

## The geometric morphometric study of Central European species of the genus *Micrasterias* (*Zygnematophyceae*, *Viridiplantae*)

Geometricko-morfometrická studie středoevropských druhů rodu *Micrasterias* (*Zygnematophyceae*, *Viridiplantae*)

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The relationships of 14 Central European species of the genus *Micrasterias* were analysed using landmark-based geometric morphometrics. The analysis of relative warps was used to depict the principal components of the variation in shape and cluster analysis to reveal the groupings of individual species within the genus. All the analysed cells were correctly placed in their appropriate species clusters on the basis of geometric morphometric data. The width of the polar lobe associated with depth of the incisions between lateral lobules is the dominant morphological trend in the data investigated.

**Key words:** *Micrasterias*, geometric morphometrics, relative warps analysis, cluster analysis, *Desmidiiales*, *Streptophyta*

### Introduction

The members of the genus *Micrasterias* Ag. ex Ralfs frequently occur in temperate wetlands world-wide. Because of their conspicuous cells, the members of the genus have been reported in floristic studies from Central Europe since the middle of the 19th century (Kützing 1849, Nägeli 1849) up till the present (Růžička 1981, Lenzenweger 1996). Their complex morphology made *Micrasterias* species suitable model organisms for investigating cytoplasmatic mechanisms in plant morphogenesis (Meindl 1993). The methods of traditional morphometrics, based on linear measurements of distances and angles, have been used previously for the taxonomic evaluation of individual *Micrasterias* taxa (Sormus & Bicudo 1974, Vyverman & Viane 1995, Gil-Gil & Bicudo 2000, Bicudo & Gil-Gil 2003). In this paper, the shape characteristics of 14 *Micrasterias* species found in the Czech Republic and Austria, out of 18 species reported from these countries in the literature (Lhotský & Rosa 1955, Růžička 1981, Lenzenweger 1996, Poulíčková et al. 2004), were used in a landmark-based geometric morphometric study of infrageneric morphological variation. In contrast to traditional morphometric methods, geometric morphometrics evaluate the dynamics of the shape as a whole. The higher statistical power of geometric morphometric methods, compared to traditional measurements, for discriminating biological entities has been demonstrated many times (e.g. Rohlf 2000, Jensen et al. 2002, Monteiro et al. 2002, Beszteri et al. 2005).

The monographs of Růžička (1981) and Lenzenweger (1996) were used for species identification, using traditional criteria. Then, the *Micrasterias* was used as a model group for evaluating the applicability of landmark-based geometric morphometrics for species delimitation.

tation and shape analysis in desmids in general. Secondly, the infrageneric interrelationships of the individual species based on geometric morphometric data were characterized.

## Material and methods

The following species were investigated:

*Micrasterias americana* (Ehrenb.) Ralfs var. *americana* – coll. by J. Šťastný from a peat bog pool on the Nature reserve Borkovická Blata, Czech Republic (transition bog), altitude 429 m a.s.l., pH = 6.0, conductivity = 150  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias apiculata* (Ehrenb.) Menegh. ex Ralfs var. *apiculata* – coll. by J. Šťastný from a peat bog pool on the National Nature Reserve Břehyně – Pecopala, Czech Republic (transition bog), altitude 273 m, pH = 6.4, conductivity = 169  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias brachyptera* P. Lundell var. *brachyptera* – coll. by J. Šťastný from a peat bog pool on the National Nature Reserve Břehyně – Pecopala, Czech Republic (transition bog), altitude 273 m, pH = 5.7, conductivity = 151  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias crux-melitensis* (Ehrenb.) Hassall. ex Ralfs var. *crux-melitensis* – coll. by J. Neustupa from a peat bog pool on the Nature reserve Borkovická Blata, Czech Republic (transition bog), altitude 429 m, pH = 6.2, conductivity = 119  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias decemdentata* (Nägeli) Archer var. *decemdentata* – coll. by R. Lenzenweger from Spechtensee (Steiermark, Austria), altitude 1050 m.

