal variety only by the presence of an additional granula at the semicell base near each of the basal angles (Coesel & Meesters 2007) which, with respect to the rather considerable morphological plasticity of *X. fasciculatum* (see e.g. Růžička 1955b), might be considered a dismissible feature. Past findings of the type variety within the Czech Republic are relatively frequent. Pascher (1903, 1906), Dechant (1914), Wünsch (1939) (last two findings as *Holacanthum fasciculatum* Franzé), Rosa (1939, 1951, 1969), Roubal (1958) and Růžička (1973) all mention findings from Bohemia, Moravian findings are recorded by Fischer (1920), Japp (1930b), Dvořák (1932) and Gessner (1932). A recent finding from Western Bohemia is mentioned by Lederer et al. (1998) from site no. 99. However, I could not confirm those records. Of note is that the taxon in question is mentioned by Lenzenweger (1997; pl. 20, fig. 5) from Austria under the name *X. fasciculatum* var. *basidentatum* (Børges.) Růžička that, however, is actually synonymous with *X. basidentatum* (see Coesel 1993).

****Staurodesmus extensus* (Borge) Teiling var. *joshuae* (Gutw.) Teiling (Fig. 284)**
Dim.: L: 18–20 µm, Bs: 35–40 µm, I: 6–7
Occ.: 1rr, 8rr, 24rr, 47rr, 114rr

My specimens correspond very well with one of the figures of this taxon in Coesel & Meesters (2007; pl. 86, fig. 22), but considering that all cells observed possessed distinctly convergent spines that are atypical for *Std. extensus*, and, moreover, no intermediate forms (as concerns the orientation of the spines) were found at sites no. 47 and 114, where both taxa mentioned co–occurred, the taxon in question should better be considered a separate species. See also the depiction by Růžička (1972; pl. 62, fig. 8, as *Arthrodesmus triangularis* Lagerh.) clearly representing the same alga.

****Staurodesmus extensus* (Borge) Teiling var. *malaccensis* (Bernard) Coesel (Figs 285–289)**
Dim.: Ls: 11–14 µm, Lc: 20–24 µm, Bs: 9.5–13 µm, Bc: 22–32 µm, I: 4.5–5.5 µm

Occ.: 24cc (in a strongly acidic, oligotrophic pool)

The morphology and ecology of my findings agree with the data of Coesel & Meesters (2007; see pl. 86, fig. 27). This taxon is distinguished from the otherwise very similar *Std. phimus* (W.B.Turner) Thomasson predominantly by its more widely rounded sinus.

***Staurodesmus lanceolatus* (W.Archer) Croasdale var. *compressus* (W. et G.S.West) Teiling (Figs 290–292)**
Syn.: *Staurastrum lanceolatum* W.Archer. var. *compressum* W. et G.S.West
Dim.: L: 19–22.5 µm, B: 20–23.5 µm, I: 6–7 µm
Occ.: 17r, 47rr, 49rr 51r, 114rr

A rare alga, new for the Czech Republic. However, the figure in Růžička (1973; pl. 14, fig. 8, as *Staurastrum brevispinum* Ralfs var. *brevispinum* f. *minimum* Lütkem.) most likely represents this same taxon.

***Staurodesmus subhexagonus* (W. et G.S.West) Coesel (Fig. 293)**
Dim.: L: 15–16 µm, Bs: 24–26 µm, I: 8–9
Occ.: 26rr

Concerning the shape of the cell body, similar to *Std. extensus* var. *joshuae* (see above), but distinguished by a slightly shorter cell length, and in particular by much shorter spines.

***Staurastrum arctiscon* (Ralfs) P.Lundell (Fig. 294)**
Dim.: L: 90–120 µm, B: 81–118 µm, I: 21–28 µm
Occ.: 46rr, 47c

This beautiful tychoplanktonic alga is, within Europe, characterized by a marked atlantic–subarctic distribution, but nowhere is it really common (Coesel & Krienitz 2008), and in Central Europe is it evidently rare (e.g. Lenzenweger 1999 mentions the first record from Austria). Only one record has been documented (Lütkemüller 1910) from the Czech Republic.

***Staurastrum bloklandiae* Coesel et Joosten (Figs 295–296)**
Dim.: L: 27–45 µm, B: 30–44 µm, I: 5–6 µm
Occ.: 15c, 16c, 27rr, 28rr, 58rr, 60rr, 108rr, 109rr

Although only described relatively recently (Coesel & Joosten 1996), this species is already known from many European countries, namely: The Netherlands, France, England, Poland, Serbia, Austria (see Meesters & Coesel 2007), Germany (Scharf 1985, as *Staurastrum* cf. *caledonense* Huber–Pestalozzi) and Slovakia (Tomaszewicz & Hindák 2008). In the Czech Republic it was found very recently by Hašler et al. (2008) and, according to my findings, it seems to be rather common in the plankton of eutrophic water bodies.

***Staurastrum bohlinianum* Schmidle (Figs 297–298)**
Dim.: L: 22.5–25 µm, B: 22.5–25 µm, I: 9–10 µm
Occ.: 61rr, 68rr

According to Lenzenweger (1997), a species with an arctic–alpine distribution, which agrees with my data, as well as with the only previous finding in the Czech Republic recorded from the Krkonoše Mts by Nováková (2004). *St. bohlinianum* might be easily confused with reduction forms of *St. polymorphum* Ralfs var. *pygmaeum* Grönblad that, however, has a different ecology preferring mesotrophic, only slightly acidic habitats situated mostly at lower altitudes.

***Staurastrum crassangulatum* Coesel (Figs 299, 400–402)**
Syn.: *St. kaiseri* Růžička (invalid homonym of *St. kaiseri* Pevalek)
Dim.: L: 36–40 µm, B: 31.5–37 µm, I: 9–10 µm
Occ.: 1rr, 5c

From similar, but more common *St. bieneanum* Rabenh. distinguished particularly by the usually thickened cell wall at the lateral angles (Fig. 299).

***Staurastrum cristatum* (Nägeli) W.Archer var. *cuneatum* Hinode (Figs 300–301)**
Dim.: L: 43–48 µm, B: 45–53 µm, I: 22–23 µm
Occ.: 1rr, 5r, 114rr

My specimens are identical with those reported under the name *St. cristatum* var. *navigiolum* (Grönblad) Coesel from a very similar, well–preserved habitat in Austria by Lenzenweger (2000a, 2000b). However, both his material, as well as mine, correspond slightly better with *St. cristatum* var. *cuneatum*, as the differences between these two taxa are rather small (see Coesel & Meesters 2007). On site no. 114, most likely, the alga discussed was scoured from

neighbouring bogs situated in Zone I of Šumava National Park.

***Staurastrum erasum* Bréb. (Figs 302, 403–404)**
Dim.: L: 33–37.5 µm, B: 37–40 µm, I: 10–11.5 µm
Occ.: 21r, 46rr, 47rr, 49rr, 51r, 79rr, 86r, 98rr, 114rr

In the Czech Republic there has been only one previous finding of this alga (Růžička 1957a) that is well distinguished from similar taxa by bowl–shaped semicells. Judging from my findings, it seems to by typical in relatively undisturbed, mesotrophic, slightly acidic to neutral ponds and pools, where it usually occurs among submerged macrophytes; it has often been reported in the literature from similar habitats (see e.g. Messikommer 1942; Růžička 1957a; Lenzenweger 1997, 2000b).

***Staurastrum eurycerum* Skuja (Figs 303–304)**
Syn.: *St. dybowskii* Wolosz.
Dim.: L: 18–26 µm, B: 32–52 µm, I: 6–8 µm
Occ.: 1c, 2rr, 9r, 10rr, 11r, 86r

Skuja (1948) considers this species euplanktonic, but from the data on its ecology provided by Lenzenweger (1997), and also from the conditions of my findings (on all sites found in plankton, but at sites no.1, 9, 10 and 86 also found in the littoral zone associated with submerged macrophyte vegetation) it would be better described as a tychoplanktonic alga.

***Staurastrum habeebense* Irénée–Marie (Figs 305–307, 405–406)**
Dim.: L: 36–45 µm, B: 24–31 µm, I: 21–23 µm
Occ.: 91r, 93cc

A remarkable species, preferring (unlike most desmids) artificial, periodically desiccating substrata, like roof and drainage gutters or garden ornaments, where it is probably widely distributed, as indicated by its relatively frequent recent findings (Belcher & Swale 1984; Williamson 2002; Coesel & Hindák 2003; Coesel et al. 2006). For a more detailed discussion of the ecology of *Staurastrum habeebense* see Coesel & Hindák (2003) and Šťastný (2008).

***Staurastrum hystrix* Ralfs (Figs 308–309)**
Dim.: Ls: 30–35 µm, Bs: 26.5–30 µm, I: 10–11.5 µm
Occ.: 4r, 24c (at both sites in a strongly acidic, oligotrophic milieu)

Rather rare, strictly acidophilous taxon, from the Czech Republic previously reported only from the Šumava Mts by Lütkemüller (1910) and by Mattauch (1936) from sampling site no. 3.

***Staurastrum lapponicum* (Schmidle) Grönblad (Figs 310–311, 407–409)**
Dim.: L: 31–38 µm, B: 31–37 µm, I: 10–13 µm
Occ.: 1r, 4rr, 5c, 8r, 9rr, 13r, 14r, 17rr, 24c, 25c, 44rr, 47rr, 48rr, 49rr, 53rr, 86r, 89c, 99c, 105rr

Considering the relatively high frequency of my findings, it is somewhat surprising that this species has not previously been mentioned in the Czech Republic. Possibly, however, it has been mistaken for some forms close to *St. punctulatum* Ralfs. At present it seems to be relatively common in mesotrophic, slightly acidic habitats.

***Staurastrum meriani* Reinsch (Fig. 312)**
Dim.: L: 42–45 µm, B: 24–25 µm, I: 15 µm
Occ.: 50r, 117r

Rather rare species of ephemeral habitats. Previous records from the Czech Republic: Pascher (1906), Fischer (1922–24), Beck–Mannageta (1929), Růžička (1956, 1957b), Roubal (1958). Apical view usually regularly hexagonal.

***Staurastrum minimum* Coesel (Fig. 313)**
Dim.: L: 15–20 µm, B: 16–22 µm, I: 4–5 µm
Occ.: 1cc, 4c, 24cc

To date, this species has been reported only from The Netherlands (Coesel 1996) and France (Kouwets 2001). It has only a few specific morphological features (Coesel & Meesters 2007), however, rather characteristic seems to be its ecology; it prefers more or less oligotrophic, acidic habitats, which corresponds well with the circumstances of my findings.

***Staurastrum oligacanthum* W.Archer (Figs 314–315)**
Dim.: L: 37.5–42.5 µm, B: 42–45 µm, I: 20–22 µm
Occ.: 1r

New for the Czech flora; however, Coesel (1997) and Lenzenweger (1997) point to the considerable similarity and relationship between this species and *St. cristatum*; it is therefore possible that some findings of *St. cristatum* within the Czech Republic refer in fact to the alga in question, particularly Rosa (1969; pl. 13, fig. 18) and Růžička (1973;

pl. 16, fig. 2).

***Staurastrum orbiculare* RALFS var. *ralfsii* W. et G.S.WEST (Fig. 316)**
Dim.: L: 31–37.5 µm, B: 27–31 µm, I: 8.5–9.5 µm
Occ.: 44r, 114rr

Previous records from the Czech Republic: JAPP (1930b), ROSA (1939), ROUBAL (1958).

***Staurastrum oxyacanthum* W.ARCHER (Fig. 317)**
Dim.: L: 26–30 µm, B: 37–48 µm, I: 9–11 µm
Occ.: 1r
This species is usually considered to be rather common (see e.g. LENZENWEGER 1997, 2003b; COESEL & MEESTERS 2007); in the Czech Republic, however, it seems to be very rare, having been reported only twice thus far (LÜTKEMÜLLER 1910, as *S. oxyacantha* W.ARCHER, HOLZER 1931).

****Staurastrum pentasterias* GRÖNBLAD (Fig. 318)**
Dim.: L: 29–31.5 µm, B: 35.5–39 µm, I: 10–13 µm
Occ.: 1rr, 14rr

A rather variable taxon, as concerns its arm–length and thickness, and cell wall ornamentation, on the other hand, the cell length and particularly the radiation of the cells (5–radiate) seem to be quite constant (RŮŽIČKA 1972) and may therefore be used as distinguishing features.

****Staurastrum podlachicum* B.EICHLER et GUTW. (Figs 319–321)**
Dim.: L: 33–39 µm, B: 37–40 µm, I: 13–15 µm
Occ.: 1rr, 114rr

Poorly known species that is considered doubtful by COESEL & MEESTERS (2007). However, the morphological characteristics of my specimens were very consistent. Moreover, no intermediate forms were found between this taxon and, for instance, the at site no.1 co–occurring *St. oligacanthum* (compare Figs 314–315 and 319–321), a variety of which *St. podlachicum* has been previously described (GRÖNBLAD 1920). Therefore, in my opinion, *St. podlachicum* represents a "good" species. Most probably, on site no. 114 the alga in question was scoured from adjacent bogs situated in Zone I of Šumava National Park, as also indicated by its finding in a minimal abundance (one cell).

***Staurastrum pungens* RALFS (Fig. 322)**
Dim.: Ls: 39–46 µm, Bs: 33–40 µm, I: 11–12.5 µm
Occ.: 1rr

A very rare taxon, in the Czech Republic found only by PASCHER (1903, 1906).

****Staurastrum quadrispinatum* W.B.TURNER (Fig. 323)**
Dim.: Ls: 28.5–35 µm, Bs: 27.5–29 µm, I: 9 µm
Occ.: 30rr

A rather rare species of oligotrophic, strongly acidic high moors (PÉTERFI 1974; LENZENWEGER 1997). Most likely, the alga occurs also in some of the high moors within the "Modravské slatě" complex.

***Staurastrum sebaldi* REINSCH (Fig. 324)**
Dim.: L: 73–87 µm, B: 75–110 µm, I: 18–22 µm
Occ.: 1rr

In the Czech Republic *St. sebaldi* is a rare species, previously reported only from four localities (LÜTKEMÜLLER 1910; DVOŘÁK 1932; ROUBAL 1939; ROSA 1951). In addition, RŮŽIČKA (1973) found the very closely related *St. traunsteineri* HUSTEDT that might be considered *St. sebaldi* as well (see e.g. LENZENWEGER 1997; p. 125 and 138).

****Staurastrum setigerum* CLEVE (Fig. 325)**
Dim.: Ls: 50–56 µm, Lc: 57–66 µm, Bs: 43–52 µm, Bc: 57–68 µm, I: 14–17 µm
Occ.: 17c, 114r

This species is easily to be distinguished from taxa with a similar ornamentation and ecology by their dimensions; *St. teliferum* RALFS is distinctly smaller, *St. polytrichum* (PERTY) RABENH. noticeably larger. Moreover, both those taxa are much more common than *St. setigerum*. At site no. 114, most likely, the alga in question is not autochtonous but scoured from adjacent bogs situated in Zone I of Šumava National Park.

***Staurastrum smithii* (G.M.SMITH) TEILING (Fig. 326)**
Syn.: *St. contortum* G.M.SMITH
Dim.: L: 37–48 µm, B: 43–54 µm, I: 6–7 µm
Occ.: 15c, 16r, 124r

Probably a quite rare planktont, preferring eutrophic waters (COESEL 1997; LENZENWEGER 2003b) that, however, is quite probably generally

confused with other planktonic *Staurastrum* species. From the Czech Republic there have been only two findings (Pascher 1903, 1906).

***Staurastrum trapezicum* Boldt (Figs 327–328, 410)**
Dim.: L: 50–62 µm, B: 55–63 µm, I: 14–16 µm
Occ.: 1r, 103r

Previously, this species was reported from the Czech Republic only by Fischer (1922–24, 1924), Holzer (1931), Růžička (1956) and Neustupa et. al. (2002), but at least Růžička's finding, from examination of his figure (Růžička 1956; pl. 5, fig. 41), represents a different species, related to *St. hirsutum* Ralfs or its var. *muricatum* (Ralfs) Kurt Först.

****Staurastrum varians* Racib. (Figs 329, 411)**
Dim.: L: 32–35 µm, B: 30–34 µm, I: 15–16 µm
Occ.: 1r

From the Czech Republic only the closely related *St. acutum* Bréb. has been reported [Rabenhorst 1868, as *St. granulosum* Ralfs var. *acutum* (Bréb.) W. et G.S.West], which differs in that it has acute lateral angles, instead of rounded as in *St. varians* (Coesel & Meesters 2007).

***Staurastrum vestitum* Ralfs (Figs 330–331)**
Syn.: *St. anatinum* Cooke et Wills f. *vestitum* (Ralfs) Brook
Dim.: L: 32–42 µm, B: 47–84 µm, I: 12–15 µm
Occ.: 1c, 4rr, 5r, 8rr, 47r

A rare, morphologically highly variable taxon of well–preserved habitats, closely related to *St. aculeatum* Ralfs as well as to *St. anatinum* (Coesel & Meesters 2007). Its only previous finding in the Czech Republic was mentioned by Japp (1930b).

***Cosmocladium constrictum* W.Archer (Fig. 332)**
Dim.: L: 13.5–15 µm, B: 8.5–9 µm, I: 7.5–8 µm
Occ.: 9c (in plankton)

Notably, in my samplings many large colonies were present, although they only seldom are built up due to the extreme delicacy of their interconnecting slime strands. It is likely that *C. constrictum* is a very rare species, but considering the fragility of their interconnecting strands they may easily be disintegrated in fixed material (Coesel 1994), and, therefore, a subsequent confusion of individual cells with some representatives of the genus *Actinotaenium* cannot be ruled out. The only previous finding of this species in the Czech Republic was mentioned by Lütkemüller (1910).

***Cosmocladium saxonicum* De Bary (Figs 333–335)**
Dim.: L: 22–24 µm, B: 16–17.5 µm, I: 7–7.5 µm
Occ.: 98m (in plankton)

There is only one previous record of this species in the Czech Republic (Lütkemüller 1910). It is probably very rare (for instance, it was only recently, see Lenzenweger 2002, 2003a, found for the first time in Austria), but, as in the case of *C. constrictum*, fixing with alcohol or formaldehyde causes, in approximately 24 hours (Coesel 2004), a degradation of the colonies due to the disintegration of the interconnecting strands, and individual cells can then be easily mistaken for some smooth–walled representatives of the genus *Cosmarium*. Interestingly, the occurrence of the representatives of the genus *Cosmocladium* seems to be markedly ephemeral; both *C. saxonicum* as well as *C. constrictum* were sampled in large numbers in the autumn of 2005, but had already completely disappeared from the sampling sites by 2006; a similar observation was mentioned by Kouwets (Kouwets, pers. com.) for *C. perissum* J.Roy et Bisset.

***Sphaerozosma aubertianum* W.West (Figs 336, 412–413)**
Dim.: L: 14–18 µm, B: 19–27 µm, I: 5.5–7.5 µm
Occ.: 47c, 83r, 88r

A rare species preferring mesotrophic, slightly acidic habitats (Růžička 1973; Kouwets 1987), from the Czech Republic until now reported only by Lütkemüller (1910) and Růžička (1973).

***Sphaerozosma filiforme* Ralfs (Figs 337, 414–415)**
Syn.: *Onychonema filiforme* (Ehrenb.) J.Roy et Bisset
Dim.: L: 10.5–12.5 µm, B: 11.5–14.5 µm, I: 4–5 µm
Occ.: 1r, 4rr, 5r, 8rr, 9rr

This species appears to have a relatively broad ecological range; in literature it is usually described from mesotrophic, slightly acidic habitats (see e.g. Růžička 1973; Coesel 1994, 1998a; Lenzenweger 2000b), however it can also withstand slightly

eutrophic and weakly alkaline conditions (FEHÉR 2003). I found it in a similar, slightly eutrophic environment at sampling site no. 1. Previous findings from the Czech Republic: ROUBAL (1939), ROSA (1969) and RŮŽIČKA (1973). ROUBAL (1958) also mentions its nineteenth–century findings by Corda and Hansgirg in Western Bohemia.

***Hyalotheca mucosa* RALFS (Fig. 338)**
Dim.: L: 14–20 µm, B: 17–20 µm
Occ.: 26c, 33rr, 42 r, 46cc, 47r

Much less common than *Hyalotheca dissiliens* RALFS. Previous findings from the Czech Republic: PASCHER (1903, 1906), DVOŘÁK (1919), JAPP (1930a), DVOŘÁK (1932), MALOCH (1937), ROUBAL (1939), WÜNSCH (1939) and ROSA (1951).

***Desmidium baileyi* (RALFS) NORDST. var. *caelatum* (KIRCHN.) NORDST. (Figs 339, 416–417)**
Dim.: L: 16–18 µm, B: 22–24 µm
Occ.: 1r, 4rr, 5r

A rare taxon; for instance, LENZENWEGER (2000a) mentions the first finding in Austria, and notes only two known sites from Switzerland; GUTOWSKI & MOLLENHAUER (1996) describe it, probably by mistake, as "a very abundant species, currently not endangered". The nominate variety of *D. baileyi* differs in having cells about as long as they are broad with almost straight lateral sides, and it is particularly known from tropical regions (COESEL & MEESTERS 2007).

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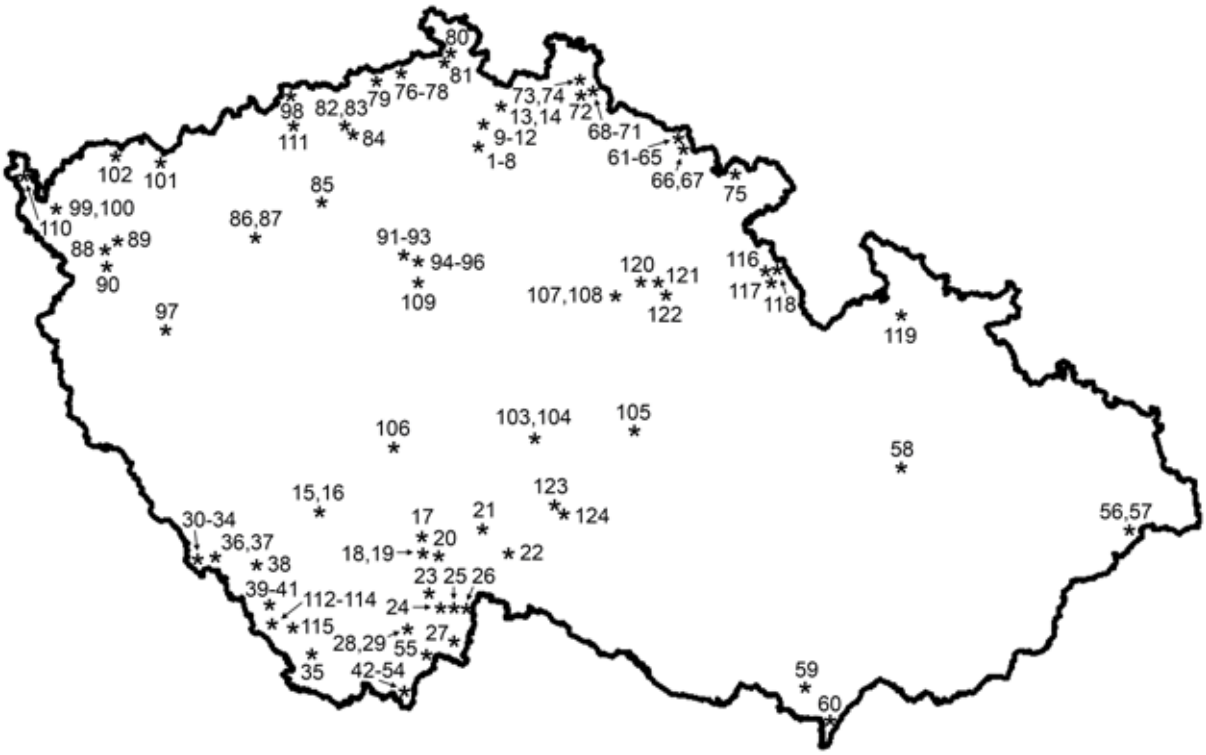
Fig. 1. Location of the sampling sites in the territory of the Czech Republic.



List of sampling sites

1. Nature reserve "Břehyně – Pecopala" – slightly eutrophic pond (50°34‘2.38“ N, 14°40‘56.05“ E; pH = 7.5–7.7, cond. = 220–234 µS.cm^{-1}) surrounded with a large complex of bogs and fens (pH at most sampling sites = 5.5–6.5, cond. = 70–220 µS.cm^{-1}).
2. Máchovo jezero (50°35‘2.13“ N, 14°38‘59.31“ E) – eutrophic pond; pH = 7.5–8.0, cond. = 212–276 µS.cm^{-1}.
3. Nature reserve "Swamp" (50°34‘48.19“ N, 14°40‘4.77“ E) – transition bog; pH = 3.5–5.4, cond. = 88–174 µS.cm^{-1}.
4. Unnamed transition bog about 350 m south–southeast from the "Swamp" Nature Reserve (50°34‘34.44“ N, 14°40‘14.45“ E); pH = 3.4–6.3, cond. = 54–156 µS.cm^{-1}.
5. Unnamed boggy pool on the southern side of "Máchovo jezero" fishpond (50°34‘39.25“ N, 14°39‘44.68“ E); pH = 5.7–6.5, cond. = 198–307 µS.cm^{-1}.
6. Unnamed transition bog on the northern side of "Máchovo jezero" fishpond (50°35‘38.69“ N, 14°38‘37.01“ E).
7. Ephemeral pool near "Břehyňský" pond (50°34‘32.27“ N, 14°40‘49.55“ E); pH = 5.8, cond. = 98 µS.cm^{-1}.
8. Nature reserve "Mariánský rybník" (50°32‘43.59“ N, 14°40‘42.71“ E) – oligomesotrophic boggy pond.

9. Držník (50°36‘39.32‘‘ N, 14°43‘23.26‘‘ E) – mesotrophic pond (part of the “Hradčanské rybníky” Nature Reserve; pH = 6.0–6.1, cond. = 185–285 µS.cm^{-1}) with a neighboring transition bog; pH = 5.5–5.8, cond. = 120–168 µS.cm^{-1}.
10. Strážovský rybník (50°36‘35.08‘‘ N, 14°45‘4.03‘‘ E) – slightly eutrophic pond (part of the “Hradčanské rybníky” Nature Reserve); pH = 6.1–6.6, cond. = 334–362 µS.cm^{-1}.
11. Hradčanský rybník (50°37‘1.55‘‘ N, 14°42‘38.55‘‘ E) – slightly eutrophic pond; pH = 6.4–6.6, cond. = 225–265 µS.cm^{-1}.
12. Hvězdovský rybník (50°38‘31.72‘‘ N, 14°47‘27.09‘‘ E) – slightly eutrophic pond.
13. Nature reserve “Rašeliniště Černého rybníka” (50°41‘22.43‘‘ N, 14°50‘30.93‘‘ E) – mesotrophic pond (pH = 6.7, cond. = 96 µS.cm^{-1}) with a neighboring transition bog.
14. Děvínský rybník (50°41‘35.67‘‘ N, 14°51‘47.64‘‘ E) – mesotrophic pond. Leg. Ladislav Hodač, Charles University, Prague.
15. Nature reserve “Řežabinec a Řežabinecké tůně” (49°15‘10.75‘‘ N, 14°5‘31.99‘‘ E) – eutrophic fishpond (pH = 8.1–10.2, cond. = 312–499 µS.cm^{-1}) with neighboring pools (pH = 6.1–7.4, cond. = 396–827 µS.cm^{-1}).
16. Podkostelní rybník (49°15‘53.63‘‘ N, 14°7‘30.95‘‘ E) – eutrophic fishpond; pH = 7.7, cond. = 357 µS.cm^{-1}.
17. Nature reserve “Borkovická blata” (49°14‘9.36‘‘ N, 14°37‘24.84‘‘ E) – transition bog; pH = 5.5–6.5, cond. = 60–160 µS.cm^{-1}.
18. Oligotrophic, acidic pool in the “Ruda” Nature Reserve (49°9‘8.29‘‘ N, 14°41‘30.66‘‘ E).
19. Nature reserve “Hliníř” (49°8‘9.2‘‘ N, 14°40‘46.54‘‘ E) – transition bog; pH = 4.4–5.8, cond. = 65–106 µS.cm^{-1}.
20. Nature reserve “Rod” (49°7‘15.94‘‘ N, 14°44‘59.42‘‘ E) – transition bog; pH = 5.5–6.2, cond. = 116–178 µS.cm^{-1}.
21. Nature reserve “Luží u Lovětína” (49°12‘20.77‘‘ N, 15°3‘18.68‘‘ E) – complex of several mesotrophic and slightly eutrophic pools.
22. Dolní Lomský rybník (49°6‘24.77‘‘ N, 15°9‘41.59‘‘ E) – slightly eutrophic fishpond.
23. Nature reserve “V Rájích” (48°59‘10.35‘‘ N, 14°42‘31.63‘‘ E) – mesotrophic spring area; pH = 5,9–7,2, cond. = 233–330 µS.cm^{-1}.
24. Pískovny Cep (48°55‘24.19‘‘ N, 14°50‘19.34‘‘ E) – complex of several oligotrophic and mesotrophic pools; pH = 4,3–7,0, cond. = 22–50 µS.cm^{-1}.
25. Nature reserve “Vizír” (48°57‘46.87‘‘ N, 14°53‘13.01‘‘ E) – slightly eutrophic pond (pH = 6,5–7,5, cond. = 110–185 µS.cm^{-1}) with a neighboring transition bog (pH = 4,0–5,5, cond. = 95–131 µS.cm^{-1}).
26. Nature reserve “Rašeliniště Pele” (48°57‘36.69‘‘ N, 14°57‘25.92‘‘ E) – transition bog; pH = 5,3–5,9, cond. = 74–112 µS.cm^{-1}.
27. Eutrophic pool in the “Horní Lužnice” Nature Reserve (48°51‘3.72‘‘ N, 14°54‘28.76‘‘ E); pH = 7.8, cond. = 212 µS.cm^{-1}
28. Krčín (48°53‘55.42‘‘ N, 14°39‘53.04‘‘ E) – eutrophic fishpond.
29. Nature reserve “Žemlička” (48°53‘29.09‘‘ N, 14°41‘21.74‘‘ E) – mesotrophic pond with a neighboring spring fen (pH = 6.3–6.8, cond. = 139–241 µS.cm^{-1}).
30. Nature reserve “Přední Mlynářská slať” (49°1‘21.30‘‘ N, 13°27‘29.53‘‘ E) – oligotrophic high moor; Zone I of Šumava National Park; pH = 3.7–4.1, cond. = 37–93 µS.cm^{-1}.
31. Shallow pool near the way from Přední Mlynářská slať to the “Roklanský” stream (49°1‘20.31‘‘ N, 13°27‘9.67‘‘ E); Zone I of Šumava National Park; pH = 5.8, cond. = 27 µS.cm^{-1}.
32. Mesotrophic pool near “Roklanský” stream (49°1‘30.06‘‘ N, 13°26‘44.74‘‘ E); Zone I of Šumava National Park.
33. Mesotrophic ditch near “Roklanský” stream (49°1‘42.71‘‘ N, 13°26‘49.47‘‘ E); Zone I of Šumava National Park.
34. Shallow, water–filled ditch near the way from Novohuťské močály to the “Březník” mountain (48°58‘18.90‘‘ N, 13°28‘3.80‘‘ E; Šumava National Park); pH = 5.3, cond. = 17 µS.cm^{-1}.
35. Small spring area near the “Pláničský rybník” Nature Reserve (48°43‘21.61‘‘ N, 14°9‘42.77‘‘ E); pH = 5.7–6.2, cond. = 96–115 µS.cm^{-1}.
36. Oligomesotrophic ephemeral pool near the “Zhůří” village (49°4‘44.18‘‘ N, 13°34‘13.35‘‘ E); Šumava National Park.
37. Oligomesotrophic ephemeral pool near the “Olšinka” stream (49°1‘15.28‘‘ N, 13°36‘38.59‘‘ E); Šumava National Park.
38. Oligomesotrophic ephemeral pool near the “Solovec” mountain (48°58‘34.79‘‘ N, 13°51‘16.33‘‘ E); Šumava Protected Landscape Area.
39. Oligomesotrophic ephemeral ditch near the “Spáleniště” mountain (48°52‘31.36‘‘ N, 13°48‘7.95‘‘ E); Šumava National Park.
40. Mesotrophic ephemeral ditch near the “Pěkná” village (48°51‘44.32‘‘ N, 13°57‘5.03‘‘ E); Šumava Protected Landcape Area.
41. Oligotrophic ephemeral ditch near the “Doupná hora” mountain (48°53‘42.84‘‘ N, 13°56‘6.56‘‘ E); Šumava Protected Landscape Area.
42. Mlýnský rybník (48°42‘38.67‘‘ N, 14°42‘43.19‘‘ E) – mesotrophic pond; Novohradské hory Mts Protected Landcape Area; pH = 5.8–6.7, cond. = 49–108 µS.cm^{-1}.
43. Oligomesotrophic ephemeral pool near the “Mlýnský” pond (48°42’33.09’’ N, 14°42’47.09’’ E); Novohradské hory Mts Protected Landcape Area; pH = 5.6, cond. = 13 µS.cm^{-1}.
44. Zlatá Ktiš (48°40’44.03’’ N, 14°42’35.92’’ E) – mesotrophic pond; Novohradské hory Mts Protected Landscape Area; pH = 6.1–6.9 , cond. = 53–81 µS.cm^{-1}.
45. Oligomesotrophic ephemeral pool near the “Zlatá Ktiš” pond (48°40‘48.09‘‘ N, 14°42‘32.57‘‘ E); Novohradské hory Mts Protected Landscape Area.
46. Small mesotrophic pond near the “Žofín” village (48°40’32.99’’ N, 14°41’33.61’’ E); Novohradské hory Mts Protected

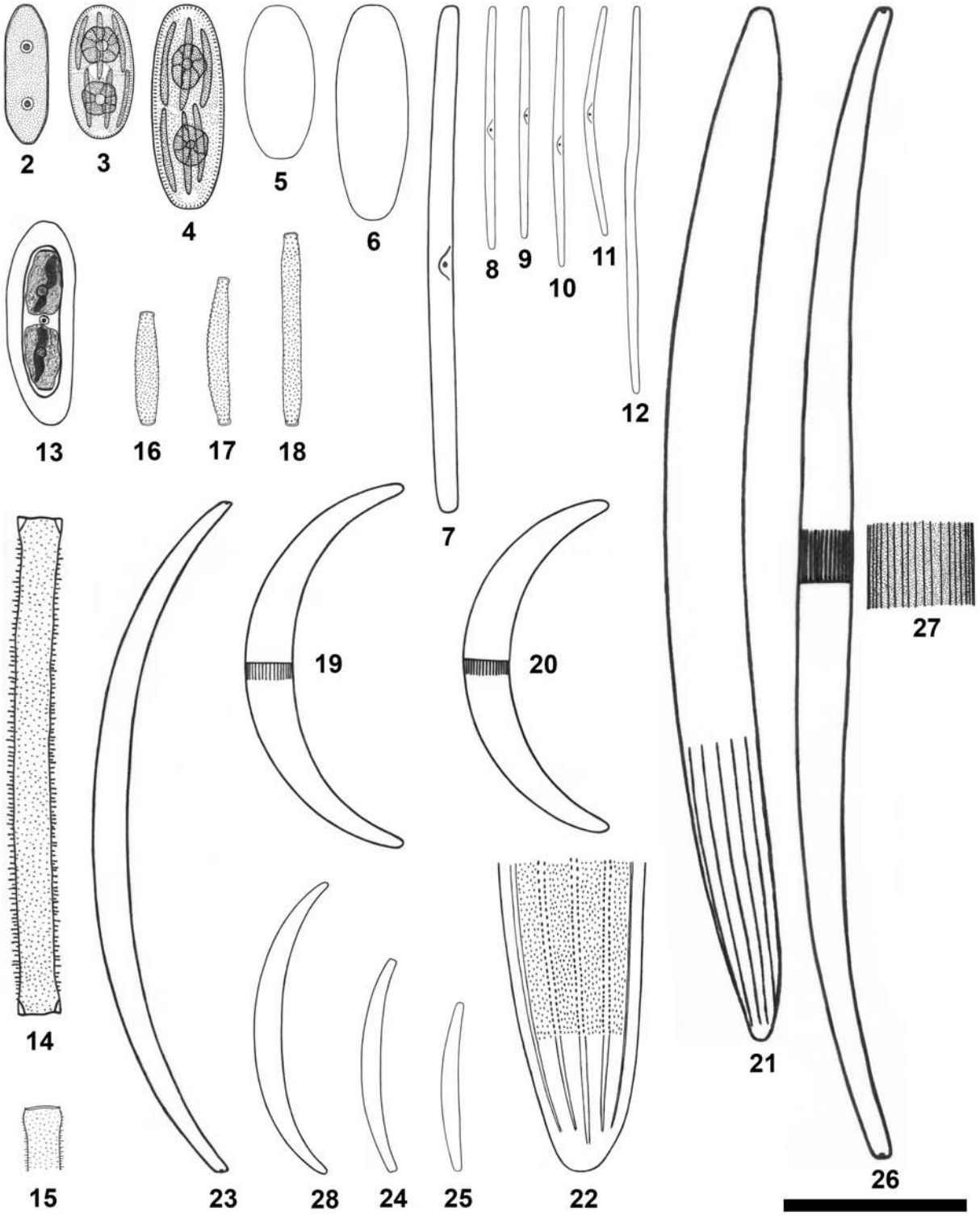
Landscape Area; pH = 6.5 cond. = 104 µS.cm^{-1}.

47. Huťský rybník (48°39’20.99’’ N, 14°40’56.36’’ E) – mesotrophic pond; Novohradské hory Mts Protected Landscape Area; pH = 5.6–6.4, cond. = 32–69 µS.cm^{-1}.
48. Uhlišťský rybník (48°38‘48.01‘‘ N, 14°39‘20.55‘‘ E) – mesotrophic pond; Novohradské hory Mts Protected Landscape Area; pH = 5.5–6.2, cond. = 36–43 µS.cm^{-1}.
49. Pohořský rybník (48°37‘4.27‘‘ N, 14°40‘26.86‘‘ E) – mesotrophic pond; Novohradské hory Mts Protected Landscape Area; pH = 5.1–6.2, cond. = 23–40 µS.cm^{-1}.
50. Wet mosses on the margin of “Pohořský” pond (48°37‘3.29‘‘ N, 14°40‘25.81‘‘ E); Novohradské hory Mts Protected Landscape Area.
51. Small mesotrophic pool about 400 m southeast from the “Pohořský” pond (48°36‘47.96‘‘ N, 14°40‘52.95‘‘ E); Novohradské hory Mts Protected Landscape Area; pH = 7.0, cond. = 47 µS.cm^{-1}.
52. Moss–covered rocks in the “Pohořský” stream (48°37‘9.25‘‘ N, 14°40‘18.79‘‘ E); Novohradské hory Mts Protected Landscape Area.
53. Kapelunk (48°36‘43.92‘‘ N, 14°42‘46.60‘‘ E) – mesotrophic pond; Novohradské hory Mts Protected Landscape Area; pH = 6.3–6.7 , cond. = 34–86 µS.cm^{-1}.
54. Shallow ephemeral pool near the “Kapelunk” pond (48°36‘37.22‘‘ N, 14°42‘42.51‘‘ E); Novohradské hory Mts Protected Landscape Area; pH = 6.2, cond. = 39 µS.cm^{-1}.
55. Nature reserve “Přesličkový rybník” (48°46‘15.63‘‘ N, 14°48‘11.14‘‘ E) – mesotrophic pond; pH = 5.9, cond. = 60 µS.cm^{-1}.
56. Oligomesotrophic ephemeral pool near the “Gruň” mountain (49°29‘32.51‘‘ N, 18°30‘22.63‘‘ E); Beskydy Protected Landscape Area; pH = 5.9, cond. = 80 µS.cm^{-1}.
57. Mesotrophic ephemeral ditch (49°30‘41.83‘‘ N, 18°28‘37.53‘‘ E) near the “Okrouhlice” mountain; Beskydy Protected Landscape Area.
58. Slightly eutrophic pool near the “Chomoutovské jezero” Nature Reserve (49°38‘46.54‘‘ N, 17°14‘35.12‘‘ E); pH = 7.9–8.1. Leg. Ludmila Hájková, Masaryk University, Brno.
59. Nature reserve “Zámecký rybník” (48°48‘29.93‘‘ N, 16°48‘34.02‘‘ E) – eutrophic fishpond; pH = 8.4, cond. = 528 µS.cm^{-1}.
60. Eutrophic ditch near the “Ranšpurk” Nature Reserve (48°40‘53.78‘‘ N, 16°58‘5.40‘‘ E).
61. Wet, moss covered granite rocks in the “Pančavský” waterfall (50°45‘39.75‘‘ N, 15°32‘42.64‘‘ E); Zone I of the Krkonoše Mts National Park; pH = 7.0, cond. = 13 µS.cm^{-1}.
62. Wet, sometimes moss–covered granite rocks in the “Úpský” waterfall (50°43‘56.018‘‘ N, 15°42‘44.678‘‘ E), Zone I of the Krkonoše Mts National Park; pH = 5.6, cond. = 12 µS.cm^{-1}.
63. Wet, moss–covered granite rocks in the “Zelený” stream (50°42‘8.25‘‘ N, 15°41‘54.28‘‘ E); Krkonoše Mts National Park; pH = 6.6, cond. = 14 µS.cm^{-1}.
64. The “Úpa” river near the town of Pec pod Sněžkou (50°43‘0.41‘‘ N, 15°43‘25.68‘‘ E); Krkonoše Mts National Park; pH = 6.2, cond. = 32 µS.cm^{-1}.
65. Small peat bog near the locality no. 64 (50°43‘0.51‘‘ N, 15°43‘25.09‘‘ E); Krkonoše Mts National Park; pH = 5.1, cond. = 14 µS.cm^{-1}.
66. Ephemeral pool near the “Černohorské rašeliniště” peat bog (50°40‘31.574‘‘ N, 15°43‘29.858‘‘ E); Krkonoše Mts National Park; pH = 5.7, cond. = 119 µS.cm^{-1}
67. Ephemeral pond near the town of Pec pod Sněžkou (50°40’51.29’’ N, 15°43’33.93’’ E); Krkonoše Mts National Park
68. Nature reserve “Na Čihadle” (50°49‘57.73‘‘ N, 15°13‘51.68‘‘ E) – oligotrophic high moor, Jizerské hory Mts Protected Landscape Area; pH = 4.3–4.4, cond. = 28–35 µS.cm^{-1}.
69. Klugeho louka – part of the “Rašeliniště Jizerky” Nature Reserve (50°49‘45.37‘‘ N, 15°20‘9.86‘‘ E) – oligotrophic high moor, Jizerské hory Mts Protected Landscape Area; pH = 4.3–4.6, cond. = 35–52 µS.cm^{-1}.
70. Vyhlídková louka – part of the “Rašeliniště Jizerky” Nature Reserve (50°49‘39.06‘‘ N, 15°19‘40.45‘‘ E) – oligotrophic high moor, Jizerské hory Mts Protected Landscape Area; pH = 4.5–4.7, cond. = 32–44 µS.cm^{-1}.
71. Tetřeví louka – part of the “Černá jezírka” Nature Reserve (50°50‘44.92‘‘ N, 15°18‘11.04‘‘ E) – oligotrophic high moor, Jizerské hory Mts Protected Landscape Area; pH = 4.1–4.5, cond. = 28–40 µS.cm^{-1}.
72. Oligotrophic ephemeral pool near the “Josefův důl” water reservoir (50°47’41.90’’ N, 15°11’49.60’’ E); Jizerské hory Mts Protected Landcape Area; pH = 4.9, cond. = 35 µS.cm^{-1}.
73. Wet, moss–covered rocks in the “Sloupský” stream, near the “Štolpišský” waterfall (50°51’4.06’’ N, 15°11’39.40’’ E); Jizerské hory Mts Protected Landscape Area.
74. Mucilaginous growths on granite rocks near locality no. 73 (50°51’1.75’’ N, 15°11’31.41’’ E); Jizerské hory Mts Protected Landscape Area.
75. Ephemeral pool in the “Adršpašsko–Teplické skály” Nature Reserve (50°36’39.48’’ N, 16°6’35.14’’ E).
76. Nature reserve “Rybník u Králova mlýna” (50°50’1.25’’ N, 14°9’20.38’’ E) – mesotrophic fishpond; Labské pískovce Protected Landscape Area; pH = 6.3–6.6, cond. = 264–286 µS.cm^{-1}.
77. Fire pond near the “Maxičky” village (50°48‘29.04‘‘ N, 14°10‘54.96‘‘ E); Labské pískovce Protected Landscape Area; pH = 6.5, cond. = 215 µS.cm^{-1}.
78. Wet rocks in an unnamed forest stream (50°49‘1.28‘‘ N, 14°10‘1.63‘‘ E); Labské pískovce Protected Landscape Area; pH = 6.0, cond. = 244 µS.cm^{-1}.
79. Mesotrophic pond near the “Libouchec” village (50°45‘59.37‘‘ N, 14°3‘32.01‘‘ E), part of the “Libouchecké rybníčky” Nature Reserve; pH = 6.6, cond = 118 µS.cm^{-1}. Leg. Sylvie Odstrčilová, Charles University, Prague.
80. Vlčí potok (50°55‘50.25‘‘ N, 14°25‘52.93‘‘ E) – periodically desiccating stream; National Park Bohemian Switzerland;

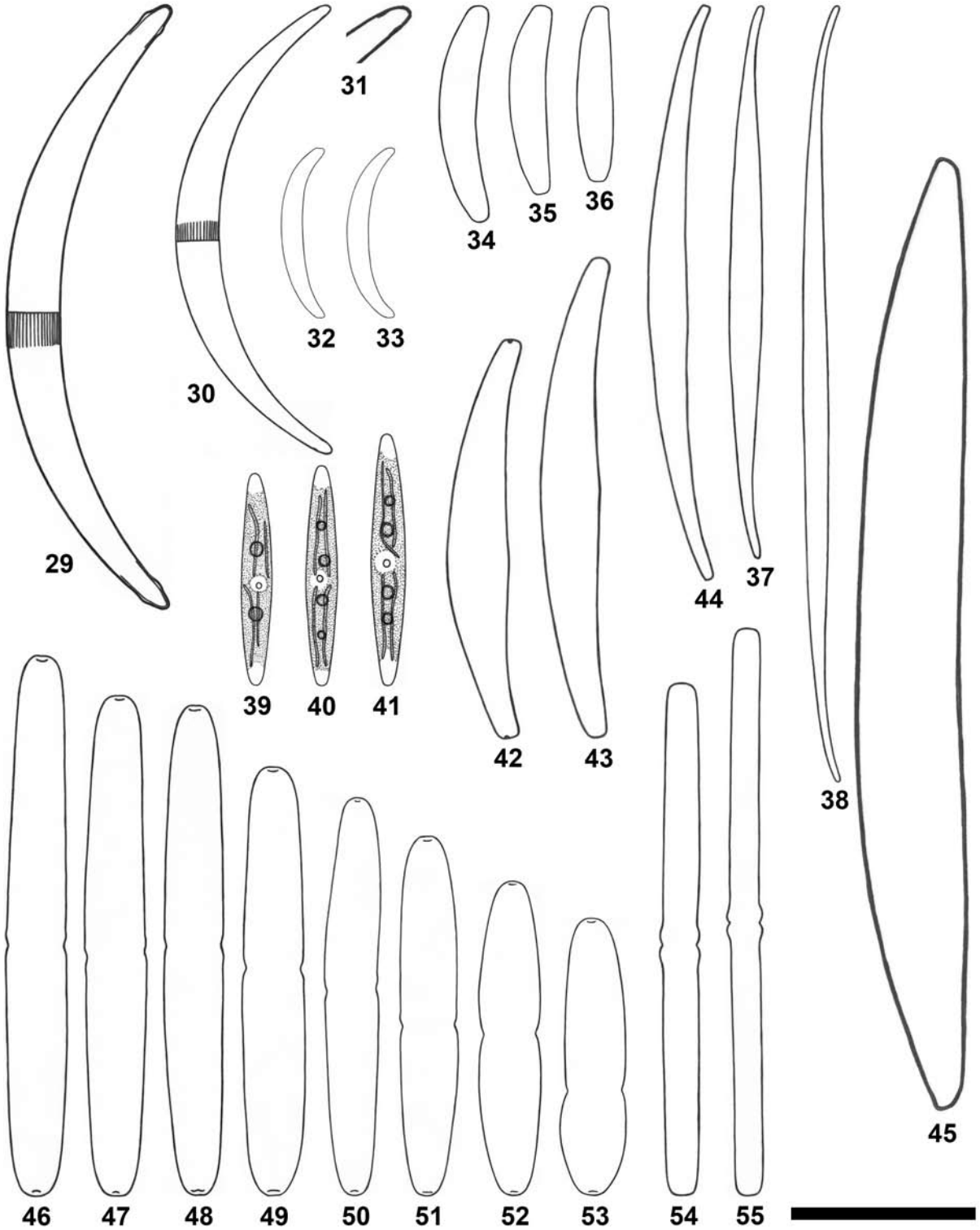
pH = 6.9–7.3, cond. = 152–162 μS.cm^{-1}. Leg. Jana Veselá, Charles University, Prague.

81. Wet sandstone rocks in the National Park Bohemian Switzerland (50°53‘20.99“ N, 14°22‘16.91“ E). Leg. Marie Pažoutová, Charles University, Prague.
82. Rašeliniště pod Bukovým vrchem (50°32’54.41” N, 13°54’18.37” E) – mesotrophic peat bog, part of the “Březina” Nature Reserve.
83. Vojenský rybník (50°32‘47.13“ N, 13°53‘47.79“ E) – mesotrophic pond; České středohoří Protected Landscape Area.
84. Wet, moss covered rocks at the top of the “Borečský vrch” Nature Reserve (50°30‘50.97“ N, 13°59‘19.54“ E). Leg. Pavel Škaloud, Charles University, Prague.
85. Nature reserve “V Bahnách” (50°10‘30.66“ N, 13°51‘44.37“ E) – mesotrophic transition bog; pH = 6.0–6.4, cond. = 132–211 μS.cm^{-1}.
86. Nature reserve “Rybníčky u Podbořánek” (50°2‘34.87“ N, 13°26‘27.69“ E) – two mesotrophic ponds; pH = 6.9–7.5, cond = 190–207 μS.cm^{-1}.
87. Oligomesotrophic ephemeral pool near the locality no. 86 (50°2‘35.11“ N, 13°26‘23.49“ E).
88. Kladský rybník (50°1‘35.88“ N, 12°40‘31.20“ E) – mesotrophic pond; Slavkovský les Protected Landscape Area; pH = 5.8. Leg. Ladislav Hodač, Charles University, Prague.
89. Mesotrophic pool in the “Upolínová louka” Nature Reserve (50°3‘59.74“ N, 12°44‘43.44“ E); Slavkovský les Protected Landscape Area. Leg. Ladislav Hodač, Charles University, Prague.
90. Mucilaginous growths on the concrete wall of an artificial pool in the town of Mariánské Lázně (49°57‘30.38“ N, 12°41‘52.41“ E). Leg. Ladislav Hodač, Charles University, Prague.
91. Periodically desiccating concrete drainage gutter near the railway corridor in the town of Roztoky u Prahy (50°9‘9.57“ N, 14°23‘50.50“ E); pH = 7.9, cond. = 175 μS.cm^{-1}.
92. Periodically desiccating concrete drainage gutter near the railway corridor in the town of Roztoky u Prahy–Žalov (50°10’4.53” N, 14°21’52.07” E).
93. Periodically desiccating concrete drainage gutter near the railway station Praha–Sedlec (50°7’52.84” N, 14°23’50.88” E).
94. Periodically desiccating concrete drainage gutter near the railway station Praha–Libeň (50°6’2.19” N, 14°30’5.37” E); pH = 7.2, cond. = 129 μS.cm^{-1}.
95. Periodically desiccating concrete drainage gutter near the railway station Praha–Běchovice (50°4’56.17” N, 14°35’34.49” E).
96. Hostivař (50°2’31.07” N, 14°32’6.21” E) – eutrophic water reservoir
97. Hracholusky (49°47’35.19” N, 13°8’39.81” E) – eutrophic water reservoir.
98. Fláje (50°41‘3.30“ N, 13°35‘6.43“ E) – mesotrophic water reservoir; pH = 7.0, cond. = 71 μS.cm^{-1}.
99. Peat bog near the “Kateřina” village (50°9’20.33” N, 12°24’33.37” E); part of the „Soos“ Nature Reserve; pH = 5.0–6.2, cond. = 65–126 μS.cm^{-1}.
100. Ephemeral pool near the locality no 99. (50°9’21.77” N, 12°24’29.45” E).
101. Shallow, oligomesotrophic pool on the margin of the “Božídarské rašeliniště” Nature Reserve (50°24’24.08” N, 12°54’45.18” E); pH = 5.4, cond. = 111 μS.cm^{-1}.
102. Nature reserve “Velký močál” (50°23’41.74” N, 12°38’17.23” E) – oligotrophic high moor; pH = 3.9, cond. = 80 μS.cm^{-1}. Leg. Jiří Neustupa, Charles University, Prague.
103. Nature reserve “Chvojnov” (49°24’23.39” N, 15°25’10.24” E) – mesotrophic spring fen.
104. Desiccating mesotrophic pool in the “Na Oklice” Nature Reserve (49°24’12.93” N, 15°23’39.03” E).
105. Shallow mesotrophic pool near the “Babín” fishpond (49°32’37.67” N, 15°53’50.73” E). Leg. Helena Bestová, Charles University, Prague.
106. Small eutrophic pond near the “Malšice” village (49°21’31.49” N, 14°34’9.11” E).
107. Wet soil and mosses on a concrete platform near the railway station Chvaletice (50°2’14.52” N, 15°25’6.13” E).
108. The Labe river near the town of Chvaletice (50°2’26.07” N, 15°21’24.18” E).
109. The Sázava river near the town of Pikovice (49°52’42.43” N, 14°25’39.25” E).
110. Wet, moss covered rocks in the “Bílý Halštrov” stream near the “Dolní Paseky” village (50°14’14.02” N, 12°14’3.01” E).
111. Squeezed mosses from a concrete platform near the inflow of “Mračný potok” stream into the Bílina river (50°32’36.84” N, 13°36’58.34” E).
112. Wet, moss covered rocks in the “Kobylí smyk” stream (48°46’36.36” N, 13°54’13.68” E); Šumava National Park.
113. Squeezed mosses from the Vltava river near the “Želnava” village (48°49’2.67” N, 13°56’52.62” E); Šumava National Park.
114. The “Lipno I” water reservoir near the “Nová Pec” village (48°47’36.03” N, 13°57’5.31” E); Šumava National Park.
115. Squeezed mosses from a concrete drainage gutter in the town of Horní Planá (48°45’48.98” N, 14°1’41.02” E); Šumava Protected Landscape Area.
116. Oligomesotrophic ephemeral pool near the town of Deštné v Orlických horách (50°17‘45.44“N, 16°20‘32.79“E); Orlické hory Protected Landscape Area.
117. Oligomesotrophic ephemeral pool near the “Karlův vrch” mountain (50°16’14.89”N, 16°22’16.97”E); Orlické hory Protected Landscape Area.
118. Nature reserve “Velká louka” (50°19’8.83”N, 16°25’28.46”E) – oligomesotrophic spring area with some mesotrophic, artificial pools; Orlické hory Protected Landscape Area.
119. Small spring area near the “Vozka” mountain (50°9’13.34”N, 17°7’4.38”E); Jeseníky Protected Landscape Area.
120. Černý Nadýmač (50°4‘25.99“N, 15°34‘54.08“E) – mesoeutrophic pond.

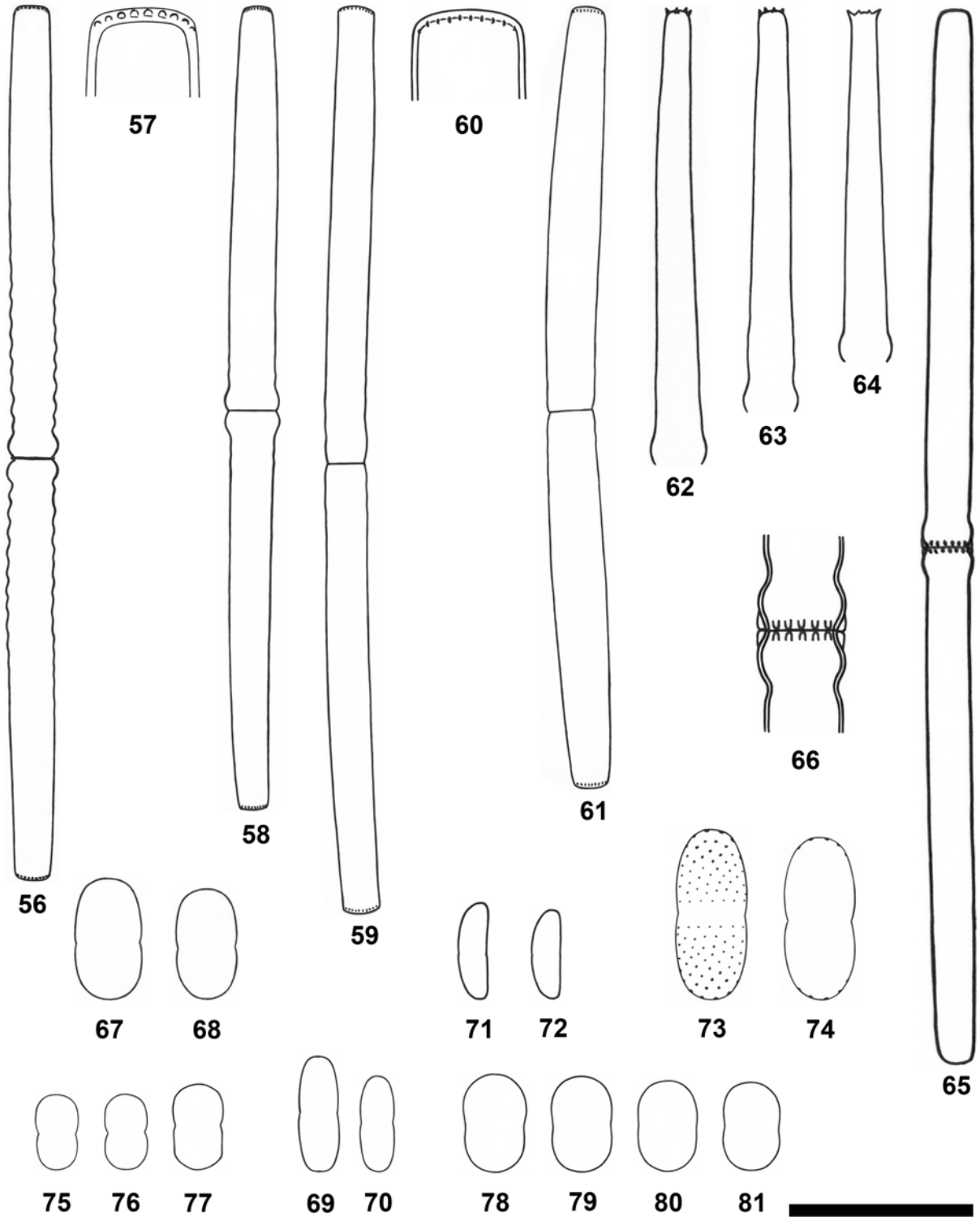
121. Mesoeutrophic sandy pool near the town of Lázně Bohdaneč (50°4‘14.16“N, 15°41‘47.42“E) filled in with *Utricularia australis* R.Br.
122. Mesoeutrophic pool in the town of Pardubice (50°2‘43.32“N, 15°46‘57.18“E) filled in with *Ceratophyllum demersum* L.
123. Nature reserve “Velký Pařezitý rybník” (49°13’43.59”N, 15°22’32.048”E) – oligomesotrophic pond with a neighboring transition bog.
124. Štěpnický rybník (49°11‘6.961“N, 15°27‘26.093“E) – eutrophic fishpond.



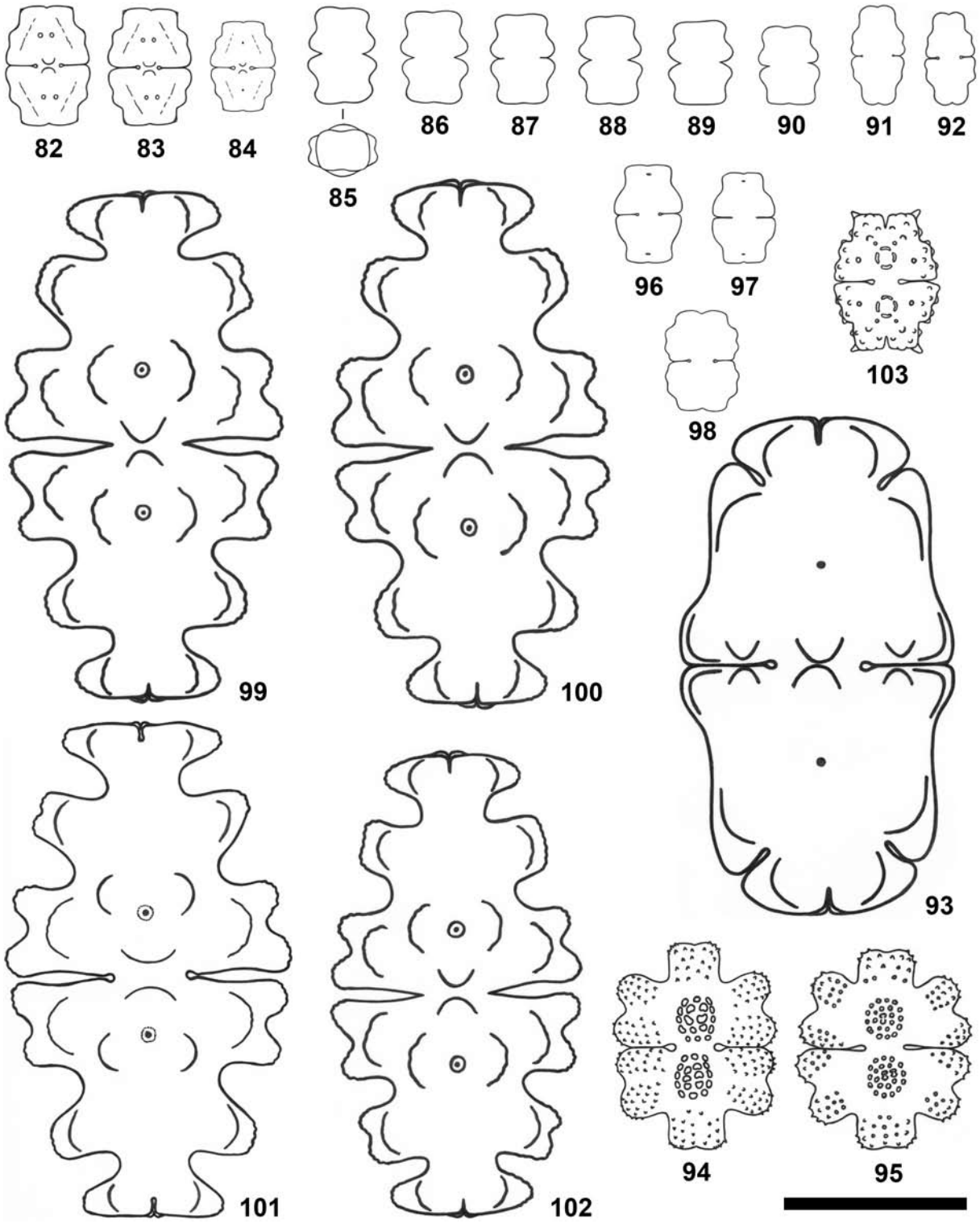
Figs 2–28. (2) *Mesotaenium caldariorum*; (3–6) *Netrium pseudactinotaenium*; (7) *Roya cambrica*; (8–12) *R. closterioides*, (12) dividing cell; (13) *Tortitaenia bahusiensis*; (14–15) *Gonatozygon aculeatum*; (16–18) *G. brebissonii* var. *alpestre*; (19–20) *Closterium archerianum* var. *pseudocynthia*; (21–22) *Cl. braunii*, (22) detail of cell wall sculpture; (23) *Cl. calosporum* var. *brasiliense*; (24–25) *Cl. cornu* var. *upsaliense*; (26–27) *Cl. delpontei*, (27) detail of cell wall sculpture; (28) *Cl. exile*. Scale bar 50 μm, 100 μm (for 21, 26).



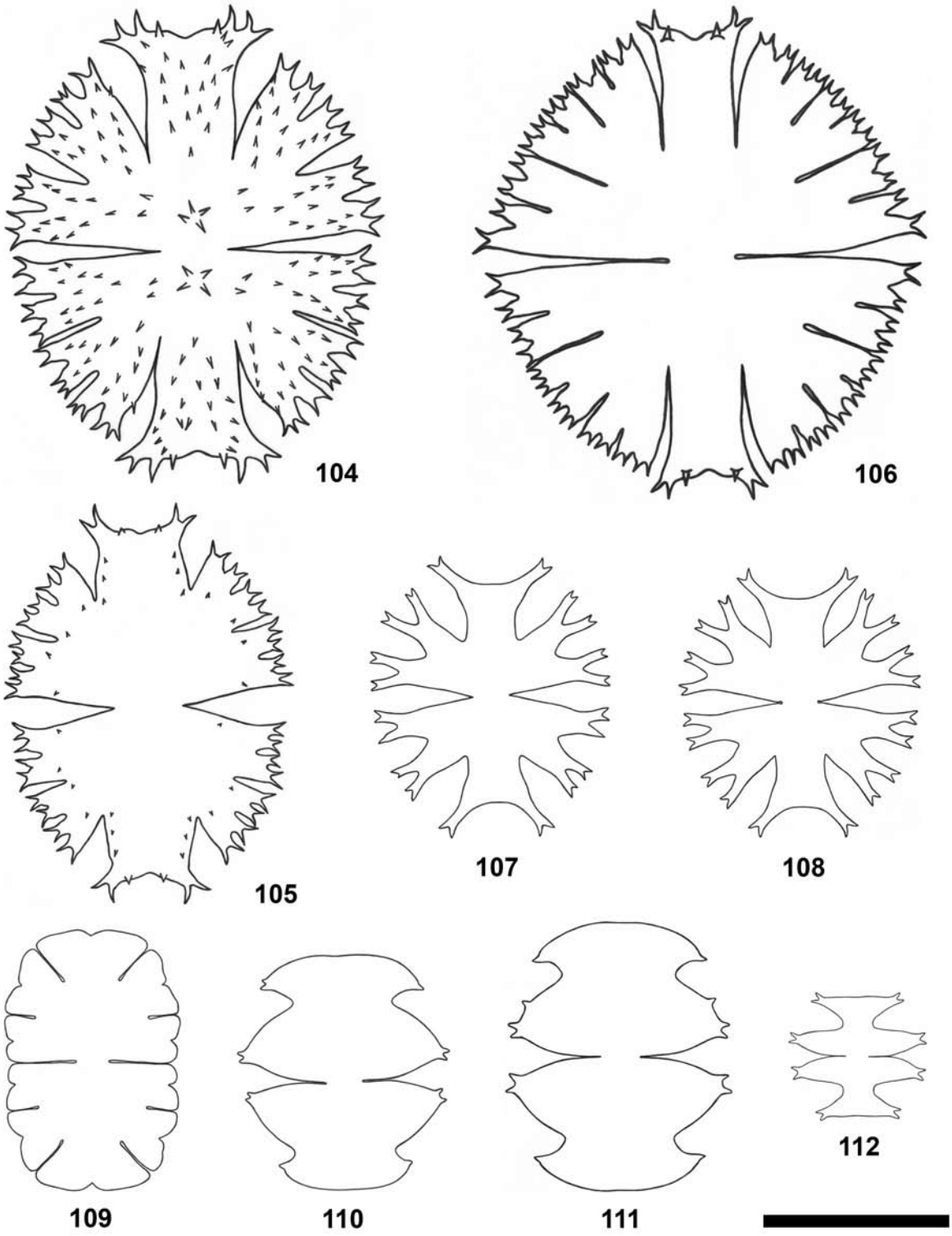
Figs 29–55. (29–31) *Closterium nematodes* var. *proboscideum*, (31) detail of the apex; (32–33) *Cl. pseudopygmaeum*; (34–36) *Cl. pusillum*; (37–38) *Cl. subulatum*; (39–41) *Cl. tortitaenoides*; (42–43) *Cl. tumidum*; (44) *Cl. tumidum* var. *nylandicum*; (45) *Cl. turgidum* var. *giganteum*; (46–53) *Haplotaenium indentatum*, morpha; (54–55) *H. rectum*. Scale bar 50 μm (for 31–44, 46–53), 100 μm (for 29, 30, 54, 55), 150 μm (for 45).



