Changes in shape of the coenobial cells of an experimental strain of *Pediastrum duplex* var. *duplex* (*Chlorophyta*) reared at different pHs

Variabilita tvaru cenobiálních buněk experimentální kultury druhu *Pediastrum duplex* var. *duplex* (*Chlorophyta*) ve vztahu k pH prostředí

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The changes in shape of the marginal coenobial cells in an experimentally cultured population of *Pediastrum duplex* Meyen var. *duplex* were investigated. The methods of landmark-based geometric morphometrics, including sliding landmarks registration, were used. The populations were cultured at 11 different pH levels and the changes in shape related to pH were studied using multivariate regression. The results of relative warps analysis revealed that morphological trends are related to both size and pH. The potential application of the results of this geometric morphometrical analysis of *Pediastrum* for biomonitoring and palaeoecological studies are discussed.

Introduction

Several species of the genus *Pediastrum*, e.g. *P. boryanum* (Turp.) Menegh. and *P. duplex* Meyen, are cosmopolitan organisms that very frequently occur in phytoplankton in eutrophic freshwater bodies (Komárek & Jankovská 2001). Pediastrum cell walls contain acetoresistant sporopolenin-like components, and as a consequence are frequently identified in fossil and subfossil samples (Jankovská & Komárek 2000). Thus, in palaeoecological studies, the *Pediastrum* species, with their clearly delimited ecological preferences, are used as bioindicators of eutrophic freshwater conditions (Jankovská & Pokorný 2002, Eilers et al. 2004, Head et al. 2005, Lezine et al. 2005, Milecka & Szeroczynska 2005).

Most of the frequently occurring *Pediastrum* species, including *P. duplex*, are well delimited taxonomically by traditional phenotypic criteria (Komárek & Jankovská 2001). However, the considerable infraspecific morphological variation has led to numerous taxonomic descriptions of individual forms and varieties in the past (see e.g. Nitardy 1914, Parra 1979, Komárek & Jankovská 2001). In most cases, as the morphological plasticity of individual taxa was not investigated in detail, their taxonomic status remains unclear.

The pH of a water environment is an important factor influencing the morphological characteristics of freshwater algae, both in experimental and natural conditions (Siver & Skogstad 1988, Alles et al. 1991, Hahn et al. 1996, Arancibia-Avila et al. 2000). In the genus *Pediastrum*, similar studies have not been conducted, but such pH-related morphological variation could be useful for palaeoecological reconstructions of the pH dynamics of freshwater lakes. In the present study current morphometric methods, namely the land-

mark-based geometric morphometrics (Zelditch et al. 2004) were used to investigate the morphological variation of the marginal coenobial cells of an experimental population of *Pediastrum duplex* var. *duplex*. Firstly, the morphological variability in the population as a whole was characterized using relative warps analysis (Rohlf 1993), which depicts the principal components of shape variation. Secondly, the size and shape variation when reared at different pH levels were investigated.

Material and methods

The investigated strain was isolated from plankton from the Vltava river in Prague, Czech Republic, in June 2004. The strain is deposited in the Culture Collection of Algae of Charles University (strain no. CAUP H 2308). In the experiment, clonal cultures were cultivated in 500 ml Erlenmeyer flasks in liquid 30% BBM for five weeks at a temperature of 21 °C, 16:8 light/dark cycle and an illumination intensity of 750 $\mu E \cdot s^{-1} \cdot m^{-2}$. The pH was adjusted with 1M NaOH to 11 different pH levels ranging from 5.5 to 10.5, i.e. each differing by 0.5. The pH level was controlled and, if need be, adjusted every 48 hours in the course of the experiment. Below pH level 5.5 no growth was observed.

At each pH level, 50 randomly selected coenobia were photographed using an Olympus BX51 light microscope and Z5060 microphotographic equipment. In each coenobium, one marginal cell was randomly selected and measured. Thus, in total 550 cells originating from different coenobia were analysed. In each cell, 26 landmarks were digitized (Fig. 1A) using TpsDig, ver. 1.40 software (Rohlf 2004a). In total, 15 landmarks were semilandmarks that were allowed to slide during the superimposition (Bookstein 1997).

The individual objects were superimposed using generalised Procrustes analysis (Bookstein 1991, Dryden & Mardia 1998) with semilandmarks allowed to slide in TpsRelw, ver. 1.40 software (Rohlf 2004b). The cells of *Pediastrum duplex* are nearly bilaterally symmetrical. Because the anterior and posterior sides of the cells do not differ, it is not possible to distinguish their respective left and right sides. Therefore, the two sides were symmetrized using the method of Klingenberg et al. (2002). This involves reflecting each of the cells (by multiplication of x-coordinates of all landmarks by –1), relabelling the paired landmarks and averaging the original and mirrored configurations in the Procrustes superimposition. The averages of original and mirrored/relabelled cells are ideal symmetrical shapes, where each half, together with landmarks lying on the median axis, bears all the information on the shape of that symmetrical object. Thus, further analysis of these symmetrized configurations involved the symmetrical part of the shape variation and omitted the asymmetry. The scatter of Procrustes superimposed objects can be seen in Fig. 1B. Then, using TpsRelw, relative warps analysis (RWA) with $\alpha = 0$ (= shape PCA) (Rohlf 1993) was performed. The extreme positions of morphospace depicted by the first few axes of RWA were illustrated as thin-plate splines from average (consensus) landmark configuration.

