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Biogeography and phenotypic plasticity in silica-scaled chrysophytes (Synurophyceae)

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LIST OF PAPERS

This thesis is based on the following papers referred to in the text by numbers:

1. Řezáčová, M. & Neustupa, J. (2007): **Distribution of the genus *Mallomonas* (Synurophyceae) - Ubiquitous dispersal in microorganisms evaluated.** *Protist* 158: 29-37.
2. Neustupa, J. & Řezáčová, M. (2007): **The genus *Mallomonas* (Mallomonadales, Synurophyceae) in several Southeast Asian urban water bodies - the biogeographic implications.** *Nova Hedwigia* 84: 249-259.
3. Řezáčová, M. (2006): ***Mallomonas kalinae* (Synurophyceae), a new species of alga from northern Bohemia, Czech Republic.** *Preslia* 78: 353-358.
4. Řezáčová-Škaloudová, M., Neustupa, J. & Němcová, Y. **Effect of temperature on the variability of silicate structures in *Mallomonas kalinae* and *Synura curtispina* (Synurophyceae).** *Nova Hedwigia Beiheft*, in press.
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Author's contribution

1. Magda Řezáčová and Jiří Neustupa planned and wrote the paper jointly. Magda Řezáčová conducted the sampling.
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4. Magda Řezáčová-Škaloudová planned the study and wrote the paper. Jiří Neustupa helped with analysis of the geometric morphometric data and Yvonne Němcová with experimental design.
5. Study was planned jointly. Anna Kynčlová and Pavel Škaloud conducted the molecular part of the study, Magda Řezáčová-Škaloudová provided geometric morphometric analysis of the scale shape data.

I declare that this thesis or any part of it was never submitted to obtain any other academic degree.

We declare the keynote participation of Magda Řezáčová-Škaloudová in this thesis, as described above.

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1 INTRODUCTION

1.1. General introduction

The silica-scaled chrysophytes are unicellular flagellates, solitary or colonial, involving in the algal classes Chrysophyceae and Synurophyceae. Members of both classes are important part of the freshwater phytoplankton and occur in all climatic zones. The class Synurophyceae is represented by the genera *Mallomonas*, *Synura*, *Tessellaria* and *Chrysodidymus*. In this thesis, I have focused on the genera *Mallomonas* Perty and *Synura* Ehrenberg.

Species of both genera have specific silica structures: scales and bristles (*Mallomonas*), or scales and spines (*Synura*). These silica structures are generated endogenously in silica deposition vesicles (SDVs) derived from the Golgi apparatus. SDVs are located along the outer surface of only one of the two chloroplasts. Scale rows overlap one another and are spiraled to the right around the cell. In a single cell there are several scale types. In *Mallomonas* there are most often three scale types: apical, body, and posterior scales. Similar sequence of scale types or the scales simply decreasing in complexity from the anterior to the posterior end occur also in *Synura* (Kristiansen 1986). The scale case is not static armour, but a dynamic structure that adjusts to the addition of new scales during both cell growth and division. Mechanisms of scale case morphogenesis in *Mallomonas adamas* attempted to explain in detail Lavau & Wetherbee (1994). Throughout interphase, a duplicate set of scales is manufactured and gradually secreted into the existing scale case in a precise sequence. The number of apical scales doubles just prior to division, apparently completing the scale duplication process. Half the parental scales are inherited by each daughter cell during division, thus recently divided cells are normally surrounded by scales. Cells divide longitudinally from the cell apex within a short period of time (minutes). In *Synura*, another model of scale case morphogenesis was proposed by Leadbeater (1990). According to his model, scales are extruded in rows, thus the scale case increases in size by addition of complete rows of scales rather than the sequential addition of single scales to existing rows.

The scales consist of a perforated basal plate with various upturned or bent parts, and a secondary ornamentation which is characteristic for the different species (Kristiansen 1986). Taxonomy of synurophytes is thus based on scale ultrastructure. Both *Mallomonas* and *Synura* scales are close to bilateral symmetry. The scale left and right sides of the some species can be discerned (for example on dome scales by asymmetric dome features). The

scales are about 1-10 μm in size, thus only few species can be surely identified using light microscope (LM). For accurate identification using electron microscope (EM) is necessary and hence transmission or scanning electron microscopes (TEM or SEM) are the standard instruments for investigations of the silica-scaled chrysophytes.

Sexual reproduction – isogamy or anisogamy - has been observed for very few species and ploidy changes during cell cycle are still unclear (Kristiansen & Preisig 2007). During or at the end of the vegetation period flagellates form resting stages (stomatocysts) which sink down into the bottom sediment and remain there until germination. The cysts are of endogenous origin and are surrounded by a silicified wall which makes them resistant. All cysts have a single pore, through which the germinating cell emerges. The pore is formed secondarily and plugged with an organic substance. Some cysts are without ornamentation, but many others are highly ornamented and the pore may be surrounded by a collar of varied construction. Both the chrysophycean cysts and scales are preserved in benthic sediments due to high resistance. The cysts are the best preserved microfossils at all and may be found throughout a whole sediment core whereas scales are less resistant and more easily dissolved. The best preserved microfossils have been found in undisturbed and laminated sediments. The oldest, exceptionally well preserved scales are known from ca. 47 Ma sediments (Middle Eocene). These sediments were deposited as the post-eruptive infill of a crater at the Giraffe Pipe kimberlite locality in the Northwest Territories of Canada (Siver & Wolfe 2005).

Knowledge of the chrysophyte occurrence and their ecological ranges is used for monitoring of the status and quality changes of water bodies. The applications in paleolimnology and paleoecology started with works done in the late 1970s and early 1980s in United States, Canada and northern Europe (Smol 1995). Paleolimnological studies have mainly been focused on monitoring eutrophication, acidification and trends in climatic changes. According to Kristiansen (2005) a good bioindicator should be taxonomically well defined and reliably identified, widely distributed, with a characteristic narrow ecological range. Just many chrysophytes, similar as many diatoms, have a wide distribution and narrow occurrence spectra which determine them as good indicators. Combinations of chrysophyte and diatom data provide more accurate paleoecological reconstructions. However, to establish correct comparisons between the numbers of diatoms and of scaled chrysophytes, the number of scales per cell of the different species must be known (Kristiansen 2005). The scales are much better indicators than the stomatocysts because they can be identified easily to species with known ecological ranges, but not provide a complete record of past chrysophyte populations as only ca. 20% of known species possess scales. Stomatocyst are formed by all chrysophyte taxa, thus potentially provide a more complete record of the entire community. On the other hand, cysts are classified only by numbers since about only one-third of

stomatocyst morphotypes have been linked to the species that produce them. A numerical system for cysts was developed in 1986 to avoid using different names for vegetative cell and cyst (Duff et al. 1995, Wilkinson et al. 2001, Kristiansen 2002). Even if cysts cannot be linked to species they can be used as bioindicators together with other microfossils due to development of the surface sediment calibration sets (“training sets”). The environmental data were related to present stomatocyst morphotypes and scales. Inferred values of pH, conductivity and trophic score for the lakes were tested against measured values and showed the agreement. The obtained values are expected to work just as well for inferring environmental values in fossil sediments (Kristiansen 2005).

1.2. Biogeography and species concept of *Mallomonas* and *Synura*

Two models concerning the distribution patterns of microorganisms, that are the subject of controversial literature discusses, have recently appeared in series of papers: the “ubiquity model” proposed by Finlay and Fenchel (Fenchel & Finlay 2004, Finlay et al. 2004) and the “moderate endemism model” published by Foissner (Foissner 2004, 2006). The main different features of both models are included in Table 1. The ubiquitous model says that all microorganisms occur everywhere the environment is suitable due to high dispersal ability and high individual numbers whereas Foissner (2008) estimated that about one third of the taxa are endemic in spite of suitable habitats in other regions. The possible reasons for existence of restricted distribution are: young species did not have sufficient time to disperse globally; species might have specific ecological requirements found only in a certain habitat or region; species might have poor dispersal capacities or might have evolved in regions not favoring wide dispersal.

Three hypotheses of different diversity pattern between unicellular and multicellular organisms were tested by Hillebrand et al. (2001). Firstly, statement reflecting the ubiquity of protist species due to high dispersal ability was partly confirmed. Relative local species richness was significantly higher than for metazoans, but the difference depended on the global species richness estimate. Secondly, they tested the hypothesis of Finlay et al. (1998), who proposed a species:area curve for protist where a low slope of the species:area relationship indicated high dispersal ability. Hillebrand et al. (2001) analyzed this relationship for a variety of taxa, but the differences with metazoans were not categorical. At last, the revealing negative impact of geographic distance on the similarity of protist communities indicates the existence of unicellular endemics or at least regionally restricted species distribution (Hillebrand et al. 2001).

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

Table 1. Comparison of the ubiquity and the moderate endemicity models (according to Foissner 2008).

The number of protist species in both models has been estimated using morphological species concept. For assessment of the distribution patterns and diversity of microorganisms is, however, undoubtedly crucial to develop a species concept reconciling morphologic, genetic, and ecological features, because there is a physiological and ecological diversity that is hidden at the morphological level and not apparent at the level of conserved genes (Weisse 2008).

DNA sequences and phylogenetic analyses were applied into taxonomy of algae in the early 1990s. Since this introduction, 18S rDNA sequence has been so tightly coupled to species identity that identical 18S rDNA sequences are sometimes considered to be evidence that otherwise genetically distinct populations belong to the same species. However, sequence divergence by itself not yields robust, phylogenetically informative species boundaries. According to Weisse (2008) evolutionary conserved genes such as 18S rDNA may underestimate protist diversity, because the variability of the ribosomal genes is different in the various protist phyla. For instance, Boenigk et al. (2005) observed different ecotypes of the chrysophyte morphospecies *Spumella* from many different environments representing by the same 18S rDNA genotype. Generally, 18S rDNA is more suitable for reconstructing

higher level relationships across the entire phylogeny whereas internal transcribed spacer (ITS) regions are useful for species- and sometimes population-level relationships. The internal transcribed spacer regions (ITS 1 and ITS 2) are located between 18S-5.8S and 5.8S-28S genes, respectively. Because ITS sequences are not under the same functional constraints as the coding regions, sequence variation accumulates more rapidly (Alverson 2008). However, there is currently no molecular “gold standard” for taxonomy and to solve the species problem using a combination of morphology, molecular sequences, physiological characteristics and ecological data is recommended.

Resolving the species problem is crucial also for the silica-scaled chrysophytes, because their species concept is still exclusively based on ultrastructure of their silica structures. However, in many cases it is questionable whether the distinguishing characters are relevant and the establishment of forms and varieties is very subjective. The investigation of the genetic pattern will thus be desirable in the future studies, and as many chrysophyte specialists have agreed (Wee 1997, Kristiansen 2001), a consistent species concept is the necessary basis for ecological and biogeographical studies. At present, the series of papers concerning biogeography of the chrysophytes, and mainly of the most species-rich genus *Mallomonas*, were published by Kristiansen and co-authors (Kristiansen & Vigna 1996, Kristiansen 2000, Kristiansen 2001, Kristiansen & Lind 2005, Kristiansen 2008). The distribution types of chrysophytes have consequently been established. Cosmopolitan and widely distributed species include about half of all silica-scaled chrysophytes. Cosmopolitan species can be found on every continent with exception of Antarctica, widely distributed are absent in one or two continents, in most cases in Australia or in Africa. Most of the northern temperate-subarctic-arctic species are restricted to Europe and Northern America, a few of them occurs only in the arctic region. Bipolar taxa occur mainly in the temperate parts of the Northern and Southern Hemisphere (mainly southernmost South America). Some taxa have only been found in one tropical region or are pantropical, several others have scattered distributions, apparently showing no relationship to climatic or other factors. Endemic species have only been found within a smaller or larger restricted area. However, their endemism, beside other factors, might be caused due to insufficient sampling (Kristiansen 2001).

Mallomonas is the ideal object for biogeographical and ecological studies because there are a lot of floristic investigations almost all over the world and all distribution records have been summarized by Kristiansen & Preisig (2007). At present, the genus *Mallomonas* comprises altogether 182 taxa (out of them 138 species) (Kristiansen & Preisig 2007, Kim & Kim 2008). About one third of *Mallomonas* taxa are cosmopolitan or widely distributed, and another one third of the taxa are considered endemic. Interestingly, the endemic taxa have not

been found in isolated habitats, such as distant oceanic islands, but most of them have been found in well investigated areas (Kristiansen 2008).

The genus *Synura* includes only 19 species, about ten of them are cosmopolitan or widely distributed (Kristiansen & Preisig 2007, Němcová et al. 2008). Additional 11 *Synura* taxa have subjectively been described on the rank of form or variety. A wide range of more or less divergent morphotypes is known especially in the *Synura petersenii* complex. Several its different morphotypes have been found mainly in northern temperate regions and have been given status of forma. *S. petersenii* f. *macracantha* has been upgraded from forma to species *S. macracantha* (Petersen & Hansen) Asmund; on the other hand *S. glabra* has been degraded from species to forma *S. petersenii* f. *glabra* (Korshikov) Kristiansen & Preisig. Other form, *S. petersenii* f. *kufferathii* is often associated with f. *petersenii* and it is difficult to delimit it because the main character of this forma – longitudinal ribs connecting the transverse struts – may be present also in scales of f. *petersenii*, i.e. the number of longitudinal ribs is very variable (Kristiansen 2007).

As it is mentioned above, a few molecular studies have been carried out on synurophytes to date. DNA sequences from the ITS/5.8S region of *Synura petersenii* strains were determined by Wee et al. (2001). They sorted 15 strains into two well-supported clades, however, they did not examine scale morphology of the investigated strains. Only one molecular study comprising *Mallomonas* and *Synura* sequence data of small subunit rDNA (SSU or 18S) of 16 taxa together with ultrastructural information of scales was published by Lavau et al. (1997). In this study, the combined phylogeny (molecular and scale) has weakly supported *Mallomonas* and *Synura* monophyletic groupings, whereas molecular or scale case-derived phylogenies alone were not able to resolve the genus *Synura* as monophyletic. Andersen (2007) published the combined gene analysis of 18S rDNA and *rbcL* sequences suggesting the monophyletic *Mallomonas* and *Synura* genera, but the *rbcL* gene alone did not support monophyly of the genera. A molecular study of several genes from three genomes (nuclear SSU and ITS, plastid *rbcL*, mitochondrial *cox3*) of *Synura petersenii* isolates, unfortunately again without any information of scale morphology, showed Boo et al. (2007). They concluded that, based on phylogenetic analyses of independent and combined datasets, this single species actually consists of several divergent lineages, where some lineages are ubiquitous and others have discontinuous distribution pattern.

1.3. Phenotypic plasticity

A phenotype is any observable character or trait of an organism: such as its morphology, development, and biochemical or physiological properties. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and possible interactions between the two. The ability of an organism with a given genotype to change its phenotype in response to changes in the environment is called phenotypic plasticity. Most classic studies of phenotypic plasticity deal with morphological plasticity, but physiological acclimation and behaviour are aspects of the phenotype nevertheless, albeit characterized by their own mechanistic basis (Pigliucci 2001). One of the first published examples of phenotypic plasticity is the phenomenon known as cyclomorphosis, a seasonal shift in morphology in the crustacean *Daphnia* (Woltereck 1909).

Phenotypic plasticity of algae has been recorded in many phyecological studies. Morphological plasticity can often be detected readily in natural populations of diatoms and desmids, because the two halves of the cell are formed at different times and, quite possibly therefore, under different environmental conditions (Mann 1999). A detailed study of morphological plasticity in the genus *Scendesmus* published Trainor (1998), who reported the specific *Scendesmus* ecomorphs that have occurred only in specific season. Neustupa et al. (2008) investigated temperature-related morphological variation of *Micrasterias rotata*. As temperatures increased, the cell size of cultured *M. rotata* generally decreased and shape of the individual temperature groups differed significantly. Natural populations were consistently similar to the low temperature cultured populations throughout the season, while the high temperature morphotypes seen in culture were not present in natural samples. A general pattern of cell size plasticity in response to temperature was proposed by Atkinson et al. (2003). At extremely low temperatures, size increases with increasing temperature. In the thermal range normally encountered by a population an inverse relationship between size and temperature is expected.

In chrysophytes, growth characteristics (i.e. physiological acclimation) and morphological plasticity of silica structures have been studied in relation to distinct environmental conditions. The influence of environmental factors, such as temperature, pH, light intensity, nutrient and Si levels, etc. have been investigated in experimental cultures in laboratory conditions. The growth characteristics have been recorded at different temperatures and pH for some *Mallomonas* and *Synura* species. Not only the individual species, but also the strains (clones) of the same species have varying optimal growth requirements. It is thus obvious, that physiological and biochemical intraspecific differences exist among geographical and seasonal clones (Lee & Kim 2007; Wee et al. 1991). If we look at the scale structure, some species have a remarkable uniformity throughout their total distribution area, while others show considerable variability (Kristiansen 2001). The effect of temperature on

the variability of scales together with the growth characteristics were investigated in three *Synura petresenii* clones by Martin-Wagenmann & Gutowski (1995). They have still been able to distinguish an individual clone under all experimental conditions, although transitions in some characters among individual clones also occurred. Gavrilova et al. (2005) also revealed different strain-specific parameters (growth and morphological) of two *Synura petresenii* strains. Changes in morphology of silica structures in *M. tonsurata* and a tendency to reduce a secondary structure at low temperature (and thus resemblance to *M. alpina* scales) reported Gutowski (1996). All these studies are based on the analysis of conventionally measured distances. At the present time, landmark based geometric morphometrics (GM) is considered the most powerful tool in biological shape analyses (Adams et al. 2004; Zelditch et al. 2004) and thus, one part of the thesis is focused on phenotypic plasticity of silica structures at different temperatures using geometric morphometric approach.

1.4. Geometric morphometrics

Morphometric methods can help decide if morphological variation is continuous, which characters can be most effectively or easily used to distinguish groups, and furthermore, they can show variation over many specimens, the range of which could not be illustrated photographically (Beszteri et al. 2005). Traditionally, morphometric data have been measurements of length, width, angles, etc., but one limitation of traditional morphometrics is that such a data contain relatively little information about shape. This classical measurement scheme can be improved using geometric morphometric methods which allow the visualization of group and individual differences, and sample variation in the space of the original specimens. Geometric morphometrics is a collection of approaches for the multivariate statistical analysis of Cartesian coordinate data, usually limited to landmark point locations. In geometric morphometrics, shape is defined as all the geometric information that remains when location, scale and rotational effects are filtered out from an object. Removing the differences between configurations that are attributable to differences in location, scale and orientation leaves only differences in shape (Zelditch et al. 2004).

The use of landmarks, which has become a central concept of morphometrics in the last two decades, has started to establish at the present time also in phycology and taxonomy of algae. For example, they have been used in taxonomic and experimental investigations of diatoms (Beszteri et al. 2005; Potapova & Hamilton 2007), synurophytes (Neustupa & Němcová 2007), and green algae (Verbruggen et al. 2005; Neustupa & Šťastný 2006; Neustupa et al. 2008). Beszteri et al. (2005) used landmark-based geometric morphometric

approaches to clarify the taxonomic identity of a centric diatom morph with an intermediate valve morphology between that of typical specimens of *Cyclotella meneghiniana* and of *Cyclotella scaldensis*. They suggested that the different morphs probably belong to three reproductively isolated species. Potapova & Hamilton (2007) studied variation of frustular morphology within the *Achnantheidium minutissimum* species complex in type populations of described taxa and in river samples. Their analysis based on shape variables and striation pattern showed that North American specimens could be more consistently classified into the six groups identified in their analysis than into historically recognized taxa. Neustupa & Němcová (2007) revealed the variation in *Mallomonas striata* scales in relation to intraspecific identification of two varieties (*M. striata* var. *striata* and *M. striata* var. *serrata*). Previously, both of these varieties had been delimited only on the basis of differences in bristles (Kristiansen 2002).

2 THE MAIN OBJECTIVES OF THE STUDY

The presented thesis focuses primarily on two issues: biogeography of *Mallomonas* species and plasticity of *Mallomonas* and *Synura* silica structures under different temperatures using geometric morphometrics. Methods of geometric morphometrics were similarly used to conclusively delimit the species concept in *Synura petersenii* complex.

The principal aims can be summarized as follows:

1. To evaluate the distribution and biogeography of *Mallomonas* species within the context of model of ubiquitous dispersal (Fenchel and Finlay 2004). *Papers 1, 2.*
2. To establish suitable *Mallomonas* and *Synura* model species for investigation of the morphological variability (plasticity). *Paper 3.*
3. To investigate the range of phenotypic scale and bristle plasticity of the model species under different environmental conditions. *Paper 4.*
4. To revise the species concept in *Synura petersenii* complex using a combination of morphological and molecular data sets. *Paper 5.*

2.1. Distribution of the genus *Mallomonas*

The distribution and biogeography of *Mallomonas* species from the point of view of the contemporary dispersal models were investigated in temperate zone in the territory of the Czech Republic (*Paper 1*) and in tropical zone in Malaysia, Singapore and Indonesia (*Paper 2*). In the Czech Republic, long term monitoring was performed in the floodplains of the upper Lužnice, where over 80% of species previously reported from the other freshwater biotopes within the country were found.

According to “ubiquity model”, the high local diversity and the high dispersal ability are indicated by a low slope of the species:area curve. We found an increase of *Mallomonas* species number in relation to the total area of available habitats on the continent and global scales. Most of the species were cosmopolitan or widely distributed, however, we have demonstrated a high probability of geographically restricted distributional pattern for several taxa.

2.2. Effect of temperature on the variability of silicate structures

Previous studies of cultured or natural populations under different conditions have shown considerable variations in scale morphology (phenotypic plasticity). To investigate the range of phenotypic scale and bristle plasticity it was necessary to establish a suitable model species. *Mallomonas kalinae* was found to be an acceptable candidate (*Paper 3*). This species is able to grow under the wide range of different conditions in laboratory, although it is not very common in natural samples. Similar properties exhibited a cultivated *S. curtispina* strain. Primarily the scale shape change related to temperature was studied, as temperature represents a leading factor influencing plasticity. Landmark based methods were used to evaluate shape change and the results were compared with previous above mentioned morphological studies (*Paper 4*).

2.3. Species concept in *Synura petersenii* complex

Taxonomy of the chrysophytes is traditionally based on ultrastructure of scales. Within *S. petersenii* a transitional morphology of scales is often recorded, but it has still remained unclear, if the variability is induced by ecological factors or if it is controlled genetically. In *Paper 5*, a polyphasic approach was applied to delimit a status of many known *S. petersenii* morphotypes. In the study, 24 strains were analyzed - 22 strains were isolated at different

localities in the Czech Republic, 2 strains were from USA. Combination of molecular phylogenetic analyses and morphological data resulted in the taxonomic revision of *S. petersenii* complex.

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Paper 1

**Distribution of the genus *Mallomonas*
(Synurophyceae) - Ubiquitous dispersal in
microorganisms evaluated.**

ORIGINAL PAPER

Distribution of the Genus *Mallomonas* (Synurophyceae) – Ubiquitous Dispersal in Microorganisms Evaluated

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The neutral dispersal model in protists was suggested as a general principle resulting in either cosmopolitan or ecologically restricted distribution of individual species. The high local diversity results in “flat” species–area curves of individual protist groups. We investigated the local and regional diversity of the genus *Mallomonas* in the alluvial plain of upper Lužnice in the Czech Republic. About 86.5% of species previously reported from all types of freshwater biotopes within the country were found in our investigated localities. However, there was a considerable increase of species numbers in relation to the total area of available habitats on the continent and global scales. In three species found in our localities, the floristic data indicate a possible geographically restricted distributional pattern. Here, we discuss possible reasons for this phenomenon.

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Key words: alluvial pools; biodiversity; biogeography; *Mallomonas*; Synurophyceae.

Introduction

The neutral model of ubiquitous dispersal of microorganisms (Fenchel and Finlay 2004; Finlay 2002; Finlay and Clarke 1999a; Finlay et al. 2002) has contributed much to our understanding of microbial diversity and distribution. Ubiquitous distributional patterns were demonstrated in different groups of protists (Fenchel and Finlay 2004; Finlay and Esteban 2001; Finlay and Fenchel 2004; Finlay et al. 2004; Wilkinson 2001). However, the generalization of the neutral model to all microorganisms still remains a controversial issue (see e.g. Coleman 2002; Foissner 1999; Lachance 2004).