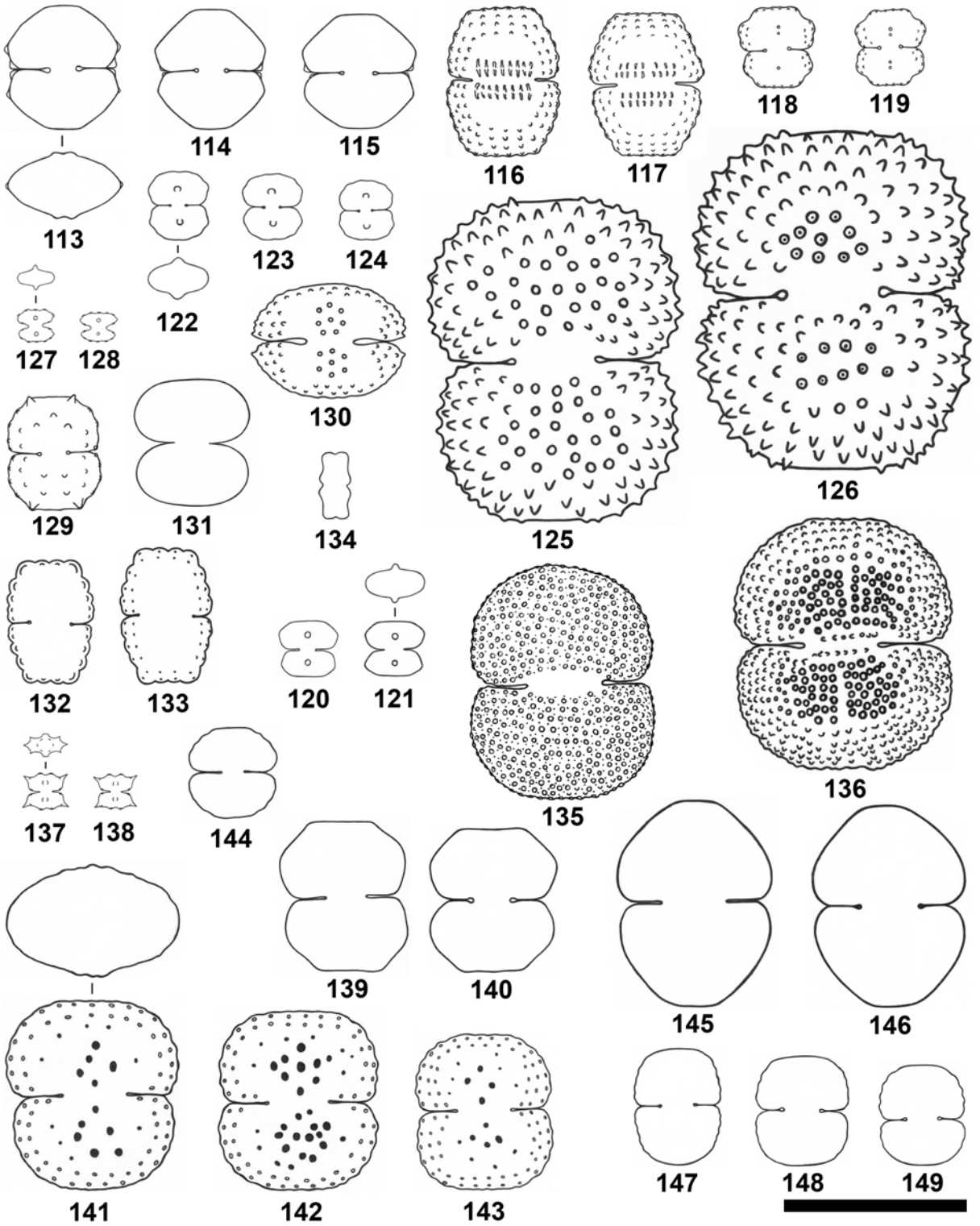
Figs 56–81. (56–58) *Pleurotaenium eugeneum*, (57) detail of the apex; (59–61) *Pl. simplicissimum*, (60) detail of the apex; (62–64) *Pl. tridentulum* (semicells); (65–66) *Docidium baculum*, (66) detail of the central part of the cell; (67–68) *Actinotaenium cruciferum*; (69–70) *A. inconspicuum*; (71–72) *A. inconspicuum* var. *curvatum*; (73–74) *A. kriegeri*; (75–77) *A. perminutum*; (78–81) *A. subsparsepunctatum*. Scale bar 30 μm (for 66–81), 50 μm (for 57, 60, 62–65), 150 μm (for 56, 58, 59, 61).



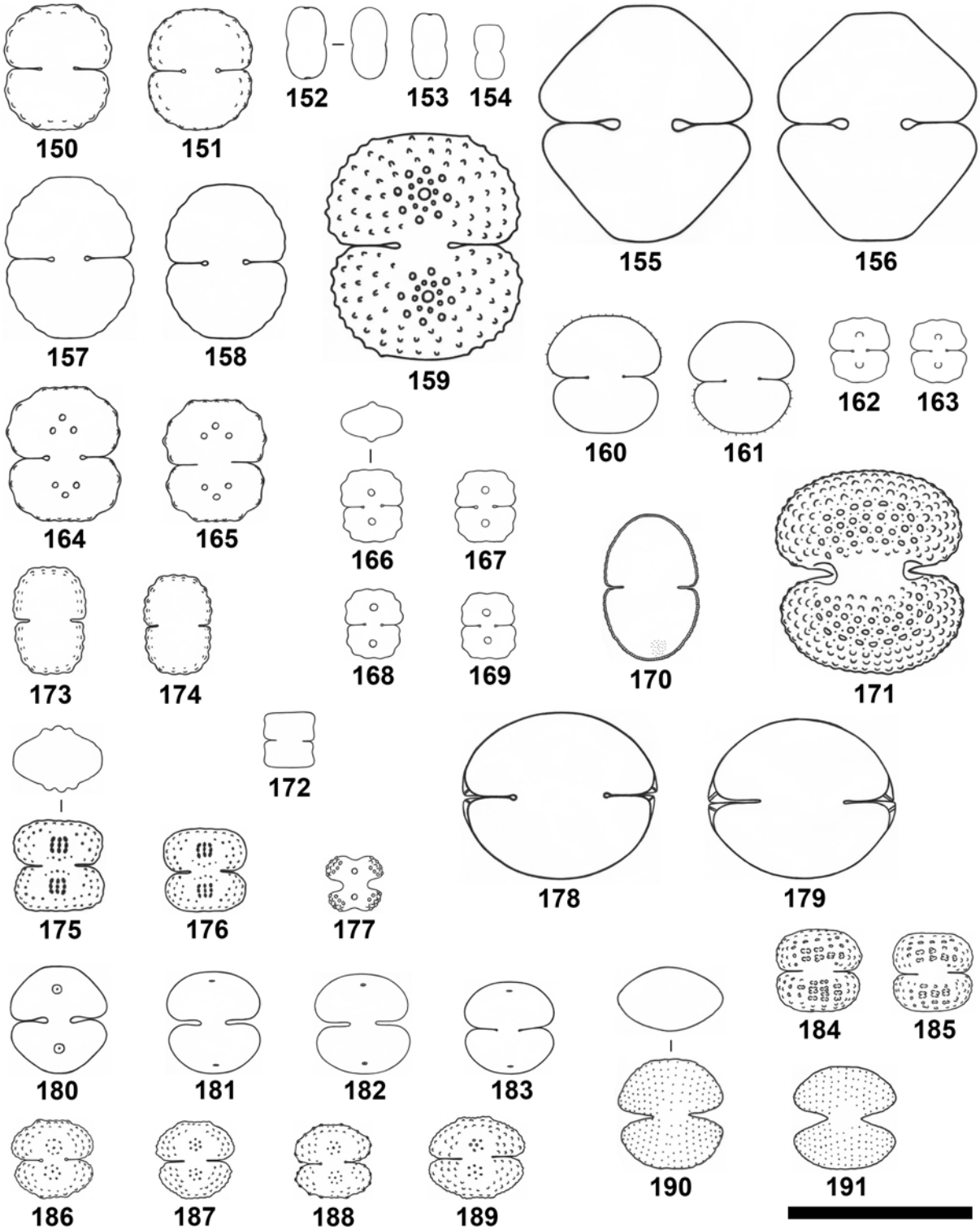
Figs 82–103. (82–84) *Euastrum biscrobiculatum*; (85–90) *E. brevisinuosum* var. *dissimile*; (91–92) *E. crassicolle*; (93) *E. crassum*; (94–95) *E. germanicum*; (96–97) *E. luetkemuelleri* var. *carniolicum*; (98) *E. montanum*; (99–102) *E. pinnatum*; (103) *E. turneri*. Scale bar 50 μm.



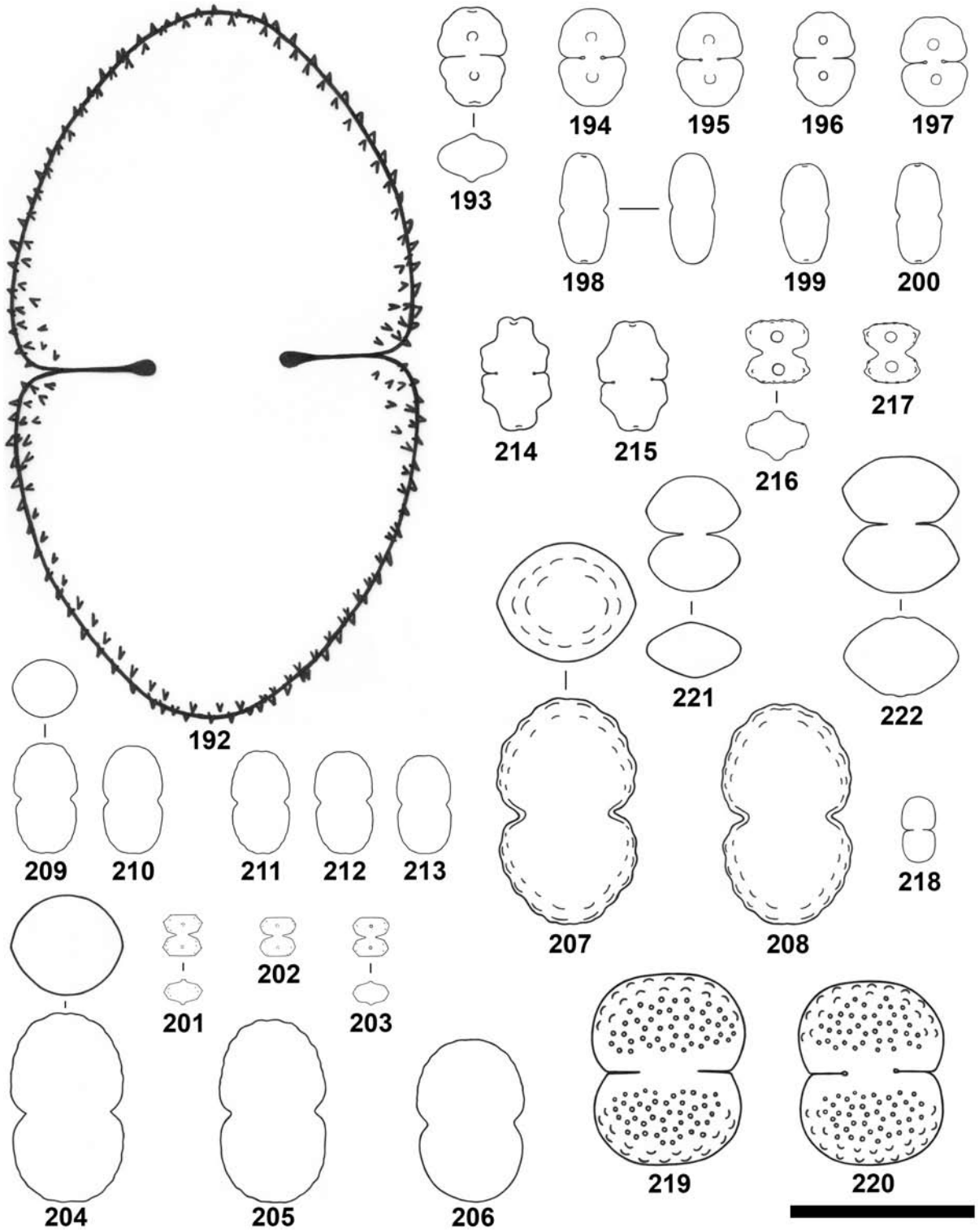
Figs 104–112. (104) *Micrasterias apiculata*; (105) *M. brachyptera*; (106) *M. fimbriata*; (107–108) *M. furcata*; (109) *M. jenneri*; (110–111) *M. oscitans*; (112) *M. pinnatifida*. Scale bar 100 µm.



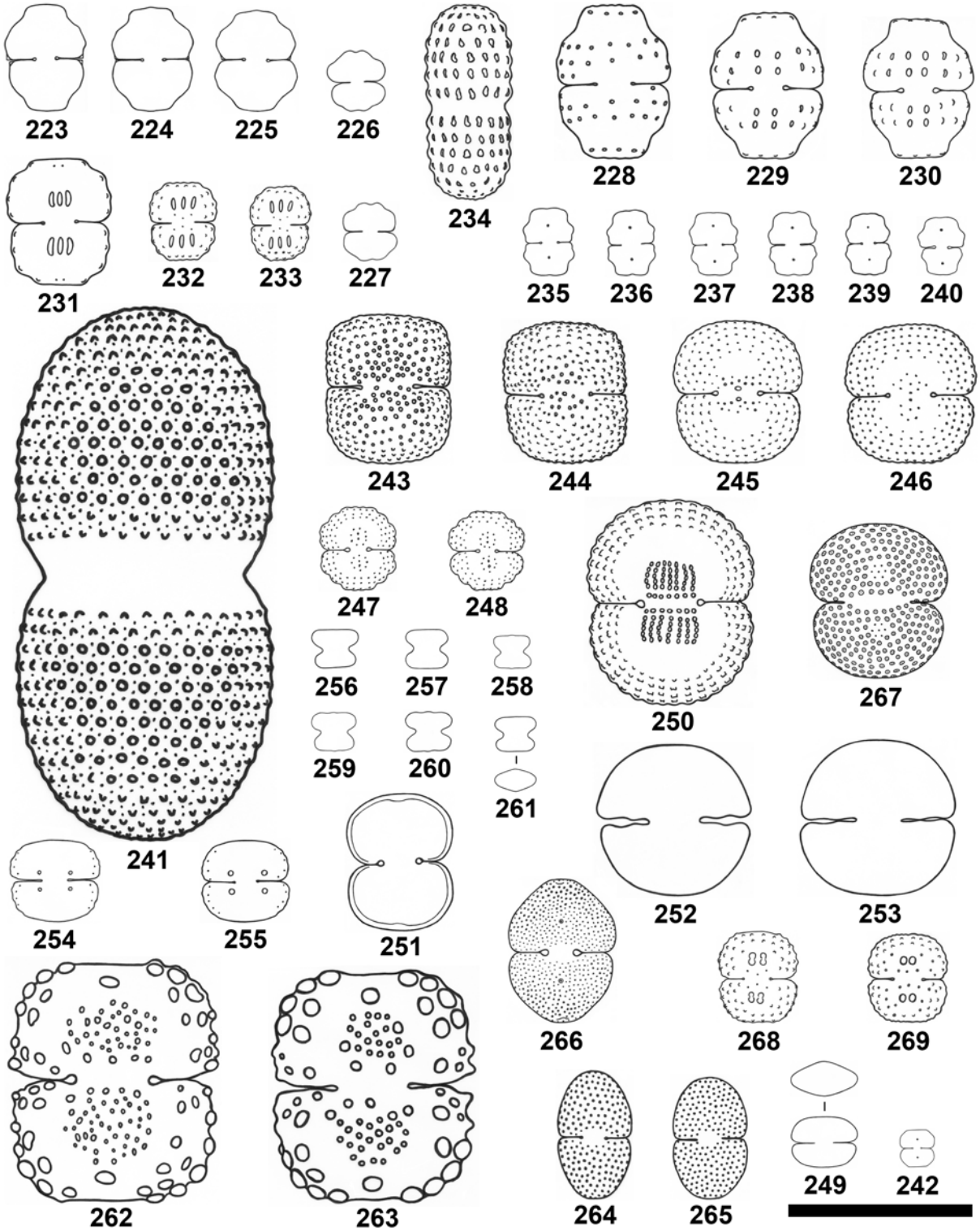
Figs 113–149. (113–115) *Cosmarium angulare*; (116–117) *C. basiornatum*; (118–119) *C. berryense*; (120–121) *C. bireme*; (122–124) *C. boitierense*; (125–126) *C. brebissonii*; (127–128) *C. carinthiacum*; (129) *C. ceratophorum*; (130) *C. commisurale* var. *acutum*; (131) *C. contractum* var. *retusum*; (132–133) *C. davidsonii*; (134) *C. decedens* var. *minutum*; (135) *C. dentiferum* var. *alpinum*; (136) *C. didymoprotupsum*; (137–138) *C. dilatatum*; (139–140) *C. eichlerianum*; (141–143) *C. fastidiosum*; (144) *C. fontigenum*; (145–146) *C. galeritum*; (147–149) *C. garrolense*. Scale bar 50 μm.



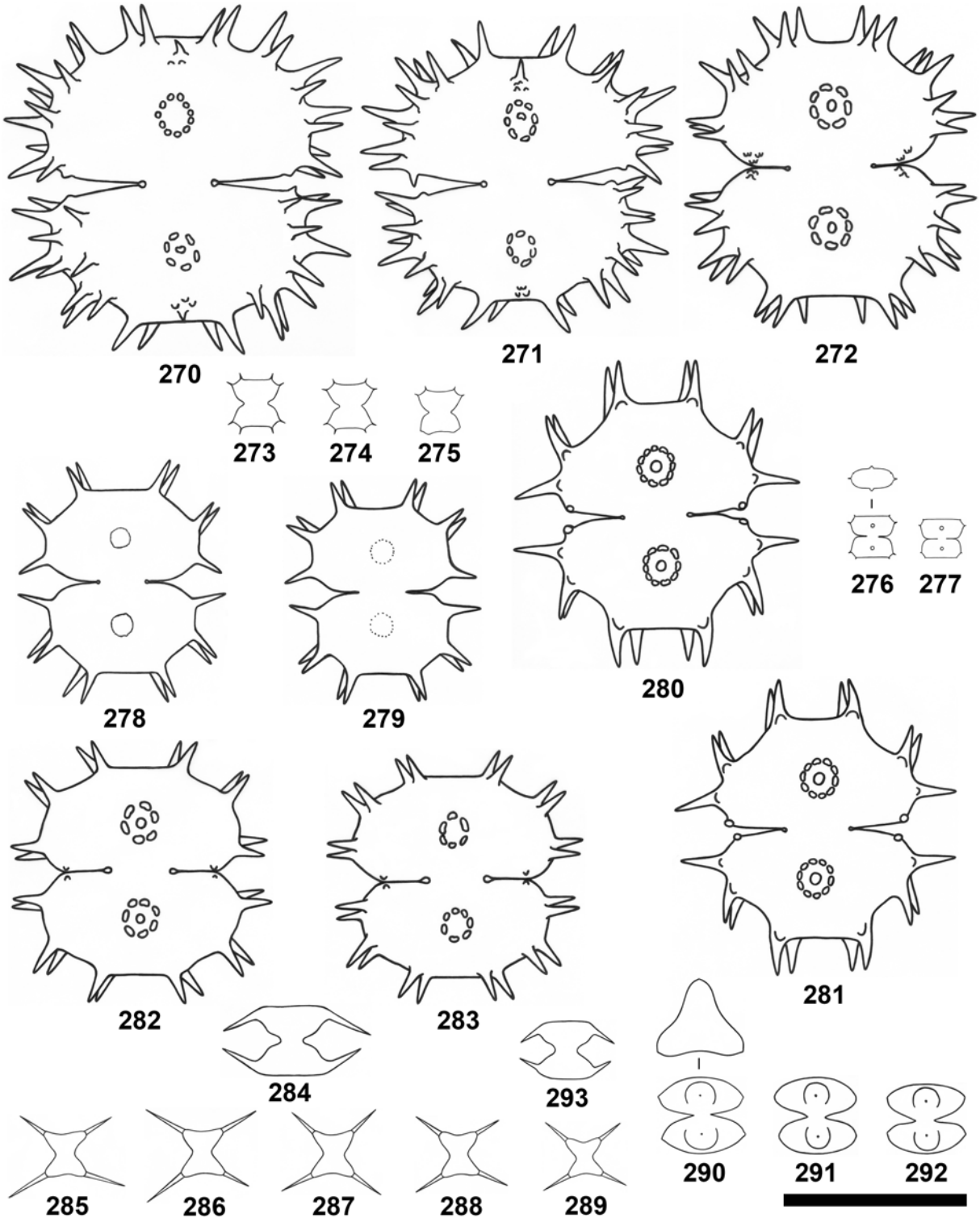
Figs 150–191. (150–151) *Cosmarium gibberulum*; (152–154) *C. goniodes* var. *subturgidum*; (155–156) *C. homalodermum*; (157–158) *C. jaoi*; (159) *C. kirchneri*; (160–161) *C. klebsii*; (162–163) *C. lagerheimii*; (164–165) *C. limnophilum*; (166–169) *C. medioretusum*; (170) *C. microsphinctum* var. *crispulum*; (171) *C. netzerianum*; (172) *C. norimbergense* var. *depressum*; (173–174) *C. notabile*; (175–176) *C. notatum*; (177) *C. novae–semliae* var. *granulatum*; (178–179) *C. obsoletum*; (180) *C. ocellatum*; (181–183) *C. ocellatum* var. *notatum*. (184–185) *C. ordinatum*; (186–188) *C. ornatulum*; (189) *C. ornatulum* var. *depressum*; (190–191) *C. orthopunctulatum*. Scale bar 50 μm.



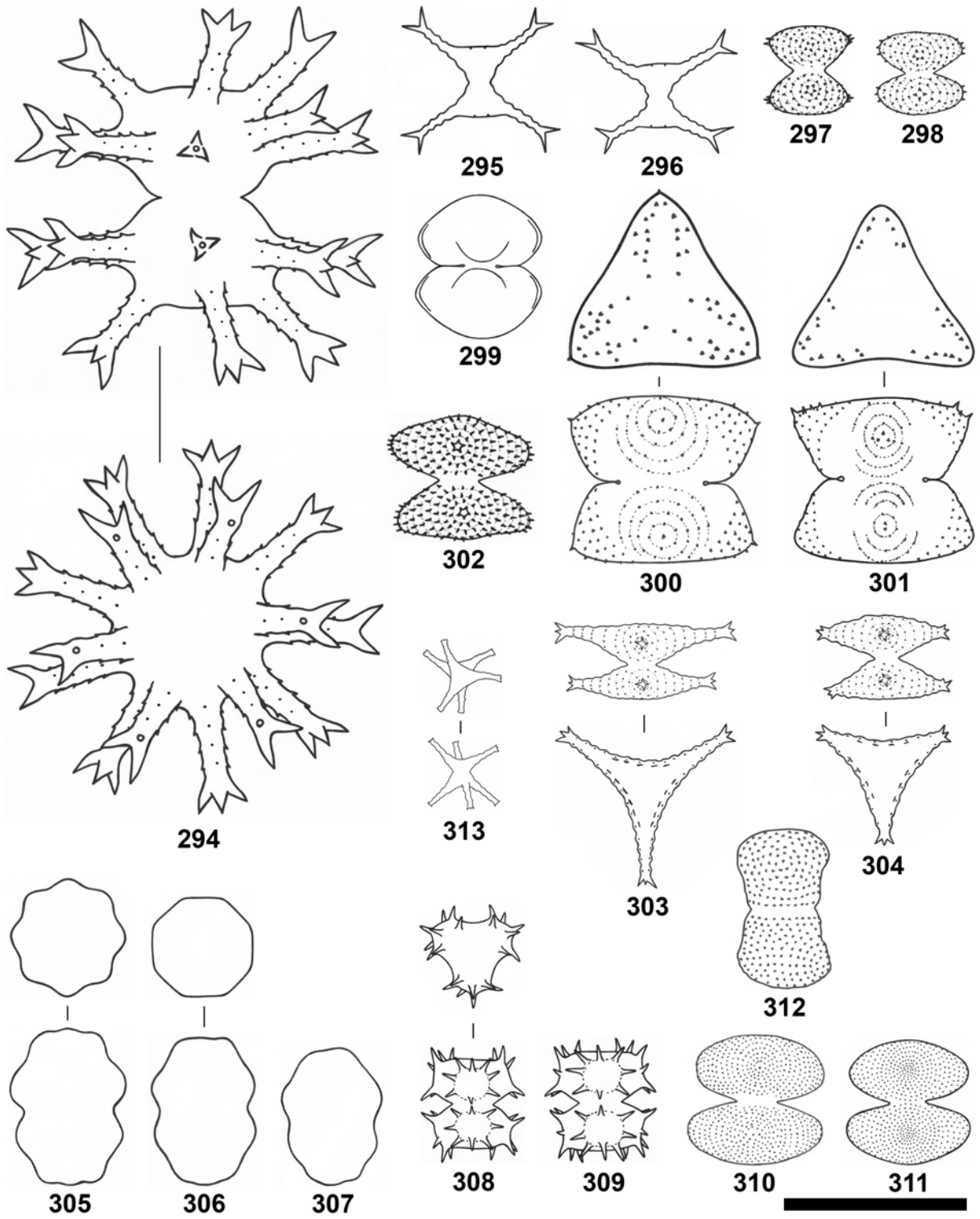
Figs 192–222. (192) *Cosmarium ovale*; (193–197) *C. paragranatoides*; (198–200) *C. parvulum* var. *undulatum*; (201–203) *C. paucigranulatum*; (204–206) *C. pericymatium*; (207–208) *C. pericymatium* var. *corrugatum*; (209–213) *C. pericymatium* var. *notabiliforme*; (214–215) *C. pokornyanum*; (216–217) *C. prominulum* var. *subundulatum*; (218) *C. pseudoexiguum*; (219–220) *C. pseudoinsigne*; (221) *C. pseudoprotuberans*; (222) *C. pseudoprotuberans* var. *sulcatum*. Scale bar 50 μm.



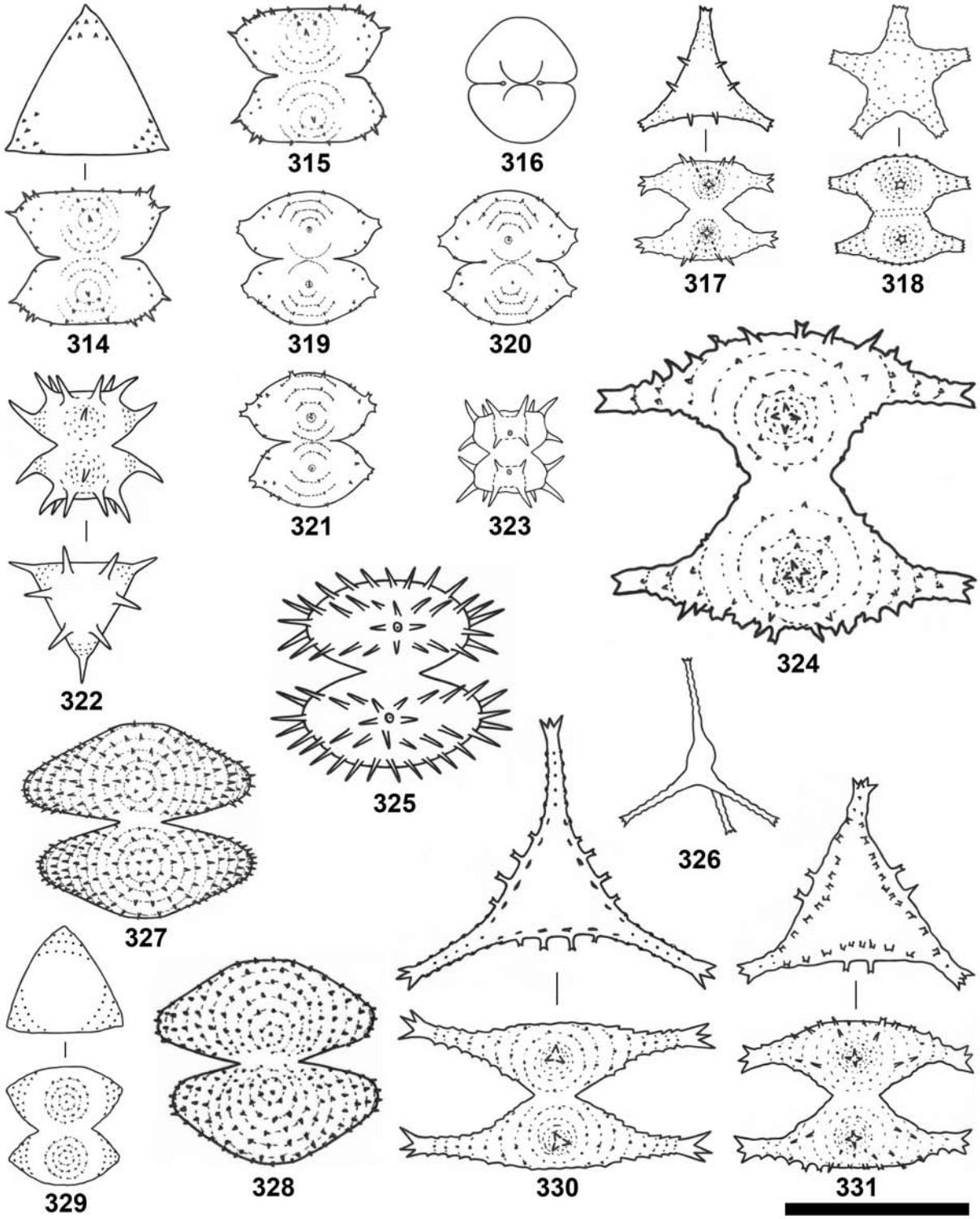
Figs 223–269. (223–225) *Cosmarium pseudoretusum*; (226–227) *C. pseudowembaerense*; (228–230) *C. retusum*; (231) *C. sexnotatum* var. *bipunctatum*; (232–233) *C. sexnotatum* var. *tristriatum*; (234) *C. simplicius*; (235–240) *C. sphyrelatum*; (241) *C. striolatum*; (242) *C. subadoxum*; (243–244) *C. subbroomei*; (245–246) *C. subbroomei* f. *isthmochondrum*; (247–248) *C. subprotumidum* var. *pyramidale*; (249) *C. subquadrans* var. *minor*; (250) *C. subspeciosum*; (251) *C. subtumidum* var. *groenbladii*; (252–253) *C. taxichondriforme*; (254–255) *C. tetrachondrum*, forma. (256–261) *C. truncatellum*; (262–263) *C. ungerianum* var. *subtriplicatum*; (264–265) *C. variolatum*; (266) *C. variolatum* var. *cataractarum*; (267) *C. varsoviense*; (268–269) *C. vogesiacum*. Scale bar 50 μm.



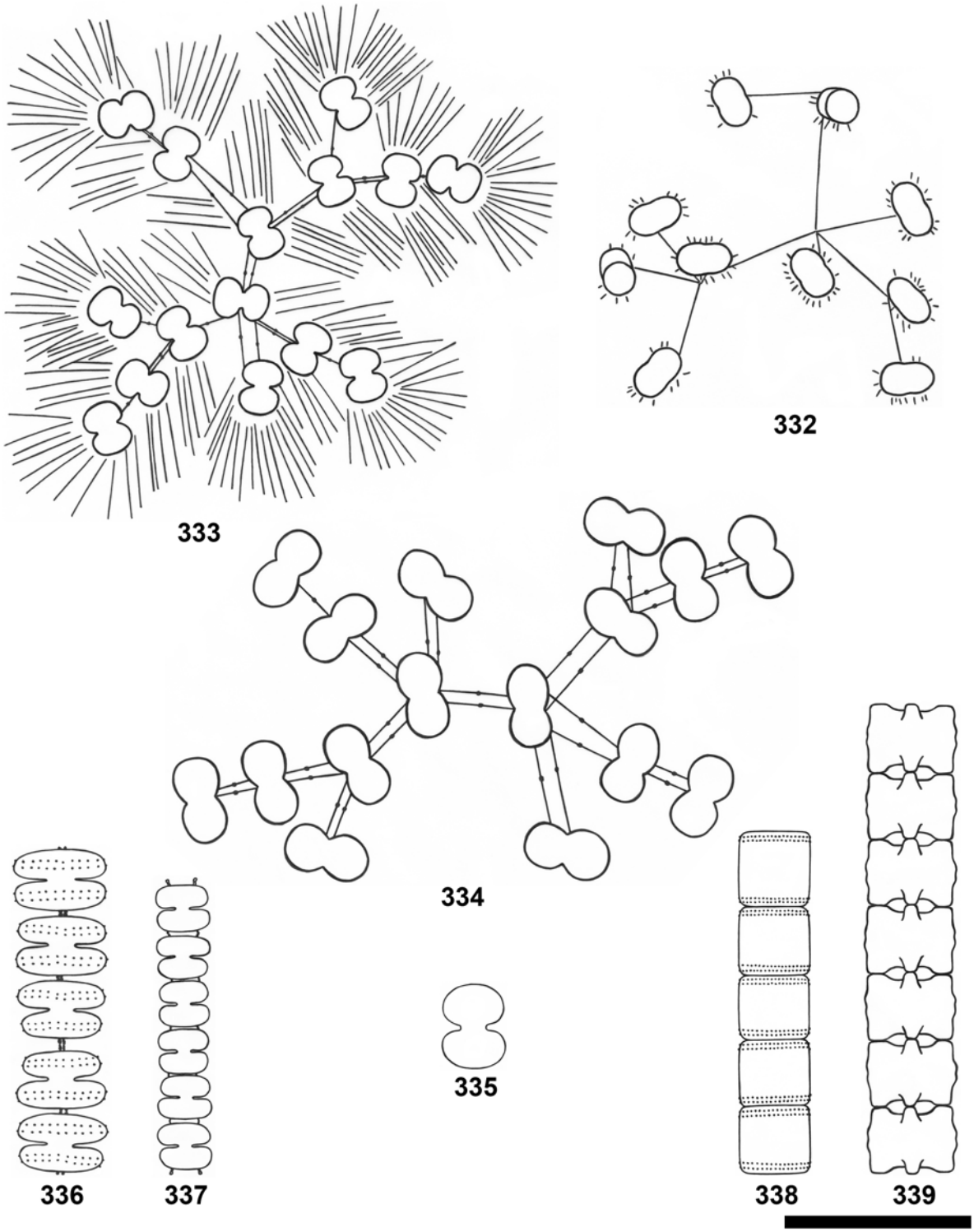
Figs 270–293. (270–271) *Xanthidium aculeatum*; (272) *X. basidentatum*; (273–275) *X. bifidum*. (276–277) *X. concinnum*; (278–279) *X. cristatum*; (280–281) *X. cristatum* var. *uncinatum* f. *polonicum*; (282–283) *X. fasciculatum* var. *oronense*; (284) *Staurodesmus extensus* var. *joshuae*; (285–289) *Std. extensus* var. *malaccensis*; (290–292) *Std. lanceolatus* var. *compressus*; (293) *Std. subhexagonus*. Scale bar 50 μm.



Figs 294–313. (294) *Staurastrum arctiscon*; (295–296) *St. bloklandiae*; (297–298) *St. bohlinianum*; (299) *St. crassangulatum*; (300–301) *St. cristatum* var. *navigiolum*; (302) *St. erasum*; (303–304) *St. eurycerum*; (305–307) *St. habeebense*; (308–309) *St. hystrix*; (310–311) *St. lapponicum*; (312) *St. meriani*; (313) *St. minimum*. Scale bar 50 µm.

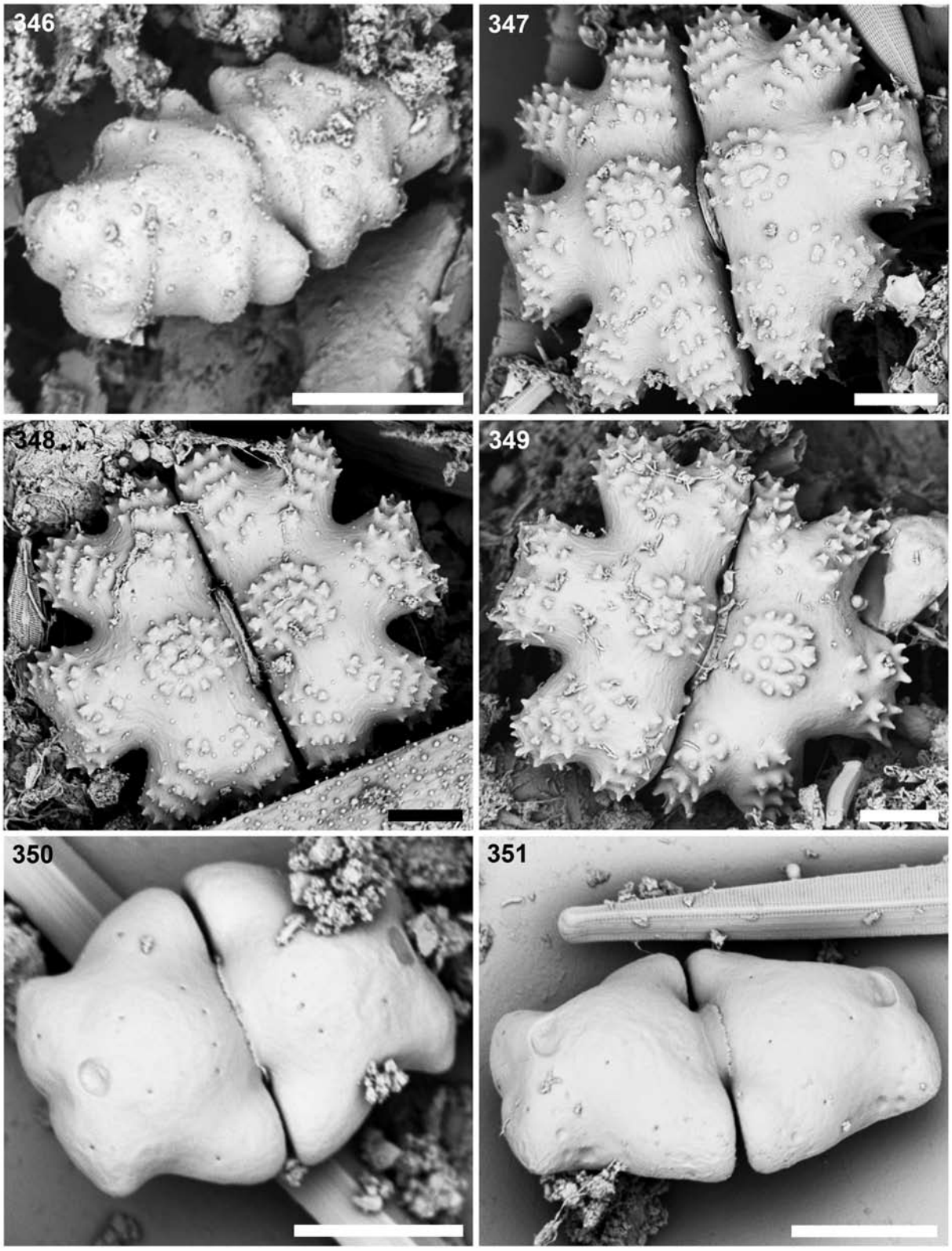


Figs 314–331. (314–315) *Staurastrum oligacanthum*; (316) *St. orbiculare* var. *ralfsii*; (317) *St. oxyacanthum*; (318) *St. pentasterias*; (319–321) *St. podlachicum*; (322) *St. pungens*; (323) *St. quadrispinatum*; (324) *St. sebaldi*; (325) *St. setigerum*; (326) *St. smithii*; (327–328) *St. trapezicum*; (329) *St. varians*; (330–331) *St. vestitum*. Scale bar 50 µm.

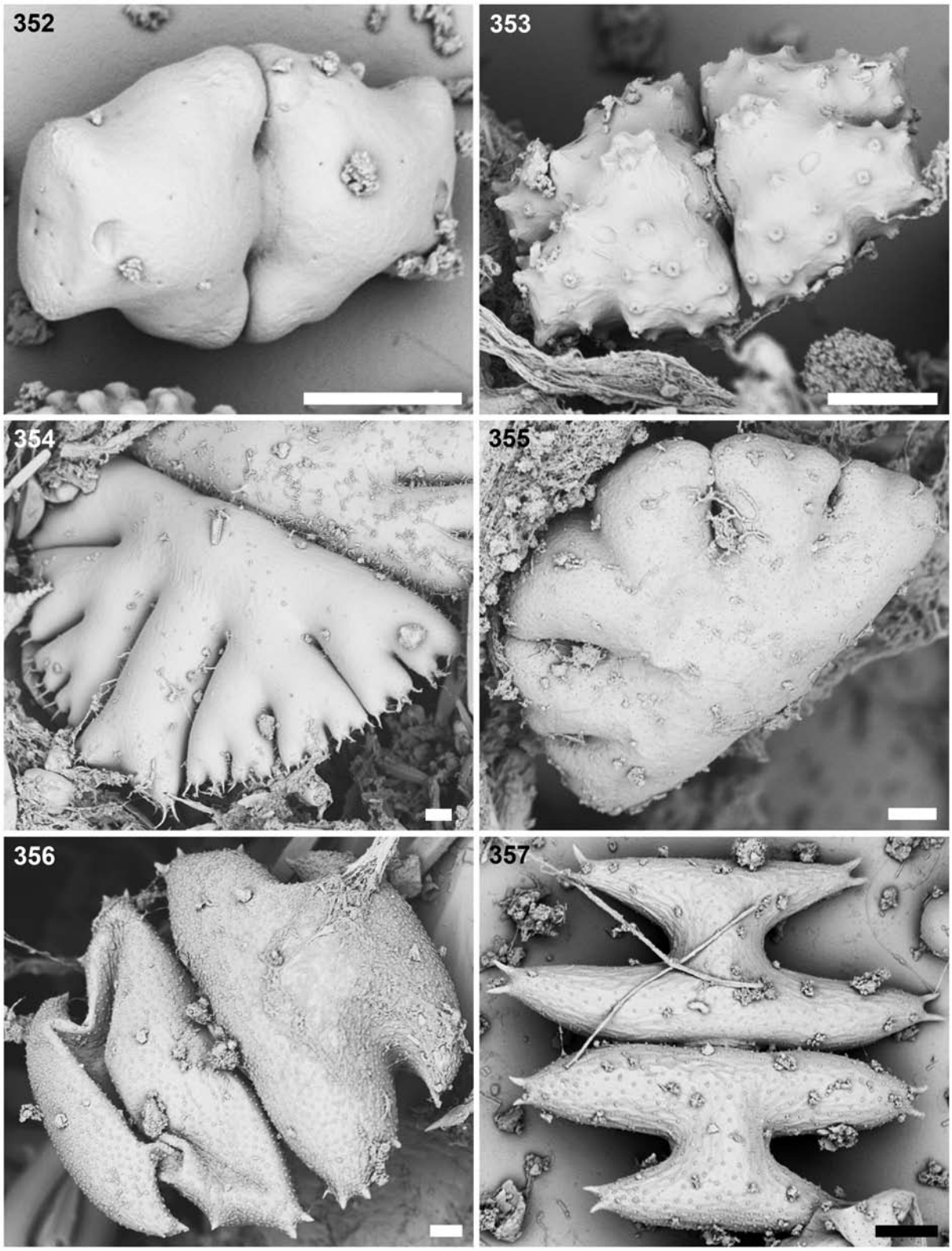


Figs 332–339. (332) *Cosmocladium constrictum*, part of a cell colony; (333–335) *C. saxonicum*; (336) *Sphaerozosma aubertianum*; (337) *Sph. filiforme*; (338) *Hyalotheca mucosa*; (339) *Desmidium baileyi* var. *caelatum*. Scale bar 50 μm, 80 μm (for Fig. 333).

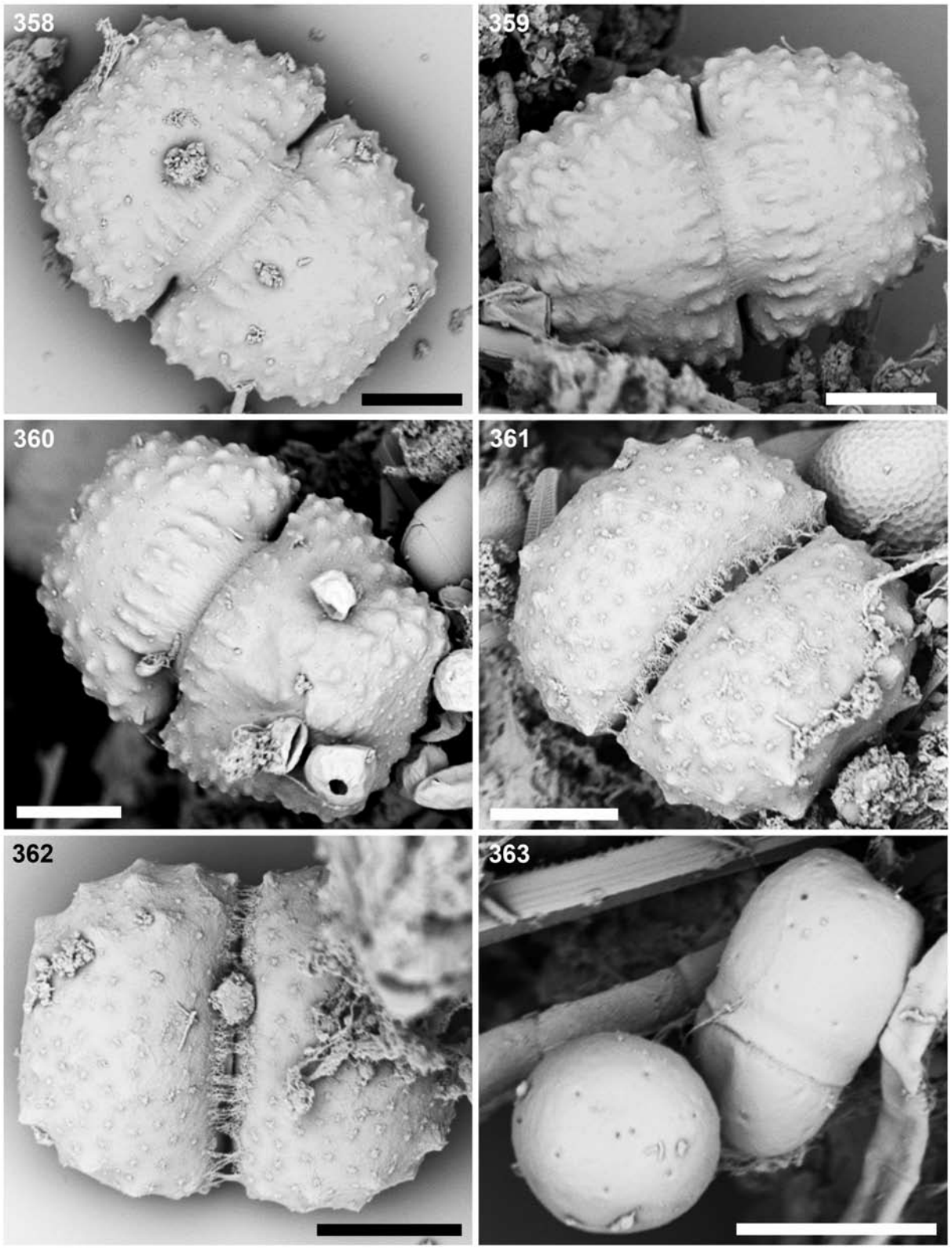
Figs 340–345. (340) *Gonatozygon aculeatum*; (341–342) *Haplotaenium indentatum* var. *latius,* morpha; (343) *Pleurotaenium simplicissimum*, apex; (344–345) *Actinotaenium inconspicuum*. Scale bar 10 µm.



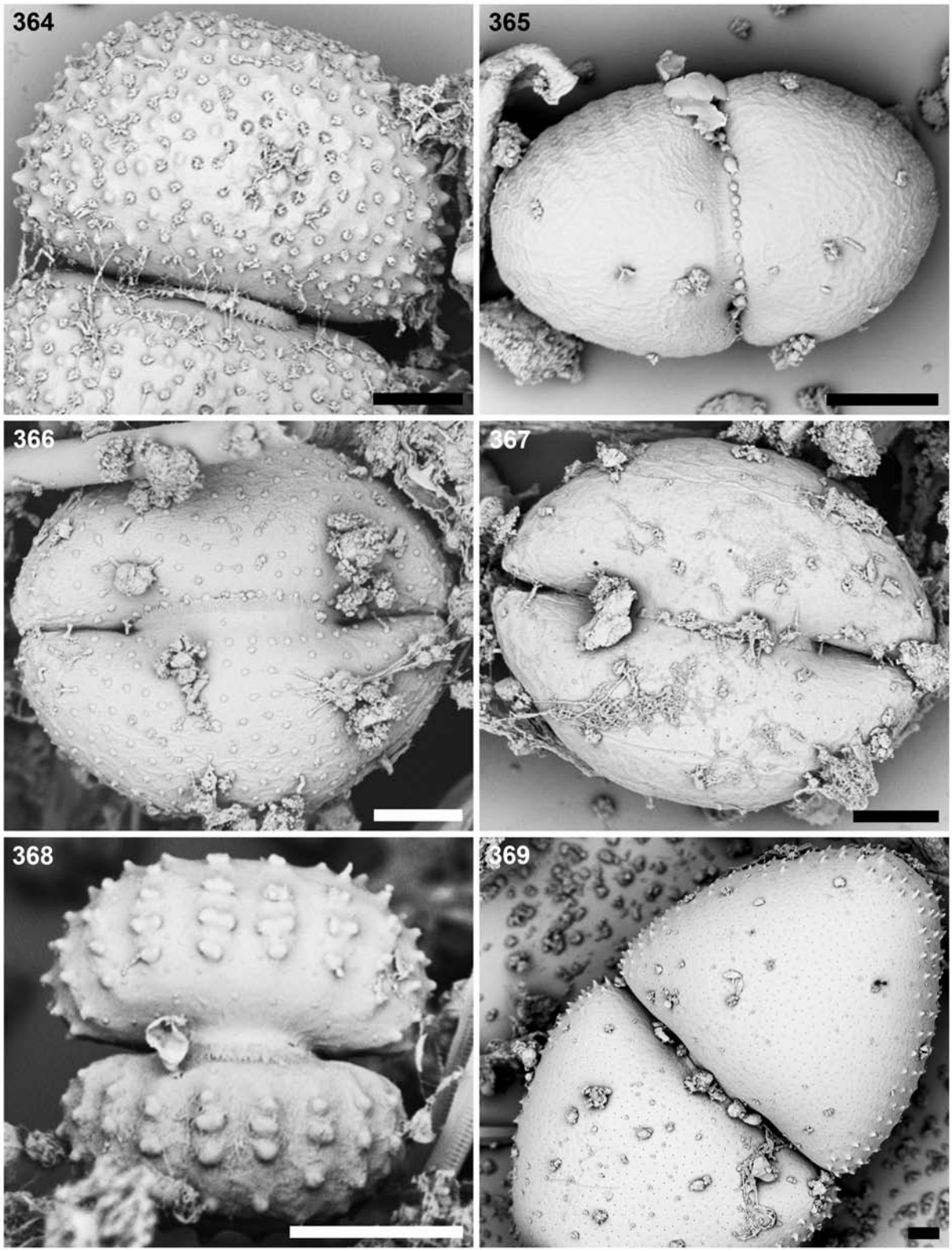
Figs 346–351. (346) *Euastrum crassicolle*; (347–349) *E. germanicum*; (350–351) *E. luetkemuelleri* var. *carniolicum*. Scale bar 10 µm.



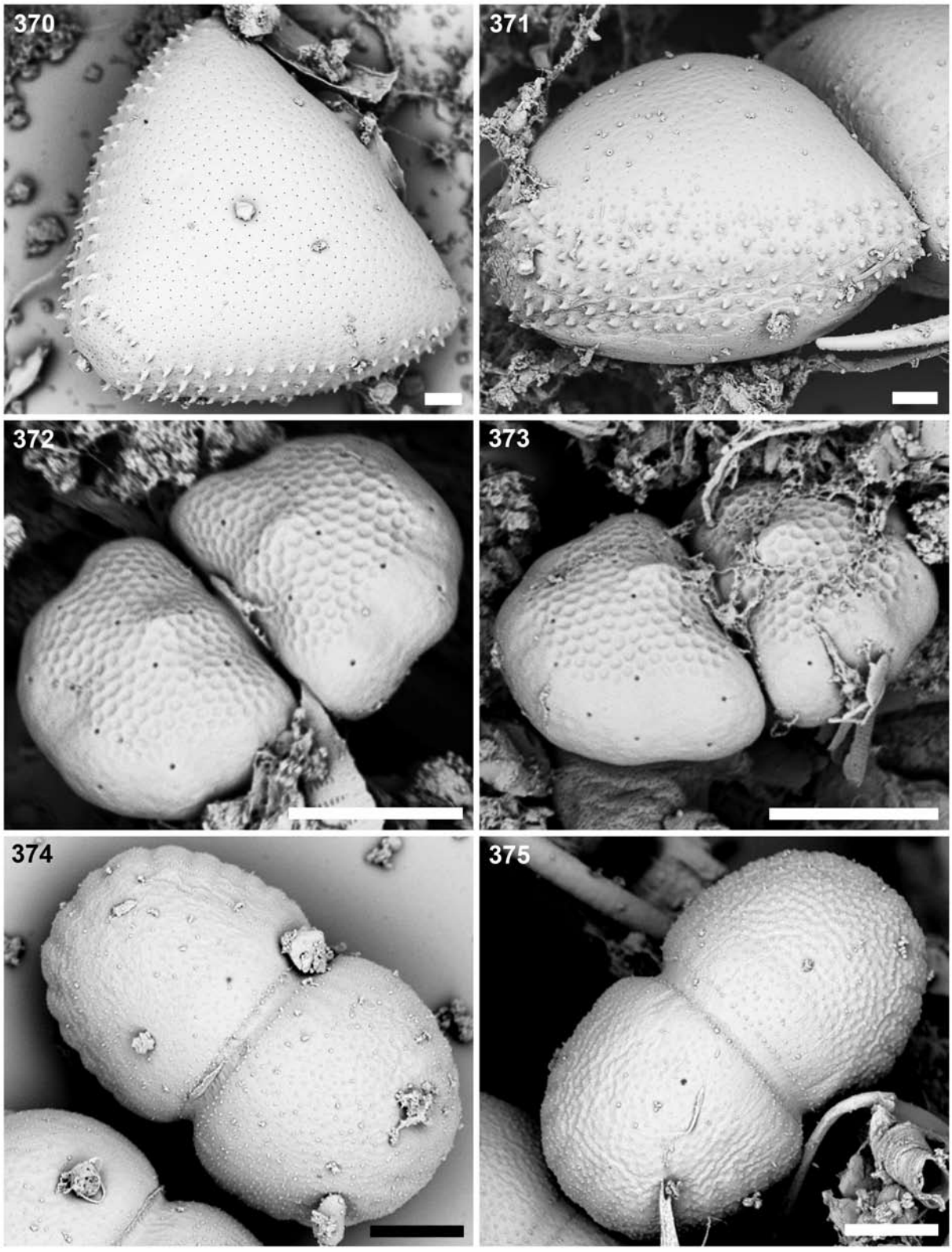
Figs (352–357). (352) *Euastrum luetkemuelleri* var. *carniolicum*; (353) *E. turneri*; (354) *Micrasterias fimbriata*; (355) *M. jenneri*; (356) *M. oscitans*; (357) *M. pinnatifida*. Scale bar 10 µm.



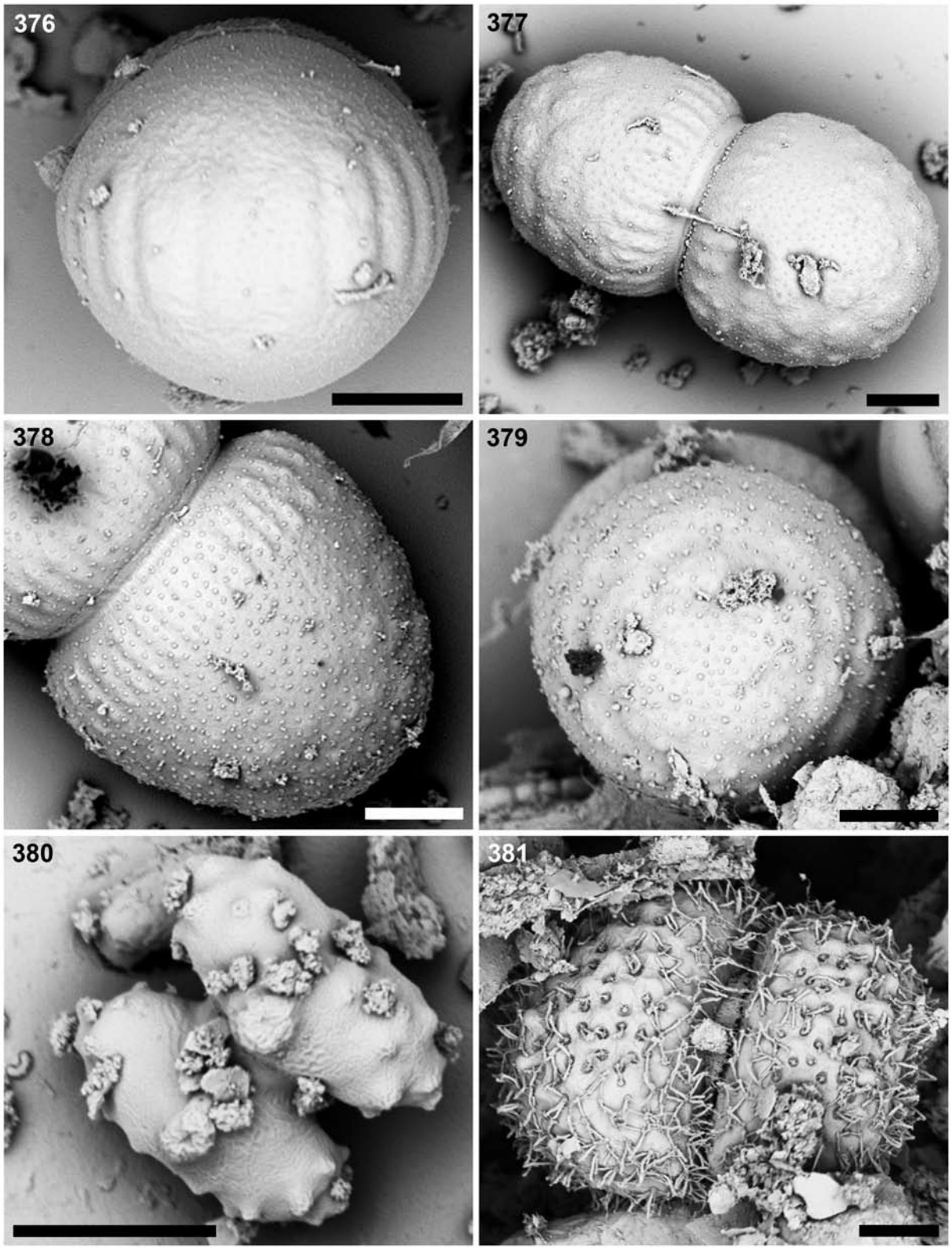
Figs 358–363. (358–360) *Cosmarium basiornatum*; (361–362) *C. ceratophorum*; (363) *C. goniodes* var. *subturgidum*. Scale bar 10 μm.



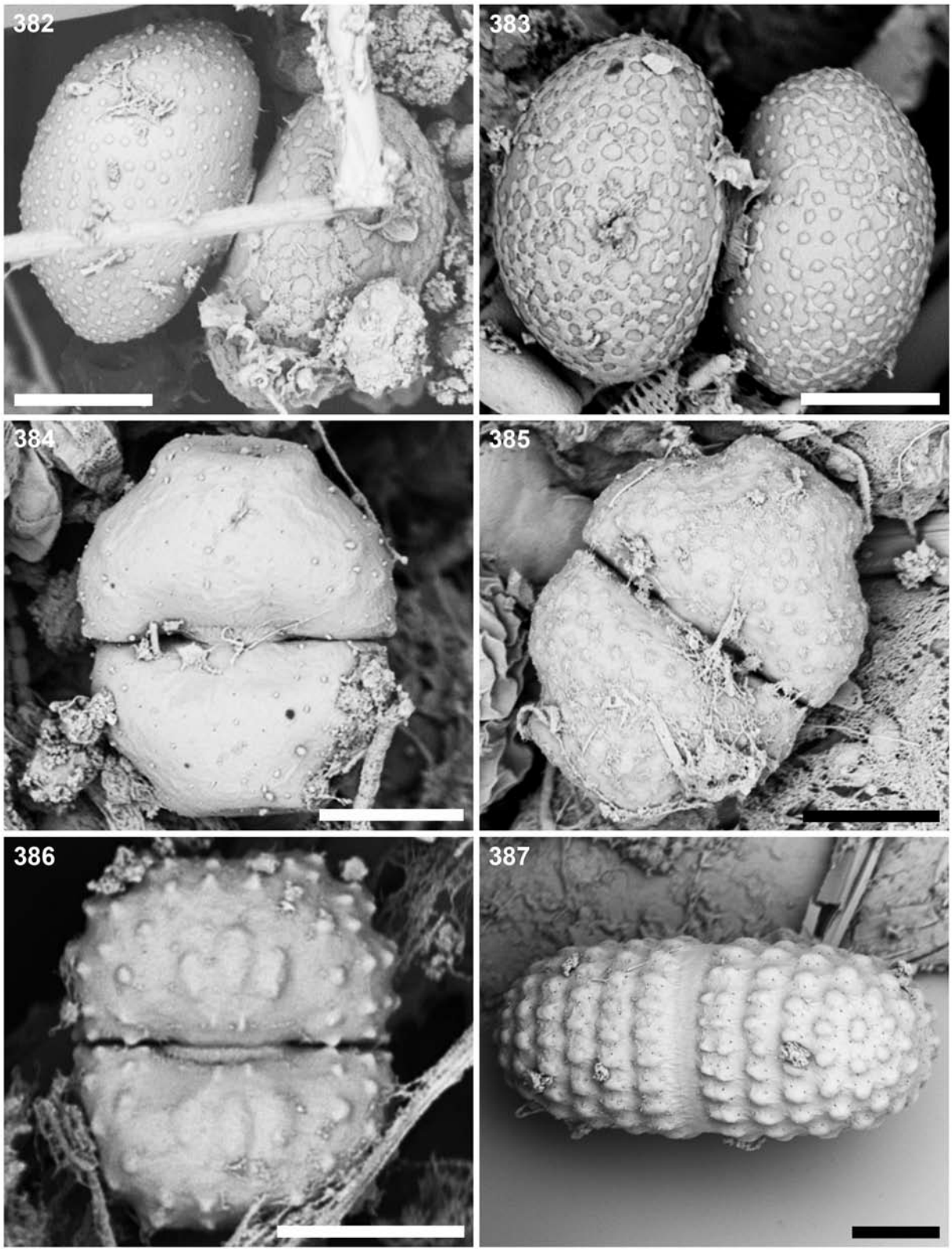
Figs 364–369. (364) *Cosmarium kirchneri*; (365) *C. microsphinctum* var. *crispulum*; (366–367) *C. obsoletum*; (368) *C. ordinatum*; (369) *C. ovale*. Scale bar 10 µm.



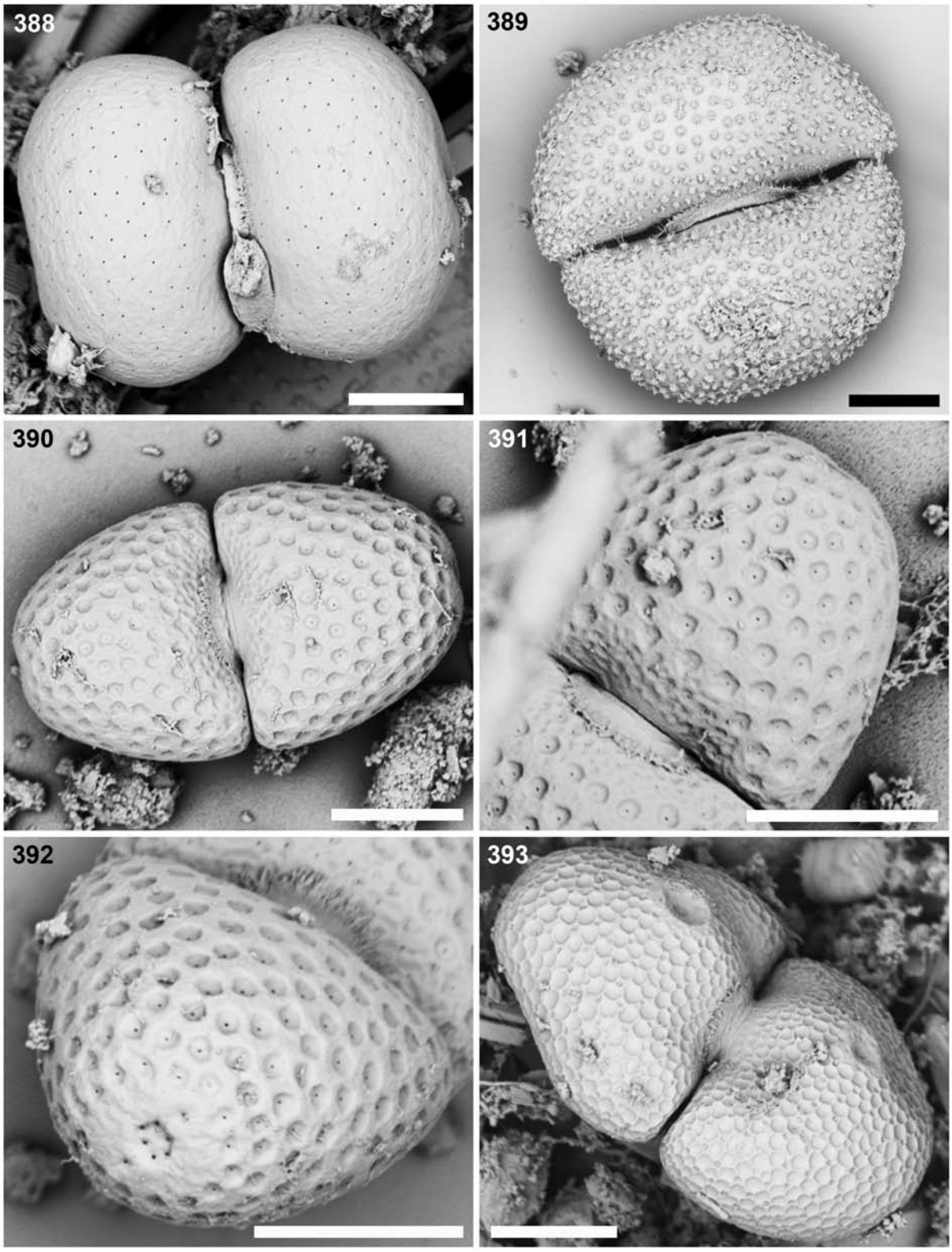
Figs 370–375. (370–371) *Cosmarium ovale*; (372–373) *C. paragranatoides*; (374–375) *C. pericymatium*. Scale bar 10 µm.