*Micrasterias denticulata* Bréb. ex Ralfs var. *angulosa* (Hantzsch.) West et G.S. West – coll. by J. Šťastný from a peat bog pool on the Nature reserve Borkovická Blata, Czech Republic (transition bog), altitude 429 m, pH = 6.0, conductivity = 150  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias fimbriata* Ralfs var. *fimbriata* – coll. by J. Šťastný from a peat bog pool on the Nature Reserve Hliníř, Czech Republic (transition bog), altitude 422 m, pH = 5.8, conductivity = 106  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias furcata* Ralfs var. *furcata* – coll. by R. Lenzenweger from Schwemm bei Walchsee (Tirol, Austria), altitude 664 m.

*Micrasterias jeneri* Ralfs var. *jeneri* – coll. by J. Šťastný from a peat bog pool on the National Nature Monument Swamp, Czech Republic (transition bog), altitude 275 m, pH = 4.1, conductivity = 64  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias papillifera* Bréb. var. *papillifera* – coll. by J. Šťastný from a peat bog pool of Nature reserve Borkovická Blata, Czech Republic (transition bog), altitude 429 m, pH = 6.2, conductivity = 147  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias radiosa* Ralfs var. *radiosa* – coll. by R. Lenzenweger from Schwemm bei Walchsee (Tirol, Austria), altitude 664 m.

*Micrasterias rotata* var. *rotata* – coll. by J. Neustupa from a peat bog pool on the Nature reserve Borkovická Blata, Czech Republic (transition bog), altitude 429 m, pH = 5.6, conductivity = 69  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias thomasi* Archer var. *notata* (Nordst.) Grönblad – coll. by J. Šťastný from a peat bog pool on the Nature reserve Borkovická Blata, Czech Republic (transition bog), altitude 429 m, pH = 6.0, conductivity = 150  $\mu\text{S}\cdot\text{cm}^{-1}$ .

*Micrasterias truncata* (Corda) Bréb. var. *truncata* – coll. by J. Šťastný from a pool on the Pískovny Cep, Czech Republic (oligomesotrophic pools), altitude 450 m, pH = 5.8, conductivity = 33  $\mu\text{S}\cdot\text{cm}^{-1}$ .

The microphotographs were taken on natural populations using an Olympus BX51 light microscope and Olympus Z5060 digital microphotographic equipment. For each species, 15 adult semicells were analysed, with a total of 210 objects included in the geometric morphometric analysis. Seventeen landmarks were defined on each semicell (Fig. 1), with 16 landmarks bilaterally symmetrically positioned and one positioned on the axis of bilateral symmetry. The positions of the landmarks were as follows: 1, 17 – the isthmus margins; 2, 16 – the lower extremities of the lower lateral lobules; 3, 15 – the upper extremities of the lower lateral lobules; 4, 14 – the bases of the lateral incisions; 5, 13 – the lower extremities of the upper lateral lobules; 6, 12 – the upper extremities of the upper lateral lobules; 7, 11 – the bases of incisions between the polar and lateral lobes; 8, 10 – the lateral margins of the polar lobes; 9 – the central incision of the polar lobe.