The core of the neutral model hypothesis can be summarized as: (1) metapopulations of free-living microorganisms are sufficiently abundant to have a world-wide distribution; (2) the extreme population numbers of individual species lead to their equable dispersion over the planet in a negligible time from an evolutionary point of view; (3) consequently, microbial species richness of individual localities is large and represents a significant proportion of global diversity; (4) consequently, the individual species occur in detectable numbers wherever suitable environmental conditions are available; (5) there are no historically determined biogeographic areas of microorganisms; and (6) the only geographic pattern that can be detected results from individual ecological requirements of species.

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Individual species are therefore cosmopolitan and they occur in detectable numbers in their favored environment (e.g. peat bog pools or eutrophic freshwater). The only biogeographic pattern that can be discerned over large areas, continents, or climatic zones is connected with temperature and, consequently, with latitude. We found species that cannot tolerate freezing and therefore occur in a pantropic pattern, or species preferring colder environments whose distribution has a bipolar pattern. Therefore, Finlay et al. (2004) attempted to disprove the presumed occurrence of some species in one hemisphere only.

A major obstacle for the neutral dispersal model is the existence of many endemic microbial species that have been reported rarely and often only from a single locality. However, the authors of the neutral model demonstrated that numerous slowly growing protist species are so rare in nature that the probability of their discovery is negligible. In these species, their findings depend on the sampling effort and undersampling is the crucial problem for evaluation of distributional patterns (Finlay and Clarke 1999b; Finlay et al. 2004). The “single-report” occurrence explains nothing about their global distribution.

In many protist groups, existing ambiguous species concepts, possibly incorporating numerous biological species within a single traditionally delimited morphospecies, have argued against the neutral dispersal model (Lachance 2004). Therefore, distributional studies focused on those microorganisms with more consistent species concepts are desirable to evaluate the neutral model hypothesis.

In this study, we concentrate on the synurophycean autotrophic flagellates of the genus *Mallomonas*, whose cells are covered with species-specific inorganic silica scales (Lavau et al. 1997; Siver 1991; Wee 1997). This makes *Mallomonas* a very good model group for evaluating the neutral dispersal model. The silica scales enable ultrastructural identification of individual species from plankton and also from the sediments of freshwater habitats. Since the 1950s, the taxonomy of the genus has been based on their ultrastructural morphology (Asmund and Kristiansen 1986; Fott 1955; Harris and Bradley 1960). To date, 176 species and infraspecific taxa have been described within the genus and almost 200 floristic and ecological studies reporting *Mallomonas* species include all kinds of freshwater habitats from around the world (Kristiansen and Lind 2005). In many species, the ecological preferences were determined and *Mallomonas* species are widely

used for biomonitoring purposes (Hartmann and Steinberg 1989; Lott and Siver 2005; Siver and Marsicano 1996).

Based on floristic reports, the biogeography of the genus *Mallomonas* was established. Presumed distributional patterns of individual taxa range from cosmopolitan, over distributions restricted to particular continents or climatic zones, to endemic (Kristiansen 2001a, b; Kristiansen and Lind 2005). More than 40 species have been considered as endemics of individual continents (including the “single-report” species). Interestingly, old freshwater lakes such as Lake Biwa in Japan or Lake Baikal in Russia, known by the high endemism of their fauna and macrophytic flora, do not contain any endemic *Mallomonas* species (Kristiansen and Lind 2005).

According to the neutral dispersal model, global distributions of microorganisms follow latitudinal or cosmopolitan patterns. Of course, given the microbial nature of *Mallomonas*, none of the floristic studies that are based on EM investigations of scales from plankton or sediment samples could claim to be a comprehensive account of all species present at the investigated localities. Rather, locally abundant species occurring in detectable numbers can be found using a floristic approach. Nevertheless, given the present findings of individual species, we can ask for the probability of their ubiquitous distribution – either worldwide or in particular climatic zones.

Let us imagine a simple example. In total, we have 10 independent floristic accounts. Seven of them are situated in a region *N* (e.g. temperate northern hemisphere), and the three remaining are in a region *S* (e.g. temperate southern hemisphere). The species *x* was reported three times – and actually, it was from the region *N* only. Now, the probability *p* that the distribution of the species *x* in temperate zones of the planet follows the neutral dispersal model is:

$$p = [Z/A] \times [(Z-1)/(A-1)] \times \dots \times [(Z-(x-1))/(A-(x-1))],$$

where *Z* is a number of independent reports (floristic studies) from a particular region (e.g. continent, hemisphere, or climatic zones), *A* is a number of all independent reports (floristic studies) worldwide, and *x* is a number of independent reports of a particular investigated species.

In our example, $p = [7/10] \times [(7-1)/(10-1)] \times [(7-2)/(10-2)] = 0.291$. Therefore, we can see that in our example the probability that the presumed pattern of the occurrence restricted to

the region *N*, based on three reports out of 10 independent studies could have emerged by chance is fairly high 29.1%. In this fashion, we can investigate probabilities of non-random distribution of those *Mallomonas* taxa, with the presumed distribution pattern, which seemingly contradicts the neutral model (Kristiansen 2002).

Here, we present our floristic data of *Mallomonas* distribution and species richness in alluvial pools of upper Lužnice in the Czech Republic, a result of a 4 year systematic study aimed at the enumeration of *Mallomonas* species. We present the species-area curve for the genus *Mallomonas* based on these results.

Results and Discussion

For systematic monitoring, we chose the T2 pool, an alluvial pool with an area of 330 m² and

maximum depth of 2 m (Pithart 1997). We took about 25 whole water and sediment samples that were analyzed for the presence of *Mallomonas* scales. The total TEM investigation time was about 100 h. Simultaneously, we investigated the *Mallomonas* species richness in the alluvial ecosystem as a whole, where the total average area of freshwater pools is about 40 ha. Six *Mallomonas* species from the area are new to the Czech Republic (*M. corymbosa*, *M. cyathellata*, *M. eoa*, *M. mangofera* f. *mangofera*, *M. lelymene*, *M. torquata*) (Fig. 1). In the T2 pool — a single small mesotrophic locality — 38 *Mallomonas* species were found (Table 1). The list of species from the T2 pool represents 84.5% of the total species number from the whole investigated alluvial complex. The whole alluvial complex (including the T2 pool) contained 86.5% of the total species number found in the Czech Republic in about

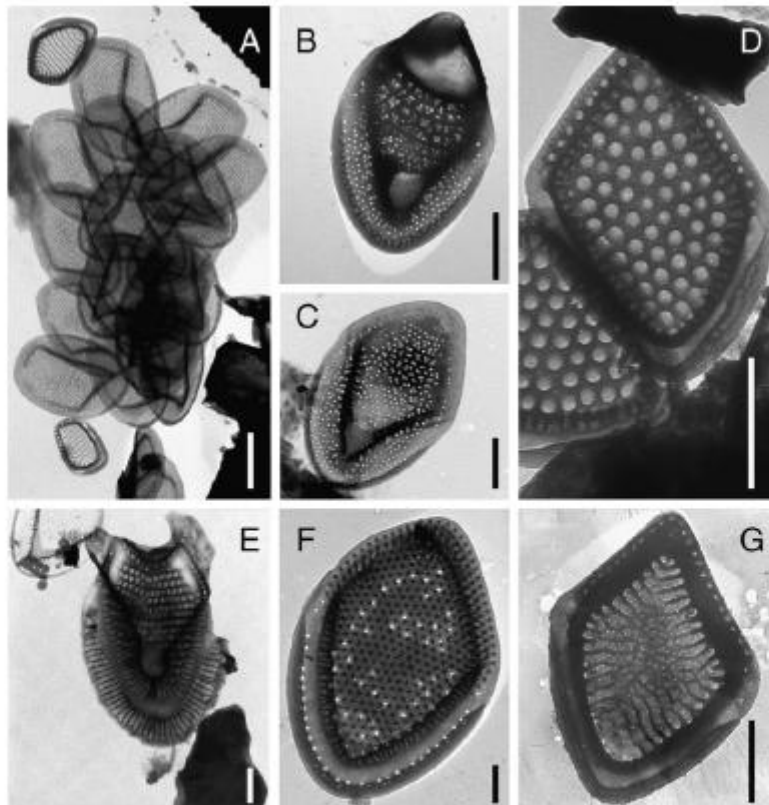


Figure 1. (A) *Mallomonas corymbosa*. Body scales. Bar: 2 μm . (B, C) *Mallomonas cyathellata*. Body scales. Bars: 1 μm . (D) *Mallomonas eoa*. Body scale. Bar: 1 μm . (E) *Mallomonas lelymene*. Body scale. Bar: 1 μm . (F) *Mallomonas mangofera* var. *mangofera* f. *mangofera*. Body scale. Bar: 0.5 μm . (G) *Mallomonas torquata* f. *torquata*. Body scale. Bar: 0.5 μm .

Table 1. *Mailomonas* species found in alluvial pools of upper Lužnice, the Czech Republic. The parameters Z , A , and x of the formula following the neutral dispersal model are given for species with northern temperate distribution. Low probabilities of bipolar distribution were found for three species (marked by an asterisk).

Taxon	T2 pool	Other alluvial pools	Distribution types	Z	A	x	Probability of bipolar distribution
<i>Mailomonas acaroides</i>	1		Widely distributed				
<i>M. actinoloma</i> var. <i>maramuresensis</i>	1		Northern temperate (arct., temp.)	119	125	14	0.483
<i>M. akrokomos</i>	1	1	Cosmopolitan				
<i>M. alata</i>	1	1	Widely distributed				
<i>M. alpina</i>	1		Cosmopolitan				
<i>M. alveolata</i>	1		Scattered				
<i>M. annulata</i>	1	1	Widely distributed				
<i>M. areolata</i>	1	1	Widely distributed				
<i>M. calceolus</i>	1	1	Widely distributed				
<i>M. caudata</i>	1	1	Widely distributed				
<i>M. corcontica</i>	1		Northern temperate (arct., temp., subtr.)	151	173	12	0.184
<i>M. corymbosa</i>	1	1	Bipolar				
<i>M. costata</i>	1		Widely distributed				
<i>M. crassiquama</i>	1	1	Widely distributed				
<i>M. cratis</i>	1		Widely distributed				
<i>M. cyathellata</i>	1	1	Widely distributed				
<i>M. doignonii</i>	1	1	Widely distributed				
<i>M. elongata</i>	1		Northern temperate (arct., temp., subtr.)	151	173	14	0.137
<i>M. eoa</i>	1	1	Widely distributed				
<i>M. flora</i>	1	1	Widely distributed				
<i>M. heterospina</i>	1	1	Widely distributed				
<i>M. intermedia</i>	1	1	Endemic to Europe				
<i>M. lelymene</i>	1	1	Scattered, but widely distributed				

<i>M. mangofera</i> f. <i>mangofera</i>	1	1	Cosmopolitan				
<i>M. matvienkoae</i>	1	1	Cosmopolitan				
<i>M. multica</i>	1	1	Northern temperate (arct., temp., subtr.)	151	173	36	0.004*
<i>M. oviformis</i>	1	1	Northern temperate (arct., temp., subtr.)	151	173	28	0.015*
<i>M. papillosa</i>	1	1	Cosmopolitan				
<i>M. parvula</i>	1	1	Widely distributed				
<i>M. paxillata</i>	1	1	Widely distributed				
<i>M. pillula</i> f. <i>valdiviana</i>	1	1	Bipolar				
<i>M. portae-ferreae</i>	1	1	Cosmopolitan				
<i>M. prora</i>	1	1	Bipolar				
<i>M. pumilio</i> var. <i>pumilio</i>	1	1	Bipolar				
<i>M. pumilio</i> var. <i>silvicola</i>	1	1	Endemic to Europe				
<i>M. punctifera</i> var. <i>punctifera</i>	1	1	Northern temperate (arct., temp., subtr.)	151	173	56	0.000*
<i>M. retifera</i>	1	1	Scattered				
<i>M. schwemmleri</i>	1	1	Northern temperate	151	173	19	0.065
<i>M. striata</i>	1	1	(arct., temp., subtr.)				
<i>M. teilingii</i>	1	1	Widely distributed	119	125	27	0.224
<i>M. tonsurata</i>	1	1	Northern temperate (arct., temp.)				
<i>M. torquata</i> f. <i>simplex</i>	1	1	Cosmopolitan	119	125	21	0.323
<i>M. torquata</i> f. <i>torquata</i>	1	1	Northern temperate (arct., temp.)				
<i>M. transsylvanica</i>	1	1	Bipolar				
<i>M. trummensis</i>	1	1	Northern temperate (arct., temp.)	119	125	15	0.457

135 localities ranging from mountainous peat bogs to alkalic eutrophic fish ponds (Němcová et al. 2002, 2003; Řezáčová et al. 2004). The corresponding species—area curve is supplemented with the data for total species number as reported from Europe and worldwide to date (Franceschini and Kristiansen 2004; Forsström et al. 2005; Kristiansen 2002; Lott and Siver 2005; Řezáčová and Škaloud 2005; Siver et al. 2005; Vigna and Siver 2003; Carty and Wujek 2003; Wujek and Ogundipe 2003; Wujek et al. 2004) (Fig. 3). The species—area curve was compiled in the same way as the species—area data as presented in Fenchel and Finlay (2004). We took the log data of the area of freshwater habitats across different scales: the T2 pool, the alluvial ecosystem of upper Lužnice (Pithart 1997), the Czech Republic (Víček 1984), Europe and the world (CIA 2006; Revenga et al. 2000), and the *Mallomonas* species number reported from floristic studies.

Comparing these data with the results of Finlay (2002) and Fenchel and Finlay (2004), we can see a similar “flat” species—area pattern in our data when followed up to the area of the Czech Republic. This pattern agrees well with the “high local diversity — low global diversity” paradigm assumed by the neutral dispersal model (Finlay 2002). The extremely low slope of the species—area curve of microorganisms in contrast to larger organisms, e.g. insects (Finlay 2002), indicates their easy and frequent dispersal leading to their ubiquitous distribution in a given area. There is a certain increase of the slope in continent-wide and global parts of the curve when compared with the corresponding curves of Fenchel and Finlay (2004). We propose two possible explanations of this phenomenon: (1) higher ecological diversification of worldwide freshwater habitats when compared to marine ecosystems; and (2) inadequate sampling at smaller scales of our curve.

Firstly, we presume that the increase of species number at continent-wide and global levels (boosting the slope of our species—area curve) is connected with the increase of available habitats — probably mainly in the subtropical and tropical localities without an annual freezing period. In our opinion, these results indicate generally higher numbers of microbial species whose distribution is restricted to a particular climatic zone in freshwater ecosystems in comparison to marine ecosystems.

Secondly, we cannot exclude that there is also an inadequate sampling of *Mallomonas* in the Czech Republic. Although this country has been

one of the centers of *Mallomonas* floristic research (Fott 1955; Němcová et al. 2003), species new to the Czech Republic are still being found during detailed EM investigations of many samples (e.g. Němcová et al. 2003; Neustupa et al. 2001; Nováková et al. 2004; Řezáčová et al. 2004).

Looking at the distributional patterns of *Mallomonas* taxa found in the investigated pools (Table 1), we see that 25 of them can be considered cosmopolitan or widely distributed according to Kristiansen (2002), which means that they were found in different continents and in different climatic zones. In addition, there are five taxa known as bipolar in their distribution — thus occurring in colder regions of the planet. The distributional patterns of these species conform to the neutral dispersal model. However, there are 10 taxa that were reported as northern temperate by Kristiansen (2002), which means that they were found in arctic/temperate to subtropical localities in the Northern Hemisphere only. Looking at the probabilities that these patterns could have emerged by chance on the basis of available floristic data (Table 1), we can see that in most of these taxa, probabilities of bipolar temperature-dependent distribution are very high (for convenience, we take the 5% probability as the highest possible level that should attract our attention to distribution of a particular taxon). Three species — *M. multiunca*, *M. oviformis*, and *M. punctifera* var. *punctifera* (Fig. 2) seemingly do not conform to the neutral dispersal model due to their highly non-random distribution in subtropic to subarctic zones of the Northern Hemisphere (Fig. 3).

Nevertheless, their reported absence from the Southern Hemisphere may be due to inadequate sampling and scarcity of studies from these regions investigating appropriate habitats conforming to ecological preferences of these species. *Mallomonas multiunca* was found in 36 independent studies ranging from subtropical to subarctic Europe, North and Central America, and Asia. It occurs at a pH range from 4.0 to over 9.0 and tolerates a wide spectrum of lake types, including oligotrophic, dystrophic, and eutrophic localities (Siver 1991; Takahashi 1978). *Mallomonas oviformis* has been reported in 28 studies from subtropical to subarctic Europe, North America, and Asia (Kristiansen 2002). Siver (1989) and Němcová et al. (2003) found *M. oviformis* mainly in alkaline and relatively eutrophic conditions at conductivities above $80 \mu\text{S cm}^{-1}$. *Mallomonas punctifera* var. *punctifera* belongs to one of the most frequently reported taxa within the genus. There are 56 reports of this species from subtropic

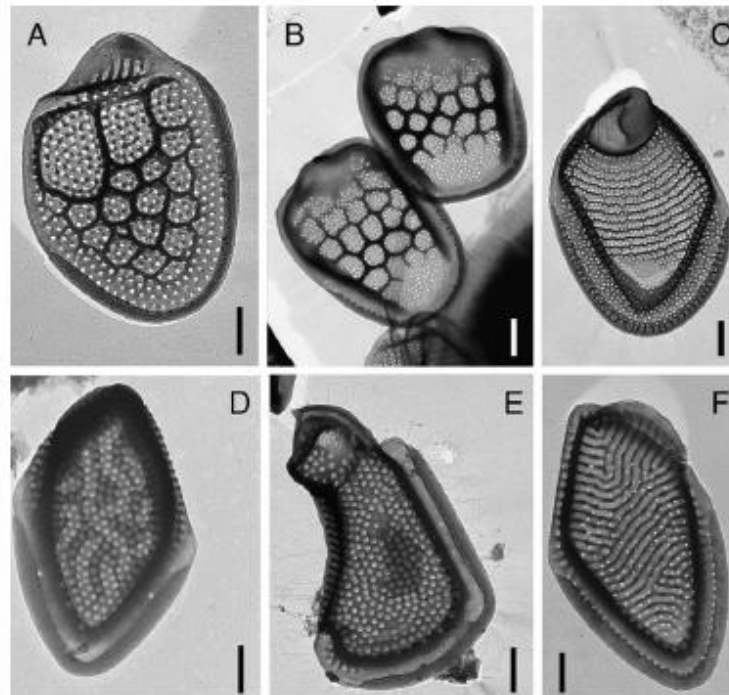


Figure 2. (A) *Mallomonas multiunca*. Body scale. Bar: 0.5 μm . (B) *Mallomonas punctifera* var. *punctifera*. Body scales. Bar: 1 μm . (C) *Mallomonas portae-ferreae*. Body scale. Bar: 1 μm . (D, E) *Mallomonas prora*. (D) Body scale. (E) Apical scale. Bars: 0.5 μm . (F) *Mallomonas schwemmlei*. Body scale. Bar: 0.5 μm .

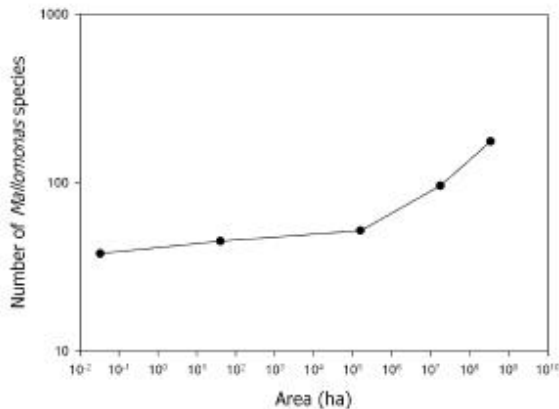


Figure 3. Species—area curve based on *Mallomonas* species from alluvial pool T2 from the whole alluvial complex of upper Lužnice, the Czech Republic, Europe, and the whole world.

to subarctic ecosystems of Europe, North America, and Asia. It is characterized as an alkaliphilic to pH-neutral species (Němcová et al. 2003;

Rojackers and Kessels 1986). A second variety of the species, *M. punctifera* var. *brasiliensis*, was found 14 times in North and South America, including the tropical equatorial localities (Kristiansen and Menezes 1998). From a taxonomic point of view, both varieties are well delimited and easily discernible (Kristiansen 2002; Kristiansen and Menezes 1998). All three taxa form relatively large and easily discernible scales.

Other *Mallomonas* species with many independent floristic reports but geographically restricted distribution were detected in subtropical/tropical ecosystems (e.g. *M. ocellata* and *M. ceylanica* in South and East Asia, *M. plumosa* in South-East Asia and Australia) and in temperate ecosystems (*M. hamata* in the Northern Hemisphere, *M. clavus* in Europe, and *M. duerschmidtiae* in arctic North America) (Kristiansen 2002; Kristiansen and Lind 2005). While it seems that, given the present amount of floristic data, the neutral model can successfully be applied to the distribution of most *Mallomonas* species (at least those found in our study), the non-neutral geographic patterns of these several species should be investigated for

possible underlying explanatory mechanisms. There could be three possible theoretical explanations of seemingly non-neutral, geographically restricted distribution in freshwater microbial organisms such as *Mallomonas*: (1) a species could be very young from an evolutionary point of view and, so far, it was not able to colonize the available habitats worldwide; (2) distributional abilities of the species could be distinctly lower than in other taxa of the genus. In *Mallomonas*, this could involve the lower survival rate of cysts and palmelloid stages that are probably the most easily dispersible stages of the life cycle (Kristiansen 2001b; Wee et al. 2005). In addition, the rate of dispersal depends on the absolute abundance of individual species so that locally rare species should disperse more slowly; and (3) there could be presently unknown environmental factors, e.g. obligatory biotic interactions with biogeographically restricted larger organisms, that "hold" a species in a restricted area and whose absence from the rest of the world results in failure of dispersal attempts.

None of these three possible explanations has ever been tested in chrysophytes or synurophytes. Nevertheless, the first two hypothetical mechanisms in fact represent the historically determined distribution. They could be addressed in specifically designed experimental studies investigating either the evolutionary age or cyst formation and their survival characteristics in individual *Mallomonas* species. If there would be any significant differences between cosmopolitan or bipolar species and species with seemingly non-neutral distribution that have many times been found within some restricted region or hemisphere, then these taxa could be considered to contradict the neutral dispersal model.

Although, we do not consider the existence of an unknown ecological factor restricting the occurrence of a species in a single continent or hemisphere as a likely phenomenon, it always remains a possibility. However, unless there is any single proved example of such a mechanism in free-living protists, such as *Mallomonas*, this explanation remains entirely speculative.

Methods

All investigated localities are alluvial pools in the floodplains of the upper Lužnice, the Czech Republic. For systematic monitoring, we chose the T2 pool, an alluvial pool with an area of 330 m² and a depth of about 2 m (Pithart 1997). The range

of pH values in the T2 pool was 6.0–7.5 and conductivity was between 150 and 240 $\mu\text{S cm}^{-1}$. In 2002 and 2003, we took 1.5 l whole water samples for investigation of synurophyte species composition monthly. In 2005, we investigated the samples of the upper 1 cm of benthic sediment for the presence of *Mallomonas* scales. In 2003, we investigated the alluvial ecosystem as a whole, with a total area of freshwater pools of about 40 ha (Pithart 1997). For this investigation, we chose 12 pools lying in an 8 km long strip along the river. The pH values were 6.0–7.3 and conductivity values were 140–280 $\mu\text{S cm}^{-1}$. All samples were prepared for examination by TEM (for methods see Řezáčová et al. 2004).

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Paper 2

The genus *Mallomonas* (Mallomonadales, Synurophyceae) in several Southeast Asian urban water bodies - the biogeographic implications.

**The genus *Mallomonas* (Mallomonadales, Synurophyceae)
in several Southeast Asian urban water bodies –
the biogeographic implications**

by

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With 12 figures and 1 table

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Abstract: We report the occurrence of 10 *Mallomonas* taxa from several urban water bodies in four Southeast Asian cities in Malaysia, Singapore and Indonesia. Apart from some cosmopolitan or pantropic species, the two presumed Asian synurophyte endemics (*Mallomonas grata* and *Mallomonas ocellata*) were found. We discuss the patterns of their geographic distribution that could contravene Finlay's neutral model of ubiquitous dispersal of microbial eukaryotes for these two species.