Then, the relationship of cell size, coenobial size and marginal cell shape to pH level was tested. The size was evaluated using centroid size (square root of the sum of squared distances from the landmarks to their centroid), which is a standard size measure in morphometric studies (Rohlf 1993, Zelditch et al. 2004). However, in taxonomic literature the size of *Pediastrum* cells is given as a width and length measurement. Therefore, paral-
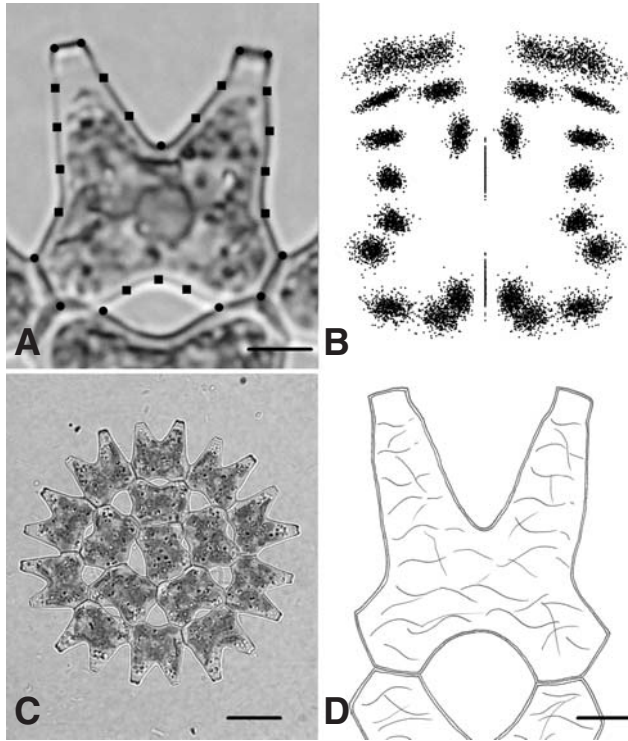
Fig. 1. – A. The cell of *Pediastrum duplex* var. *duplex* with the position of landmarks. Squares indicate semilandmarks. Bar = 5 μm. – B. The scatter of *Procrustes* superimposed landmarks configurations. – C. A coenobium of *Pediastrum duplex* var. *duplex*. Bar = 20 μm. – D. The cell wall sculpturing of the investigated strain. Bar = 5 μm.

lel tests on this traditional measurement and, in addition, the regressions of width and length of the cells to centroid size to assess their relation were conducted. The width and length of the investigated cells were evaluated using TpsDig, ver. 2.04 (Rohlf 2005). The coenobial size was measured as the diameter of individual coenobia (Komárek & Jankovská 2001). The univariate regressions of the centroid size, cell width and length and the coenobial size to pH was conducted using PAST, ver. 1.33. morphometric software (Hammer et al. 2001).

The multivariate regression of shape variables (represented by partial warps and the uniform component) to pH was conducted using TpsRegr, ver. 1.29 software (Rohlf 2004c). Two multivariate regressions were conducted. Firstly, the significance of the relation between shape and pH with size uncontrolled was tested. Secondly, whether the cells grown at particular pH levels differ in shape with size as a covariate was tested. The linearity of the relationship between variables was evaluated in TpsRegr by checking the relation of individual shape variables (partial warps) to the independent variable. Departures from linearity were not registered and the significance of the regression model in TpsRegr was tested using Wilk's λ and Goodall's F-ratio using 1000 permutations (Rohlf 2004c, Sheets et al. 2004). The Goodall's F is analogous to the ratio of explained variance to unexplained variance in regression (Rohlf 2004c). The percentage of variation unexplained

by the multivariate regressions was used as an overall measure of fit for the comparison of different independent variables (Rohlf 2004c). The positions typical for individual pH levels and extreme positions of regression model were illustrated using thin-plate splines from consensus landmark configuration.