The landmarks were digitised in TpsDig ver. 1.40. (Rohlf 2004a); the individual objects were superimposed using the generalized Procrustes analysis that standardizes the size of the objects and optimizes their rotation and translation so that the distances between corresponding landmarks are minimized (Bookstein 1991, Dryden & Mardia 1998, Zelditch et al. 2004) in TpsRelw, ver. 1.40 (Rohlf 2004b). The TpsSmall ver. 1.19. software (Rohlf 1998a) was used to assess the correlation between Procrustes and the Kendall tangent space distances to ensure that the amount of shape variation in a data set is small enough to allow statistical analyses to be performed in the linear tangent space, approximating the Kendall shape space, which is non-linear (see e.g. Rohlf 1998b for details). *Micrasterias* cells are bilaterally symmetrical and because the anterior and posterior sides of a cell do not differ, it is impossible to distinguish their respective left and right sides. Therefore, the two sides were symmetrized (Klingenberg et al. 2002). This involved reflecting the cells (by multiplication of x-coordinates of all landmarks by  $-1$ ), the re-labelling of the paired landmarks and averaging the original and mirrored configurations in the Procrustes superimposition. The averages of original and mirrored/relabelled cells are ideal symmetric shapes, where each half, together with landmarks lying on median axis, includes all the information on the shape of a symmetric object. Thus, further analysis of these symmetrized configurations omitted any asymmetry. Then, using TpsRelw, the relative warps analysis (RWA) with  $\alpha = 0$  (= PCA of Procrustes residuals) were performed (Rohlf 1993). The extreme positions of a shape space depicted by first few axes of RWA were illustrated as thin-plate splines (transformation grids) from average (consensus) landmark configuration. The scores of objects on the first 10 axes were used for the cluster analysis in PAST ver. 1.33 (Harper et al. 2001) and Statistica 5.1 package to demonstrate the interrelationships among the objects analysed. The shape features typical for individual species were illustrated using thin-plate splines from consensus landmark configuration to the average configuration of each species in TpsSpline ver. 1.20 (Rohlf 2004c). The size was evaluated using centroid size, a standard size measure in morphometric studies (Dryden & Mardia 1998, Zelditch et al. 2004), that is defined as the square root of the sum of squared distances from the landmarks to their centroid.

## Results

The positions of individual landmarks in mutually corresponding positions on individual semicells of the species are illustrated in Fig. 1. The scatter of all 210 objects, after Procrustes superimposition, can be seen in Fig. 2. The variation in these specimens in shape