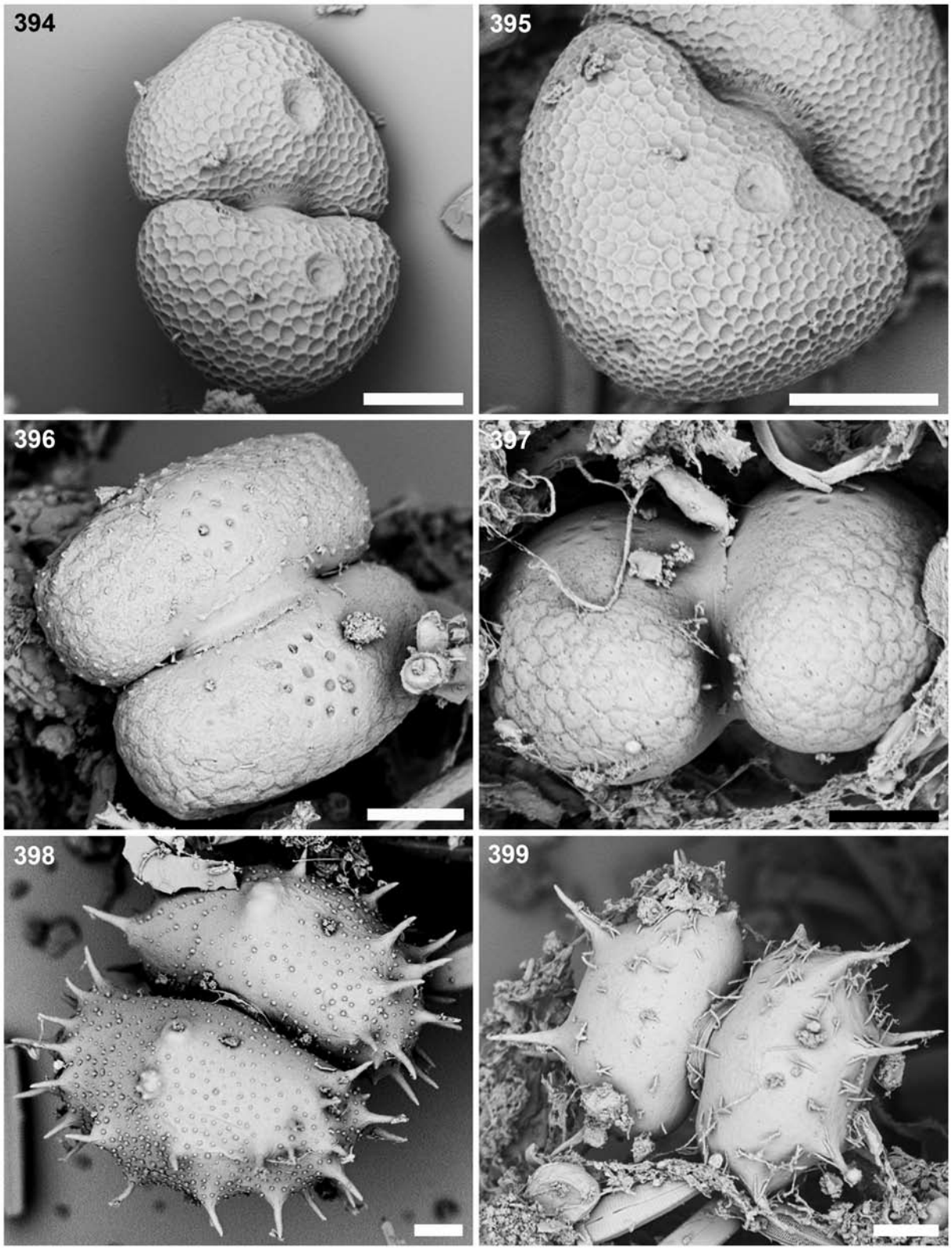
Figs 376–381. (376) *Cosmarium pericymatium*, apical view; (377–379) *C. pericymatium* var. *corrugatum*, (379) apical view; (380) *C. prominulum* var. *subundulatum*; (381) *C. pseudoinsigne*. Scale bar 10 µm.



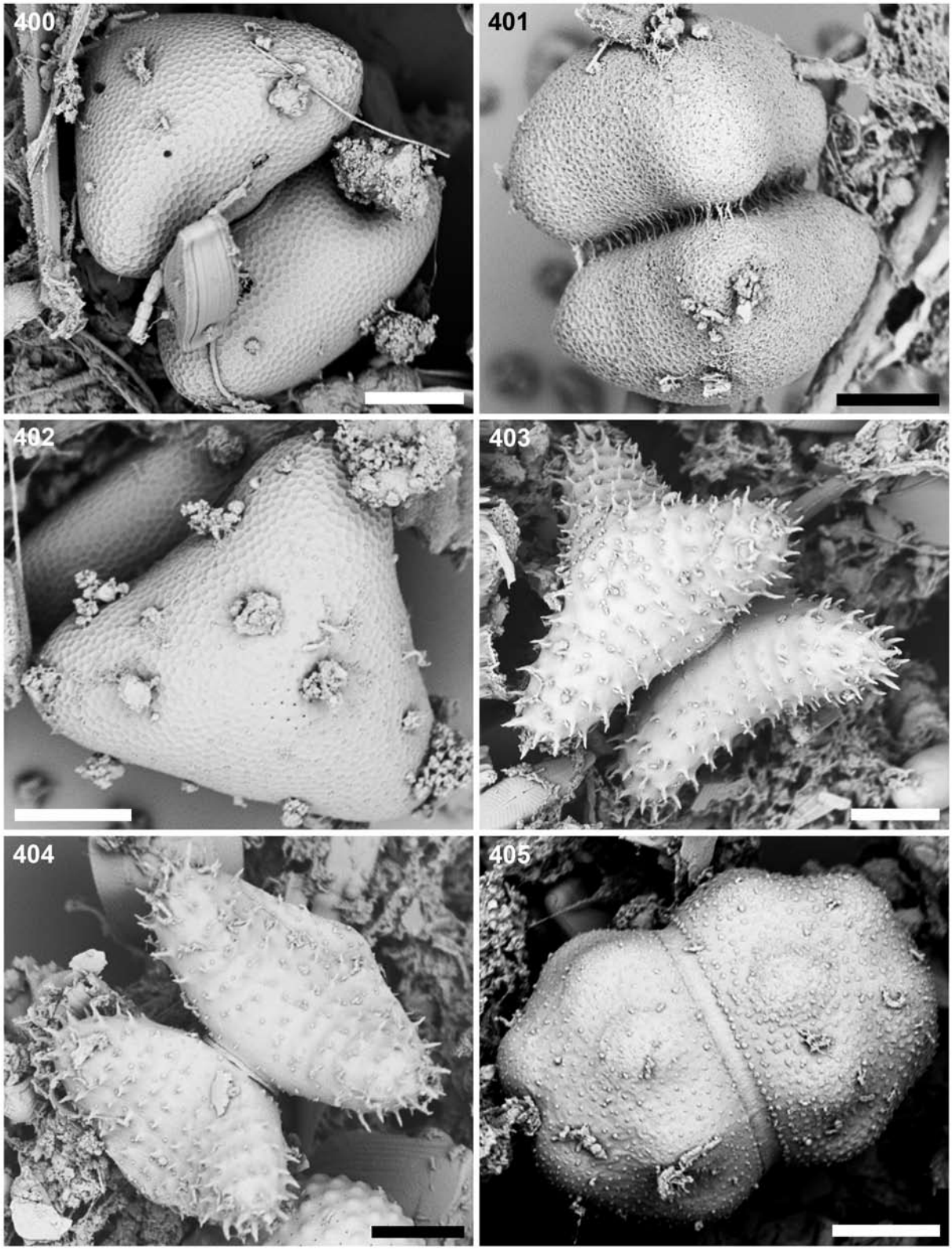
Figs 382–387. (382–383) *Cosmarium pseudoprotuberans*; (384–385) *C. pseudoretusum*; (386) *C. sexnotatum* var. *tristriatum*; (387) *C. simplicius*. Scale bar 10 µm.



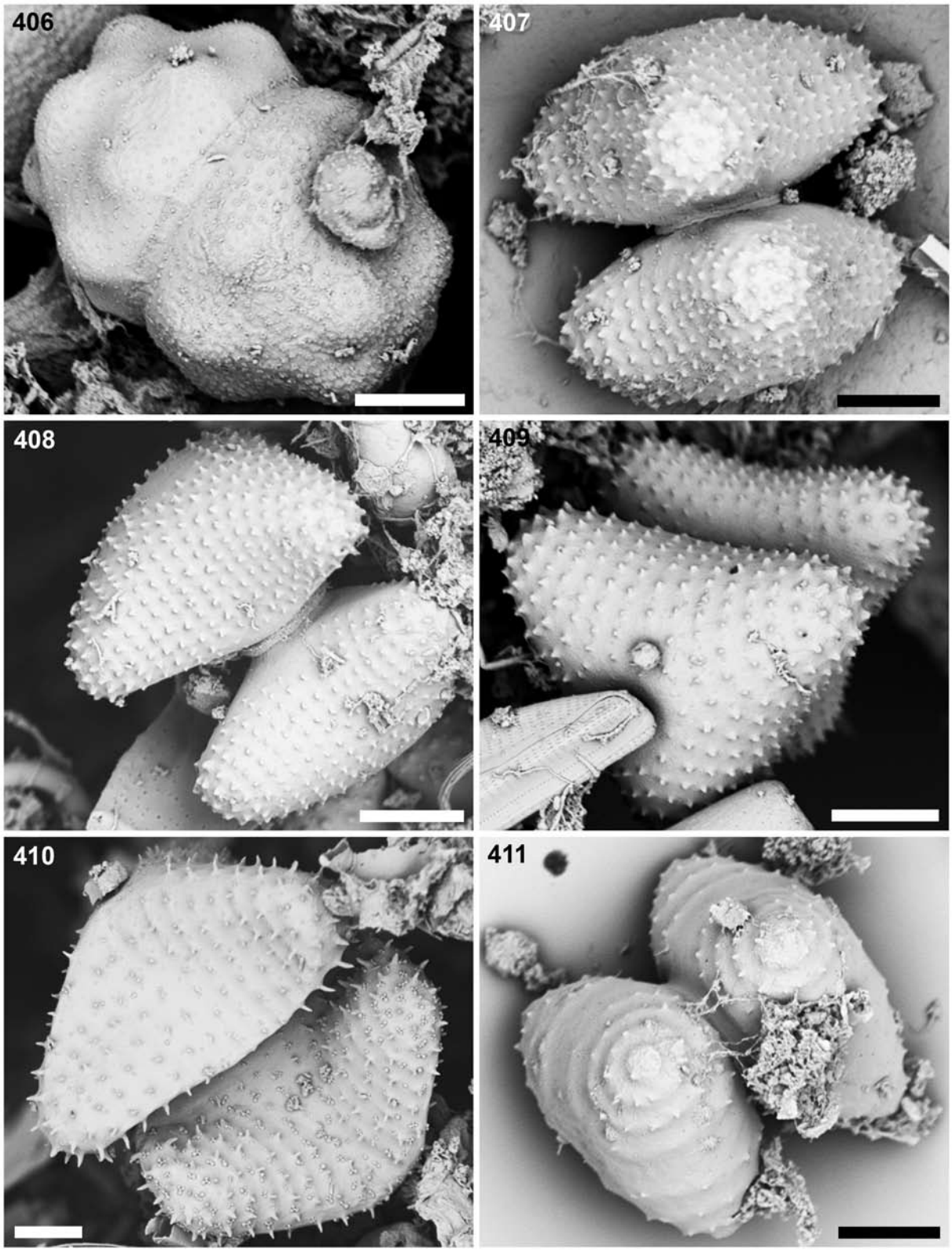
Figs 388–393. (388) *Cosmarium subtumidum* var. *groenbladii*; (389) *C. taxichondriforme*; (390–392) *C. variolatum*; (393) *C. variolatum* var. *cataractarum*. Scale bar 10 µm.



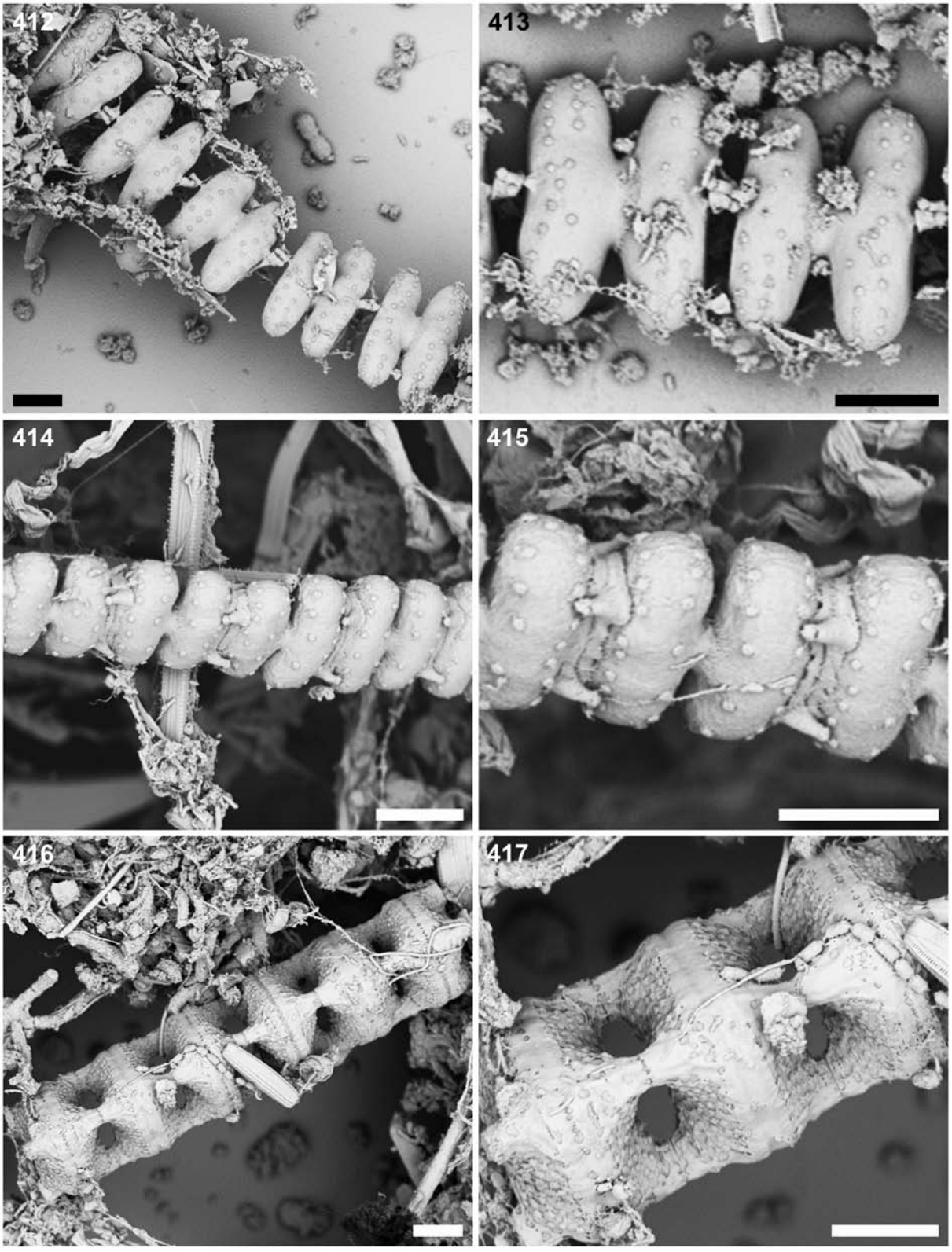
Figs 394–399. (394–395) *Cosmarium variolatum* var. *cataractarum*; (396–397) *C. varsoviense*, (396) cell with collapsed apices; (398) *Xanthidium aculeatum*; (399) *X. cristatum*. Scale bar 10 µm.



Figs 400–405. (400–402) *Staurastrum crassangulatum*, (402) apical view; (403–404) *St. erasum*; (405) *St. habeebense*. Scale bar 10 µm.



Figs 406–411. (406) *Staurastrum habeebense*; (407–409) *St. lapponicum*; (410) *St. trapezicum*. (411) *St. varians*. Scale bar 10 µm.



Figs 412–417. (412–413) *Sphaerozosma aubertianum*; (414–415) *Sph. filiforme*; (416–417) *Desmidium baileyi* var. *caelatum*. Scale bar 10 μm.

Table 1. List of all taxa found with their indicative notations [(TRPH) Trophy, (oli) oligotrophic, (mes) mesotrophic, (eu) eutrophic; (ACID) Acidity, (aci) acidic, (neu) neutral, (alk) alkalic; (LF) life form, (ben) benthic, (atm) atmophytic, (pla) planktonic; (R) rarity within the Czech Republic; (S) ecological sensitivity. For details see Introduction].

	TRPH	ACID	LF	R	S
Actinotaenium cruciferum (De Bary) Teiling	oli	aci	ben–atm	3	
Actinotaenium cucurbita (Ralfs) Teiling	oli	aci	ben–atm		1
Actinotaenium curtum (Ralfs) Teiling	mes–oli	aci–neu	atm–ben	1	
Actinotaenium diplosporum (P.Lundell) Teiling	mes–oli	aci	ben–atm	1	1
Actinotaenium diplosporum var. *americanum* (W. et G.S.West) Teiling	mes	aci	ben–atm	2	
Actinotaenium inconspicuum (W. et G.S.West) Teiling	oli–mes	aci	ben–atm	2	
Actinotaenium inconspicuum var. *curvatum* Kouwets	oli–mes	aci	ben–atm	3	
Actinotaenium kriegeri (Messik.) Kouwets	oli–mes	aci	ben–atm	3	
Actinotaenium obcuneatum (W.West) Teiling	oli	aci	ben–atm	3	
Actinotaenium perminutum (G.S.West) Teiling	oli–mes	aci	ben–atm	3	3
Actinotaenium silvae – nigrae (Rabanus) Kouwets et Coesel	oli	aci	ben–atm	1	2
Actinotaenium silvae – nigrae var. *parallelum* (Willi Krieg.) Kouwets et Coesel	oli	aci	ben–atm	2	2
Actinotaenium subsparsepunctatum (Grönblad) Coesel	oli	aci	ben–atm	3	
Actinotaenium turgidum (Ralfs) Teiling	mes	aci	ben		2
Bambusina brebissonii Kütz.	oli	aci	ben		2
Closterium abruptum W.West	mes–oli	aci	ben–atm	1	
Closterium acerosum Ralfs	eu–mes	alk–aci	ben		
Closterium acerosum var. *elongatum* Bréb.	eu–mes	alk–aci	ben–pla		
Closterium acerosum var. *minus* Hantzsch	eu–mes	aci–alk	ben		
Closterium aciculare T.West	eu–mes	alk–neu	pla	1	
Closterium acutum Ralfs	oli–eu	aci–alk	ben–pla		
Closterium acutum var. *variabile* (Lemmerm.) Willi Krieg.	eu–mes	neu–alk	pla		
Closterium angustatum Ralfs	mes–oli	aci	ben	1	3
Closterium angustatum var. *sculptum* (Racib.) Růžička	mes–oli	aci	ben	3	3
Closterium archerianum Cleve	mes	aci	ben	2	2
Closterium archerianum var. *pseudocynthia* Růžička	mes	aci–neu	ben	2	2
Closterium attenuatum Ralfs	mes	aci	ben	1	2
Closterium baillyanum (Ralfs) Bréb.	oli–mes	aci	ben	1	2
Closterium baillyanum var. *alpinum* (Viret) Grönblad	oli–mes	aci	ben	1	2
Closterium braunii Reinsch	mes	aci	ben	3	3
Closterium calosporum Wittr.	mes	aci	ben	1	1
Closterium calosporum var. *brasiliense* Børges.	mes–oli	aci	ben	3	3
Closterium calosporum var. *maius* (W. et G.S.West) Willi Krieg.	mes–eu	neu	ben	3	
Closterium closterioides (Ralfs) A.Louis et Peeters	mes–oli	aci	ben	3	3
Closterium closterioides var. *intermedium* (J.Roy et Bisset) Růžička	mes–oli	aci	ben	3	3
Closterium cornu Ralfs	oli–mes	aci	ben–atm		
Closterium cornu var. *upsaliense* Nordst.	oli–mes	aci	ben–atm	2	
Closterium costatum Ralfs	mes	aci	ben	1	2
Closterium costatum var. *borgei* (Willi. Krieg.) Růžička	mes	aci	ben	1	2
Closterium cynthia De Not.	mes	aci	ben	1	2

Table 1 Cont.

	TRPH	ACID	LF	R	S
Closterium delpontei (G.A.Klebs) Wolle	mes	aci	ben	3	3
Closterium dianae Ralfs	mes	aci	ben		1
Closterium dianae var. *arcuatum* (Bréb.) Rabenh.	mes	aci	ben	1	2
Closterium dianae var. *minus* Hieron.	mes	aci	ben	1	1
Closterium dianae var. *pseudodianae* (J.Roy) Willi Krieg.	mes	aci	ben	3	2
Closterium dianae var. *rectius* (Nordst.) De Toni	mes	aci	ben	3	2
Closterium didymotocum Ralfs	mes	aci	ben	2	3
Closterium directum W.Archer	oli–mes	aci	ben	2	2
Closterium ehrenbergii Ralfs	eu–mes	alk–aci	ben		
Closterium exile W. et G.S.West	oli	aci–neu	ben–atm	3	
Closterium gracile Ralfs	mes–oli	aci	ben		2
Closterium gracile var. *elongatum* W. et G.S.West	mes	aci	ben	2	2
Closterium idiosporum W. et G.S.West	mes–eu	aci–neu	ben	1	
Closterium incurvum Bréb.	mes–eu	aci–alk	ben		
Closterium intermedium Ralfs	mes–oli	aci	ben		1
Closterium juncidum Ralfs	oli–mes	aci	ben	1	2
Closterium juncidum var. *brevius* (Rabenh.) J.Roy	oli–mes	aci	ben	1	2
Closterium kuetzingii Bréb.	mes	aci–neu	ben		
Closterium leibleinii Ralfs var. *boergesenii* (Schmidle) Skvortsov	eu	alk–neu	ben	1	
Closterium limneticum Lemmerm.	eu	alk–neu	pla		
Closterium limneticum var. *fallax* Růžička	eu	alk–neu	pla	1	
Closterium limneticum var. *tenue* Lemmerm.	eu	alk–neu	pla		
Closterium lineatum Ralfs	mes	aci	ben	1	2
Closterium lineatum var. *elongatum* (Rosa) Croasdale	mes	aci	ben	1	2
Closterium lineatum var. *costatum* Wolle	mes	aci	ben	3	2
Closterium littorale F.Gay	eu–mes	alk–neu	ben–pla	2	
Closterium lunula Ralfs	mes	aci	ben		1
Closterium moniliferum Ralfs	eu–mes	alk–aci	ben		
Closterium navicula (Bréb.) Lütkem.	mes–oli	aci	ben		
Closterium nematodes Joshua var. *proboscideum* W.B.Turner	mes	aci	ben	3	2
Closterium parvulum Nägeli	mes	aci–neu	ben		
Closterium praelongum Bréb.	mes–eu	aci–alk	ben–pla		
Closterium praelongum var. *brevius* (Nordst.) Willi Krieg.	mes–eu	aci–alk	ben		
Closterium pritchardianum W.Archer	mes–eu	aci–neu	ben–pla		
Closterium pronum Bréb.	oli–mes	aci	ben		1
Closterium pseudolunula Borge	mes–eu	aci–neu	ben	2	
Closterium pseudopygmaeum Kouwets	oli–mes	aci	ben–atm	3	
Closterium pusillum Hantzsch	oli	aci	atm–ben	3	
Closterium ralfsii Ralfs	mes	aci	ben	3	2
Closterium ralfsii var. *hybridum* Rabenh.	mes	aci	ben	1	2
Closterium regulare Bréb.	mes	aci–alk	ben	2	2
Closterium rostratum Ralfs	mes	aci	ben–atm		
Closterium setaceum Ralfs	oli–mes	aci	ben	1	2

Table 1 Cont.

	TRPH	ACID	LF	R	S
Closterium strigosum BRÉB.	eu–mes	alk–neu	pla–ben	2	
Closterium strigosum var. *elegans* (G.S.WEST) WILLI KRIEG.	eu–mes	neu	ben	2	
Closterium striolatum RALFS	oli	aci	ben		
Closterium sublaterale Růžička	mes	aci–neu	ben	1	
Closterium submoniliferum WORON.	eu–mes	alk–aci	ben		
Closterium subulatum (KÜTZ.) BRÉB.	eu–mes	neu–alk	ben	3	1
Closterium tortitaenoides COESEL	oli	aci	ben	3	2
Closterium tumidulum F.GAY	eu	alk–neu	ben–pla		
Closterium tumidum JOHNS.	oli	aci	ben–atm	2	
Closterium tumidum var. *nylandicum* GRÖNBLAD	oli	aci	ben–atm	3	
Closterium turgidum RALFS	mes	aci	ben	1	2
Closterium turgidum var. *giganteum* (NORDST.) DE TONI	mes	aci–neu	ben–pla	3	2
Closterium venus RALFS	eu–mes	neu–alk	ben–pla		
Cosmarium abbreviatum RACIB.	mes–eu	neu–alk	ben–pla	3	
Cosmarium amoenum RALFS	oli–mes	aci	ben	1	2
Cosmarium anceps P.LUNDELL	oli	aci	atm–ben	1	
Cosmarium angulare JOHNS.	eu	alk	ben	3	2
Cosmarium angulosum BRÉB.	mes	aci	ben	2	3
Cosmarium angulosum var. *concinnum* (RABENH.) W. et G.S.WEST	mes	aci	ben	3	3
Cosmarium basiornatum (GRÖNBLAD) COESEL	oli–mes	aci	atm–ben	2	
Cosmarium berryense KOUWETS	eu	alk	ben	3	2
Cosmarium bioculatum RALFS	mes	aci	ben		
Cosmarium bioculatum var. *depressum* (SCHAARSCHM.) SCHMIDLE	mes–eu	aci–alk	ben–pla		
Cosmarium bireme NORDST.	mes	aci	ben	3	3
Cosmarium biretum RALFS	eu	alk	ben–pla	2	
Cosmarium biretum var. *trigibberum* NORDST.	eu	alk	ben–pla	2	
Cosmarium blytii WILLE	mes–oli	aci	ben	3	3
Cosmarium blytii var. *novae – sylvae* W. et G.S.WEST	mes–oli	aci	ben	1	2
Cosmarium boeckii WILLE	mes–eu	aci–alk	ben		1
Cosmarium boitierense KOUWETS	mes–eu	aci–neu	ben	3	2
Cosmarium botrytis RALFS	mes–eu	aci–neu	ben	2	
Cosmarium botrytis var. *gemmiferum* (BRÉB.) NORDST.	mes–eu	neu–aci	ben	2	
Cosmarium botrytis var. *mediolaeve* W.WEST	mes–eu	aci–neu	ben	1	
Cosmarium botrytis var. *tumidum* WOLLE	mes–eu	neu	ben	1	2
Cosmarium brebissonii RALFS	mes–oli	aci	ben	3	2
Cosmarium caelatum RALFS	oli–mes	aci	atm–ben		
Cosmarium carinthiacum LÜTKEM.	oli–mes	aci	ben	3	3
Cosmarium ceratophorum LÜTKEM.	mes	aci	ben	3	
Cosmarium commisurale RALFS var. *acutum* BRÉB.	mes	aci	ben	3	
Cosmarium connatum RALFS	mes	aci	ben	1	2
Comarium conspersum RALFS var. *latum* (BRÉB.) W. et G.S.WEST	mes	aci	ben	2	3
Cosmarium contractum KIRCHN.	mes–oli	aci	ben	2	3
Cosmarium contractum var. *ellipsoideum* (ELFVING) W. et G.S. WEST	mes–oli	aci	ben	2	3

Table 1 Cont.

	TRPH	ACID	LF	R	S
Cosmarium contractum var. *minutum* (Delponte) Coesel	mes–eu	aci–alk	ben		
Cosmarium contractum var. *retusum* (W. et G.S.West) Willi Krieg. et Gerloff	mes	aci	ben	3	3
Cosmarium contractum var. *rotundatum* Borge	mes–oli	aci	ben	3	3
Cosmarium crenatum Ralfs	oli–mes	aci	atm–ben	1	
Cosmarium crenatum var. *bicrenatum* Nordst.	oli–mes	aci	atm–ben	2	
Cosmarium crenulatum Nägeli	mes	aci–neu	ben	1	1
Cosmarium cucumis Ralfs	mes	aci	ben	2	3
Cosmarium cyclicum P.Lundell	mes–oli	aci	atm–ben	2	
Cosmarium cyclicum var. *arcticum* Nordst.	mes–oli	aci	atm–ben	2	
Cosmarium davidsonii J.Roy et Bisset	oli	aci	atm–ben	3	
Cosmarium debaryi W.Archer	mes	aci	ben	1	2
Cosmarium decedens (Reinsch) Racib.	oli	aci	atm–ben	3	
Cosmarium decedens var. *apertum* Willi Krieg. et Gerloff	oli	aci	atm–ben	3	
Cosmarium decedens var. *minutum* (Gutw.) Willi Krieg. et Gerloff	oli–mes	aci	atm–ben	3	
Cosmarium denboeri Meesters et Coesel	eu	neu–alk	pla–ben	2	
Cosmarium dentiferum Nordst. var. *alpinum* Messik.	oli	aci	ben–atm	3	
Cosmarium depressum (Nägeli) P.Lundell	mes	aci–neu	ben	1	2
Cosmarium depressum var. *planctonicum* Reverdin	mes	neu	pla–ben	2	
Cosmarium dickii Coesel	mes	aci–neu	ben	1	2
Cosmarium didymoprotupsum W. et G.S. West	eu–mes	neu–eu	ben	3	2
Cosmarium difficile Lütkem.	mes	aci	ben		1
Cosmarium dilatatum Järnefelt et Grönblad	eu–mes	neu	pla	3	
Cosmarium eichlerianum (Grönblad) Messik.	mes	aci	ben	3	3
Cosmarium fastidiosum W. et G.S. West	mes	aci	ben	3	3
Cosmarium fontigenum Nordst.	mes	aci–neu	ben	3	2
Cosmarium formosulum Hoff	eu–mes	aci–alk	ben–pla		
Cosmarium furcatospermum W. et G.S. West	mes–eu	neu	ben	3	
Cosmarium galeritum Nordst.	oli–mes	aci	ben–atm	3	
Cosmarium garrolense J.Roy et Bisset	oli–mes	aci	ben–atm	3	
Cosmarium gibberulum Lütkem.	mes	neu–aci	ben	2	2
Cosmarium goniodes W. et G.S. West var. *subturgidum* W. et G.S. West	mes–oli	aci	ben	1	2
Cosmarium granatum Ralfs	mes–eu	neu–aci	ben		
Cosmarium holmiense P.Lundell	mes	aci	ben	3	
Cosmarium holmiense var. *hibernicum* (W.West) Schmidle	mes–oli	aci	ben–atm	3	
Cosmarium holmiense var. *integrum* P.Lundell	mes–oli	aci	atm–ben		
Cosmarium homalodermum Nordst.	oli–mes	aci	ben–atm	3	
Cosmarium hornavanense Gutw.	mes–oli	aci	ben–atm	3	
Cosmarium hornavanense var. *dubovianum* (Lütkem.) Růžička	mes–eu	neu–alk	ben	2	
Cosmarium humile (F.Gay) Nordst.	mes–eu	aci–alk	ben		2
Cosmarium impressulum Elfving	mes	aci–neu	ben		
Cosmarium jaoi Kouwets	eu	alk–neu	ben	3	2
Cosmarium kirchneri Børges.	mes	aci	ben	3	3

Table 1 Cont.

	TRPH	ACID	LF	R	S
Cosmarium kjellmanii WILLE	eu	alk–neu	ben–pla	2	
Cosmarium klebsii GUTW.	mes–eu	aci–alk	ben	3	2
Cosmarium laeve RABENH.	eu–mes	alk–aci	ben–pla		
Cosmarium laeve var. *octangulare* (WILLE) W. et G.S. WEST	eu–mes	alk–aci	ben–pla		
Cosmarium lagerheimii GUTW.	mes–eu	aci–neu	ben	3	2
Cosmarium limnophilum SCHMIDLE	mes–eu	aci–neu	ben	3	3
Cosmarium margaritatum (P.LUNDELL) J.ROY et BISSET	mes	aci	ben	3	3
Cosmarium margaritiferum RALFS	mes	aci	ben	1	2
Cosmarium medioretusum COESEL	mes	aci	ben	3	3
Cosmarium meneghinii RALFS	mes–eu	neu–aci	ben	1	
Cosmarium microsphinctum NORDST.	oli–mes	aci	atm–ben	3	
Cosmarium microsphinctum var. *crispulum* NORDST.	oli–mes	aci	atm–ben	3	
Cosmarium moniliforme RALFS var. *panduriforme* (HEIMERL) SCHMIDLE	mes	aci	ben	2	2
Cosmarium netzerianum SCHMIDLE	oli	aci	ben–atm	3	
Cosmarium norimbergense REINSCH var. *depressum* (W. et G.S.WEST) WILLI KRIEG. et GERLOFF	oli–mes	aci–neu	ben	3	
Cosmarium notabile BRÉB.	mes–oli	aci–neu	atm–ben		
Cosmarium notatum (GRÖNBLAD) COESEL	mes	aci	ben	3	3
Cosmarium novae – semliae WILLE var. *granulatum* (SCHMIDLE) SCHMIDLE	oli–mes	aci	ben	3	
Cosmarium obliquum NORDST.	oli	aci	ben–atm	2	
Cosmarium obsoletum (HANTZSCH) REINSCH	mes–oli	aci	ben	3	3
Cosmarium obtusatum SCHMIDLE	mes–eu	neu–alk	ben		
Cosmarium ocellatum B.EICHLER et GUTW.	mes	aci	ben	3	2
Cosmarium ocellatum var. *notatum* (NORDST.) WILLI KRIEG. et GERLOFF	mes	aci	ben	3	3
Cosmarium ochthodes NORDST.	mes	aci	ben	1	1
Cosmarium ordinatum (BØRGES.) W. et G.S.WEST	mes–oli	aci	ben	3	3
Cosmarium ornatulum COESEL	eu	alk	pla	1	
Cosmarium ornatulum var. *depressum* COESEL	eu	alk	pla	3	
Cosmarium ornatum RALFS	mes–oli	aci	ben	1	2
Cosmarium orthopunctulatum SCHMIDLE	oli	aci	ben–atm	1	
Cosmarium ovale RALFS	mes	aci	ben	3	3
Cosmarium pachydermum P.LUNDELL	mes	aci	ben	1	2
Cosmarium pachydermum var. *aethiopicum* W. et G.S.WEST	mes	aci	ben	2	2
Cosmarium paragranatoides SKUJA	mes	aci	ben	3	3
Cosmarium parvulum BRÉB. var. *undulatum* SCHMIDLE	oli	aci	atm–ben	3	
Cosmarium paucigranulatum BORGE	mes	neu	ben	3	
Cosmarium perforatum P.LUNDELL	mes	aci	ben	3	3
Cosmarium pericymatium NORDST.	oli	neu–aci	atm–ben	2	
Cosmarium pericymatium var. *corrugatum* BROOK	oli	neu	atm–ben	3	
Cosmarium pericymatium var. *notabiliforme* INSAM et KRIEGER	oli	neu	atm–ben	3	
Cosmarium phaseolus RALFS	mes	aci	ben	3	
Cosmarium phaseolus var. *elevatum* NORDST.	mes	aci	ben	3	2
Cosmarium pokornyanum (GRUNOW) W. et G.S.WEST	mes	aci	ben–atm	3	

Table 1 Cont.

	TRPH	ACID	LF	R	S
Cosmarium polygonum (Nägeli) W.Archer var. *depressum* Messik.	mes	aci–neu	ben		1
Cosmarium portianum W.Archer	mes	aci–neu	ben	1	2
Cosmarium praemorsum Bréb.	mes–eu	neu	ben	3	1
Cosmarium prominulum Racib. var. *subundulatum* W. et G.S.West	oli–mes	aci	ben	3	3
Cosmarium protractum (Nägeli) De Bary	eu	alk	ben	3	2
Cosmarium pseudoexiguum Racib.	mes	aci	ben	3	2
Cosmarium pseudoinsigne Prescott	eu–mes	alk–neu	ben	3	2
Cosmarium pseudonitidulum Nordst. var. *validum* W. et G.S.West	oli	aci–neu	ben–atm	3	
Cosmarium pseudoornatum B.Eichler et Gutw.	mes	aci	ben	2	2
Cosmarium pseudoprotuberans Kirchn.	mes	aci	ben	3	3
Cosmarium pseudoprotuberans var. *sulcatum* (Nordst.) Coesel	mes	aci	ben	3	3
Cosmarium pseudopyramidatum P.Lundell	oli–mes	aci	ben	2	2
Cosmarium pseudoretusum F.Ducell	mes	aci	ben	3	3
Cosmarium pseudowembaerense Kouwets	eu	alk	pla–ben	3	
Cosmarium punctulatum Bréb. var. *subpunctulatum* (Nordst.) Børges.	mes–eu	aci–alk	ben		
Cosmarium pygmaeum W.Archer	oli	aci	ben	2	2
Cosmarium pyramidatum Ralfs	oli–mes	aci	ben	2	2
Cosmarium pyramidatum var. *stenonotum* (Nordst.) Klebs	oli–mes	aci	ben	3	2
Cosmarium quadratulum var. *boldtii* (Messik.) Willi Krieg. et Gerloff	mes	aci	ben	1	2
Cosmarium quadratum Ralfs	mes	aci	ben		
Cosmarium quadrum P.Lundell	mes	aci	ben	1	3
Cosmarium quadrum var. *sublatum* (Nordst.) W. et G.S.West	mes	aci	ben	3	3
Cosmarium ralfsii Ralfs	oli	aci	ben	3	3
Cosmarium ralfsii var. *montanum* Racib.	oli	aci	ben	3	3
Cosmarium rectangulare Grunow	mes	aci	ben	2	2
Cosmarium regnellii Wille	mes–eu	aci–alk	ben		
Cosmarium regnesii Reinsch	mes	aci	ben	2	2
Cosmarium reniforme (Ralfs) W.Archer	eu–mes	aci–alk	ben		
Cosmarium reniforme var. *compressum* Nordst.	eu–mes	aci–alk	ben		
Cosmarium retusum (Perty) Rabenh.	mes	aci	ben	3	3
Cosmarium sexnotatum Gutw. var. *bipunctatum* (Wolosz.) Coesel	mes	aci	ben	3	3
Cosmarium sexnotatum var. *tristriatum* (Lütkem.) Schmidle	oli–mes	aci	ben	3	3
Cosmarium simplicius (W. et G.S.West) Grönblad	mes–oli	aci	atm–ben	3	2
Cosmarium sinostegos Schaarschm. var. *obtusius* Gutw.	mes	aci	ben	3	2
Cosmarium speciosum P.Lundell	oli–mes	aci	atm–ben	3	
Cosmarium speciosum var. *simplex* Nordst.	oli–mes	aci	atm–ben	1	
Cosmarium speciosum var. *tumidum* Schmidle	oli–mes	aci	atm–ben	3	
Cosmarium sphagnicolum W. et G.S.West	oli	aci	ben	3	2
Cosmarium sphyrelatum Coesel	mes	aci	ben	3	3
Cosmarium sportella Bréb. var. *subnudum* W. et G.S.West	mes	aci	ben	1	
Cosmarium striolatum (Nägeli) W.Archer	mes	aci	ben	3	3
Cosmarium subadoxum Grönblad	mes	aci	ben	3	
Cosmarium subbroomei Schmidle	eu–mes	neu–alk	ben	3	
Cosmarium subbroomei f. *isthmochondrum* Coesel	eu–mes	neu–alk	ben	3	2

Table 1 Cont.

	TRPH	ACID	LF	R	S
Cosmarium subcostatum NORDST.	mes	neu	ben	3	
Cosmarium subcostatum var. *minus* (W. et G.S.WEST) KURT FÖRST.	mes	aci–alk	ben		
Cosmarium subcrenatum HANTZSCH	mes	aci	ben–atm	3	
Cosmarium subcucumis SCHMIDLE	mes	aci	ben		
Cosmarium subgranatum (NORDST.) LÜTKEM.	mes–eu	aci–alk	ben		
Cosmarium subgranatum var. *borgei* WILLI KRIEG.	mes–eu	aci–alk	ben		
Cosmarium subprotumidum NORDST.	eu–mes	alk–aci	ben	1	2
Cosmarium subprotumidum var. *pyramidale* COESEL	mes–eu	aci–neu	ben	3	2
Cosmarium subquadrans W. et G.S.WEST var. *minor* SYMOENS	oli	aci	ben	3	3
Cosmarium subspeciosum NORDST.	mes	aci	ben	3	2
Cosmarium subspeciosum var. *transiens* MESSIK.	oli–mes	aci	ben–atm	1	
Cosmarium subtumidum NORDST.	mes–oli	aci	ben	2	2
Cosmarium subtumidum var. *groenbladii* CROASDALE	mes	aci	ben	3	3
Cosmarium taxichondriforme B.EICHLER et GUTW.	mes	aci	ben	3	3
Cosmarium tenue W.ARCHER	mes	aci–neu	ben		
Cosmarium tetrachondrum P.LUNDELL forma	mes	aci	ben	3	3
Cosmarium tetraophthalmum RALFS	mes	aci–neu	ben		
Cosmarium thwaitesii RALFS var. *penioides* KLEBS	mes	aci–neu	ben	1	
Cosmarium tinctum RALFS	oli–mes	aci	ben	1	2
Cosmarium tinctum var. *subretusum* MESSIK.	oli–mes	aci	ben	2	2
Cosmarium trachypleurum P.LUNDELL var. *minus* RACIB.	mes	aci	ben	3	3
Cosmarium truncatellum PERTY	oli	aci	ben	3	3
Cosmarium turpinii BRÉB. var. *podolicum* GUTW.	mes–eu	aci–alk	ben	1	2
Cosmarium ungerianum (NÄGELI) DE BARY var. *subtriplicatum* W. et G.S.WEST	mes	aci	ben	3	3
Cosmarium variolatum P.LUNDELL	mes	aci	ben	3	3
Cosmarium variolatum var. *cataractarum* RACIB.	mes–eu	aci–alk	ben	3	2
Cosmarium varsoviense RACIB.	mes	aci	ben	2	2
Cosmarium vexatum W.WEST	mes–eu	neu–alk	ben	2	
Cosmarium vexatum var. *concavum* SCHMIDLE	mes	aci	ben	3	
Cosmarium vogesiacum LEMAIRE	oli–mes	aci	ben	3	
Cosmarium wittrockii P.LUNDELL	eu–mes	neu–alk	ben	2	2
Cosmocladium constrictum W.ARCHER	mes	aci	pla	3	
Cosmocladium saxonicum J.ROY et BISSET	mes	neu	pla	3	
Cylindrocystis brebissonii DE BARY	oli	aci	ben–atm		
Cylindrocystis crassa DE BARY	oli	aci	atm	3	
Cylindrocystis gracilis I.HIRN	oli	aci	ben–atm		
Desmidium aptogonum KÜTZ.	mes	aci–neu	ben	2	2
Desmidium baileyi (RALFS) NORDST. var. *caelatum* (KIRCHN.) NORDST.	mes	aci	ben	3	3
Desmidium grevillei (RALFS) DE BARY	mes–oli	aci	ben	2	3
Desmidium swartzii RALFS	mes	aci–neu	ben		1
Docidium baculum RALFS	mes–oli	aci	ben	3	3
Euastrum ansatum RALFS	mes–oli	aci	ben		1
Euastrum ansatum var. *rhomboidale* F.DUCELL.	mes–oli	aci	ben	3	3

Table 1 Cont.

	TRPH	ACID	LF	R	S
Euastrum bidentatum Nägeli	mes	aci–neu	ben		
Euastrum bidentatum var. *speciosum* (Boldt) Schmidle	mes	aci–neu	ben	1	
Euastrum binale Ralfs	oli	aci	ben	3	
Euastrum binale var. *gutwinskii* (Schmidle) Homfeld	oli	aci	ben		
Euastrum biscrobiculatum (Wolosz.) Coesel	mes	aci	ben	3	3
Euastrum brevisinuosum (Nordst.) Kouwets var. *dissimile* (Nordst.) Kouwets	oli	aci	atm–ben	3	
Euastrum crassicolle P.Lundell	oli–mes	aci	ben–atm	3	
Euastrum crassum Ralfs	oli	aci	ben	3	3
Euastrum denticulatum F.Gay	mes	aci	ben	1	2
Euastrum dubium Nägeli	oli–mes	aci	ben	3	
Euastrum dubium var. *ornatum* Wolosz.	mes	aci	ben	2	2
Euastrum elegans Ralfs	mes	aci	ben	1	2
Euastrum gayanum De Toni	mes–oli	aci	ben		1
Euastrum germanicum (Schmidle) Willi Krieg.	eu–mes	aci–alk	ben–pla	2	2
Euastrum humerosum Ralfs	oli–mes	aci	ben		2
Euastrum humerosum var. *affine* (Ralfs) G.C.Wallich	oli–mes	aci	ben	3	2
Euastrum insigne Ralfs	oli	aci	ben	2	3
Euastrum insulare (Wittr.) J.Roy	mes	aci–neu	ben	2	2
Euastrum luetkemuelleri F.Ducell. var. *carniolicum* (Lütkem.) Willi Krieg.	oli–mes	aci	ben	3	3
Euastrum montanum W. et G.S.West	mes–oli	aci	ben	3	
Euastrum oblongum Ralfs	mes	aci	ben		2
Euastrum pectinatum Ralfs	mes	aci	ben	1	2
Euastrum pinnatum Ralfs	mes	aci	ben	3	3
Euastrum pulchellum Bréb.	mes	aci	ben	3	3
Euastrum subalpinum Messik.	oli–mes	aci	ben–atm	2	
Euastrum subalpinum var. *crassum* Messik.	oli–mes	aci	ben–atm	3	
Euastrum turneri W.West	mes	aci	ben	3	3
Euastrum verrucosum Ralfs	mes	aci	ben	1	2
Euastrum verrucosum var. *alatum* Wolle	mes	aci	ben	1	2
Gonatozygon aculeatum Hastings	mes	aci	ben	3	3
Gonatozygon brebissonii De Bary	mes	aci	ben	1	2
Gonatozygon brebissonii var. *alpestre* Růžička	oli–mes	aci	ben–atm	3	
Gonatozygon kinahanii (W.Archer) Rabenh.	mes–eu	aci–neu	ben	1	1
Gonatozygon monotaenium De Bary	mes–eu	aci–neu	ben–pla	1	1
Haplotaenium indentatum Kouwets var. *latius* Kouwets morpha	oli	aci	ben	3	3
Haplotaenium minutum (Ralfs) Bando	oli	aci	ben	2	3
Haplotaenium rectum (Delponte) Bando	oli–mes	aci	ben	2	3
Hyalotheca dissiliens Ralfs	mes	aci–neu	ben		
Hyalotheca dissiliens var. *tatrica* Racib.	oli	aci	ben	2	2
Hyalotheca mucosa Ralfs	mes	aci	ben	2	1
Mesotaenium caldariorum (Lagerh.) Hansg.	mes	aci–neu	atm–ben	3	
Mesotaenium degreyi W.B.Turner	oli	aci	atm–ben	3	
Mesotaenium endlicherianum Nägeli	oli	aci	atm–ben	2	

Table 1 Cont.

	TRPH	ACID	LF	R	S
Mesotaenium macrococcum (KÜTZ.) J.ROY et BISSET	oli	aci	atm		
Mesotaenium macrococcum var. *minus* (DE BARY) COMPÈRE	oli	aci	atm–ben	1	
Micrasterias americana RALFS	mes	aci	ben–pla	1	2
Micrasterias apiculata RALFS	mes	aci	ben	3	3
Micrasterias brachyptera P.LUNDELL	mes	aci	ben	3	3
Micrasterias crux – melitensis RALFS	mes	aci–neu	ben–pla	1	2
Micrasterias denticulata RALFS	mes	aci	ben	3	2
Micrasterias denticulata var. angulosa (HANTZSCH) W. et G.S.WEST	mes	aci	ben	2	2
Micrasterias fimbriata RALFS	mes	aci	ben	2	3
Micrasterias furcata RALFS	mes	aci	ben	3	3
Micrasterias jenneri RALFS	oli	aci	ben	3	3
Micrasterias oscitans RALFS	oli	aci	ben	3	3
Micrasterias papillifera RALFS	mes	aci	ben	1	2
Micrasterias papillifera var. *pseudomurrayi* LAPORTE	mes	aci	ben	3	2
Micrasterias pinnatifida RALFS	mes	aci	ben	3	3
Micrasterias rotata RALFS	mes	aci	ben	1	2
Micrasterias thomasiana W.ARCHER	mes–oli	aci	ben	3	1
Micrasterias thomasiana var. *notata* (NORDST.) GRÖNBLAD	mes–oli	aci	ben		1
Micrasterias truncata RALFS	oli–mes	aci	ben		1
Micrasterias truncata var. *bahusiensis* WITTR.	oli–mes	aci	ben	2	1
Micrasterias truncata var. *quadrata* BULNH.	oli	aci	ben	3	1
Micrasterias truncata var. *semiradiata* (NÄGELI) WOLLE	oli–mes	aci	ben	1	1
Netrium digitus ITZIGS. et ROTHE	oli–mes	aci	ben		
Netrium interruptum (RALFS) LÜTKEM.	mes	aci	ben	2	2
Netrium interruptum var. *minor* (BORGE) WILLI KRIEG.	oli–mes	aci	ben–atm	3	
Netrium oblongum (DE BARY) LÜTKEM.	oli	aci	ben–atm	1	1
Netrium pseudactinotaenium COESEL	oli	aci	ben	3	3
Penium cylindrus RALFS	oli–mes	aci	ben		
Penium exiguum W.WEST	mes–oli	aci	ben	3	3
Penium margaritaceum RALFS	mes–oli	aci	ben	1	1
Penium polymorphum PERTY	oli	aci	ben	2	2
Penium spirostriolatum J.BARKER	mes–oli	aci	ben	1	2
Pleurotaenium archeri DELPONTE	mes	aci	ben	3	2
Pleurotaenium coronatum (RALFS) RABENH.	mes	aci	ben	3	2
Pleurotaenium coronatum var. *fluctuatum* W.WEST	mes	aci	ben	2	2
Pleurotaenium crenulatum (RALFS) RABENH.	mes	aci	ben		
Pleurotaenium ehrenbergii (RALFS) DE BARY	mes	aci	ben		1
Pleurotaenium eugeneum (W.B.TURNER) W. et G.S.WEST	mes	aci	ben	3	2
Pleurotaenium nodulosum (RALFS) DE BARY	mes	aci	ben	3	3
Pleurotaenium simplicissimum GRÖNBLAD	mes	aci	ben	3	3
Pleurotaenium trabecula NÄGELI	mes	aci–neu	ben	1	1
Pleurotaenium tridentulum (WOLLE) W.WEST	oli	aci	ben	3	3
Pleurotaenium truncatum (RALFS) NÄGELI	mes	aci	ben	1	2
Roya cambrica W. et G.S.WEST	mes	aci	ben	3	
Roya closterioides COESEL	mes	aci	ben	2	2

Table 1 Cont.

	TRPH	ACID	LF	R	S
Roya obtusa (Bréb.) W. et G.S.West	oli	aci	atm–ben		
Roya obtusa var. *anglica* (G.S.West) Willi Krieg.	oli	aci	atm–ben		
Sphaerozosma aubertianum W.West	mes	aci	ben	3	3
Sphaerozosma filiforme Ralfs	mes	aci–neu	ben	3	3
Spirotaenia condensata Ralfs	mes–oli	aci	atm–ben		
Spirotaenia endospira (Bréb.) W.Archer	oli–mes	aci	atm–ben	3	
Spirotaenia erythrocephala Itzigs.	oli–mes	aci	atm–ben	3	
Spondylosium planum (Wolle) W. et G.S.West	mes–eu	neu	pla–ben	3	
Spondylosium pulchellum W.Archer	oli–mes	aci	ben	1	2
Staurastrum aculeatum Ralfs	mes	aci	ben	3	3
Staurastrum alternans Ralfs	mes	aci–neu	ben		1
Staurastrum arctiscon (Ralfs) P.Lundell	mes	aci	pla	3	3
Staurastrum arcuatum Nordst.	eu–mes	alk–neu	pla	3	
Staurastrum avicula Ralfs	mes–eu	aci–neu	ben–pla	1	1
Staurastrum bieneanum Rabenh.	mes	aci	ben	1	1
Staurastrum bloklandiae Coesel et Joosten	eu	alk	pla	1	
Staurastrum bohlinianum Schmidle	oli	aci	ben	3	
Staurastrum boreale W. et G.S.West	mes	aci	ben		
Staurastrum borgeanum Schmidle	oli–mes	aci	ben	3	
Staurastrum brachiatum Ralfs	oli–mes	aci	ben	2	2
Staurastrum brebissonii W.Archer in A.Pritch.	mes	aci	ben	3	3
Staurastrum capitulum Ralfs	oli–mes	aci	ben–atm	2	
Staurastrum chaetoceras (Schröder) G.M.Smith	eu	alk	pla		
Staurastrum controversum Ralfs	mes	aci	ben	1	2
Staurastrum crassangulatum Coesel	mes	aci	ben	3	3
Staurastrum cristatum (Nägeli) W.Archer	mes	aci	ben	2	2
Staurastrum cristatum var. *cuneatum* Hinode	mes	aci	ben	3	3
Staurastrum cyrtocerum (Bréb.) Ralfs	mes	aci	ben	2	1
Staurastrum dilatatum Ralfs	mes	aci	ben	3	2
Staurastrum dispar Bréb.	mes	aci	ben		1
Staurastrum echinatum Ralfs	oli	aci	ben–atm	3	
Staurastrum erasum Bréb.	mes	aci–neu	ben	2	2
Staurastrum eurycerum Skuja	eu–mes	neu–alk	pla–ben	2	2
Staurastrum furcatum (Ralfs) Bréb.	oli	aci	ben	2	2
Staurastrum furcatum var. *aciculiferum* (W.West) Coesel	oli	aci	ben	2	2
Staurastrum furcigerum (Ralfs) W.Archer	mes	aci–neu	ben–pla	1	2
Staurastrum gracile Ralfs	mes	aci	ben		
Staurastrum habeebense Irénée-Marie	oli	neu	atm–ben	3	
Staurastrum hirsutum Ralfs	oli	aci	ben		
Staurastrum hirsutum var. *arnellii* (Boldt) Coesel	oli	aci	ben	3	
Staurastrum hirsutum var. *muricatum* (Ralfs) Kurt Först.	oli	aci	ben		
Staurastrum hystrix Ralfs	oli	aci	ben	3	3
Staurastrum inflexum Bréb.	mes–oli	aci–neu	ben		1

Table 1 Cont.

	TRPH	ACID	LF	R	S
Staurastrum kouwetsii COESEL	mes	aci	ben	1	2
Staurastrum lapponicum (SCHMIDLE) GRÖNBLAD	mes	aci	ben	1	2
Staurastrum lunatum RALFS	mes	aci	ben	2	1
Staurastrum manfeldtii DELPONTE	mes	aci–neu	ben	2	2
Staurastrum margaritaceum RALFS	oli	aci	ben		
Staurastrum meriani REINSCH	oli	aci	ben–atm	3	
Staurastrum micron W. et G.S.WEST	oli–mes	aci	ben	2	1
Staurastrum minimum COESEL	oli–mes	aci	ben	3	3
Staurastrum muticum RALFS	mes	aci	ben	1	2
Staurastrum oligacanthum W.ARCHER	mes	aci	ben	3	3
Staurastrum orbiculare RALFS	mes	aci	ben	3	3
Staurastrum orbiculare var. *depressum* J.ROY et BISSET	mes–eu	neu–aci	ben		
Staurastrum orbiculare var. *extensum* NORDST.	oli–mes	aci	ben–atm	2	
Staurastrum orbiculare var. *ralfsii* W. et G.S.WEST	mes	aci	ben	3	
Staurastrum oxyacanthum W.ARCHER	mes	aci	ben	3	3
Staurastrum pentasterias GRÖNBLAD	mes	aci	ben	3	2
Staurastrum pileolatum BRÉB.	oli	aci	ben–atm	2	
Staurastrum pingue TEILING	eu–mes	alk–neu	pla		
Staurastrum planctonicum TEILING	eu–mes	alk–neu	pla		
Staurastrum podlachicum B.EICHLER et GUTW.	mes	aci	ben	3	3
Staurastrum polymorphum RALFS	mes	aci	ben		
Staurastrum polymorphum var. *pygmaeum* GRÖNBLAD	mes	aci	ben	1	2
Staurastrum polytrichum (PERTY) RABENH.	mes	aci	ben	1	2
Staurastrum proboscideum (RALFS) W.ARCHER var. *productum* MESSIK.	mes	aci	ben	2	1
Staurastrum punctulatum RALFS	oli	aci	ben		
Staurastrum punctulatum var. *muricatiforme* SCHMIDLE	oli	aci	ben–atm	2	
Staurastrum punctulatum var. *pygmaeum* (RALFS) W. et G.S.WEST	oli	aci	ben–atm	3	
Staurastrum pungens RALFS	mes	aci	ben	3	3
Staurastrum pyramidatum W.WEST	oli	aci	ben	3	3
Staurastrum quadrispinatum W.B.TURNER	oli	aci	ben	3	3
Staurastrum retusum W.B.TURNER var. *boreale* W. et G.S.WEST	eu–mes	neu–alk	ben	3	2
Staurastrum scabrum RALFS	oli	aci	ben	3	3
Staurastrum sebaldi REINSCH	mes	aci	ben	3	3
Staurastrum sebaldi var. *gracile* MESSIK.	mes	aci	ben	3	3
Staurastrum senarium RALFS	mes	aci	ben	3	2
Staurastrum setigerum CLEVE	mes	aci	ben	3	2
Staurastrum sexcostatum RALFS	oli–mes	aci	ben–atm	2	
Staurastrum sexcostatum var. *productum* W.WEST	mes	aci	ben	1	
Staurastrum simonyi HEIMERL	oli	aci	ben	1	2
Staurastrum simonyi var. *gracile* LÜTKEM.	oli	aci	ben	3	2
Staurastrum simonyi var. *semicirculare* COESEL	oli	aci	ben	1	2
Staurastrum smithii (G.M.SMITH) TEILING	eu	alk	pla	3	
Staurastrum spongiosum RALFS	oli–mes	aci	ben–atm	3	2
Staurastrum spongiosum var. *perbifidum* W.WEST	oli–mes	aci	ben–atm	3	2

Table 1 Cont.

	TRPH	ACID	LF	R	S
Staurastrum striatum (W. et G.S.West) Růžička	mes	aci–neu	ben		
Staurastrum striolatum (Nägeli) W.Archer	mes	aci	ben	2	2
Staurastrum subarcuatum Wolle	mes	aci	ben–pla	3	2
Staurastrum subavicula (W.West) W. et G.S.West	mes–oli	aci	ben	2	2
Staurastrum teliferum Ralfs	mes	aci	ben		2
Staurastrum tetracerum Ralfs	mes–eu	neu–aci	ben		1
Staurastrum tetracerum var. *subexcavatum* Grönblad	eu	alk	pla	2	
Staurastrum trapezicum Boldt	mes	aci	ben	3	3
Staurastrum varians Racib.	mes	aci	ben	3	3
Staurastrum vestitum Ralfs	mes	aci	ben	3	3
Staurodesmus brevispina (Ralfs) Croasdale	mes	aci–neu	ben	3	2
Staurodesmus convergens (Ralfs) S.Lill.	mes	aci	ben	1	2
Staurodesmus cuspidatus (Ralfs) Teiling	mes	aci–neu	ben–pla	1	1
Staurodesmus cuspidatus var. *divergens* (Nordst.) Coesel	eu–mes	alk–neu	pla–ben		
Staurodesmus dejectus (Ralfs) Teiling	mes	aci–neu	ben	1	1
Staurodesmus dejectus var. *apiculatus* (Bréb.) Teiling	mes	aci–neu	ben	1	1
Staurodesmus dejectus var. *robustus* (Messik.) Coesel	mes	neu	ben	3	1
Staurodesmus dickiei (Ralfs) S.Lill.	mes	aci	ben	2	2
Staurodesmus dickiei var. *circularis* (W.B.Turner) Croasdale	mes	aci	ben	3	2
Staurodesmus extensus (Borge) Teiling	mes	aci–neu	ben–pla	1	1
Staurodesmus extensus var. *isthmosus* (Heimerl) Coesel	oli–mes	aci	ben	2	2
Staurodesmus extensus var. *joshuae* (Gutw.) Teiling	oli–mes	aci	ben	3	2
Staurodesmus extensus var. *malaccensis* (Bernard) Coesel	oli	aci	ben	3	2
Staurodesmus extensus var. *vulgaris* (B.Eichler et Racib.) Croasdale	mes	aci	ben		1
Staurodesmus glaber (Ralfs) Teiling	mes–oli	aci	ben		2
Staurodesmus incus (Bréb.) Teiling	oli–mes	aci	ben	1	
Staurodesmus lanceolatus (W.Archer) Croasdale var. *compressus* (W. et G.S.West) Teiling	mes–oli	aci	ben	3	3
Staurodesmus mucronatus (Bréb.) Croasdale	mes	aci	ben	3	2
Staurodesmus omearae (W.Archer) Teiling	mes–oli	aci	ben		
Staurodesmus patens (Nordst.) Croasdale	mes	neu–aci	ben	1	2
Staurodesmus spencerianus (Mask.) Teiling	oli	aci	ben	3	2
Staurodesmus subhexagonus (W. et G.S.West) Coesel	oli–mes	aci	ben	3	2
Teilingia excavata (Ralfs) Bourr.	mes	aci	ben	1	1
Teilingia granulata (J.Roy et Bisset) Bourr.	mes–eu	aci–alk	ben–pla		1
Tetmemorus brebissonii Ralfs	oli	aci	ben	2	2
Tetmemorus brebissonii var. *minor* De Bary	oli	aci	ben	2	2
Tetmemorus granulatus Ralfs	mes–oli	aci	ben		1
Tetmemorus laevis Ralfs	oli	aci	ben–atm		
Tetmemorus laevis var. *minutus* (De Bary) Willi Krieg.	oli	aci	ben–atm	1	
Tortitaenia bahusiensis (Nordst. et Lütkem.) Coesel	oli–mes	neu	atm–ben	3	
Tortitaenia obscura (Ralfs) Brook	oli–mes	aci	atm–ben		
Xanthidium aculeatum Ehrenb.	mes–oli	aci	ben	3	
Xanthidium antilopaeum Kütz.	mes	aci–neu	ben–pla	1	1
Xanthidium antilopaeum var. *laeve* Schmidle	oli	aci	ben–pla	2	3

Table 1 Cont.

	TRPH	ACID	LF	R	S
Xanthidium antilopaeum var. *herbidarum* W. et G.S.WEST forma *javanicum* (NORDST.) COESEL	mes	aci–neu	ben–pla	2	1
Xanthidium antilopaeum var. *planum* ROLL	mes	aci	ben–pla	2	1
Xanthidium armatum RALFS	oli	aci	ben	2	3
Xanthidium basidentatum (BØRGES.) COESEL	mes	aci	ben	3	3
Xanthidium bifidum (BRÉB.) DEFLANDRE	mes	aci	ben	3	3
Xanthidium concinnum W.ARCHER	mes	aci	ben	3	3
Xanthidium cristatum RALFS	mes	aci	ben	3	3
Xanthidium cristatum var. *uncinatum* RALFS forma *polonicum* GUTW.	mes	neu	ben	3	3
Xanthidium fasciculatum RALFS var. *oronense* W. et G.S.WEST	mes	aci	ben	3	3
Xanthidium octocorne RALFS	mes–oli	aci	ben	1	2

Paper II

Cosmarium gauthierae sp. nov. (Conjugatophyceae, Desmidiales) from an ephemeral pool in South-West Macedonia

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Cryptogamie, Algol., 2008, 29 (3): 255-260

Cosmarium gauthierae sp. nov. (Conjugatophyceae, Desmidiales) from an ephemeral pool in South-West Macedonia

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Abstract – A new desmid species with slightly twisted cells and asymmetrical ornamentation, *Cosmarium gauthierae* Štastný & Neustupa, is described from an ephemeral pool in south-western mountains of the Republic of Macedonia. It is morphologically close to asymmetrical forms of *C. dimaziforme*, *C. onychonema* and *C. pseudotaxichondrum* to which it has been compared.

***Cosmarium* / Desmidiales / Republic of Macedonia / ephemeral habitats / phytobenthos**

Résumé – ***Cosmarium gauthierae* sp. nov. (Conjugatophyceae, Desmidiales) d'une mare temporaire du sud-ouest de la Macédoine.** Une nouvelle espèce de desmidiées à cellules un peu tordues et à ornamentation asymétrique, *Cosmarium gauthierae* Štastný & Neustupa, est décrite d'une mare temporaire des montagnes du sud-ouest de la Macédoine. Cette nouvelle espèce est morphologiquement voisine de formes de *C. dimaziforme*, *C. onychonema* et *C. pseudotaxichondrum* auxquelles elle est comparée.

***Cosmarium* / Desmidiales / République de Macédoine / habitats éphémères / phytobenthos**

INTRODUCTION

Ephemeral pools are one of the least studied desmid habitats. However, these localities often contain interesting and little known desmid species (see e.g. Růžička, 1964, 1967; Kouwets, 1997; Williamson, 1999, 2000; Coesel *et al.*, 2006). In April 2007 the second author visited the Ohrid lake region, including the Galičica Mountains. in the south-western part of the Republic of Macedonia. In an ephemeral pool on humic soil formed on a karstic field basement we found a very interesting benthic desmid community that included a taxon that we now propose for taxonomic description as a new species within the genus *Cosmarium* Ralfs.

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MATERIALS AND METHODS

The investigated karstic field is located in Galičica Mountains, approximately 1500 m from the eastern shores of Ohrid lake, at an altitude of 990 m a.s.l. (40°59'15" N, 20°48'50" E). The investigated pool is located near the St. Spas chapel in the middle of the field. The pool was filled by water from melted snow from the adjacent mountain slopes. The climatic conditions of the locality are influenced by the nearby reservoir of the deep Ohrid lake and involve dry and hot sub-mediterranean summers with temperatures regularly > 30°C. However, there is a relatively short winter with freezing temperatures and a permanent snow cover, melting usually in late February and March. The vegetation in the immediate vicinity of the sampling locality involves submediterranean deciduous forests of *Quercus frainetto*, *Q. cerris*, *Q. pubescens*, *Carpinus orientalis* and *Juniperus communis* with common *Anemone blanda* in the undergrowth (Medwecka-Kornaś *et al.*, 1986). The water depth in the pool was up to 10 cm at the time of sampling. The surroundings of the locality and the massif of the Galičica Mountains are not inhabited and the only recent anthropogenic activity consists of limited cattle breeding in the non-forested parts of the area.

The locality was sampled on 12 April 2007. Benthic samples were fixed using 2% formaldehyde and examined using an Olympus BX51 light microscope with Olympus Camedia C – 5050Z digital photomicrographic camera. Line drawings were made using a drawing apparatus.

RESULTS AND DISCUSSION

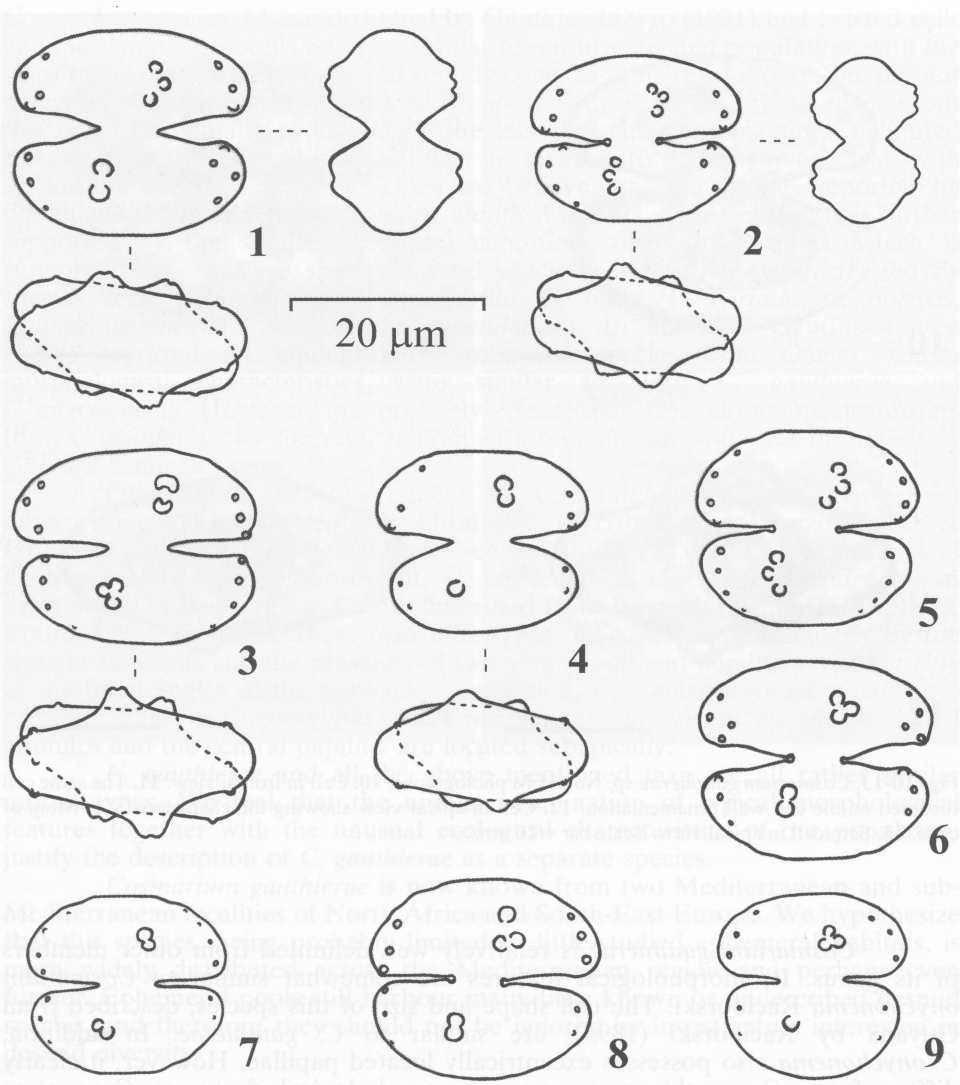
Cosmarium gauthierae Šťastný *et* Neustupa, species nova

Cellulae paulo latiores quam longiores, aspectu frontali fere ellipticae, profunde constrictae, sinu plus minusve aperto; semicellulae a fronte visae late trapeziformes vel ellipticae apice truncato vel obtuso, lateribus saepe undulatis 3-4 granulis parvis intramarginalibus ornatis, pariete frontale 2-3 granulis ornato, leviter asymmetrice a centro dispositis sinistrorsum, a vertice visae ellipticae, mutue tortae fere 20°, a latere visae fere circulares. Cellularum longitudo 18.5-24 µm, latitudo 21-27.5 µm, crassitudo 13-16.5 µm, longitudo latitudine ratio 0.8-1,0, latitudo isthmi 5.5-7.5 µm.