For the multivariate regression of shape in relation to pH with the variation due to size controlled, the method of Zelditch et al. (2004) was used. The linearity of the relation of shape to centroid size for the complete data set and, subsequently, for each group separately, was checked. There was a linear relation between shape variables and centroid size. Then, a multivariate test of common slopes to test the null hypothesis that relation of shape to size is the same at all pH levels was performed. However, there was a significant interaction between the covariate (centroid size) and the factor (pH level). This means that the difference in shape of the cells at different pH levels is not a simple consequence of their difference in size. Thus, following Zelditch et al. (2004) the significance of the relation of shape to pH at a specified cell size in the complete data set was tested. The mean centroid size for the whole data set was used for this purpose. The IMP geometric morphometrics software package (Sheets 2002) for the multivariate regression of shape relative to centroid size in each group (each individual pH level in our case) was used. Then, the shape of theoretical populations with mean centroid size standardized to the mean centroid size for the whole data set was predicted by regression for each of the 11 groups (pH levels). Finally, using these models, size standardized populations for individual pH levels, a regression of shape on pH was conducted for the complete data set. Statistical tests equivalent to those conducted with uncontrolled size data were done (the significance evaluated by permutation tests on Wilk's λ and Goodall's F-ratio) and the results illustrated by thin-plate splines.

To demonstrate the relation of the shape of marginal cells to their size, a multivariate regression of shape variables to centroid size (this test in fact represented the evaluation of the effect of ontogeny on shape of the organism) and coenobial size, was conducted using TpsRegr, ver. 1.29 software. The results were illustrated by thin-plate splines of extreme positions of the data from an average (consensus) landmark configuration. To illustrate the results, all the thin-plate splines were used to create a theoretical model of the cells using "unwarping to fixed configuration" function of TpsSuper, ver. 1.13 (Rohlf 2004d).

Results

The morphology of the strain corresponds to the taxonomic description of *P. duplex* var. *duplex*. The coenobia were circular, with perforations between cells, (40–) 65–120 (–150) µm in diameter, composed of 8–64 cells (Fig. 1C). The cells were quadratic in outline, with concave sides. The dimensions of cells were (8–) 12.5–23.5 (–31) × (6.5–) 8.5–20.0 (–25.5) µm. The marginal cells had two distally narrowing marginal lobes with a distinct V-incision. The polar parts of the marginal lobes were composed of short cylindrical processi, from which long, thin plasmatic bristles radiated on some cells. The cell wall was smooth or, occasionally, very finely sculptured with irregular net-like structures (Fig. 1D).

The RWA ordination diagrams are presented in Fig. 2 and the respective shape changes associated with individual axes illustrated in Fig. 3. The first relative warp (= first principal component) accounted for 45.7% of the total variability in the data set. This shows the shape dynamics that is related to the angle between the marginal lobes. This variation in shape is
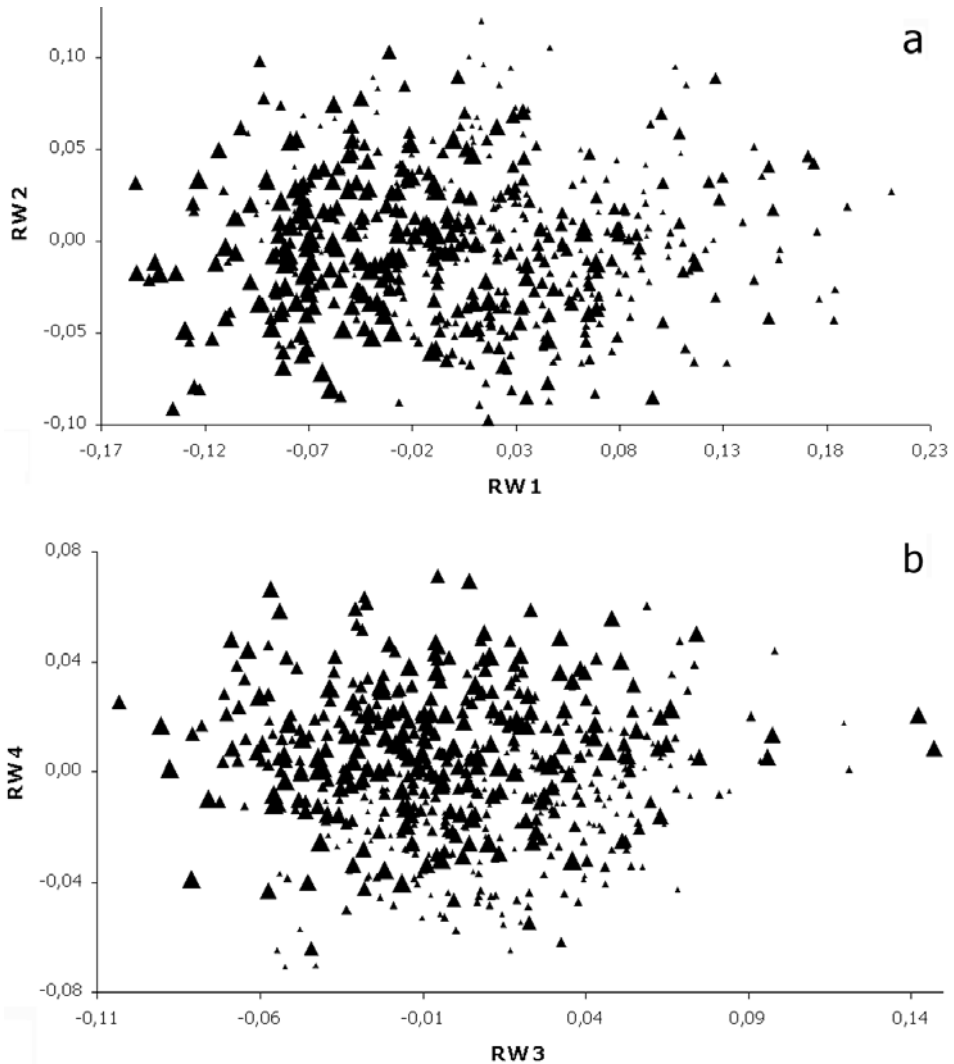
Fig. 2. – The RWA ordination diagrams. The increasing dimensions of the triangles indicate increasing pH level.
a – RW1 × RW2; b – RW3 x RW4.