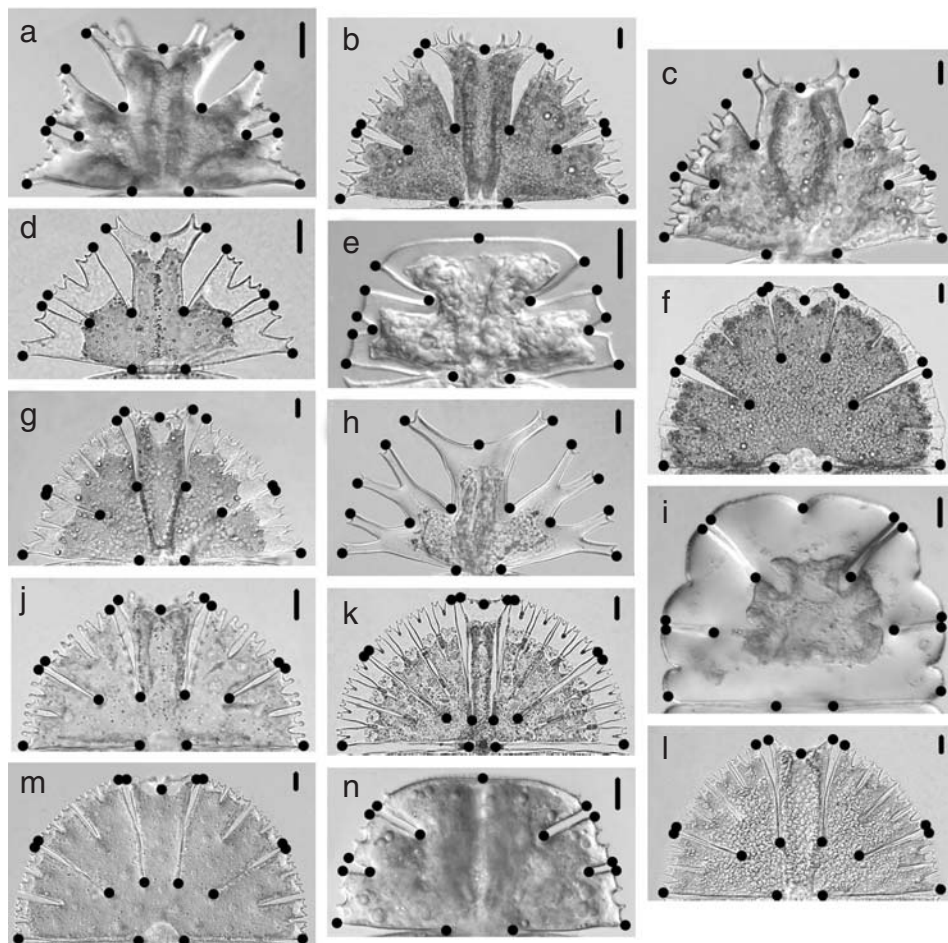


Fig. 1. – The semicells of the species investigated with depicted landmarks. a – *Micrasterias americana* var. *americana*, b – *M. apiculata* var. *apiculata*, c – *M. brachyptera* var. *brachyptera*, d – *M. crux-melitensis* var. *crux-melitensis*, e – *M. decemdentata* var. *decemdentata*, f – *M. denticulata* var. *angulosa*, g – *M. fimbriata* var. *fimbriata*, h – *M. furcata* var. *furcata*, i – *M. jeneri* var. *jeneri*, j – *M. papillifera* var. *papillifera*, k – *M. radiosa* var. *radiosa*, l – *M. rotata* var. *rotata*, m – *M. thomasiana* var. *notata*, n – *Micrasterias truncata* var. *truncata*. Bar = 10  $\mu$ m.

space was sufficiently small to allow the use of the tangent plane approximation and interpretation of the results (correlation revealed by TpsSmall:  $r = 0.99$ ). The RWA ordination diagram and respective shape changes associated with individual axes are illustrated in Fig. 3. The first relative warp (= first principal component) accounted for most of the morphological variation in the data set (75.4% of the total variability). It described the change in shape connected with the widening of the polar lobe together with the tendency of more shallow incisions in cells with wide polar lobes. The second relative warp, describing the second most important morphological trend in the data set, not correlated with the variation revealed by the first axis, accounted for 12.4% of the total variability. It described the morphological trend associated with the incisions between polar and lateral lobes. The



Fig. 2. – The scatter of Procrustes superimposed landmark configurations.

cells with shallow incisions had negative scores on second RW and there was a conspicuous deepening of these incisions with positive RW2 values. The RW1  $\times$  RW2 ordination diagram demonstrated obvious clustering of the shape space into groups representing individual morphospecies of *Micrasterias* (Fig. 3).

The general clustering pattern of all the variation contained in the shape data (i.e. with size effect removed), was illustrated by a single tree of UPGMA analysis. The scores of individual objects on the first 10 relative warps, which accounted for 99.4% of the total morphological variation, were used (Fig. 4). All of the 14 species clustered into well delimited groups with no object misclassified. The data set divided into two main groups corresponding primarily to the width of the polar lobe (Fig. 5). In the group with a broad polar lobe, *Micrasterias truncata* and *M. decemdentata* are clustered tightly together as a sister group to the cluster of six species. The latter is composed of three subclusters representing *Micrasterias jenneri*, the group of *Micrasterias brachyptera*, *M. apiculata* and *M. cruxmelitensis* and the group of *Micrasterias furcata* and *M. americana*. The group of six species with a narrow polar lobe divided into two clusters. One group consisting of *Micrasterias radiosa* and *M. thomasiana* typically has a very deep incision between the polar and lateral lobes. The second consists of four species – *Micrasterias denticulata* and a cluster of three species with unequal lateral lobules (*Micrasterias rotata*, *M. fimbriata* and *M. papillifera*).

The centroid size differed considerably between species with that of *M. decemdentata* approximately 3.5 times smaller than that of *M. denticulata* and *M. rotata*, the largest species in the data set (Fig. 6). The joint effect of size and shape on the clustering of individual populations was evaluated by a UPGMA of both the shape and centroid size data. The UPGMA demonstrated that 5 specimens were misclassified. Otherwise, the topology of the resulting tree was similar to that based only on shape. The clustering of *Micrasterias thomasiana* and