Introduction

The members of the class Synurophyceae, whose cells are covered with species-specific inorganic silica scales, are widely considered model organisms for studies of microalgal distribution and biogeography (Siver 1991, Kristiansen 2000, 2001, 2002, Kristiansen & Lind 2005). In this study, we concentrate on the synurophycean flagellates of the genus *Mallomonas*. Up to present, about 175 of species and infraspecific taxa have been described within this genus (Kristiansen 2002). There are now about 200 floristic and ecological studies reporting *Mallomonas* species composition in localities from all over the world, from all kinds of freshwater habitats (Siver 1991, Kristiansen 2002, Němcová et al. 2003). For numerous species, some ecological preferences were detected and *Mallomonas* species are now widely used for biomonitoring purposes (Smol et al. 1984, Hartmann & Steinberg 1989; Siver et

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al. 1996, Nováková et al. 2004). Mass occurrences of *Mallomonas* species in freshwater phytoplankton have mostly been reported from temperate and subarctic ecosystems (Eloranta 1995, Nicholls 1995). Even in tropical and subtropical ecosystems, however, there are now numerous studies documenting the occurrence of *Mallomonas* in these types of biotopes (e.g. Cronberg 1989, Vyverman & Cronberg 1993, Kristiansen & Menezes 1998, Wei & Yuan 2001, Wujek & Bicudo 2004, Menezes et al. 2005, Vigna et al. 2005).

Biogeography of microalgae has recently become a frequently discussed topic with the formulation of the neutral model of ubiquitous microorganismal dispersal (Finlay 2002, Fenchel & Finlay 2004; Finlay & Fenchel 2004; Finlay et al. 2004). The core of the neutral model hypothesis can be summarized as follows:

- The metapopulations of free-living microorganisms are sufficiently abundant to have a world-wide distribution and these extreme population numbers of individual species lead to their equable dispersion over the planet in a short evolutionary time.
- There are no historically determined biogeographic areas in microorganisms and the only geographic pattern that can be detected results from ecological requirements of individual species (e.g. temperature requirements).

Therefore, the relative frequency of the occurrence of an individual microbial species within a particular climatic zone on different continents or hemispheres should be similar according to the neutral model. Ubiquitous distributional patterns were demonstrated in different algal and protozoan groups (e.g. by Finlay & Clarke 1999, Finlay et al. 1999, Esteban et al. 2001, Wilkinson 2001, Fenchel & Finlay 2004), but the generalization of the neutral model to all eukaryotic microorganisms still remains a controversial issue (e.g. Foissner 1999; Lachance 2004). In the genus *Mallomonas*, the patterns of distribution were demonstrated for many species (Kristiansen 2000, 2001, 2002, Kristiansen & Lind 2005). The presumed distributional patterns suggested for individual taxa include those with cosmopolitan distribution, those with distributions restricted to particular climatic zones, and those which are endemic to individual continents or regions. However, according to the neutral dispersal model, the global distributions of species should follow presumably the latitudinal (based on general temperature gradient) or cosmopolitan patterns. Given the microbial nature of *Mallomonas*, none of the floristic studies that are based on EM investigations of scales from plankton or sediment samples, can claim to represent a comprehensive account of all species present at investigated localities. Rather, only the locally abundant species occurring in detectable numbers can be found using the floristic approach. Nevertheless, given the state of knowledge of the world distribution of *Mallomonas*, we can ask for the approximate probability of their ubiquitous distribution - either world-wide or within particular climatic zones.

The tropical and subtropical *Mallomonas* flora has now been reported in more than 70 floristic and ecological studies and 30 taxa are hitherto known only from tropical or subtropical climatic zones (Kristiansen 2002, Wujek et al. 2004, Vigna et al. 2005, Wujek & Dziedzic 2005). In this paper we concentrate on several urban water bodies in the equatorial region of Southeast Asia that

were sampled in the years 2003-2005 in the course of field studies concerned primarily with terrestrial algae (Neustupa 2005). We have carefully investigated the whole water samples aiming at the enumeration of *Mallomonas* species occurring in phytoplankton of the localities and at the evaluation of their distributional patterns.

Material and methods

The 1000 ml whole water samples were taken at individual localities. The samples were immediately fixed with standard Lugol's solution. Water temperature and pH were measured in the field using a Hannah field pH-meter. The samples were rinsed in distilled water in a centrifuge, dried on to Formvar coated copper grids and shadowcast with chromium (Rezáčová et al. 2004). The grids were examined with Philips T 300 and JEOL 1011 transmission electron microscopes.

The samples were taken at the following localities:

- 1) a pool in Bogor Botanical Garden, West Java, Indonesia, altitude 200 meters a.s.l., area ca 100 m², pH 7.8, temperature 29°C, coll. 8.2. 2003.
- 2) a pool in Woodlands city area, Singapore, altitude 15 meters a.s.l., area ca 400 m², pH 7.2, temperature 25°C, coll. 4.2. 2003.
- 3) an artificial lake in Singapore Botanical Garden, altitude 45 meters a.s.l., area ca 1 ha, pH 6.8, temperature 31°C, coll. 31.1. 2005.
- 4) a pool in Chinese Bonsai Garden, Singapore, altitude 30 meters a.s.l., area ca 100 m², pH 8.3, temperature 30°C, coll. 31.1. 2005.
- 5) an artificial lake in Tasik Perdana city park, Kuala Lumpur, Malaysia, altitude 120 meters a.s.l., area ca 2.5 ha, pH 8.0, temperature 28°C, coll. 1.2. 2003.
- 6) an artificial lake in Seremban city park, Negeri Sembilan province, Malaysia, altitude 150 meters a.s.l., area ca 2 ha, pH 7.1, temperature 26°C, coll. 29.1. 2005.

Results

In total, 10 *Mallomonas* species were found in the investigated samples (Table 1).

Mallomonas cyathellata Wujek & Asmund

Figs 1, 2

The scales with characteristic irregularly shaped pits on the secondary shield were observed. The observed struts on proximal border resemble *M. cyathellata* var. *chilensis* Dürschmidt, but the rear scales with hare's ear-like protuberances were not observed, so that we report the occurrence of this alga on the species level only. This species was repeatedly reported from tropical to temperate localities worldwide (Kristiansen 2002), with a higher incidence in warm waters.

Mallomonas grata Takahashi in Asmund & Takahashi

Figs 3, 4

We observed the body and collar scales with several more or less clearly demarcated subcircular depressions on the shield well corresponding with scales from the original description of this species (Takahashi 1963). So far, this species was reported in 10 floristic studies from Asia (Japan, China and India) (Kristiansen 2002). The recent

Table 1. List of species found at the investigated localities (for the description of localities - see section Material and methods). The numbers given in columns indicate the total number of scales observed.

	1	2	3	4	5	6
<i>Mallomonas cyathellata</i> Wujek & Asmund					4	
<i>Mallomonas grata</i> Takahashi in Asmund & Takahashi		5			1	
<i>Mallomonas mangofera</i> Harris & Bradley var. <i>mangofera</i> f. <i>reticulata</i> Cronberg					1	
<i>Mallomonas mangofera</i> Harris & Bradley var. <i>sulcata</i> Dürschmidt				2		
<i>Mallomonas matvienkoeae</i> (Matvienko) Asmund & Kristiansen var. <i>matvienkoeae</i> f. <i>matvienkoeae</i>				8	10	
<i>Mallomonas matvienkoeae</i> (Matvienko) Asmund & Kristiansen var. <i>grandis</i> Dürschmidt & Cronberg			6			1
<i>Mallomonas multisetigera</i> Dürschmidt				11		
<i>Mallomonas ocellata</i> Dürschmidt & Croome					2	
<i>Mallomonas rasilis</i> Dürschmidt		6				
<i>Mallomonas tonsurata</i> Teiling em. Krieger var. <i>tonsurata</i>	12	7		3		2

floristic account of this species from Belize (Carty & Wujek 2003) we consider as dubious as the presented scales do not correspond to *M. grata*.

Mallomonas mangofera Harris & Bradley var. *mangofera* f. *reticulata* Cronberg
Fig. 5

The identification of this taxon was based on the presence of characteristic raised ridges connecting the papillae on the shield (Kristiansen 2002).

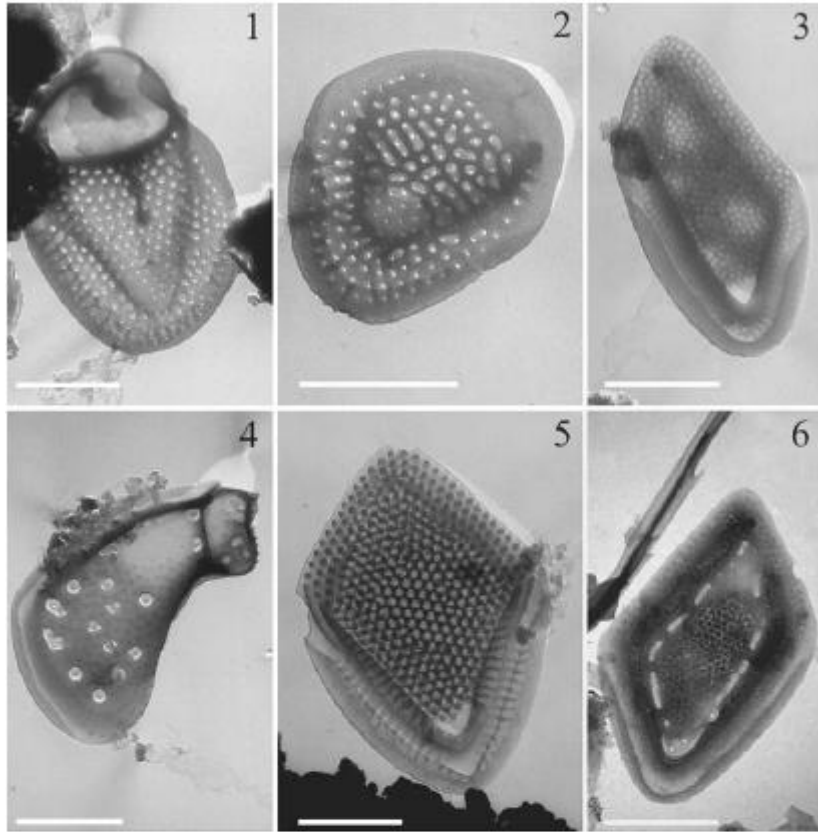
Mallomonas mangofera Harris & Bradley var. *sulcata* Dürschmidt Fig. 6

This variety of *M. mangofera* is characterized mainly by the presence of grooves along the margin of the shield (Kristiansen 2002).

The varieties and forms of *M. mangofera* belong to the most frequently reported *Mallomonas* taxa from the tropics. However, it has also been found in the summer season in several temperate localities. *Mallomonas mangofera* var. *mangofera* f. *reticulata* was reported in seven studies from South America, Africa and Asia (Kristiansen 2002). So far, *M. mangofera* var. *sulcata* was reported in three studies from subtropical Japan and South America (Dürschmidt 1983, Ito 1990, 1991).

Mallomonas matvienkoeae (Matvienko) Asmund & Kristiansen var. *matvienkoeae* f. *matvienkoeae* Fig. 7

One of the most common, cosmopolitan *Mallomonas* species occurring in tropical to subarctic ecosystems.



Figs 1-2. *Mallomonas cyathellata*. Fig. 1. Body scale, Fig. 2. Rear scale. Scale bars = 1 μ m. Figs 3-4. *Mallomonas grata*. Fig. 3. Body scale. Scale bar = 1 μ m. Fig. 4. Collar scale. Scale bar = 2 μ m. Fig. 5. *Mallomonas mangofera* var. *mangofera* f. *reticulata*. Body scale. Scale bar = 2 μ m. Fig. 6. *Mallomonas mangofera* var. *sulcata*. Body scale. Scale bar = 2 μ m.

Mallomonas matvienkoeae* (Matvienko) Asmund & Kristiansen var. *grandis
 Dürschmidt & Cronberg Fig. 8

This variety is characteristic by the presence of large subcircular pores on the proximal area of the scales and by larger scale dimensions. So far, this taxon was reported in 18 studies from tropical ecosystems world-wide (Kristiansen 2002, Franceschini & Kristiansen 2004, Wujek & Dziedzic 2005).

- Mallomonas multisetigera* Dürschmidt** Fig. 9
 A cosmopolitan species that occurs more frequently in subtropical and tropical ecosystems, but that was several times reported also from temperate Europe and North America (Kristiansen 2002).
- Mallomonas ocellata* Dürschmidt & Croome** Fig. 10
 The scales are characterized by the large eye-like pits with centrally thickened area of the base plate. So far, this species was reported only from two Asian regions - subtropical Japan (Ito 1990, 1991) and Malaysia (Dürschmidt & Croome 1985).
- Mallomonas rasilis* Dürschmidt** Fig. 11
 A cosmopolitan species, with about 66% of reported findings from tropical and subtropical localities, but reported from temperate biotopes, too (Kristiansen 2002).
- Mallomonas tonsurata* Teiling em. Krieger var. *tonsurata*** Fig. 12
 One of the most frequent, cosmopolitan *Mallomonas* species. Gutowski (1996) reported the temperature dependent morphological variability of scales in an experimental strain of this species with short and wide scales developing characteristically in warm water. The scales found in our localities were very similar to Gutowski's (1996) warm water morphotypes.

Discussion

Altogether 10 *Mallomonas* taxa were found. Five taxa clearly occur world-wide from temperate to tropical ecosystems. Three of these taxa (*M. cyathellata*, *M. multi-setigera* and *M. rasilis*) are warm water organisms occurring more frequently in subtropical and tropical regions. Both infraspecific taxa of *M. mangofera* found in the investigated localities and *M. matvienkoae* var. *grandis* are probably organisms with pantropic distribution. However, *M. mangofera* var. *mangofera* f. *reticulata* and *M. mangofera* var. *sulcata* are very rare taxa and, in addition, their taxonomic status is still unclear. *Mallomonas matvienkoae* var. *grandis*, with frequent reports from tropical regions of Central and South America, Africa and Asia, is a typical tropical taxon and its scales can be used as bioindicators of tropical climate in palaeoecological investigations.

Two species (*M. grata* and *M. ocellata*) seem to be geographically restricted and therefore are of special interest in the context of evaluation of the neutral dispersal model in synurophytes. So far, both these species have been found only in Asian localities. *Mallomonas ocellata* was only reported from subtropical and tropical localities. In total, there are now 74 floristic studies from all types of freshwater localities in subtropical or tropical ecosystems world-wide (Kristiansen 2002, Wujek et al. 2002, Wujek & Ogundipe 2003, Franceschini & Kristiansen 2004, Wujek et al. 2004, Wujek & Dziejczak 2005). From these floristic investigations, 18 studies were carried out in Asia. *Mallomonas ocellata* was found in four independent studies (including this paper) (Kristiansen 2002).

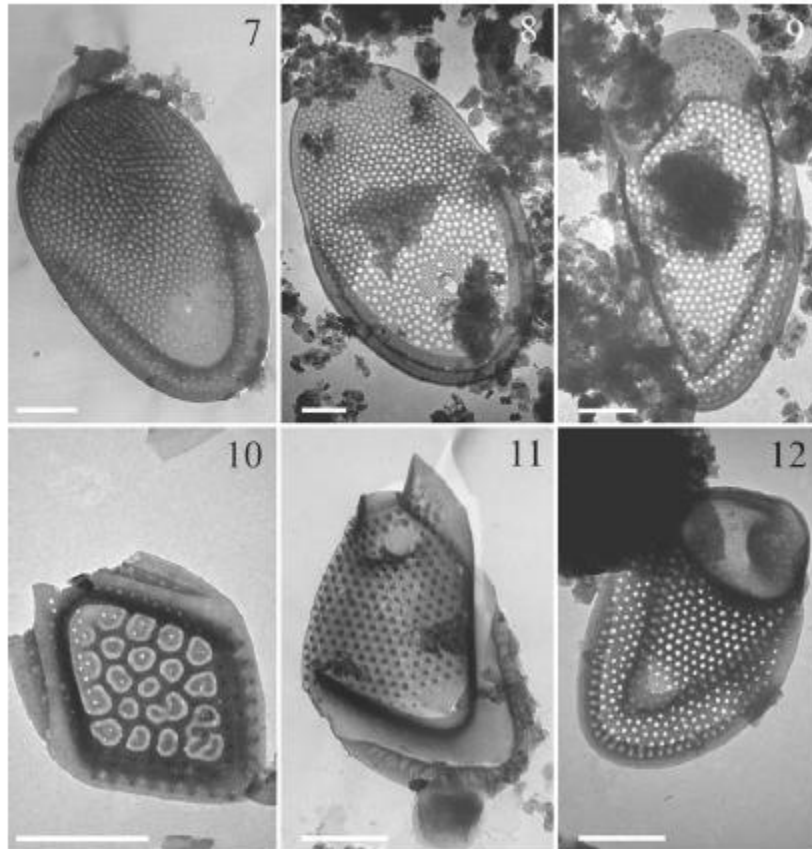


Fig. 7. *Mallomonas matvienkoeae* var. *matvienkoeae* f. *matvienkoeae*. Body scale. Scale bar = 1 μ m. Fig. 8. *Mallomonas matvienkoeae* var. *grandis*. Body scale. Scale bar = 1 μ m. Fig. 9. *Mallomonas multisetigera*. Body scale. Scale bar = 1 μ m. Fig. 10. *Mallomonas ocellata*. Body scale. Scale bar = 1 μ m. Fig. 11. *Mallomonas rasilis*. Apical scale. Scale bar = 1 μ m. Fig. 12. *Mallomonas tonsurata* var. *tonsurata*. Body scale. Scale bar = 1 μ m.

The second species, *Mallomonas grata*, was reported in 11 independent investigations from Asian localities ranging from typical temperate (winter phytoplankton in north China - Kristiansen 1989) to subtropical and tropical climate (India - Wujek & Saha 1996). In total, there are now 177 studies from these climatic zones world-wide (27 of them are from Asia). On the basis of these data, we can ask for the probability that these seemingly geographically restricted patterns of distribution in both species have emerged by chance. In other words, we ask for the probability that the

distribution of a particular species follows the neutral model. Thus, on the global scale, the distribution should be based mostly on temperature gradient, not reflecting any geographic barriers within the climatic zone and the species should occur with approximately equal frequency over different continents.

In *Mallomonas ocellata*, we have 74 independent floristic accounts (A). Out of them, 18 studies were conducted in Asia - a particular region of interest (Z). The species - *M. ocellata* - was found four times (x) in a region Z . The probability that this pattern have emerged merely by chance is:

$$p = [Z/A] \cdot [(Z-1)/(A-1)] \cdot \dots \cdot [(Z-(x-1))/(A-(x-1))],$$

in *Mallomonas ocellata* it is:

$$p = (18/74) \cdot (17/73) \cdot (16/72) \cdot (15/71) = 0.0026$$

Thus, the probability that this species occurs with equal frequency in subtropical and tropical ecosystems over different continents and that the presumed distributional pattern restricted to Asia have emerged by chance is fairly small. The *Mallomonas grata* situation is even more pronounced. The probability that this species occurs with equal frequency over different continents and that the Asian distributional pattern has emerged by chance is negligible 8.8×10^{-10} .

Of course, we suppose in these calculations that in the floristic studies the authors look with generally the same attention for the possible occurrence of *M. ocellata* and *M. grata* irrespective of the continent of investigation. Actually, this needs not to be the case. The phenomenon of more frequent reports in floristic studies of some *Mallomonas* species that has either been recently described or reported as endemic for some region was documented by Kristiansen (2001) and Kristiansen & Lind (2005). However, at least in *M. ocellata*, which is very well morphologically delimited and quite conspicuous species, we need not to presume that it could have been overlooked in non-Asian floristic studies. The *Mallomonas grata* situation is perhaps more complicated, because the morphological delimitation of the species is not so straightforward as in *M. ocellata* (Kristiansen 2002). Thus, we cannot exclude that some findings of this species might be overlooked or misidentified. The recent report of *M. grata* from Belize (Carty & Wujek 2003) that we consider as unclear and non-convincing on the basis of published data serves as an example of the somewhat complicated taxonomic status of this species.

However, on the basis of recent knowledge both *Mallomonas ocellata* and *Mallomonas grata* can be suspected as true Asian endemics. Their geographic distribution probably could contravene the neutral dispersal model and may be geographically restricted across different climatic zones in a single continent. We can now pose the question - which factors can restrict their distribution? In general, we can hypothesize about ecological or evolutionary factors. Either these organisms have significantly lower dispersal abilities than other freshwater microalgae (e.g. lower cyst survival rates than other synurophytes) or they can be young species from an evolutionary point of view that were not yet able to colonize the suitable biotopes elsewhere in the world. Ecophysiological culture studies and molecular phylogenetic data should be of much use in future investigations of this topic. Molecular phylogenetic data should only be

acquired from identified cultured material. However, no strains of these species are available so far. Thus, the isolation and cultivation of such geographically restricted species should be of crucial importance for future research progress in this field. At the same time, the further floristic data on distribution of *Mallomonas* flagellates in freshwater localities - especially in the tropics - will be very useful for our understanding of real biogeographic patterns of these organisms.

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Paper 3

***Mallomonas kalinae* (Synurophyceae), a
new species of alga from northern Bohemia,
Czech Republic.**

***Mallomonas kalinae* (Synurophyceae), a new species of alga from northern Bohemia, Czech Republic**

Mallomonas kalinae, nový druh řasy ze třídy Synurophyceae ze severních Čech

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Řezáčová M. (2006): *Mallomonas kalinae* (Synurophyceae), a new species of alga from northern Bohemia, Czech Republic. – Preslia 78: 353–358.

A new species of *Mallomonas*, *M. kalinae*, is described from a small peaty pool Ostrov in the Bohemian Switzerland National Park (northern Bohemia). The species is located in the section *Papillosae* and its morphological characteristics are closest to *M. rasilis*, *M. calceolus*, *M. binocularis* and *M. paxillata*. However, it can be distinguished from these species by differences in scale and bristle morphology. It was previously reported from different parts of the world, but its taxonomic status remained unrecognized due to the lack of a detailed investigation of cultured material. The taxonomy and distribution of the species are discussed and compared with information in the literature.

Key words: algae, Czech Republic, *Mallomonas*, new species, Synurophyceae

Introduction

The *Mallomonas* species described in this paper, *M. kalinae* sp. nov., belongs to the section *Papillosae*. Together with other *Mallomonas* species (Nováková et al. 2004) it was collected in May 2001 from a peat bog situated in the Bohemian Switzerland National Park, northern Bohemia. Previously, similar scales were recorded as *M. cf. rasilis* from Malaysia, Australia and Papua New Guinea (Dürschmidt & Croome 1985, Vyverman & Cronberg 1993), and as *M. rasilis* from Australia and Central Europe (Croome & Tyler 1988, Hartmann & Steinberg 1989). The description of this taxon is based on studies using both electron and light microscopy.

Material and methods

The material was collected from a small peaty pool (depth of about 20 cm, water temperature 16 °C, pH 5.5, conductivity 56 µS/cm) at the locality Ostrov, 3 km NE of the Tisá village (14°02'43,9" N, 50°48'35,4" E, see Nováková et al. 2004) in the Bohemian Switzerland National Park, on 31 May 2001. The species was isolated by pipetting and culturing in Erlenmeyer flasks in DY IV medium (Andersen 1997) under laboratory conditions at room temperature and natural daylight conditions.

To prepare the cells for scanning electron microscopy (SEM), about 5 ml of culture suspension was filtered using a Millipore polycarbonate filter (3.0 µm pore size). Cells were fixed at room temperature in 1% OsO₄ buffered with the culture medium for 1 hour in the Millipore column. The fixative was then diluted with distilled water and the cells on the filter were dehydrated through a graded ethanol series. The filter with cells attached was

transferred subsequently to a vial of 100% ethanol and was critical point-dried with CO₂ (Bal-Tec CPD 030). The dried filter was mounted with double-stick tape to a glass coverslip mounted onto an aluminium stub and then sputter-coated with platinum-palladium for 30 seconds using a model JEOL JFC 2300 HR. The samples were observed using a JEOL JSM-6400 scanning electron microscope operated by the Institute of Biology, University of Copenhagen.