typical of *P. duplex* and related mainly to the number of cells within individual coenobia (L. Hodač, unpublished data). The negative position on this axis, associated with a narrow angle between the lobes, is typical of coenobia with a large number of cells, whereas wide angles are typical of marginal cells of coenobia with few cells. The second relative warp accounted for 16.4% of the total morphological variability. This axis is negatively correlated with the relative length of the cell associated with parallel widening of the marginal lobes in negative positions of RW2. The third relative warp accounted for 14.5% of the total variability. This RW describes the widening of the central part of cells associated with pronounced shortening and widening of both the basal and marginal lobes. The fourth relative warp
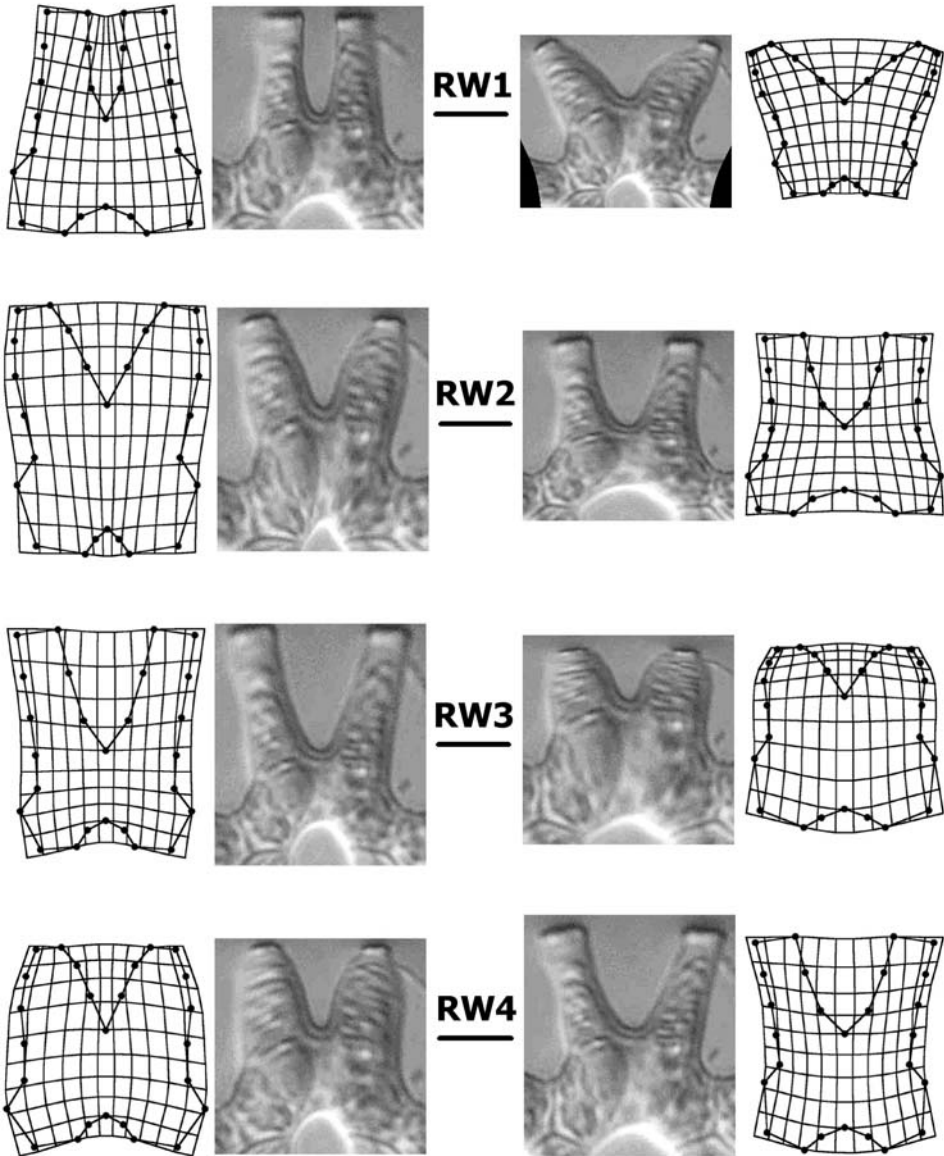
Fig. 3. – The shape changes associated with extreme positions along individual axes of RWA.