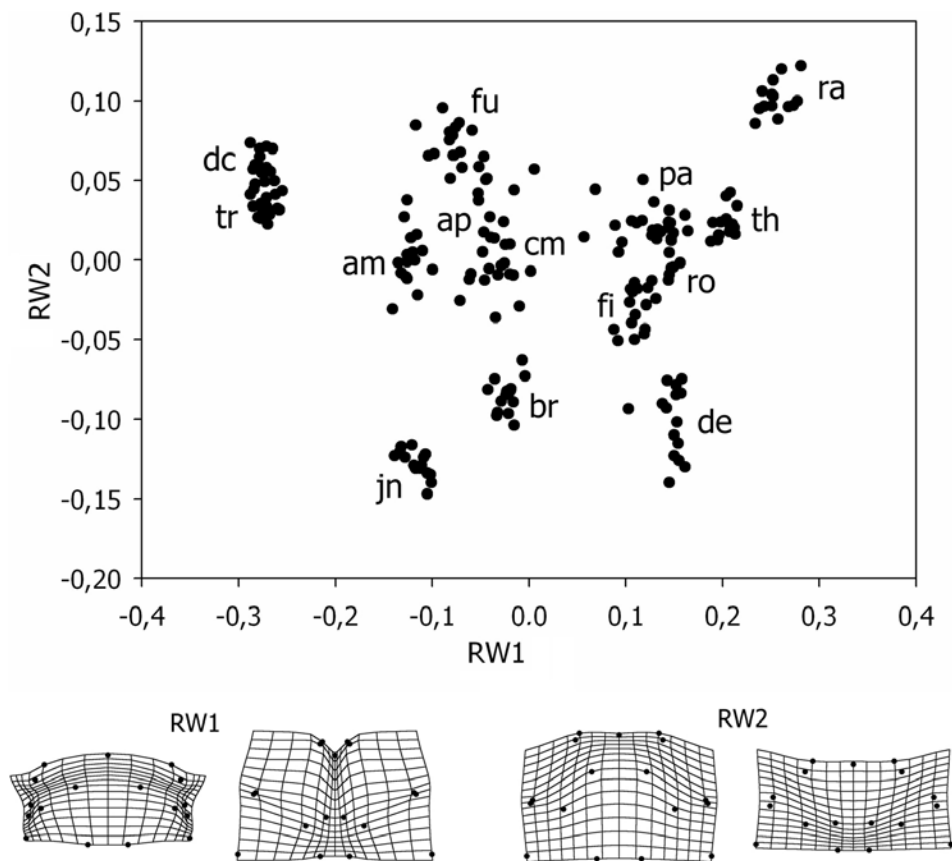


Fig. 3. – The RW1  $\times$  RW2 ordination diagram that accounted for 87.8% of the total variation. The changes in shape at the negative and positive extremes of the RW1 and RW2 axes are illustrated as thin-plate splines. The positions of individual species on the ordination diagram are indicated by following abbreviations: am – *Micrasterias americana*; ap – *M. apiculata*; br – *M. brachyptera*; cm – *M. crux-melitensis*; dc – *M. decemdentata*; de – *M. denticulata*; fi – *M. fimbriata*; fu – *M. furcata*; jn – *M. jenneri*; pa – *M. papillifera*; ra – *M. radiosa*; ro – *M. rotata*; th – *M. thomasiana*; tr – *M. truncata*.

*M. radiosa*, and of *M. rotata*, *M. papillifera* and *M. fimbriata*, was similar to that revealed by the UPGMA of shape. In addition, *M. decemdentata* and *M. truncata* were in a single cluster and *M. crux-melitensis*, *M. furcata*, *M. americana* and *M. jenneri* were grouped together. However, the analysis of the combined shape and size data resulted in *Micrasterias apiculata* and *M. brachyptera* clustering with *M. rotata* / *papillifera* / *fimbriata* rather than with *M. crux-melitensis* / *furcata* / *americana*, as was the case when the effect of size was controlled for.