Holotypus hic designatus: Macedonia, Galičica Mountains, periphyton of an ephemeral pool, 990 m a.s.l., leg. *J. Neustupa*, 12.4. 2007. Holotype is deposited in PRC.

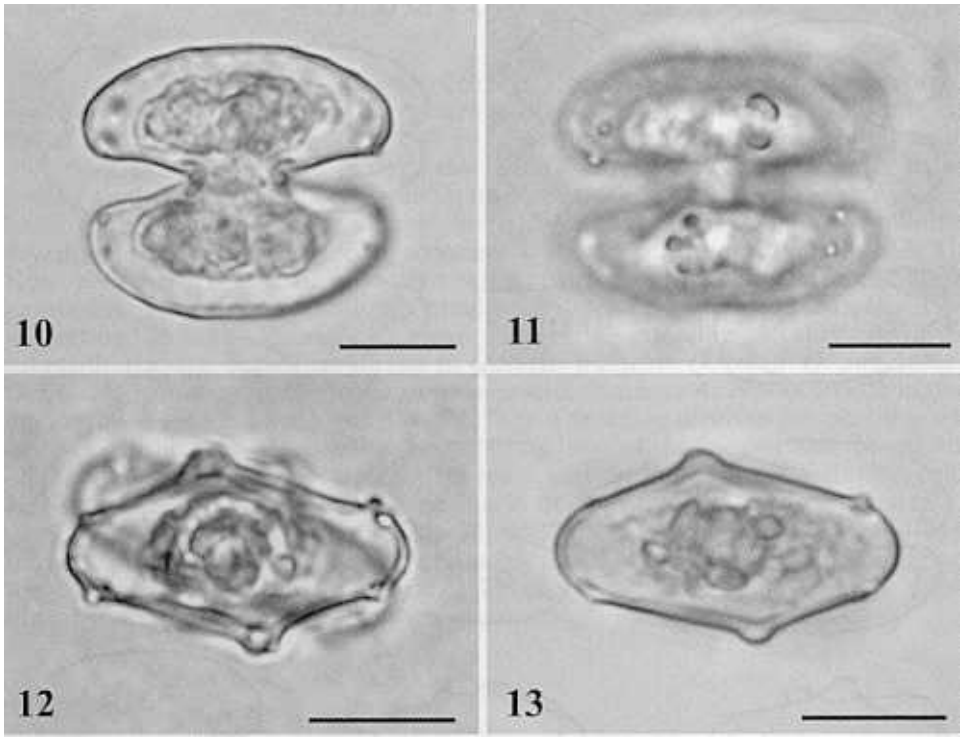
Etymology: Described in honour of Lucienne Gauthier-Lièvre, who conducted extensive investigations of algae in Mediterranean wetlands and mountainous habitats of North Africa, and also contributed to the knowledge of asymmetrical desmids.

The vegetative cells are slightly wider than long; in frontal view they are approximately elliptical, with a deep median constriction and with a more or less open sinus (Figs 1-10). The cells are twisted at the sinus through about 20°, which is best seen in apical views (Figs 1-4, 12). In frontal views, the semicells are broadly trapeziform to nearly elliptical with a truncate or rounded apex and often with some undulations at the lateral margins. A group of two to three papillae is



Figs 1-9. *Cosmarium gauthierae* sp. nov.

regularly visible left of the centre in one semicell and right of the centre in the other semicell. The lateral parts of the semicells bear three or four small intramarginal granules (Figs 1-9, 11). In apical views the semicells are elliptical; in lateral views they are approximately circular, with central tubercles distinctly visible in both apical and lateral view. In apical views the intramarginal granules are also usually visible (Figs 1-4, 12-13). The vegetative cells are 18.5-24 µm long, 21-27.5 µm wide and 13-16.5 µm thick. The isthmus is 5.5-7.5 µm wide. The length:width ratio is 0.8-1.



Figs 10-13. *Cosmarium gauthierae* sp. Nov., LM photographs. **10.** Cell in frontal view. **11.** The same cell focussed on the cell wall ornamentation. **12.** Cell in apical view showing the characteristic twisting of cells. **13.** Semicell in apical view. Scale bar = 10 μm.

Cosmarium gauthierae is relatively well delimited from other members of its genus. Its morphological features are somewhat similar to *Cosmarium onychonema* Raciborski. The cell shape and size of this species, described from Guyana by Raciborski (1895), are similar to *C. gauthierae*. In addition, *C. onychonema* also possesses excentrically located papillae. However, it clearly differs from *C. gauthierae* in several morphological features. *Cosmarium onychonema* lacks the intramarginal granules and regularly has only a single central papilla (Raciborski, 1895; Prescott *et al.*, 1981). In addition, *C. onychonema* lacks the twisting of cells, which is one of the prominent discriminating features of *C. gauthierae*. Krieger & Gerloff (1969) described *Cosmarium onychonema* var. *africanum* based on the drawings of Schmidle (1898) and Borge (1928). This variety differs from *C. gauthierae* in all the above mentioned discriminative characters of a type variety of *C. onychonema*. In addition, it has more elliptical semicells that do not conform to the morphological features of *C. gauthierae*.

Gauthier-Lièvre (1931) found a desmid population in an ephemeral wetland in the Mediterranean region of Algeria that she determined as *C. onychonema*. However, Krieger & Gerloff (1969) suggested that her material probably does not fit into *C. onychonema* and could represent an undescribed

Cosmarium species. Material studied by Gauthier-Lièvre (1931) had twisted cells and the shape of the cells was very similar to our investigated population, with the sinus being more widely open and slightly concave in its central part. She did not detect any intramarginal granules which are, however, present in our species, but this could be actually explained by the fact that she only examined a limited number of cells and had no possibility to investigate the empty cell wall with discernible cell wall features. Thus, we believe that the species reported by Gauthier-Lièvre (1931) was probably identical to ours. This hypothesis is further supported by the similar ecological conditions of both localities, which is supported also by their similar desmid species composition (characterized by species such as *Cosmarium commisurale*, *C. blytii*, *C. corbula*, *C. botrytis*, *C. impressulum* and *Staurastrum punctulatum*). In addition, Gauthier-Lièvre (1958) reported an unidentified *Cosmarium* species from Congo, whose morphological characteristics were similar to both *C. gauthierae* and *C. onychonema*. However, this probably undescribed *Cosmarium* species differs from *C. gauthierae* by the regular granulation of the cell wall and the regularly elliptical semicell shape.

Other taxa that are similar to *C. gauthierae* include *Cosmarium dimaziforme* (Grönbl.) Scott & Grönblad, described from Brazil (Scott & Grönblad, 1957; for the original figure see Grönblad, 1945) and some varieties of *C. pseudotaxichondrum* Nordstedt, in particular *C. pseudotaxichondrum* var. *asymmetricum* Bourrelly & Couté, described from French Guyana (Bourrelly & Couté, 1982). However, these taxa differ from *C. gauthierae* particularly by the straight semicells and the presence of two very prominent papillae on either side of the basal angles of the semicells. In addition, *C. dimaziforme* lacks excentric papillae, while in *C. pseudotaxichondrum* var. *asymmetricum* the intramarginal granules and the central papillae are located subapically.

C. gauthierae and all the above mentioned taxa are all rather similar morphotypes. We feel that the unique combination of typical morphological features together with the unusual ecological characteristics of our population justify the description of *C. gauthierae* as a separate species.

Cosmarium gauthierae is now known from two Mediterranean and sub-Mediterranean localities of North Africa and South-East Europe. We hypothesize that this species, being probably limited to little-studied ephemeral habitats, is more widely distributed across the Mediterranean region and perhaps even further. Ephemeral pools still harbour many little known or undescribed desmid species, and therefore they should not be ignored by investigators interested in desmid diversity.

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Paper III

New and remarkable desmids (Zygnematophyceae, Streptophyta) from Europe: taxonomical notes based on LM and SEM observations

Jan Ġ Šastný & Frans Kouwets

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New and remarkable desmids (Zygnematophyceae, Streptophyta) from Europe: taxonomical notes based on LM and SEM observations

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Abstract: In the present paper, the morphology and taxonomy of seven desmid taxa collected in various European habitats is discussed, mainly on the basis of scanning electron microscopic observations of cell wall sculpturing. Four taxa (*Actinotaenium riethii*, *Closterium pseudocostatum*, *Cosmarium discrepans* and *C. hostensiense*) are newly described and the name of one taxon (*Cosmarium lenzenwegeri*) is recombined. In addition, the morphology of *Cosmarium cataractarum* and *C. cinctutum* is described in greater detail, confirming their status as independent species.

Key words: *Actinotaenium,* cell wall sculpture, *Closterium, Cosmarium*, Desmidiales, ephemeral habitats, morphology, new species, scanning electron microscopy, taxonomy

Introduction

Traditional desmid taxonomy is mainly based on differences in cell morphology and wall sculpturing (e.g. Brook 1981). However, since about the last two decades, molecular research has increasingly, and sometimes controversially been turning desmid taxonomy upside down, especially at the level of genus and higher (e.g. McCourt et al. 2000; Gontcharov et al. 2003; Neustupa & Škaloud 2007; Gontcharov 2008; Hall et al. 2008; Gontcharov & Melkonian 2005, 2008, 2010). Only very recently molecular methods have also been used in the evaluation of taxonomic concepts of traditional, morphology–based taxa at the level of species and variety, resulting in remarkable taxonomic changes (Neustupa et al. 2010, 2011; Nemjová et al. 2011).

However, assuming a careful and critical application, the traditional analysis of cell morphology may still provide a lot of important and useful taxonomical data. Some general morphological characters are prominent, well defined and clearly visible under the light microscope, and may even distinguish between genera. Other characters are very variable, or inconspicuous and less well visible, such as cell wall sculpturing and pore patterns. The morphological variability ranging from apparently smooth to profusely sculptured makes the desmid cell wall perfectly suited for studies using scanning electron microscopy (SEM; see, e.g., Couté & Tell 1981; Coesel 1984). Therefore, in addition to the regular line drawings after light microscopical observations in the present study we also used a scanning electron microscope to clearly illustrate the discriminative morphological characters of the individual taxa.

Extensive sampling of desmids by the first author in the Czech Republic (Šťastný 2010) and also in several other European countries revealed a number of remarkable forms that apparently needed a more detailed study to clear up their taxonomy. In the present paper we discuss seven of them.

Materials and Methods

The samples were microscopically analyzed using an Olympus BX51 light microscope, and line drawings were made using a drawing tube.

For scanning electron microscopy (SEM) acetone–washed glass coverslips (10 or 12 mm in diameter) were placed on a heating block, and coated three times with a poly–L–lysine solution (Sigma, 1:10 in distilled water) to ensure better adhesion of the desmid cells. After cooling, a drop of the formaldehyde–fixed

material was placed on the glass and when almost dry, it was transferred into 30% acetone and dehydrated by an acetone series (30, 50, 70, 90, 95, 99% and 2x in 100%, 10 minutes each). Finally, the cells were dried to critical–point with liquid CO_2 subsequently sputter coated with gold and examined using a Phenom Desktop or JEOL 6380 LV scanning electron microscope.

Environmental variables (pH, conductivity) were measured either with a Combo HI 98129 (HANNA, Germany) portable instrument or with a WTW 330 pH–meter and WTW LF 315 conductometer (WTW, Germany). The list of all sampling sites is given in Table 1.

Results and Discussion

Closterium pseudocostatum Šťastný et Kouwets sp. nov. (Figs 1–9, 22–27)

Descriptio: cellulae moderate curvatae, media parte rectis, cingulis veris, ad utrumque polum sensim attenuatis, apicibus late tholiformibus, membrana ochroa costata striis circiter 2–6 / 10 µm. Cellularum longitudo 326–540 µm, latitudo 34–41 µm, apex 11–14 µm, long./lat. circiter 9–15.

Description: cells only slightly curved, in the midregion straight and cylindric, towards the ends gradually attenuated, with more or less conical, cowl– or dome–shaped apices. Cell wall with true girdle bands, brownish, more or less coarsely costate (2–6 str./10 µm).

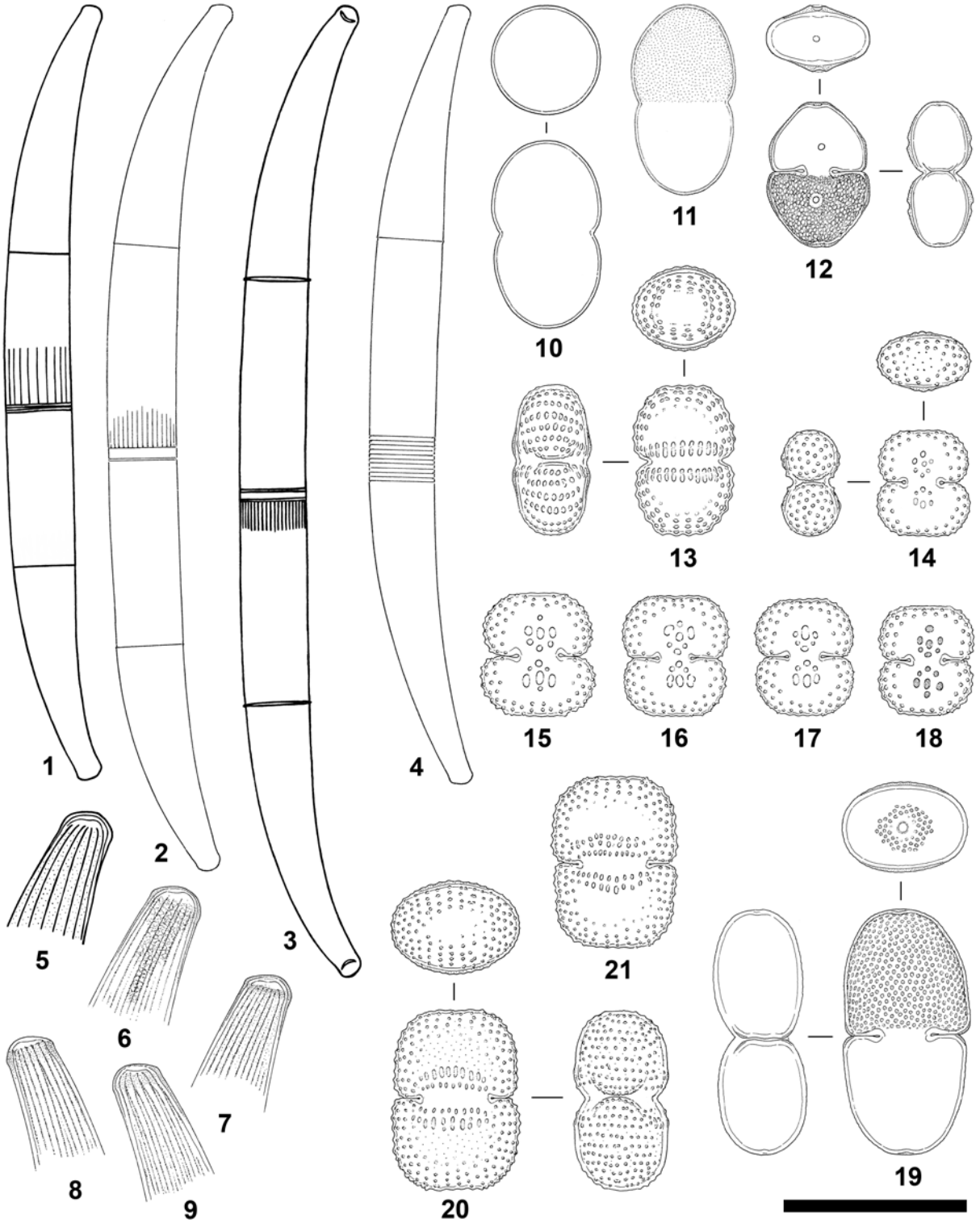
Dimensions: length 326–540 µm, width 34–41 µm, length/width ratio approximately 9–15.

Type locality: site 2 (see Table 1), leg. J. Šťastný, 17.9. 2009. The holotype is deposited in PRC (Hic designatus).

Taxonomy: *Closterium pseudocostatum* at first glance resembles *C. costatum* Ralfs. Corresponding characters are especially the coarsely costate cell wall [although the rib–like striae are usually not so much pronounced as in typical *C. costatum* and in some populations cells with a denser striation, similar to *C. costatum* var. *borgei* (Willi Krieg.) Růžička, may occur (see e.g. Figs. 2, 3, 25 and 27)] and the distinctive cowl–shaped apex. However, there are also some remarkable differences in comparison with *C. costatum*, namely the somewhat less regular and rather weak curvature and particularly the presence of true girdle bands in all cells of the populations presently studied. True girdle bands differ from false or pseudo girdle bands in that they are formed more orderly: they develop following a vegetative cell division as a straight extension of the youngest semicell. In full–grown cells there is only one per semicell, each of approximately the same length (Lütkemüller 1902). Pseudo girdle bands, on the contrary, vary in place, length and number, and may even be slightly curved. Not all *Closterium* species do form girdle bands, and there is a striking and fundamental difference in the way of cell–division in *Closterium* species with or without true girdle bands (Růžička 1977). In the original description of *C. costatum* (Ralfs 1848, p. 170, tab. 29, fig. 1) no mention is made of any girdle bands at all. However, in later desmid monographs (e.g. Růžička 1977; Förster 1982; Coesel & Meesters 2007) the occasional presence of pseudo girdle bands was reported; most remarkably, Krieger (1937) even ranks *C. costatum* among the girdle band species!

Forms very similar to the present material have been reported from Arctic Norway by Scharf (1981, Fig. 15) and from Austria by Lenzenweger (1996, pl. 6:2), both as *C. costatum*. Other findings probably representing *C. pseudocostatum* come from France (Deflandre 1927, fig. 3) and from south Germany (Förster 1964, tab. 2, figs 7–8), both labeled as *C. costatum* var. *westii*. Moreover, Brook & Williamson (2010) present a semicell of a form with a (true?) girdle band that they consider similar to var. *westii*. This variety has been described by Cushman (1905) referring to figures of W. & G.S. West (1896, pl. 13: 23–24) from North America, showing two cells of an unnamed forma of *C. costatum* that are only faintly curved, have truncate apices and clear pseudo girdle bands, which are not mentioned in the text. Later, Cushman (1908, pl. 3: 14) provided an additional, somewhat poor and atypical figure of his new variety. Next, also from North America, a much more characteristic figure of a form of *C. costatum* with a pseudo girdle band is presented as var. *westii* by Borge (1909). Referring to Borge (*l.c.*), Grönblad (1920) reported *C. costatum* var. *westii* from central Finland, but presented a figure of a cell with a rather long, straight girdle band. However, a constant character of the forms mentioned above is that they only measure 230–300 × 24–32 µm. Later, the concept of var. *westii* has been further confused by Krieger (1937; compare Růžička 1977) and therefore, we consider this variety very doubtful.

The last record of a form possibly representing *C. pseudocostatum*, is reported by John & Williamson (2009, p. 32, Pl. 4A) from the west of Ireland. They labeled their specimen as



Figs 1–21. (1–9) *Closterium pseudocostatum*, (1–3) typically developed mature cells with two girdle bands and a various striation density, (4) young daughter cell with only one girdle band, (5–9) details of apices; (10–11) *Actinotaenium riethii*; (12) *Cosmarium cataractarum*; (13) *C. cinctutum*; (14–18) *C. discrepans*; (19) *C. hostensiense*; (20–21) *C. lenzenwegeri*. Scale bar 100 µm (Figs 1–4), 50 µm (Figs 5–21).

C. costatum var. *erectum* Grönblad. This variety has been described by Krieger (1937) after a drawing sent to him by Grönblad that represented a cell with a straight middle part and measured 350 × 42 µm. Unfortunately, Krieger (*l.c.*) did not reproduce this figure, no mention is made of girdle bands and this variety is also considered very doubtful.

On the basis of the observations mentioned above, we consider the description of *Closterium pseudocostatum* as a new species as fully justified since raising one of the doubtful varieties mentioned to species level seems not appropriate. Unfortunately, no details are available on the molecular background of the formation of girdle bands, and on the phylogenetic relation between the groups with and without these structures. It seems therefore a matter of speculation whether the present form has evolved from a typical *C. costatum*, or rather has evolved in parallel and independently, including the mutual morphological features (the coarsely costate cell wall and the typical cowl–shaped apex).

Cells of *C. pseudocostatum* with a denser striation are somewhat similar to *C. intermedium* Ralfs, whose cells, however, are narrower and on average also shorter (approximately 200–400 × 20–30 µm) and lack the characteristic cowl–shaped apex.

Ecology and distribution: *Closterium pseudocostatum* prefers mesotrophic, slightly acidic habitats and judging from the nature of its Czech (Břehyně–Pecopala NR, Swamp NR) and Austrian (the very desmid–rich "Schwemm" near Walchsee peat bog, see Lenzenweger 2000; Šťastný & Lenzenweger 2008) sampling sites, its occurence seems to be limited to well–preserved biotopes with a high desmid diversity.

Actinotaenium riethii Šťastný et Kouwets sp. nov. (Figs 10–11, 28–30)

Descriptio: cellulae cylindricae, a fronte visae late ellipticae, circiter 1 3/4 longiores quam latae, in medio parum constrictae, apicibus late rotundatis, a vertice visae circulares, membrana subtilissime poribus ornata. Cellularum longitudo 39–54 µm, latitudo 25–31 µm, long./lat. circiter 1.55–1.95.

Description: cells approximately cylindrical with a slight median constriction, sinus very shallow and widely open. Apices broadly rounded, lacking any apical indentation or a distinct pore. Chloroplast stelloid, with 10–11 longitudinal ridges (4–5 visible in frontal view). Apical view circular. Cell wall densely set with fine pores.

Dimensions: length 39–54 µm, breadth 25–31 µm, length/breadth ratio approximately 1.55–1.95.

Type locality: site 4 (see Table 1), leg. J. Šťastný, 18.4. 2007. The holotype is deposited in PRC (Hic designatus).

Etymology: the new species is described in honour of Prof. Alfred Rieth who conducted an extensive study into diverse aspects of the biology of the form in question and presented arguments in favour of their description as a separate species.

Taxonomy: the *Actinotaenium* in question has been found on a number of periodically desiccating sites in the Czech Republic (the type locality and others) and could not be identified with any species described so far. The only paper reporting forms being probably identical with the newly described species is that by Rieth (1985). He described many aspects of the morphology and biology of two *Actinotaenium* forms he had found in a habitat very similar to the Czech sites ("von Regenwasser gespeisten, temporären Wegerandpfützen"). One of these taxa ("Form I" in Rieth *l.c.*) is obviously identical with our material. Rieth (*l.c.*) provisionally identified his material as *A. curtum* (Ralfs) Teiling var. *globosum* Wille, originally described as *Penium curtum* Brébisson var. *globosum* by Wille (1879, tab. 13, fig. 72) from the Novaja Zemlja archipelago. However, at once he presented numerous arguments for raising this variety to the rank of species. Although the description presented by Wille (*l.c.*) is rather poor and confusing (no apical view, no chloroplast details), our material as well as that described by Rieth (1985) obviously represent a taxon different from *A. curtum* var. *globosum*. As dimensions Wille (1879) gave 31–38 × 24–28 µm, whereas Rieth's (39–54 × 25–31 µm) as well as our specimens (41.5–51.5 × 26–29 µm) were much larger. The species in question has also nothing to do with the nominate variety of *A. curtum*, as the observations of Rieth (1985, under "Form II") convincingly demonstrate. *A. curtum* clearly differs by distinctly fusiform, comparatively narrower cells with an apical indentation bearing a distinct pore, and by cell wall that is much less densely set with pores than in *A. riethii* (Šťastný, pers. observation). Therefore, in our opinion the description of *A. riethii* as a new species is fully justified.

Ecology and distribution: *Actinotaenium riethii* is well characterized by its striking ecology. It is typical for periodically desiccating, usually

Table 1. List of sampling sites.

No.	Locality	Geographic coordinates	Details concerning habitats
1	Pond on the margin of the Schwemm Nature Reserve (NR), Austria	47°39‘16.50‘‘N, 12°17‘24.69‘‘E	meso–eutrophic; pH = 6.8, cond. = 184 µS. cm^{-1}
2	Pool in the Břehyně–Pecopala NR, Czech Republic	50°35‘1.10‘‘N, 14°42‘11.85‘‘E	mesotrophic; pH = 6.0, cond. = 166 µS. cm^{-1}
3	Pool in the Břehyně–Pecopala NR, Czech Republic	50°35‘1.47‘‘N, 14°43‘0.05‘‘E	oligo–mesotrophic; pH = 5.5, cond. = 113 µS. cm^{-1}
4	Periodically desiccating concrete drainage gutter in the town of Roztoky u Prahy, Czech Republic	50°9‘9.57‘‘N, 14°23‘50.49‘‘E	pH = 7.9, cond. = 175 µS. cm^{-1}
5	Mucilaginous growths on granite rocks in the Vlašim chateau park, Czech Republic	49°42‘29.57‘‘N, 14°52‘58.78‘‘E	n.d.
6	Boggy pool in the northern part of the Swamp NR, Czech Republic	50°35‘37.93‘‘N, 14°38‘37.70‘‘E	oligo–mesotrophic; pH = 5.2, cond. = 40µS. cm^{-1}
7	Držník pond, part of the Hradčanské rybníky NR, Czech Republic	50°36‘40.19‘‘N, 14°43‘15.90‘‘E	mesotrophic; pH = 5.5–6.0, cond. = 120–285 µS. cm^{-1}
8	Bohdanečský pond, Czech Republic	50°5‘18.44‘‘N, 15°39‘54.38‘‘E	slightly eutrophic; pH = 7.4
9	Ephemeral pool near the Mlýnský pond, Novohradské hory Mts, Czech Republic	48°42‘33.09‘‘N, 14°42‘47.09‘‘E	oligo–mesotrophic; pH = 5.6, cond. = 13µS. cm^{-1}
10	Periodically desiccating pool near the town of Hostens, France	44°29‘55.18‘‘N, 0°38‘22.51‘‘W	oligo–mesotrophic
11	Moorland pool north of Hostens, France	44º32‘42.37‘‘N, 0º40‘04.49‘‘W	oligo–mesotrophic
12	Rivulet in Marais du Cla, near “Le Gat Mort”, northeast of Hostens, France	44º30‘36.72‘‘N, 0º36‘45.05‘‘W	oligo–mesotrophic
13	L’Étang Bleu, France	48º01‘55.15‘‘N, 2º10‘44.25‘‘W	mesotrophic
14	Lac de Lamoura, France	46º23‘40.20‘‘N, 5º58‘47.25‘‘E	mesotrophic
15	Mucilaginous growths on a concrete platform near the Vidrenjak river, Serbia	43°02‘42.17‘‘N, 20°16‘27.24‘‘E	n.d.
16	Ephemeral pool near the way from the Remetské Hámre village to the Morské oko lake, Slovakia	48°52‘30.84‘‘N, 22°12‘27.23‘‘E	oligo–mesotrophic

man–made substrata like, e.g., concrete drainage gutters, concrete platforms, etc. where it may co–occur in particular with *A. curtum*, *Cosmarium pericymatium* var. *pericymaticum* and var. *corrugatum* and *Staurastrum habeebense*, a drought–resistant desmid community typical for this type of habitats (ŠŤASTNÝ 2008). Here, it can be easily confused with *C. pericymatium* whose cells, however, are not omniradiate and therefore have different frontal and lateral views (compare, e.g., WILLIAMSON 2000, tab. 2, fig. 7). Most likely also the reports of *A. cucurbita* by BROOK (2001, found together with *C. pericymatium* and *S. habeebense*) from a sun–dial and by WILLIAMSON (2002, in a community with *C. pericymatium* var. *pericymatium* and var. *corrugatum*, *S. habeebense* and *A. curtum*) from a stone birdbath actually represent *A. riethii*.

***Cosmarium cataractarum* (RACIBORSKI) EICHLER 1895 (Figs 12, 33–37)**

Basionym: *Cosmarium variolatum* LUNDELL var. *cataractarum* RACIBORSKI [1889, pl. 5(1), fig. 3]

Morphology and taxonomy: the cells are longer than broad with a deep, linear and closed sinus. Semicells are in outline pyramidal with rounded angles, straight to slightly convex sides and a slightly concave apex. In apical and lateral view they are elliptical, in apical view a slight median inflation is present. Cell wall coarsely scrobiculated and with a large scrobicula in the semicell centre.

Dimensions: length 37–42 µm, breadth 26–29 µm, thickness 17–19 µm, isthmus 7.5–9 µm; length/breadth ratio approximately 1.30–1.40.

Cosmarium cataractarum, found in sample from sites 7, 8 and 14, had originally been described as *C. variolatum* var. *cataractarum* by RACIBORSKI (1889, pl. 5, fig. 3) from southern Poland. RACIBORSKI (*l.c.*) obviously related his new form with *C. variolatum* LUNDELL on the basis of the similarity of the cell wall sculpture. The nominate variety of *C. variolatum* is also characterized by a coarsely scrobiculated cell wall and, according to the original description, var. *cataractarum* should only differ in the presence of a large scrobicula in the centre of each semicell (which, however, may rarely be reduced or even lacking; see Figs 36–37).

Under the light microscope the cell wall sculpture of both taxa indeed appears roughly the same (compare figs 264–5 and 266 in ŠŤASTNÝ 2010 or figs. 2 and 3 on pl. 49 in LENZENWEGER 1999). However, the use of SEM revealed remarkable differences. In *C. variolatum*, the cell wall sculpture consists of equally large and evenly distributed scrobiculae, each of them bearing a distinct pore (see Figs 31–32 after material from site 3). On the other hand, in var. *cataractarum*, the scrobiculae are generally more shallow, unevenly large (the larger being usually situated in the apical and lateral parts of the semicells), more densely distributed over the cell wall and the pores are located between them (see Figs 33–37). Generally, the cell wall sculpture in *C. variolatum* var. *cataractarum* more resembles that in *C. pseudovariolatum* GRÖNBLAD (see COUTÉ & TELL 1981, pl. 14, figs 3–6) than that in *C. variolatum*.

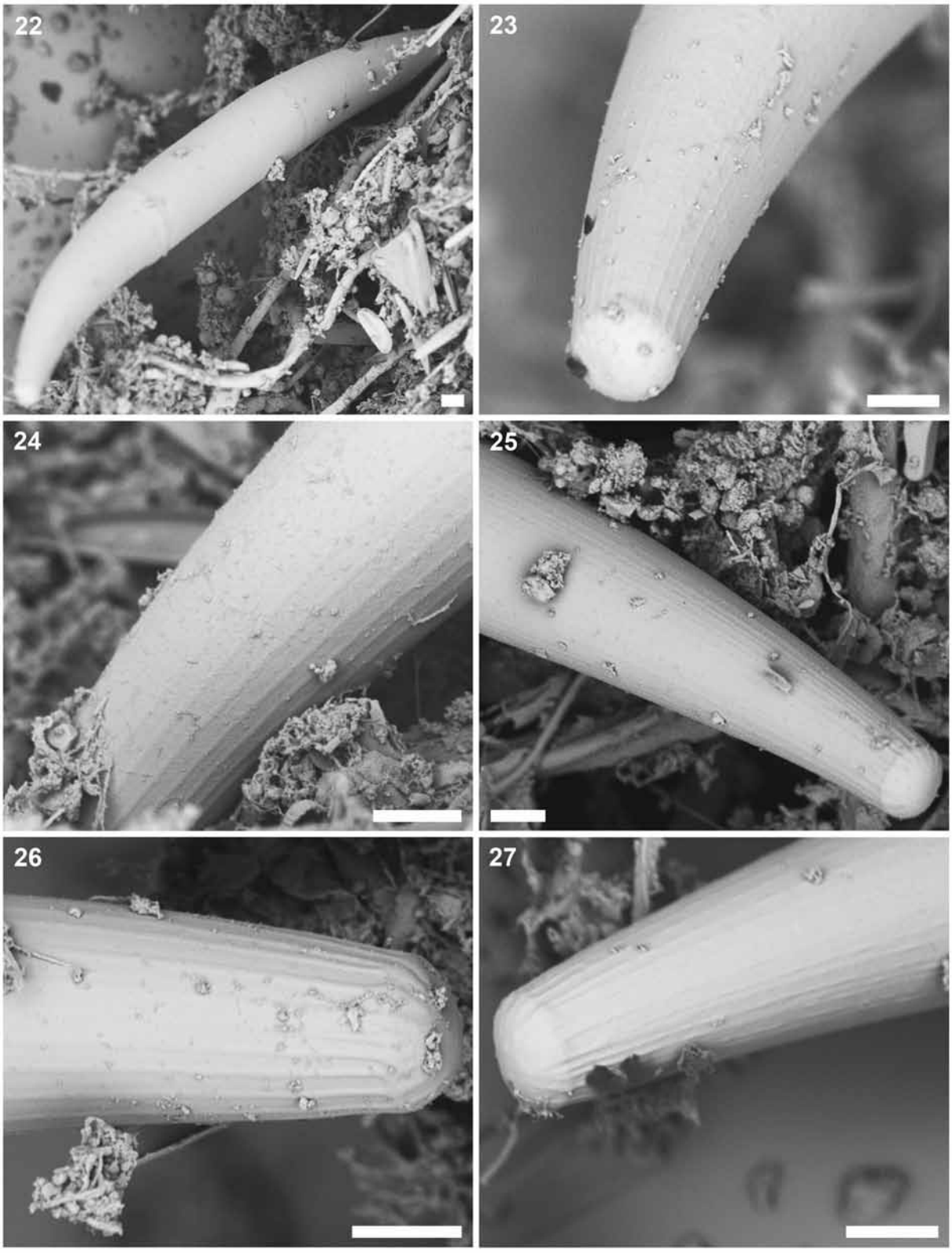
In addition, the ecological demands of the nominate variety of *C. variolatum* and var. *cataractarum* are quite different. The nominate variety is a rare species of mesotrophic, slightly acidic bogs, while var. *cataractarum* is a characteristic form from the tychoplankton of greater, meso– to slightly eutrophic and neutral to slightly alkaline water bodies (see e.g. ŠŤASTNÝ 2010).

Despite all the above–mentioned morphological and ecological differences, in desmidiological literature *C. cataractarum* is still generally considered a variety of *C. variolatum*. Only EICHLER (1895) treated it as a separate species, but his view has not been generally accepted. However, our observations fully support EICHLER's (*l.c.*) opinion and confirm the status of *C. cataractarum* as a separate species.

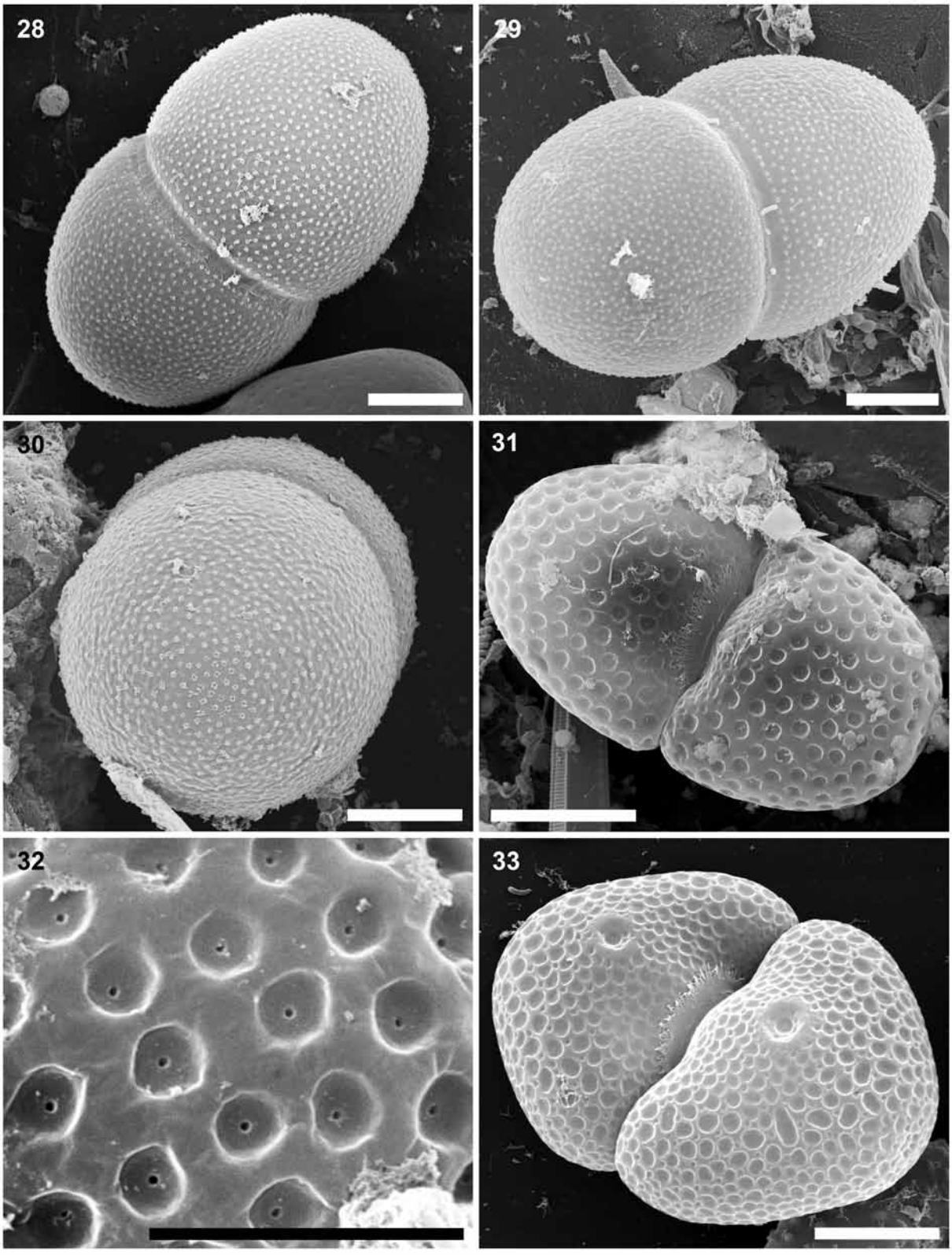
***Cosmarium cinctutum* NORDSTEDT 1875 (Figs 13, 42–49)**

Morphology and taxonomy: the cells in our material, coming from site 5, are longer than broad with a very shallow, open sinus. Semicells are in outline trapeziform with convex lateral sides and undulate–crenate margins. In both apical and lateral view they are elliptical. Cell wall ornamentation is composed of radiating series of intramarginal and marginal, flat granulae and in addition of a horizontal band of 8–12 (mostly 10) elongate, irregular verrucae just above the isthmus (Figs 13, 42–47). The cells are 36–45 µm long, 25–29 µm wide and 20–23 µm thick. The isthmus is 18.5–21.5 µm wide. The length/width ratio is approximately 1.40–1.55.

Our material showed some similarities with *C. basiornatum* (GRÖNBLAD) COESEL. This species



Figs 22–27. *Closterium pseudocostatum*: (22) whole cell, (23) detail of the same cell, (24–25) parts of cells with a less dense (24) and more dense (25) striation, (26–27) details of apices. Scale bar 10 μm.



Figs 28–33. (28–30) *Actinotaenium riethii*, (30) apical view; (31–32) *Cosmarium variolatum*, (32) detail of cell wall sculpture; (33) *C. cataractarum*. Scale bar 10 µm, 5 µm (Fig. 32).

was originally described by Grönblad (1926) as a variety of *C. davidsonii* J. Roy & Bisset and rightly made a separate species by Coesel (1998). However, a closer look revealed several subtle differences between *C. basiornatum* and our specimen. *C. basiornatum* is characterized by truncate–pyramidate semicells with straight or only faintly convex sides, a very moderate, largely closed sinus, and an ornamentation of short, radiating series of comparatively large granules leaving the semicell centre smooth. In addition a supra–isthmial ornamentation of 6–8 longitudinal series of mostly two (seldom fusing) elongate warts is present. Also between this horizontal band and the radiating series near the basis a smooth zone is present (see Figs 38–39 after material from site 9; compare also Coesel 1998, pl. 11: 39–40; Šťastný 2010, figs 116–117). Dimensions are 37–41.5 × 27–31 µm; isthmus 15–17 µm. The length/width ratio is approximately 1.28–1.37.

Our material, on the other hand, has slightly more rounded semicells, an even more shallow, gaping sinus, and a different supraisthmial ornamentation that merges with the radiating series of granules near the basis. Finally, it has a higher length/breadth ratio. These characters rather match the diagnosis of *C. cinctutum*, given by Nordstedt (1875). Unfortunately, Nordstedt only presented the figures of two semicells, one agreeing with our material, one significantly broader which possibly is an artifact (Nordstedt *l.c.* pl. 7: 20a and 20a', respectively). As dimensions he gave 40 × 28–33 µm, the larger width agreeing with his broader form.

Most remarkably, despite the above mentioned differences Coesel & Meesters (2007) without argumentation consider *C. basiornatum* a synonym of *C. cinctutum*, which name accordingly would have priority. However, in our opinion, both species should be kept apart.

C. cinctutum should also be compared with a few other species. *C. tumens* Nordstedt clearly differs by its characteristic supraisthmial ornamentation composed of 3–4 transversal rows of small granulae (Figs 40–41). In addition it has somewhat greater dimensions (in the Serbian population from site 15 cell dimensions were: length 43–52 µm, width 30–35 µm, thickness 24.5–26.5 µm, isthmus width 19–21 µm).

A second rather similar form is *C. speciosum* var. *rostafinskii* (Gutwiński) W. et G.S. West, originally described as *C. rostafinskii* ["Rostafińskii"] by Gutwiński (1890; see also Gutwiński 1892). However, general morphology and ornamentation of this form shows more affinity with *C. basiornatum* (compare also Skuja 1928). Croasdale (1962) described the slightly smaller *C. cinctutum* var. *reductum*, but this variety does not seem very closely related although the figures are somewhat confusing (Croasdale *l.c.* pl. 5: 80–82). Finally, Raciborski (1889) reported a form of *C. cinctutum* from a locality near Dresden, unfortunately without figures, measuring 36–47 × 26–30 µm, thickness 21–22 µm and isthmus 21–25 µm.

Ecology and distribution: as far as could be traced, up till now only one other more or less reliable record of *C. cinctutum* has been published. Kossinskaja (1936) mentioned the species presenting a detailed drawing after material collected near the Gulf of Jenisej, in Arctic Russia (Kossinskaja *l.c.* pl. 3: 6). However, her – single – figure seems slightly different from the present material concerning the shape of the isthmus and the development of the lateral crenations. The dimensions of her material are near the lower end of the range given above. *C. cinctutum* apparently is a rare, predominantly Arctic–Alpine species, presumably preferring hemi–atmophytic habitats.

***Cosmarium discrepans* Šťastný et Kouwets sp. nov. (Figs 14–18, 52–57)**

Descriptio: cellulae subparvae, paullo longiores quam latae, medio sinu anguste lineato profunde constrictae; semicellulae subtrapezi– subreniformes, angulis basalibus late rotundatis, superioribus obtuso–rotundatis, dorso truncatae, a vertice visae ellipticae, a latere visae subrotundatae; membrana intra margine granulis seribus irregulariter dispositis ornatis, supra isthmum granulo paullo majore ornatae, apicem versus granulis 2 minoris, granulis verruciformis plerumque geminatis 3, interdum granulo singulo ornatae. Cellularum longitudo 29–34 µm, latitudo 24–29 µm, crassitudo 16.5–18.5 µm, long./lat. circiter 1.10–1.25.

Description: the cells are slightly longer than broad, with a deep, linear and closed sinus. The semicells are approximately oval in outline with rounded basal angles and convex lateral sides that rather merge with the truncate apex. The cell wall ornamentation is composed of radiating series of small intramarginal/marginal granulae and a characteristic central ornamentation, consisting of a uniquely arranged rosette of wart–like granules. Between central and marginal ornamentation a smooth zone is present. The central ornamentation is rather variable, generally showing a prominent

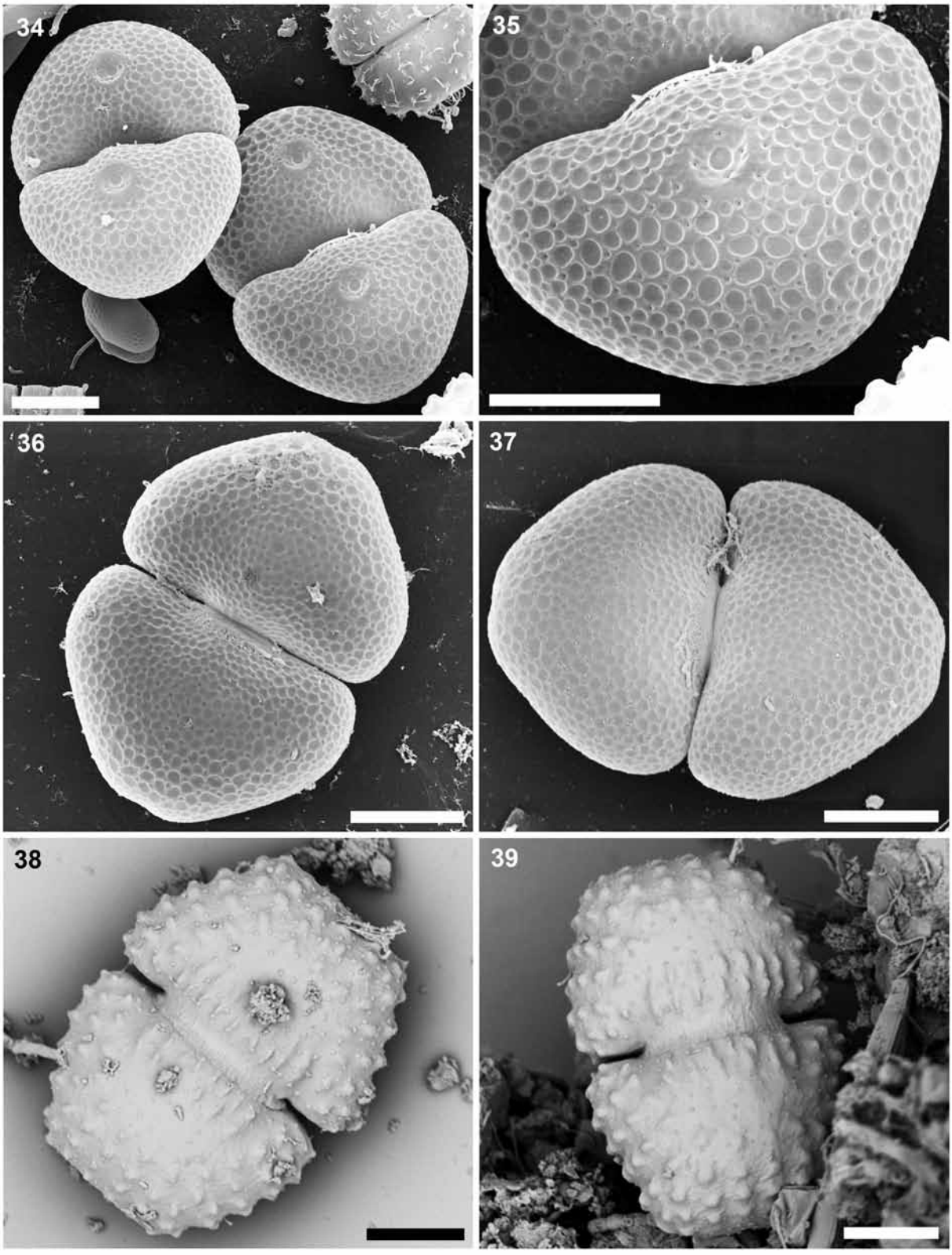
supraisthmial granule with, more apically, on either side a somewhat smaller granule. Above this a more or less horizontal series of three elongate warts is situated. These warts, and particularly the central one, show a strong tendency to subdivide, so that the total number of granulae may vary between six and nine and usually comprises seven. The variability of the central sculpture is particularly well visible in Figs 52–57. The apical view is ellipsoid with a slight median inflation, the lateral view is (sub)circular (see Fig. 14).
Dimensions: length 29–34 µm, breadth 24–29 µm, thickness 16.5–18.5 µm, isthmus 9–11 µm; length/breadth ratio approximately 1.10–1.25.
Type locality: site 6 (see Table 1), leg. J. Šťastný, 22.9. 2006. The holotype is deposited in PRC (Hic designatus).
Etymology: the epith "*discrepans*" means "different" pointing to the fact that this new species obviously has been considered a form of *C. punctulatum*.
Taxonomy: *Cosmarium discrepans* rather closely resembles *C. punctulatum* BRÉB. var. *subpunctulatum* (NORDST.) BØRGESEN, originally described as *C. subpunctulatum* by NORDSTEDT (1887, p. 160) from New Zealand. Searching the literature several reports of this taxon were found that are almost certainly identical with *C. discrepans*. Compare in particular the forms in COMPÈRE (1980, fig. 48) from the Belgian part of the Ardennes highlands, in WILLIAMSON (1992, figs 19, 4) from the Shetland Islands and also the interesting find of WILLIAMSON (2004, pl. 3, fig. 7) from the Indonesian Island Sulawesi. Most probably also the findings of W. & G.S. WEST (1897, pl. 6, fig. 19, as *C. subpunctulatum* NORDSTEDT) from South England and DUBOIS–TYLSKI (1969, pl. 1, fig. 20) from the French part of the Ardennes highlands represent the taxon in question.

The above–mentioned records are generally specified to originate from acidic and oligotrophic environments. On the other hand, *C. punctulatum* var. *subpunctulatum* is a common species of meso–eutrophic, slightly acidic to slightly alkaline waters (e.g. COESEL & MEESTERS 2007, ŠŤASTNÝ 2010). In addition to this striking difference in ecology of both taxa there is also a difference in cell wall ornamentation. The granulae in *C. punctulatum* var. *subpunctulatum* are generally somewhat more delicate than those in *C. discrepans* and in particular the central ornamentation of the semicells is very different. In *C. punctulatum* var. *subpunctulatum* this central ornamentation is composed of a horizontal row of 4 or 5, rather small supraisthmial granulae with more apically a group of 6–9, variably arranged and sometimes geminate granulae (compare NORDSTEDT 1888, pl. 5, fig. 8, see also Figs 50–51 after material from site 1).
Ecology and distribution: *Cosmarium discrepans* was found by the first author in several localities in northern Bohemia (the type locality and some others) and northern Belgium (De Teut NR). The second author encountered it almost simultaneously on several sites in the west of France (Bretagne, Aquitaine, sites 11, 12, 13). All sampling sites were generally acidic and oligo(–meso)trophic and the species in question often occurred in high numbers. Most likely it is a rather rare, but widely distributed species of oligo–mesotrophic, acidic, well preserved habitats.

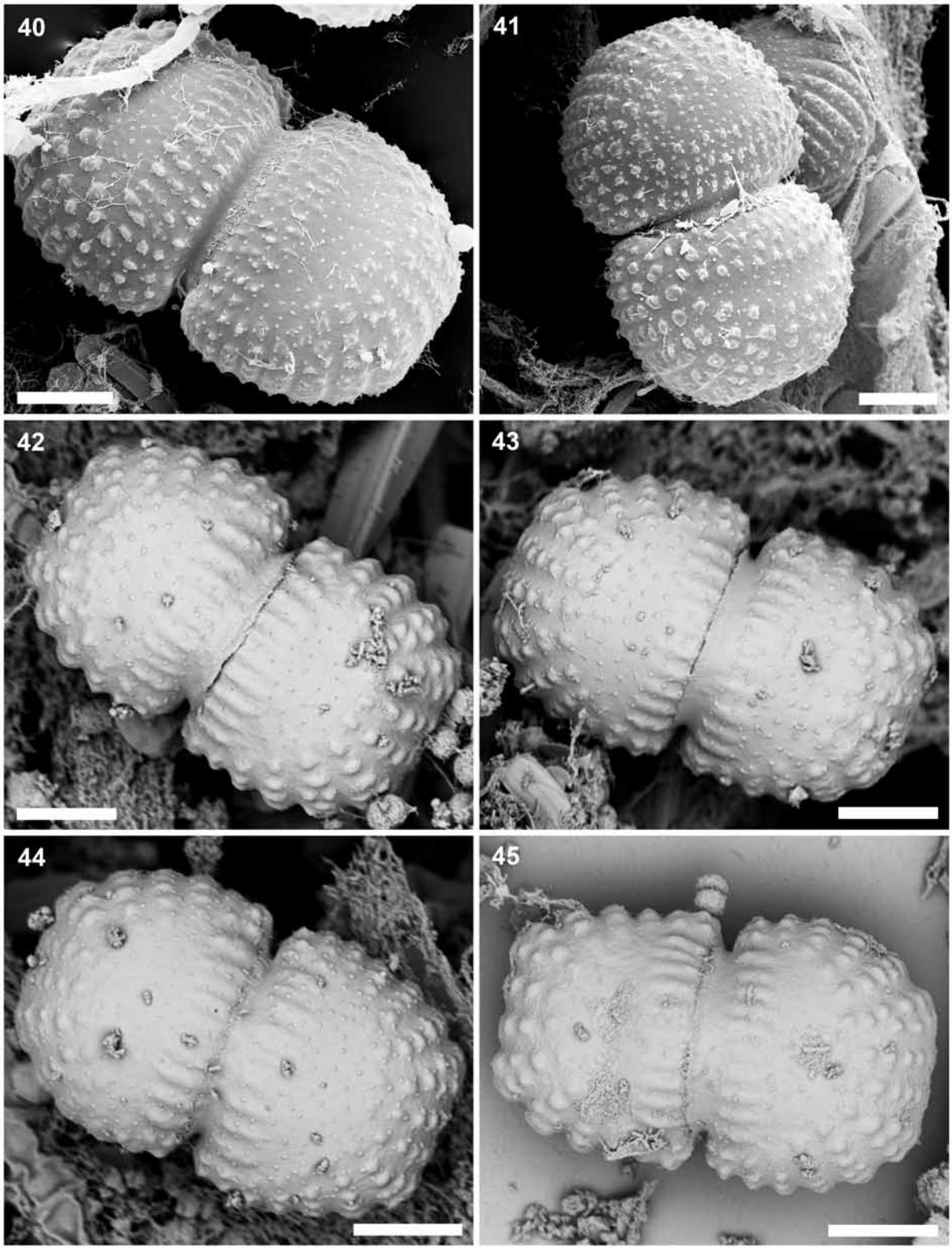
***Cosmarium hostensiense* ŠŤASTNÝ et KOUWETS sp. nov. (Figs 19, 58–61)**
Descriptio: cellulae a fronte visae ellipticae, profunde constrictae, sinu anguste lineari, apicibus subtruncatis–rotundatis in medio insectis, a vertice visae late ovatae, a latera visae compresse ovatae; membrana scrobiculata, scrobiculis poris ornatis. Cellularum longitudo 53–67 µm, latitudo 30–35 µm, crassitudo 23–25 µm, long./lat. circiter 1.75–2.00

Description: cells in outline truncately elliptic, about twice as long as broad, deeply constricted; basal angles rather acutely rounded, sides convex, straight to occasionally slightly concave and gradually tapering towards the truncately rounded apex. Cell wall evenly and densely covered with marked scrobiculae. Dimensions: length 53–67 µm, breadth 30–35 µm, thickness 23–25 µm, isthmus 12.5–14 µm; length/breadth ratio approximately 1.75–2.00.
Type locality: site 10 (see Table 1), leg. J. Šťastný, 12.3. 2009. The holotype is deposited in PRC (Hic designatus).
Etymology: the new species is named after Hostens, the French district where the type locality is situated.
Taxonomy: in a sample from locality 10, the first author found yet another desmid that is generally associated with *C. variolatum*, *i.e.* a form currently known as *C. variolatum* var. *skujae*.

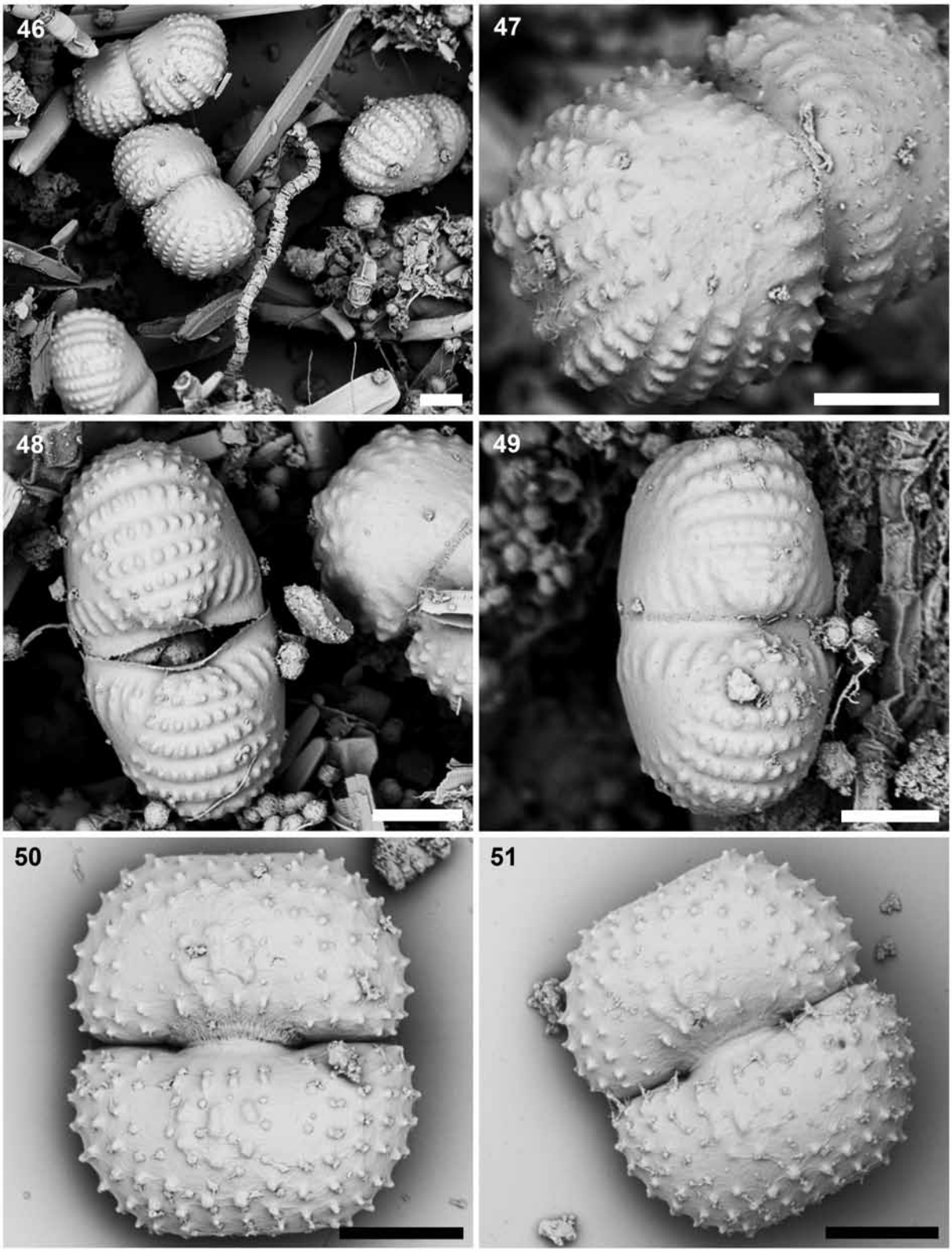
This rather conspicuous variety had originally been described by CROASDALE in CROASDALE & FLINT (1988, p. 112, pl. 36, fig. 13), after a figure published by SKUJA (1976)



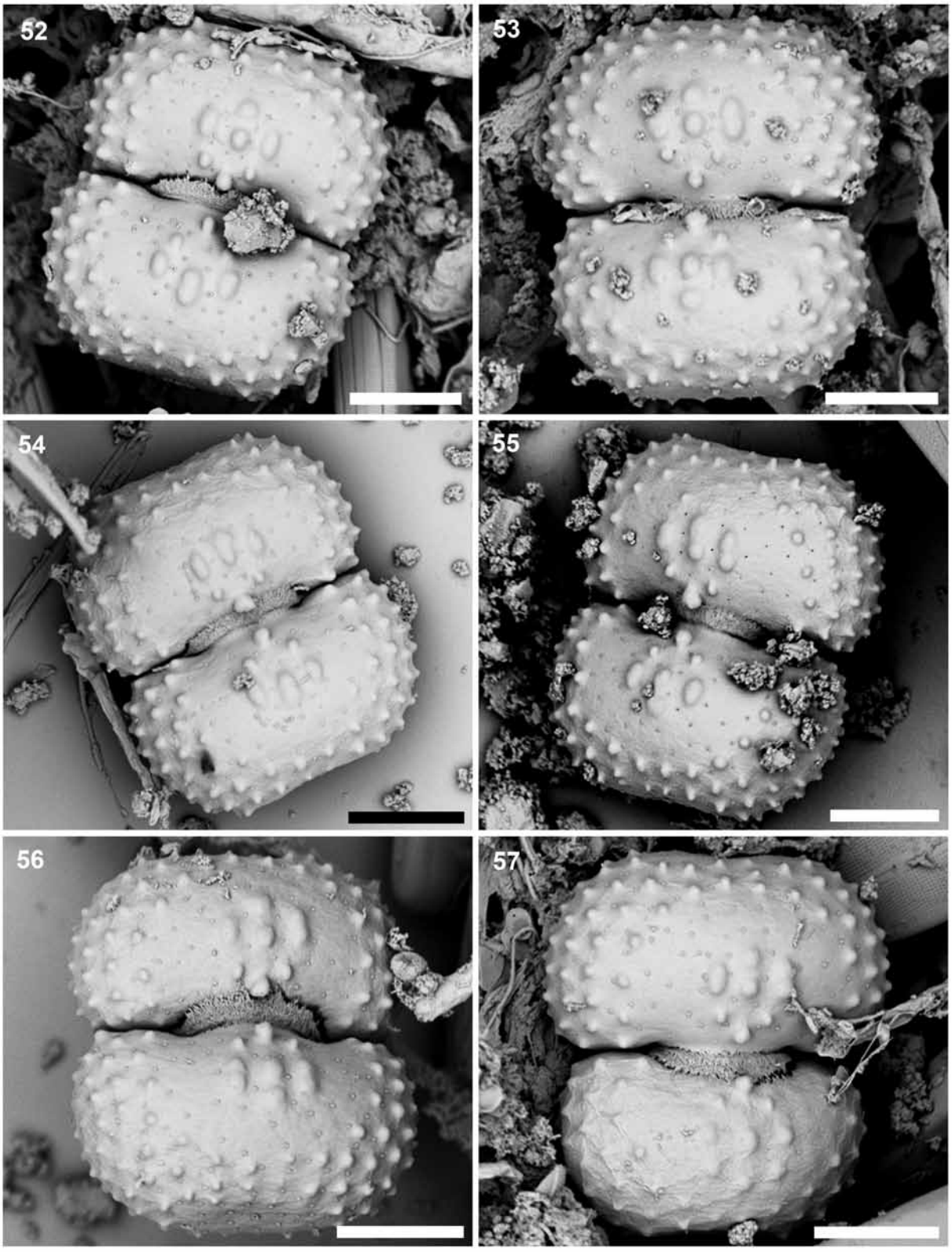
Figs 34–39. (34–37) *Cosmarium cataractarum*, (34) typically developed cells, (35) detail of cell wall sculpture, (36–37) anomalous cells with very weakly developed (upper semicell in 36) or completely lacking central scrobiculae; (38–39) *C. basiornatum*. Scale bar 10 μm.



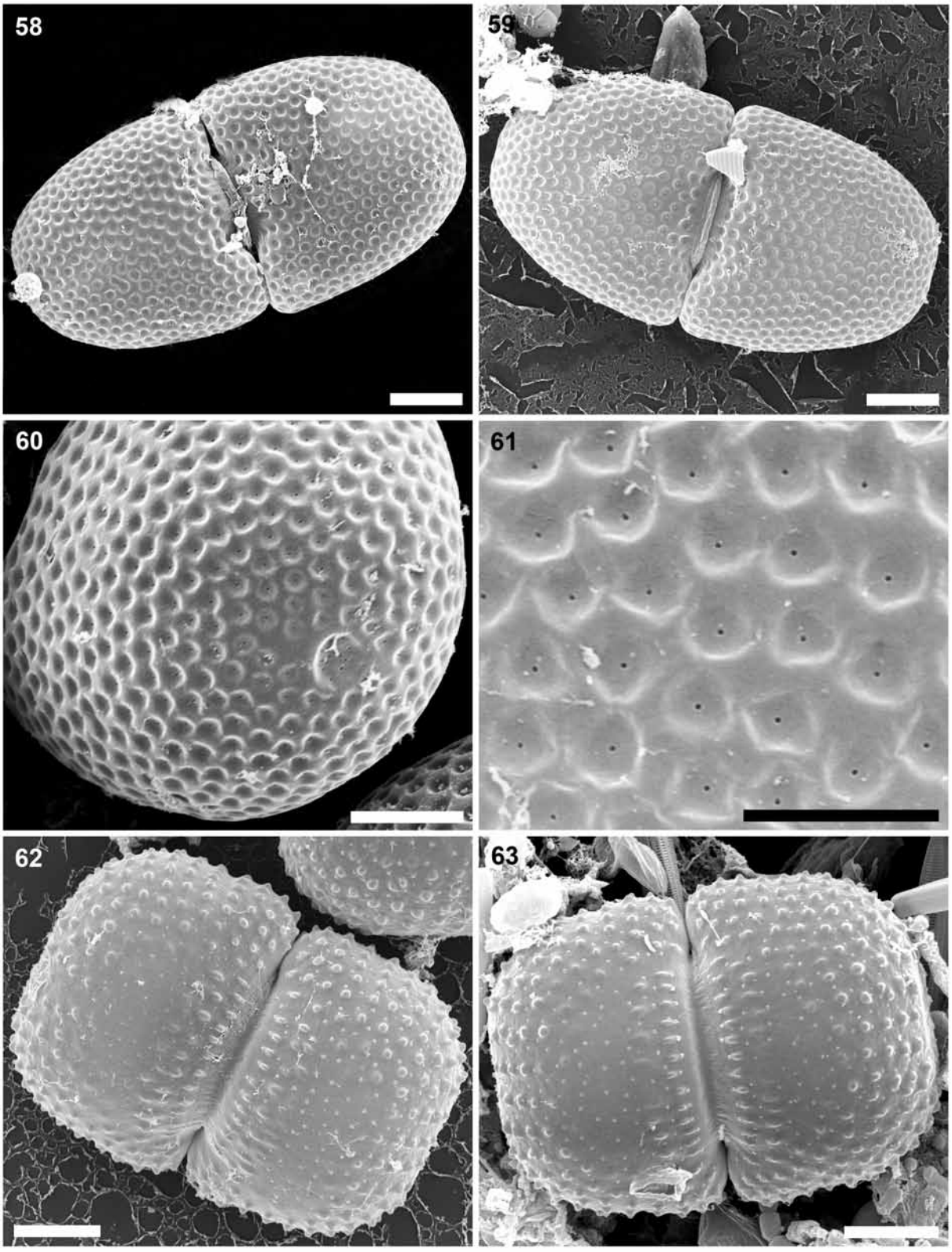
Figs 40–45. (40–41) *Cosmarium tumens*; (42–45) *C. cinctutum*. Scale bar 10 µm.