accounted for 7.0% of the shape variation in the data set and this axis describes the trend of widening marginal lobes with largely unchanged basal lobes. The ordination diagrams (Fig. 2) indicate that the pH level is associated with the shape trends depicted by several relative warps (notably RW1, RW3 and RW4). The cells from low pH conditions mostly shared positive RW1, positive RW3 and negative RW4 values.
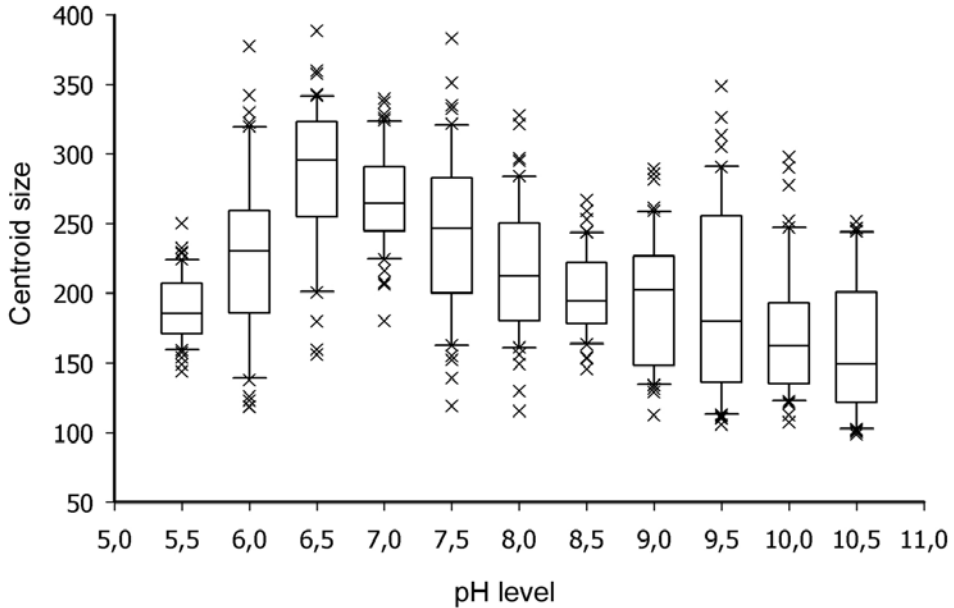
Fig. 4. – The size values of the marginal cells of individuals reared at different pH.
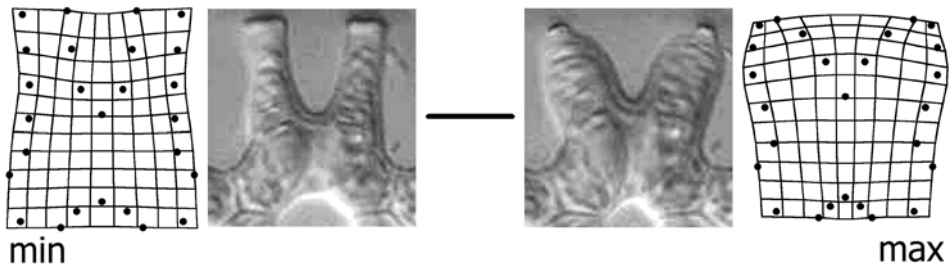

Fig. 5. – The changes in shape associated with cell size. The extreme configurations are illustrated.

Centroid size appeared to be significantly related to both width ($R^2 = 0.33$, $P < 0.001$) and length ($R^2 = 0.37$, $P < 0.001$) of the cells. The size of the cells from particular pH-levels is illustrated in Fig. 4. The regression revealed a significant correlation between the centroid size of the cells and pH of the environment [$R^2 = 0.37$, P (uncorr.) < 0.001]. Generally, the cells tended to be smaller at higher pH levels. Nevertheless, at the lowest pHs (5.5 and 6.0) the cells were also comparatively small. There were similar correlations between cell width and pH [$R^2 = 0.41$, P (uncorr.) < 0.001] and between cell length and pH [$R^2 = 0.39$, P (uncorr.) < 0.001], too. The coenobial diameter was less correlated with pH-level than the dimensions of the cells. However, the regression was significant [$R^2 = 0.13$, P (uncorr.) $= 0.003$].