## Discussion

Geometric morphometrics have been used in algal research to study various taxonomic (Pappas et al. 2001, Neustupa 2004, 2005, Beszteri et al. 2005, Verbruggen et al. 2005a, b)

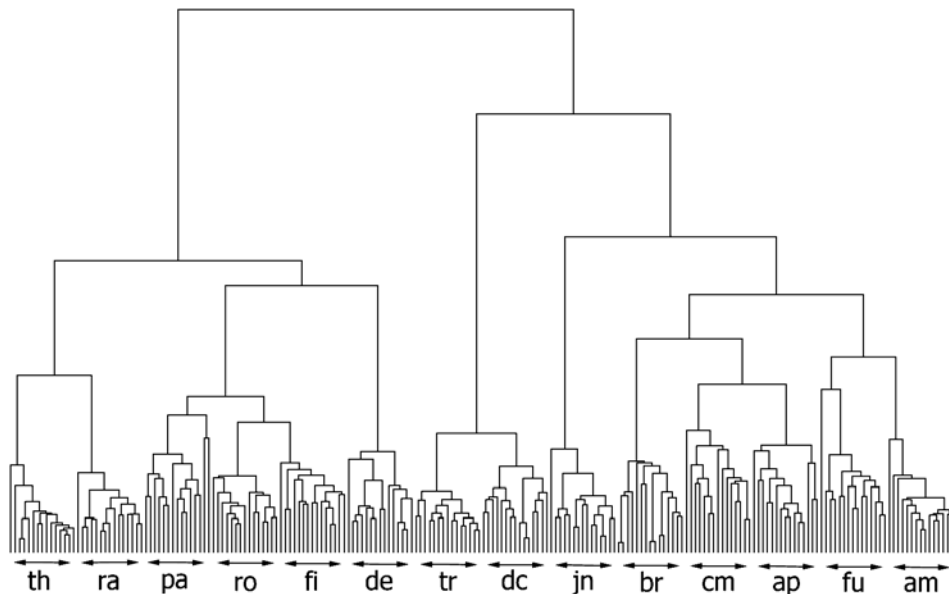


Fig. 4. – The UPGMA dendrogram of the shape data for the 210 objects analysed. The position of individual species is indicated by abbreviations (see Fig. 3).

and ecological (Neustupa & Hodač 2005, Řezáčová & Škaloud 2005) problems. In this study, populations of particular *Micrasterias* species, identified using traditional criteria, were also correctly separated by a landmark-based geometric morphometric analysis. The relative warps analysis and cluster analysis of shape data revealed the single most important trend dividing the species into two groups, the width of the polar lobe and depth of the incisions between individual lobes and lobules. This pattern accords with some classical attempts at an infrageneric classification of the genus *Micrasterias* using traditional morphological criteria. Ralfs (1848) delimited several sections within the genus. *Micrasterias radiosa*, *M. rotata*, *M. papillifera* and *M. fimbriata* form a cluster in the analysis reported here and were included in one particular section of Ralfs's (1848) classification. Similarly, *Micrasterias truncata* and *M. crux-melitensis*, clustered in a particular subgroup, and are also classified in a particular section of Ralfs's scheme. In addition, the analysis revealed some taxa with very similar shapes that are also considered to be closely related in terms of traditional taxonomy. *Micrasterias decemdentata* and *M. truncata*, which clustered as a separate group of two closely related species in this study, were sometimes treated as varieties of the same species in the past (Playfair 1908). On the other hand, some close taxonomic relationships between species suggested in classical taxonomic monographs on the genus are not supported by geometric morphometrics. West & West (1905) treated *M. apiculata*, *M. brachyptera* and *M. fimbriata* as varieties of the same species. However, the close morphological relationship of *M. fimbriata* to *M. apiculata* and *M. brachyptera* was not supported by this analysis. At the same time, *M. crux-melitensis* and *M. furcata*, considered to be closely related species by Růžička (1981), in this analysis clustered in different subgroups. *Micrasterias furcata* clustered with *M. americana*, because of the ex-