For transmission electron microscopy (TEM), the samples were gently rinsed with distilled water in a centrifuge, dried on to Formvar coated copper grids and shadowcast with chromium (Němcová et al. 2002). The grids were examined using a transmission electron microscope Philips T 300 operated by the Department of Botany, Charles University, Prague.

The LM photographs were taken using Olympus Z300 microphotograph equipment attached to an Olympus BX51 light microscope. The strain (number B 601) is kept in The Collection of Algae of Charles University, Prague, Czech Republic (CAUP) (Nováková & Neustupa 2005).

Results

Mallomonas kalinae Řezáčová spec. nova

Latin diagnosis: Cellulae ellipsoidae, 15.0–17.5 µm longae, 8.0–9.5 µm latae, squamis cupuliferis tectae. Squamae tripartitae (3.7–3.9 × 1.7–2.0 µm), cupula parva aut sine papillis dissipatis ornata. Scutum papillis aequidistanter dispositis ornatum. Pori singuli sive bini circumvallati in angulo cristae v-formis dispositi. Costae submarginales distinctae adulti. Limbi inornati. Squamae postremae circiter 2.5 µm longae, 1.3 µm latae, squamis corporis minores. Setae 4.1–7.3 µm longae, exigue curvatae et acutae. In parte anteriore cellulae setae basaliter extensae ad apicem acutum attenuatae. Cystae ignotae.

Iconotypus in stagno turfoso, Ostrov apud Tisá, distr. Ústí nad Labem, Bohemia, die 31 Martii anni 2001, inventus. Iconotypus Figura mea 2c.

The epithet is dedicated in honour of the phycologist Tomáš Kalina, Czech Republic.

Cells are ellipsoidal (15.0–17.5 × 8.0–9.5 µm) and are covered with domed scales each bearing a bristle (Fig. 1c, some bristles were damaged during fixation). The scales (3.7–3.9 × 1.7–2.0 µm) are tripartite with a small and smooth dome. The shield is marked with small papillae, which are regularly spaced and arranged in rows. Distinct base plate pore or occasionally two pores are situated in the proximal area of the shield at the base of the V-rib. This area is mostly devoid of papillae (Figs 2a, b, c). Anterior submarginal ribs are well developed. The posterior and anterior flanges are smooth, the posterior flange is bordered with a smooth upturned rim. The rear scales are smaller than the body scales (ca. 2.5 × 1.3 µm). Bristles are 4.1–7.3 µm long, smooth, slightly curved and pointed. Cysts were not observed.

Discussion

Mallomonas kalinae belongs to the section *Papillosae* Asmund et Kristiansen and within that section is similar to *M. rasilis* Dürrschmidt, *M. calceolus* Bradley, *M. binocularis* Siver and *M. paxillata* (Bradley) Péterfi et Momeu (Kristiansen 2002). The species most similar to *M. kalinae* is *M. rasilis*, and therefore it was designated as *M. cf. rasilis* in earlier

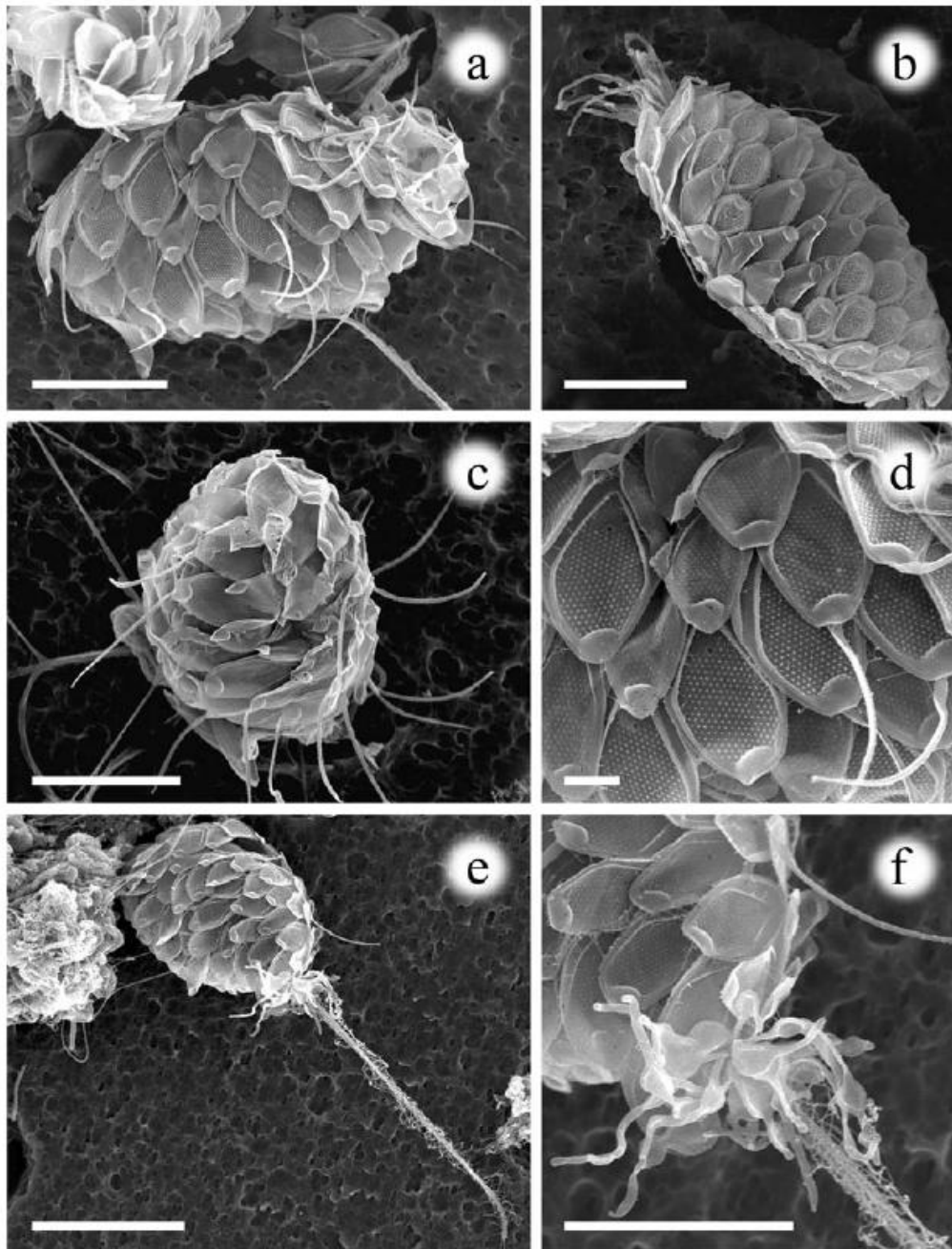


Fig. 1. – *Mallomonas kalinae* sp. nov. (SEM): a–c – Entire cells. Scale bars indicate 5 μ m; d – Group of body scales. Scale bar indicates 1 μ m; e – Entire cell with one flagellum. Scale bar indicates 10 μ m; f – Detail of deformed scales. Scale bar indicates 5 μ m.

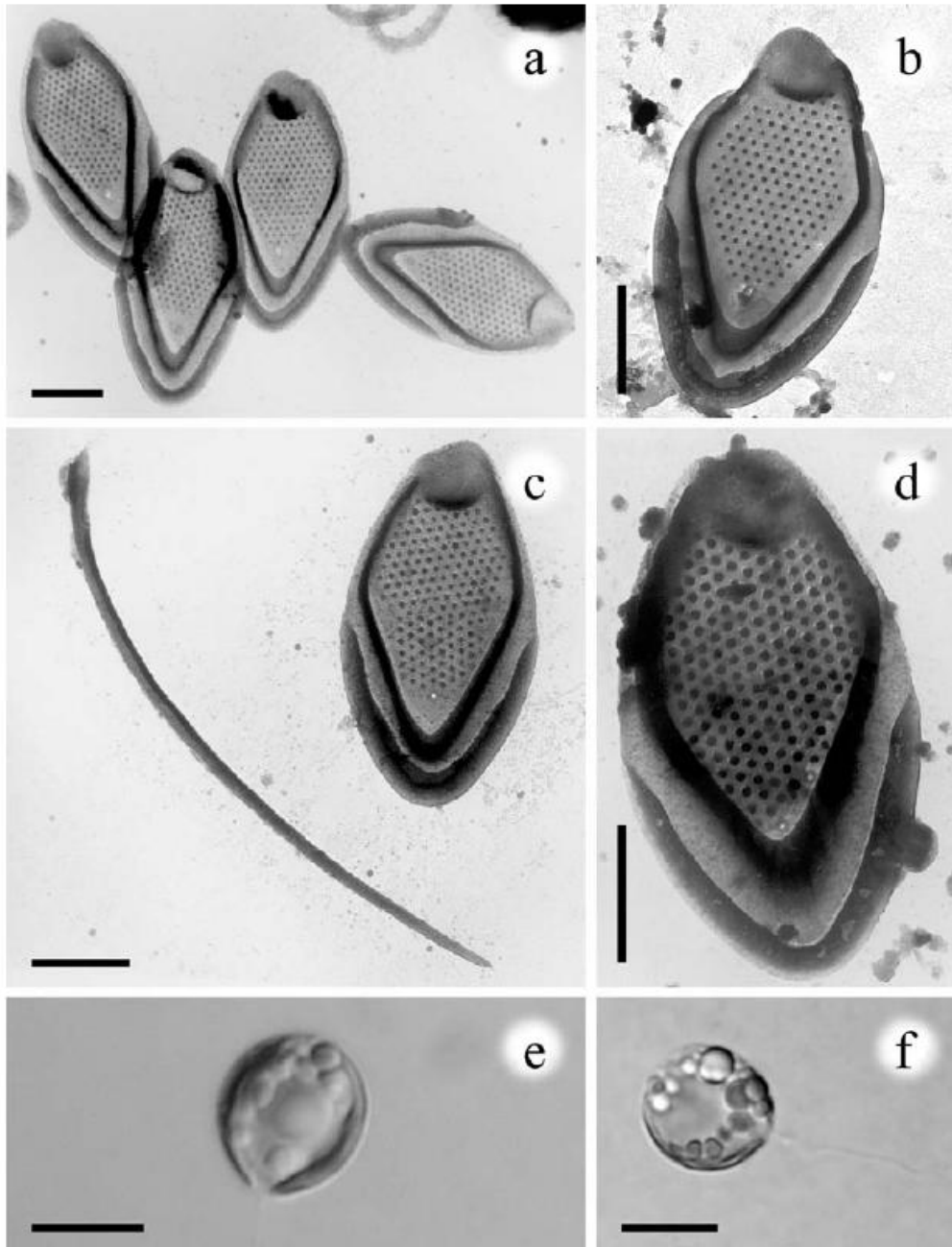


Fig. 2. – *Mallomonas kalinae* sp. nov. (TEM, LM): a – Group of body scales. Scale bar indicates 5 μm; b – Body scale with reduced anterior flanges. Scale bar indicates 5 μm; c – Body scale and bristle. Scale bar indicates 5 μm; d – Body scale with two pores. Scale bar indicates 5 μm; e–f – Cells from the culture. Scale bars indicates 10 μm.

studies (Dürschmidt & Croome 1985, Vyverman & Cronberg 1993). The scales of *M. kalinae* differ from those of *M. rasilis* Dürschmidt in having well-developed anterior submarginal ribs, lacking papillae on the anterior flanges and having only smooth proximal borders. The scales are also narrower than those of *M. rasilis*. In addition, the bristles of *M. rasilis* are unilaterally serrate with short pointed teeth and therefore unlike those of *M. kalinae*. Vyverman & Cronberg (1993) record that their *M. cf. rasilis* designated cells had scales with the dome on one side half covered with papillae. The cells are more elongate ($23.0\text{--}23.9 \times 5.6\text{--}6.4 \mu\text{m}$) with slightly broader and longer scales ($4.2 \times 2.1\text{--}2.6 \mu\text{m}$) and somewhat longer bristles ($9.5\text{--}12.0 \mu\text{m}$) than those of *M. kalinae*. In spite of this, these cells are considered to be *M. kalinae* due to the presence of certain structural features, especially scales with distinct anterior submarginal ribs. The shield on the scales of *M. calceolus* Bradley is marked with widely spaced and scattered papillae instead of the densely and regularly spaced papillae present on *M. kalinae* scales. Scales of *M. calceolus* also lack the pore in the angle of the V-rib and the distal ends of the bristles are bifurcate. *Mallomonas kalinae* also resembles *M. binocularis* Siver in having bristles and papillae on the shield. However, *M. binocularis* lacks anterior submarginal ribs and at the base of the V-rib has two conspicuous pores resembling a pair of eyes (Siver 2002). Interestingly, several scales of *M. kalinae* with two pores have also been found (Fig. 2d), but these are in a different position than those of *M. binocularis*. Siver (2002) discussed the significance of the rimmed pores within Section *Papillosae*, specifically in the case of *M. papillosa* and *M. binocularis*, and emphasized their importance for specific delimitation. As was demonstrated here, populations can include a few cells bearing some atypical scales. Finally, *M. kalinae* differs from *M. paxillata* (Bradley) Péterfi et Momeu in the absence of a well developed anterior submarginal rib that extends past the dome forming a forward-pointing “tooth”, and in lacking papillae on the anterior flanges. In addition, the scales of *M. kalinae* have a pore in the base plate and the distal ends of bristles lack bifurcate tips.

Interestingly, at the anterior end of some cultivated *M. kalinae* cells peculiar structures develop (Figs 1b, e, f). These structures are probably variously distorted and deformed scales, enlarged at the base and narrowing towards the blunt distal end (Fig. 1f). In the genus *Mallomonas* similar apical structures are known only in *M. retrorsa* (Siver 1991). However, their shape is different; being paddle shaped and widest at the distal end instead of the proximal as in *M. kalinae*. These peculiar structures in *M. kalinae* were observed only in older cultures, but it cannot be excluded that they also appear in nature.

Mallomonas kalinae has previously been found (and identified as *M. cf. rasilis* or *M. rasilis*) in oligotrophic and dystrophic waters rich in humic acids at temperatures between 26 and 28 °C, pH 5.6 and conductivity of 16 $\mu\text{S}/\text{cm}$ in Malaysia and Australia (Dürschmidt & Croome 1985), Australia (Croome & Tyler 1988) and Papua New Guinea (Vyverman & Cronberg 1993). Hartmann & Steinberg (1989) found single scales in a sample from former West Germany from water with a temperature of 11.4 °C, pH 7.3 and conductivity of 122 $\mu\text{S}/\text{cm}$. Thus, *M. kalinae* appears to prefer slightly acidic and oligotrophic water bodies and rather high temperatures. It is probably a widely distributed, but rarely occurring species. Unlike many other *Mallomonas* taxa, this acidophilic species grows well in culture, which makes it a suitable model organism for investigating the morphological variation and ontogenesis of scales (Gutowski 1996, Siver & Skogstad 1988). Including *M. kalinae*, the *Mallomonas* flora of the Czech Republic now comprises 42 taxa (Němcová et al. 2003, Nováková et al. 2004, Řezáčová et al. 2004).

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Souhrn

Nový druh rodu *Mallomonas*, *M. kalinae* sp. nov., je popsán z malé rašelinné tůně, z lokality Ostrov v NP Českosaské Švýcarsko (severní Čechy). Na základě morfologických charakteristik se tento druh řadí do sekce *Papillosae* a podobá se druhům *M. rasilis*, *M. calceolus*, *M. binocularis* a *M. paxillata*. Od těchto druhů se odlišuje ornamentací šupin a strukturou ostnů. Byl již zaznamenán v několika floristických publikacích z různých částí světa, avšak s nejistým určením. Detailnější informace o struktuře křemičitých struktur přineslo až studium bičíkovce udržovaného v kultuře v laboratorních podmínkách. Taxonomie a rozšíření tohoto druhu je dále diskutována a porovnána s literárními údaji.

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Paper 4

Effect of temperature on the variability of silicate structures in *Mallomonas kalinae* and *Synura curtispina* (Synurophyceae).

Effect of temperature on the variability of silicate structures in *Mallomonas kalinae* and *Synura curtispina* (Synurophyceae)

by

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With 14 figures and 4 tables

Řezáčová-Škaloudová M., J. Neustupa & Y. Němcová: Effect of temperature on the variability of silicate structures in *Mallomonas kalinae* and *Synura curtispina* (Synurophyceae).

Abstract: Taxonomy of the class Synurophyceae is based on the morphology of the siliceous cell coat, especially of the scales that are found in samples of freshwater plankton or in sediments and examined by electron microscope (EM). Members of this class are known to be powerful indicators of environmental conditions and are often used for biomonitoring and paleoecological studies. We investigated the effect of temperature on scale shape using landmark-based geometric morphometric methods in two synurophycean species *Mallomonas kalinae* Řezáčová and *Synura curtispina* (Petersen & Hansen) Asmund. Clones of these species were grown at different temperatures (5, 10, 15, 20, 25, and 30°C) in batch culture experiments. We found statistically significant differences in the shape and size variation of silicate structures corresponding to temperature changes, although a substantial part of shape variation was associated with the position of scales on the cell. The range of shape variation was characterised by wide rounded vs. tapered scales, and further by the extent of the dome area, and the secondary layer. Highly asymmetrical apical *Mallomonas* scales occurred only at low temperatures (10 and 15°C). We also revealed a tendency for scale size to be reduced with increasing temperature; significant differences were found both in size of scales and in length of bristles or spines. Additionally, the present study provides evidence that geometric morphometrics is a powerful tool in analyses of temperature induced shape variation of silica scales.

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Introduction

During the last decade, many studies on relationships among environmental temperature, organismal growth, shape plasticity, and cell size in several taxa have been published. The temperature-dependent plasticity of body size has been observed in bacteria, protists, plants, and animals (e.g. Atkinson & Sibly 1997; Partridge & Coyne 1997; Kindleman et al. 2001; Angilletta et al. 2004; Yom-Tov & Geffen 2006). In this study, we have investigated the effect of water temperature on shape and plasticity of silicate structures in two synurophycean species. Members of the class Synurophyceae (Heterokontophyta) are characterized by silica scales that cover their cellular body. Taxonomy of this group has been based on morphology of scales and bristles since the 1950`s, when EM became available. Most synurophycean species are classified into two genera. The genus *Mallomonas* Perty includes unicellular species with about 30 to 150 scales per cell, depending on the species (Siver 1991). The bases of bristles are connected to the apical part of the scales (dome), but both bristles and scales develop independently in cells (Siver 1991; Wee 1997). The genus *Synura* Ehrenberg is colonial and, depending on the species, the number of scales ranges from approximately 50 to 100 per a single cell (Wee 1997). A number of synurophyte species are known to occur only in specific habitats that are generally characterized by temperature, pH, conductivity or trophic conditions. Therefore, synurophytes are frequently used in limnological and paleolimnological biomonitoring studies. In this respect, the study of morphological variation of siliceous structures could represent important information for biomonitoring studies (Smol 1995). Siver & Skogstad (1988) found a distinct relationship between temperature and the two morphological types (helmet and serrated) of siliceous bristles in population of *Mallomonas crassisquama*. The serrated bristles were typical for cold water, whereas, helmet bristles occurred exclusively in a warm water environment. In laboratory conditions, the effect of temperature on the variability of scales was investigated in *Synura petersenii* (Martin-Wagenmann & Gutowski 1995) and in *Mallomonas tonsurata* (Gutowski 1996). All these studies were based on the analysis of conventionally measured distances (length, width, angles) of structurally homologous points on silica-scale structures. Here, we use methods of landmark-based geometric morphometrics for the analysis of temperature-dependent differences in *Mallomonas kalinae* and *Synura curtispina* scales. Geometric morphometrics is based on high-dimensional multivariate analyses of shape variables that retain all of the geometric information contained within the data and provides informative visualizations that are frequently not possible to obtain with alternative methods (Slice 2005). Geometric

morphometric methods have recently become the most useful tool for the evaluation of biological shape in many branches of organismal biology (Adams et al. 2004).

In phycology, geometric morphometric methods were applied in studies of taxonomy of morphologically closely related diatoms (Beszteri & Medlin 2005; Potapova & Hamilton 2007), and in several studies dealing with green algae (Neustupa & Hodač 2005; Verbruggen et al. 2005; Neustupa & Škaloud 2007). In synurophytes, Neustupa & Němcová (2007) investigated variation in scales of *Mallomonas striata* using geometric morphometric analyses of natural populations and data from the literature. They found significant differences in the shape of scales occurring world-wide in two varieties – *M. striata* var. *striata* and *M. striata* var. *serrata* Harris & Bradley. Previously, both of these varieties had been delimited only on the basis of differences in bristles (Kristiansen 2002).

This report considers the effect of temperature on silicate structures (both scales and bristles) of clonal populations of *Mallomonas kalinae* and *Synura curtispina*. The aim of this study is to describe the main trends in scale shape variation, and to document these changes in relation to temperature.

Materials and Methods

Mallomonas kalinae was isolated from a peaty pool in the Czech Republic and is deposited in the Culture Collection of Algae at Charles University in Prague (CAUP B601). *Synura curtispina* (SAG 29.92) was obtained from the Culture Collection of Algae at the University of Göttingen. Both unialgal batch cultures were grown in DY IV medium (Andersen et al. 1997). Stock cultures were grown at 25°C under continuous light. Initially, about 50 cells of each flagellate were transferred into Erlenmeyer flasks during their exponential phase of growth. Experiments were carried out simultaneously in different chambers at temperatures of 5, 10, 15, 20, 25, and 30°C on a 16:8 light:dark cycle at 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for one month. Lugol's fixed algal suspensions were then prepared for transmission electron microscopy (TEM) and examined with a JEOL 1010. For both species, about 60 randomly selected scales from each population growing at each of the temperatures, and which allowed for the delimitation of the landmarks, were photographed at the same magnification. This investigation included three to five electronmicroscopical grids per given temperature. Cultures at the lowest temperatures (5°C for *M. kalinae*, 5 and 10°C for *S. curtispina*) contained low numbers of scales due to poor cell growth, thus they were not included in the analyses. Altogether, there were 319 *Mallomonas* dome-bearing scales and 264 *Synura* scales bearing spines suitable for the landmark-based geometric

morphometrics. Further, 60 randomly selected *Mallomonas* bristles from each of the temperatures were photographed at the same magnification.

The shape dynamics of silica scales were characterized with the landmark-based geometric morphometrics methods. Twenty-three landmarks for *M. kalinae* and nineteen landmarks for *S. curtispina* were digitalized on each of the scales (Figs 1, 2) using the TpsDig ver. 1.40 programme (Rohlf 2004a). Sixteen (for *M. kalinae*) and thirteen (for *S. curtispina*) of the landmarks were allowed to slide along the outline (semilandmarks). The scales of both flagellates are almost bilaterally symmetrical, the left and right sides of *M. kalinae* scales can be distinguished by asymmetric dome features. This is not true for *S. curtispina* scales, so the landmarks in mirror positions were symmetrised using the method recommended by Klingenberg et al. (2002). This involves reflecting each of the scales (by multiplication of x-coordinates of all landmarks by -1), re-labelling paired landmarks, and averaging the original and mirrored configurations in a Procrustes superimposition. The averages of original and mirrored/re-labelled scales are ideal symmetric shapes in which each half, together with landmarks lying on the median axis, bears all the information on the shape of that symmetric object. Thus, further analysis of these symmetrised configurations involves only the symmetric part of the shape variation and omits the asymmetric part. In symmetrised configurations, all the shape information is included in the coordinates of one half of the paired landmarks plus the landmarks lying on the median axis (Neustupa & Němcová 2007). The halved configurations were used for the canonical variates analysis; however, the thin-plate splines of extreme positions were made using entire configurations for better graphical illustration in TpsRegr ver. 1.28. (Rohlf 2003; Zelditch et al. 2004).