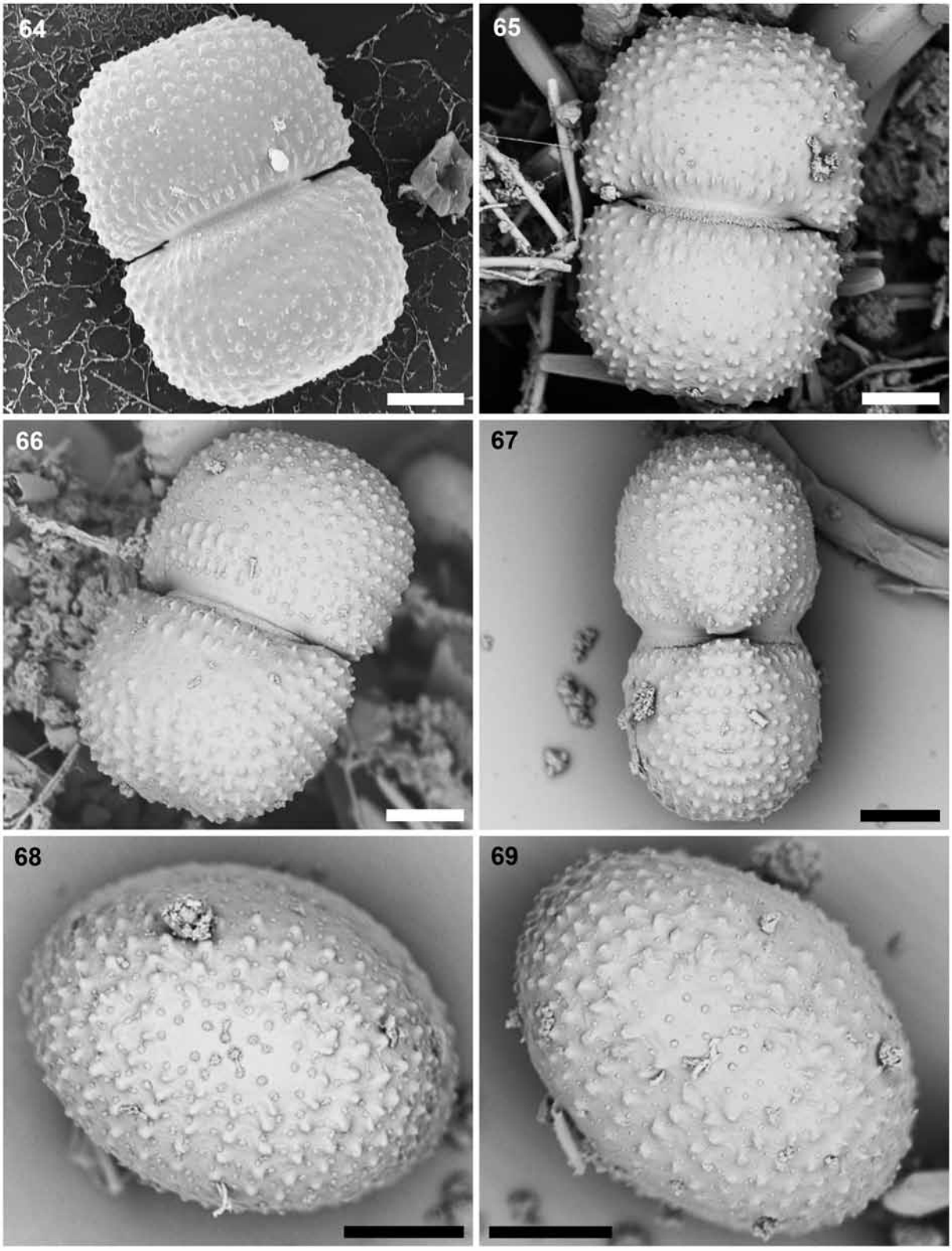
Figs 46–51. (46–49) *Cosmarium cinctutum*, (47) detail of the apical part of a cell, (48–49) lateral view; (50–51) *C. punctulatum* var. *subpunctulatum*. Scale bar 10 µm.



Figs 52–57. (52–57) *Cosmarium discrepans*. Scale bar 10 μm.



Figs 58–63. (58–61) *Cosmarium hostensiense*, (60) detail of the apical part of a semicell, (61) detail of cell wall sculpture; (62–63) *C. lenzenwegeri*. Scale bar 10 μm, 5 μm (Fig. 61).



Figs 64–69. (64–69) *Cosmarium lenzenwegeri*, (67) lateral view, (68–69) apical views. Scale bar 10 µm.

under *C. variolatum* var. *extensum* Nordstedt (Skuja *l.c.*, Pl. 9, Figs. 6–7). Last mentioned variety had previously been transferred to *C. pseudopyramidatum* P. Lundell by Krieger & Gerloff (1965, p. 127), apparently on the basis of similarities in general cell wall sculpture and morphology (compare Nordstedt 1887, p. 161, 1888, p. 55, pl. 6:3a). However, Skuja's form obviously is not related to Nordstedt's var. *extensum*, inducing Croasdale to retain it in *C. variolatum*, giving it the new varietal name *skujae*. By doing so, she in fact described it as a new taxon, unfortunately without providing a Latin diagnosis and indicating a type, so that her description is invalid according to ICBN Articles 36.2 and 37.1 (McNeill et al. 2006).

The present material provided the opportunity to study this taxon with SEM, in order to reveal its cell wall sculpture and clarify its systematic position. Contrary to *C. cataractarum*, the cell wall of the present form has a pattern very similar to the nominate variety of *C. variolatum*, consisting of rather coarse scrobiculae with a central pore (see Fig. 61). However, in our opinion the general cell morphology – including dimensions – of the present form is too much different from that of *C. variolatum* to merely consider it a variety of the latter (compare also Kouwets 2008). Moreover, as with *C. cataractarum* there is a striking difference in the ecology between both taxa and, therefore, it is proposed to classify it as a separate species. Since a *C. skujae* has already been described by Krieger & Gerloff (1965), a new name must be chosen, and we propose the name *Cosmarium hostensiense*.

Ecology and distribution: *Cosmarium hostensiense* obviously prefers acidic, more or less oligotrophic, often periodically desiccating water bodies. For instance in the west of Ireland it is one of the most common and often dominant desmids in this type of habitats (Šťastný, personal observations; see also John & Williamson 2009).

***Cosmarium lenzenwegeri* Šťastný et Kouwets nom. nov. et stat. nov. (Figs 20–21, 62–69)**

Synonym: *Cosmarium subbroomei* Schmidle var. *taylorii* Croasdale (1956, pl. 16, figs 12–13)

Etymology: the new name of the present form is given in honour of Prof. Rupert Lenzenweger who conducted extensive investigations into desmids of higher altitudes, particularly in the Austrian Alps.

Morphology and taxonomy: the cells are distinctly longer than broad, in frontal view rather oblong–rectangular with a relatively shallow, linear, closed sinus and broadly rounded apical angles (Figs 20–21, 62–65). The semicells are somewhat subrectangular with rounded basal angles and parallel to slightly convergent, straight to slightly concave sides and a straight apex. In apical view they are elliptical; in lateral view subelliptic. The cell wall ornamentation is composed of radiating series of small granules running across the sides and by 2–3 transversal rows of elongate granules just above the isthmus (Figs 20–21, 62–66). The cells are 45–52.5 µm long, 33–37.5 µm wide and 24–26 µm thick. The isthmus is 16.5–20 µm wide. The length/width ratio is approximately 1.3–1.4.

The present, conspicuous, but unfamiliar *Cosmarium* form has been found in great abundance in an ephemeral pool in eastern Slovakia (site 16). Scrutinizing the literature for figures of this form only the report of *Cosmarium subbroomei* Schmidle var. *taylorii* ["*Taylorii*"], described by Croasdale (1956) from Alaska, came into consideration for the identification. This variety is morphologically almost identical with our material, differing basically only in its slightly greater dimensions, measuring 57–58 × 47–48 µm. Moreover, although Croasdale (*l.c.*) does not mention any explicit details about the habitat her variety was found ("On stones, branches, etc., in brooks and rivers"), some of the accompanying species (e.g. *Cosmarium holmiense* var. *integrum*, *C. pokornyanum*, *C. speciosum* var. *simplex*) point to a (hemi–)atmophytic habitat type, similar to our sampling site. A superficially related form seems to be *C. diplodesmium*, described from northern Sweden by Skuja (1964, p. 214, pl. 36, fig. 20), but this form is slightly smaller and more coarsely granulated.

However, in our opinion Croasdale's (*l.c.*) form has no relationship with *C. subbroomei* Schmidle. Moreover, *C. subbroomei* is generally considered a very dubious taxon. When examining Schmidle's original figures of this species (Schmidle 1893, pl. 5, figs 22–24), it seems obvious that he included two markedly different taxa, one represented by Fig. 22, the other by Figs. 23–24. In their "British Desmidiaceae", W. & G.S. West (1912, pl. 100, fig. 10) only gave a copy of Schmidle's fig. 22, at the same time stating that they had never seen a form exactly like it (W. & G.S. West *l.c.*, p. 24). Therefore, to

preclude any taxonomic confusion, we propose to raise *C. subbroomei* var. *taylorii* to the rank of a separate species. Since a *Cosmarium taylorii* has already been described by Carter [1935, as "Taylori", synonym *Actinotaenium taylorii* (Carter) Teiling], a new name has to be choosen as to avoid the creation of a later homonym.

Ecology and distribution: *Cosmarium lenzenwegeri* obviously is a very rare (hemi–) atmophytic species, most probably with an arctic–alpine distribution.

Conclusions

The present study confirms the usefulness of SEM revealed morphological cell wall characteristics for the description and delimitation of desmid species. Although molecular research suggests a much more complicated taxonomy (e.g. Gontcharov & Melkonian 2008, 2011), cell morphology still is an important character for routine identification and SEM apparently is a welcome additional tool. This has also been concluded by other authors (e.g. Coesel 1984; Gontcharov et al. 2002; Neustupa et al. 2010) and its full potential obviously hasn't been realized yet. This especially concerns smaller forms with few morphological characteristics where it may be used for the delimitation of (pseudo)cryptic species. As already stressed by Coesel (1984) differences in cell wall sculpturing should be of equal importance in the identification of taxa as, e.g., differences in form and arrangement of cell wall ornamentation such as granules or spines. The significance of cell wall sculpture for the taxonomy of desmids should be further investigated.

The present results also draw attention to the actual biodiversity question, trying to assess the overall global number of desmid taxa. On one hand they illustrate the often desultory description of infraspecific desmid taxa by many authors (*Cosmarium variolatum* var. *cataractarum*, *C. subbroomei* var. *taylorii*; compare Kouwets 2008), paying no attention to conspicuous distinctive morphological characters. On the other hand they show that in cases where a description of a new species would be fully justified (*Closterium pseudocostatum*, *Cosmarium discrepans*) such distinctive characters are frequently overlooked or simply ignored and obviously considered to be part of the variability of the species in question.

It is clear that the cases discussed in the present paper only represent the "tip of the iceberg" and that many of the infraspecific desmid taxa described so far should be better considered separate species. This trend has been convincingly demonstrated in several recent papers (e.g. Neustupa et al. 2010, 2011; Nemjová et al. 2011). In our opinion, it can be assumed that the estimation of the overall global desmid species number by Hoshaw et al. (1990, 15000 spp.) is much more realistic than for instance that by Cranwell et al. (1990, 1500 spp.) and probably much higher than the recent number of desmid taxa described (ca. 4000; Gerrath 1993).

Finally, the present results also demonstrate that ephemeral pools, where several taxa discussed above have been found, certainly still include a number of little known or undescribed desmid species and should not be ignored in investigations of desmid diversity.

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Paper IV

Polyphasic evaluation of *Xanthidium antilopaeum* and *Xanthidium cristatum* (Zygnematophyceae, Streptophyta) species complex

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POLYPHASIC EVALUATION OF *XANTHIDIUM ANTILOPAEUM* AND *XANTHIDIUM CRISTATUM* (ZYGNEMATOPHYCEAE, STREPTOPHYTA) SPECIES COMPLEX[1]

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We investigated twenty-six strains of *Xanthidium antilopaeum* Kütz. and seven strains of *X. cristatum* Ralfs isolated from various European localities or obtained from public culture collections. A combination of molecular, geometric morphometric, and morphological data were used to reveal the patterns of the phylogenetic and morphological differentiation of these taxonomically very complicated desmid taxa. The molecular data based on *trn*G^{ucc} and ITS rDNA sequences illustrated the monophyly of both the complexes, which indicated that their traditional morphology-based discriminative criteria, such as the different number of spines, may generally continue to be considered relevant. The single exception was *X. antilopaeum* var. *basiornatum* B. Eichler et Raciborski, which was positioned outside the *X. antilopaeum/cristatum* clade. The independent status of this taxon was also confirmed on the basis of the geometric morphometric data, so that we concluded that it probably represents a separate species. Within *X. cristatum* complex, the traditional varieties *X. cristatum* var. *cristatum* Ralfs, *X. cristatum* var. *uncinatum* Ralfs, and *X. cristatum* var. *scrobiculatum* Scott et Grönblad turned out to be separate taxa. Conversely, *X. cristatum* var. *bituberculatum* Lowe lacked any taxonomical value. Our data on *X. antilopaeum* illustrated extensive phylogenetic as well as phenotypic variability within this species complex. However, our data did not result in any unambiguous pattern that would allow sound taxonomic classification. Finally, we also found out that the morphologically peculiar *Staurastrum tumidum* Ralfs belongs to the genus *Xanthidium* based on the combined *rbc*L + *cox* III data set. Consequently, this species was formally transferred to this genus.

Key index words: **Desmidiales; geometric morphometrics; molecular phylogeny; taxonomy; *Xanthidium***

Desmids belong to the Zygnematophyceae, the largest and the most diverse group of streptophyte green algae (Gontcharov and Melkonian 2005). They are known for their high morphological variation that led to the description of over 4,000 species (Gontcharov et al. 2003, Gontcharov 2008). As inhabitants of freshwater wetlands, desmids belong to the most important phytobenthic groups in these habitats, in terms of both species richness and biomass (Coesel and Meesters 2007). Therefore, they have been frequently used in various kinds of ecological and biomonitoring studies (Coesel 2001, 2003, Pals et al. 2006, Krasznai et al. 2008, Neustupa et al. 2009, 2011a). Their suitability and reliability for these purposes essentially requires reliable species concepts. However, the traditional desmid taxonomy, based entirely on morphological features, has recently been challenged by molecular data. Molecular research has increasingly, and sometimes controversially, been turning desmid taxonomy upside down, especially at the generic level (McCourt et al. 2000, Gontcharov et al. 2003, Gontcharov and Melkonian 2005, 2008, 2010, 2011, Gontcharov 2008, Hall et al. 2008, Škaloud et al. 2011). Most of the traditional species-rich genera were found to be polyphyletic or paraphyletic (Gontcharov and Melkonian 2005, 2008, Gontcharov 2008). However, while we now know that most of the traditional generic concepts have been inadequate, little information is available on the validity of traditional desmidiacean species concepts. In many desmids, the extensive morphological variation within the range of traditionally defined species led to the descriptions of numerous infraspecific taxa, whose taxonomic validity remained largely unresolved (Kouwets 2008). Several studies based on crossbreeding experiments (Blackburn and Tyler 1987, Denboh et al. 2003) indicated that concepts of at least some desmid species have been too broad, implicating some degree of pseudocryptic or cryptic species diversity. This opinion has been further supported by several recent studies that used a combination of molecular, morphological, and

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geometric morphometric methods that revealed surprising microevolutionary differentiation within several desmid species. The studies on *Micrasterias crux-melitensis/M. radians* (Neustupa et al. 2010) and *M. truncata* complexes (Nemjová et al. 2011) illustrated pseudocryptic diversity within these traditional desmid taxa. Several morphologically defined infraspecific taxa were shown to be artificial and probably lacking any taxonomic value, but some of the other varieties apparently represented independent species. Another study that focused on the *M. rotata/M. fimbriata* species complex (Neustupa et al. 2011b) revealed phylogenetic homogeneity of traditional *M. rotata* but it also illustrated that European populations of *M. fimbriata* actually belong to two species lineages. It has to be noted that in the above-mentioned studies, individual species-level phylogenetic lineages were always found to be morphologically perfectly identifiable, both by careful microscopic analysis, as well as by geometric morphometrics. However, these studies comprised only the representatives of the morphologically most conspicuous genus *Micrasterias*, while there are still no phylogenetic data on the validity of traditional species concepts in other desmidiacean genera, often disposing much less morphological discriminative characters than the above-mentioned genus.

The genus *Xanthidium* Ralfs is a widely distributed, well-known desmid genus with more than 110 morphologically delimited species (Gontcharov and Melkonian 2011). It was characterized by biradiate cells with multiform semicells, provided with a series of spines usually confined to the semicell angles (Coesel and Meesters 2007). The type species of the genus *Xanthidium* has not yet been designated (Guiry and Guiry 2012). The genus as a whole was found to be polyphyletic on the basis of molecular phylogenetic analysis (Gontcharov and Melkonian 2011). However, several of the traditional "core" *Xanthidium* species, such as *Xanthidium antilopaeum*, *X. cristatum*, *X. subhastiferum*, and *X. brebissonii*, were recovered in a single well-supported phylogenetic lineage within Desmidiaceae (Gontcharov and Melkonian 2011). Conversely, *X. octocorne* Ralfs was found to be part of a strongly supported *Staurodesmus* lineage (Gontcharov and Melkonian 2011). Conversely, Gontcharov et al. (2003) and Gontcharov and Melkonian (2011) illustrated a possible sister relationship of *Staurastrum tumidum* Ralfs to the "core" *Xanthidium* lineage.

In the present study, we evaluated the phylogenetic and morphological patterns in the well-known traditional species *X. antilopaeum* Kütz. and *X. cristatum* Ralfs. These species belong to one of the most frequently occurring representatives of the genus, and *X. antilopaeum* has even been considered to be one of the most frequently occurring desmids in the temperate European peatlands (Lenzenweger 1997). According to the traditional morphological criteria, *X. cristatum* can be distinguished from *X. antilopaeum* by the different number of cellular spines, possessing a total of 10 instead of eight spines per semicell that are typically present in *X. antilopaeum* (West and West 1912). Both taxa, however, exhibit notable morphological variation that led to the taxonomic description of numerous infraspecific taxa, differing in the arrangement of the marginal spines and the central pattern of scrobiculae (Coesel and Meesters 2007). In total, 93 infraspecific taxa have been described in *X. antilopaeum* and 42 in *X. cristatum* (Guiry and Guiry 2012). Many of these traditional subspecific taxa may have no phylogenetic value, and *X. antilopaeum* and *X. cristatum* have therefore been considered to belong to the taxonomically most problematic desmids (Prescott et al. 1982). Coesel (2005) suggested that some of the *X. antilopaeum* varieties are so different from the nominate variety that they might be considered species of their own. Therefore, we focused on infraspecific variation within these two morphologically very diverse species. In total, we examined 26 strains of *X. antilopaeum* and 7 strains of *X. cristatum*. The strains were isolated from natural samples obtained from across Europe, or they had been obtained from public culture collections. Morphology of the strains was ascertained, and, if possible, they were identified to the variety level. In addition, morphological diversity of strains was assessed by geometric morphometrics and by performing scanning electron microscopy (SEM). Geometric morphometrics is a quantitative method for the evaluation of biological shapes that has recently been used for the morphological variation analyses in various groups of microalgae (e.g., Beszteri et al. 2005, Potapova and Hamilton 2007, Veselá et al. 2009), including desmids (Neustupa and Šťastný 2006, Neustupa et al. 2010, 2011b, Nemjová et al. 2011). SEM has also been considered to be a key additional tool for the examination of the richly sculptured cell wall of desmids (Coesel 1984, Gontcharov et al. 2002). The gene sequence data of four molecular markers (ITS rDNA, *cox*III, *rbc*L, and *trn*G^{ucc}) were analyzed to evaluate the phylogenetic position of strains and to test for the monophyly and phylogenetic differentiation of the two investigated traditional species.

In summary, we primarily asked whether *X. antilopaeum* and *X. cristatum* consist of phylogenetically homogenous entities, i.e., whether the traditional morphology-based discriminative characters (the different number of spines per semicell) may still be considered to be relevant. Moreover, we aimed to resolve the inner phylogenetical structure within both complexes, and we attempted to identify morphological peculiarities of the newly recognized species-level lineages. Finally, we intended to corroborate the unusual phylogenetic position of *S. tumidum* by sequencing our own isolate of this species.

MATERIALS AND METHODS

Isolation and cultivation of strains and LM and SEM observations. For this study, we isolated 21 strains of *X. antilopaeum* and *X. cristatum* and 1 strain of *S. tumidum* from various European wetlands (Table 1). In addition, 12 strains of *X. antilopaeum* and *X. cristatum* were obtained from public culture collections such as Sammlung von Conjugaten-Kulturen, University Hamburg (SVCK) and Culture Collection of Algae, University of Vienna (ASW), nowadays deposited in the Culture Collection of Algae at the University of Cologne (CCAC; Table 1). The clonal strains were isolated by single-cell

TABLE 1. Origin and description of strains.

Strain no.	Taxon name	Origin	Geographic coordinates
H 21	*Xanthidium antilopaeum* var. *antilopaeum*	Grand Étang de Biscarosse, Aquitaine, France	44°23′13.60″N, 1°11′18.90″W
H 23	*Xanthidium antilopaeum* var. *antilopaeum*	Huťský pond, Czech Republic	48°39′20.99″ N, 14°40′56.36″ E
H 25	*Xanthidium antilopaeum* var. *antilopaeum*	Étang de Cazaux, Aquitaine, France	44°30′39.20″N, 1°11′25.15″W
H 27	*Xanthidium antilopaeum* var. *antilopaeum*	Borkovická blata peat bog, Czech Republic	49°14′9.36″ N, 14°37′24.84″ E
H 29	*Xanthidium antilopaeum* var. *antilopaeum*	Pískovny Cep I sandpits, Czech Republic	48°55′6.119″N, 14°53′3.058″E
H 31	*Xanthidium antilopaeum* var. *antilopaeum*	Pískovny Cep I sandpits, Czech Republic	48°55′6.119″N, 14°53′3.058″E
H 32	*Xanthidium antilopaeum* var. *antilopaeum*	Bastemose, Bornholm, Denmark	55°07′37.63″N, 14°56′42.15″E
SVCK 28	*Xanthidium antilopaeum* var. *antilopaeum*	Fischteiche-Wolkersdorf near Frankenberg, Germany	—
SVCK 281	*Xanthidium antilopaeum* sensu lato	Burnham's Swamp near Falmouth, USA	—
SVCK 343	*Xanthidium antilopaeum* sensu lato	Pitztal, Austria	—
SVCK 105	*Xanthidium antilopaeum* sensu lato	Fokstumyr northeast from Dombas, Norway	62°07′18.49″N, 9°15′32.27″E
ASW 07105	*Xanthidium antilopaeum* sensu lato	Reservoir for drinking water, Jakobshaven, Greenland	—
ASW 07106	*Xanthidium antilopaeum* sensu lato	Reservoir for drinking water, Jakobshaven, Greenland	—
ASW 07107	*Xanthidium antilopaeum* sensu lato	Pool in the tundra, St. Matthew Island, Bering Sea, USA	—
H 26	*Xanthidium antilopaeum* sensu lato	A peaty pool near Maumwee Lough, Ireland	53°28′27.30″N, 9°32′38.52″W
H 17	*Xanthidium antilopaeum* var. *basiornatum*	Schwemm near Walchsee peat bog, Austria	47°39′34.52″N, 12°17′50.51″E
H 22	*Xanthidium antilopaeum* var. *depauperatum*	Eirk Lough, Kerry, Ireland	51°56′25.36″N, 9°37′37.85″W
H 20	*Xanthidium antilopaeum* var. *laeve*	A peaty pool near Maumwee Lough, Ireland	53°28′27.30″N, 9°32′38.52″W
SVCK 147	*Xanthidium antilopaeum* var. *minneapoliense*	A pool northeast from Raleigh, USA	—
H 30	*Xanthidium antilopaeum* var. *polymazum*	An unnamed lake, Connemara, Ireland	53°34′10.60″N, 9°48′29.57″W
H 10	*Xanthidium antilopaeum* var. *incrassatum*	Břehyně wetland, Czech Republic	50°34′59.72″N, 14°42′17.76″E
H 19	*Xanthidium antilopaeum* var. *incrassatum*	Schwemm near Walchsee peat bog, Austria	47°39′34.52″N, 12°17′50.51″E
H 15	*Xanthidium antilopaeum* var. *planum*	Marienteich, Czech Republic	50°32′43.53″N, 14°40′39.44″E
H 18	*Xanthidium antilopaeum* var. *planum*	Schwemm near Walchsee peat bog, Austria	47°39′34.52″N, 12°17′50.51″E
H 28	*Xanthidium antilopaeum* var. *planum*	An unnamed lake near Upper Lake, Kerry, Ireland	51°57′57.66″N 9°35′49.24″W
SVCK 74	*Xanthidium antilopaeum* var. *planum*	Peaty pool south from Koli, Finland	—
H 59	*Xanthidium cristatum* var. *cristatum*	Mariánský pond, Czech Republic	50°32′43.53″N, 14°40′39.44″E
ASW 07060	*Xanthidium cristatum* var. *cristatum*	An unnamed bog, Finland	—
ASW 07101	*Xanthidium cristatum* var. *cristatum*	A peat bog near Tamsweg, Salzburg, Austria	—
H 08	*Xanthidium cristatum* var. *scrobiculatum*	The Long Range, Kerry, Ireland	51°59′51.04″N, 9°33′3.81″W
H 11	*Xanthidium cristatum* var. *scrobiculatum*	An unnamed lake, Connemara, Ireland	53°34′10.60″N, 9°48′29.57″W
ASW 07102	*Xanthidium cristatum* var. *scrobiculatum*	Skarsvag, Norway	—
H 12	*Xanthidium cristatum* var. *uncinatum*	Břehyně wetland, Czech Republic	50°35′4.27″N, 14°42′24.95″E
H 76	*Staurastrum tumidum*	Dvořiště peat bog, Czech Republic	49°4′14.09″N, 14°38′59.31″E

pipetting. To achieve relatively rapid growth of the strains, they were cultured in the MES-buffered DY IV liquid medium at 24°C and continuously illuminated at 5–15 μmol photons $\cdot m^{-2} \cdot s^{-1}$ from 18 W cool fluorescent tubes (Philips TLD 18W/33, Royal Philips Electronics, Amsterdam, Netherlands). Microphotographs of the cells were obtained using an Olympus BX51 (Olympus, Tokyo, Japan) light microscope with an Olympus Z5060 digital camera. For SEM, the acetone-washed glass coverslips were heated, and coated three times with a poly-L-lysine solution (1:10 in deionized water) to ensure appropriate cell adhesion. A drop of the formaldehyde-fixed cell suspension was transferred into 30% acetone, and dehydrated by using an acetone series. Subsequently, the cells were dried to a critical point by using liquid CO_2. Finally, they were sputter coated with gold (Bal-Tec Sputter Coater SCD 050, Capovani Brothers Inc., Sconia, NY, USA) and examined using a JEOL 6380 LV (JEOL Ltd., Tokyo, Japan) scanning electron microscope.

DNA isolation, amplification, and sequencing. After centrifugation of desmid cells in 2 mL tubes, 100–200 mL of the InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA) was added to the pellet. Then, the cells were mechanically disrupted by shaking for 5 min with glass beads (3 mm in diameter; Sigma-Aldrich, St. Louis, MO, USA) in Mixer Mill MM 400 (RETSCH GmbH, Haan, Germany). Subsequently, the solution was incubated at 56°C for 30 min, vortexed for 10 s, and heated at 99°C for 8 min. After another vortexing step, the tubes were centrifuged at 16,000 rcf for 2 min. Finally, the supernatant (DNA) was diluted to a concentration of 10 ng $\cdot \mu L^{-1}$ for use in polymerase chain reaction (PCR) amplification. Four molecular markers were amplified by PCR: chloroplast *rbc*L and *trn*G^{ucc}, mitochondrial *cox*III, and nuclear ITS rDNA. The PCR reaction in total volume of 20 μL contained 13.1 μL of sterile Mili-Q water, 2 μL of AmpliTaq Gold® 360 Buffer 10 × (Applied Biosystems, Life technologies, Carlsbad, CA, USA), 2.2 μL of $MgCl_2$ (25 mM), 0.4 μL of dNTP mix (10 mM), 0.25 μL of each primer (25 nM), 0.6 μL of 360 GC enhancer, 0.2 μL of AmpliTaq Gold® 360 DNA Polymerase, and 1 μL of DNA (10 ng $\cdot \mu L^{-1}$). The PCR amplification was performed in either a Touchgene Gradient Thermal Cycler (Krackeler Scientific, Albany, NY, USA) or an XP thermal cycler (Bioer, Tokyo, Japan). Amplification primers and cycling conditions are listed in Table 2 and Table 3. The PCR products were stained using bromophenol blue loading dye, quantified on 1% agarose gel, stained with ethidium bromide, and cleaned with the JETQUICK PCR Purification Kit (Genomed, Löhne, Germany), according to the manufacturer's protocol. The purified amplification products were sequenced using an Applied Biosystems (Seoul, Korea) automated sequencer (ABI 3730 × l) at Macrogen Corp. in Seoul, Korea. The sequencing reads were assembled and edited using the SeqAssem programme (Hepperle 2004).

Sequence alignment. Three different alignments were constructed for the phylogenetic analyses: (i) a concatenated *rbc*L + *cox*III alignment of 101 desmid sequences selected to encompass all the main phylogenetic lineages of the family, including *Xanthidium* sequences, determined in this study; (ii) a *trn*G^{ucc} alignment of 32 newly determined *Xanthidium* sequences; and (iii) an ITS rDNA alignment comprising 27 new *Xanthidium* sequences. The list of all the sequences involved in phylogenetic analyses, including the GenBank accession numbers, is given in Table S1 in the Supporting Inforamtion. The final *rbc*L + *cox*III alignment was generated as follows: First, we downloaded 45 core *rbc*L + *cox*III sequences from the GenBank database that encompassed most of the Desmidiales lineages according to Hall et al. (2008). Then, we added 35 *rbc*L sequences selected according to the desmid phylogeny recently published by Gontcharov and Melkonian (2011) to cover the remaining Desmidiales lineages. Finally, 5 *rbc*L and 14 *cox*III sequences determined in this study together with six closely related sequences revealed by BLAST searches were added to the alignment and manually aligned in MEGA 4. The concatenated 1,870-bp matrix contained 101 taxa and was 88% filled for the *rbc*L data and 59% filled for the *cox*III data. The *trn*G^{ucc} and ITS rDNA sequences were manually aligned in MEGA 4 (Kumar et al. 2008). ITS sequences were aligned with the help of their rRNA secondary structure information, constructed using the mfold computer program, ver. 2.3 (Zuker 2003). All alignments are available in Table S1.

Table 2. List of primers used for PCR amplification and sequencing.

Primer name	Primer sequence (5′–3′)	Reference
*rbc*L		
rbcL-Pleurot-F	GGT TAA AGA TTA TAG ACT TAC	Škaloud et al. (2012)
rbcL-Pleurot-R	CCT TGA CGA GCA AGA TCA CG	Škaloud et al. (2012)
*trn*G^{ucc}		
trnG-F	AGC GGG TAT AGT TTA GTG GT	Neustupa et al. (2010)
trnG-R	GGT AGC GGG AAT CGA ACC CGC	Neustupa et al. (2010)
*cox*III		
COX-ZYG-F3	TTA CTG GAG GTG GCA CAC TT	Škaloud et al. (2011)
COX-ZYG-R2	TCC ATG AAA TCC AGT AGC TAA G	Škaloud et al. (2011)
ITS rDNA		
ITS1	TCC GTA GGT GAA CCT GCG G	White et al. (1990)
ITS4	TCC TCC GCT TAT TGA TAT GC	White et al. (1990)
ITS2N	TCG CTG CGT TCT TCA TC	Beck et al. (1998)
ITS3N	GAT GAA GAA CGC AGC GA	Beck et al. (1998)

Model selection and phylogenetic analyses. A suitable partitioning strategy and partition-specific substitution models for the *rbc*L + *cox*III data set were selected in a multi-step process (Verbruggen et al. 2010, Škaloud et al. 2012). The BIC-based model selection procedure selected the following partitioning strategy: (i) First and second codon position of *rbc*L (GTR + Γ); (ii) third codon position of *rbc*L (GTR + Γ); (iii) second codon position of *cox*III (HKY + Γ); and (iv) third codon position of *cox*III (GTR + Γ). The most appropriate substitution models for the *trn*G^{ucc} and ITS rDNA data sets were estimated using the Akaike information criterion (AIC) with PAUP/MrModeltest 1.0b (Nylander 2004). The phylogenetic trees were inferred with Bayesian inference (BI) using MrBayes, version 3.1 (Ronquist and Huelsenbeck 2003). The analysis of the *rbc*L + *cox*III data set was carried out on a partitioned data set using a covarion-like substitution model and the strategy selected during the multi-step process described above. The calculation was performed on the 86 unique sequences. The analysis of the *trn*G^{ucc} data set was performed on the 14 unique sequences by using a GTR + I substitution model. The analysis of the ITS rDNA region was carried out on a partitioned data set by using a SYM + Γ model for ITS1, a GTR + Γ model for ITS2, and a JC model for 5.8 rRNA partition. The calculation was performed on 19 unique sequences. All the parameters were unlinked among partitions. In the BI analyses, two parallel Markov chain Monte Carlo (MCMC) runs were carried out for 3 million generations,

TABLE 3. PCR cycling conditions.

Region	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Final extension
*rbc*L	94°C (4 min)	94°C (1 min)	54°C (1 min)	72°C (2.0 min)	35×	72°C (10 min)
*trn*G^{ucc}	94°C (2 min)	94°C (1 min)	62°C (1 min)	72°C (1.5 min)	40×	72°C (10 min)
*cox*III	95°C (2 min)	94°C (1 min)	50°C (1 min)	72°C (1.5 min)	37×	72°C (10 min)
ITS rDNA	94°C (5 min)	94°C (1 min)	50°C (1 min)	72°C (1.5 min)	35×	72°C (10 min)

each with one cold and three heated chains. Trees and parameters were sampled for every 100 generations. Convergence of the two cold chains was checked and "burn-in" was determined using the "sump" command. Bootstrap analyses were performed by maximum likelihood (ML) and weighted parsimony (wMP) criteria using GARLI, version 0.951 (Zwickl 2009) and PAUP*, version 4.0b10 (Swofford 2002), respectively. ML analyses consisted of rapid heuristic searches (100 pseudo-replicates) using automatic termination (genthreshfortopoterm command set to 100,000). The wMP bootstrapping (1,000 replications) was performed using heuristic searches with 100 random sequence addition replicates, Tree bisection reconnection swapping, random addition of sequences (the number limited to 10,000 for each replicate), and gap characters treated as a fifth character state. The weight to the characters was assigned using the rescaled consistency index on a scale of 0–1,000. New weights were based on the mean of the fit values for each character over all of the trees in memory.

Morphometric analyses. For each strain, 20–25 adult semicells were randomly chosen for geometric morphometric investigation. In total, 10 fixed landmarks were depicted in different positions on the semicells, including the tips of the spines. In addition, 12 sliding landmarks, depicting outline curves along the semicell outlines were also digitalized (Fig. 1). For the details of the landmark digitalization on desmid semicells and description of different landmark types, see Neustupa et al. (2010) and Nemjová et al. (2011). The TPS-series software (available at http://life.bio.sunysb.edu/morph/) was used for most of the morphometric analyses. The generalized Procrustes analysis (GPA) standardized the size and optimized their rotation and translation so that the distances between the corresponding landmarks of the investigated objects were minimized (Zelditch et al. 2004). Correlation between Procrustes and the Kendall tangent space distances was assessed 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 hence, we proceeded with further statistical analyses. The landmark configurations of the semicells were symmetrized according to Klingenberg et al. (2002). The principal component analysis (PCA) of geometric morphometric data was conducted on the entire set of 805 semicells acquired from 33 *Xanthidium* strains. Scores of the objects on the non-zero principal component (PC) axes were used for the canonical variates analysis (CVA) aimed at separation of individual phylogenetic lineages. The significance of this analysis was assessed by the Wilk's λ in PAST, ver. 2.13 (Hammer et al. 2001). The leave-out cross-validation tests were also conducted to test for the group assignment of individual semicells on the basis of geometric morphometric data. Similarly, PCA was separately conducted on the set of seven *X. cristatum* strains and shape separation of individual semicells into three groups defined by molecular data was tested by the canonical discrimination analysis. The characteristic cell shapes of individual groups were reconstructed in TpsSuper, ver. 1.14. The Procrustes distances in shape of individual semicells were plotted against the Kimura 2-parameter genetic distances acquired from the molecular data. The genetic distances among strains evaluated by the ITS1, ITS2, ITS1 + 2, and *trnG*^{ucc} markers were used in four separate linear regression analyses. The F-tests for the equality of regression slopes between pairs of individual analyses were used to test for the differences in correlation of shape characteristics of semicells and genetic distances evaluated by different molecular markers.

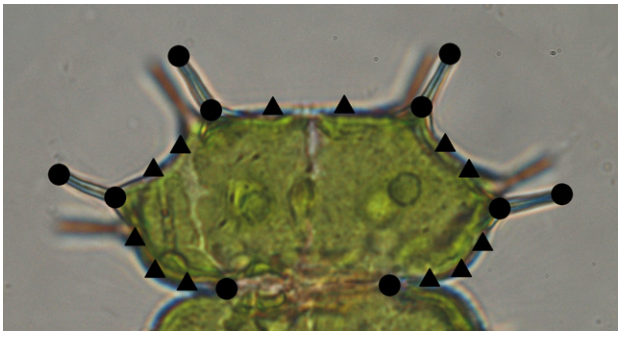

FIG. 1. Position of landmarks (circles) and semi-landmarks (triangles) on a semicell of *Xanthidium antilopaeum*.

RESULTS

Morphology of strains. The cells of all the investigated *Xanthidium* strains were readily identifiable as either *X. antilopaeum* or *X. cristatum*. Within *X. cristatum*, the cells of all strains had 10 spines per semicell, divided into four pairs and two singles (Fig. 2, A–C). However, within the traditional species boundaries, there still were remarkable differences in the morphology of individual strains (Figs. 2–5). Most of these differences corresponded to taxonomic definitions of traditional subspecific taxa, such as individual varieties. The strains representing *X. cristatum* var. *cristatum* Ralfs (H 59, ASW 07060, and ASW 07101) had relatively small cells (~40 × 35 μm without spines) and either one or two tubercles in the semicell center (Fig. 4A). In some cells, the tubercles were strongly reduced or even lacking. Conversely, cells of *X. cristatum* var. *uncinatum* Ralfs (H 12) were distinctly larger than the nominate variety (~60 × 55 μm without spines) and they differed by the upwards curved paired spines and the characteristic central ornamentation consisting of a circle of large granules (Fig. 4B). Finally, the characteristic feature of strains representing *X. cristatum* var. *scrobiculatum* Scott et Grönblad (H 08, H11, and ASW 07102) was their central ornamentation that was built of a variable number of shallow pits (scrobiculae; Fig. 4C).

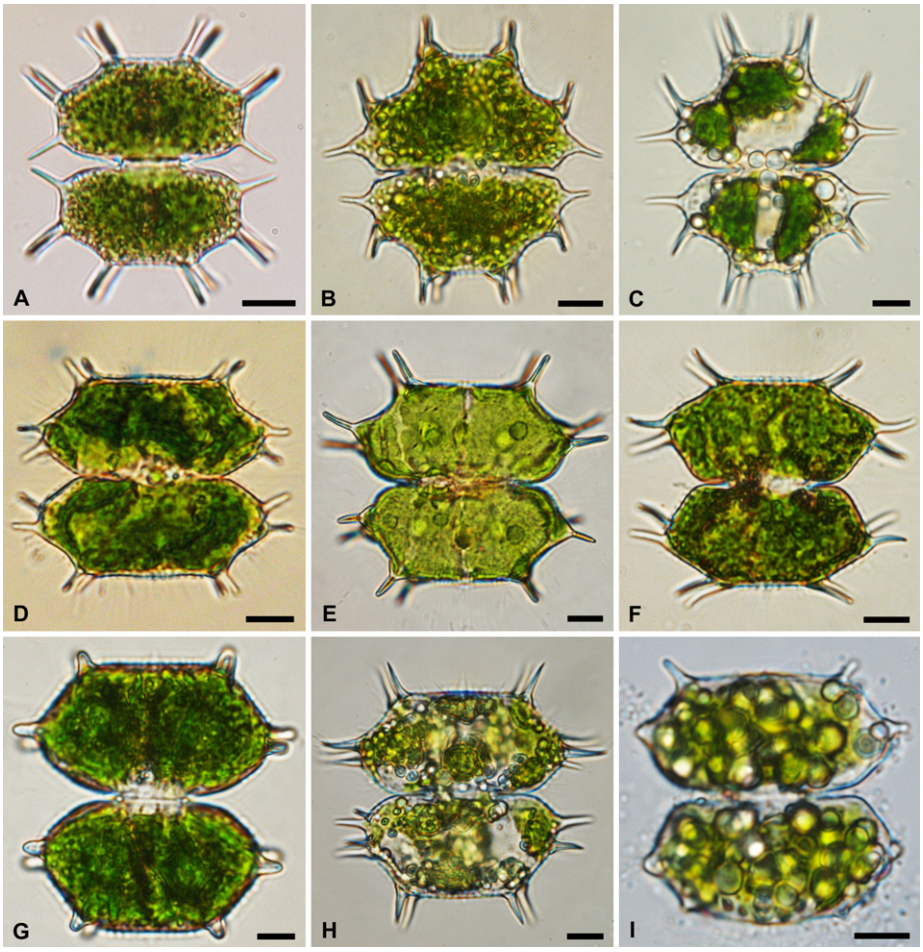

FIG. 2. LM pictures of selected strains. (A) *Xanthidium cristatum* var. *cristatum*, strain H 59. (B) *X. cristatum* var. *uncinatum*, strain H 12. (C) *X. cristatum* var. *scrobiculatum*, strain H 08. (D) *X. antilopaeum* var. *antilopaeum*, strain H 32. (E) *X. antilopaeum* var. *basiornatum*, strain H 17. (F) *X. antilopaeum* var. *planum*, strain H 18. (G) *X. antilopaeum* var. *laeve*, strain H 20. (H) *X. antilopaeum* var. *polymazum*, strain H 30. (I) *X. antilopaeum* var. *minneapoliense*, strain SVCK 147. Scale bar = 10 μm.

Within *X. antilopaeum*, all the strains had eight spines per semicell, but they were mutually different because of the arrangement of the spines, the pattern of the central scrobiculae, and the cell dimensions. Most of the strains (H 21, H 23, H 25, H 27, H 29, H 31, H 32, and SVCK 28) fitted well into the diagnosis of the nominate variety of *X. antilopaeum*. This has been characterized by comparatively wide cells and the central ornamentation consisting of an approximately elliptical group of shallow pits (Fig. 4D). The morphology of strain H 17 perfectly corresponded to that of *X. antilopaeum* var. *basiornatum* B. Eichler et Raciborski. This taxon has been distinguished on the basis of spines with a stout base, by a distinct central protuberance, and particularly by a horizontal series of 12–14 supraisthmial shallow pits located immediately above the isthmus (Fig. 4E). In *X. antilopaeum* var. *planum* Roll (H 15, H 18, H 28, and SVCK 74) both the apical and lateral spines were downwards curved and the central ornamentation was built of a central protuberance surrounded by a circle of shallow pits (Fig. 4F). The single strain identified as *X. antilopaeum* var. *laeve* Schmidle (H 20) was characterized by large and comparatively long cells (~75 × 60 μm without spines) with a relatively open sinus. The cell wall was either smooth or a central group of several shallow pits was present (Fig. 4G). *Xanthidium antilopaeum* var. *polymazum* Nordst. (H 30) was differentiated by conspicuous ornamentation consisting of a transversal, subapical arc of granules (Fig. 4H). *Xanthidium antilopaeum* var. *minneapoliense* Wolle (SVCK 147) was typical by the presence of a subapical arc of granules and two additional spines (one on either face of the cell). However, it should be noted that these spines were often reduced to conspicuous granules (Fig. 4I) or they were even lacking in some cells. Two strains of *X. antilopaeum* var. *incrassatum* (Grönblad) Förster (H 10 and H 19) differed from the nominate variety by rather large cells (~65 × 65 μm without spines) and the cell wall thickening in the central region of the semicells. Other parts of the cell wall were either smooth, or the subapical group of indistinct shallow pits was present

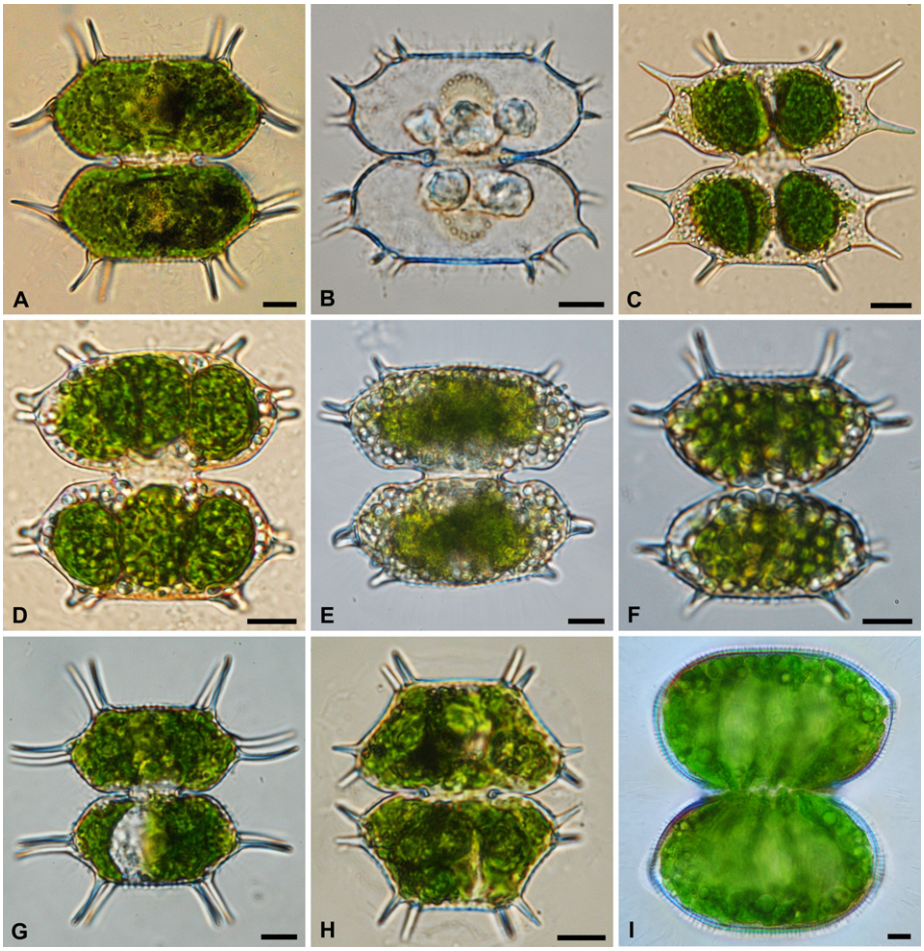

FIG. 3. LM pictures of selected strains. (A) *Xanthidium antilopaeum* var. *incrassatum*, strain H 19. (B) *X. antilopaeum* var. *depauperatum*, strain H 22. (C) *X. antilopaeum* sensu lato, strain ASW 07106. (D) *X. antilopaeum* s.l., strain ASW 07107. (E) *X. antilopaeum* s.l., strain SVCK 105. (F) *X. antilopaeum* s.l., strain SVCK 281. (G) *X. antilopaeum* s.l., strain SVCK 343. (H) *X. antilopaeum* s.l., strain H 26. (I) *Staurastrum tumidum*, strain H 76. Scale bar = 10 μm.

(Fig. 5A). Finally, *X. antilopaeum* var. *depauperatum* W. et G.S.West (strain H22) was characterized by the central ornamentation composed of two concentric rings of distinct pits surrounding a middle granula (Fig. 5B), and by obtuse lateral angles of the semicells. Conversely, another typical feature of this variety, a conspicuous variation in the number, length, and arrangement of spines (West and West 1912) was not observed. The other investigated strains (ASW 07105, ASW 07106, ASW 07107, SVCK 105, SVCK 281, SVCK 343, and H 26) fitted well into morphological species definition of *X. antilopaeum*, but they could not be determined to belong to any of the traditional varieties (Figs. 3 and 5). Morphology of our *S. tumidum* isolate (strain H 76) corresponded with the morphological delimitation of this species (Fig. 3I). LM and SEM pictures of all the strains are available online as supplementary material.

Molecular phylogenetic analyses. The Bayesian phylogenetic tree constructed on the basis of the partitioned *rbcL* + *cox*III data set (Fig. 6) inferred all the investigated *Xanthidium* strains in a single, well-resolved clade (BI/ML/MP support 1.00/98/98). Among the traditionally recognized *Xanthidium* species (*X. antilopaeum*, *X. cristatum*, *X. hastiferum*, *X. subhastiferum*, and *X. brebissonii*), this clade also comprised *S. tumidum*, a species having smooth cells lacking a series of spines that was considered typical for the genus *Xanthidium*. The *rbc*L sequences of our isolate H 76 and the strain SVCK 85 (accession no. AJ553972.1) were identical, corroborating the correct placement of *S. tumidum* within the *Xanthidium* clade. All but one of the *X. antilopaeum* strains were closely related, inferred in a single clade together with *X. cristatum*, *X. hastiferum*, and *X. subhastiferum*. The exception was *X. antilopaeum* var. *basiornatum* that formed a well-resolved clade together with *S. tumidum*. *Xanthidium armatum*, a conspicuous desmid species characteristic by stout, bi- or trifurcate spines, was recognized in a sister position to all the *Xanthidium* taxa. The relation of *X. armatum* to other *Xanthidium* taxa was moderately supported (BI/ML/MP support 0.98/-/77). Our

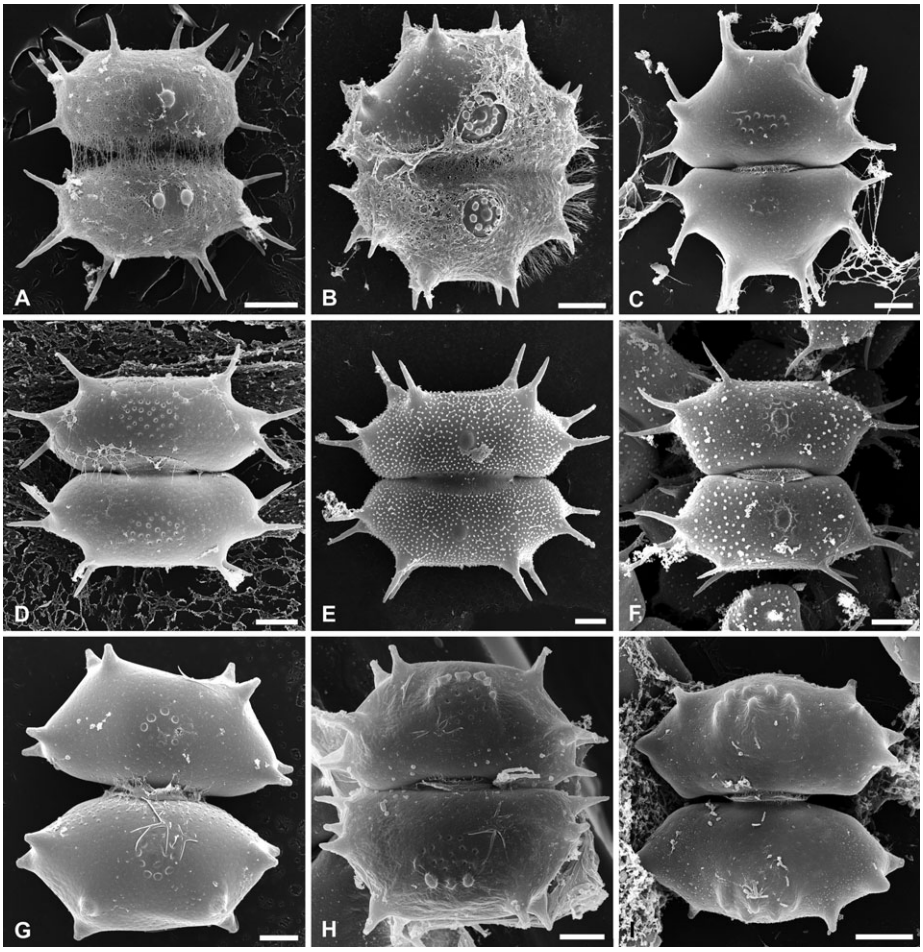

FIG. 4. SEM pictures of selected strains. (A) *Xanthidium cristatum* var. *cristatum*, strain H 59. (B) *X. cristatum* var. *uncinatum*, strain H 12. (C) *X. cristatum* var. *scrobiculatum*, strain H 11. (D) *X. antilopaeum* var. *antilopaeum*, strain H 29. (E) *X. antilopaeum* var. *basiornatum*, strain H 17. (F) *X. antilopaeum* var. *planum*, strain H 28. (G) *X. antilopaeum* var. *laeve*, strain H 20. (H) *X. antilopaeum* var. *polymazum*, strain H 30. (I) *X. antilopaeum* var. *minneapoliense*, strain SVCK 147. Scale bar = 10 μm.

phylogenetic analysis thus recognized the monophyly of almost all the *Xanthidium* species with known molecular data. The single exception was *X. octocorne*, a small desmid with cells characteristically possessing eight long spines. This species was nested within the firmly supported "*Staurodesmus* 1" lineage (*sensu* Gontcharov 2008), distantly related to the *Xanthidium* clade.

To better resolve the phylogenetic relationships of the *X. antilopaeum* and *X. cristatum* strains, we conducted further analyses of two fast-evolving loci, i.e., plastid *trn*G^{ucc} and nuclear ITS rDNA (Fig. 7). The sequencing of *trn*G^{ucc} was successful in all the 32 investigated strains; however, high-quality ITS rDNA sequences were obtained for only 27 of them. Despite repeated DNA isolation and PCR amplification, poor sequencing products were constantly obtained for the strains H18, H22, H27, H28, and SVCK 343, so that these were omitted from the ITS rDNA phylogenetic analysis. A comparison of the phylogenetic trees, estimated separately for the *trn*G^{ucc} and ITS rDNA data sets, resulted in the detection of several reciprocal monophyletic clades that were concordant between the unlinked loci. However, an obvious incongruence was detected in the topology of the uppermost clades in Figure 7 (see below). In general, both single-loci trees were congruent in resolving two major lineages corresponding to *X. antilopaeum* and *X. cristatum*. Within the latter lineage, three well-resolved clades were determined, corresponding to the traditional subspecific taxa *X. cristatum* var. *cristatum*, *X. cristatum* var. *scrobiculatum*, and *X. cristatum* var. *uncinatum*. Within the *X. antilopaeum* complex, four clades were reciprocal monophyletic: (i) the traditional subspecific taxon *X. antilopaeum* var. *planum*, (ii) a clade comprising *X. antilopaeum* var. *incrassatum* and two morphologically distinct strains SVCK 105 and SVCK 343, (iii) a clade containing morphologically about similar taxa *X. antilopaeum* var. *polymazum* and *X. antilopaeum* var. *minneapoliense*, and (iv) a poorly resolved clade of *X. antilopaeum* var. *laeve* and *X. antilopaeum*, strain H26. Three remaining *X. antilopaeum* clades inferred from the *trn*G^{ucc} data

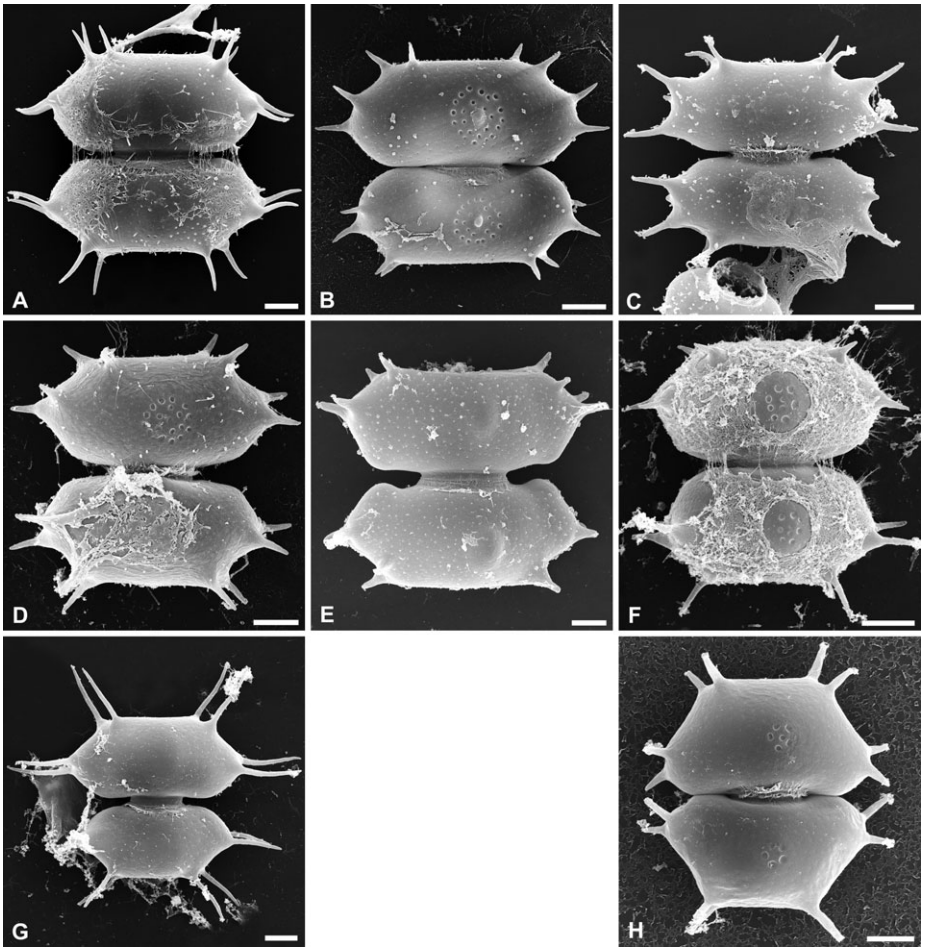

FIG. 5. SEM pictures of selected strains. (A) *Xanthidium antilopaeum* var. *incrassatum*, strain H 19. (B) *X. antilopaeum* var. *depauperatum*, strain H 22. (C) *X. antilopaeum* sensu lato, strain ASW 07106. (D) *X. antilopaeum* s.l., strain ASW 07107. (E) *X. antilopaeum* s.l., strain SVCK 105. (F) *X. antilopaeum* s.l., strain SVCK 281. (G) *X. antilopaeum* s.l., strain SVCK 343. (H) *X. antilopaeum* s.l., strain H 26. Scale bar = 10 μm.

were either polyphyletic or unresolved in the ITS rDNA phylogram. *Xanthidium antilopaeum* var. *antilopaeum* strains were resolved monophyletic in the ITS rDNA phylogenetic tree, but formed two distinct clades in the *trn*G^{ucc} phylogeny. Similarly, three *X. antilopaeum* strains ASW07105, ASW07106, and ASW07107 had identical *trn*G^{ucc} sequences, but were recovered in two different clades in the ITS rDNA tree.

Morphometric analyses. The CVA of geometric morphometric data of the entire set of 805 semicells (Fig. 8A) was analyzed to evaluate separation of *X. antilopaeum, X. cristatum* and *X. antilopaeum* var. *basiornatum* that formed three different phylogenetic lineages. The ordination resulted in their highly significant separation (Wilk's $\lambda = 0.094$, $P < 0.0001$). The leave-one-out cross-validation tests illustrated that the canonical discriminant function correctly classified 97.9% of the *X. antilopaeum* semicells. In addition, there was 99.4% correct classification of *X. cristatum*, and 100% correct classification of *X. antilopaeum* var. *basiornatum* semicells. The separate analysis of 163 semicells focused on the *X. cristatum* cluster (Fig. 8B) revealed strong shape separation of three phylogenetic lineages within this traditional morphospecies (Wilk's $\lambda = 0.015$, $P < 0.0001$). The cross-validation tests illustrated the 100% correct classification of the *X. cristatum* var. *uncinatum* and *X. cristatum* var. *cristatum* semicells; whereas there were 98.7% of the *X. cristatum* var. *scrobiculatum* semicells correctly classified on the basis of geometric morphometric data.

The linear regression analyses of morphometric versus genetic distances illustrated that the distance in shape of semicells was significantly related to their genetic distances (Fig. 9). However, this relation was relatively weak in the ITS1, ITS2, and ITS1 + two regions. Conversely, a stronger relation was revealed between the Procrustes distance and the Kimura 2-parameter distance evaluated by the sequences of the *trn*G^{ucc} plastid encoded marker. This difference in relation of morphology and sequence data evaluated by different molecular markers was also confirmed by the F-tests for the equality of regression

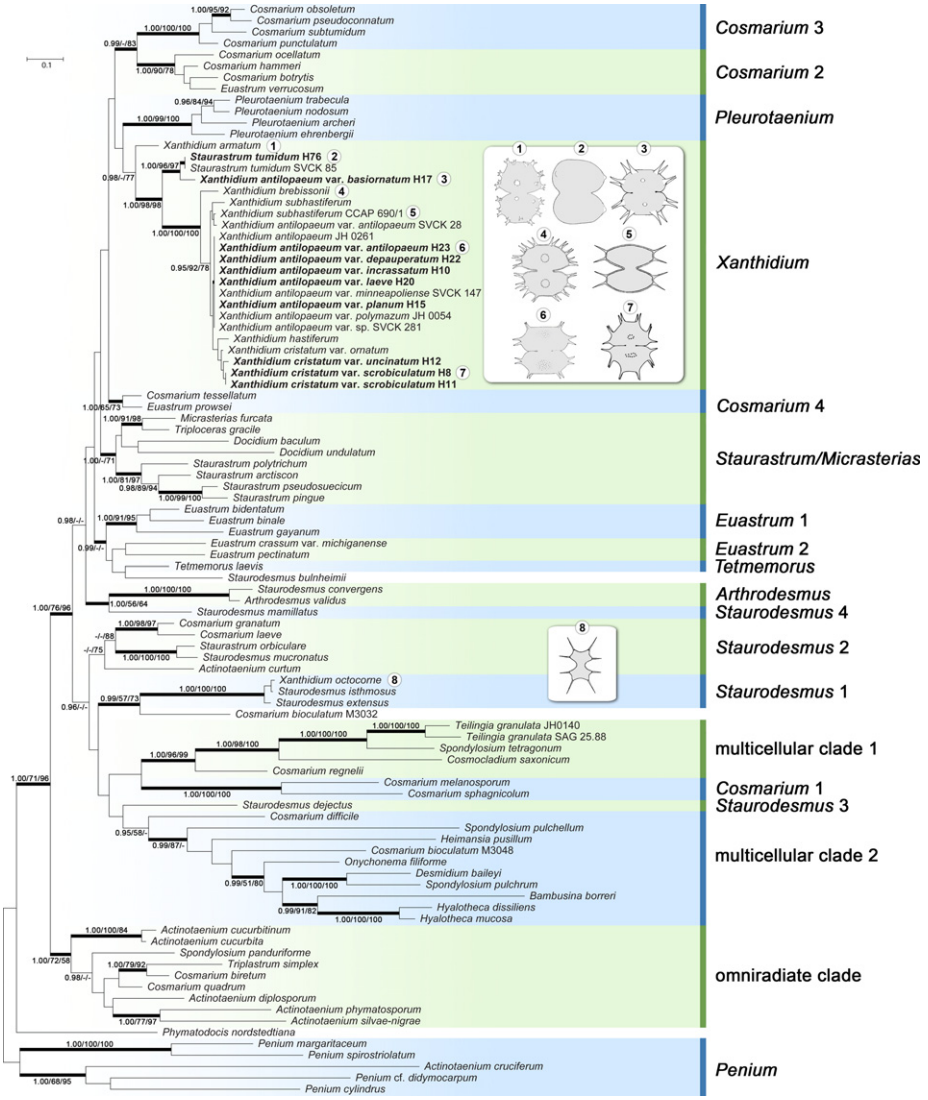

FIG. 6. Bayesian analysis based on the combined and partitioned *rbc*L + *cox*III data set of Desmidiaceae. Four partitions were analyzed separately by using a GTR + Γ model for the first and second codon positions of the *rbc*L gene, GTR + Γ model for the third codon position of the *rbc*L gene, HKY + Γ model for the first and second codon positions of the *cox*III gene, and GTR + Γ model for the third codon position of the *cox*III gene, under the covarion model. Values at the nodes indicate statistical support estimated by three methods —MrBayes posterior-node probability (left), maximum-likelihood bootstrap (in the middle), and maximum-parsimony bootstrap (right). Thick branches represent nodes receiving the high PP support (≥ 0.99). Species affiliation to 19 desmid clades *sensu* Gontcharov and Melkonian (2011) is indicated. Morphology of the selected investigated strains is given in the boxes and linked to corresponding sequences by numbers. Scale bar shows the estimated number of substitutions per site.

slopes among individual analyses. The slope of *trn*G^{ucc}-based regression analysis differed significantly from slopes of all the three remaining analyses (F = 11.65–13.48, $P = 0.0003$–0.0007). On the other hand, the differences among slopes of regression analyses based on different parts of the ITS region were insignificant (F = 0.08–0.99, $P > 0.05$).

DISCUSSION

Our molecular phylogenetic analyses based on the partitioned *rbcL* + *cox*III data set confirmed the monophyly of almost all the traditional *Xanthidium* species examined, including our target taxa *X. antilopaeum* and *X. cristatum*. This has previously been indicated by other authors as well (Hall et al. 2008, Gontcharov and Melkonian 2011). The single exception was *X. octocorne*, which was nested within the firmly supported "*Staurodesmus* 1" clade (*sensu* Gontcharov 2008). Since this strongly supported lineage also contains *Staurodesmus triangularis* (Lagerheim) Teiling (Gontcharov and Melkonian 2011), the type species of the genus *Staurodesmus* (Compère 1977), we decided to establish a new combination *Staurodesmus octocornis* (see chapter Taxonomical consequences). This separation of *X. octocorne* from *Xanthidium* is a logical consequence of its longtime recognized controversial taxonomical position.

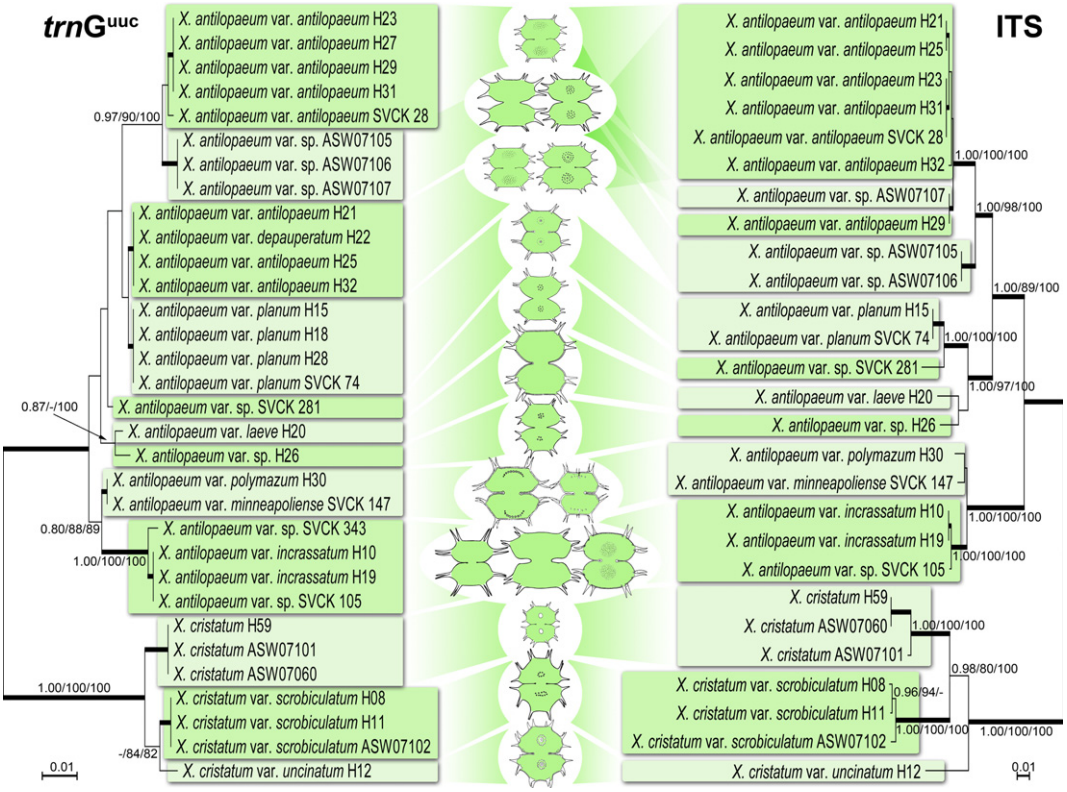

FIG. 7. Phylogenetic trees of *Xanthidium cristatum* and *X. antilopaeum* taxa derived from Bayesian analyses of chloroplast *trn*G^{ucc} and nuclear ITS rDNA sequences, using a GTR + I model for the *trn*G^{ucc} *rbc*L gene, GTR + Γ model for the ITS1 region, GTR + I model for the ITS2 region, and the JC model for the 5.8 rDNA region. Values at the nodes indicate statistical support estimated by three methods—MrBayes posterior-node probability (left), maximum-likelihood bootstrap (in the middle), and maximum-parsimony bootstrap (right). Thick branches represent nodes receiving the highest PP support (1.00). A scale bar shows the estimated number of substitutions per site. See text for details of discordance between the two topologies.