The shape of the cells was significantly related to centroid size (Wilk's $\lambda = 0.282$, P-value after 1000 permutations $= 0.001$; Goodall's F-ratio $= 89.2$, P-value after 1000 per-
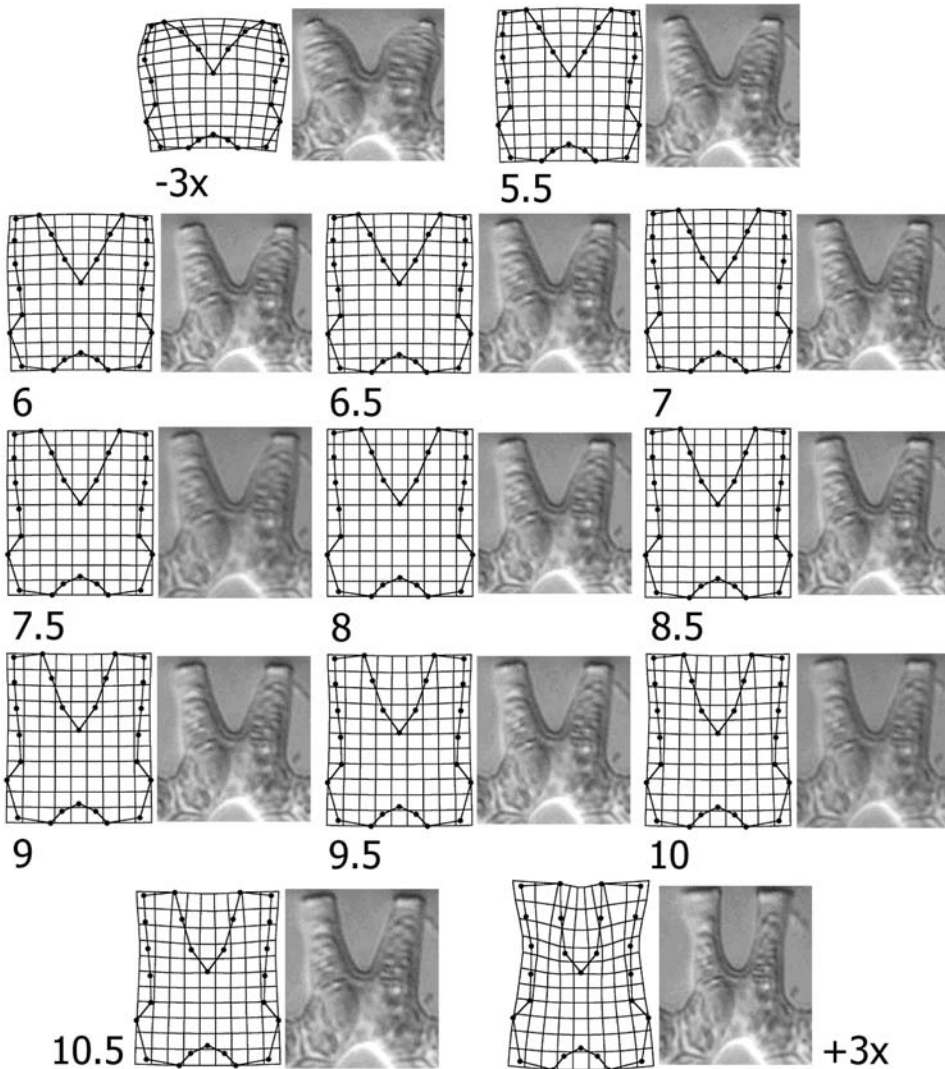
Fig. 6. – The landmark configurations and cell shapes reconstructed from a multivariate regression of shape variables on pH. The shapes typical of particular pH levels and of three times magnified extremes of the regression model are illustrated.