The Procrustes superimpositions and relative warps analyses (shape PCA) with parameter α set to 0 (Rohlf 1993) were performed with the programme TpsRelw ver. 1.39 (Rohlf 2004b). The extreme positions of the individual relative warps axes were presented as deformation grids allowing the visualisation of principal trends of shape variation. The canonical variates analysis programme CVAGen6 (Sheets 2002) was used to find the set of axes that allows for the greatest possible ability to discriminate among groups. The tests of significance of the canonical variate axes in CVAGen6 are all based upon the Wilk's lambda (λ) value and on the Bartlett's test. In *Mallomonas*, a distinctly delimited clump of apical scales (for details see results) was removed from the input data for the CVA, in order to find other features that discriminated among groups.

TpsSuper ver. 1.13 (Rohlf 2004c) was used to reconstruct the shape of scales at different temperatures. Only one scale was chosen for shape reconstruction, and consensus for each of the temperatures was used as a fixed reference. For the shape reconstruction of apical scales, one apical scale was chosen and consensus of all apical scales was used as a fixed reference.

Size of the scales is given as centroid size, which is defined as square root of the sum of squared distances from the landmarks to their centroid. Differences among centroid sizes of scales were tested using a one-way ANOVA and Tukey's pairwise comparisons in PAST ver. 1.40 (Hammer et al. 2001). A multivariate regression testing the influence of two independent variables (centroid size and temperature) on shape variables was computed using TpsRegr ver. 1.28. Lengths of both *Mallomonas* bristles and *Synura* spines were measured conventionally. Again, differences among the values of a length were tested using a one-way ANOVA and Tukey's pairwise comparisons in PAST ver. 1.40 (Hammer et al. 2001).

Results

Mallomonas kalinae

The first three relative warps explained 71.5% of the variance in shape (RW1 36.4%, RW2 19.8%, RW3 15.3%). The position of scales in the space of the first two warps is shown in a PCA plot (Fig. 3). A distinctly delimited clump of scales, corresponding to the apical scales, is enclosed in the ellipse and a consensus of these scales is reconstructed. The thin-plate splines of extreme positions corresponding to the first two relative warps from each of the temperatures are depicted in Fig. 4. The extreme values of the first axis that described the shape variation of scales are related to their position on the cell. Asymmetrical scales with a dome placed more to the right, towards the centre of symmetry, correspond to apical scales, whereas dome placed more to the left, is typical for rear scales (Fig. 5). As is obvious from the thin-plate splines of extreme positions, highly asymmetrical apical *M. kalinae* scales were found only at low temperatures (10 and 15°C). Slightly asymmetrical apical scales were revealed at higher temperatures (25 and 30°C), and the most homogenous appearance of scales was observed at 20°C. In the CVA, there were four significant canonical variates ($\lambda = 0.0554$, $p < 0.0001$; $\lambda = 445.7959$, $p < 0.0001$; $\lambda = 0.4482$, $p = 0.0001$; $\lambda = 0.6916$, $p = 0.0001$), that separated scales from each temperature treatment. The main distinguishing characteristics among groups were the extent of scale elongation (rounded vs. tapered), and the extent of dome area (Fig. 6). The scales originating at 10, 25, and 30°C were more rounded than scales originating at 15 and 20°C. At 10°C, the area of the dome was clearly smaller, although the overall scale appearance was rather robust. The shape reconstruction of scales at particular temperatures corresponding to above mentioned shape changes are depicted in Fig. 7. Distinctly larger scales were found at 10°C, as is obvious from box plots of scales centroid sizes (Fig. 8), including a test of significance (Table 1). In multivariate regression, both temperature and centroid size explained a small amount (11.03%, $p <$

0.00001) of shape variation, and residual variation was larger for temperature. Significantly shorter bristles were revealed at 25 and 30°C (Fig. 9, Table 2).

Synura curtispina

The first three relative warps accounted 93.6% of shape variation (RW1 61.9%, RW2 21.0%, RW3 10.7%). The thin-plate splines of extreme positions from each of the temperatures corresponding to the first two relative warps are depicted in Fig. 10. The narrow scales from the caudal part of the cell were associated with the first axis (RW1) at 20, 25 and 30°C, whereas caudal scales grown at 15°C were related to the second axis (RW2). In the CVA, there were three distinct canonical variates ($\lambda = 0.3480$, $p < 0.0001$; $\lambda = 0.6184$, $p = 0.0001$; $\lambda = 0.8186$, $p = 0.0001$). The distinguishing characteristics among groups were the change from wide and rounded scales to narrow oval scales, i. e. the breadth of scales, as well as the extent of a secondary layer covering the scales. It also depended on how tapered both the proximal and distal parts of the scales (Fig. 11). The shape reconstruction of scales, showing a narrowing trend with increasing temperature, is depicted in Fig. 12. The range of centroid sizes, including a test of significance (Fig. 13, Table 3), shows that the smallest scales appeared at 25°C. In multivariate regression, both temperature and centroid size explained 23.09% of shape variation ($p < 0.0001$), and residual variation was larger for centroid size. The pattern of spine lengths correspond to the centroid sizes of scales (Fig. 14, Table 4). The shortest spines were found at 25°C, the longest spines occurred at 15°C.

Discussion

In both studied species, the substantial shape variation of scales could be explained by their position on the cell. The pattern of an inverse relationship between size of siliceous structures and temperature was highly visible in the thermal range of 10-25°C. Generally, the scales became narrower, bristles and spines were shorter with increasing temperature. Both in *M. kalinae* and *S. curtispina* a small increase in scale size was observable at 30°C. However, since we also detected several deformed scales at this highest temperature, this increase of scale size was probable caused by thermal stress.

To date, there are only a few studies elucidating the range of morphological variability of siliceous structures in different conditions within individual species or populations of synurophytes. Several experiments have been hitherto performed with *Mallomonas tonsurata*, *M. striata*, *Synura petersenii* and *S. echinulata* (Martin-Wagenmann & Gutowski 1995;

Gutowski 1996; Hahn et al. 1996; Gavrilova et al. 2007; Neustupa & Němcová 2007; Němcová et al. - see this volume). Gutowski (1996) cultivated a *Mallomonas tonsurata* clone at different temperatures (5, 10, 15, 20, and 25°C) under continuous light. Then, she measured main scale and bristle characteristics including: length and breadth of scale, length and breadth of dome (for dome-bearing scales), and length and breadth of anterior area (for domeless scales). A multiple range test showed these significant changes: the scales were shorter at 15, 20, and 25°C, with a tendency to have a larger dome. However, the domeless scales had the tendency to develop a smaller anterior area (Gutowski 1996). These significant changes in *M. tonsurata* correspond to the main distinguishing characteristics among groups revealed by CVA in *M. kalinae* (Fig. 6), i. e. rounded vs. tapered scales, and the extent of the dome area. *Mallomonas kalinae* scales, similar to those of *M. tonsurata*, were distinctly larger at 10°C than at other investigated temperatures. Similar trends in response to temperature were found in bristles of both species. Gutowski (1996) found two types of bristles at 5°C - shorter apical scale bristles and longer lateral scale bristles. Above 15°C lateral bristles have a tendency to shorten and both bristle types were indistinguishable at 25°C. This phenomenon is also in accordance with our data on *M. kalinae* bristles, which are only one type. *M. kalinae* bristles were significantly shorter at 25 and 30°C than at lower temperatures. Length of bristles was correlated with size of scales, which is probably caused by construction restrictions. Martin-Wagenmann & Gutowski (1995) investigated three clones of *Synura petersenii*, in relation to temperature, and reported changes in scale morphology only at 5 and 20°C. In addition, *S. petersenii* is considerably distinct from *S. curtispina* in the construction of the elements of its scales, therefore, it is possible to compare only the overall shape and/or size of the scales. Interestingly, Martin-Wagenmann & Gutowski (1995) found that only the breadth (not the length) of the scales of the *S. petersenii* f. *petersenii* clone was significantly influenced by temperature. The f. *petersenii* clone developed narrower scales at higher temperature. The same pattern was revealed by our *S. curtispina* scales.

Summarizing the mentioned literary data and our investigations, a tendency to reduce size of siliceous structures with increasing temperature seems to be a general attribute of *Synura* and *Mallomonas* species. Even if we did not investigate the cell size and growth characteristics of the strains, we suppose that the reduction of scale size could be related to the higher growth cell rate. Higher temperatures result in faster cell growth, and subsequently in smaller size of dividing cells. This phenomenon, known as a temperature-size rule, is applicable for the majority of ectotherms (Angiletta & Dunham 2003). However, the additional investigations focused on the relationships between the cell size and temperature will be necessary to confirm the relevance of temperature-size rule in chrysophytes. In addition, nutrient limitation can also result in larger cells and has a significant effect on the development of siliceous

structures. Hahn et al. (1996) reported shape change of *M. tonsurata* scales and bristles in relation to phosphorus, nitrogen and silica content in medium.

The suggested relationships among shape, size and temperature are considered to be critical primarily from the paleoecological point of view. Chrysophyte microfossils have considerable potential in paleoclimatic reconstructions, especially with heightened interest in possible future climate modifications (Smol 1995). Temperature dependent morphological variability (mainly length of bristles and spines, size of scales) could potentially be a good indicator in the reconstruction of climatic histories.

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Table 1. Tukey’s pairwise comparison of centroid sizes of *Mallomonas kalinae* scales (n – number of scales, significant differences on a 0.1% level with three asterisks and on a 1% level with two asterisks).

n	t (°C)	Tukey’s pairwise comparison				
		10	15	20	25	30
68	10	-	***	***	***	***
60	15		-	-	**	-
64	20			-	-	-
65	25				-	-
62	30					-

Table 2. Tukey’s pairwise comparison of lengths of *Mallomonas kalinae* bristles (n – number of bristles, significant differences on a 0.1% level with three asterisks and on a 1% level with two asterisks).

n	t (°C)	Tukey’s pairwise comparison				
		10	15	20	25	30
60	10	-	-	**	***	***
60	15		-	-	***	***
60	20			-	***	***
60	25				-	-
60	30					-

Table 3. Tukey’s pairwise comparison of centroid sizes of *Synura curtispina* scales (n – number of scales, significant differences on a 0.1% level with three asterisks and on a 5% level with one asterisk).

n	t (°C)	Tukey’s pairwise comparison			
		15	20	25	30
60	15	-	*	***	***
60	20		-	***	-
60	25			-	***
58	30				-

Table 4. Tukey’s pairwise comparison of lengths of *Synura curtispina* spines (n – number of spines, significant differences on a 0.1% level with three asterisks).

n	t (°C)	Tukey’s pairwise comparison			
		15	20	25	30
67	15	-	***	***	***
67	20		-	***	-
67	25			-	***
67	30				-

Figs 1-2. Positions of landmarks (white circles) and semilandmarks (black squares). Fig. 1. *Mallomonas kalinae*. Fig. 2. *Synura curtispina*. Scale bars 1 μm .

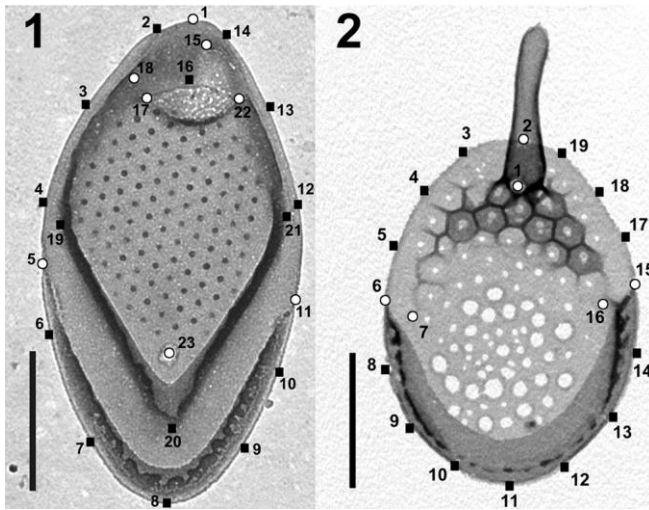


Fig. 3. Scatter plot from RWA (shape PCA) of 319 *Mallomonas kalinae* scales originating from five different temperatures. Distinctly asymmetrical apical scales associated with RW1 are enclosed in the ellipse, and depicted as their consensual reconstruction.

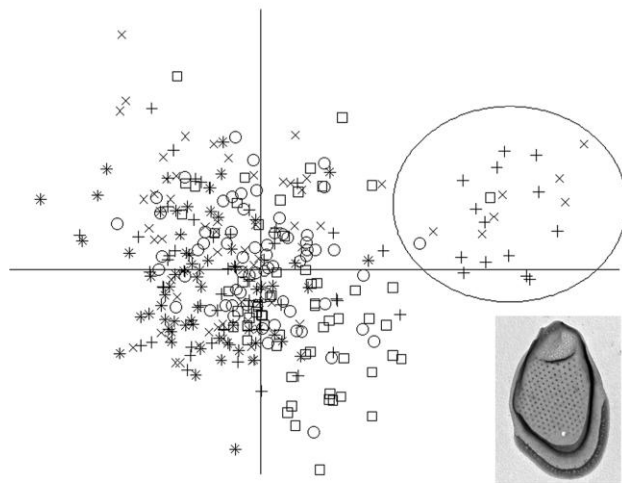


Fig. 4. *Mallomonas kalinae* - the thin-plate splines of extreme positions of the first two relative warps (RW) at given temperatures. Highly asymmetrical apical scales appeared only at 10 and 15°C. Slightly asymmetrical scales were revealed at higher temperatures (25 and 30°C) on both the first and second axis. The most homogenous appearance of scales was found at 20°C.

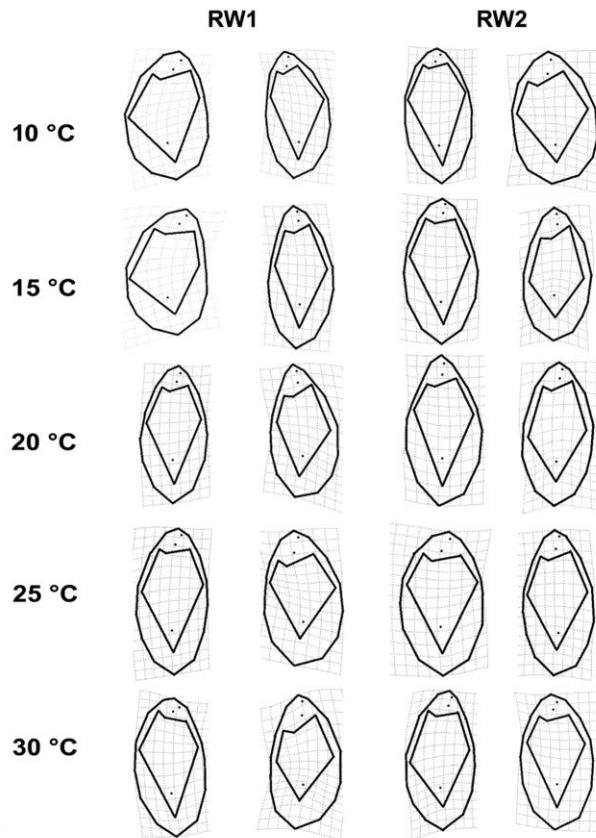


Fig. 5. *Mallomonas kalinae* cell at 10°C. Apical scales (white arrows) and rear scales (black arrows) corresponding to the first relative warp. Scale bar 5 μm.



Fig. 6. Scatter plot from CVA of 296 *Mallomonas kalinae* scales originating from five different temperatures.

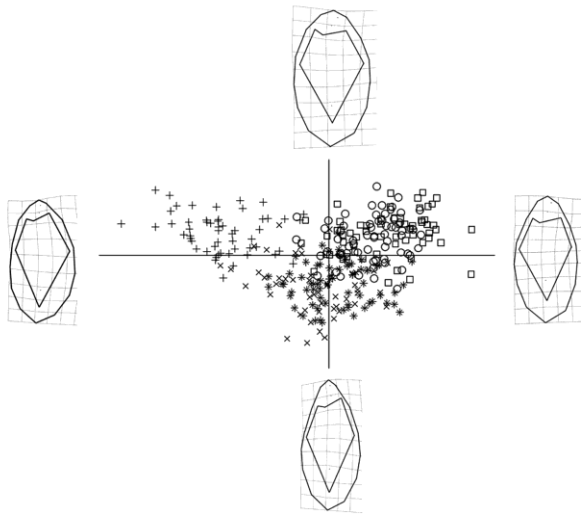


Fig. 7. Shape reconstruction in *Mallomonas kalinae*. The scales arising at 20°C appear the narrowest with a slightly tapering dome. Very similar scales are seen at 15°C. At 25, 30 and especially at 10°C the scales are distinctly broader and more rounded. A rim of the basal plate seems to be rather shorter at lower temperature (10 and 15°C) indicating an inverse trend than that of *Synura curtispina* (see Fig. 12).

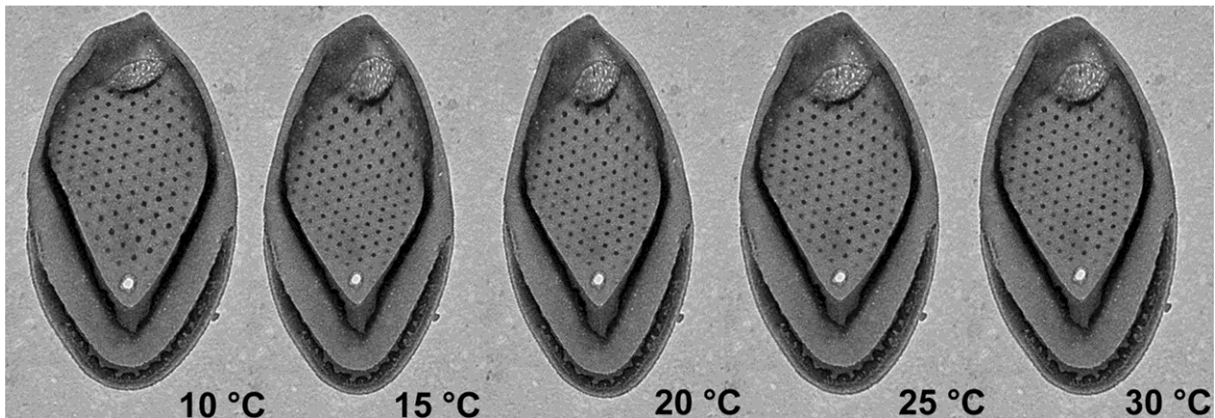


Fig. 8. Box plots of centroid sizes of *Mallomonas kalinae* scales.

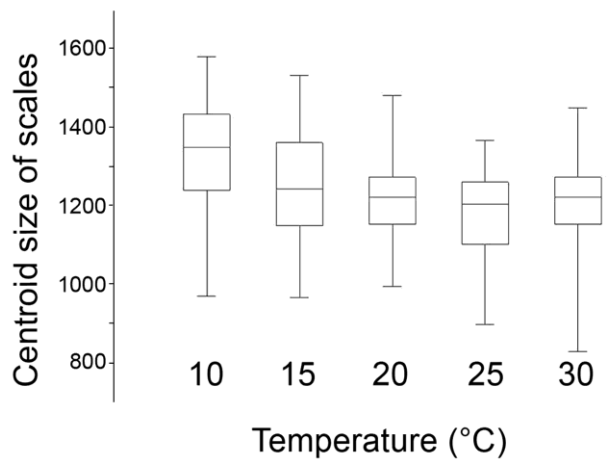


Fig. 9. Box plots of lengths of *Mallomonas kalinae* bristles.

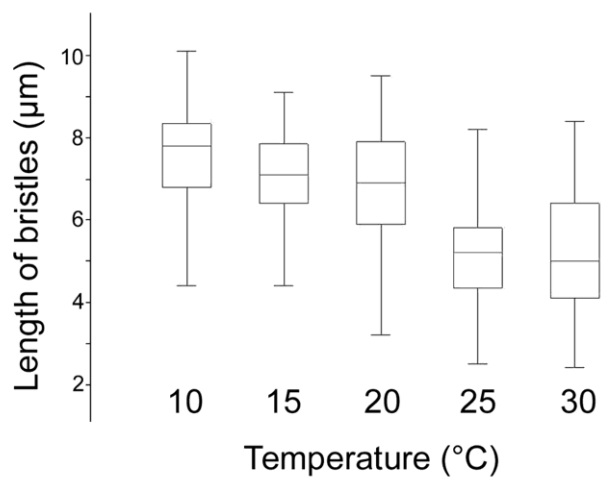


Fig. 10. *Synura curtispina* - the thin-plate splines of extreme positions of the first two relative warps (RW) at given temperatures. The scales shape change is connected with their position on the cells. The narrow scales are from the caudal part of the cell. This phenomenon holds for the first axis (RW1) at 20, 25 and 30°C, but only at 15°C for the second axis (RW2). The first axis (RW1) demonstrates dominant occurrence of the broadly oval scales at 15°C. In contrast, the narrowly oval scales are typical for higher temperatures.

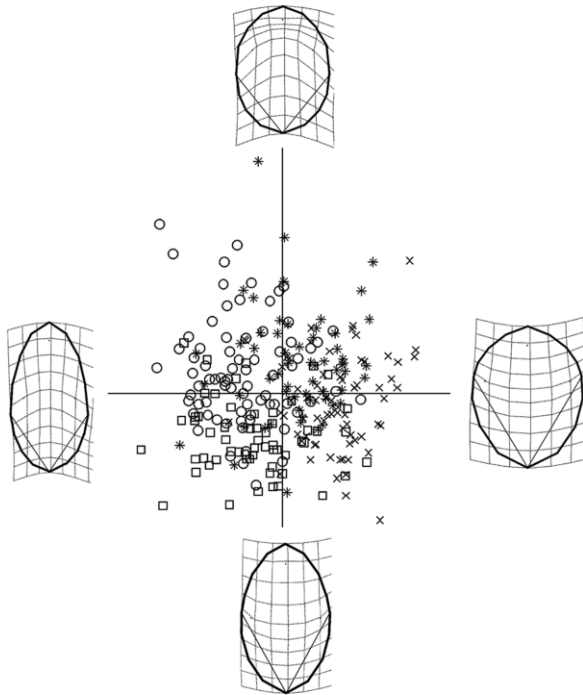


Fig. 11. Scatter plot from CVA of 264 *Synura curtispina* scales originating from four different temperatures.

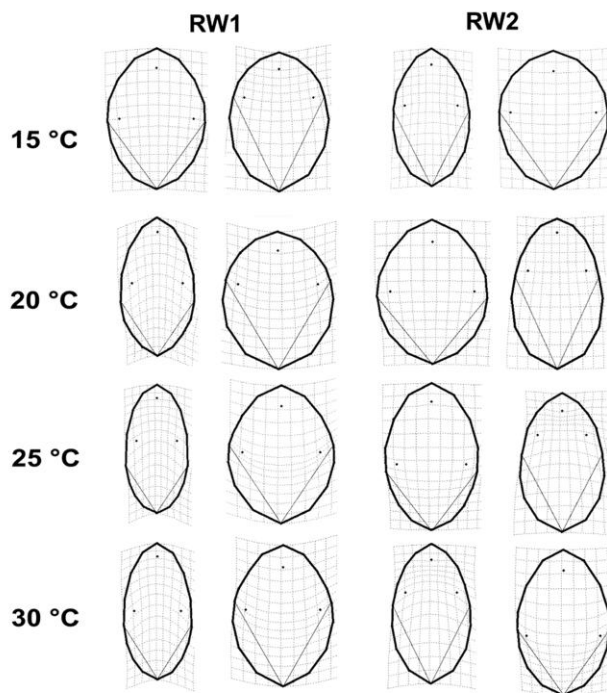


Fig. 12. Shape reconstruction in *Synura curtispina*. The scales gradually become narrower with increasing temperature. At 15 and 20°C, the rim of the basal plate slightly exceeds half of the scale and the extent of a secondary layer is less than at 25 and 30°C.

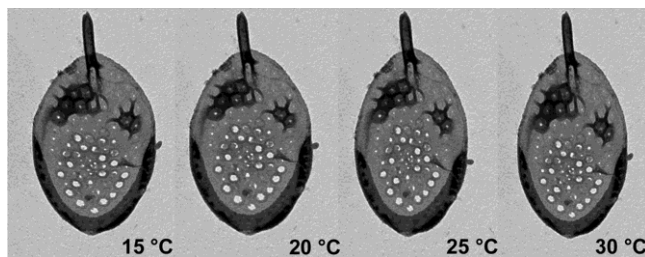


Fig. 13. Box plots of centroid sizes of *Synura curtispina* scales.