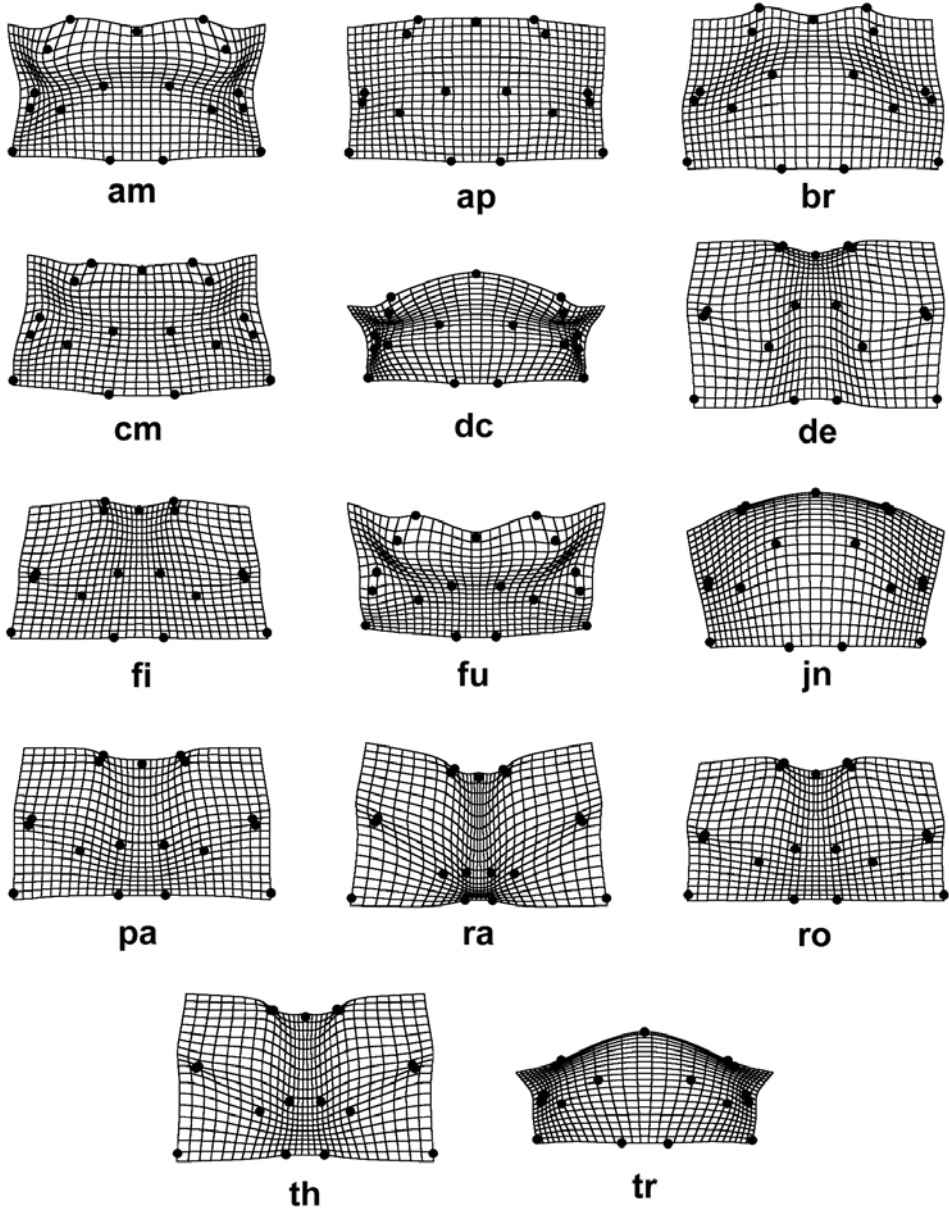


Fig. 5. – The thin-plate splines illustrating the changes in shape obtained from the overall consensus of average configurations of each species. For abbreviations see Fig. 3.

tremely broad incisions between the polar and lateral lobes indicated by the lateral compression of the grid (Fig. 5). *Micrasterias thomasiana* var. *notata*, which is classified as a variety of *M. denticulata* in some classical studies (e.g. Nordstedt 1888), was not included in the same subgroup as this species. The more pronounced incisions between the polar and lateral lobes (see Fig. 5) clustered *M. thomasiana* together with *M. radiosa*.