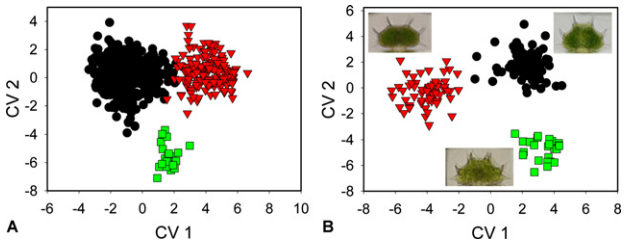

FIG. 8. Multivariate analyses of geometric morphometric data. (A) The ordination plot of first and second axes of the canonical variates analysis (CVA) of all the 805 investigated objects aimed at morphological separation of three phylogenetic lineages. Circles, *X. antilopaeum* sensu lato; triangles, *X. cristatum* sensu lato, squares, *X. basiornatum*. (B) The ordination plot of first and second axes of the canonical variates analysis (CVA) separating three phylogenetic species of the traditional *X. cristatum* lineage. Triangles, *X. cristatum* var. *cristatum*; circles, *X. cristatum* var. *scrobiculatum*; squares, *X. cristatum* var. *uncinatum*.

Xanthidium octocorne does not match the morphological characteristics of the genus, having no central protuberance and only a single series of spines. It is likely that some other *Xanthidium* species with a similar morphology, such as *X. smithii*, *X. impar*, or *X. bifidum*, may in fact also be a part of the *Staurodesmus* lineage. However, this hypothesis requires further molecular phylogenetic confirmation.

The analysis of the *rbcL* + *cox*III data set also confirmed the unexpected phylogenetic position of *S. tumidum*, which was already suggested by Gontcharov et al. (2003). The phylogenetic analysis of two different *S. tumidum* strains significantly inferred both isolates into the above-mentioned robust *Xanthidium* clade. Having three- or four-radiate, smooth cells lacking a series of spines typical for the genus, this large and conspicuous desmid matches the generic diagnosis of *Xanthidium* even less than *X. octocorne* (e.g., Coesel and Meesters 2007). However, a possible evolutionary relationship between *S. tumidum* and *X. armatum* has already been suggested by Meindl (1986) and Höftberger and Meindl (1993) on the basis of their similar, peculiar way of nuclear migration during the semicell morphogenesis. Such a morphological peculiarity within a well-supported monophyletic genus may be no exception among Desmidiales. Recently, Škaloud et al. (2011) illustrated that three species, originally belonging to the morphologically distinct genera *Cosmarium*, *Triploceras*, and *Staurodesmus* actually belong to the genus

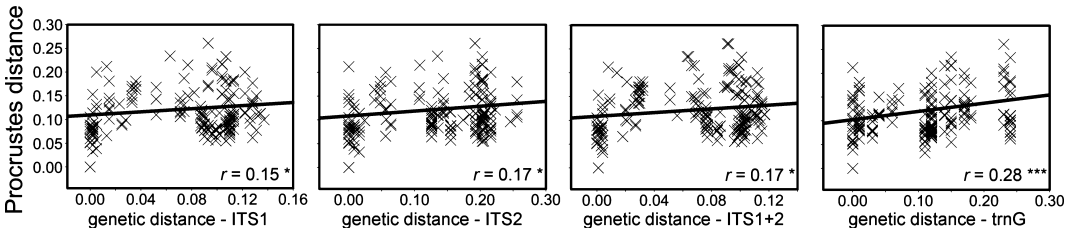

FIG. 9. Linear correlation analyses of morphometric differences among strains (Procrustes distance) and their genetic distances evaluated by the ITS1, ITS2, ITS1 + 2 and trnG^{UCC} regions. The linear correlations are represented by Pearson's *r* values. Significances are given by the *P*-values: *** $P < 0.001$, * *P*-value 0.01–0.05.

Micrasterias. The last species *M. dickiei* (Ralfs) Škaloud et al. is morphologically relatively similar to *S. tumidum*, also having three-radiate, smooth-walled cells (Coesel and Meesters 2007). Škaloud et al. (2011) illustrated hypothetic evolutionary transformation of a *M. pinnatifida*-like ancestor into *M. dickiei*. This supposedly proceeded from the occasional 3-radiate form of *M. pinnatifida* by shape simplification that usually accompanies formation of 3-radiate forms (e.g., Teiling 1956). A similar evolutionary scenario might have taken place in *S. tumidum*. Triradiate morphs of *Xanthidium* taxa have occasionally been reported (West and West 1912) and several of them were even described as separate taxa (e.g., Lundell 1871, Irénée-Marie 1939, Williamson 2002). The accompanying shape simplification would explain the loss of spines during the morphological evolution of *S. tumidum* from a *Xanthidium*-like ancestor. Similar to *X. octocorne*, the taxonomic position of *S. tumidum* has been the subject of some controversy in the past. Besides the genus *Staurastrum*, it was attributed to the genus *Staurodesmus* (e.g., Lenzenweger 1997). In addition, Wille (1890) included it in his newly created genus *Pleurenterium*. This taxon was originally described by Lundell (1871) as a subgenus of *Staurastrum*, on the basis of a different, parietal form of chloroplasts. Gay (1884) proposed to classify *Pleurenterium* as a section of *Xanthidium* on the basis of similar, parietal chloroplasts of both taxa. He also pointed out to the occasional occurrence of three-radiate forms in other species of the genus *Xanthidium*. Interestingly, presented phylogenetic data fully supported these early taxonomic studies. It is a matter of speculation whether some other morphologically similar *Staurastrum* taxa, that have been separated from *Staurastrum* into *Pleurenterium* on the basis of their parietal chloroplasts, such as *S. longispinum* (Lagerheim 1888) and *S. grande* (Wille 1890), may also belong to *Xanthidium*.

The analyses of plastid *trn*G^{ucc} and nuclear ITS rDNA resulted in trees resolving two major lineages corresponding to the traditional taxa *X. antilopaeum* and *X. cristatum* (Fig. 7). This suggests that the traditional morphology-based discriminative criteria of both species (the different number of spines per semicell) may generally still be considered relevant. However, our phylogenetic analyses also suggested that *X. antilopaeum* var. *basiornatum* does not belong to the *X. antilopaeum*/*X. cristatum* clade and forms a well-resolved independent clade together with *S. tumidum*. This would imply that *X. antilopaeum* var. *basiornatum*, although possessing eight spines per semicell like other *X. antilopaeum* taxa, in fact represents an additional separate species of the genus *Xanthidium*. This hypothesis was also corroborated by subsequent geometric morphometric analysis that unequivocally separated populations of *X. antilopaeum*, *X. cristatum*, and *X. antilopaeum* var. *basiornatum* (Fig. 8A). Therefore, we propose that the last taxon should be established as a separate species (see Taxonomical consequences). It should be noted that Förster (1983) classified *X. antilopaeum* var. *basiornatum* as a separate variety of *X. canadense* (Joshua) Förster. However, since there is a clear morphological difference between both taxa, Förster's opinion has not been generally accepted, and *X. antilopaeum* var. *basiornatum* has in reputable desmid monographs (e.g., Lenzenweger 1997) still been kept as a variety of *X. antilopaeum*. We can see that the taxonomic dilemma concerning the correct classification of this taxon has also been reflected by its isolated phylogenetic position. However, the molecular data, together with the morphometric analysis, unequivocally indicated that *X. basiornatum* should be treated as a separate species.

The molecular data revealed three well-resolved clades within traditional *X. cristatum* that corresponded to the traditional varieties *X. cristatum* var. *cristatum*, *X. cristatum* var. *scrobiculatum*, and *X. cristatum* var. *uncinatum*. This classification pattern was also confirmed by the geometric morphometric analysis that illustrated highly significant semicell shape differences among these three taxa (Fig. 8B). These results, together with the fact that the general morphology of *X. cristatum* var. *uncinatum* and *X. cristatum* var. *scrobiculatum* is clearly different from the nominate variety (compare Figs. 2, A–C and 4, A–C, see also Appendix S1 in the Supporting Information), led us to the conclusion that they should be regarded as separate species (see Taxonomical consequences). Conversely, *X. cristatum* var. *bituberculatum*, described by Lowe (1923), which differs from the nominate variety basically by the presence of two tubercles instead of one in the central area of the semicells, should not be considered a separate taxon. As already mentioned above, the form of central

ornamentation in *X. cristatum* may be extremely variable. The single strain ASW 07060 had either one tubercle or the central ornamentation was completely lacking. Semicells with either one or two tubercles were also found in strains ASW 07101 and H 59. The latter strain even had numerous Janus cells with the semicells corresponding either to *X. cristatum* var. *bituberculatum* or to *X. cristatum* var. *cristatum* (Fig. 4A, see also Appendix S1). Therefore, traditional *X. cristatum* var. *bituberculatum* should be considered an ecomorph of *X. cristatum* sensu stricto.

Comparing the lineages that were delineated within the traditional *X. antilopaeum* complex using the chloroplast and nuclear sequences, we observed a large degree of congruence. Nearly all the *trn*G^{ucc} clusters were resolved as monophyletic lineages in the ITS rDNA data set (Fig. 7). However, incongruence among loci was detected in the topology of strains H 21, H 22, H 23, H 25, H 27, H 29, H 31, H 32, SVCK 28, ASW 07105, ASW 07106, and ASW 07107. The most likely explanation of this phenomenon is that the branching order of the "species tree" might differ from that of the "genes tree" because of incomplete lineage sorting from an ancestral polymorphic gene pool (Pamilo and Nei 1988, Rosenberg 2003). Accordingly, the conflicting topologies could be caused by different rates of loci coalescence and the stochastic processes of loci differentiation (Carstens and Knowles 2007). The observed mismatch among gene trees indicated rather rapid diversification within the *X. antilopaeum* complex that could not be unambiguously differentiated by analyzing the single loci. Conversely, analyses of multiple loci and multiple sequences per loci could give a more complete picture of phylogenetic divergence among closely related species within the *X. antilopaeum* complex. Unfortunately, since the life histories inferred by the *trn*G^{ucc} and the ITS rDNA data sets are not congruent, we cannot resolve the real "species tree" without analyzing additional rapidly evolving genetic loci. Nevertheless, we have several indications that favored the *trn*G^{ucc} evolutionary scenario over the ITS rDNA topology. First, the clades inferred by the *trn*G^{ucc} phylogeny showed an apparent biogeographic pattern, suggesting the distributional differences of the recently diverged species (see next). Second, reliability of the plastid *trn*G^{ucc} data has been supported by the population genetic theory, which predicts that inferences from nuclear DNA are often confounded by their four times longer coalescent time in comparison with organelle DNA (Moore 1995). Consequently, the failure to obtain high-quality ITS rDNA sequences for all the *X. antilopaeum* strains could be explained by the existence of many paralogous copies of the ITS rDNA locus, which have not yet been sufficiently homogenized by concerted evolutionary processes (Rich et al. 1997). Finally, reliability of the *trn*G^{ucc} data set to resolve the real "species tree" has also been supported by the significantly better linear correlation between the matrices of morphological differences and the *trn*G^{ucc}-based genetic distances among *X. antilopaeum* strains, as compared with the ITS rDNA data set (Fig. 9). These results indicated the limited gene flow among the lineages as delineated by *trn*G^{ucc}-based phylogeny, resulting in subtle morphological differences among the recently diversified species. Summing up, the *trn*G^{ucc} sequences could provide good molecular markers for the species delimitation in *X. antilopaeum* species complex, even though they were less variable than the ITS rDNA sequences. The usefulness of the *trn*G^{ucc} intron sequences for resolving intraspecific diversity within various desmid species complexes has recently been also shown by Neustupa et al. (2010, 2011b), Nemjová et al. (2011), and Škaloud et al. (2012). We therefore believe that the *trn*G^{ucc} marker should be considered a good candidate for a green algal barcode, in particular for Streptophytes (Shaw et al. 2005, Hall et al. 2010). Furthermore, the phylogenetic tree inferred from the *trn*G^{ucc} sequences could provide insights into the morphological evolution within the *X. antilopaeum* species complex. For example, the rapid morphological diversification has been observed in a morphologically very diverse group of *X. antilopaeum* var. *incrassatum* and strains SVCK 105 and SVCK 343. On the other hand, genetically identical taxa *X. antilopaeum* var. *polymazum* and *X. antilopaeum* var. *minneapoliense* shared a great deal of morphological similarity (Fig. 4, H–I). Already Grönblad (1921) pointed out to the fact that the accessory subapical spines in *X. antilopaeum* var. *minneapoliense*, which are the only distinguishing characteristic between both these varieties, may very often be reduced, or even absent, and that these two varieties would better be merged together. As our morphological observations fully confirmed the Grönblad's (1921) data, we can conclude that *X. antilopaeum* var. *minneapoliense* may most probably only represent the ecomorph of *X. antilopaeum* var. *polymazum*. Despite this, we feel it is premature to propose any formal taxonomic changes within the *X. antilopaeum* complex (other than var. *basiornatum*) unless the *trn*G^{ucc} phylogeny is supported by additional molecular markers.

Recent polyphasic studies, employing light microscopic, ultrastructural, morphometric and molecular phylogenetic methods, that evaluated the validity of desmidiacean species concepts, particularly in the genus *Micrasterias* (Neustupa et al. 2010, 2011a,b, Nemjová et al. 2011), illustrated apparent pseudocryptic diversity in a number of traditional species. Several morphologically defined infraspecific taxa were shown to be artificial, but, on the other hand, several traditional varieties obviously represented independent species. Our data revealed a pattern similar to the above-mentioned studies. Although several examples of ill-defined traditional subspecific taxa were found (*X. cristatum* var. *bituberculatum* and *X. an-*

tilopaeum var. *minneapoliense*), the major part of morphological variability among the investigated strains was phylogenetically relevant. Consequently, several infraspecific taxa should now be established as separate species. This is fully in accordance with the view of Kouwets (2008) who stated, "In desmids, to achieve a feasible taxonomy, 'stable' varieties should generally be given the status of a separate species. The taxonomic status of 'forma' should be abandoned altogether. Morphological variations induced by the environment should merely be characterized as 'ecomorpha' without any taxonomic status." It is obvious, however, that in this respect, phylogenetic species-level desmid taxonomy is still at the beginning.

Brook (1981) estimated that more than 6,000 species of desmids have been described from all parts of the world. However, the recent studies on phylogenetic species concepts in desmid taxa (Neustupa et al. 2010, 2011a,b, Nemjová et al. 2011, Škaloud et al. 2012, this study) unanimously illustrated that some traditional morphological species (such as *M. truncata*, *M. fimbriata*, *Pleurotaenium ehrenbergii*, or *X. cristatum*) actually consist of at least two or three phylogenetic species recognizable using morphometric methods. Conversely, the number of traditional taxa that were found phylogenetically homogenous (such as *M. rotata* or *Pleurotaenium archeri*) was distinctly lower. Therefore, we believe that with the wide employment of the molecular methods in species-level taxonomy of Desmidiales, about twice-as-many species may be recognized than previously described on the basis of purely morphological data.

The above-mentioned studies on species concepts of desmids also revealed a clear geographic signal among several investigated species indicating that the actual species diversity of these relatively large protists may be related to either the patterns of their geographic distribution or the macroscale climatic factors. The phylogenetic structure of the *M. cruxmelitensis/M. radians* complex (Neustupa et al. 2010) reflected the origin of strains, separating the European, African, and Asian strains. Nemjová et al. (2011) concluded that the Australian strains of the traditional *M. truncata* probably represented a separate species. Neustupa et al. (2011b) illustrated that the two firmly delimited phylogenetical lineages within the conspicuous desmid species *M. fimbriata* exhibited largely disparate geographic distribution patterns across Europe. Although we only analyzed European and North American isolates, our results certainly do not contradict the above-mentioned observations. The phylogenetic tree based on the *trn*G^{ucc} data set indicated several examples of possible geographical restriction among *Xanthidium* phylogenetic taxa. All the three strains of traditional *X. antilopaeum* (ASW 07105, ASW 07106, and ASW 07107) that originated from arctic regions (Greenland, St. Matthew Island in the Bering Sea, respectively) were inferred in a single clade (Fig. 7). In addition, the phylogenetic analysis also separated the central European and western European representatives of *X. antilopaeum* var. *antilopaeum* (Fig. 7), indicating a geographic pattern very similar to that observed in two phylogenetic species of traditional *M. fimbriata* (Neustupa et al. 2011a,b). The differences in distribution pattern may also be anticipated in three phylogenetic taxa of *X. cristatum*. Whereas the nominate variety and var. *uncinatum* are relatively common all across Europe (West and West 1912, Lenzenweger 1997, Kouwets 1999, Abdelahad et al. 2003, Coesel and Meesters 2007, Šťastný 2010), we could not find any reliable report of morphotypes corresponding to var. *scrobiculatum* outside the western European regions typical with the oceanic climate.

Taxonomic consequences. On the basis of the data presented in this study, some taxonomical changes appeared to be necessary. By the transfer of *X. octocorne* to *Staurodesmus*, the genus *Xanthidium* formally remains monophyletic. Since a type species of *Xanthidium* has not yet been designated (Ralfs 1848, Guiry and Guiry 2012), and the definition of a type species is crucial for the nomenclatural identity of a genus, we state, in accordance with the ICBN article 37.3 (McNeill et al. 2006), that *X. cristatum*, one of the species that have originally been included into the genus *Xanthidium* by Ralfs (1848), should be the type species of this genus.

Xanthidium Ralfs 1848, Brit. Desmidieae, p. 111.

Type species (here designated): *Xanthidium cristatum* Ralfs 1848, Brit. Desmidieae, p. 115, Pl. 19, Fig. 3A–C.

Synonym: *Xanthidium cristatum* var. *bituberculatum* Lowe 1923, Canadian Arctic Exped. 4: p. 27, Fig. 4.

Staurodesmus octocornis (Ralfs) Stastny, Skaloud et Neustupa **comb. nov.**

Basionym: *Xanthidium octocorne* Ralfs 1848, Brit. Desmid., p. 116, Pl. 20, Fig. 2.

Synonym: *Arthrodesmus octocornis* (Ralfs) Archer in Pritchard 1861, Hist. Inf., p. 736.

Xanthidium tumidum (Ralfs) Stastny, Skaloud et Neustupa **comb. nov.**

Basionym: *Staurastrum tumidum* Ralfs 1848, Brit. Desmid., p. 126, Pl. 21, Fig. 6.

Synonyms: *Staurodesmus tumidus* (Ralfs) Teiling, 1967, Ark. F. Bot., II, 6(11), p. 578, Pl. 22, Fig. 1; *Pleurenterium tumidum* (Bréb.) Wille 1890, Engler & Prantl, Nat. Pflanzenfam. 1890, p. 11.

Xanthidium basiornatum (B.Eichler et Raciborski) Stastny, Skaloud et Neustupa **comb. nov. et stat. nov.**

Basionym: *Xanthidium antilopaeum* var. *basiornatum* B.Eichler et Raciborski 1893, Rozpr. Akad. Umiej. Wydz. Mat.-Przyr. Krakowie, II, 26, p.125, Pl. 3, Fig. 31.

Synonym: *Xanthidium canadense* (Joshua) Förster var. *basiornatum* (B.Eichler et Raciborski) Förster 1983, Algol. Studies 33, p. 378, Fig. 4.

Xanthidium uncinatum (Ralfs) Stastny, Skaloud et Neustupa **comb. nov. et stat. nov.**

Basionym: *Xanthidium cristatum* var. *uncinatum* Ralfs 1848, Brit. Desmid., p. 115, Pl. 19, Fig. 3D–F.

Xanthidium scrobiculatum (Scott et Grönblad) Stastny, Skaloud et Neustupa **comb. nov. et stat. nov.**

Basionym: *Xanthidium cristatum* var. *scrobiculatum* Scott et Grönblad 1957, Acta Soc. Sci. Fennicae, II, B, 2(8), p. 30, Pl. 17, Figs 3–6.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. List of all sequences involved in the phylogenetic analyses, including the GenBank accession numbers for the *rbc*L, *cox*III, *trn*G^{ucc}, and ITS rDNA genes. Strain number abbreviations: ACOI - Coimbra Collection of Algae, Portugal; ACKU - Algal Culture Collection of Kyonggi University, Kyonggi, Korea; CCAP - Culture Collection of Algae and Protozoa, Oban, Scotland, UK; JH - John Hall private collection; M - Culture Collection Melkonian, University of Cologne, Germany (strains available from CCAC); NIES - National Institute for Environmental Studies, Japan; SAG - Sammlung von Algenkulturen der Universität Göttingen, Germany; SVCK - Sammlung von Conjugaten-Kulturen, Germany; UTEX - Culture Collection of Algae at University of Texas, USA.

Appendix S1. LM and SEM pictures of all the *Xanthidium* strains studied.

Paper V

Molecular phylogeny of baculiform desmid taxa (Zygnematophyceae)

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ORIGINAL ARTICLE

Molecular phylogeny of baculiform desmid taxa (Zygnematophyceae)

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Abstract The baculiform, rod-like morphotypes belong to several phylogenetic lineages within Desmidiaceae (Zygnematophyceae, Viridiplantae). Some, for example the genus *Pleurotaenium*, form independent lineages, but reductive evolution of complicated desmid cells toward baculiform morphology also occurred in individual lineages, for example *Micrasterias*. In this genus, the rod like *Triploceras* forms evolved from more complex ancestors. In this study, we tested for an independent position of the subtropical and tropical genus *Triplastrum*, previously separated from *Triploceras* on the basis of morphological data. In addition, monophyly of *Pleurotaenium* was also investigated with multiple isolates corresponding to seven species of this genus, including the morphologically dissimilar *P. nodosum* and *P. ovatum*. Finally, two isolates of *Docidium baculum* were also investigated. Molecular phylogenetic analysis of concatenated *rbcL* + *cox*III sequence data implied that the baculiform taxa investigated were in three distantly related positions within Desmidiaceae. The genus *Triplastrum* proved to be unrelated to *Triploceras*, because it clustered in the "omniradiate" lineage of Desmidiaceae among morphologically dissimilar taxa. The genus *Pleurotaenium* was monophyletic, but *P. ovatum* was recovered in a weakly supported sister position to all the other members of the genus. The *trn*G^{ucc} phylogeny of *Pleurotaenium* taxa concurred with the *rbcL* + *cox*III phylogram, and generally revealed the poor morphological concepts of some species in this genus. The most common taxa *P. ehrenbergii* and *P. trabecula* were resolved as polyphyletic because their strains were distributed among several strongly supported clades. However, strains of *P. nodosum* and *P. archeri* formed separate, well supported lineages within the genus.

Keywords Desmids · Molecular phylogeny · Zygnematophyceae · *Docidium* · *Pleurotaenium* · *Triplastrum*

Introduction

Desmidiales are the single most species-rich group of microalgae of the Streptophyta evolutionary lineage of green plants (Viridiplantae). They belong to a group of conjugating green algae (Zygnematophyceae) that have recently been revealed to be the closest evolutionary relatives of embryophytes (Wodniok et al. 2011). More than 6,000 species of desmids have been described in freshwater habitats worldwide (Brook 1981). Desmids are especially abundant in the phytobenthos of acidic wetlands (Coesel and Meesters 2007). Four families of Desmidiales (Desmidiaceae, Gonatozygaceae, Peniaceae, and Closteriaceae) have been recognised on the basis of morphological and cytological data. A single family, Desmidiaceae, contains approximately 90 % of the species described so far. However, the generic taxonomy of this group has recently been virtually deconstructed because molecular phylogenetic studies illustrated non-monophyly of many traditional genera (Gontcharov et al. 2003; Gontcharov and

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Melkonian 2005, 2011; Gontcharov 2008; Hall et al. 2008). Gontcharov and Melkonian (2008, 2011) discovered that Desmidiaceae consists of at least 22 independent phylogenetic lineages that may form the basis of the newly defined monophyletic genera within this important microalgae group. However, molecular data are still not available for several traditional genera with distinct morphology; therefore, their taxonomic status remains uncertain. Moreover, Hall et al. (2008) and Gontcharov and Melkonian (2011) reported that some desmid taxa with distinctly different morphology may form a single phylogenetic group. An interesting example of this phenomenon among desmid genera is represented by the genus *Triploceras* J. W. Bailey. This genus is characterised by elongated baculiform cells dotted with numerous knot-like projections (i.e., verrucae). However, molecular data indicated it is nested within the *Micrasterias* lineage (Škaloud et al. 2011), where most taxa are characterised by flat, richly ornamented cells divided to form polar and lateral lobes (Krieger 1939; Růžička 1981; Coesel and Meesters 2007). Interestingly, the morphology of *Triploceras* is vaguely similar to that of aradiate morphs of *Micrasterias* C. Agardh ex Ralfs, i.e., cells with reduced lateral lobes which may occasionally appear in *Micrasterias* populations exposed to stressful environmental conditions (Kallio and Heikkilä 1969). In addition to *Triploceras*, baculiform desmid morphotypes belong to several other independent lineages within Desmidiaceae. Gontcharov and Melkonian (2011) defined three phylogenetic lineages corresponding to the traditional genera, namely *Pleurotaenium* Nägeli, *Haplotaenium* T. Bando, and *Docidium* Brébisson ex Ralfs, characterised by rod-like morphology of the cells. However, the phylogenetic position of further baculiform desmid genera, for example *Triplastrum* Iyengar and Ramanathan, *Ichthydontum* A. M. Scott and Prescott, or *Ichthyocercus* West and G. S. West remained unknown. These taxa almost exclusively occur in tropical and subtropical habitats and are currently not available in cultures. Molecular data are also lacking for some morphologically very distinct species of the genus *Pleurotaenium*, for example *P. nodosum* (F. M. Bailey) P. Lundell, and *P. ovatum* (Nordstedt) Nordstedt. Monophyly of these species with other *Pleurotaenium* taxa cannot be regarded as straightforward. Moreover, the presence of prominent knot-like projections on the surface of *P. nodosum* cells makes this species somewhat similar to the genus *Triploceras*. Therefore, testing for monophyly of the traditional genus *Pleurotaenium* may definitely be of interest for desmidiacean taxonomy.

The genus *Triplastrum* was introduced by Iyengar and Ramanathan (1942) to accommodate species that were formerly classified into the genus *Triploceras* but lacked typical knot-like projections (verrucae). Populations of this genus so far have only been reported in warmer regions of the Old World (Coesel 1996). Turner (1892) analysed the collections made by G. C. Wallich in India and described *Triploceras abbreviatum* W. B. Turner from samples collected in Raniganj (NW of Kolkata). His specimens had four plastids and 3 or 4 terminal cell lobes, each with a single minute lateral spine. Allorge (1924) described *Triploceras simplex* P. Allorge from Lac de Grand-Lieu in western France. This species was characterised by two plastids and two spines on the tips of terminal lobes. A few years later Kisselev (1930), cited after Kossinskaja (1960), described *Triploceras spinulosum* Kisselev from a rice field in Uzbekistan. This species differed from the two former species by having two plastids but bearing up to four spines on the apical lobes (Kossinskaja 1960). Iyengar and Ramanathan (1942), apparently unaware of Kisselev's study, created a new genus *Triplastrum* and also described a new species *T. indicum* Iyengar and Ramanathan with morphology almost identical to that of *T. spinulosum*. They also transferred *T. abbreviatum* and *T. simplex* into this newly formed genus. Hinode (1952), unaware of the Iyengar and Ramanathan (1942) study, reported *T. simplex* from Shikoku Island in southern Japan. He concluded that morphological differences between *T. abbreviatum* and *T. simplex* were negligible and therefore created a new combination, *T. abbreviatum* var. *simplex*. Hinode (1952) was also the first to illustrate fine punctuation of the cell wall in his specimens, and he also reported irregularly undulated zygospores. Gauthier-Lièvre (1960) reported *Triplastrum spinulosum* from several tropical African localities in Mali, Chad, and Uganda. She concluded that morphological differences between *T. indicum* and *T. spinulosum* are negligible and combined the former name as a variety of the later species *T. spinulosum* var. *indicum*. However, she also created a new variety, *T. spinulosum* var. *africanum*, for her specimens with a somewhat narrower subapical part of semicells. Couté and Rousselin (1975) reported a number of localities of *T. spinulosum* from the Niger river basin. The number of terminal spines in their populations varied, and they concluded that all the varieties of *T. spinulosum* should be regarded as synonymous. However, these varieties were not put into synonymy until Claassen's (1977) study of *T. spinulosum* from the Transvaal, South Africa. She observed cells with three or four terminal lobes, each bearing 2–4 spines. The cells had 2–4 and occasionally even up to six plastids. Islam and Akter (2005) reported *T. abbreviatum* with 2–4 plastids and with two spines on the terminal lobes from tea gardens in Dhaka, Bangladesh. Kouwets (1998) confirmed the occurrence of *Triplastrum* in South West France. He identified his specimens as *T. spinulosum* var. *indicum* and reported that the cells had 3–4 apical lobes, terminating in 3–4 spines. His figure depicted a cell with four plastids.

Coesel and Van Geest (2008) reported *T. abbreviatum* from the Okavango delta in Botswana. Notably, they concluded that there are possibly no essential morphological differences between all the *Triplastrum* species described so far and suggested that the genus may in fact be monospecific.

For this study, we isolated a clonal strain of *Triplastrum* from Lac de Cazaux, Aquitaine, France, in addition to 27 strains of other baculiform desmid species that belong to different traditional genera (*Pleurotaenium* and *Docidium*). Consequently, this study principally addressed two questions:

1. Is the genus *Triplastrum* really different from the genus *Triploceras*? Does it form a separate lineage within Desmidiaceae, warranting its classification as separate genus?
2. Do all the strains classified in the traditional genus *Pleurotaenium* really form a monophyletic lineage? Is there any (pseudo) cryptic species diversity within this frequently occurring genus?

Materials and methods

Isolation and cultivation of strains; light microscope (LM) and scanning electron microscope (SEM) observations

The natural populations of the genus *Triplastrum* were observed in plankton samples taken from the Lac de Cazaux (Aquitaine, France) on 4 October 2009 and 15 September 2010. The origin of all the other baculiform strains isolated in this study is given in Table 1. The strains were isolated by single-cell pipetting into unialgal cultures, which were grown in MES (morpholinoethanesulfonic acid)-buffered DY IV liquid medium (Andersen et al. 1997) at 24 °C and continuously illuminated at 5–15 µmol photons m^{-2} s^{-1} from 18 W cool fluorescent tubes (Philips TLD 18 W/33; Royal Philips Electronics, Amsterdam, The Netherlands). The clonal strain of *Triplastrum simplex* isolated in this study was deposited as strain no. K 1101 in the Culture Collection of Algae of Charles University in Prague (CAUP), see http://botany.natur.cuni.cz/algo/caup.html. Microphotographs of the cells were taken with an Olympus (Tokyo, Japan) BX51 light microscope with an Olympus Z5060 digital camera. For SEM, the acetone-washed glass coverslips were heated and coated three times with a poly(L-lysine) solution (1:10 in deionised water) to ensure appropriate cell adhesion. A drop of the formaldehyde-fixed cell suspension was put on the coverslip, transferred into 30 % acetone, and dehydrated in an acetone series. Subsequently, the cells were dried to a critical point with liquid carbon dioxide (CO_2). Finally, they were sputter coated with gold and examined with a Jeol 6380 LV SEM.

DNA isolation, polymerase chain reaction (PCR), and DNA sequencing

After centrifugation of desmid cells in 2 mL tubes, 100–200 mL of InstaGene matrix (Bio-Rad Laboratories) was added to the pellet. The cells were then mechanically disrupted by shaking for 5 min in the presence of glass beads (3 mm diameter; Sigma–Aldrich) in Mixer Mill MM 400 (Retsch, Haan, Germany). Subsequently, the solution was incubated at 56 °C for 30 min, vortex mixed for 10 s, and heated at 99 °C for 8 min. After vortex mixing a second time, the tubes were centrifuged at 12,000 rpm for 2 min, and the supernatant was directly used as a PCR template. Three molecular markers were amplified by PCR: chloroplast *rbc*L and *trn*G^{ucc} and mitochondrial *cox*III. The PCR reaction in a total volume of 20 µL contained 13.1 µL sterile Milli-Q water, 2 µL AmpliTaq Gold® 360 buffer 10× (Applied Biosystems, Life technologies, Carlsbad, CA, USA), 2.2 µL $MgCl_2$ (25 mM), 0.4 µL dNTP mix (10 mM), 0.25 µL of each primer (25 nM), 0.6 µL 360 GC enhancer, 0.2 µL AmpliTaq Gold® 360 DNA polymerase, and 1 µL DNA (10 ng μL^{-1}). The *rbc*L gene was amplified using newly designed primers rbcL-Pleurot-F (5′-GGT TAA AGA TTA TAG ACT TAC-3′) and *rbcL*-Pleurot-R (5′-CCT TGA CGA GCA AGA TCA CG-3′). The *trn*G^{ucc} marker was amplified using the primers designed by Neustupa et al. (2010): trnG-F (5′-AGC GGG TAT AGT TTA GTG GT-3′) and trnG-R (5′-GGT AGC GGG AAT CGA ACC CGC-3′). Finally, amplification of *cox*III marker was performed using primers designed by Škaloud et al. (2011): COX-ZYG-F3 (5′-TTA CTG GAG GTG GCA CAC TT-3′) and COX-ZYG-R2 (5′-TCC ATG AAA TCC AGT AGC TAA G-3′). The *rbc*L, *trn*G^{ucc}, and *cox*III markers were amplified in either a Touchgene gradient thermal cycler (Krackeler Scientific, Albany, NY, USA) or an XP thermal cycler (Bioer, Tokyo, Japan), starting with initial denaturation at 94 °C for 4/2/2 min, followed by 35/40/37 cycles of denaturing at 94 °C for 1 min, annealing at 54/62/50 °C for 1 min, and elongation at 72 °C for 2.5/1.5/3 min, with a final extension at 72 °C for 10 min, respectively. The PCR products were stained with bromophenol blue loading dye, quantified on 1 % agarose gel, stained with ethidium bromide, and cleaned with the Jetquick PCR purification kit (Genomed, Löhne, Germany) in accordance with the manufacturer's procedure. The purified amplification products were sequenced using an Applied Biosystems (Seoul, Korea) automated sequencer (ABI 3730xl) at Macrogen in Seoul, Korea. Sequencing reads were assembled and edited by use of SeqAssem software (Hepperle 2004).

Table 1 List of strains used in the study and their origin

Identity	Strain designation	Locality	Geographic coordinates
Docidium baculum	C6	Břehyně wetland, Czech Republic	50°34′58.58″N; 14°42′10.96″E
Docidium baculum	I1	A pool near Derrycunihy, Kerry, Ireland	51°57′57.66″N; 9°35′49.24″W
Pleurotaenium archeri	H50	Schwemm peat bog near Walchsee, Austria	47°39′19.35″N; 12°17′22.71″E
Pleurotaenium archeri	H51	Schwemm peat bog near Walchsee, Austria	47°39′19.35″N; 12°17′22.71″E
Pleurotaenium crenulatum	592	Grand Étang de Biscarrosse, Aquitaine, France	44°23′13.92′'N; 01°11′32.68″W
Pleurotaenium ehrenbergii	579	A pool near Hostens, Aquitaine, France	44°29′54.83″N; 00°38′19.06″W
Pleurotaenium ehrenbergii	737	Lough an Oileáin, Connemara, Ireland	53°27′38.91″N; 9°32′35.90″W
Pleurotaenium ehrenbergii	H1	A pool in Břehyně wetland, Czech Republic	50°35′01.17″N; 14°43′00.68″E
Pleurotaenium ehrenbergii	H13	Étang de Cazaux, Aquitaine, France	44°30′34.49″N; 1°11′39.12″W
Pleurotaenium ehrenbergii	H55	The mesotrophic sandpit pool near Cep, Czech Republic	48°55′04.03″N; 14°52′56.72″E
Pleurotaenium ehrenbergii	Q12	A bog near Étang Hardy, Aquitaine, France	43°43′12.99″N; 1°22′07.93″W
Pleurotaenium ehrenbergii	Q15	A pool close to Lac de Cazaux, Aquitaine, France	44°30′28.70″N; 1°11′57.30″W
Pleurotaenium ehrenbergii	Q19	A pool close to Lac de Cazaux, Aquitaine, France	44°30′28.70″N; 1°11′57.30″W
Pleurotaenium nodosum	I3	Long Range lake, Kerry, Ireland	51°59′51.04″N; 9°33′3.81″W
Pleurotaenium nodosum	I8	Lough na hEasléime, Connemara, Ireland	53°26′15.48″N; 9°32′36.78″W
Pleurotaenium nodulosum	H37	A peaty pool near Loughanillaun lake, Connemara, Ireland	53°27′36.63″N; 9°32′35.27″W
Pleurotaenium sp.	H14	Étang de Cazaux, Aquitaine, France	44°30′34.49″N; 1°11′39.12″W
Pleurotaenium sp.	H38	Bastemose wetland, Bornholm, Denmark	55°07′37.63″N; 14°56′42.15″E
Pleurotaenium sp.	H39	Schwemm peat bog near Walchsee, Austria	47°39′19.35″N; 12°17′22.71″E
Pleurotaenium sp.	H41	Étang Hardy, Aquitaine, France	43°43′01.64″N; 1°21′45.82″W
Pleurotaenium sp.	J-E9	A flower basin in Katano Kamoike, Kaga, Japan	36°19′17.23″N; 136°17′37.26″E
Pleurotaenium sp.	Q18	A littoral zone of Lac de Cazaux, Aquitaine, France	44°31′08.32″N; 1°10′44.69″W
Pleurotaenium ovatum	J2	Midorogaike pond, Kyoto, Japan	35°03′26.66″N; 135°46′06.13′'E
Pleurotaenium trabecula	634	Étang Hardy, Aquitaine, France	43°43′04.19″N; 01°21′57.15″W
Pleurotaenium trabecula	655	A pool near Hostens, Aquitaine, France	44°31′07.08″N; 00°36′51.18″W
Pleurotaenium trabecula	748	A pool near Derrycunihy, Kerry, Ireland	51°57′57.66″N; 9°35′49.24″W
Pleurotaenium trabecula	H40	The mesotrophic sandpit Cep, Czech Republic	48°55′04.03″N; 14°52′56.72″E
Triplastrum simplex	Q13	The littoral zone of Lac de Cazaux, Aquitaine, France	44°31′08.32″N; 1°10′44.69″W

Sequence alignment, model selection, and phylogenetic analysis

Two different alignments were constructed for the phylogenetic analysis:

1. a concatenated *rbc*L + *cox*III alignment of 104 desmid sequences selected to encompass all Desmidiaceae lineages, including *Triplastrum*, *Docidium*, and *Pleurotaenium* sequences, determined in this study; and
2. a *trn*G^{ucc} alignment of 24 *Pleurotaenium* sequences, which were all determined in this study.

The list of all sequences involved in phylogenetic analysis, including the GenBank accession numbers, is given in online resource 1. The *trn*G^{ucc} sequences were manually aligned in MEGA 4 (Kumar et al. 2008). The final *rbc*L + *cox*III alignment was generated as follows. First, we downloaded 82 core *rbc*L + *cox*III sequences from GenBank database to encompass most of the Desmidiales lineages, using the phylogeny published by Hall et al. (2008) as a guide. We then added 35 additional *rbc*L sequences selected according to the desmid phylogeny recently published by Gontcharov and Melkonian (2011) to cover the remaining Desmidiales lineages. Finally, 19 *rbc*L and 20 *cox*III sequences determined in this study together with seven closely related sequences revealed by BLAST searches were added to the alignment and manually aligned in MEGA 4. The final concatenated matrix contained 104 taxa, which was 1,885-bp long, and was 98 % filled for the *rbc*L data and 58 % filled for the *cox*III data. The matrix is available from the corresponding author.

A suitable partitioning strategy and partition-specific substitution models for the *rbc*L + *cox*III dataset were selected in a multi-step process (Verbruggen et al. 2010). Initially, a guide tree was obtained by conducting a second-level maximum-likelihood (ML) search on the unpartitioned dataset with an HKY + Γ_8 model by using

Treefinder (Jobb 2008). The dataset was then divided by four different partitioning strategies, combining different codon position segmentation. For each partition present in these partitioning strategies, 12 different nucleotide substitution models were evaluated (F81, HKY, GTR, and their combinations with Γ, I, and Γ + I). Subsequently, Bayesian information criterion (BIC) calculations were performed for all four potential partitioning strategies, assuming the guide tree and evaluated models for each partition. This BIC-based model selection procedure selected the following partitioning strategy with four partitions (receiving the lowest BIC score):

1. first and second codon position of *rbc*L (GTR + Γ);
2. third codon position of *rbc*L (GTR + Γ);
3. second codon position of *cox*III (HKY + Γ); and
4. third codon position of *cox*III (GTR + Γ).

The most appropriate substitution model for the *trn*G^{ucc} dataset was estimated by use of the Akaike information criterion (AIC) with PAUP/MrModeltest 1.0b (Nylander 2004).

The phylogenetic trees were inferred with Bayesian inference (BI) by using MrBayes version 3.1 (Ronquist and Huelsenbeck 2003). Analysis of the *rbc*L + *cox*III dataset was carried out on a partitioned dataset by using the strategy selected during the multi-step process described above. All parameters were unlinked among partitions. In the BI analyses, two parallel Markov chain Monte Carlo (MCMC) runs were carried out for 3 million generations each with one cold and three heated chains. Trees and parameters were sampled for every 100 generations. Convergence of the two cold chains was checked and "burn-in" was determined by use of the "sump" command. Bootstrap analysis was performed by ML and weighted parsimony (wMP) criteria by using Treefinder and PAUP version 4.0b10 (Swofford 2002), respectively. ML bootstrap values were obtained by running ML bootstrap analysis (100 replicates) under the partitioned *rbc*L + *cox*III and unpartitioned *trn*G^{ucc} datasets. The wMP bootstrapping (1,000 replicates) was performed using heuristic searches with 100 random sequence addition replicates, TBR swapping, and random addition of sequences (the number was limited to 10,000 for each replicate). The weight to the characters was assigned using the rescaled consistency index on a scale of 0–1,000. New weights were based on the mean of the fit values for each character over all of the trees in memory.

Results

The *Triplastrum* cells from Lac de Cazaux had typical baculiform morphology. The cells were 65–90 µm long and 8.5–13.0 µm broad. Cells with subapical narrowing frequently occurred in the natural samples (Fig. 1a). Conversely, the cultured populations had rather straight rod like cells (Fig. 1b), even if some cells with subapical narrowing were also present. There were 2–4 lobed plastids per cell, each with a centrally located pyrenoid (Fig. 1a–c). The cell wall was covered with minute pores (Fig. 1d, e), and a 2–3 µm thick sheath envelope was also observed (Fig. 1c). The apical parts of the cells were typically elongated into three terminal lobes (Fig. 1f–i). However, four apical lobes were observed on many cells (Fig. 1j). Interestingly, some semicells had triradiate outlines down to the isthmus level (Fig. 1f, g). The spines were present on the tips of the terminal lobes. Most often, there were two spines per lobe (Fig. 1g, h, j), but their number varied from one to three in the natural samples and in the cultured clonal populations (Fig. 1g–j).

Morphology of the other investigated strains corresponded to the well-known desmid morphospecies with baculiform cells (for identification, see Table 1). The *Docidium* cells were characterised by elongated cylindrical, more or less straight cells, with a shallow sinus and truncate, or truncately rounded, and smooth apices (Fig. 2a). The whorl of longitudinal, granule-like plications—a characteristic feature of this genus—was also present (Fig. 2b). The cells of the genus *Pleurotaenium* differed by the absence of this whorl of plications, by parietal chloroplasts, and by the whorl of granules or tubercles at the apex (Fig. 2c–k). In total, representatives of seven *Pleurotaenium* species have been found: *P. ovatum* (Fig. 2d), *P. ehrenbergii* (Fig. 2e), *P. crenulatum* (Fig. 2f), *P. trabecula* (Fig. 2g), *P. archeri* (Fig. 2h), *P. nodosum* (Fig. 2i), and *P. nodulosum* (Fig. 2j). *Pleurotaenium ovatum* was clearly distinguished from all the other species of the genus by the thick, oval-shaped cells with 2.5–3.5:1 length-to-width ratio. The cells were 270–320 µm long and 90–130 µm broad. *P. ehrenbergii* had cylinder-shaped semicells and there were 4–6 distinct granules along the semicells apices. The cells of this morphospecies were 250–450 µm long and 15–30 µm broad. *P. crenulatum* had, typically, 350–450 µm long and 35–45 µm broad cells distinctly tapering toward the apex, which bore 6–8 small granules. The strains of *P. trabecula* were typically slightly tapering toward the smooth, rounded apices; their cells were 350–750 µm long and 30–50 µm broad. *P. archeri* was distinguished by a characteristic deep constriction above the basal inflation. The apices of this species had 4–7 marginal granules. The cells of this species were 450–800 µm long and 35–55 µm broad. The two investigated strains of *P. nodosum* were clearly distinguished by 4–5 rings of conspicuous projections in each semicell. Finally, *P. nodulosum* was identified by relatively thick cylinder-shaped cells (450–550 µm long and 45–60 µm

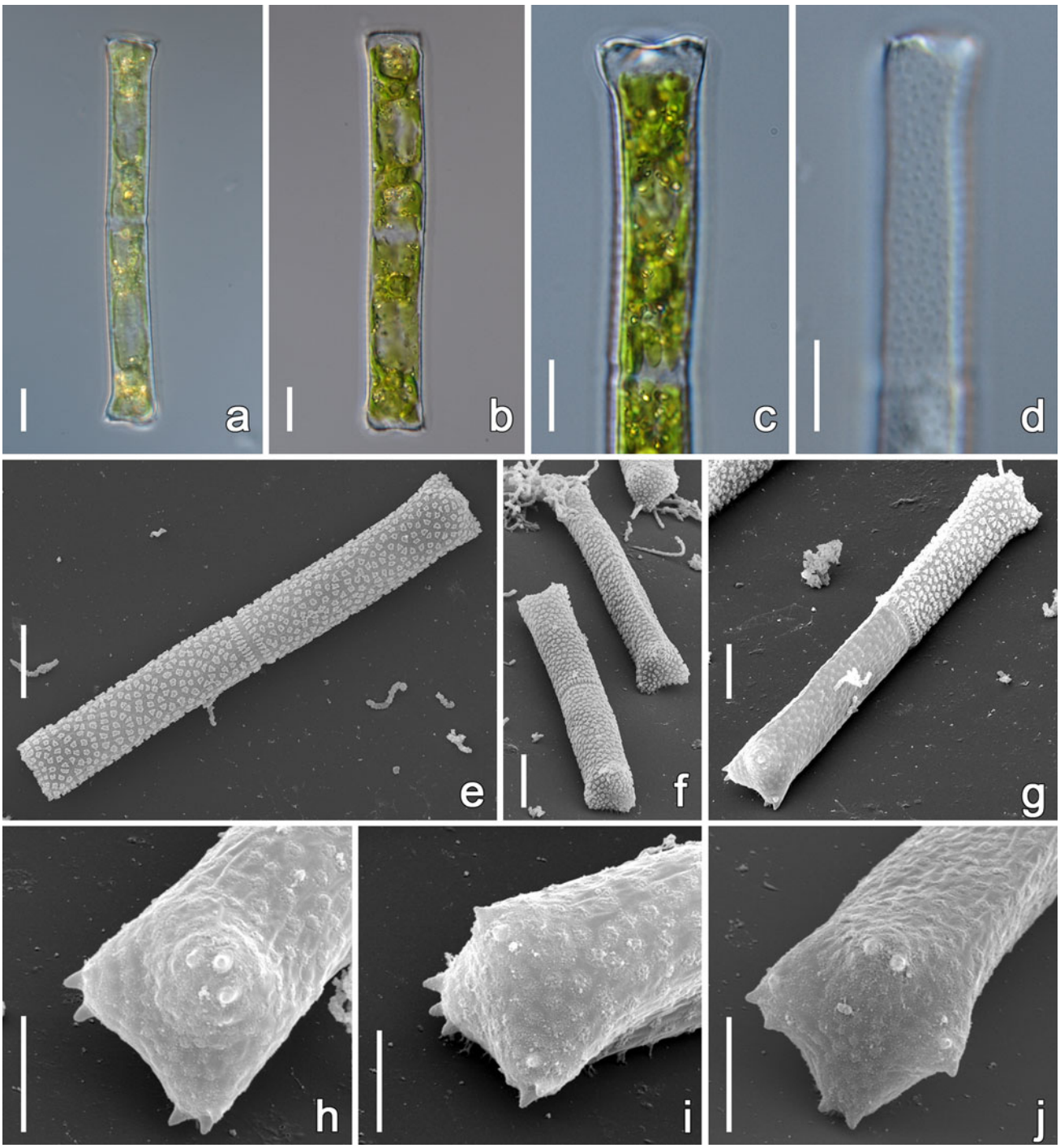


Fig. 1 Morphology of *Triplastrum simplex* (Q13). **a** Morphology of a cell found in the natural sample, **b** morphology of a cultured cell. Note the sheath envelope surrounding the cell, **c** detail of one semicell containing two plastids with distinct pyrenoids, **d** empty semicell showing cell-wall ornamentation, **e** cell-wall ornamentation, **f** apical parts of two *Triplastrum* cells, typically elongated into three terminal lobes (note triradiate outline of the rear cell), **g** triradiate *Triplastrum* cell, **h** three terminal lobes bearing two or three spines, **i** terminal lobes bearing three spines, **j** four terminal lobes. *Scale bar* = 10 μm (Fig. 1a–g) or 5 μm (Fig. 1h–j)

broad) and by a ring of small granules that were typically visible along the semicell apices. Five additional *Pleurotaenium* strains could not be allocated with certainty to any of the previously described species on the basis of the traditional morphological delimitation criteria, for example semicell shape, dimensions, or cellular apex ornamentation (Fig. 2k). Therefore, these strains were tentatively labelled as *Pleurotaenium* sp.

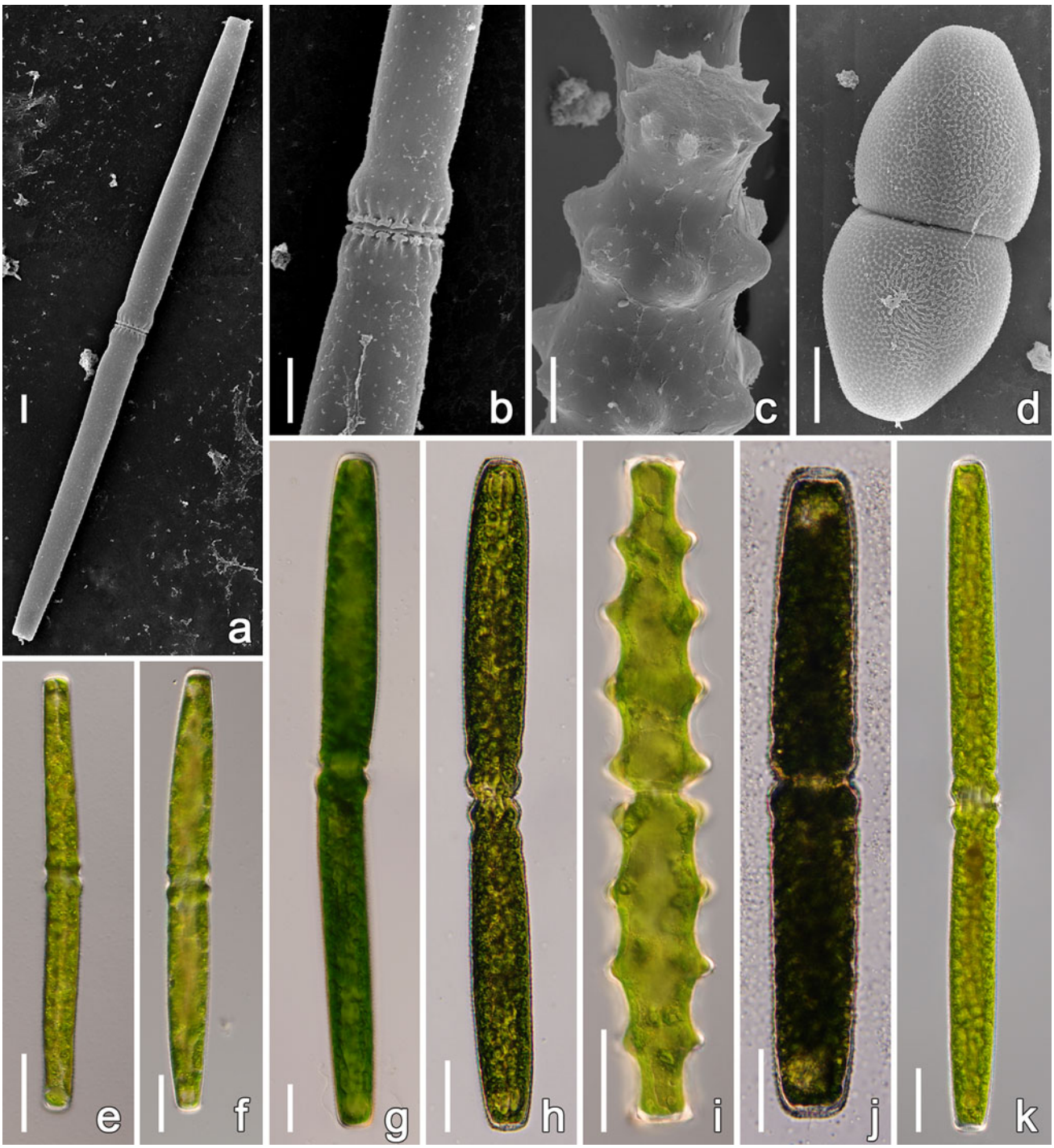


Fig. 2 Morphology of baculiform taxa *Docidium* and *Pleurotaenium*. **a**, **b** *Docidium baculum* (I1), **a** overall morphology, **b** middle of the cell showing a whorl of longitudinal, granule-like plications at the basal semicell inflations. **c**–**k** *Pleurotaenium*, **c** apex of the cell bearing a whorl of tubercles (I3), **d** *P. ovatum* (J2), **e** *P. ehrenbergii* (579), **f** *P. crenulatum* (592), **g** *P. trabecula* (655), **h** *P. archeri* (H50), **i** *P. nodosum* (I8), **j** *P. nodulosum* (H37), **k** *Pleurotaenium* sp. (J–E9). *Scale bar* = 10 µm (Fig. 2**a**–**c**) or 50 µm (Fig. 2**d**–**k**)

The concatenated *rbcL* + *cox*III phylogenetic tree constructed by Bayesian inference on partitioned datasets inferred the investigated baculiform desmid species in three distantly related positions within Desmidiaceae (Fig. 3). *Triplastrum simplex* was nested within the firmly supported "omniradiate" lineage (sensu Gontcharov 2008), in a sister position to a morphologically very different taxon, *Spondylosium luetkemuelleri* Grönblad. The two strains of *Docidium baculum* were highly similar in their nucleotide sequences, forming a distinct lineage inferred

within the *Staurastrum/Micrasterias* clade (Fig. 3). This lineage was distantly related to two other baculiform strains, *Docidium undulatum* and *Haplotaenium minutum*, albeit with relatively low statistical support (BI/ML/MP support 0.86/–/88). Finally, all *Pleurotaenium* strains were inferred as closely related to each other, supporting the monophyly of the genus revealed by Hall et al. (2008) and Gontcharov and Melkonian (2011). With the single exception of the morphologically distinct *P. ovatum*, all the other *Pleurotaenium* strains formed a single, strongly supported lineage within Desmidiaceae. *P. ovatum* was placed in a sister position to the *Pleurotaenium* lineage but with very low statistical support (0.76/–/–).

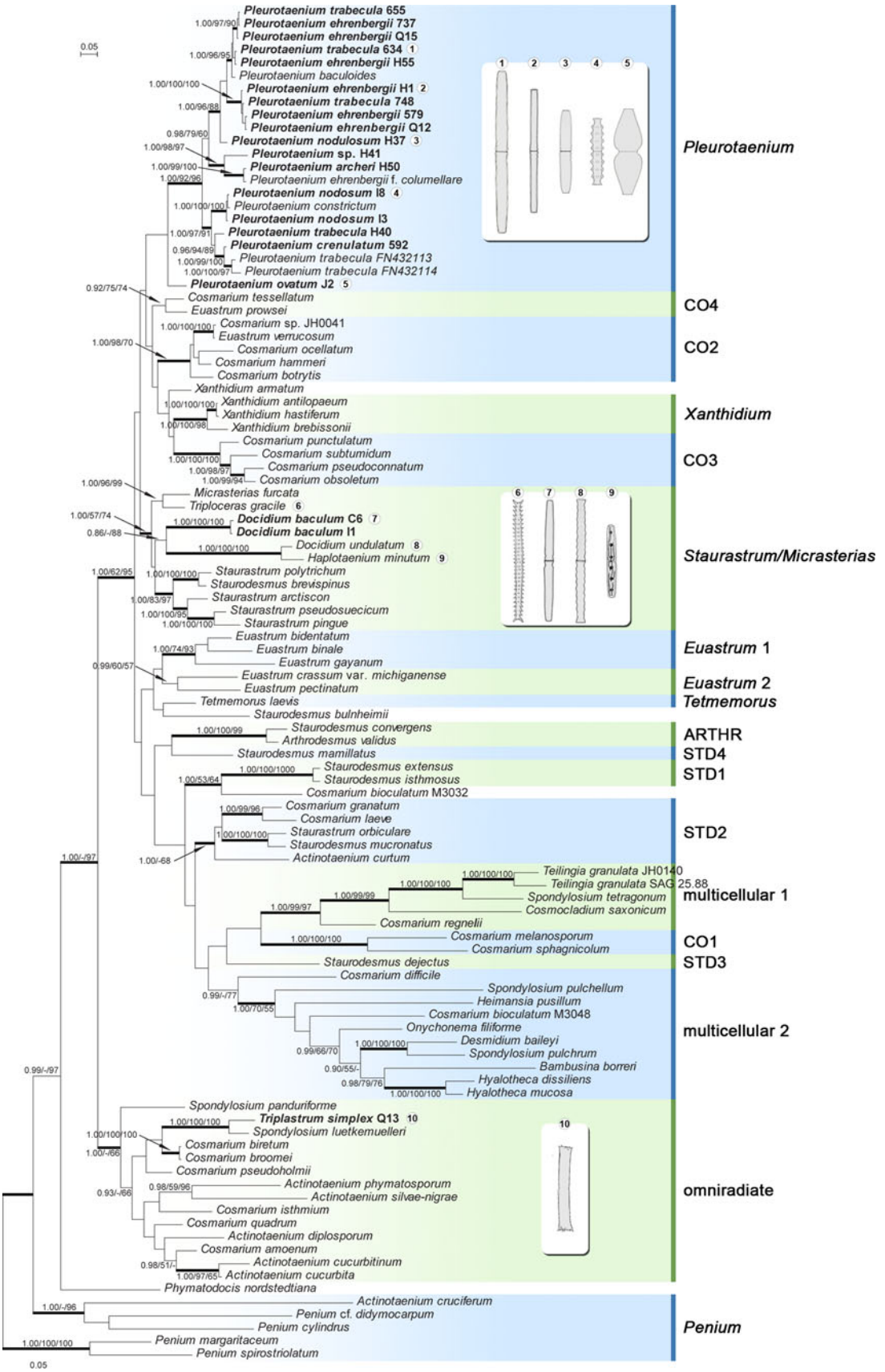
To resolve better phylogenetic relationships among *Pleurotaenium* strains, we also analysed the *trn*G^{ucc} sequences obtained for 24 *Pleurotaenium* strains (Fig. 4). The *trn*G^{ucc} phylogeny was in agreement with that of the *rbcL* + *cox*III phylogram, generally revealing poor species concept in this genus. The most common species of the genus *P. ehrenbergii* and *P. trabecula* were resolved as polyphyletic because their strains were distributed among several strongly supported clades in the tree. However, two strains of *Pleurotaenium nodosum*, typically with the prominent knot-like projections (Fig. 2i), formed a separate firmly supported lineage within the genus. Similarly, the two strains of *Pleurotaenium archeri* also clustered together and formed a distinct clade of the *Pleurotaenium* lineage.

Discussion

In total, four species (i.e., *T. abbreviatum*, *T. indicum*, *T. simplex*, and *T. spinulosum*) have been described within the genus *Triplastrum*. However, their separate status remained unclear because reliable characters for their discrimination are lacking. Růžička (1977) suggested that the number of plastids may possibly be used for species delimitation. However, there were 2–4 plastids in our samples including the clonal strains. Similar variation in plastid number was also observed by Claassen (1977) so this character can hardly be regarded as sufficiently stable for species discrimination. At the same time, overall cell shape, especially subapical narrowing of semicells, was used for distinguishing *T. spinulosum* var. *africanum* by Gauthier-Lièvre (1960). However, Couté and Rousselin (1975) reported high variability of this feature and suggested that *T. spinulosum* var. *africanum* may be conspecific with other varieties of this species. We certainly cannot disprove their view. In our material, the straight rod like cells and the cells with narrowed subapical parts were both present. Most taxonomic value was attributed to the varying number of apical spines on terminal cell lobes among different morphologically defined species (Krieger 1937; Hinode 1952; Gauthier-Lièvre 1960). However, we ascertained that this character was highly variable both in natural populations and cultures. The number of spines varied from one to three, with most lobes bearing two apical spines. Therefore, our strains could be identified as *T. simplex*, in agreement with the original description of Allorge (1924) from Lac de Grand-Lieu close to Nantes (Pays de la Loire, France). However, Kouwets (1998) identified his specimens from nearby Aquitaine as *T. spinulosum* var. *indicum* because the cells frequently had 3–4 terminal spines on the apical lobes. Interestingly, in our samples the cells bearing more than two spines were sometimes also present. Therefore, our observations certainly do not contradict the hypothesis of Coesel and Van Geest (2008) that all findings of *Triplastrum* may be of a single species. However, bearing in mind that cryptic and pseudocryptic species differentiation may apparently not be rare among desmids (Gontcharov and Melkonian 2008; Neustupa et al. 2010; Nemjová et al. 2011; Neustupa et al. 2011), we would not like to formally synonymise these traditional species before molecular data are available for populations from different parts of the world. We should note that this matter may further be complicated by the fact that Iyengar and Ramanathan (1942) did not formally establish a type species for the genus *Triplastrum*.

Our concatenated *rbcL* + *cox*III phylogenetic tree of Desmidiaceae (Fig. 3) generally concurred with previously published desmidiacean phylogenies (Gontcharov 2008; Hall et al. 2008; Gontcharov and Melkonian 2011). The family Desmidiaceae consisted of approximately 20 independent lineages, partly corresponding to individual traditional genera. The traditional genus *Triplastrum* forms a part of the "omniradiate" lineage of Desmidiaceae as defined by Gontcharov and Melkonian (2008, 2011). Because this lineage consists of species with profoundly different morphology, traditional taxonomy placed them into different genera. The only character shared by many of the strains included in this lineage was the omniradiate symmetry of cells in their apical view. The morphology of

Fig. 3 Bayesian analysis based on the combined and partitioned *rbc*L + *cox*III dataset of Desmidiaceae. Four partitions were analysed separately by using a GTR + Γ model for the first and second codon positions of the *rbc*L gene, GTR + Γ model for third codon position of the *rbc*L gene, HKY + Γ model for first and second codon positions of the *cox*III gene, and GTR + Γ model for third codon position of the *cox*III gene. *Values* at the nodes indicate statistical support estimated by three methods—MrBayes posterior-node probability (*left*), maximum-likelihood bootstrap (*middle*), and maximum-parsimony bootstrap (*right*). Thick branches represent nodes receiving the highest PP support (1.00). Species affiliation to 19 desmid clades sensu Gontcharov and Melkonian (2011) is indicated. Morphology of the selected investigated strains is given in the boxes and linked to corresponding sequences by numbers. *Scale bar* shows the estimated number of substitutions per site


Pleurotaenium trabecula 655
Pleurotaenium ehrenbergii 737
Pleurotaenium ehrenbergii Q15
Pleurotaenium trabecula 634
Pleurotaenium ehrenbergii H55
Pleurotaenium baculoides
Pleurotaenium ehrenbergii H1
Pleurotaenium trabecula 748
Pleurotaenium ehrenbergii 579
Pleurotaenium ehrenbergii Q12
Pleurotaenium nodulosum H37
Pleurotaenium sp. H41
Pleurotaenium archeri H50
Pleurotaenium ehrenbergii f. columellare
Pleurotaenium nodosum I8
Pleurotaenium constrictum
Pleurotaenium nodosum I3
Pleurotaenium trabecula H40
Pleurotaenium crenulatum 592
Pleurotaenium trabecula FN432113
Pleurotaenium trabecula FN432114
Pleurotaenium ovatum J2
Pleurotaenium
Cosmarium tessellatum
Euastrum prowsei
CO4
Cosmarium sp. JH0041
Euastrum verrucosum
Cosmarium ocellatum
Cosmarium hammeri
Cosmarium botrytis
CO2
Xanthidium armatum
Xanthidium antilopaeum
Xanthidium hastiferum
Xanthidium brebissonii
Xanthidium
Cosmarium punctulatum
Cosmarium subtumidum
Cosmarium pseudoconnatum
Cosmarium obsoletum
CO3
Micrasterias furcata
Triploceras gracile
Docidium baculum C6
Docidium baculum I1
Docidium undulatum
Haplotaenium minutum
Staurastrum polytrichum
Staurodesmus brevispinus
Staurastrum arctiscon
Staurastrum pseudosuecicum
Staurastrum pingue
Staurastrum/Micrasterias
Euastrum bidentatum
Euastrum binale
Euastrum gayanum
Euastrum 1
Euastrum crassum var. michiganense
Euastrum pectinatum
Euastrum 2
Tetmemorus laevis
Tetmemorus
Staurodesmus bulnheimii
Staurodesmus convergens
Arthrodesmus validus
ARTHR
Staurodesmus mamillatus
STD4
Staurodesmus extensus
Staurodesmus isthmosus
STD1
Cosmarium bioculatum M3032
Cosmarium granatum
Cosmarium laeve
Staurastrum orbiculare
Staurodesmus mucronatus
Actinotaenium curtum
STD2
Teilingia granulata JH0140
Teilingia granulata SAG 25.88
Spondylosium tetragonum
Cosmocladium saxonicum
multicellular 1
Cosmarium regnellii
Cosmarium melanosporum
Cosmarium sphagnicolum
CO1
Staurodesmus dejectus
STD3
Cosmarium difficile
Spondylosium pulchellum
Heimansia pusillum
Cosmarium bioculatum M3048
Onychonema filiforme
Desmidium baileyi
Spondylosium pulchrum
Bambusina borreri
Hyalotheca dissiliens
Hyalotheca mucosa
multicellular 2
Spondylosium panduriforme
Triplastrum simplex Q13
Spondylosium luetkemuelleri
Cosmarium biretum
Cosmarium broomei
Cosmarium pseudoholmii
Actinotaenium phymatosporum
Actinotaenium silvae-nigrae
Cosmarium isthmium
Cosmarium quadrum
Actinotaenium diplosporum
Cosmarium amoenum
Actinotaenium cucurbitinum
Actinotaenium cucurbita
omniradiate
Phymatodocis nordstedtiana
Actinotaenium cruciferum
Penium cf. didymocarpum
Penium cylindrus
Penium margaritaceum
Penium spirostriolatum
Penium
0.05

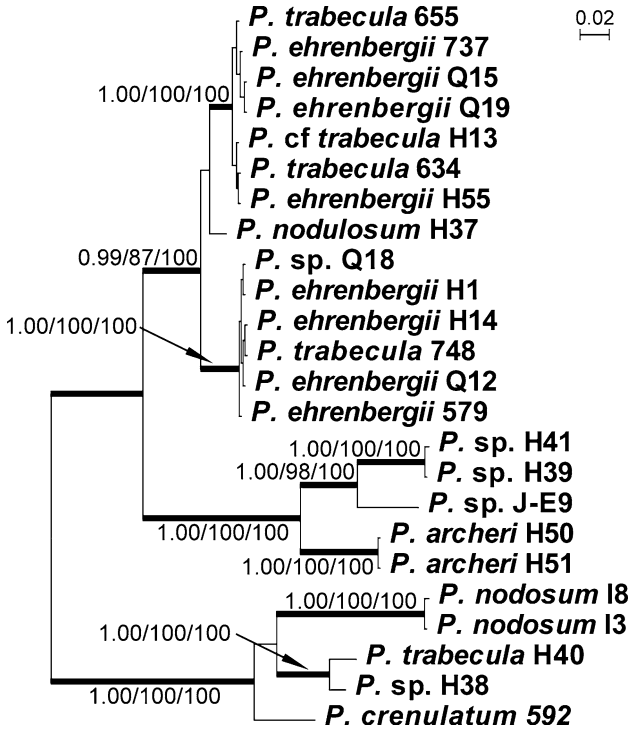


Fig. 4 Bayesian analysis of *Pleurotaenium trn*G^{ucc} sequences, using a GTR + Γ nucleotide substitution model. *Values* at the nodes indicate statistical support estimated by three methods—MrBayes posterior-node probability (*left*), maximum-likelihood bootstrap (*middle*), and maximum-parsimony bootstrap (*right*). *Thick branches* represent nodes receiving the highest PP support (1.00). *Scale bar* shows the estimated number of substitutions per site

the genus *Triplastrum* seems to corroborate this basic morphological delimitation, because the cells usually have cylindrical outlines, differentiating into 3 or 4 terminal lobes (Claassen 1977; Kouwets 1998; this study). However, our SEM investigations occasionally revealed that more or less distinctly triradiate *Triplastrum* semicells may also be formed (Fig. 1f, g). Gontcharov and Melkonian (2008, 2011) suggested that the "omniradiate" lineage may be defined as a separate genus on the basis of their morphology (cylindrical cell outline) and the presence of non-homoplasious synapomorphy consisting of two substitutions in the spacer of helix 25 in the SSU rRNA molecule. However, our morphological investigations did not detect any simple morphological criterion for delimitation of this lineage. Therefore, should the "omniradiate" lineage of Desmidiaceae eventually be described as a separate genus, it may be defined solely on the basis of molecular data. Interestingly, *Triplastrum* was recovered in a sister position to a sequence depicted as *Spondylosium luetkemuelleri* by Gontcharov and Melkonian (2011). It was originally described by Grönblad (1938) from Finland to accommodate natural populations of distinctly *Cosmarium*-like desmids that formed loose filaments resembling other members of the filamentous genus *Spondylosium* Brébisson ex Kützing. However, this species is morphologically entirely different from *Triplastrum* and from the other members of the "omniradiate" clade. Unfortunately, neither clonal strain nor a microphotograph drawing or natural sample of desmid

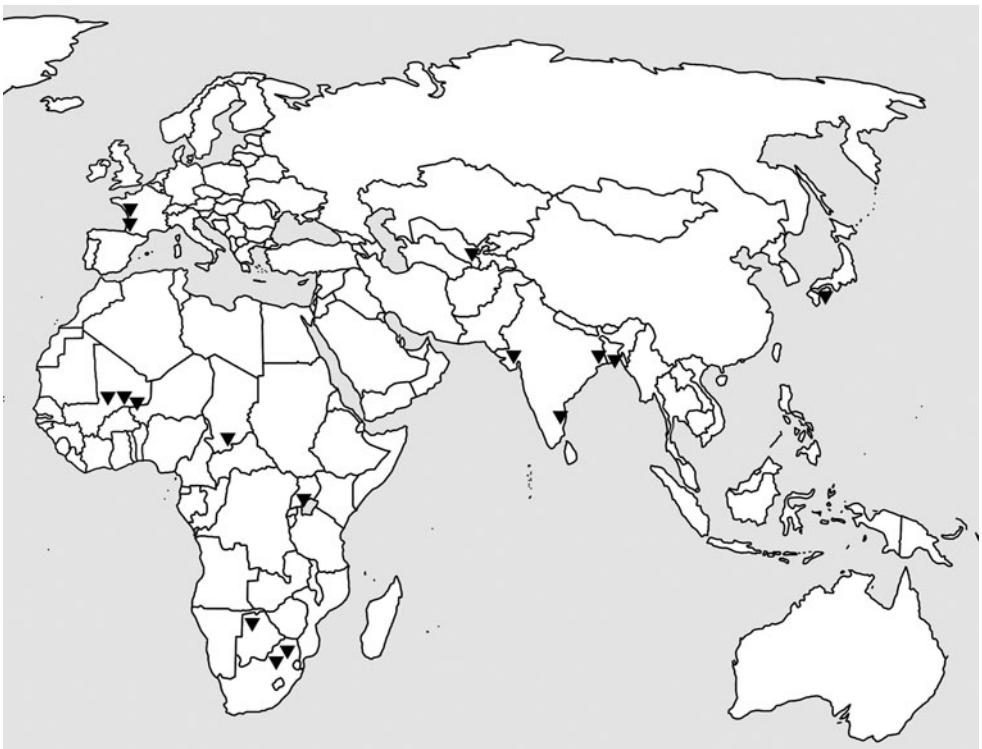


Fig. 5 Geographic distribution of the genus *Triplastrum* showing its presumed occurrence in warmer regions of the old world

related to the *rbcL* sequence (FN432115) deposited by Gontcharov and Melkonian (2011) is available. The original locality of their sampling is also unknown. Therefore, taxonomic affiliation of the closest known relative of *Triplastrum simplex* remains speculative. Molecular characterization of additional *S. luetkemuelleri* strains or natural populations is sorely needed for elucidation of this interesting and rather improbable relationship.