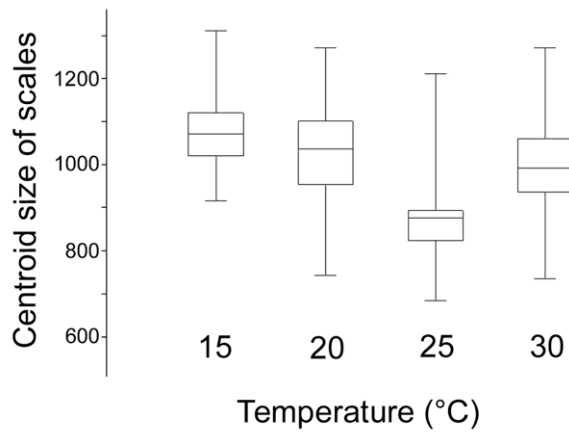
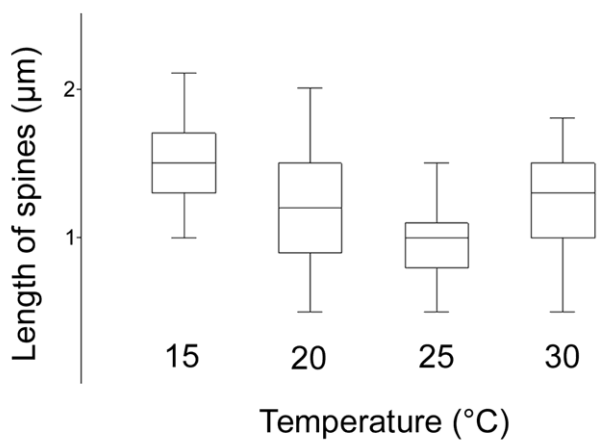


Fig. 14. Box plots of lengths of *Synura curtispina* spines.



Paper 5

**Molecular diversity and species concept in
Synura petersenii complex (Synurophyceae,
Heterokontophyta).**

Molecular diversity and species concept in *Synura petersenii* complex

(Synurophyceae, Heterokontophyta)

by

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With 9 figures and 2 tables

Anna Kynčlová, Pavel Škaloud & Magda Škaloudová: Molecular diversity and species concept in *Synura petersenii* complex (Synurophyceae, Heterokontophyta).

Abstract: Taxonomy of the species *Synura petersenii* is traditionally based on morphology of the silica scales covering its cells. In the past, many changes were made in the classification of the various morphotypes found in this species. In this study, we used a polyphasic approach to clarify the species concept of *S. petersenii*, and to insure that the results were as reliable as possible. Utilizing ITS regions analysis of clonal cultures of *S. petersenii* from different Czech localities, strains were divided into six distinct clades. Identification of compensatory base changes (CBCs) and hemi-CBCs of the six clades unequivocally confirmed the results of our ITS analysis. Furthermore, a morphological analysis revealed unambiguous differences in features of the scale structure among the six clades. All of the three species concepts (ITS rDNA phylogeny, CBC, and scale morphology) produced clearly congruent results. By means of this polyphasic approach, *S. petersenii* and *S. glabra* were shown to be different species, and additionally, four new species (*S. americana*, *S. macropora*, *S. reticulata* and *S. oculea*) were identified.

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Introduction

Synura petersenii Korshikov is the most frequently encountered taxon in the Synurophyceae (Wee 1982, Siver 1987, Pichrtová et al. 2007). It is a colonial freshwater golden-brown flagellate with characteristic silica scales covering its cell surface. The scales, and the features of their delicate ornamentation, have always been the most important characteristics for delimitation of this taxon, as well as for all the other *Synura* species.

The silica scales, which can be preserved in sediment for an extended period of time, together with its well known ecological preferences make *Synura* a very useful species for biomonitoring and a valuable source of information for paleolimnology. For these kinds of studies, an unambiguous species definition and clear morphological characterization is necessary.

S. petersenii is a member of sectio *Petersenianae*; identification of species in this section is based upon scale features such as keel shape, or presence and number of ribs and their interconnections. However, these characteristics have always been somewhat uncertain in the *Synura petersenii* complex, because a transitional morphology of scales was often recorded (Kristiansen 1986, Sandgren et al. 1996). Therefore, whether to treat the distinct morphotypes as a single species, or to what ranks they should be assigned remained ambiguous. This was the case for the *glabra* morphotype in the *S. petersenii* complex. This morphotype was first described by Korshikov (1929) as a new species, *Synura glabra*, although, Korshikov himself doubted whether he should give it the rank of species or of variety. In the same paper, Korshikov describes *Synura petersenii* as well. The main difference between these two species is the scale shape and the presence or absence of ribs, which are well developed in *S. petersenii* and much reduced or absent in *S. glabra*. In 1941, Huber-Pestalizzi united the two species under the name *S. petersenii*, and morphotype *glabra* was ranked as a variety; later, it was given the status of forma (Kristiansen & Preisig, 2007). *S. petersenii* became a species rich in formae. Besides f. *petersenii* and *glabra*, it is important to mention f. *kufferathii* (Petersen & Hansen, 1958), which is characterized by a well developed network of ribs and strong silification.

However, not even the designation of many forms was able to do sufficient justice to the rich morphological variability of *S. petersenii*, and so there were always doubts, and clues suggesting that *S. petersenii* could in fact be considered a complex of species (Řezáčová & Škaloud 2005).

The concern was that the *Synura petersenii* species concept was based exclusively on morphological data. There was no molecular analysis until the study of Wee et al. (2001) that revealed significant intraspecific variability in ITS regions of *S. petersenii*. However, the

molecular data have not been compared with scale morphology, and thus, it remains impossible to interpret the role of scale morphology in the *S. petersenii* species concept.

Therefore, in this study we have used three different approaches: morphological, phylogenetic, and CBC to shed light on the *S. petersenii* species concept. By means of this polyphasic method we have confirmed and elucidated the cryptic diversity of this species.

Materials and methods

Samples were taken by a 25 µm mesh plankton net at different localities in the Czech Republic. Water temperature, pH and conductivity were measured in the field using Hanna Combo pH & EC meter. Unialgal cultures were obtained by the micropipetting of one *Synura* colony, and were subsequently cultivated in micro-plates in DY IV medium (Andersen et al. 1997) at a temperature of 15 °C, in daylight illumination (cooling box Helkama C5G). Apart from newly established cultures, two strains were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton in Maine, USA – CCMP 862, 866. The origins of investigated strains, including accession numbers of sequences, are given in Table 1.

Volumes of 200 µl of exponentially growing cultures were removed from the micro-plates by pipetting into a 1ml Eppendorf tubes, which were kept frozen (-20 °C) overnight prior to polymerase chain reaction (PCR). Thawed cultures were directly used as a template to amplify ITS rDNA region, using terminal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3'; White et al. 1990) and Kn4.1 (5'-TCA GCG GGT AAT CTT GAC TG-3'; Wee et al. 2001). All PCR were performed in 20 µl reaction volumes (15.1 µl sterile Milli-Q Water, 2 µl 10' PCR buffer (Sigma), 0.4 µl dNTP (10 µM), 0.25 µl of primers (25 pmol/ml), 0.5 µl Red Taq DNA Polymerase (Sigma) (1U/ml), 0.5 µl of MgCl₂, 1 µl of DNA (not quantified). PCR was performed in a XP thermal cycler (Bioer). PCR amplification began with 35 cycles of denaturing at 94 °C for 1 min, annealing at 54 °C for 1 min and elongation at 72 °C for 1 min 30 s, with a final extension at 72 °C for 10 min. The PCR products were quantified on a 1% agarose gel stained with ethidium bromide and cleaned either with the JetQuick PCR Purification Kit (Genomed) or with QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's protocols. The purified amplification products were sequenced with the PCR primers at Macrogen, Inc. (Seoul, Korea, <http://dna.macrogen.com>).

ITS sequences were visually aligned on the basis of their rRNA secondary structure information (see below) with MEGA 3.1 (Kumar et al. 2004). Positions with deletions in a majority of sequences were removed from the alignment, resulting in an alignment comprising 496 base positions. The phylogenetic trees were inferred by maximum likelihood

(ML) and weighted parsimony (wMP) criteria using PAUP*, version 4.0b10 (Swofford 2002), and by Bayesian inference (BI) using MrBayes version 3.1 (Ronquist & Huelsenbeck 2003). A substitution model was estimated using the Akaike Information Criterion (AIC) with PAUP/MrModeltest 1.0b (Nylander 2004). Accordingly, the GTR+ Γ model was deemed best. Maximum likelihood analyses consisted of heuristic searches with 1,000 random sequence addition replicates and Tree Bisection Reconnection swapping. Reliability of the resulting topology was tested using bootstrap analysis (100 replications) consisting of heuristic searches with 10 random sequence addition replicates, Tree bisection reconnection swapping, and a rearrangement limit of 5,000 for each replicate. The wMP bootstrapping 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. In BI analysis, two parallel MCMC runs were carried out for 2 million generations, each with one cold and three heated chains employing the above-stated evolutionary model. Trees and parameters were sampled every 100 generations. Convergence of the two cold chains was checked and burn-in was determined using the "sump" command.

The secondary structures of ITS rDNA sequences were constructed using the mfold computer program (version 2.3; Walter et al. 1994; Zuker 2003), with folding temperature set to 25°C. The common secondary structure was created using RnaViz (version 2; De Rijk et al. 2003) and used to identify compensatory base changes (CBCs) and hemi-CBCs.

Preparations for TEM were made one month after the isolation of the colony. All samples were dried onto formvar coated copper grids, rinsed with distilled water and examined using a transmission electron microscope JEOL 1010. Scale morphology of all isolates corresponding to the arrangement of the clades was identified. Two representative strains from each of the clades were chosen and photographed for morphological analysis and delimitation of their discriminative features. In total, 120 morphological characters of representative scales were measured (10 scales per strain). The characteristics measured were: scale length to width ratio, keel pore area, base plate pore area, and foramen area; observations for the presence, and shape of a keel tip were also made. To obtain the area of keel pore and base plate pore, ten pores of each scale were measured and the data were averaged. All the characteristics were measured in Adobe Photoshop Elements 5.0.

All *Synura* cells used for geometric morphometric analysis were cultivated for one month under the same conditions. Altogether, 353 scales from 11 strains were photographed under the same magnification. Twenty-four landmarks were defined on each of the investigated scales using the TpsDig ver. 2.05 (Rohlf 2004a) programme to delimit outlines of the basal plate and the keel. Twenty of the semilandmarks were allowed to slide along the outline

(Fig. 1). Landmark configurations were superimposed by generalized Procrustes analysis (Bookstein 1991) in tpsRelw ver.1.42 (Rohlf 2004b) and canonical variate analysis (CVA)/Manova was performed in IMP (Sheets 2002).

Results

ITS rDNA phylogeny

The different tree construction methods produced similar trees, resolving identical, highly supported clades, as well as their relationships. The trees differed only in the organization of their inner branches within a clade. To assess the root placement of the ingroup, the second alignment, including the sequence of *Synura uvella* AF308847.1, was produced on the base of common ITS1 and ITS2 secondary structures, and analyzed separately. The analysis revealed basal positions of clades, including strains: S 8.1, S 9.1, S 9.2 and S 14.1, with moderate support (tree not shown, alignment can be downloaded at http://botany.natur.cuni.cz/algo/align/01_Synura_petersenii.fas). The maximum likelihood (ML) phylogram, rooted with the above-mentioned clade, is presented in Fig. 2. In this phylogeny, the *S. petersenii* strains clustered together into six groups: (1 – referred to further in the text as *S. americana*) GenBank sequences AF308837-AF308844, AF308846; (2 – *S. macropora*) S 5.1, S 5.2, S 5.3, and S 14.2; (3 – *S. petersenii*) S 1.1, S 1.2, S 1.3, S 4.19, S 6.4, S 6.5, S 7.7, S 16.2, and GenBank sequences AF308832- AF308836; (4 – *S. reticulata*) S 7.10 and S 10.2; (5 – *S. oculatea*) S 15.3, S 15.5, and S 15.9; and (6 – *S. glabra*) S 8.1, S 9.1, S 9.2, and S 14.1. Just a single GenBank sequence AF308845 possessed a separate position, related to clades 1 and 2. All six groups of sequences received high statistical support, with a MrBayes PP ≥ 0.99 , ML bootstrap ≥ 86 , and MP bootstrap ≥ 96 . Moreover, results also indicated a highly supported clustering of clades 1 and 2; as well as clades 1, 2, and 3.

ITS2 rDNA secondary structure and compensatory base changes

In all six of the *S. petersenii* clades compared, a common overall organization of the ITS2 rDNA secondary structure could be identified (Fig. 3). The secondary structure was comprised of three paired regions (helices I-III), with helix I the most conservative region having no observed nucleotide changes. In two clades (1 and 3), we detected a slight intraclade ITS2 variation (up to 4 nucleotide changes per clade). However, this variation was concentrated either on the loop region, or the nucleotide change broke a pairing between the nucleotides in the helix region. Thus, neither compensatory base changes (CBCs; nucleotide changes at both sides of paired bases) nor hemi-CBCs (change at only one side of nucleotide pair, but still preserving pairing) were present between sequences from the same clade

(Coleman 2000, 2003). In contrast to absolute absence of intraclade (hemi-)CBCs, the number of (hemi-)CBCs varied from 3 to 10 among the different clades (Fig. 3B). The highest number of (hemi-)CBCs was determined between clades 2 and 4, differing by three CBCs and seven hemi-CBCs. The regions with the most extensive interclade variation, including all three CBCs observed, were helix II, and the basal region of helix III.

Morphological analyses

The important scale characters can be seen in Fig 4. Results of the morphological analysis are given in Fig. 5. The length to width ratio divides the clades into two distinct groups, one of them consisting of the clades with rounded scales: length to width ratio = 1.3-1.8 (clade 1, 2 and 6), and the second one including the clades with long and narrow scales: length to width ratio = 1.8-2.4 (clade 3, 4 and 5). Compared to the other clades, clade 5 has a notably large foramen (77,650-243,550 nm²). Base plate pores of clade 3 and 5 are rather small (250-697 nm²), while in clade 2 they are quite large (2,235-4,618 nm²), and in the case of the other clades (1, 4 and 6) they are medium-sized (174-2,027 nm²). Clade 2 has a large average keel pore area (5,658-14,685 nm²), while keel pores of clade 1 and 6 are medium-sized (1,163-7,792 nm²), and keel pores of clade 3 are small (1,587-3,985 nm²). Size of the keel pores of clade 4 varied from medium-sized to large (4,287-9,690 nm²). The keel tip of most of the clades is acute. Clade 5 has a rounded keel tip, sometimes with a very thin tip or teeth on top. The morphological analyses revealed distinct characteristic combinations of features for each of the clades (see Discussion, Table 2).

Geometric morphometrics

All six genetically delimited clades were found to be distinguishable on the bases of morphological characters. Our analysis of 353 *S. petersenii* scales from 11 strains was based on geometric morphometric data. Statistically significant differences among all analyzed strains were revealed by Manova/CVA analysis (five significant canonical variates: $\lambda = 0.0122$; $p < 0.0001$; $\lambda = 0.1276$; $p < 0.0001$; $\lambda = 0.3143$; $p < 0.0001$; $\lambda = 0.5305$; $p < 0.0001$; $\lambda = 0.8095$; $p = 0.0029$). Along the first CV axis there are three groups separated from each other by the shape of their outline and keel (Fig. 6). *S. glabra* clade has more rounded scales with a less developed keel. Basal plates of clades 1 and 2 are similarly rounded, but have an augmented keel and the rim of the scales is typically longer. The last group has more prolonged and rather lanceolate scales, with a wide and well-developed keel.

Taxonomic conclusions

The phylogeny presented in Fig. 2 is in accordance with the distribution of (hemi-)CBCs in the ITS2 rDNA secondary structure, and the morphological, as well as morphometric, data. Thus, three independent species concepts (namely: phylogenetic, CBC, and morphological) corroborate each other and validate the splitting of *Synura petersenii* into six species.

Synura americana Kynčlová, Škaloud & Řezáčová-Škaloudová sp. nov. (Figs 7A-D)

LATIN DIAGNOSIS: Squamae ovatae, 1.4-3.6 μm longae et 0.8-2.1 μm latae. Carina cum cuspile acuta. Multae costae praesentes. Pori tabellae basalis medii (diameter 15-34 nm), pori carinae etiam medii (diameter 38-86 nm). Foramen parvus (diameter 83-274 nm). Squamae posteriores longae, angustae et simpliores.

HOLOTYPE: *Synura americana* strain CCMP 862, frozen material deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Figure 7A is an illustration of the holotype.

TYPE LOCALITY: Winter's Creek, Keeweenaw County, Michigan, USA (47.2917N 88.0721W, 189 m asl).

ETYMOLOGY: (Latin) americana, American; so far this species has only been found in the North American continent.

DIAGNOSIS: Scales are oval (1.9 x 1.2 μm). Keel terminates in an acute keel tip, especially in the apical scales. Numerous struts connect the keel with scale edge. Base plate pores are medium-sized (diameter 15-34 nm), as are the keel pores (diameter 38-86 nm). Foramen is small (diameter 83-274 nm). Rear scales are long and narrow with somewhat reduced structure.

Synura macropora Kynčlová, Škaloud & Řezáčová-Škaloudová sp. nov. (Figs 7E-I)

LATIN DIAGNOSIS: Squamae ovatae, 2.6-3.3 μm longae et 1.5-2.1 μm latae. Carina cum cuspile parva acuta. Costae parvae aut absentes. Pori tabellae basalis magni (diameter 53-77 nm), pori carinae etiam magni (diameter 85-137 nm). Foramen parvus (diameter 156-330 nm). Squamae posteriores parviores et rotundae.

HOLOTYPE: *Synura macropora* strain S 14.2, frozen material deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Figure 7E is an illustration of the holotype.

TYPE LOCALITY: Peat bog, Swamp NR, North Bohemia, Czech Republic (50.5760N 14.6700E, 267 m asl).

ETYMOLOGY: (Latin) macropora, with large pores; remarkably large keel pores and base plate pores are typical for this species.

DIAGNOSIS: Scales are oval (3 x 1.8 µm), keel without keel tip or ending with a small acute keel tip. Both the base plate pores and keel pores are large (diameter 53-77 nm and 85-137 nm, respectively). Foramen is small (diameter 156-330 nm). Ribs are reduced or nearly absent. Rear scales are smaller and rounded.

Synura petersenii Korshikov 1929; Arch. Protistenk. 67, pp. 283-5, figs 37-38 (iconotype) emend. Kynčlová, Škaloud & Řezáčová-Škaloudová (Figs 8A-D)

SYNONYM: *Synura petersenii* Korshikov f. *kufferathii* Petersen & Hansen 1958; Biol. Medd. Kgl. Dan. Vid. Selsk. 23, p. 10, fig. 5.

EPITYPE: *Synura petersenii* strain S 6.4, frozen material deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Figure 8A is an illustration of the epitype.

EMENDED DIAGNOSIS: Body scales are oblong (4.1 x 2 µm), keel ending with an acute keel tip, especially in the apical scales. Pores of the base plate are small (diameter 19-30 nm), keel pores are relatively small as well (diameter 45-71 nm). Foramen is relatively small (diameter 244-358 nm). Numerous ribs, which are often interconnected, extend from the keel to the edge of the scale. Number of the interconnecting ribs is highly variable. Rear scales are much smaller and narrow with somewhat reduced structure.

Synura reticulata Kynčlová, Škaloud & Řezáčová-Škaloudová sp. nov. (Figs 8E-I)

LATIN DIAGNOSIS: Squamae lanceolatae, 2.6-3.7 µm longae et 1.4-1.9 µm latae. Carina cum cuspidate acuta. Multae costae praesentes. Pori tabellae basalis medii (diameter 25-51 nm), pori carinae medii vel magni (diameter 66-100 nm). Foramen parvum (diameter 190-319 nm). Squamae posteriores multo parviores, angustae et simpliciores.

HOLOTYPE: *Synura reticulata* strain S 7.10, frozen material deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Figure 8E is an illustration of the holotype.

TYPE LOCALITY: Babín pool, Žďárské vrchy PLA, Czech Republic (49.5422N 15.8969E, 568 m asl).

ETYMOLOGY: (Latin) reticulata, reticulose; keel pores of this species are so large and closely arranged that it appears as if the keel was formed from a reticulate structure.

DIAGNOSIS: Body scales are oblong-lanceolate (3.3 x 1.6 µm). Keel with an acute keel tip, especially in the apical scales. Base plate pores are medium-sized (diameter 25-51 nm), keel pores are medium to large-sized (diameter 66-100 nm). Foramen is small (diameter 190-319 nm). Numerous ribs extend from the keel to the edge of the scale. Rear scales are much smaller and narrow with somewhat reduced structure.

Synura oculatea Kynčlová, Škaloud & Řezáčová-Škaloudová sp. nov. (Figs 9A-D)

LATIN DIAGNOSIS: Squamae ovatae vel lanceolatae, 3.0-3.9 µm longae et 1.6-1.9 µm latae. Carina squamarum corporearum sine cuspidate aut cum cuspidate parva, carina squamarum anteriorum cum cuspidate tereti, nonnumquam cum denti angusto. Multae costae praesentes. Pori tabellae basalis parvi (diameter 18-22 nm), pori carinae etiam parvi (diameter 47-70 nm). Foramen squamarum corporearum magnopere grandis (diameter 315-557 nm). Squamae posteriores multo parviores, angustae et simpliores.

HOLOTYPE: *Synura oculatea* strain S 15.3, frozen material deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Figure 9A is an illustration of the holotype.

TYPE LOCALITY: Peat bog, Úpské rašeliniště, Krkonoše NP, Czech Republic (50.7362N 15.7091E, 1450 m asl).

ETYMOLOGY: (Latin) *oculea*, goggle-eyed; scale foramens of this species are notably large.

DIAGNOSIS: Body scales are oblong-lanceolate (3.5 x 1.8 µm), keel of the body scales without keel tip or with a very reduced one, keel tips on apical scales are rounded, sometimes with a very thin tip or teeth on top. Foramen of the body scales is remarkably large (diameter 315-557 nm). Both base plate and keel pores are small (diameter 18-22 nm and 47-70 nm, respectively). Numerous ribs extend from the keel to the edge of the scale. Rear scales are much smaller and narrow with somewhat reduced structure.

Synura glabra Korshikov 1929; Arch. Protistenk. 67, p. 285, figs 59-65 (iconotype) emend. Kynčlová, Škaloud & Řezáčová-Škaloudová (Figs 9E-I)

SYNONYMS: *Synura petersenii* Korshikov var. *glabra* Huber-Pestalozzi non. Prov. 1941; Phytoplankton Süsw. 2, p. 144; *Synura petersenii* Korshikov f. *glabra* Kristiansen & Preisig 2007; Süswasserflora von Mitteleuropa, vol 1, part 2, Synurophyceae, p.118.

EPITYPE: *Synura glabra* strain S 14.1, frozen material deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Figure 9E is an illustration of the epitype.

EMENDED DIAGNOSIS: Scales are oval (3 × 2 μm), less silicified. Keel is less developed, usually without keel tip or ending with a small acute keel tip. Ribs are somewhat reduced or often absent. Base plate pores are medium-sized (diameter 29-40 nm), keel pores are medium-sized (diameter 66-100 nm). Foramen is small (diameter 144-322 nm). Rear scales are smaller and oval.

Key to the taxa

1a Scales oval, length to width ratio about 1.5

2a Base plate pores large (diameter 53-77 nm) *macropora*

2b Base plate pores medium-sized (diameter 15-40 nm)

3a Keel and ribs more developed, ratio of base plate and keel area 3.6-5.2 ...*americana*

3b Keel and ribs less developed, ratio of base plate and keel area 5.7-9.8*glabra*

1b Scales oblong-lanceolate, keel well developed, length to width ratio approximately 2

2a Small foramen (diameter 144-322 nm), body and especially apical scales with an acute spine

3a Keel pores small (diameter 45-71 nm) *petersenii*

3b Keel pores medium-sized to large (diameter 74-111 nm) *reticulata*

2b Large foramen, (diameter 315-557 nm), body scales with a very reduced or absent spine, apical scales with rounded spine *oculea*

Discussion

Synura petersenii, as well as most other silica-scaled chrysophytes, is identified based exclusively on the morphology of its silica scales as observed under electron microscopy. Even if the slight morphological differences led several authors to establish new varieties and formae within *S. petersenii*, these are presently regarded as attributes of intraspecific variability, rather than separate species (Kristiansen & Preisig 2007). However, the congruence of ITS rDNA sequence divergence, distribution of (hemi-)CBCs in the ITS2 rDNA secondary structure, geometric morphometric data, and morphological separation of *S. petersenii* clones used in this study points to the presence of several species in *Synura petersenii* sensu lato. Generally, three independent species concepts concur to divide the studied clones into species.