mutations = 0.001, 86.1% unexplained). The shape change associated with centroid size, corresponding to allometric changes during the ontogeny of the organism, is illustrated in Fig. 5. The morphological dynamics involving presumably the marginal cell lobes are obvious. In large cells, the angle between the marginal lobes is wide, and the lobes tend to be more rounded and wide. In small cells, the angle is narrower and the marginal lobes straight. The other parts of the cell do not change with size. In addition, there was
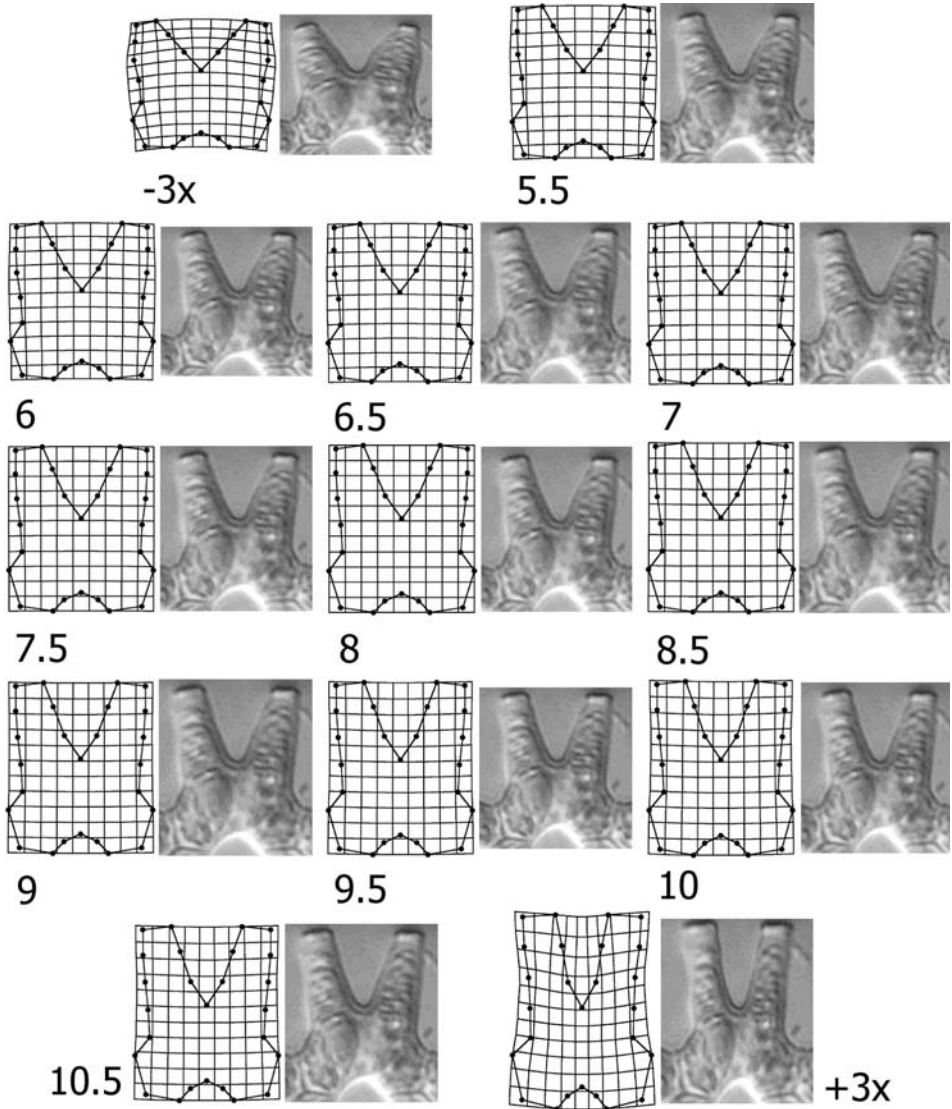
Fig. 7. – The landmark configurations and cell shapes reconstructed from a multivariate regression of average centroid size standardized shape variables on pH. The shapes typical of particular pH levels and of three times magnified extremes of the regression model are illustrated.

a weaker, albeit still significant, relation between shape and coenobial size (Wilk's λ = 0.853, P-value after 1000 permutations = 0.003; Goodall's F-ratio = 5.6, P-value after 1000 permutations = 0.002, 98.9% unexplained).

To test for the relation between shape and pH, a multivariate regression was conducted and a significant relation found (Wilk's λ = 0.502, P-value after 1000 permutations = 0.001; Goodall's F-ratio = 53.4, P-value after 1000 permutations = 0.001, 91.2% unex-

plained). The principal change in shape associated with pH (Fig. 6) is from wide basal parts of marginal lobes and a wider angle between these lobes at low pHs, to narrower basal parts and narrower angles at high pHs. On the other hand, the distal parts of the marginal lobes tend to be wider at higher pHs. In addition, the basal lobes tend to be wider in cells from higher pHs. Furthermore, the relation between shape and pH, with variation due to size controlled, was tested. Because there is a significant relation between shape and centroid size, a test for common slopes was conducted, to test the hypothesis that the relation between change in shape and size is the same at all pH levels. However, this null hypothesis was rejected (Wilk's $\lambda = 0.267$, P-value < 0.001), which means that the rate of change in shape differed with pH. In other words, the change in cell shape associated with pH was not a simple consequence of the change in size. Subsequently, the relation was tested for cells in each group adjusted to average centroid size at a given pH. The width and length values corresponding to the average centroid size value (214.5) were: width 19.6 μm, length 15.4 μm. This is the cell size typical of natural populations of the species studied (Komárek & Jankovská 2001). The test of the relation between shape standardized to mean centroid size and pH yielded significant results (Wilk's $\lambda = 0.494$, P-value after 1000 permutations = 0.001; Goodall's F-ratio = 59.3, P-value after 1000 permutations = 0.001, 90.3% unexplained) (Fig. 7). In general, the changes in shape associated with pH appeared relatively similar to that for the uncontrolled effect of size. However, the main difference is the little change that occurred in the marginal cell lobes. They are narrower at low pH and, at the same time, their distal parts do not widen with increase in pH.