Geographic distribution of many desmids, which is based on numerous floristic accounts, has been well documented (Coesel 1996; Coesel and Krienitz 2008). The genus *Triplastrum*, being distinctly different from other desmids, could possibly not be overlooked for individual investigations. Conversely, it has repeatedly been reported from regions where it is probably widely distributed, for example the Niger basin (Gauthier-Lièvre 1960; Couté and Rousselin 1975), eastern parts of India and Bangladesh (Iyengar and Ramanathan 1942; Islam 1980; Islam and Akter 2005), or western and southwestern France (Allorge 1924; Kouwets 1998; this study). Published records restrict the currently known distribution of *Triplastrum* to Africa, Asia, and Europe (Fig. 5). However, in Europe, a continent with most detailed desmidiacean distribution data available, any future reports of *Triplastrum* outside Aquitaine or other adjacent regions of Western France would be rather surprising. The genus *Triplastrum* is, apparently, a warm-water lineage, and it is probably missing from regions with pronounced annual freezing periods. Even the northern-most findings from France (Allorge 1924; Kouwets 1998; this study), southern Japan (Hinode 1952), or Uzbekistan (Kisselev 1930) originated from localities with warm temperate or subtropical climate. In Lac de Cazaux, it has always been encountered in late summer phytoplankton, i.e., in a period with the highest water temperatures. In cultures, it virtually did not grow at temperatures below 20 °C, and growth was apparently more rapid in temperatures above 25 °C. Undoubtedly, the most natural locations of *Triplastrum* are in tropical habitats. However, in many tropical locations the genus still remains rare and infrequent (Turner 1892; Couté and Rousselin 1975).

The individual lineages of baculiform desmids did not form a clade within Desmidiaceae. Therefore, we can conclude that the rod like morphology evolved repeatedly several times within desmids. Our study confirmed the moderately supported lineage including species traditionally allocated to *Docidium* and *Haplotaenium*. Gontcharov and Melkonian (2011) also illustrated monophyly of these taxa on the basis of *rbcL* sequence data but with no bootstrap support. Interestingly, our strains of *Docidium baculum* from Ireland and the Czech Republic were recovered in a sister position to the firmly supported clades of *Docidium undulatum* and *Haplotaenium minutum*. Therefore, we suggest that the traditional genus *Haplotaenium* may, in fact, be nested within *Docidium*.

In accordance with previous results of Hall et al. (2008) and Gontcharov and Melkonian (2011), the broadly sampled traditional genus *Pleurotaenium* was revealed as a monophyletic lineage within Desmidiaceae. Even the morphologically distinct species *P. nodosum* and *P. ovale* were recovered within this *Pleurotaenium* generic lineage. Interestingly, clearly higher species diversity within *Pleurotaenium* was indicated by the *trn*G^{ucc}-based phylogenetic tree than may be apparent on the basis of morphological data. Two traditional species with relatively few discriminating morphological features, *P. ehrenbergii and P. trabecula*, were found to be polyphyletic, and they will have to be split into several phylogenetic species in the future. There were at least two well-defined phylogenetic species-level lineages including strains with both *P. ehrenbergii* and *P. trabecula* morphology. Likewise, an additional independent clade that included a single strain of *P. trabecula* and an unidentified *Pleurotaenium* sp. strain was also recovered. However, these individual species lineages may also be morphologically discerned after a more detailed study of a large number of strains. Meanwhile, we can conclude that traditional morphological characters distinguishing *P. trabecula* and *P. ehrenbergii*, for example apex morphology and presence or absence of apex granules, are probably not valid. Similar pseudocryptic species diversity was recently identified in the genus *Micrasterias* (Neustupa et al. 2010, 2011). However, individual species-level lineages were defined on the basis of their biogeographic and micro-morphological differences. These characters might also be useful for distinguishing phylogenetic species of the genus *Pleurotaenium*.

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Paper VI

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

Jiří Neustupa, Jan Šťastný, Katarína Nemjová, Petra Mazalová, Emma Goodyer, Aloisie Poličková, Pavel Škaloud & Jana Veselá

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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)

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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}

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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

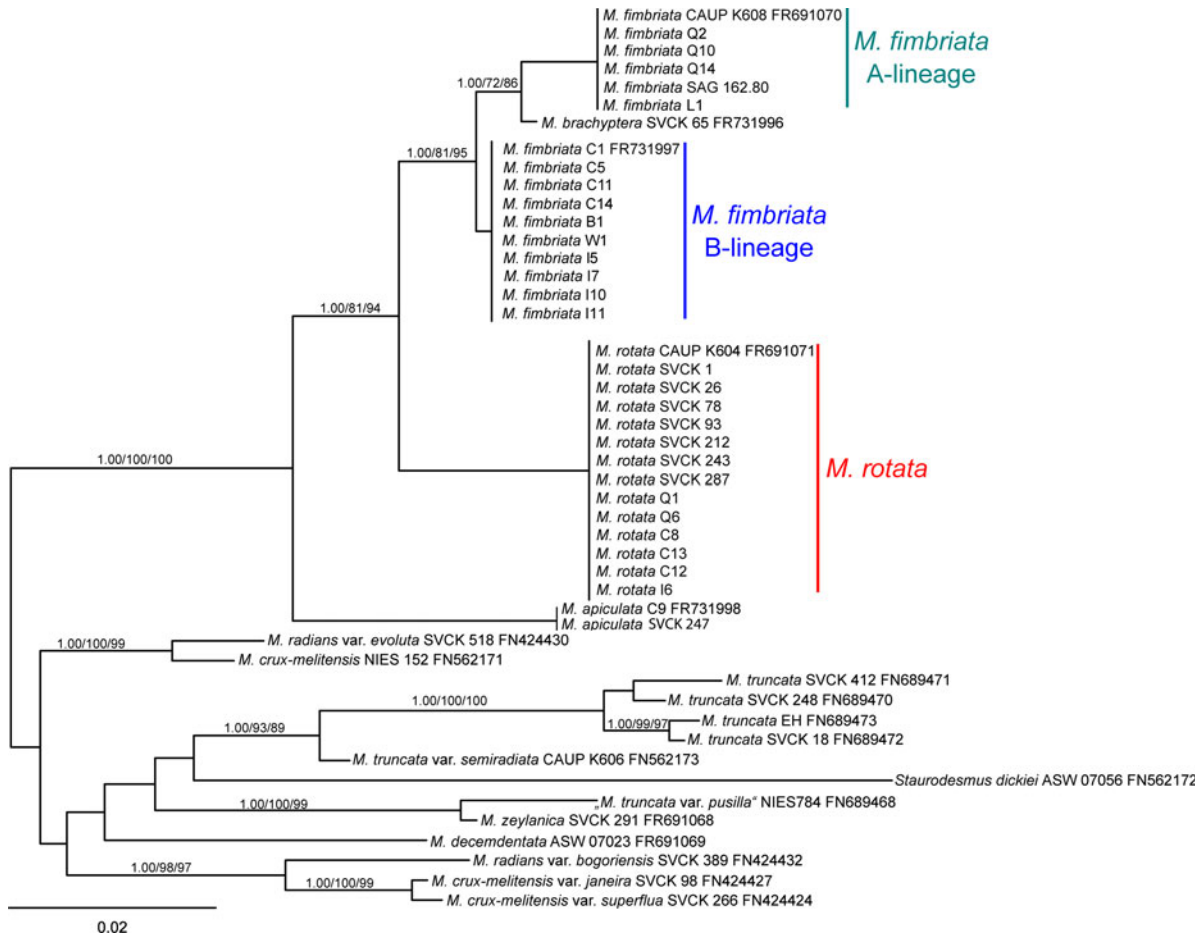


Fig. 1 Unrooted Bayesian phylogenetic tree of *trnG*^{ucc} sequences. The *scale bar* shows the estimated number of substitutions per nucleotide. The posterior probabilities lower then 0.70 and bootstrap support levels below 50% are omitted. The indicators of statistical significance are provided as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

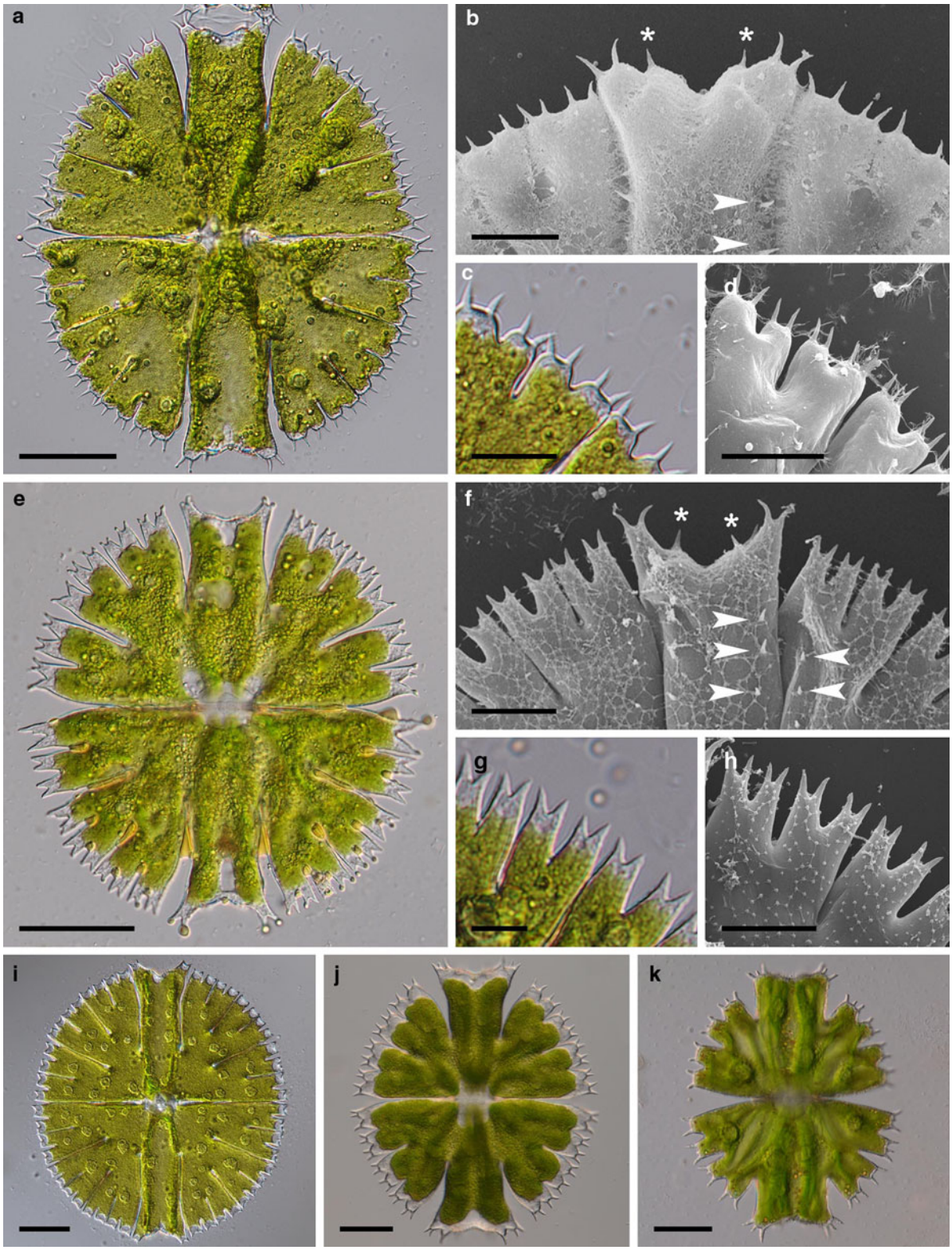
Morphology and geometric morphometrics

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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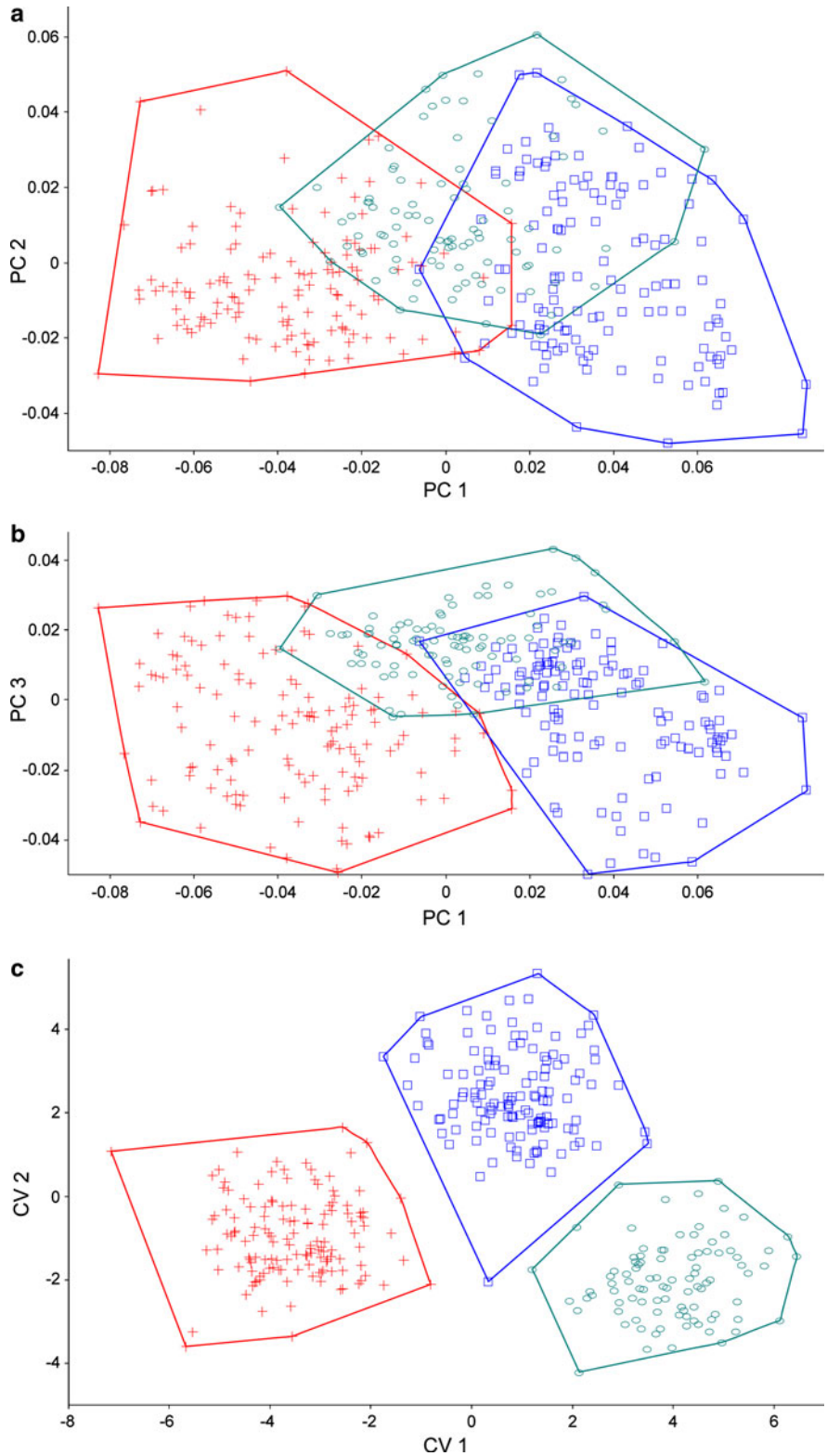

Fig. 3 The PCA and CVA ordination plots of geometric morphometric data of *Micrasterias rotata*, and *M. fimbriata* (A- and B-lineages) semicells. The PC1 versus PC2 (**a**), PC1 versus PC3 (**b**), and CV1 versus CV2 plots (**c**) are depicted. *Crosses*: *M. rotata*, *ellipses*: A-lineage of *M. fimbriata*,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

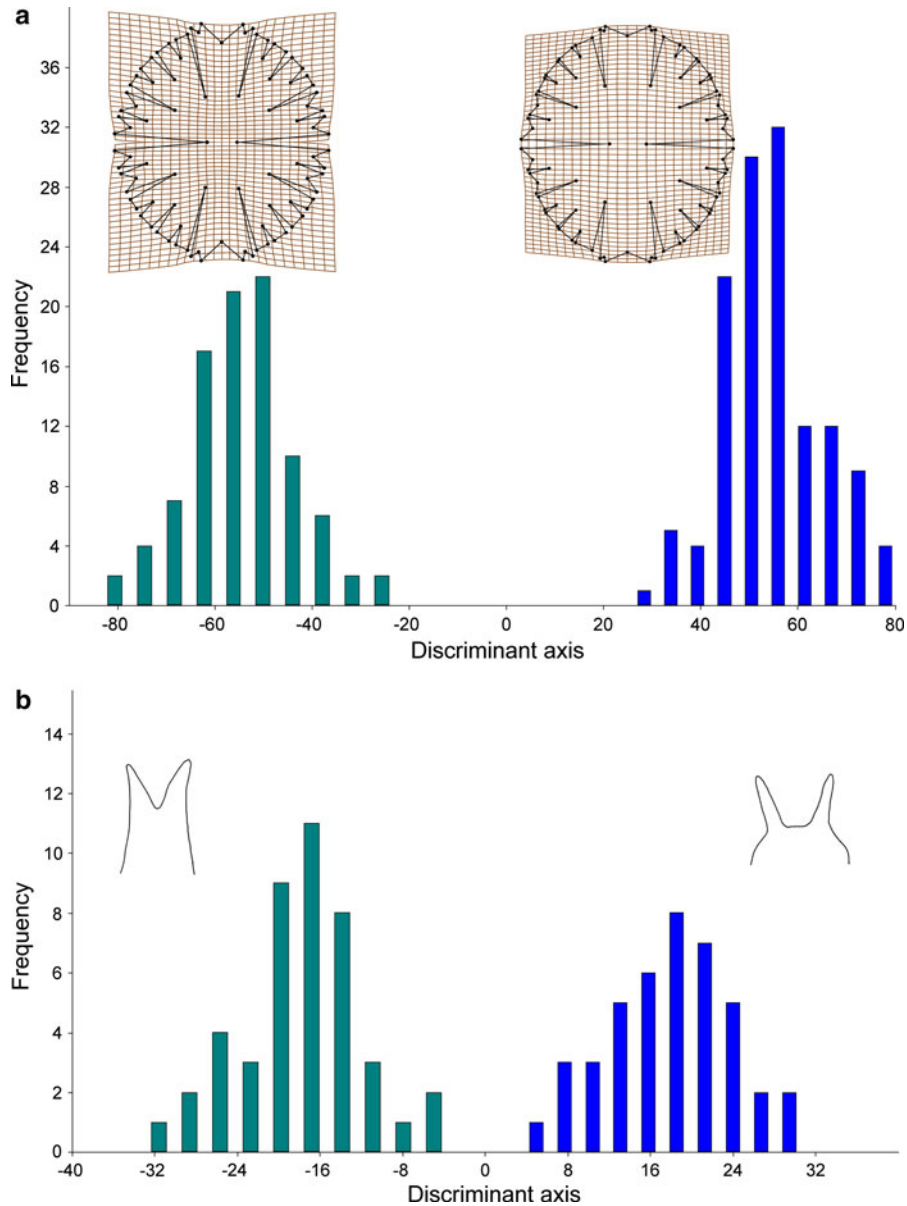

Fig. 4 The linear discrimination analyses of geometric morphometric data of *M. fimbriata* strains. The landmarks analyses of semicells (**a**), and terminal lobules (**b**) are depicted. The A-lineage of *M. fimbriata* is depicted in *left bars*, the B-lineage in *right bars*

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

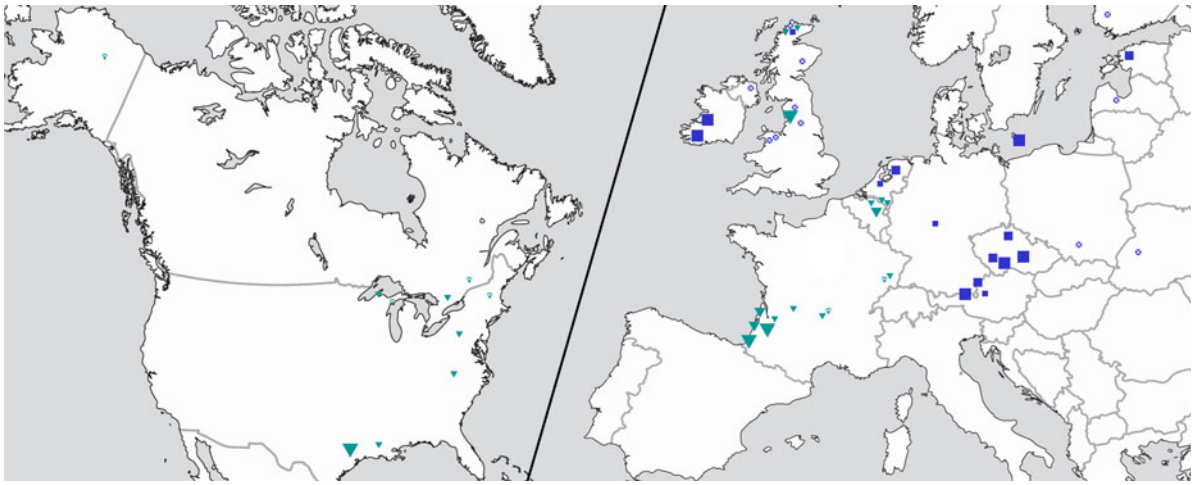

Fig. 5 The *map* illustrating presumptive distribution of A- and B-lineages of *M. fimbriata* in Europe and North America. *Triangles* represent A-lineage and *squares* represent B-lineage findings. *Large symbols* indicate localities of clonal strains, *middle-sized symbols* indicate localities of investigated 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.

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Paper VII

Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*

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Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*

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SUMMARY

The morphological and molecular differentiation of the *Micrasterias truncata* (Corda) ex Bréb species complex was investigated. In total, 17 strains traditionally assigned to *M. truncata* were isolated from different European localities (Czech Republic, southwest France, Ireland), and obtained from public culture collections. In addition, strains of the morphologically similar species, *M. decemdentata* (Nägeli) W. Archer and *M. zeylanica* F. E. Fritsch, were also included. Molecular phylogenetic analysis based on trnG^{ucc} intron sequences revealed five well supported clades. Two Australian strains assigned to *M. truncata* var. *pusilla* G. S. West formed a lineage sister to *M. zeylanica*. This was evident from a concatenated phylogeny based on small subunit rDNA and trnG^{ucc} intron sequences. The isolated position of these strains was also illustrated by parallel landmark-based geometric morphometric analysis of cell shapes. The strains NIES 783 and NIES 784 probably represent a separate species. Particular analysis, including additional strains, is needed to resolve the relationship inside this lineage. The second phylogenetic lineage, containing two strains of *M. truncata* var. *semiradiata* (Kützing) Wolle, was also different from other strains on the basis of morphometric data. We suggest recognizing this variety as a separate species, *Micrasterias semiradiata* L.A. Brébisson ex F. T. Kützing. The remaining three clades formed a firmly supported group of the 'core' *M. truncata* recognized by both molecular markers. However, neither any morphological, morphometric, nor geographical pattern was detected among members of these three clades. This pattern could be caused by a relatively recent origin of these lineages that may represent a sympatric, truly cryptic species. Strains attributable to traditional morphologically defined variety *M. truncata* var. *neodamensis* were nested within the 'core' *M. truncata*.

Key words: cryptic diversity, *Desmidiales*, geometric morphometrics *Micrasterias truncata*, small subunit rDNA, species concept, trnG^{ucc} intron.

INTRODUCTION

Desmids belong to the Zygnematophyceae, a class that represents the most diversified algal streptophycean lineage (Lewis & McCourt 2004). They are known for their high morphological diversity and about 4000 species were described (Gontcharov 2008). Almost all of the species were traditionally defined on the basis of morphological characters. However, cross-breeding experiments illustrated that many traditional morphospecies, such as the *Closterium ehrenbergii* Meneghini ex Ralfs/ *moniliferum* Ehrenberg ex Ralfs complex (Denboh *et al*. 2003), or *Micrasterias thomasiana* W. Archer (Blackburn & Tyler 1987), comprise several reproductively isolated possibly cryptic entities. Recently, desmid taxonomy has gained significant new insights through the application of molecular methods. Most of the traditional species-rich genera have been found to be polyphyletic (e.g. *Cosmarium, Staurastrum* or *Euastrum*) or paraphyletic (e.g. *Micrasterias*) (Gontcharov & Melkonian 2005, 2008; Gontcharov 2008). A more robust outline of the phylogenetic relationships of the Desmidiales has begun to emerge on the basis of multigene molecular studies of the group (Gontcharov *et al*. 2004; Hall *et al*. 2008). Although many of the morphological generic characters were shown to be artificial, the validity of traditional desmidiacean species concepts is still puzzling. Denboh *et al*. (2003) illustrated considerable phylogenetic differentiation within a lineage corresponding to traditional *Closterium ehrenbergii*. However, the morphological differences among lineages within the traditional morphospecies were not evaluated. The only study that concentrated on molecular species concepts in the genus *Micrasterias* illustrated pseudocryptic diversity within the traditional species *M. crux-melitensis* Ralfs and *M. radians* W. B. Turner (Neustupa *et al*. 2010).

The genus *Micrasterias* is a widely distributed and well-known desmid genus, characterized by complex semicells divided into three lobes (Krieger 1939; Prescott *et al*. 1977; Růžička 1981). In the present study, we concentrated on the phylogenetic and morphological structures within the traditional species *M. truncata*.

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This species is one of the most frequently encountered desmids (Růžička 1981; Coesel & Meesters 2007), and a number of traditional intraspecific taxa have been described. Their morphology differs from the type variety of the species, but, in many cases, intermediate forms have been reported (Prescott *et al*. 1977). In total, approximately 51 intraspecific taxa have been described (Guiry & Guiry 2010). However, Růžička (1981), in his treatise of European desmids, accepted just five of them and concluded that many of the previously established varieties and forms may simply represent ecomorphs or dichotypic specimens. The species as a whole has a world-wide distribution, but some of the varieties apparently have restricted distribution, e.g. *M. truncata* var. *pusilla* in (sub-) tropical regions (Krieger 1939; Prescott *et al*. 1977). Several species, such *as M. zeylanica* and *M. decemdentata*, are morphologically similar to *M. truncata* and their phylogenetic and taxonomic relation is unclear (Tyler 1970; Růžička 1981).

In this study, we focused on infraspecific variation within this morphologically diverse species. We examined 17 *M. truncata* strains that were isolated from natural samples or acquired from public culture collections. The strains were morphologically evaluated according to the traditional criteria, and, if possible, identified to variety level. We used the polyphasic approach to assess phylogenetic diversity in morphologically defined *M. truncata*, and investigated gene sequence data of two independent molecular markers. The plastid encoded RNA-Gly transfer gene intron ($trnG^{ucc}$) has previously been used in a phylogenetic study of the *Micrasterias crux-melitensis*/*radians* species complex (Neustupa *et al*. 2010), and shown to be useful for species delimitation. In addition, we used the small subunit (SSU) rDNA sequences to identify the phylogenetic relationships among *M. truncata* lineages and other related *Micrasteria*s species. In parallel, morphological diversity was assessed using geometric morphometrics and scanning electron microscopy (SEM). Geometric morphometrics is a quantitative method for the evaluation of biological shapes, based on the Procrustes superimposition (Zelditch *et al*. 2004). It has recently been used in several studies investigating morphological variation in microalgae, e.g. diatoms (Rhode *et al*. 2001; Beszteri *et al*. 2005; Potapova & Hamilton 2007), as well as in the genus *Micrasterias* (Neustupa & Škaloud 2007; Neustupa *et al*. 2008, 2010). Summing up, we asked the following questions:

1 Do the clonal strains of *M. truncata* morphologically correspond to individual varieties described by traditional taxonomy (mostly on the basis of uncultured material)?
2 Is this traditionally defined species phylogenetically homogenous? If not, can the individual lineages be morphologically recognized?
3 Do they follow some obvious geographical distributional patterns?

MATERIALS AND METHODS

Isolation and cultivation of strains, LM and SEM observations

Strains of *M. truncata* were obtained from four culture collections (SVCK, CAUP – our own isolates, ASW and NIES), and complemented with single-cell isolates from different natural habitats (Table 1). The cultures for morphometric comparisons were initiated with 10–15 cells and grown for 6 weeks in 100 mL Erlenmeyer flasks in liquid oligotrophic medium used in the CAUP culture collection (for details see http://botany.natur.cuni.cz/algo/caup-media.html). Strains were maintained at temperatures of 20°C to 22°C and continuously illuminated at 40 μmol m^{-2} s^{-1} from 18 W cool fluorescent tubes (Philips TLD 18W/33). Microphotographs were taken under an Olympus BX51 (Olympus, Tokyo, Japan) light microscope with an Olympus Z5060 camera. Projection of light microscopic images was made using the Deep Focus 3.0 module implemented in the QuickPHOTO CAMERA 2.3 software. For scanning electron microscopy (SEM) acetone-washed glass coverslips were placed on a heating block, and coated three times with a poly-L-lysine solution (1:10 in deionized water) to ensure appropriate adhesion of the cells. Then, a drop of the formaldehyde-fixed cell suspension was placed on the glass, transferred into 30% acetone, and dehydrated by an acetone series. Subsequently, cells were dried to a critical-point with liquid CO_2. Finally, they were sputter coated with gold and examined with a JEOL 6380 LV scanning electron microscope.

Geometric morphometrics

For each strain, we photographed 50 randomly chosen mature semicells. In total, 33 fixed or sliding landmarks were depicted on each semicell. In addition, landmarks were digitized on semicells of nine taxa illustrated in several authoritative taxonomic monographs (Krieger 1939; Růžička 1981). We included all the varieties of *M. truncata* recognized by Růžička (1981). In addition, we analyzed the type drawing of *M. truncata* var. *pusilla*, and figures of *M. decemdentata* and *M. zeylanica* (primarily for comparison with the NIES 783 and NIES 784 strains).

For most of the geometric morphometric analyses the TPS-series software (Rohlf 2008) was used. The positions of landmarks were digitized in TpsDig, ver. 2.12. The configurations of landmarks from 850 cells belonging to 17 strains were superimposed by the generalized Procrustes analysis (GPA) in TpsRelw, ver.

Table 1. List of the strains used in this study

Strains	Species	Location	Accession number trnG^{ucc}	SSU rDNA	Geographic coordinates	Isolation date
†SVCK 18	*Micrasterias truncata*	Wildes Moor Schwabstedt by Husum, Germany	FN689472	FN689475	54°25′N 09°15′E	1962
SVCK 51	*M. truncata*	Zeller Loch by Fulda, Germany	Same as FN689471	–	50°30′46.78″N 09°37′15.37″E	1963
SVCK 248	*M. truncata* var. *neodamensis*	Moore um Pfronten im Allgäu, Germany	FN689470	FN689474	–	1970
SVCK 291	*M. zeylanica*	Victoria or New South Wales, Australia	FR691068	FN689479	–	–
SVCK 359	*M. truncata*	King, England	Same as FN689470	–	–	1952
SVCK 412	*M. truncata* var. *neodamensis*	Laguna de Mucubaj¡, Paramo de Mucubaj, Merida, Venezuela	FN689471	FN689476		1995
‡NIES 783	*M. truncata* var. *pusilla*	Sydney, Centennial Park, Australia	Same as FN689468	–	–	1.9.1988
NIES 784	*M. truncata* var. *pusilla*	Near Cairns Queensland, Australia	FN689468	FN689478	–	09/1988
EH	*M. truncata*	Etang Hardy bog, Aquitaine, France	FN689473	–	43°43′08.83″N 01°22′09.75″W	03/2009
HS1	*M. truncata*	Pools by Hostens, Aquitaine, France	Same as FN689472	–	44°29′54.67″N 00°38′19.02″W	03/2009
HS2	*M. truncata*	Pools by Hostens, Aquitaine, France	Same as FN689471	–	44°29′55.03″N 00°38′22.31″W	03/2009
HS3	*M. fimbriata*	Pools by Hostens, Aquitaine, France	FR691070	–	44°30′00.34″N 00°37′53.58″W	03/2009
§CAUP K606	*M. truncata* var. *semiradiata*	Borkovická Blata bog, Czech Republic	FN562173	AM419211	49°14′10.89″N 14°37′21.23″E	05/2006
CAUP K604	*M. rotata*	Benthos of flooded quarry pools near Cep village, Czech Republic	FR691071	AM419209	48°55′24.86″N 14°50′20.95″E	08/2005
SL	*M. truncata*	Studna u Lužné bog, Czech Republic	Same as FN689470	–	50°06′22.74″N 12°16′36.53″E	05/2009
KT1	*M. truncata*	Kateřina bog, Czech Republic	Same as FN689470	–	50°09′16.23″N 12°24′28.13″E	05/2009
KT2	*M. truncata*	Kateřina bog, Czech Republic	Same as FN689470	–	50°09′16.23″N 12°24′28.13″E	05/2009
BR1	*M. truncata*	Břehyně wetland, Czech Republic	Same as FN689472	–	50°35′02.90″N 14°42′11.10″E	05/2009
BR2	*M. truncata* var. *semiradiata*	Břehyně wetland, Czech Republic	FN689469	–	50°34′36.91″N 14°42′42.04″E	05/2009
WL	*M. truncata*	The White Lakes, Ireland	Same as FN689472	–	53°26′12.42″N, 9°54′45.13″W	03/2009
¶ASW 07023	*M. decemdentata*	An unnamed peat bog, Austria	FR691069	FN689477	–	–

Strain designations (SVCK, NIES, CAUP and ASW) indicate the source culture collection: †SVCK – Sammlung von Conjugaten-Kulturen. ‡NIES Microbial Culture Collection, Japan. §CAUP Culture Collection of Algae of Charles University in Prague. ¶ASW Culture Collection of Algae, University of Vienna; nowadays deposited in the Culture Collection of Algae at the University of Cologne (CCAC).

1.42. This widely used method standardizes the size and optimizes the rotation and translation of the objects (landmark configurations) so that the distances among corresponding landmarks are minimized (Bookstein 1991; Zelditch *et al*. 2004). The sliding landmarks are allowed to slide along the abscissa connecting adjacent landmarks in the course of GPA so that they can be used for delimitation of outlines (Zelditch *et al*. 2004; Mitteroecker & Gunz 2009). 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 statistical analyses (Zelditch *et al*. 2004). In the dataset of landmark configurations from 850 *M. truncata* semicells this correlation was very high ($r = 0.999$), so we proceeded with further analyses. The symmetrization of left and right semicell halves was conducted by the standard method of Klingenberg *et al*. (2002), in the same way as in previous geometric morphometric studies of Desmidiales (Neustupa & Škaloud 2007; Neustupa *et al*. 2010).

The principal component analysis (PCA) of the entire set resulted in 62 axes describing the shape variation. The averages of the individual 17 strains were subjected to non-metric multidimensional scaling ordination analysis (NMDS), using the Euclidean distance matrix in PAST, ver. 1.90 (Hammer *et al*. 2001). The resulting two-dimensional ordination plot was used for graphical representation of the overall morphological relations among strains. The mean configurations of individual strains, and the comparative figures from the taxonomic monographs were used for the cluster analysis (using Ward's clustering method). The morphological separation between strains belonging to the *M. truncata* lineage and those of the *M. truncata* var. *semiradiata* lineage was tested by linear discrimination analysis (LDA) of the shape data depicted by the PC axes. The GPA-aligned shape data of the objects were regressed on the discriminant axis to illustrate the shapes features distinguishing these two lineages.

DNA isolation, PCR reaction, sequencing

Following disintegration of the mucilage enveloping the cells by ultrasonification, cell disintegration and DNA extraction was performed according to Neustupa *et al*. 2010 using the Invisorb Spin Plant Minikit (Invitek GmbH, Berlin, Germany). The trnG^{ucc} and SSU rDNA markers were amplified in 20 μL volumes of 13.9 μL of sterile Mili-Q water, 2 μL of $MgCl_2$ (25 μM), 2 μL of polymerase chain reaction (PCR) Buffer 10 × (Applied Biosystems, Life technologies, Carlsbad, CA, USA), 0.4 dNTP (10 μM), 0.25 μL of each primer, 0.2 μL of AmpliTaq polymerase (5 U μL^{-1}) and 1 μL of DNA (not quantified). The trnG^{ucc} marker was amplified using the primers designed by Neustupa *et al*. (2010): trnG-ucc-F 5′-AGCGGGTATAGTTTAGTGGT-3′, and reverse trnG-ucc-R 5′-GGTAGCGGGAATCGAACCCGC-3′. The SSU rDNA gene was amplified using a combination of primers 18S-F (5′-AACCTGGTTGATCCTGCCAGT-3′; Katana *et al*. 2001) and 18 L (5′-CACCTACGGAAACC TTGTTACGACTT-3′; Hamby *et al*. 1988). The trnG^{ucc} and SSU rDNA markers were amplified in Touch Gene Gradient Cycler (Krackeler Scientific, Albany, NY, USA) and Mastercycler epgradientS (Eppendorf, Hamburg, Germany), starting with an initial denaturation at 94°C for 2/5 min, followed by 40/35 cycles of denaturing at 94°C for 1 min, annealing at 62/56°C for 1 min and elongation at 72°C for 1.5/2.5 min, and closed by a final extension at 72°C for 10 min, respectively. Amplified DNA was quantified on 1% agarose gel stained by ethidium bromide, the DNA was stained with bromophenol blue loading dye and cleaned with the JetQuick PCR Purification Kit (Genomed, Löhne, Germany) according to manufacturer's protocol.

The PCR products were sequenced using either the same primers used for PCR (trnG^{ucc}) or the set of nested standard primers (SSU rDNA; Katana *et al*. 2001) by Macrogen Inc., Seoul, Korea on an automatic 3730XL DNA sequencer using BigDye terminator cycling conditions. The SSU rDNA marker was amplified for selected strains after checking the molecular diversity in a more variable trnG^{ucc} marker. The sequences are available in the National Center for Biotechnology Information (NCBI) database (for accession numbers see Table 1).

Sequence alignment and phylogenetic analyses

Sequences were assembled and edited using Seqassem (Hepperle 2004). Subsequently, trnG^{ucc} sequences were aligned using the ClustalW algorithm implemented in the Mega 4.0 (Tamura *et al*. 2007) with default parameters using the IUB DNA weight matrix. After deleting identical sequences, the alignment stability was further assessed through comparison of ClustalW alignments produced under different gap opening/extension penalties using SOAP v.1.2 alpha 4 (Löytynoja & Milinkovitch 2001). The opening gap penalties were incrementally adjusted from 7 to 20 by steps of 2.5. The gap extension penalties were adjusted from 2 to 10 by steps of 1.5. The final trnG^{ucc} alignment comprised 16 sequences of 727 characters.

Sequences of the SSU rDNA were aligned manually on the basis of their secondary structure (Denboh *et al*. 2001). The final alignment comprised 20 sequences of 1679 characters. Substitution models for both trnG^{ucc} and SSU rDNA alignments were estimated by Akaike Information Criterion (AIC) using the PAUP/MrMtGui v1.0b (Nylander 2004). The general reversible model for a proportion of invariable sites (GTR + I) was selected as being the best for the trnG^{ucc} dataset,

whereas for the analysis of SSU rDNA, the PAUP/MrMtGui v1.0b selected the GTR + I + G model.

The phylogenetic trees were inferred with Bayesian inference (BI) using MrBayes version 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). To better infer the phylogenetic relationships among species within the genus *Micrasterias*, the trnG^{ucc} sequences were concatenated with SSU rDNA data. The concatenated sequences originated from the same desmid strains, except in the case of *M. fimbriata* and *Staurodesmus dickiei* (Ralfs) S. Lillieroth, where the sequences of strain ASW 07026 (AJ428098.1) and HS3 (FR691070.1) were merged, respectively SVCK 38 (AJ428101.1) and ASW 07056 (FN562172.1). The concatenated dataset was divided into three partitions. The trnG^{ucc} partition was estimated using the selected model for trnG^{ucc}. The SSU rDNA dataset was further partitioned into stems and loops based on the secondary structure model published by Denboh *et al*. (2001). Different substitution models were selected for stem and loop regions. For the loop regions, a 4-state, single-nucleotide substitution model was selected; while for the paired stem regions, the doublet model (a 16-state RNA stem substitution model of Schöniger & von Haeseler 1994) was selected (Verbruggen & Theriot 2008). Two parallel runs were carried out for 5×10^6 (trnG^{ucc}) and 10^7 (concatenated dataset) 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 set for the 'sumt' and 'sump' commands to a number that was 25% of the total number of trees.

Bootstrap analyses were performed under maximum likelihood (ML) and maximum parsimony (MP) criteria. The ML analyses were performed in PhyML 3.0 (Guindon & Gascuel 2003) using the GTR + I + G model for concatenated dataset and GTR + I model for trnG^{ucc} dataset. The methods of subtree pruning re-grafting and the nearest neighbor interchange were used to improve individual topologies. Statistical support for resulting topologies was assessed by bootstrapping with 100 pseudo replicates of the original dataset. The MP analyses were conducted in the Mega 4.0 (Tamura *et al*. 2007). The MP bootstrap values of the consensus tree were inferred from 10 000 replicates.

The obtained phylogenetic trees were displayed in FigTree v1.3.1 (Rambaut 2009) and Mega 4.0 (Tamura *et al*. 2007). Finally, the phylogenetic trees were graphically adjusted in Adobe Illustrator v 13.0.1.

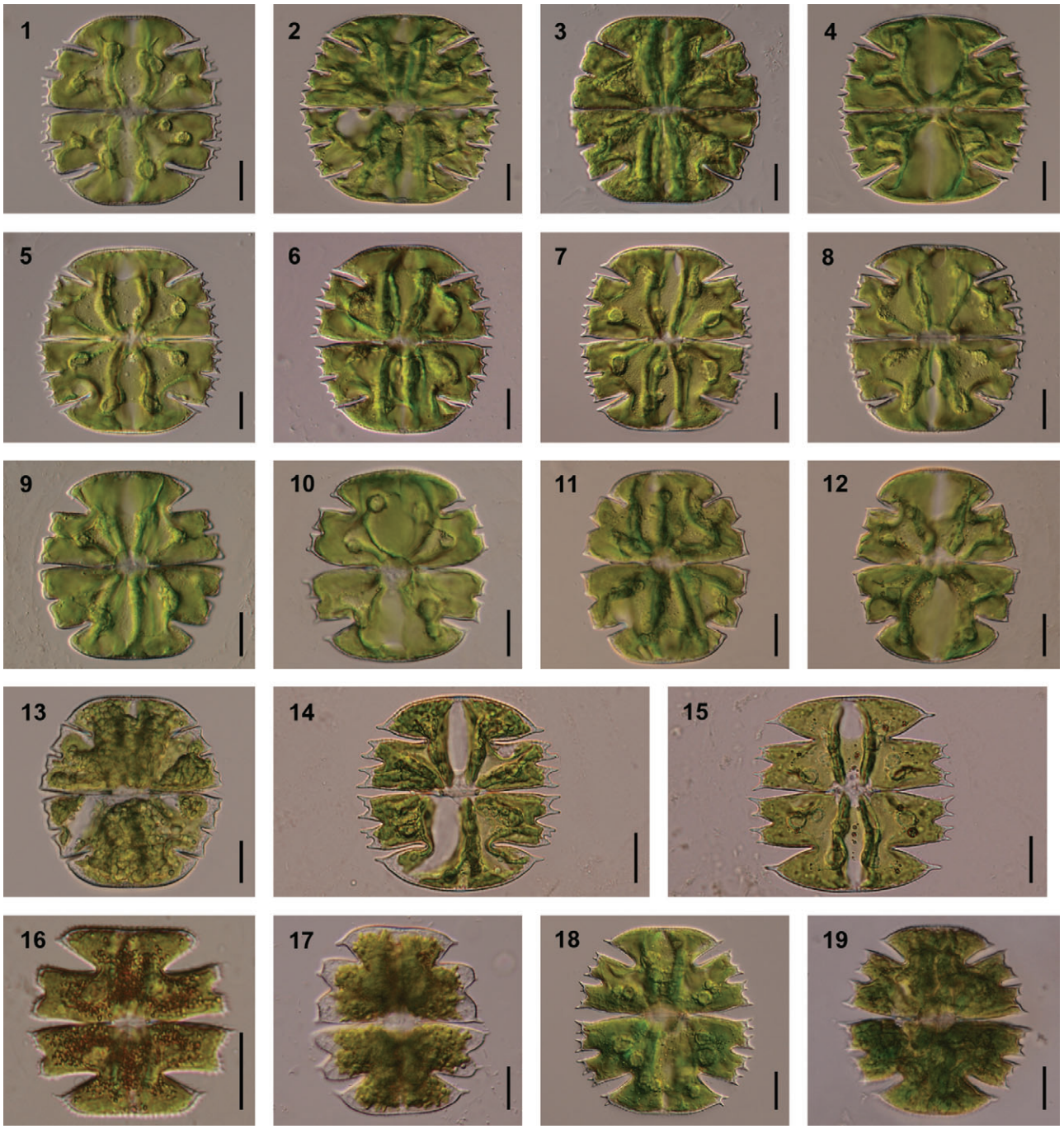
RESULTS

Morphology

Most of the investigated strains (see Table 1), originally assigned as *Micrasterias truncata*, fitted well into the taxonomic definition of this broadly perceived species (Figs 1–13). However, there was a notable exception of the two Australian strains (NIES 783 and NIES 784) originally designated as *M. truncata* var. *pusilla* (Figs 14,15). These strains consistently produced semicells with compressed lateral lobes. These developed second order lobules, their upper margins were almost horizontal, and sometimes slightly undulated. The polar lobe was widely elliptical, with single spikes on both lateral margins. The dimensions of their cells were approximately 60–85 μm × 55–75 μm, i.e. smaller than in other *M. truncata* strains. These morphological features corresponded more to *Micrasterias decemdentata* (Fig. 16) or *M. zeylanica* (Fig. 17) (Krieger 1939; Tyler 1970; Prescott *et al*. 1977). *M. truncata* var. *pusilla*, described by West (1914), refers to cells with much wider lateral lobes, trapezoid-shaped polar lobes, and, typically, with a narrower subpolar angle (Krieger 1939; Prescott *et al*. 1977). Therefore, we decided to compare these NIES strains with cultures of *M. decemdentata* and *M. zeylanica* using molecular sequence data, as well as geometric morphometric comparisons to figures published in taxonomic monographs. The strains CAUP K606 and BR2 broadly corresponded to *Micrasterias truncata* var. *semiradiata* (Figs 18,19). This variety characterized dentate apices of lobes and lobules, by widely opened sinus and subpolar incisions, and by distinctly trapezoid-shaped polar lobes (Růžička 1981; Coesel & Meesters 2007). Two other strains (SVCK 248, SVCK 412) were originally assigned to *Micrasterias truncata* var. *neodamensis* (A. Braun) W. Krieger (Figs 11,12). This variety is characteristic by shallow lobulation of their lateral lobes so that, in most cases, just the first order lobules are developed. In addition, the polar lobe is more or less rounded, bearing typically just a single spike at the lateral extremities. Both of these strains corresponded to this variety, and there were also additional strains (originally assigned as simply *M. truncata* – WL, SVCK 18, SVCK 51) that were similar to *M. truncata* var. *neodamensis* also (Figs 9,10,13). The remaining strains seemed to correspond to the type variety of the species, *Micrasterias truncata* var. *truncata* (Figs 1–8). However, we still observed a considerable variation in some morphological features among the strains, such as in the angle of sinus and of subpolar incisions, and in width of the polar lobes. The cell walls of all the strains were invariably provided with the pore-linked mucilage extrusions (Figs 20–25).

Geometric morphometrics

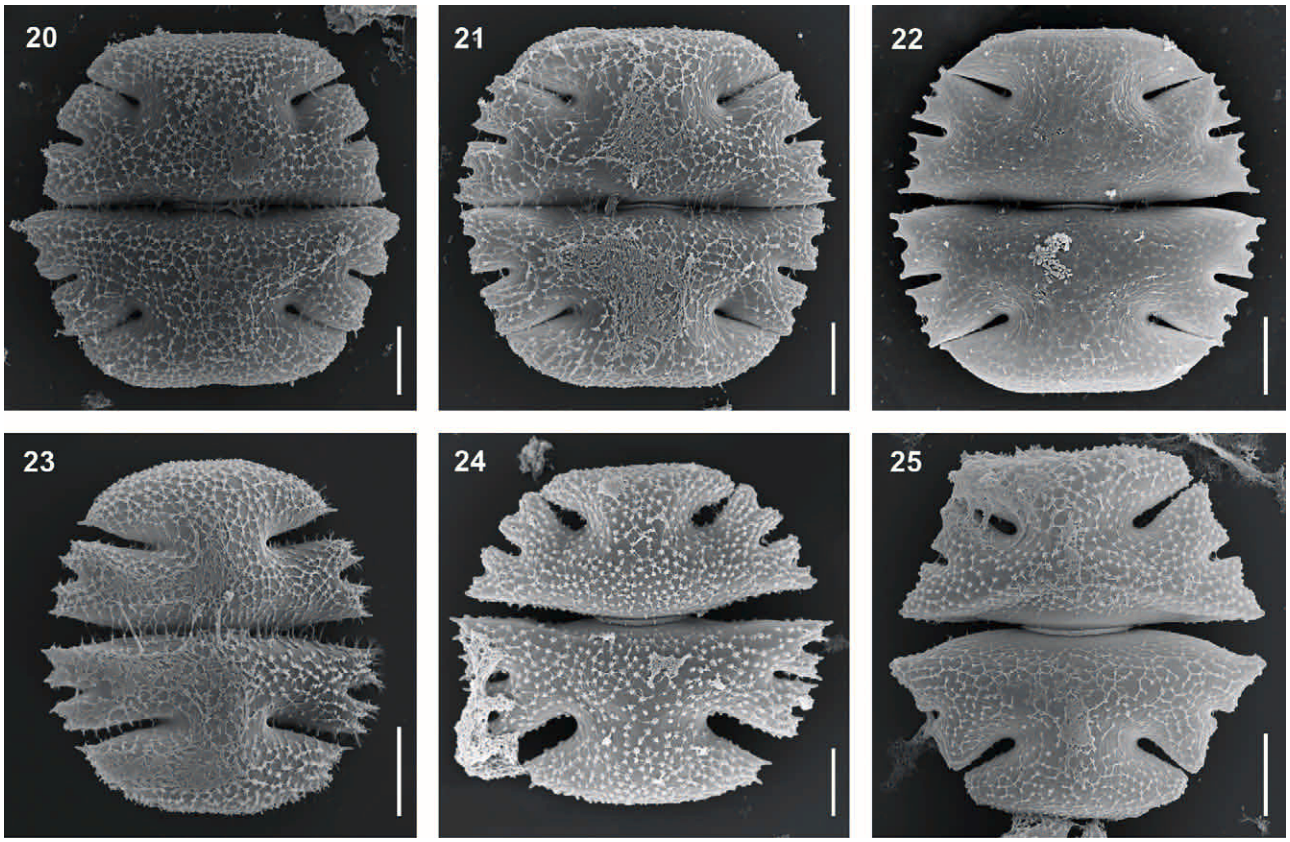
We analyzed the shape distances among the investigated 17 strains acquired from the culture collections and isolated from nature. The non-metric multidimensional scaling ordination plot illustrated overall morphological separation of the NIES 783 and 784 strains



Figs 1–19. Light micrographs of investigated *Micrasterias* strains. 1–8. *Micrasterias truncata* var. *truncata*. 1. SVCK 359. 2. EH. 3. HS1. 4. HS2. 5. SL. 6. KT1. 7. KT2. 8. BR1. 9–13. *Micrasterias truncata* var. *neodamensis*. 9. SVCK 18. 10. SVCK 51. 11. SVCK 248. 12. SVCK 412. 13. WL. 14, 15. '*Micrasterias truncata* var. *pusilla*'. 14. NIES 783. 15. NIES 784. 16. *Micrasterias decemdentata* ASW 07023. 17. *Micrasterias zeylanica* SVCK 291. 18, 19. *Micrasterias truncata* var. *semiradiata*. 18. CAUP K606. 19. BR2. Scale bar = 20 μm.

(Fig. 26). The strains corresponding to traditional *M. truncata* var. *semiradiata* (BR2, CAUP K606) were located on the other side of the ordination space. The rest of the strains formed a loose group located in the centre of the ordination space. Among them, the strains with reduced lobulation, broadly corresponding to *M. truncata* var. *neodamensis* (strains WL, SVCK 248, SVCK 412, SVCK 18, SVCK 51), were vaguely separated from the *M. truncata* var. *truncata*-like strains along the second ordination axis.

The cells of NIES 783 and NIES 784 proved to be similar to cells of *M. decemdentata* ASW 07023 and to



Figs 20–25. Scanning electron micrographs of investigated *Micrasterias* strains. 20–22. *Micrasterias truncata* var. *truncata*. 20. EH. 21. BR1. 22. KT1. 23. '*Micrasterias truncata* var. *pusilla*' NIES 783. 24, 25. *Micrasterias truncata* var. *semiradiata* CAUP K606. Scale bar = 20 μm.

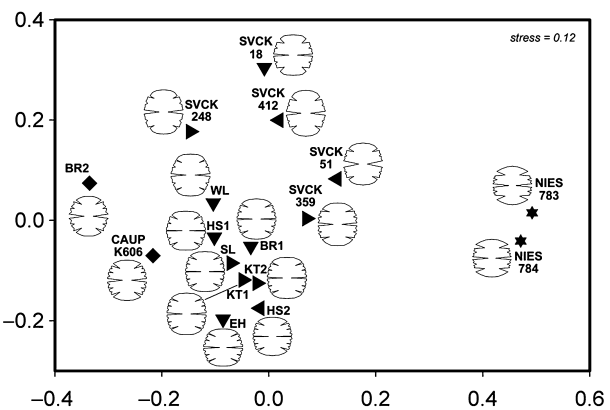


Fig. 26. The non-metric multidimensional scaling (NMDS) ordination diagram of the geometric morphometric data. The centroids of individual clonal populations are illustrated, and their shapes reconstructed from the landmark data. The symbols correspond to individual lineages as reconstructed by the phylogenetic analysis of trnG^{ucc} sequences. *M. truncata* var. *semiradiata* (◆), *M. truncata* var. *pusilla* (★), the core *M. truncata* lineages (▼/◀/►).

M. zeylanica SVCK 291, as illustrated by the similarity dendrogram based on the landmark data (Fig. 27). In addition, they were also comparable to figures of these species from the literature, and to the shape of *M. truncata* var. *bahusiensis* Wittrock (Růžička 1981). The BR2 and CAUP K606 strains were most similar to published drawings of *M. truncata* var. *semiradiata* and *M. truncata* var. *pusilla*, respectively (Prescott *et al.* 1977). All the SVCK strains of *M. truncata*, together with the HS1 and WL isolates, clustered with *M. truncata* var. *neodamensis*. Conversely, isolates from the Czech Republic and southwest France were most similar to *M. truncata* var. *truncata*, and also to the two traditional varieties – *M. truncata* var. *quadrata* and *M. truncata* var. *crenata* (Ralfs) Grönblad.

Among the investigated strains, the shape separation of CAUP K606 and BR2, attributed to *M. truncata* var. *semiradiata*, from the rest of *M. truncata* isolates was not entirely apparent from the results of the ordination analyses (e.g. Fig. 26). Therefore, we proceeded with the discrimination analysis specifically evaluating their shape differences. This discrimination analysis was highly significant ($P < 10^{-6}$). In total, 99.6% of the semicells were correctly assigned into their phylogenetic

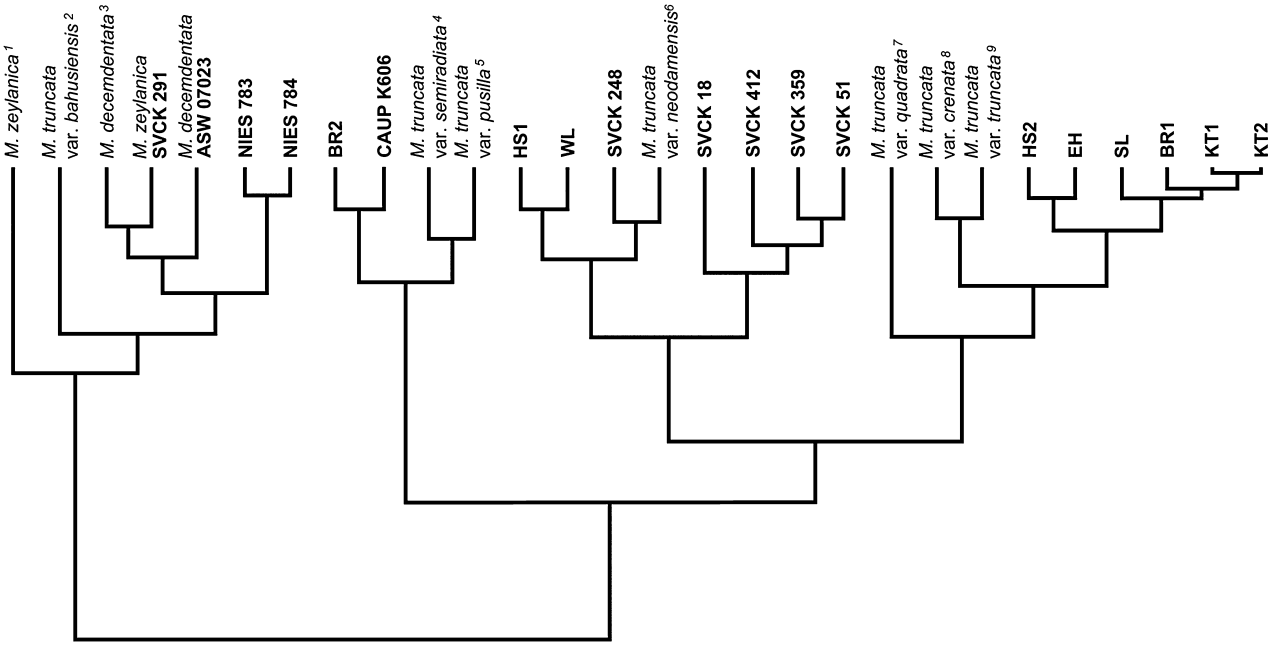


Fig. 27. The similarity dendrogram of centroids of investigated clonal populations (in bold) and published figures (in italic) based on the geometric morphometric data. The data for individual strains are included in Table 1. The following figures from the literature were used: 1. *Micrasterias zeylanica*, Krieger (1939), Tf. 101, Figure 13; 2. *M. truncata* var. *bahusiensis*, Růžička (1981), Tf. 96, Figure 1; 3. *M. decemdentata*, Růžička (1981), Tf. 94, Figure 5; 4. *M. truncata* var. *semiradiata*, Růžička (1981), Tf. 94, Figure 15; 5. *M. truncata* var. *pusilla*, Krieger (1939), Tf. 102, Figure 9.; 6 *M. truncata* var. *neodamensis*, Krieger (1939), Tf. 103, Figure 8; 7. *M. truncata* var. *quadrata*, Růžička (1981), Tf. 96, Figure 16; 8. *M. truncata* var. *crenata*, Růžička (1981), Tf. 96, Figure 8; 9. *M. truncata* var. *truncata*, Růžička (1981), Tf. 95, Figure 1.

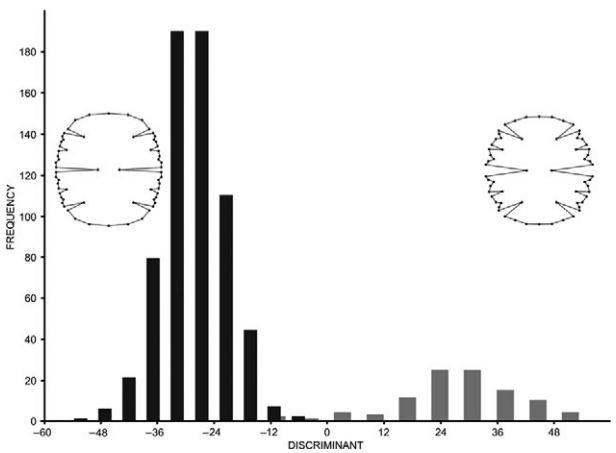


Fig. 28. The linear discrimination analysis of the geometric morphometric data distinguishing the *Micrasterias truncata* var. *semiradiata* (grey bars) from the core *M. truncata* (black bars). The shapes characteristic for marginal values of the discriminant axis were reconstructed from the landmark data.

groups, leaving only three out of the 750 objects misidentified by the discriminant function (Fig. 28). The shape characteristics were reconstructed for illustration of the morphological features that distinguish both lineages (Fig. 28). The *M. truncata* var. *semiradiata* cells differed from the other *M. truncata* strains primarily by their narrow, trapezoid-shaped polar lobes. In addition, the angle between polar and lateral lobes was clearly more open in *M. truncata* var. *semiradiata* strains than in the other investigated *M. truncata* cultures. Similarly, shape separation of traditional *M. truncata* var. *truncata* and *M. truncata* var. *neodamensis* was also tested. This linear discrimination analysis yielded a highly significant separation ($P < 10^{-6}$), and 98% of the semicells were correctly classified. In total, 13 out of the 650 objects were misidentified by the discriminant function.

Molecular phylogeny

The final trnG^{ucc} alignment contained 558 sites without polymorphism and 107 parsimony informative sites out of a total of 727 nucleotide characters. The GC content was rather low, only 31.7%. The trnG^{ucc} based phylogeny of 17 investigated strains and related taxa revealed that organisms affiliated to *M. truncata* formed three well supported clades (Fig. 29). One of them consisted of NIES 783/784 strains with identical trnG^{ucc} sequences, closely related to the strain SVCK 291 of *M. zeylanica*. The second lineage contained two cultures with identical trnG^{ucc} sequences, corresponding to *M. truncata* var. *semiradiata* (CAUP K606 and BR2). The

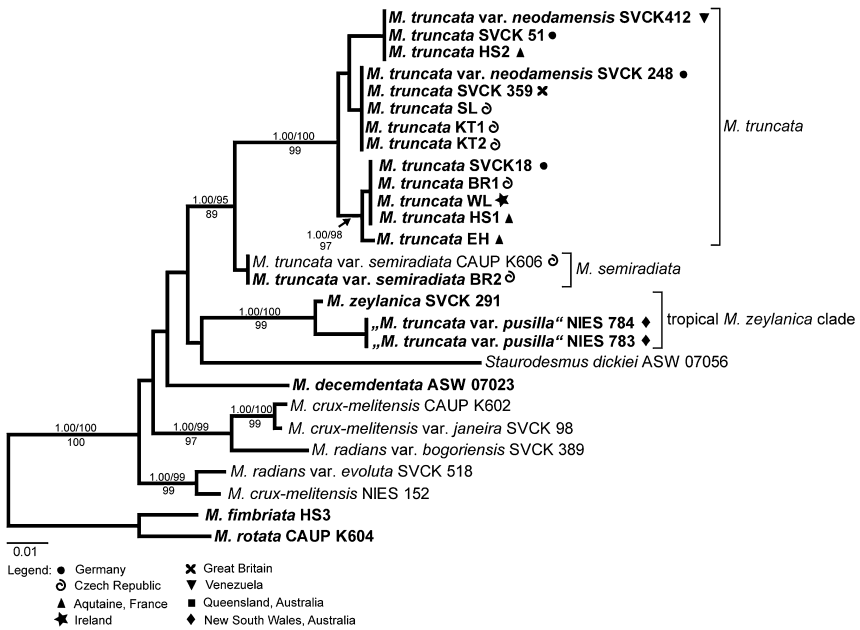


Fig. 29. Bayesian phylogenetic tree of *Micrasterias truncata* trnG^{ucc} sequences. The sequences of *Micrasterias rotata* and *Micrasterias fimbriata* were used as an outgroup. The scale bar shows the estimated number of substitutions per nucleotide. Names printed in bold represent sequences obtained in this study. The posterior probabilities lower then 0.70 and bootstrap support levels below 50% are omitted. The indicators of statistical significance are provided Bayesian posterior probability/maximum likelihood bootstrap support upon the branch and below the branch maximum parsimony bootstrap support. The geographic origin of investigated strains are provided.

third highly supported clade included all the other investigated *M. truncata* strains. However, this clade was further divided into three clearly delimited subclades. The strains within each of these three subclades had identical sequences, with the single exception of the EH strain that differed by a single nucleotide substitution. The phylogenetic relationship among the three groups of the broad *M. truncata* clade remained unclear, due to a lack of bootstrap support for the sister position of groups containing SVCK 248 and SVCK 412 strains. The isolates belonging to these three groups showed neither geographical, nor morphological patterns. The strains originating from different European localities were morphologically indistinguishable, and even the single strain from Venezuela was identical to the other members of the phylogenetic group.