Firstly, according to the phylogenetic species concept, each species is defined by the unique combination of character states in their ITS sequences. In fact, the ITS rDNA region has now become the single most frequently utilized DNA region in taxonomic studies of protists, due to its ability to discriminate accurately between biological species. For example, ITS

sequences were recently used in discovery of genetic variability and hidden diversity within several genera of green algae (Kroken & Taylor 2000, Lewis & Flechtner 2004, Vanormelingen et al. 2007) or diatoms (Behnke et al. 2004, Lundholm et al. 2006, Amato et al. 2007, Vanormelingen et al. 2008). As compared to the above-mentioned reports, the comparable differences in ITS rDNA sequences led authors either to describe separate clades as new species, or at least to consider them as separate species entities. Secondly, the presence of at least one compensatory base change (CBC) or hemi-CBC in the secondary structure of a spacer region ITS2 has been correlated with the occurrence of two different species in the sense of a biological species concept, i.e. the presence of reproductive barriers between the organisms (Coleman 2000). Recently, this hypothesis has been supported in various groups of protists, especially in diatoms (e.g. Coleman & Mai 1997, Behnke et al. 2004, Amato et al. 2007, Casteleyn et al. 2007, Müller et al. 2007). Comparing the secondary structures of ITS2 rRNA molecules, we always found at least 3 (hemi-)CBCs between strains of different species, and no (hemi-)CBC between any pair of strains belonging to the same species, without exception. According to the above-mentioned CBC calculations, all described species should be sexually incompatible, and thus represent distinct biological species. It will be interesting to perform crossing experiments among the strains of the same and different *Synura* species to test Coleman's hypothesis in Synurophyceae, and conclusively confirm the biological nature of the described species. Thirdly, there is an incontrovertible congruence between the morphology of the clones and their position in the ITS rDNA phylogeny. Members of different species are distinguished by both conventional morphological characters and modern geometric morphometric analyses (Table 2; Fig. 6). Thus, the newly delimited *Synura* species are not cryptic, and could be distinguished by a detailed comparison of the discriminative features presented, i.e. using a morphological species concept.

To objectively determine the morphological differences among the species, we have cultivated all clones under the same conditions. However, because we studied only those populations growing in culture, we have no information about morphological variability, which may occur in nature. Indeed, in a natural sample the differences could be less distinctive due to ecomorphic variability. The dependence of scale variability on environmental conditions was studied by Martin-Wagenmann & Gutowski (1995) and Gavrilova et al. (2005). In the latter study, the morphology of silica scales was investigated in different pH conditions. Although the scale dimensions significantly differed in various conditions, their length/width ratio remained the same. Similarly, the base foramen diameter, as well as both base plate pore and keel pore areas were stable under all conditions studied. Considerable variation was observed in the silicification of scales, shape of central ridge, and scale rim size. Sandgren et al. (1996) found out that the basic ornamentation pattern of *Synura*

scales cannot be influenced by manipulating silica availability. Martin-Wagenmann & Gutowski (1995) investigated the changes in scale morphology related to temperature and culture age in three *S. petersenii* clones. Concurring with the findings of Gavrilova et al. (2005), the areas of base foramen, basal plate pores and keel pores were not influenced by various experimental conditions. Moreover, they found significant morphological differences between individual strains allowing them to be distinguished from one another regardless of experimental conditions. By comparing the presented microphotographs of silica scales with our morphological data, we were able to unambiguously assign all three investigated clones to *S. petersenii* (clone I), *S. glabra* (clone II) or *S. macropora* (clone III). All measured morphological data (i.e. scale dimension, length/width ratio, the area of base foramen, basal plate pore and keel pore size) fit well into our species descriptions.

Summarizing, our investigations, as well as data in the literature, demonstrate the stability of the proposed morphological features for species recognition, and thus, the applicability and validity of a morphological species concept. However, due to the fact that our morphological comparisons were of a high number of scales retrieved from clonal culture, species determination based on single scale morphology of a natural sample could be misleading. For example, according to Wagenmann & Gutowski's investigations (1995), some scales of *S. petersenii* growing at 5 °C have an oblong to oval shape and could resemble *S. americana*. Therefore, for the morphological determination of species, we recommend comparing the morphology of several randomly chosen silica scales per population, instead of examining only a single scale.

In the present study, the *Synura* clones investigated were isolated from localities situated only in the Czech Republic. Despite this narrow geographical range, we discovered four new species previously unknown as individual species or as formae within *S. petersenii* s.l. Further, we verified the existence of *S. glabra*, first described by Korshikov (1929), as a separate species. According to scale variability and morphological similarities with *S. petersenii*, this species was considered to be a variety (Huber-Pestalozii 1941) or even a forma (Kristiansen & Preisig 2007). The most formidable attempt to abolish *S. glabra* was presented by Hällfors & Hällfors (1988), who described *S. petersenii* var. *glabra* as a weakly silicified ecomorph of *S. petersenii*, having no taxonomic status. However, our data, both morphological as well molecular is in agreement with the observations of Wagenmann & Gutowski (1995), and strongly supports the specific status of *S. glabra*. In addition to *S. petersenii* s.str. and *S. glabra*, whose scales' description and pictures can be frequently found in the literature, we can now confidently review certain previously published reports on *S. petersenii* single scales and assign them to one to the newly described species. For example, *S. reticulata* was found by Couté & Franceschini (1998; Fig. 79) or Řezáčová & Škaloud

(2005; Fig. 34); reports of silica scales of *S. macropora* were published by Martin-Wagenmann & Gutowski (1995; Figs 17-23) or Kristiansen & Preisig (2007; Fig. 233b). Hence, several authors previously published reports based on the scales of newly described species, however, these were considered as pure intraspecific variability within *S. petersenii* s.l.

Our results underscore the large hidden diversity in the *S. petersenii* species complex. How many species we can expect to exist in nature that, by general morphology of silica scales, resemble *S. petersenii* s.l.? Because our data originated in a limited geographical area, we suppose that many additional species will be found and described on the basis of molecular and morphological investigation of clones isolated from diverse geographical areas. The morphological data retrieved from a number of floristic studies confirm the highly probable existence of a large number of hidden species that differ slightly in the morphology of their silica scales. Some of these organisms were described as different formae (Asmund 1968, Vigna 1979, Cronberg & Kristiansen 1980, Siver 1987, 1988, Kristiansen et al. 1997), and some morphologically unique scales were simply reported, without any taxonomic conclusions drawn (Kristiansen 1992, Řezáčová & Škaloud 2005). According to our results, only small morphological differences in silica scale structure can be found among these particular species. Thus, we can assume that all above-mentioned formae probably represent separate species, and in fact, have the same taxonomic rank, such as: *S. petersenii*, *S. glabra*, *S. macracantha* or *S. australiensis*. However, we do not consider it appropriate at present to confer upon them the rank of species on the basis of single, i.e. morphological, species concept. Only the combination of morphological and molecular data obtained from the clonal cultures could accurately discriminate between morphological variability and the existence of unique species. For example, we found the silica scales that morphologically correspond to *S. petersenii* f. *kufferathii* (Petersen & Hansen 1958) in several clonal cultures of *S. petersenii* s.str. Measurement of scale dimensions and pore diameters of *S. petersenii* f. *kufferathii* iconotypus confirmed that this forma represent only a morphological variability within *S. petersenii*.

Conclusions and prospects

Using a combination of morphological and molecular data, *Synura petersenii* clones could be divided into six groups, representing separate species – *S. petersenii* s.str., *S. glabra*, *S. americana* spec. nov., *S. macropora* spec. nov., *S. reticulata* spec. nov. and *S. oculea* spec. nov. In contrast to using the single species concept, multiple congruent lines of evidence provide stronger support for lineage separation, and will lead to the establishment of more

robust species boundaries (de Queiroz 2007). In this paper, we present the taxonomic conclusions based on the absolute congruence of three independent species concepts: morphological, phylogenetic, and CBC. According to our investigations, divergence in ITS rDNA generally correlate with the occurrence of at least three hemi(-CBCs) in the ITS2 secondary structure, and with slight, but clearly defined morphological differences in the silica scales. We suggest that future studies on *S. petersenii* s.l. clones will discover more hidden species and reveal the actual species diversity within the *S. petersenii* complex. The delimitation and morphological differentiation of these species will highly increase the value of *S. petersenii* as an ecological indicator in biomonitoring and paleolimnological studies. Further investigation of dispersal potencies could clarify obvious differences in biogeography of newly established species (e.g. *S. americana* restricted to the North America). Detailed investigation of niche preferences or seasonal fluctuation could explain the sympatric occurrence of particular species (e.g., *S. petersenii* and *S. reticulata* were isolated from the same sample taken from a Babín pool). Finally, further studies of molecular diversity in Synurophyceae will be able to determine whether similar hidden diversity is also present in other *Synura* species, or if it is restricted to *S. petersenii*.

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Table 1. Strains included in this study with their source localities, and GenBank accession numbers of the ITS rDNA sequence data.

Taxon	Strain identifier	Collection informaton	Accession No.
<i>S. macropora</i>	S 5.1	Aluvial pool, Modřany, Prague, Czech Republic	FM178494
<i>S. macropora</i>	S 5.2	Aluvial pool, Modřany, Prague, Czech Republic	FM178495
<i>S. macropora</i>	S 5.3	Aluvial pool, Modřany, Prague, Czech Republic	FM178496
<i>S. macropora</i>	S 14.2	Peatbog, Swamp NR, North Bohemia, Czech Republic	FM178497
<i>S. petersenii</i>	S 1.1	Zlatá stoka canal, Třeboň, Czech Republic	FM178498
<i>S. petersenii</i>	S 1.2	Zlatá stoka canal, Třeboň, Czech Republic	FM178499
<i>S. petersenii</i>	S 1.3	Zlatá stoka canal, Třeboň, Czech Republic	FM178500
<i>S. petersenii</i>	S 6.4	Aluvial pool, Horní Lužnice NR, South Bohemia, Czech Republic	FM178501
<i>S. petersenii</i>	S 6.5	Aluvial pool, Horní Lužnice NR, South Bohemia, Czech Republic	FM178502
<i>S. petersenii</i>	S 4.19	Kladský pond, West Bohemia, Czech Republic	FM178503
<i>S. petersenii</i>	S 7.7	Babín pool, Žďárské vrchy PLA, Czech Republic	FM178504
<i>S. petersenii</i>	S 16.2	Xerr pond, South Bohemia, Czech Republic	FM178505
<i>S. reticulata</i>	S 7.10	Babín pool, Žďárské vrchy PLA, Czech Republic	FM178506
<i>S. reticulata</i>	S 10.2	Huťský pond, Novohradské hory, South Bohemia, Czech Republic	FM178507
<i>S. oculea</i>	S 15.3	Peatbog, Úpské rašeliniště, Krkonoše NP, Czech Republic	FM178508
<i>S. oculea</i>	S 15.5	Peatbog, Úpské rašeliniště, Krkonoše NP, Czech Republic	FM178509
<i>S. oculea</i>	S 15.9	Peatbog, Úpské rašeliniště, Krkonoše NP, Czech Republic	FM178510
<i>S. glabra</i>	S 8.1	Kyjský pond, Prague, Czech Republic	FM178511
<i>S. glabra</i>	S 9.1	Confluence of the Morava and Dyje rivers, South Moravia, Czech Republic	FM178512
<i>S. glabra</i>	S 9.2	Confluence of the Morava and Dyje rivers, South Moravia, Czech Republic	FM178513
<i>S. glabra</i>	S 14.1	Peatbog, Swamp NR, North Bohemia, Czech Republic	FM178514

Table 2. Summary of characteristic combinations of morphological features for each of the 6 clades.

Clade number	Length/ width	Fora- men diame- ter (nm)	Average base plate pore diame-ter (nm)	Average keel pore diameter (nm)	Scale/ keel area ratio	Keel tip
clade 1 (<i>S. americana</i>)	< 1.8	< 180	13 - 25	36 - 44	< 6	acute
clade 2 (<i>S. macropora</i>)	< 1.8	< 180	> 25	> 44	< 6	acute
clade 3 (<i>S. petersenii</i>)	> 1.8	< 180	< 13	< 36	< 6	acute
clade 4 (<i>S. reticulata</i>)	> 1.8	< 180	13 - 25	40 - 56	< 6	acute
clade 5 (<i>S. oculatea</i>)	> 1.8	> 180	< 13	< 36	< 6	rounded
clade 6 (<i>S. glabra</i>)	< 1.8	< 180	13 - 25	36 - 44	> 6	acute

Fig 1. Position of landmarks (circles) and semilandmarks (squares).

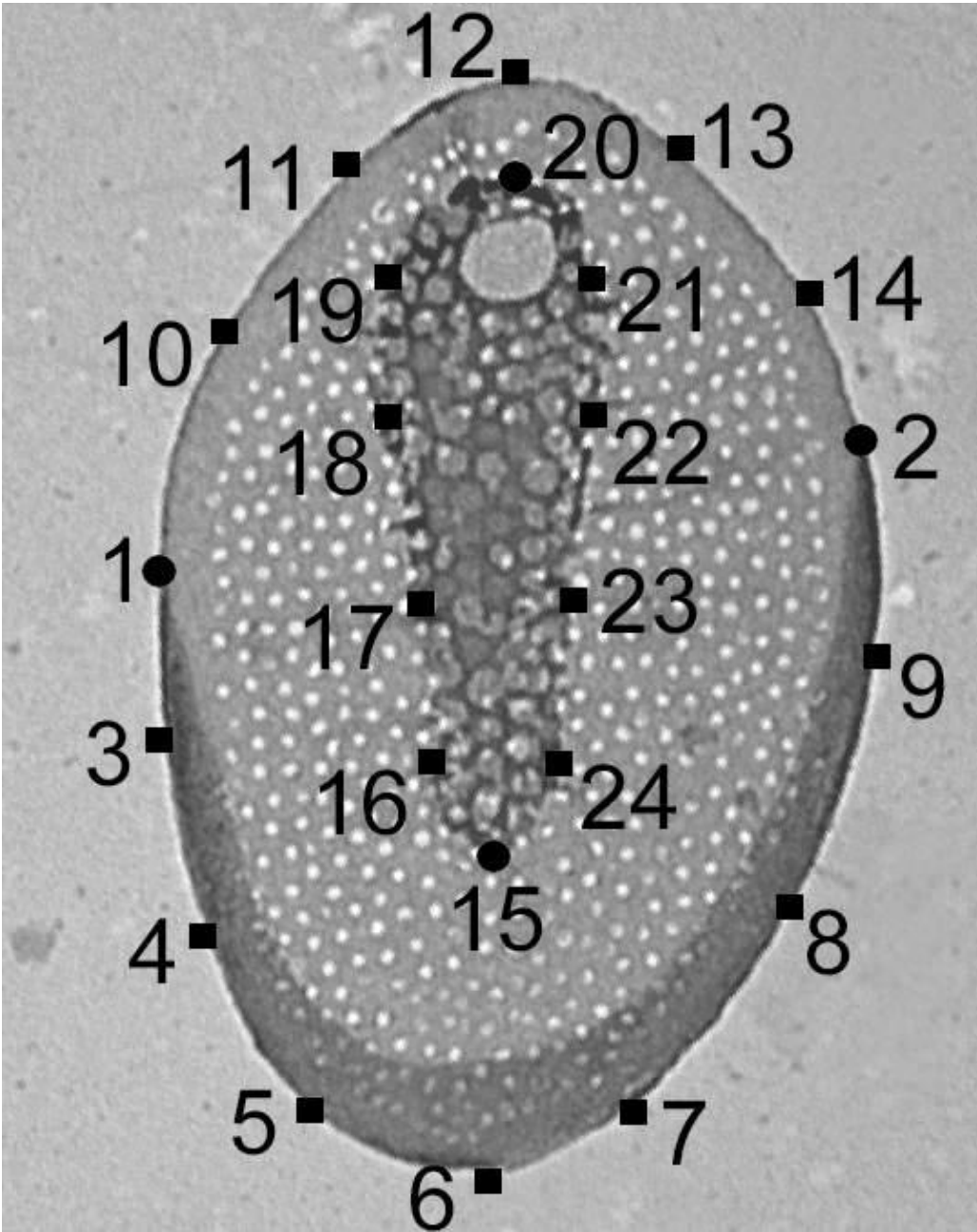


Fig 2. Maximum likelihood tree based on ITS sequences. Values at the nodes indicate statistical support estimated by three methods - ML bootstrap (top left), MP bootstrap (top right) and MrBayes posterior node probability (lower). ITS sequences determined in this study are given in bold face. Scale bar – substitutions per site.

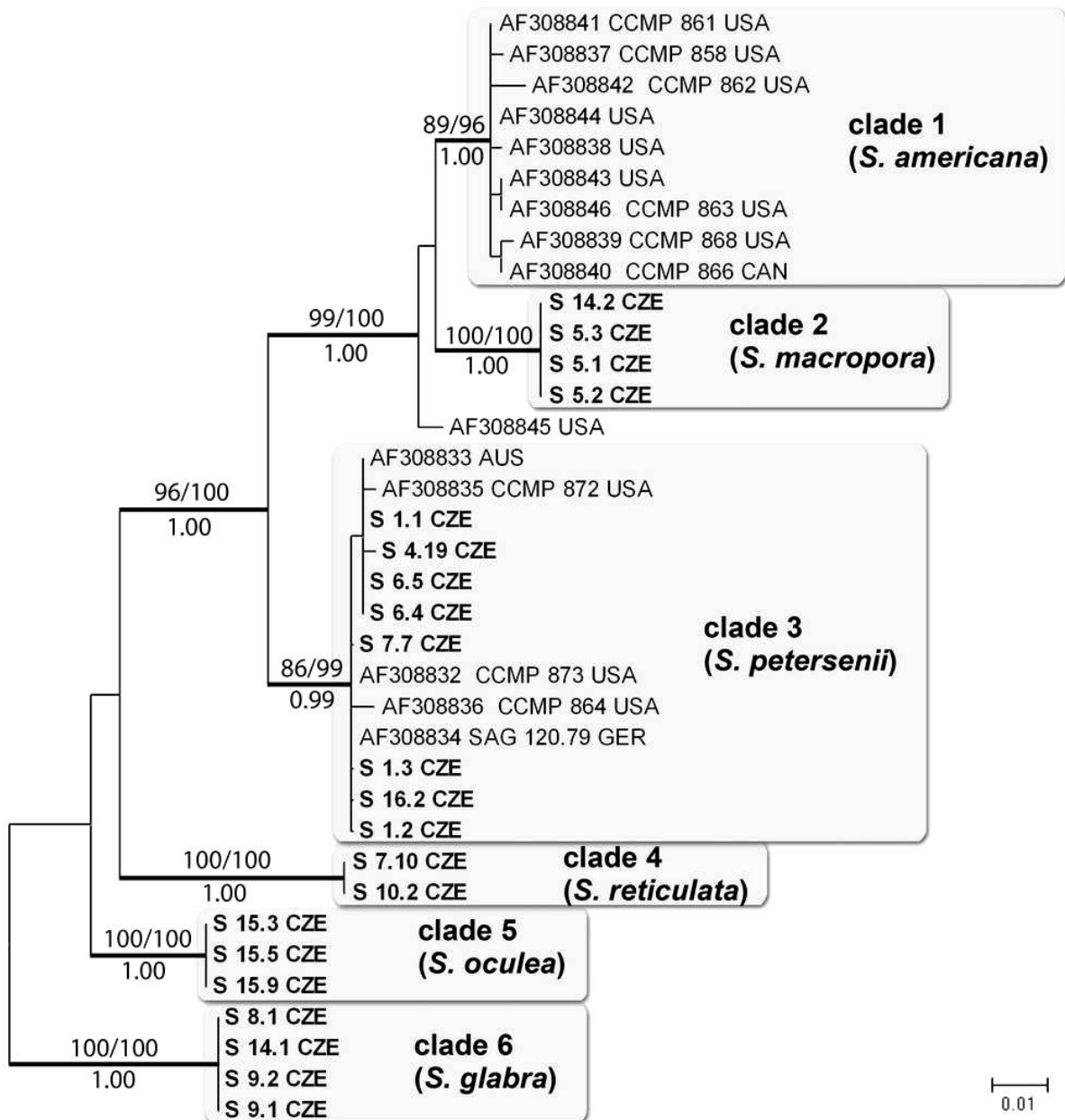


Fig. 3. A. Predicted secondary structures of the ITS2 transcripts of *Synura petersenii* (strain S 1.3). Base changes between the different *S. petersenii* s.l. genotypes are indicated: the base pair marked in a dark-grey box indicates compensatory base changes (CBCs); base pairs marked in grey boxes indicate hemi-CBCs; single base changes are marked in circles; changes of the helix parts are indicated in large boxes. Affiliation of (hemi-)CBCs to particular clades is marked as white numbers in black spots. **B.** Numbers of (hemi-)CBCs differing each clade pair.

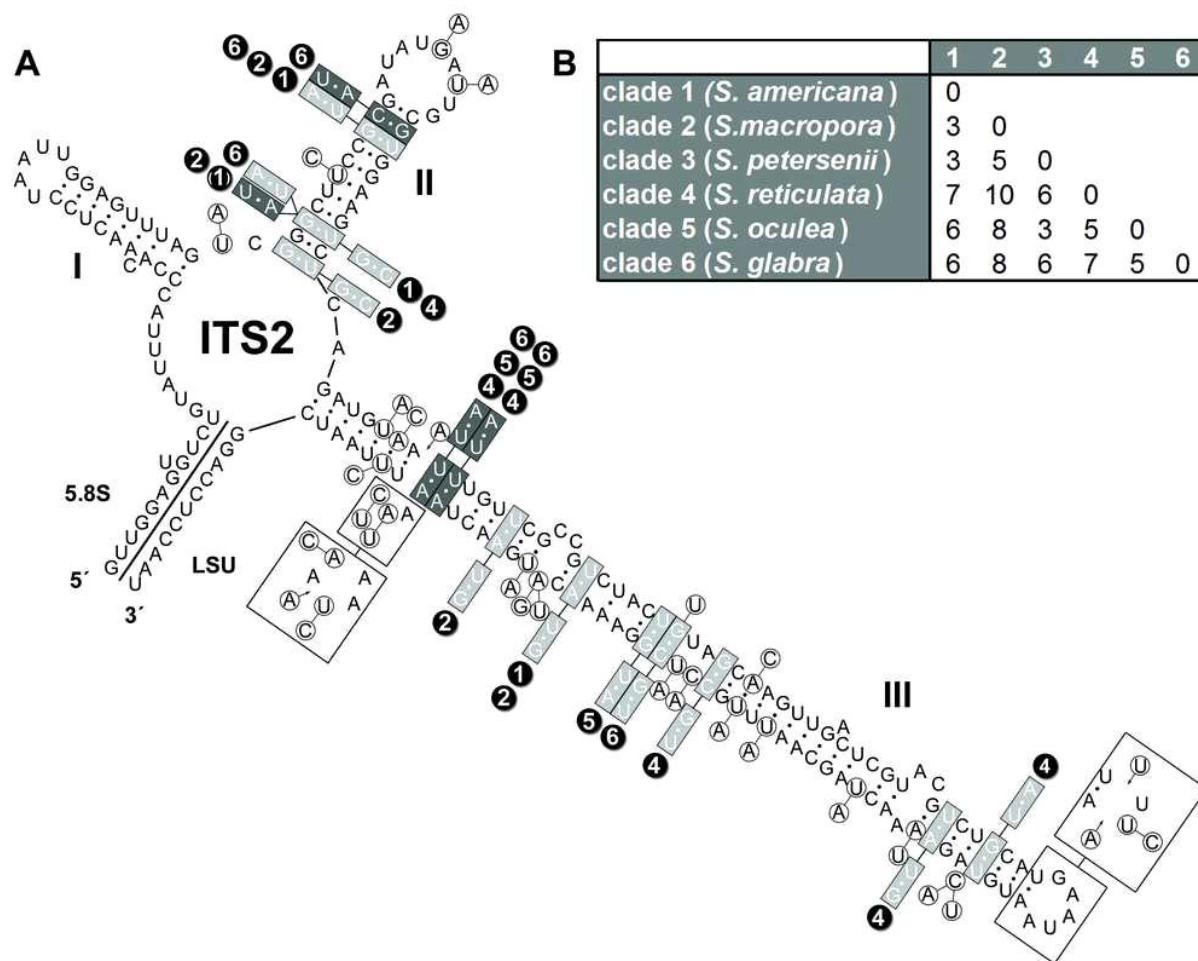


Fig. 4. Characteristic features of *S. petersenii* s.l. scale.