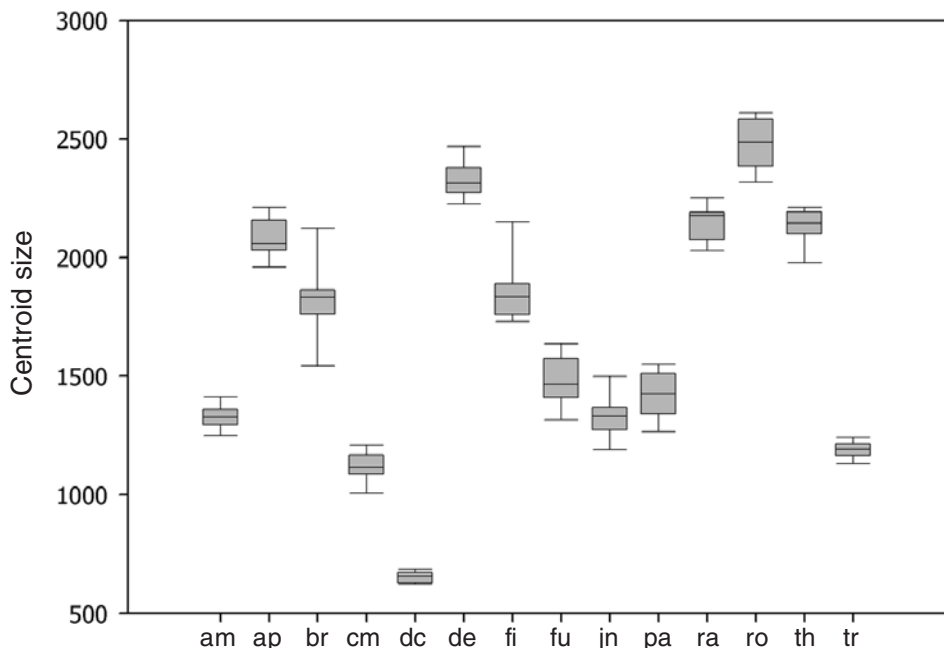


Fig. 6. – The centroid sizes of species investigated.

The centroid sizes of the species were comparatively similar to traditional width and length data (Prescott et al. 1975, Růžička 1981, Lenzenweger 1996). The UPGMA of the combined shape and size data revealed a similar pattern to the analysis of the shape data with the effect of size removed. However, the relatively large species, *M. apiculata* and *M. brachyptera*, clustered with the largest *Micrasterias* species, including *M. rotata*, *M. fimbriata* and *M. denticulata*, which are characterized by a narrow polar lobe. On the basis of shape data alone, *M. brachyptera* and *M. apiculata* were placed in the cluster consisting mostly of small species with a wide polar lobe. The analysis of the combined shape and size data did not cluster all the specimens correctly into groups corresponding to their species affiliations. Thus, the landmark analysis of shape data with the effect of size removed (Fig. 4) is recommended as the more reliable method for the differentiation of *Micrasterias* populations into traditional species clusters.

The current morphological generic concept in *Desmidiaceae* is widely considered to be artificial (Prescott et al. 1975, Růžička 1977), which has been confirmed by recent molecular phylogenetic analyses (Gontcharov et al. 2003, Gontcharov & Melkonian 2005). Very little molecular data exist for the genus *Micrasterias* (with only three species sequenced and 18S rDNA sequences available for only *M. fimbriata* and *M. crux-melitensis*), but the published phylogenetic reconstructions suggest paraphyly in this genus (Gontcharov et al. 2003). At the species level, molecular methods might provide an independent data set for delimitating individual populations and species. Consequently, the morphological characteristics, captured by the landmark analysis, and the morphometric reconstruction of the relationships between individual species will provide the data for a comparative investigation of phylogenetic relationships.

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## Souhrn

Pomocí metod geometrické morfometrie jsme studovali vzájemné vztahy 14 středoevropských druhů rodu *Micrasterias*. Pro znázornění hlavních morfologických trendů jsme použili analýzu relativních warpů a pro znázornění skupin jednotlivých druhů na základě morfometrických dat také shlukovací analýzu. Všechny analyzované buňky byly ve shlukovací analýze tvarových dat správně umístěny ve skupinách odpovídajících jednotlivým druhům. Jako hlavní trend v našem zkoumaném datovém souboru jsme zjistili morfologickou dynamiku polárního buněčného laloku spojenou se změnou hloubky zářezů mezi laterálními podlaloky.

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