All available SSU rDNA sequences of related species (Neustupa *et al*. 2010) were used to infer broader phylogeny. The sequences of *Euastrum humerosum* Ralfs and *Cosmarium depressum* (Nägeli) P. Lundell were used as outgroups. The alignment contained 2123 sites without polymorphism and 283 variable sites out of a total 2406 sites. The GC content was higher than in trnG^{ucc} alignment, 44.5%.

The phylogram (Fig. 30) demonstrated a better resolution of the relationships within the 'core' *M. truncata* clade, than the single trnG^{ucc} marker. The strain SVCK 248 was closely related to SVCK 18, but not to the clade of SVCK 412. The phylogenetic tree also supported the sister position of *M. truncata* var. *semiradiata* to the *M. truncata* clade. The Bayesian analysis, as well as the maximum likelihood and maximum parsimony analyses, reconstructed the Australian '*M. truncata* var. *pusilla*' as closely related to *M. zeylanica*, but not to the morphologically very similar *M. decemdentata*. The doublet-model based Bayesian phylogeny moderately resolved the positions within *M. crux-melitensis*, *M. radians*, *M. decemdentata* and *Staurodesmus dickiei;* however, maximum likelihood and maximum parsimony does not support this branching order.

DISCUSSION

The 17 investigated strains appeared to be highly divergent, on the basis of morphometric, as well as molecular data. The Australian strains from the NIES culture collection (assigned to *M. truncata* var. *pusilla*) were obviously different from the rest of the *M. truncata* isolates. Their morphological differences were illustrated by the geometric morphometric analysis that placed these two strains outside of the true *M. truncata* cluster, apart from the published figures of *M. truncata* var. *pusilla*. The NIES strains, on the other hand, appeared to be more similar in shape to cultures and published figures of *M. decemdentata* and *M. zeylanica*. Indeed, the molecular phylogenetic analysis confirmed the position of these Australian strains to be different from other *M. truncata* isolates. Interestingly, they were closely related to *M. zeylanica* SVCK 291, also originating from Australia. Nevertheless, the European *M. decemdentata* (strain ASW 07023) was also morphologically very similar to NIES 783/784, but it appeared be only distantly related to these Australian strains. These results suggest that there may be a more complex phylogenetic structure among species of this morphological complex. Moreover, there have been several reports of morphotypes similar to the *M.*

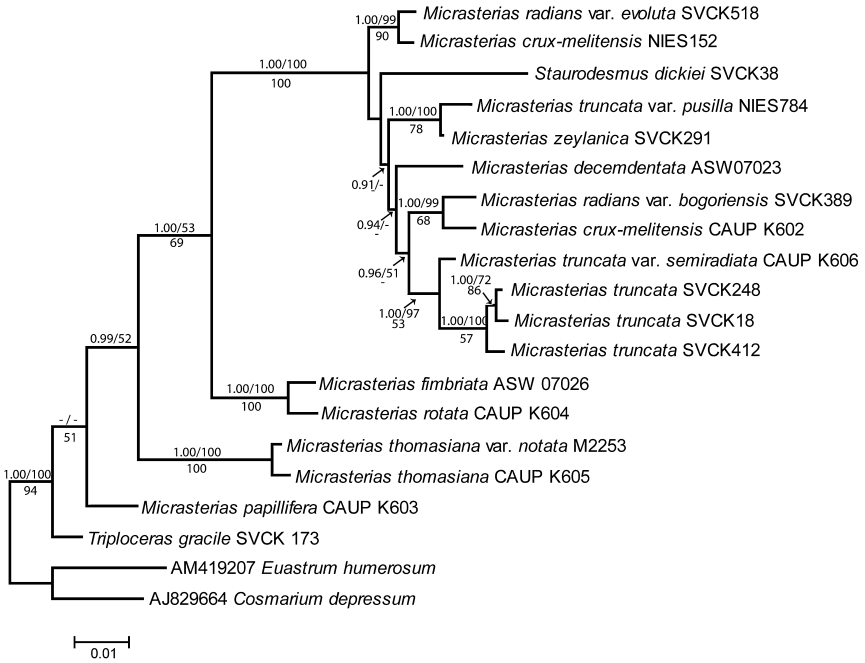


Fig. 30. The phylogenetic tree obtained through Bayesian analysis of concatenated data set of small subunit rDNA and trnG^{ucc} sequences. The sequences of *Cosmarium depressum* and *Euastrum humerosum* were used as an outgroup. The scale bar shows the number of substitutions per nucleotide. The posterior probabilities lower then 0.70 and bootstrap support levels below 50% are omitted. The indicators of statistical significance are provided upon the branch in following order: Bayesian posterior probability/maximum likelihood bootstrap support, below the branch is displayed maximum parsimony bootstrap support.

zeylanica/NIES 783/784 complex from Australia (Playfair 1908; Scott & Prescott 1958; Tyler 1970), and the actual diversity of this lineage may be higher. However, this topic was beyond the scope of the present study, and future revision of this complex should be based on a comprehensive analysis of numerous Australian isolates. For now, we can conclude that the NIES 783 and NIES 784 strains, identified originally as *M. truncata* var. *pusilla*, probably represent a separate species.

There were several well supported phylogenetic lineages among the strains morphologically attributed to *M. truncata*. The two strains (BR2 and CAUP K606), identified morphologically as *M. truncata* var. *semiradiata*, formed an independent lineage in both phylogenetic trnG^{ucc} and concatenated trees. Both these strains originated from localities within the Czech Republic, but this morphological variety has been reported from numerous localities across Europe (Kouwets 1999; Coesel & Meesters 2007) and therefore is likely to be widely distributed. The morphological discrimination between *M. truncata* var. *semiradiata* cells and other *M. truncata* isolates was confirmed by the discrimination analysis of the geometric morphometric data. In fact, the high degree of morphological separation, as illustrated by the discrimination analysis, should make it possible to identify members of this lineage in natural samples using the traditional morphological criteria defining the original *M. truncata* var. *semiradiata*. The species name *Micrasterias semiradiata* was taxonomically described by Kützing (1849), and later re-classified as the variety of broadly perceived *M. truncata* by Wolle (1884). On the basis of phylogenetic and morphologic differences ascertained by our analyses, we propose that this taxon should once again be considered as a separate species, rather than a variety of *M. truncata*.

All the other investigated *M. truncata* strains belonged to the single well-supported lineage reconstructed by the trnG^{ucc} intron phylogenetic tree. Morphologically, the strains from SVCK collection, and the HS1 and WL isolates broadly corresponded to traditional *M. truncata* var. *neodamensis* according to their limited lobulation, and somewhat rounded polar lobes. The other strains isolated from the Czech Republic and from Aquitaine, France, were most similar to *M. truncata* var. *truncata*. These two varieties were also unambiguously discriminated on the basis of geometric morphometric data. However, the trnG^{ucc} molecular marker illustrated that the members of this lineage were further diversified into three subclades. Individual strains within these clades were phylogenetically homogenous in the trnG^{ucc} sequences irrespective of their geographic origin. Interestingly, the single strain from Venezuela (SVCK 412) also clustered into one of these three *M. truncata* clades, and did not differ from the temperate European isolates. In addition, members of these three clades were morphologically indistinguishable. They resembled either *M. truncata* var. *neodamensis*, or the type variety of the species, but this morphological disparity was not congruent with the molecular data. We propose that these lineages may represent a sympatric species, possibly of relatively recent origin. However, we were not able to find any morphological or biogeographical pattern that could possibly be used for their taxonomic delimitation. We cannot preclude that these lineages will be described as

separate, truly cryptic species in the future. Their separate species status, however, will need to be confirmed by other features, including for instance, reproductive isolation and analyses of additional fast evolving molecular markers.

The present study demonstrated the usefulness of trnG^{ucc} marker for resolving intraspecific diversity within *M. truncata* species complex. By contrast, SSU rDNA seemed to be of limited use for determining relationships among closely related *Micrasterias* species, as rather low sequence divergence was detected. The non-coding intron sequences of plastid trnG^{ucc} gene have occasionally been used in phylogenetic studies of higher plants (e.g. Pedersen & Hedenäs 2003; Shaw *et al*. 2005). However, in streptophyte algae it was used only recently by Neustupa *et al*. (2010), who inferred the species relationship in *Micrasterias crux-melitensis/M. radians* species complex. In that study, the plastid trnG^{ucc} phylogeny was shown to be highly congruent with the nuclear ITS2 rDNA phylogram. Because of some drawbacks that could dismiss the phylogenetic utility of multiple-copy nuclear sequences (Álvarez & Wendel 2003), and a very high success in PCR amplification of the trnG^{ucc} intron, we recommend the use of this low-copy plastid marker for studies investigating phylogenetic differentiation of closely related desmid species.

Recently, taxonomic study of desmids has been significantly improved and advanced through the application of molecular methods that demonstrated the polyphyletic status of most traditional genera (see Introduction). At the species level, Neustupa *et al*. (2010) illustrated that the phylogenetic structure of *Micrasteras crux-melitensis/M. radians* complex reflected the morphological data to a large extent, and that, in some cases, the phylogenetic species lineages corresponded more accurately to traditional subspecific taxa. At the same time, the phylogenetic structure also reflected the geographic origin of strains (Neustupa *et al*. 2010). Similarly, in the present study we detected that traditional *M. truncata* var. *semiradiata* and *M. truncata* var. *pusilla* should be regarded as a separate species. However, the lack of any morphological or geographical signal in delimitation of three cryptic subclades of the *M. truncata* lineage has been revealed. The isolates belonging to these lineages resemble at least two traditional subspecific taxa, and they probably occur sympatrically across the European continent. Similar cryptic phylogenetic differentiation has also been ascertained in some other protistan groups, such as diatoms (Kooistra *et al*. 2008; Quijano-Scheggia *et al*. 2009), foraminifers (Pawlowski *et al*. 2008) or green flagellates (Foulon *et al*. 2008). In most of these groups, such cryptic lineages have not been formalized as separate species, as yet. Fenchel and Finlay (2006) even suggested that the protistan species should, in general, be defined at higher phylogenetic levels encompassing mostly widely distributed lineages with ecological and phenotypic differentiation. We also do not suggest that the cryptic subclades of the *M. truncata* lineage should be described as separate taxa at this time. However, their existence illustrates that the traditional, well known and frequently encountered desmid morphospecies, such as *M. truncata*, may actually represent cryptic phylogenetic complexes.

This study demonstrated that geometric morphometric methods may be useful for taxonomic revisions of species as the majority of the desmid descriptions were based on the iconotypes. In addition, this approach was quite powerful to define the morphological discrimination features of the *M. semiradiata* and *M. truncata* lineages. Correct species concepts, and development of appropriate methods for species delimitation could be highly useful as the desmids diversity data have been frequently used in numerous ecological applications (Coesel 2003; Krasznai *et al*. 2008; Neustupa *et al*. 2009).

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Paper VIII

The molecular phylogenetic and geometric morphometric evaluation of *Micrasterias crux-melitensis / M. radians* species complex

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THE MOLECULAR PHYLOGENETIC AND GEOMETRIC MORPHOMETRIC EVALUATION OF *MICRASTERIAS CRUX-MELITENSIS/ M. RADIANS* SPECIES COMPLEX[1]

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We investigated nine strains of the *Micrasterias crux-melitensis* (Ehrenb.) Hassall ex Ralfs and *M. radians* W. B. Turner species complex. A combination of molecular, morphological, and geometric morphometric data was used to reveal the patterns of their phenotypic and phylogenetic differentiation. The molecular data based on internal transcribed spacer (ITS) rDNA, glycine transfer RNA (*trnG*^{uuc}) intron, and SSU rDNA sequences revealed three phylogenetic lineages. One of them comprised the six European and North American strains that were morphologically identified as *M. crux-melitensis*. Phenotypic data illustrated high morphological variability of strains within this genetically homogenous lineage that spanned several traditional infraspecific taxa, including strains corresponding to *M. crux-melitensis* var. *janeira* (Racib.) Grönblad and *M. crux-melitensis* var. *superflua* W. B. Turner, whose morphometric characteristics profoundly differed. Three strains of *M. radians* formed two separate phylogenetic lineages corresponding to traditional varieties *M. radians* var. *evoluta* (W. B. Turner) Willi Krieg. and *M. radians* var. *bogoriensis* (C. J. Bernard) G. S. West. The morphological types corresponding to the former variety have, so far, only been reported from Africa. Therefore, we cannot preclude that geographic isolation may play a role in species differentiation of relatively large freshwater protists, such as *Micrasterias*.

Key index words: **Desmidiales; geometric morphometrics; *Micrasterias*; molecular phylogenetics; Streptophyta; taxonomy**

Abbreviations: **AIC, Akaike information criterion; BI, Bayesian inference; CAUP, Culture Collection of Algae of Charles University in Prague; CVA, canonical variates analysis; GPA, generalized Procrustes analysis; ILD, incongruence length difference test; ITS, internal transcribed spacer; ML, maximum likelihood; PCA, principal component analysis; SVCK, Sammlung von Conjugaten-Kulturen am Institut für Allgemeine Botanik der Universität Hamburg; trnG, glycine transfer RNA gene; wMP, weighted maximum parsimony**

The members of the green algal genus *Micrasterias* are some of the most conspicuous protists. Similarly to other Desmidiaceae, their cells are composed of two semicells divided by a narrow isthmus that contains the centrally located nucleus. Semicells of *Micrasterias* are typically further divided into several lobes and lobules resulting in highly complex cells. Most *Micrasterias* species actually have the highest level of cell complexity, evaluated by their deviation from ideal globularity, among the Desmidiales (Neustupa et al. 2009). Since the 19th century, ~110 species and subspecific taxa have been recognized. The extraordinarily rich taxonomic history of the genus *Micrasterias* has been summarized by Krieger (1939) and Prescott et al. (1977). The European taxa were compiled by Růžička (1981), who considered many subspecies and forms, originally described on the basis of slight morphological differences, as synonyms of broadly perceived plastic taxa. Consequently, species concept within this genus has not been stable, despite a relative abundance of morphological markers in comparison to most other protist groups. The species complex of *M. crux-melitensis* and *M. radians* represents a well-known example of indistinct species boundaries within a genus.

M. crux-melitensis is one of the most frequently occurring species of this genus. It prefers phytobenthos of mesotrophic, slightly acidic wetlands, and it has been reported worldwide, typically from temperate habitats or from high-altitude localities of tropical ecosystems (Vyverman 1992, Coesel 1996). Notable morphological variation in this species was reported and resulted in the description of several varieties and forms on the basis of different morphology of vegetative cells (Krieger 1939, Růžička 1981). However, most of these subspecific taxa were defined solely on the basis of quantitative differences in depth of the incisions between lobes and lobules, cell dimensions, or their degree of lobulation. Notably, *M. crux-melitensis* var. *janeira* was defined by its shallow incisions between lobes. On the other hand, *M. crux-melitensis* var. *superflua* was characterized by deep incisions and the lobules of third order on vegetative cells, whereas lobulation of up to second order was reported for the type variety (Krieger 1939). Růžička (1981) synonymized most of these varieties and forms of *M. crux-melitensis* and proposed the single, broadly perceived and

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morphologically variable *M. crux-melitensis*. The second species of this morphological complex, *M. radians,* differs primarily by slightly greater cell dimensions, by deeper incisions between lobes and lobules, as well as by a presumably tropical distribution (Krieger 1939, Scott and Prescott 1961). Vyverman and Viane (1995) investigated morphometric variation (based on conventional measurements of distances and angles) of different populations of traditional *M. crux-melitensis* and *M. radians* from Papua New Guinea. The main difference between the typical *M. crux-melitensis* and *M. radians* populations relied on the relative length of the incisions, differences in cell dimensions, and also in the shape of a cell's apex. The apex was generally narrower and U-shaped in *M. radians*, but more opened and wider in populations of *M. crux-melitensis*. However, there was a rather gradual shift in morphology from typical *M. radians* in lowland localities to typical *M. crux-melitensis* in high-altitude Papua New Guinea samples. Therefore, Vyverman and Viane (1995) hypothesized that traditional *M. crux-melitensis* and *M. radians* may actually belong to a single species with temperature-related morphological variation reflecting the altitudinal gradient of the tropical ecosystems.

Taxonomy of desmids has recently undergone major changes based on molecular phylogenetic analyses (McCourt et al. 2000, Gontcharov 2008, Gontcharov and Melkonian 2008, Hall et al. 2008). Most major desmid genera were found to be polyphyletic (Gontcharov and Melkonian 2005, 2008). Thus far, there is limited taxon sampling available for species of the genus *Micrasterias*, but the present data suggest that the genus might be paraphyletic (Gontcharov et al. 2003, Hall et al. 2008). Interestingly, *Triploceras gracile* Bailey, which is morphologically quite distinct from *Micrasterias* and rather resembles some aradiate mutants of *Micrasterias* (i.e., cells without lateral semicell lobes, Pickett-Heaps 1975), was repeatedly nested within the genus *Micrasterias* in molecular phylogenetic studies (Gontcharov 2008, Hall et al. 2008).

Most of the molecular phylogenetic investigations of Desmidiales were concentrated on reconstruction of major lineages corresponding to families and orders. Few studies have specifically evaluated taxonomic concepts of traditional morphology-based desmid species. Denboh et al. (2001) demonstrated monophyly of several morphologically closely similar *Closterium* species groups. However, Denboh et al. (2003) illustrated higher phylogenetic diversity within the *Closterium moniliferum/ehrenbergii* species complex than was expected on the basis of traditional taxonomic identification. In addition, they demonstrated reproductive isolation between individual clades that indicated possible cryptic or pseudocryptic species diversity within the investigated complex. A similar study based on mating experiments between geographically distant, but morphologically very similar isolates of *Micrasterias thomasiana* W. Archer, revealed the existence of several reproductively isolated mating groups (Blackburn and Tyler 1987). Gontcharov and Melkonian (2008) illustrated a probable pseudocryptic or cryptic diversity in two strains of *Cosmarium punctulatum* Bréb. that were morphologically very similar but were positioned in distant parts of the phylogenetic tree of Desmidiaceae. Similarly, two strains of *Staurodesmus extensus* (Borge) Teiling were also only distantly related and apparently polyphyletic (Gontcharov and Melkonian 2008). These scarce data may indicate that traditional concepts of at least some desmid species have been too broad, and there may be conspicuous pseudocryptic or cryptic species diversity. Consequently, the estimates of total species richness of ~3,200 species in Desmidiales (Gontcharov 2008) might be underestimated, and the traditional species concepts should be revised utilizing molecular and quantitative morphometric methods.

In the present study, we evaluated the phylogenetic position of nine strains of *M. crux-melitensis* and *M. radians* on the basis of molecular data in order to test for the monophyly and phylogenetic differentiation of this complex. In addition, morphology of these strains was analyzed using geometric morphometric methods and SEM of cells. The variation in shape among strains, and eventual phylogenetic groups, was revealed. We asked whether the investigated strains, originating from geographically distant locations, would be homogenous in highly variable nuclear and chloroplast sequences, thus indicating probable cosmopolitanism of broadly perceived species comprising the traditional *M. crux-melitensis* and *M. radians*. Alternatively, we attempted to identify morphological and possible distributional differences among phylogenetic lineages within the investigated complex.

MATERIALS AND METHODS

Origin and cultivation of strains, LM and SEM observations. The investigated strains were acquired from algal culture collections at the Hamburg (SVCK) and Prague (CAUP) universities (Table 1). Cultures were initiated with an inoculum of 10–15 cells and grown for 6 weeks in 100 mL Erlenmeyer flasks containing liquid oligotrophic medium developed by the Culture Collection of Algae of Charles University of Prague (CAUP) (http://botany.natur.cuni.cz/algo/caup.html). Strains were maintained at temperatures of 20°C and illuminated at 40 μmol photons $\cdot$ m^{-2} $\cdot$ 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. Microphotographs were taken on an Olympus BX51 light microscope with Olympus Z5060 digital microphotographic equipment (Olympus Corporation, Tokyo, Japan). Projection of light microscopic images was made using the Deep Focus 3.0 module (Promicra s.r.o, Prague, Czech Republic) implemented in the QuickPHOTO CAMERA 2.3 software (Promicra s.r.o). For SEM, the acetone-washed glass coverslips (10 mm in diameter) were placed on a heating block and coated three times with a poly-L-lysine solution (1:10 in

TABLE 1. Origin and description of strains.

Strain no.	Taxon name	Origin	Accession nos.		
			ITS	*trnG*	SSU
SVCK 266	*M. crux-melitensis* Ralfs var. *superflua* W. B. Turner	Kiebitzmoor peat bog near Hamburg, Germany	FN424415	FN424424	–
SVCK 431	*M. crux-melitensis* Ralfs var. *crux-melitensis*	N. Deming Pond, Itasca State Park in Minnesotta, USA	FN424416	FN424425	–
SVCK 128	*M. crux-melitensis* Ralfs var. *superflua* W. B. Turner	A pond in Rheinland, Germany	FN424417	FN424426	–
SVCK 98	*M. crux-melitensis* Ralfs var. *janeira* (Raciborski) Grönblad	A bog near Korvanen, Finland	FN424418	FN424427	–
CAUP K602	*M. crux-melitensis* Ralfs var. *crux-melitensis*	Borkovicka Blata peat bog in South Bohemia, Czech Republic	FN424419	FN424428	–
CAUP K607	*M. crux-melitensis* Ralfs var. *crux-melitensis*	Brezina bog in North Bohemia, Czech Republic	FN424420	FN424429	–
SVCK 518	*M. radians* W. B. Turner var. *evoluta* (W. B. Turner) Willi Krieg.	Lake Ol Bolossat, Kenia	FN424421	FN424430	FN424433
SVCK 519	*M. radians* W. B. Turner var. *evoluta* (W. B. Turner) Willi Krieg.	Lake Ol Bolossat, Kenia	FN424422	FN424431	–
SVCK 389	*M. radians* Ralfs var. *bogoriensis* (C. J. Bernard) Willi Krieg.	Kuching, Malaysia	FN424423	FN424432	FN424434

distilled water) to ensure better adhesion of cells. After cooling, a drop of the formaldehyde-fixed cell suspension was placed on the glass, and when almost dry, it was transferred into 30% acetone and dehydrated by an acetone series (10 min successively in 30, 50, 70, 90, 95, 99% and 2x in 100%). Finally, cells were dried to a critical point with liquid CO_2, subsequently sputter-coated with gold (Bal-Tec Sputter Coater SCD 050, Capovani Brothers Inc., Sconia, NY, USA), and examined using the JEOL 6380 LV scanning electron microscope (JEOL Ltd., Tokyo, Japan).

DNA isolation, amplification, and sequencing. DNA was extracted from the strains listed in Table 1. After centrifugation in an MPW 223 centrifuge (MPW Med. Instruments, Warsaw, Poland), algal cells were mechanically disrupted by shaking in the presence of glass beads (0.5 mm diameter, Sigma-Aldrich, St. Louis, MO, USA). Genomic DNA was extracted using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) following the manufacturer's instructions. The ITS rDNA regions were amplified by PCR using the algal-specific primer nr-SSU-1780-5′ (5′-CTGCGGAAGGATCATTG-ATTC-3′; Piercey-Normore and DePriest 2001) and a universal primer ITS4-3′ (5′-TCCTCCGCTTATTGATATGC-3′; White et al. 1990). Additionally, the *trnG*^{uuc} intron was amplified using newly designed primers trnG-uuc-F-5′ (5′-AGCGGGT-ATAGTTTAGTGGT-3′) and trnG-uuc-R-3′ (5′-GGTAGCGGG-AATCGAACCCGC-3′). The new primers were designed based on a published chloroplast genome of *Staurastrum punctulatum* (GenBank accession no. AY958085; Turmel et al. 2005). All PCRs were performed in 20 μL reaction volumes (15.6 μL sterile Milli-Q Water [Millipore Corp., Bedford, MA, USA], 2 μL 10′ PCR buffer [Sigma-Aldrich], 0.4 μL dNTP [10 μM], 0.25 μL of primers [25 pmol · mL^{-1}], 0.5 μL Red Taq DNA Polymerase [Sigma-Aldrich] [1 U · mL^{-1}], and 1 μL of DNA [not quantified]). PCR reactions were performed in either an XP thermal cycler (Bioer, Tokyo, Japan) or a Touchgene gradient cycler (Techne, Cambridge, UK). PCR amplification of the ITS rDNA began with an initial denaturation at 95°C for 5 min and was followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 54°C for 1 min and elongation at 72°C for 1 min, with a final extension at 72°C for 7 min. Identical conditions were used for the amplification of the *trnG*^{uuc} intron, except that an annealing temperature of 66°C was used. The PCR products were quantified on a 1% agarose gel stained with ethidium bromide and cleaned either with the JetQuick PCR Purification Kit (Genomed, Löhne, Germany) or with QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocols. The purified amplification products were sequenced with the PCR primers with an Applied Biosystems (Seoul, Korea) automated sequencer (ABI 3730xl) at Macrogen Corp. in Seoul, Korea. Sequencing reads were assembled and edited using SeqAssem software (SequentiX, Klein Raden, Germany). After checking the diversity in ITS rDNA and *trnG*^{uuc} intron sequences, SSU rDNA regions were amplified for selected strains. PCR amplification was performed as described in Neustupa and Škaloud (2007). In total, nine ITS rDNA, nine *trnG*^{uuc} intron, and two SSU rDNA sequences were generated and submitted to GenBank (Table 1).

Sequence alignment. ITS2 rDNA and *trnG*^{uuc} intron sequences were manually aligned in MEGA 4 (Kumar et al. 2008). The *trnG*^{uuc} intron sequences were all of equal length (750 bases); their alignment was straightforward and unambiguous. However, ITS2 rDNA sequences showed considerable variability that made it difficult to create precise alignment. Thus, the common ITS2 rRNA secondary-structure transcript has been constructed using the mfold program (version 2.3; Walter et al. 1994, Zuker 2003) to align the sequences correctly. The final alignment (374 bases) was generated in 4SALE (Seibel et al. 2006, 2008), according to the secondary-structure information (Fig. S1 in the supplementary material). The final SSU rDNA alignment was generated as follows. First, we downloaded 280 aligned sequences of Desmidiales from the SILVA database, version 98 (Pruesse et al. 2007); then, the alignment was reduced to Desmidiaceae and analyzed by the neighbor-joining (NJ) method in PAUP*, version 4.0b10 (Nylander 2004). On the basis of an inferred phylogenetic tree and literary data (Gontcharov et al. 2003, Gontcharov and Melkonian 2008, Hall et al. 2008), the alignment was reduced to 63 sequences that were selected to encompass all Desmidiaceae lineages. In addition, the long-branched *Cosmarium* sequences AJ428113, AJ428114, AY964133, and AY964135 were removed to avoid the long-branch attraction (LBA) effect in phylogenetic analyses. Finally, two new SSU rDNA *Micrasterias* sequences, together with one closely related sequence revealed by BLAST searches, were added to the alignment and aligned with the help of the published SSU rRNA secondary-structure transcript of the

genus *Closterium* (Denboh et al. 2001). The final alignment thus comprises 62 sequences (Table S1 in the supplementary material). Ambiguously aligned regions (loops at the end of stem 17 and between the stems 45 and 46) and positions with deletions in most sequences were removed from the alignment, resulting in an alignment comprising 1,725 base positions. All of the alignments were submitted to TreeBASE (ID: SN4729).

Phylogenetic analyses. The phylogenetic trees were inferred with Bayesian inference (BI) using MrBayes version 3.1 (Ronquist and Huelsenbeck 2003). The most appropriate substitution model was estimated for each data set using the Akaike information criterion (AIC) with PAUP/MrModeltest 1.0b (Nylander 2004). In the BI analysis, two parallel Markov chain Monte Carlo (MCMC) runs were carried out for three million generations, each with one cold and three heated chains. Bootstrap analyses were performed by maximum-likelihood (ML) and weighted parsimony (wMP) criteria using PAUP*, version 4.0b10. ML bootstrap analysis (100 replications) consisting of heuristic searches with 10 random sequence addition replicates, tree bisection-reconnection (TBR) swapping, and a rearrangement limit of 5,000 for each replicate. The wMP bootstrapping (1,000 replications) was performed using heuristic searches with 100 random sequence addition replicates, TBR swapping, random addition of sequences (the number limited to 10,000 for each replicate), and gap characters treated as a fifth character state. Congruence between ITS2 rDNA and $trnG^{uuc}$ intron data sets was tested using the incongruence length difference (ILD) test (Farris et al. 1995), as implemented by the partition homogeneity test in PAUP* (heuristic search, simple addition, TBR branching swapping, 100,000 replicates).

Morphometric analyses. For each strain, 50 adult semicells were randomly chosen and photographed. On each semicell, 31 structurally corresponding landmarks and semilandmarks were depicted (Fig. 1). Whereas the landmarks were placed in fixed positions, the semilandmarks (nos. 10, 11, 15, 17, 21, and 22) were allowed to slide along the abscissa connecting adjacent landmarks (Zelditch et al. 2004). For most of the geometric morphometric analyses, the TPS-series software (available at http://life.bio.sunysb.edu/morph/) was used. Positions of landmarks and length and width of the cells were digitized in TpsDig, ver. 2.12. The landmark configurations of the entire set of 450 objects were superimposed by generalized Procrustes analysis (GPA) in TpsRelw, ver. 1.42. This widely used method standardizes the size of the object and optimizes the rotation and translation so that the distances between corresponding landmarks of investigated objects are minimized (Bookstein 1991, Zelditch et al. 2004). 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 statistical analyses (Zelditch et al. 2004). The correlation of Procrustes and Kendall shape spaces was very high ($r = 0.999$), so we proceeded with further analyses. The *Micrasterias* semicells are bilaterally symmetrical, and their anterior and posterior sides do not differ (Neustupa and Škaloud 2007). As the asymmetry of semicells was not of interest in this study, cells were symmetrized prior to analysis following the standard formula of Klingenberg et al. (2002). This involved reflecting the landmark configurations (by multiplying the *x*-coordinates in all landmarks by −1). Then, the paired landmarks in the reflected copy were relabeled, and the original and mirrored configurations were averaged in the general Procrustes superimposition (Klingenberg et al. 2002). A principal component analysis (PCA) of geometric morphometric data was conducted on the entire set of 450 semicells. Scores of the objects on the all nonzero 29 principal component (PC) axes were used for canonical variate analysis (CVA) in PAST, ver. 1.89 (Hammer et al. 2001), to test for the differences in shape of individual strains. In addition, scores on PC axes were used for the two-group linear discrimination analyses between all pairs of investigated strains. Significance of the difference between group mean shape configurations was assessed by the Hotelling's T^2 test. In parallel, the *K*-means clustering, a method based on the nonhierarchical clustering of multivariate data into a specified number of groups (Bishop 1995), was used to illustrate relative differences in shape of the individual strains. The *K*-means clustering into two groups was conducted in pairs of all investigated strains. Number of cells classified out of the original pattern of 50 versus 50 objects from two strains in each pair indicated their overall shape similarity. *K*-means clustering pattern identical with the underlying origin of cells from two strains indicated clear separation of groups, while a high number of cells placed into the wrong group illustrated the close relationship of two groups on the basis of their landmark-based shape data.

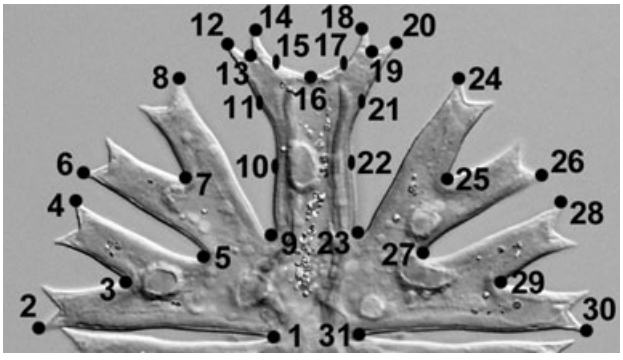

FIG. 1. Position of structurally corresponding landmarks on *Micrasterias* cells.

RESULTS

Phylogenetic analyses. The genetic diversity of nine *Micrasterias* strains was determined using the ITS2 of rDNA and the group II intron sequences of the plastid gene that encodes transfer RNA-Gly ($trnG^{uuc}$). Results of genotype and haplotype groupings among the strains are illustrated in Figure 2. These two markers differed considerably in the amount of their variation. The ITS2 rDNA showed a higher average genetic distance (Kimura 2-parameter model) between the lineages (0.168) than the $trnG^{uuc}$ intron (0.032). The ITS2 rDNA sequences were so variable that the alignment could only be established with the aid of the common ITS2 rRNA secondary structure transcript (Fig. S1). On the other hand, the alignment of $trnG^{uuc}$ intron sequences was straightforward and unambiguous, allowing us to analyze the phylogeny of nine investigated *Micrasterias* strains, together with the three related strains (*M. crux-melitensis* NIES 152, GenBank accession no. FN562171; *M. truncata* CAUP K606, FN562173; and *Staurodesmus dickiei* ASW 07056, FN562172). In general, genotype groups observed in the ITS2 rDNA sequences (Fig. 2A) corroborated the haplotype groupings observed in the $trnG^{uuc}$ intron sequences (Fig. 2B). The congruence of both

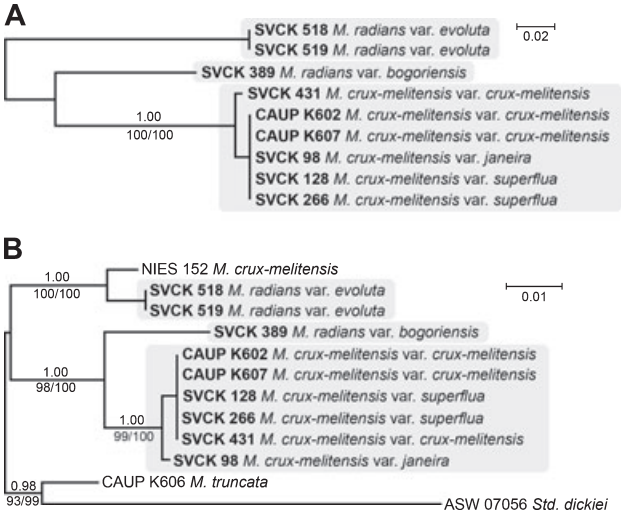

FIG. 2. Unrooted Bayesian analyses based on ITS2 rDNA (A) and *trnG*^{uuc} intron (B) sequences using a GTR model for ITS2, and a GTR + I model for *trnG*^{uuc} data set. Values at the nodes indicate statistical support estimated by three methods: MrBayes posterior node probability (upper), maximum-likelihood bootstrap (bottom left), and maximum-parsimony bootstrap (bottom right). Three lineages recognized from the phylogenetic analyses are highlighted by gray boxes. Scale bars = expected number of substitutions per site. GTR, generalized time reversible; ITS, internal transcribed spacer; trnG, glycine transfer RNA gene.

phylogenies was supported by the statistically significant partition homogeneity test (ILD test; $P = 0.52$). Generally, three lineages could be established from the phylogenetic analyses: (i) a large lineage comprising all the European and the single North American *M. crux-melitensis* isolates (CAUP K602, CAUP K607, SVCK 98, SVCK 128, SVCK 266, and SVCK 431, respectively); (ii) the single *M. radians* strain originating from Southeast Asia (SVCK 389); and (iii) two *M. radians* isolates of tropical African origin (SVCK 518 and SVCK 519). The single difference between the ITS2 and *trnG*^{uuc} phylogenies pertained to the genetic identity of the European strains. The ITS2 analysis showed the divergence of SVCK 431, whose sequence differed by two substitutions and one insertion from that of the other strains in the European lineage. By contrast, the *trnG*^{uuc} analysis revealed a single substitution in the SVCK 98 sequence that differentiated it from the rest of the strains. Moreover, the *trnG*^{uuc} data revealed the monophyly of *M. crux-melitensis/radians* complex (BI/ML/MP support 0.98/93/99). However, the strains pertaining to the same traditional morphospecies did not cluster together (Fig. 2B). According to the *trnG*^{uuc} sequences, European/North American *M. crux-melitensis* isolates formed a well-supported clade (1.00/98/100) with Asian *M. radians* SVCK 389. Similarly, the pair of African *M. radians* isolates significantly clustered with *M. crux-melitensis* NIES 152 (1.00/100/100).

Although both ITS2 rDNA and *trnG*^{uuc} markers were very useful to reveal genetic relationships among nine investigated *Micrasterias* strains, they were too variable for determining phylogenetic position of the three recognized lineages within the genus *Micrasterias* and Desmidiaceae as a whole. Therefore, SSU rDNA sequences of the selected representative strains (CAUP K602, SVCK 389, and SVCK 518) were compared with the SSU rDNA data set of 59 desmid species (Fig. 3). All three lineages formed a well-supported independent clade, together with *M. crux-melitensis* NIES 152, *M. truncata* CAUP K606, and *S. dickiei* SVCK 38. The strain of *M. crux-melitensis* CAUP K602 belonging to the European/North American cluster formed a clade (74/95/0.99) with the Asian *M. radians* SVCK 389, which did not include the African *M. radians* SVCK 518 (Fig. 3). Although the exact position within Desmidiaceae remained ambiguous, BI, ML, and MP analyses (latter two not shown) consistently showed all other *Micrasterias* sequences as the next closest relatives to this clade. However, neither the relationship with any of these other *Micrasterias* species nor the lineage encompassing the genus as a whole received significant internal branch support.

Morphology of cells. The cells of all strains were readily identifiable into *M. crux-melitensis/M. radians* species complex (Fig. 4). However, there were apparent differences in morphology of individual strains. Cell dimensions profoundly differed (Fig. 5, A and B). The strains SVCK 266, SVCK 431, and SVCK 98 consistently had the smallest cells, while cells of the SVCK 128, CAUP K602, CAUP K607, and SVCK 518 strains were ~35% to 45% larger. Moreover, the degree of lobulation differed among strains. Most strains had well-developed second-order lobules present in all semicells (Fig. 4, A, B, E–I), with the notable exception of the SVCK98 strain, whose second-order lobules were only weakly developed (Fig. 4D). At the same time, this strain had the relatively smallest cells (Fig. 5, A and B), and it corresponded well to the *M. crux-melitensis* var. *janeira*, according to the traditional taxonomy (Krieger 1939). On the other hand, the SVCK128 strain of *M. crux-melitensis* var. *superflua* had third-order lobules present on most semicells (Fig. 4C) and relatively large cells (Fig. 5, A and B), so that it fit well into the description of this variety. The second strain, originally assigned as *M. crux-melitensis* var. *supeflua*, SVCK 266, was different from SVCK 128 as it had considerably smaller cells and most semicells did not develop the third-order lobules. The SVCK 431, CAUP K602, and K607 fit into descriptions of the type variety *M. crux-melitensis* var. *crux-melitensis*, even if their cell dimensions differed among strains. In contrast to other strains, the SVCK 518 and SVCK 519 had a pair of spines on each side of their polar lobes (Fig. 6, A–C). However, these polar lobe spines were not always symmetrically developed on all semicells, and, sometimes, there was just a single asymmetrical spine present (Fig. 6B). This characteristic presence

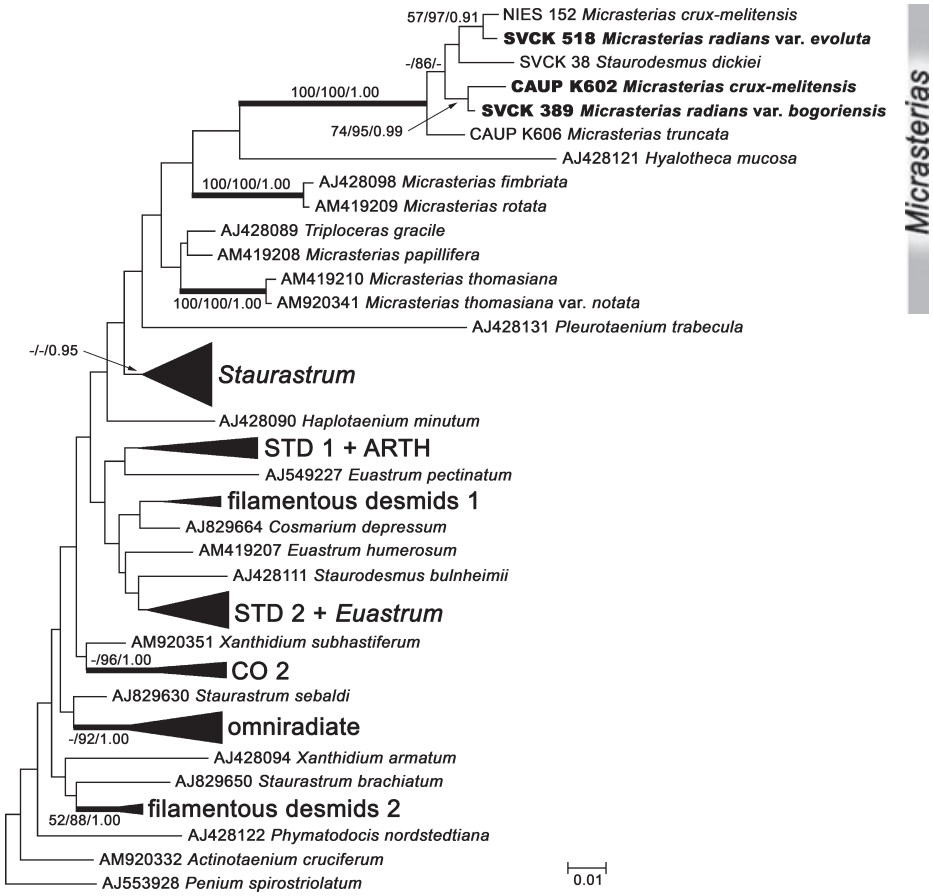

FIG. 3. Bayesian analysis of SSU rDNA sequences of *Micrasterias* species and other representatives of Desmidiaceae using a GTR + I + Γ model. Values at the nodes indicate statistical support estimated by three methods: maximum-likelihood bootstrap (left), maximum-parsimony bootstrap (in the middle), and MrBayes posterior node probability (right). Thick branches represent nodes receiving the highest PP support (1.00). The sequences representing three lineages recognized from the phylogenetic analyses of ITS2 rDNA and $trnG^{uuc}$ intron sequences (Fig. 2) are shown in bold. Collapsed groups of sequences correspond to clades proposed by Gontcharov and Melkonian (2008). The tree is rooted with *Penium spirostriolatum* AJ553928. Scale bar = expected number of substitutions per site. GTR, generalized time reversible; ITS, internal transcribed spacer; PP, posterior probability; trnG, glycine transfer RNA gene.

of polar lobe spines, as well as the long furcate lobules of their semicells, led us to identify these strains as *M. radians* var. *evoluta* (W. B. Turner) Willi Krieg. according to traditional criteria (Krieger 1939). The SVCK 389 strain of *M. radians* var. *bogoriensis* (C. J. Bernard) Willi Krieg. fit well into the taxonomic description of this variety, mainly by the presence of long, slightly curved spines on tips of the polar lobes and lateral lobules (Fig. 6F). Furthermore, the third-order lobules were sometimes present, in accordance with the taxonomic delimitation of this variety. The cell walls of all the investigated strains were consistently provided with pore-linked mucilage extrusions (Fig. 6, D–F), and no differences among strains were detected in this genus-wide feature.

Geometric morphometrics. The PCA of the symmetrized Procrustes-superimposed landmark data yielded 29 nonzero PC axes. The first PC axis spanned 59.5% of the total variation. Semicells varied from being deeply lobed, with a narrow polar lobe in negative values, to compact shapes with shallow incisions and a wide polar lobe in positive marginal positions along the first PC axis (Fig. 7). Consequently, the strains SVCK 518, SVCK 519, and SVCK 389, which were characterized by deeply lobed semicells of the *M. radians* type were positioned close to the negative margin on PC1. Conversely, the SVCK 98 strain had the most positive PC1 scores corresponding to its compact semicells with shallow incisions (Fig. 8, A and B). The second PC axis, which spanned 10.44% of the total shape variation, was related to width of the upper lateral lobule. Semicells with negative scores on PC2 had narrow upper lateral lobules and open incisions, while semicells with positive PC2 scores were characterized by closed incisions and wide upper lateral lobules (Figs. 7; 8, A and B). This axis primarily separated strains SVCK 518, SVCK 519, SVCK 389, and SVCK 98 positioned mostly in negative PC2 values from the rest of the strains.

The CVA illustrated the separation of strains on the basis of their shape characteristics (Fig. 8, C and D). The overall separation among strains was highly significant (Wilk's $\lambda = 1.46e10^{-5}$, $P = 0$). The SVCK 98 strain was the most dissimilar from all other strains. In addition, the three strains SVCK 518, SVCK 519, and SVCK 389, assigned originally as *M. radians,* were grouped together and more or less separated from the remaining strains (Fig. 8, C and D). The pair comparisons revealed that all strains were mutually statistically significantly different in their mean shape characteristics (Table 2). The *P*-value of the Hotelling's T^2 tests was $<10^{-5}$ in all

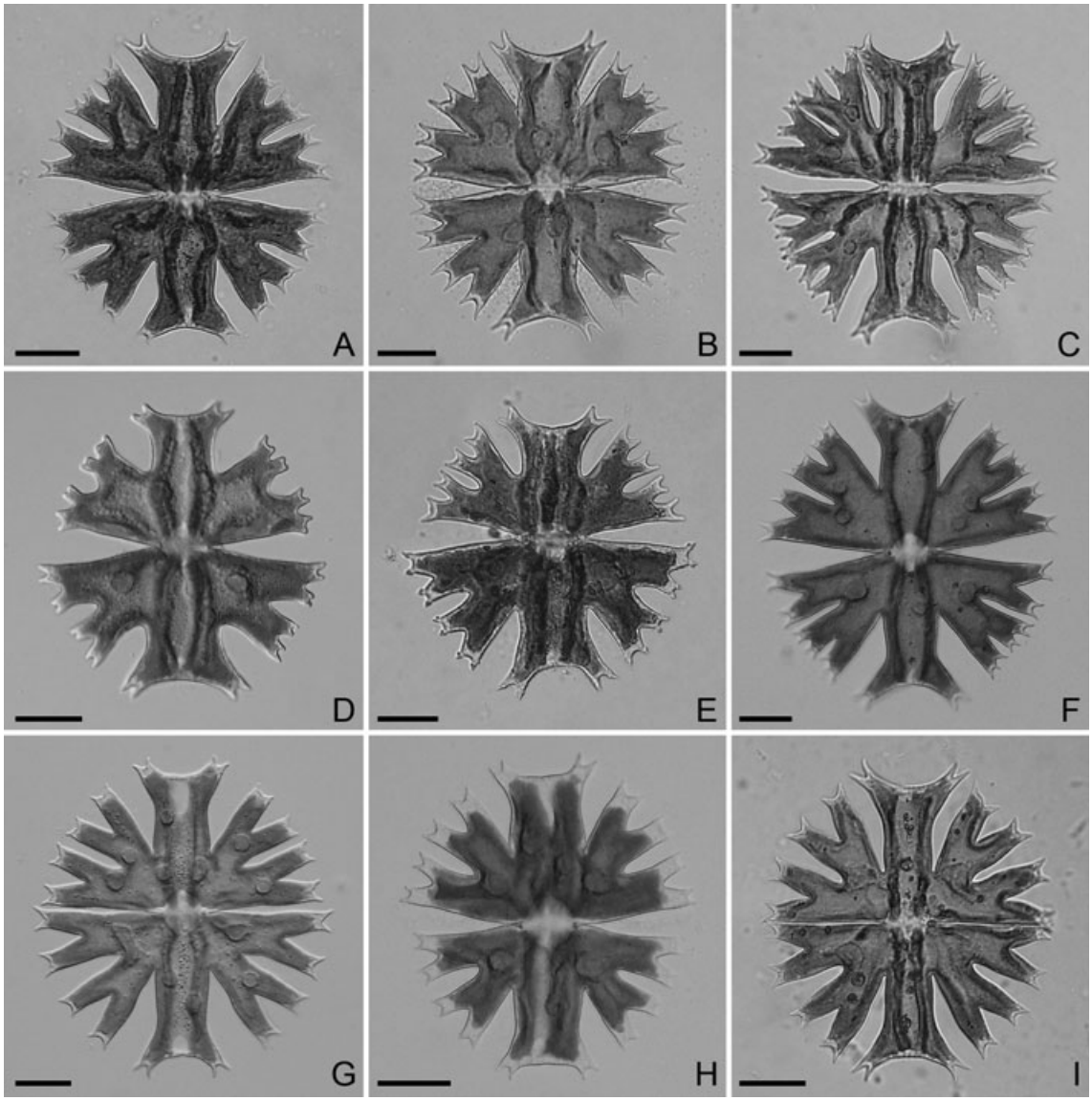

FIG. 4. Morphology of *Micrasterias* strains. (A) *M. crux-melitensis* var. *superflua*, strain SVCK 266. (B) *M. crux-melitensis* var. *crux-melitensis*, strain SVCK 431. (C) *M. crux-melitensis* var. *superflua*, strain SVCK 128. (D) *M. crux-melitensis* var. *janeira*, strain SVCK 98. (E) *M. crux-melitensis* var. *crux-melitensis*, strain CAUP K602. (F) *M. crux-melitensis* var. *crux-melitensis*, strain K 607. (G) *M. radians* var. *evoluta*, strain SVCK 518. (H) *M. radians* var. *evoluta*, strain SVCK 519. (I) *M. radians* var. *bogoriensis*, strain SVCK 389. Scale bar = 20 μm.

pairs, and there were 100% correctly classified cells in most of the pair comparisons. The *K*-means clustering algorithm illustrated a higher number of objects classified across the original group assignment (Table 2). In this respect, the pairs of *M. crux-melitensis* strains from the Czech Republic (CAUP K602, K607) and the pair of African *M. radians* var. *evoluta* strains SVCK 518 and SVCK 519 had the most similarly shaped semicells.

DISCUSSION

Our molecular phylogenetic analyses clearly did not confirm taxonomic homogeneity of the *M. crux-melitensis*/*M. radians* species complex. The phylogenetic inferences based on the nuclear ITS2 rDNA and chloroplast $trnG^{uuc}$ intron sequences consistently demonstrated that the investigated nine strains clustered into three different lineages. The phylograms of both molecular markers were highly congruent, except for slight differences in the relationships of the *M. crux-melitensis* strains (Fig. 2). However, because the differences were based on just a few substitutional changes in the rapidly evolving molecular markers, they may correspond to an among-population structure of a single species. This slight incongruence in genetic variability between the two molecular markers within the European

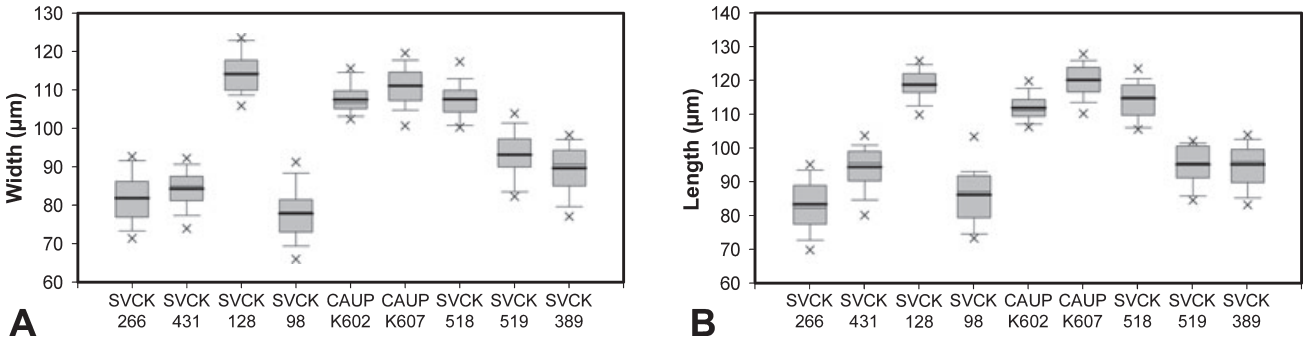

FIG. 5. Size data of investigated strains: (A) width and (B) length of cells.

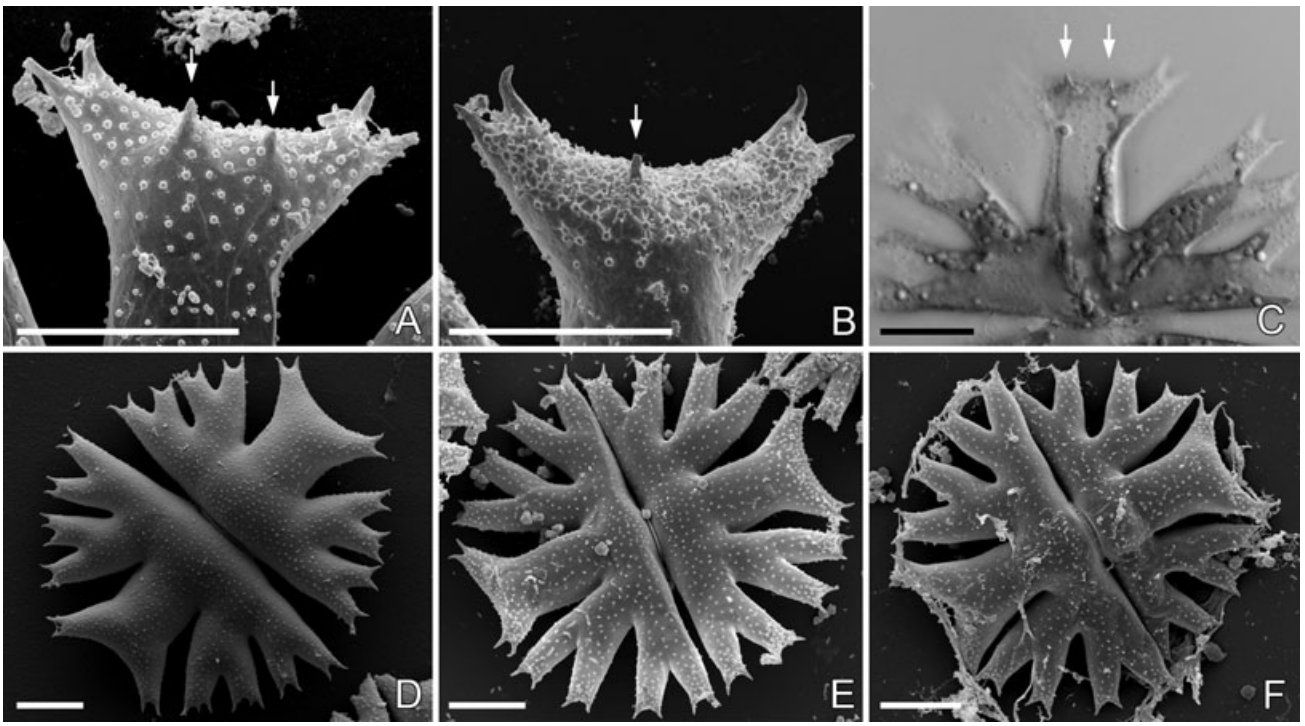

FIG. 6. Morphology of *Micrasterias* strains. (A–B) Scanning electron microscopic images of the polar lobes with prominent spines in *M. radians* var. *evoluta*: (A) SVCK 518 and (B) SVCK 519. (C) Projection of two light microscopic images visualizing the spines in the polar lobe, SVCK 518. (D–F) SEM images of whole cells illustrating the consistent nodulation of the cell walls: (D) CAUP K607, (E) SVCK 518, and (F) SVCK 389. Spines present in the polar lobes are marked by arrows. Scale bar = 20 µm.

lineage clearly illustrates the importance of using several independent molecular markers for inferring phylogenies of closely related organisms.

The SSU rDNA phylogeny unambiguously grouped all the investigated strains into the highly supported clade within Desmidiaceae (Fig. 3). In addition to several *Micrasterias* sequences, this lineage also included the single sequence of the morphologically completely different *S. dickiei* (strain SVCK 38, accession no. AJ428101). Based on the substantial morphological dissimilarity between *Micrasterias* species and *S. dickiei*, we decided to resequence this species to disprove possible contamination or misidentification of strain SVCK 38. Since the *Staurodesmus* strain SVCK 38 is no longer available in the culture collections, we acquired the SSU rDNA and $trnG^{uuc}$ intron sequences from the *S. dickiei* strain ASW 07056 (GenBank accession no. FN562174). The obtained SSU rDNA sequence indeed differed from AJ428101 by just a single substitution change. Therefore, we confirmed the phylogenetic position of *S. dickiei* among the *Micrasterias* species, as previously reported by Gontcharov et al. (2003). Such close relationship between the members of the genus *Micrasterias* and other morphologically dissimilar desmid species was already reported by Hall et al. (2008), who illustrated the position of *Triploceras gracile* among the

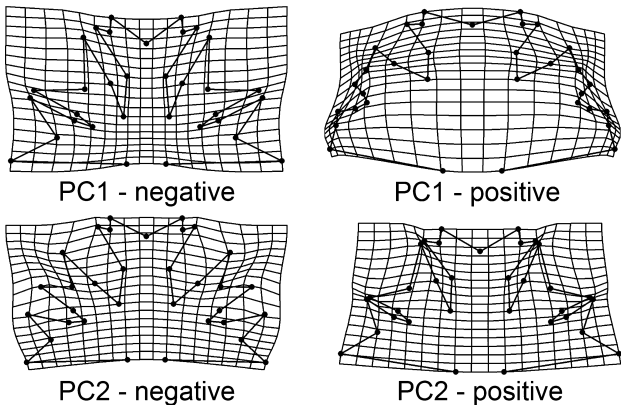

FIG. 7. The deformation grids illustrating shapes reconstructed at the extreme positions of first and second axes of the principal component analysis of the entire investigated set.

Micrasterias species. This phenomenon may indicate repeated reductive morphological evolution of morphologically different desmid species from *Micrasterias*-like ancestors. The SSU rDNA phylogeny did not reveal internal structure of the well-supported clade that encompassed the *M. crux-melitensis/radians* sequences, *S. dickiei*, and *Micrasterias truncata*. However, the phylogenetic inference based on the $trnG^{uuc}$ intron sequences clustered all the *M. crux-melitensis/radians* sequences into a well-supported monophyletic lineage (BI/ML/MP support 0.98/93/99), sister to the pair of *M. truncata* and *S. dickiei* (Fig. 2B).

In Desmidiaceae, the SSU rDNA phylogenies yielded rather weak resolution and support of internal branches (Besendahl and Bhattacharya 1999, Denboh et al. 2001, Gontcharov et al. 2003). However, several recent phylogenetic studies that combined various nuclear, chloroplast, and mitochondrial markers resulted in increased phylogenetic resolution and resolved inconsistencies among particular single-gene phylogenies (Gontcharov et al. 2004, Gontcharov and Melkonian 2008, Hall et al. 2008). Although we used the single-gene phylogeny, the Bayesian inference of SSU rDNA sequences was generally consistent with phylogenies of Desmidiaceae based on concatenated data sets (Gontcharov and Melkonian 2008, Hall et al. 2008). The close relationship of *Micrasterias* and *Staurastrum* lineages was demonstrated, although it was not supported by statistical tests (Fig. 3).

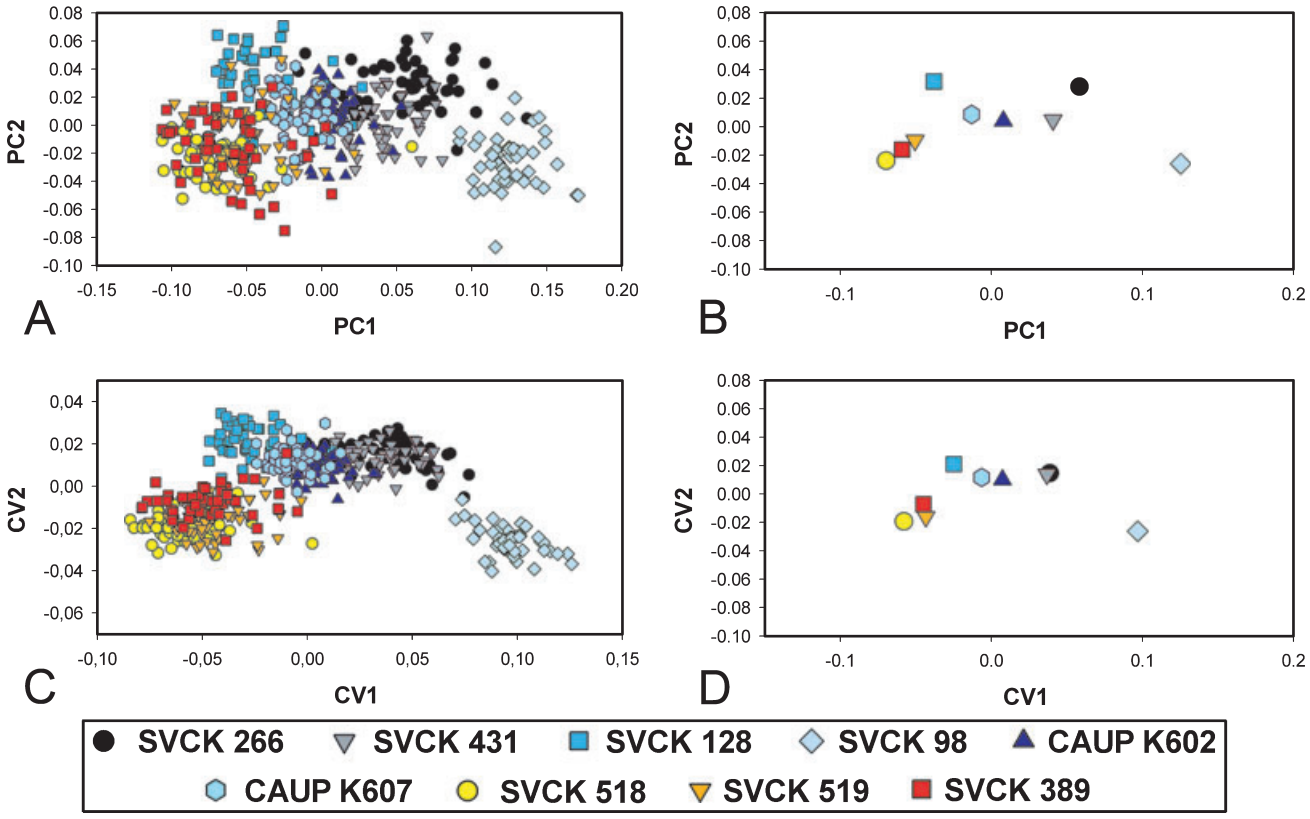

FIG. 8. Multivariate analyses of geometric morphometric data. (A) The ordination plot of first and second axes of the principal component analysis (PCA) of all the 450 investigated objects. (B) Position of the group centroids in the ordination plot of first and second axes of the PCA of all the 450 objects. (C) The ordination plot of first and second axes of the canonical variates analysis (CVA) aimed at separation of individual groups. (D) Position of the group centroids in the ordination plot of first and second axes of the CVA aimed at separation of individual groups.

TABLE 2. The results of multivariate statistical tests. The upper triangle corresponds to proportion of objects correctly assigned to their appropriate groups in linear discrimination analyses. The lower triangle corresponds to the number of displaced objects in two-group *K*-means clustering.

	SVCK 266	SVCK 431	SVCK 128	SVCK 98	CAUP K602	CAUP K607	SVCK 518	SVCK 519	SVCK 389
SVCK 266	–	100	100	100	100	100	100	100	100
SVCK 431	17	–	100	100	100	100	100	100	100
SVCK 128	4	2	–	100	100	100	100	100	100
SVCK 98	3	0	0	–	100	100	100	100	100
CAUP K602	14	5	3	0	–	99	100	100	100
CAUP K607	8	2	6	0	35	–	100	100	100
SVCK 518	1	1	2	1	1	1	–	99	100
SVCK 519	2	1	2	0	4	6	33	–	100
SVCK 389	1	3	0	0	1	3	2	5	–

Comparison of phylogenetic results with morphological and morphometric data provides evidence for the probable unreliability of some traditionally delimited subspecific taxa of *M. crux-melitensis*. This species, represented in our study by strains from temperate Europe and North America, was genetically homogenous, despite its high morphological variability. In fact, SVCK 98, morphologically corresponding to *M. crux-melitensis* var. *janeira*, and SVCK 128, corresponding to *M. crux-melitensis* var. *superflua*, occupied opposite ends of the overall morphospace of the investigated complex. Despite this morphological dissimilarity, they were phylogenetically homogenous. Therefore, we can certainly not reject the hypothesis of Růžička (1981), who suggested that subspecific varieties of *M. crux-melitensis* bear no taxonomic value and should be synonymized into a single taxon. On the other hand, an evident pseudocryptic diversity was illustrated for *M. radians*. Therefore, we can reject the hypothesis of possible conspecificity of *M. crux-melitensis* and *M. radians* (Vyverman and Viane 1995). The pair of African strains (SVCK 518 and SVCK 519) formed a tight cluster separate from the Asian SVCK 389, which remained in an isolated phylogenetic position within the investigated complex. The overall cell-shape properties of these three strains were relatively similar as they occupied adjacent or overlying parts of the morphospace (Fig. 8). However, they clearly differed in the pattern of spine formation on cells. The African strains formed spines on central parts of semicell polar lobes that corresponded to traditionally delimited *M. radians* var. *evoluta*. On the other hand, the SVCK 389 strain had conspicuous spines, mainly on lateral tips of polar lobes, and occasionally formed the third-order semicell lobules corresponding to the taxonomic delimitation of *M. radians* var. *bogoriensis*. Based on the degree of their phylogenetic divergence and presence of the unambiguous discriminating morphological characters, we suppose that these two lineages are in fact separate species. However, we would be reluctant to describe these species formally on the basis of an investigation of only three strains. Evidently, further natural populations corresponding to *M. radians* var. *evoluta* and to *M. radians* var. *bogoriensis* should be sampled and sequenced before taxonomic conclusions can be made. Nevertheless, our study demonstrated that *M. radians* is heterogenous and clearly nonmonophyletic. This species has mainly been reported in floristic studies from tropical and subtropical habitats worldwide, and the morphology of these findings was typically documented by illustrations or microphotographs. *M. radians* var. *evoluta*, unambiguously illustrated with spines in the central parts of polar lobes, which morphologically corresponds to our SVCK 518/519 phylogenetic lineage, was reported, for example, from Congo (Van Oye 1957), Mali (Couté and Tell 1981), Uganda (Lind 1971), Mozambique (Rino 1972), South Africa (Williamson 1994), and Madagascar (Bourelly and Manguin 1949, Coesel 2003). Turner (1892) described this taxon from East India, but no illustration was provided. Therefore, we can speculate that occurrence of the SVCK 518/519 lineage, corresponding to the traditional *M. radians* var. *evoluta*, may be limited to tropical regions of Africa, with most reports stemming from the sub-Saharan region. *M. radians* var. *bogoriensis* was taxonomically characterized by the presence of third-order lobules and by conspicuous spines on polar lobe tips (Krieger 1939). This taxon has frequently been reported from tropical and subtropical Asia and Australia (e.g., North Australia, Scott and Prescott 1958, Indonesia, Scott and Prescott 1961). In addition, Franceschini (1992) illustrated the *M. radians* specimen from tropical South America, morphologically similar to *M. radians* var. *bogoriensis*. The variety has further been reported from this region by Heckman (1998). The single European report of *M. radians* var. *bogoriensis* from Aquitaine, France (Capdevielle 1982), should be considered as uncertain, because the illustrated specimens, characterized solely by the presence of the third-order lobules, may very well correspond to *M. crux-melitensis* var. *superflua*. This is known from several European localities and belongs to the temperate *M. crux-melitensis* lineage according to our molecular data.

In conclusion, we have established that morphology may not provide a reliable species identification, even in morphologically very complex protists such as *Micrasterias*. The strains of *M. crux-melitensis*,

originating from European and North American localities, encompassed a large part of the observed shape variation within the investigated complex, but this phenotypic variability was not reflected in the molecular data. On the other hand, morphologically similar, subtropical and tropical *M. radians* strains from Africa and Asia, corresponding to traditional subspecific taxa, were revealed to be of separate lineages. These results suggest that there may be more *Micrasterias* species than indicated solely by morphological data. In addition, the actual species diversity of these relatively large protists may be related to the patterns of their geographic distribution, or to the climatic factors, rather than to purely morphological features, as indicated, for example, by the continent-wide European distribution of the morphologically diverse *M. crux-melitensis* lineage.

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Supplementary Material

The following supplementary material is available for this article:

Figure S1. Predicted secondary structures of the ITS2 transcripts of three *Micrasterias* taxa: *M. crux melitensis* CAUP K602 (accession no. FN424419), *M. radians* var. *evoluta* SVCK 518 (accession no. FN424421), and *M. radians* var. *bogoriensis* SVCK 389 (accession no. FN424423).

Table S1. Origin and description of strains included in SSU rDNA analysis (Fig. 3).

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