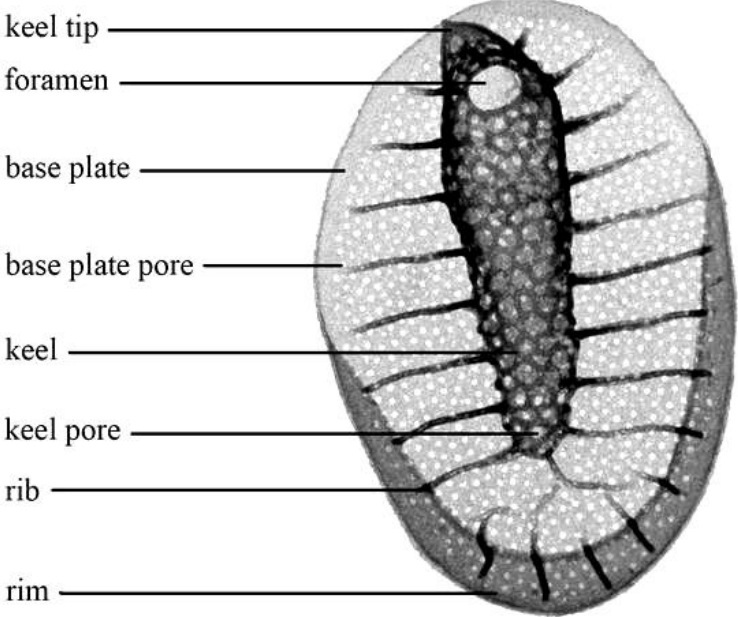


Fig. 5. Box plots of morphometric data of *Synura petersenii* s.l. scales. The morphometric data comprise length to width ratio of the scales, foramen area, average base plate pore area, average keel pore area and base plate to keel area ratio. The grey areas indicate 25% and 75% percentiles, the line within the fields is the median. The error bars indicate the 10% and 90% percentiles.

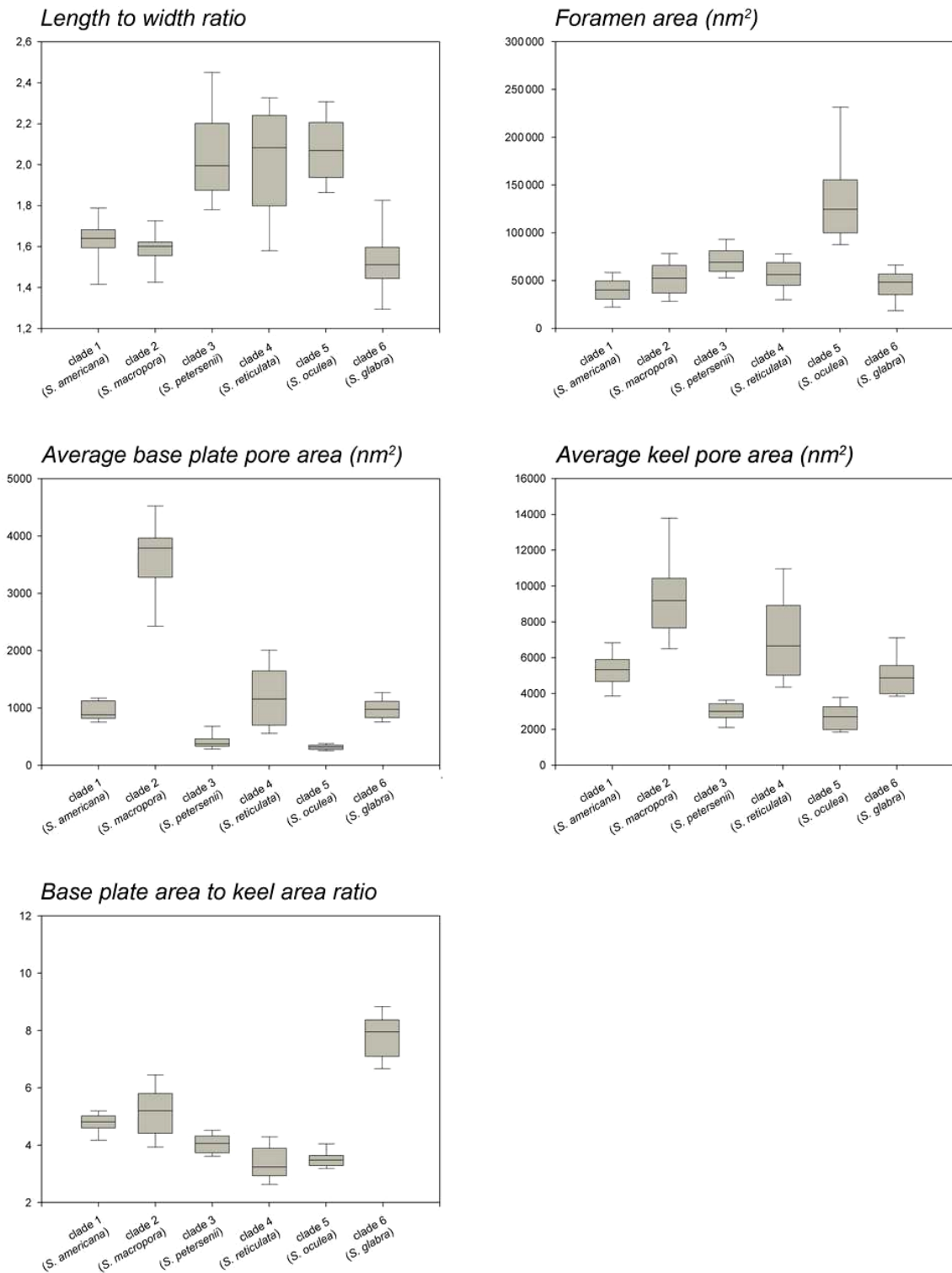


Fig. 6. The scatter plot of Manova/CVA analysis of 353 *S. petersenii* scales. Individual clades are grouped along the first CV axis and mean landmark configurations of three distinct groups are depicted.

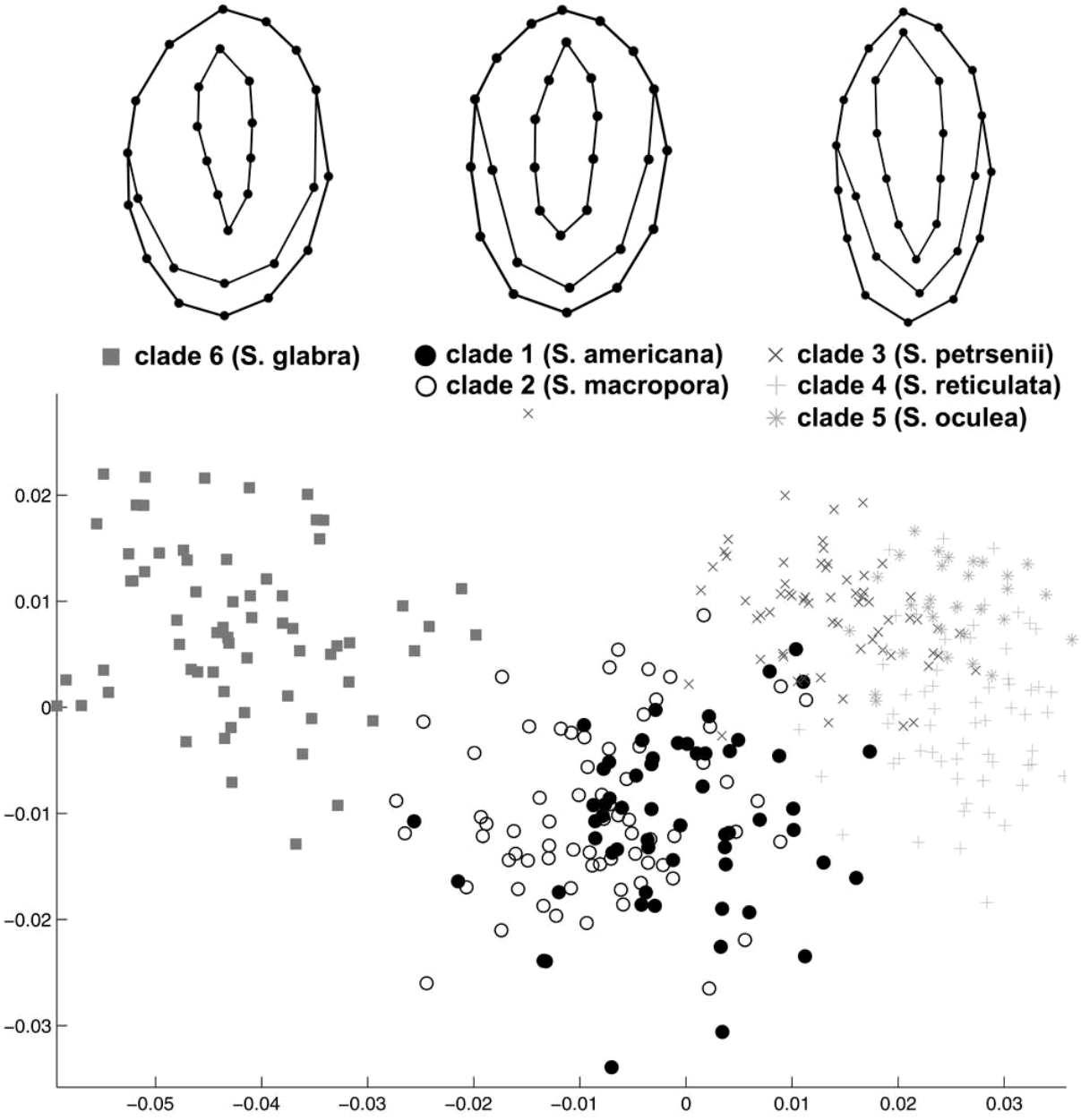


Fig. 7. TEM of the scales of clades 1 and 2. (A - D) clade 1 (*S. americana*). (E - I) clade 2 (*S. macropora*).

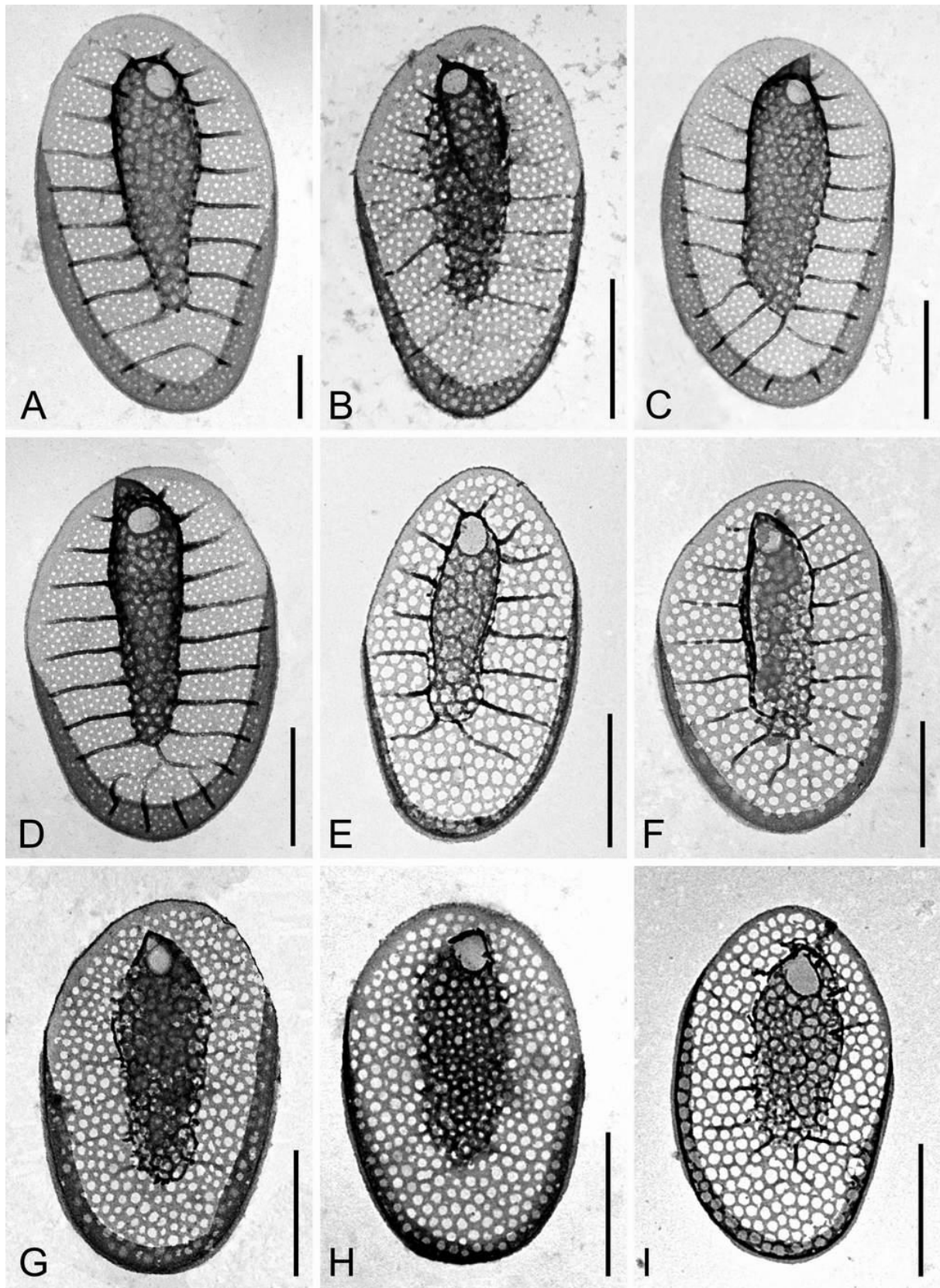


Fig. 8. TEM of the scales of clades 3 and 4. (A - D) clade 3 (*S. petersenii*). (E - I) clade 4 (*S. reticulata*).

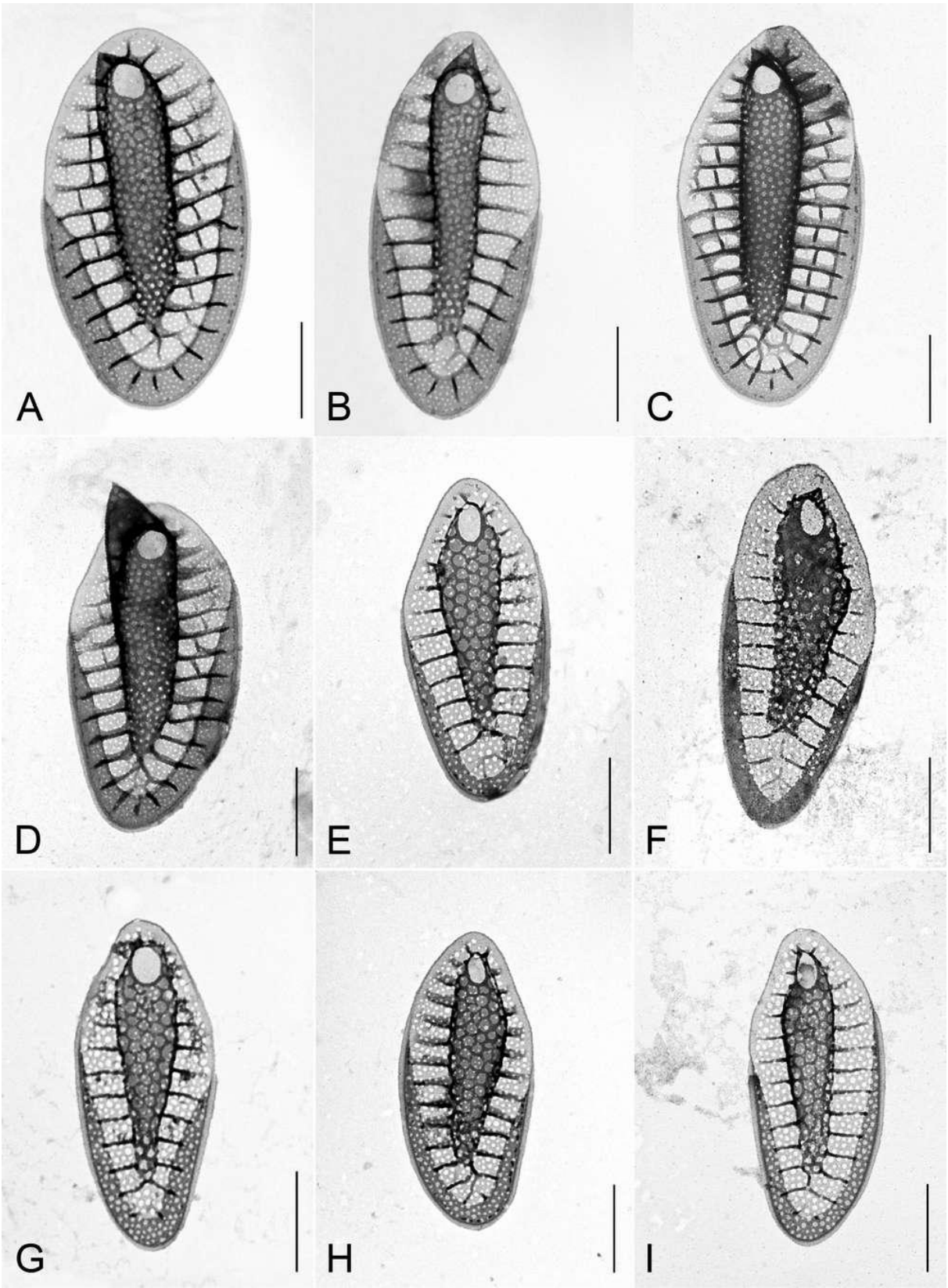
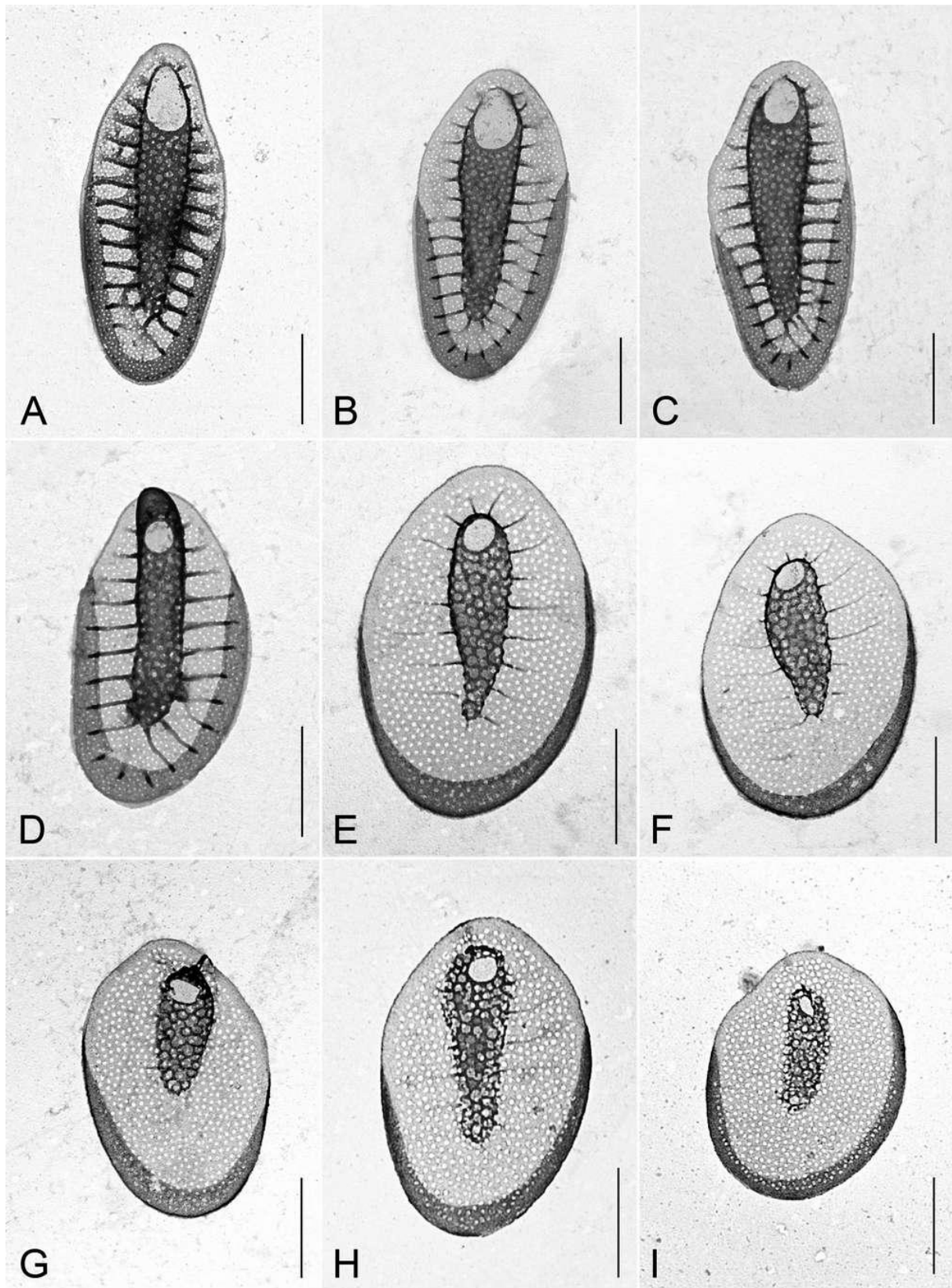


Fig. 9. TEM of the scales of clades 5 and 6. (A - D) clade 5 (*S. oculatea*). (E - I) clade 6 (*S. glabra*).



5 CONCLUSIONS

Results of the *Papers 1* and *2* summarizing *Mallomonas* distribution rather support the “moderate endemicity model” of Foissner. The most of *Mallomonas* species were cosmopolitan or widely distributed, but we also found several species with geographically restricted occurrence contradicting the “ubiquity model”. Besides two Asian endemics there are three *Mallomonas* species (*M. multiunca*, *M. oviformis*, and *M. punctifera* var. *punctifera*) not conforming to ubiquity model due to their highly nonrandom distribution in subtropic to subarctic zones of the Northern Hemisphere. Several others examples of *Mallomonas* species with restricted distribution are given in literature.

Mallomonas kalinae was isolated and described from a peaty pool in North Bohemia (*Paper 3*). This strain together with the *Synura curtispina* strain was used for investigation of scale and bristle plasticity under different temperatures (*Paper 4*). Changes in morphology of silicate structures in relation to temperature were significant, although the large part of variability was caused by different position on the cell. Bristles become significantly shorter with increasing temperature. An inverse relationship between size of scales and temperature corresponding to the temperature-size rule was found. The main scale characters were stable under all temperatures.

A combination of morphological and molecular approaches for clarification of the *Synura petersenii* species concept was used in *Paper 5*. Three investigated characteristics (ITS rDNA phylogeny, compensatory base changes occurring in the secondary structure transcript of ITS rRNA (CBCs), and scale morphology) shown congruent results, and the hidden diversity in the *S. petersenii* species concept was demonstrated. It is now obvious, that there are genetic pattern behind the many forms and varieties previously described in *S. petersenii*. Although some scale variability may be caused by ecological parameters, the main scale characters are stable. *S. petersenii* clones were divided into six groups, representing separate species – *S. petersenii* s.str., *S. glabra*, *S. americana* spec. nov., *S. macropora* spec. nov., *S. reticulata* spec. nov. and *S. oculatea* spec. nov. Many other described forms in *S. petersenii* distributed all over the world will probably represent separate species, but further gene sequences and morphological studies are necessary for understanding their status.

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Publications

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