Discussion

The RWA revealed a pattern in the morphological variation of a population of *P. duplex*. The most important morphological trend was a widening in the angle between the marginal cell lobes. Given the circular shape of *Pediastrum* coenobia, the angle between the marginal cell lobes depends mainly on the number of cells within a coenobium, with a wider angle between marginal cell lobes in coenobia composed of few cells and a narrower angle between those in coenobia with many cells. RW2 described the morphological dynamics involved in the widening of the marginal lobes. Both these morphological trends are associated with the allometric changes occurring in cell shape with size during the development of the organism (Figs 3, 5).

Different measures of size correlated significantly with the pH-level at which the populations were grown. However, the measures of cell size (centroid size, cell width and length) proved to be more strongly correlated with pH-level than coenobial size and shape of the cells less strongly correlated with coenobial size than cell size. Both these results indicate the higher relevance of marginal cell size than coenobial size in the changes in shape. Both multivariate regressions of shape on pH-level revealed significant non-random correlations, which were confirmed by permutation tests. The results of multivariate regression of shape data standardized to mean centroid size demonstrated that the morphological variation connected with pH-level cannot be ascribed only to changes in cell size, as it also affects shape characteristics. The shape changes correlated with pH in this size-standardized data set indicated the pattern that is not directly associated with change in size. In comparison with the shape data not controlled for size the prominent morphologi-

cal trends associated with marginal lobes and the dominant effect of longitudinal elongation at higher pH values are less prominent. Thus, changes in shape are most directly associated with pH-level.

However, both multivariate regressions of shape on pH-level failed to explain more than about 10% of the variation in the data. The pH-level definitely affected cell shape, but was not the dominant factor explaining the changes in shape. Nevertheless, given the significant results, it was possible to demonstrate the principal morphological trend associated with changing pH. The morphology associated with low pH resembles that of *P. duplex* var. *rotundatum* Lucks, one of the infraspecific taxa described without knowledge of the variation in natural populations. Similar trends were also depicted by the third and fourth relative warp axes that correlated with pH change, so it is likely that *P. duplex* var. *rotundatum* probably represents the ecomorph that occurs most frequently in low pH environments. Interestingly this investigation did not reveal any significant trend in shape that could be related to the transition between the type variety and a very frequently occurring *P. duplex* var. *gracillimum* W. et G. S. West, with generally thinner cells and long pronounced lobes. Either the shape in change from the type variety to *P. duplex* var. *gracillimum* is induced by some unknown environmental factor, or both varieties are real taxonomic entities, as presumed by Komárek & Jankovská (2001).

Given the rather low proportion of cell shape variation explained by pH-level, it is premature to use this data for inferring the pH of natural biotopes, e.g. in palaeoecological studies. However, there are significant correlations, with clear, albeit subtle, morphological trends in shape. Therefore, the pH-correlated population variability in *Pediastrum* should be investigated at a broader scale in the future, in order to assess its potential for the biomonitoring of pH changes in natural habitats. The shape vs. pH relationships should be tested on natural populations of *P. duplex* to ascertain the relation between changes in shape and key environmental factors (e.g. pH, conductivity, nutrients, average temperature, size of the water body, the water retention time, size/depth ratio and others). Such investigations, using partial least squares analysis (Rohlf & Corti 2000), should be able to demonstrate the covariation of changes in shape with environmental factors and compare with the pattern of pH related variability revealed in this study.

In addition, similar studies need to be conducted on other common *Pediastrum* species (e.g. *P. boryanum*, *P. simplex*) to ascertain whether they show similar trends in morphospace dynamics in different environments. *Pediastrum boryanum*, whose marginal cells have a similar morphology may show a similar pattern, with the first principal axis of shape variation reflecting the angle between two marginal cell lobes. However, in *P. simplex*, where only one marginal lobe develops, this pattern should, obviously, be missing. Thus, this species is likely to show a different type of morphospace pattern.

Once there are credible data on shape variation and its correlation with pH-level in both cultured and natural populations for at least these three common species, then there is a real possibility of developing a complex and accurate model for inferring the pH of natural water bodies. In this way, it should be possible to develop a framework for the environmentally related morphospace dynamics in *Pediastrum*, which could be used in biomonitoring and palaeoecological applications.

In palaeoecology and bioindication research, however, there have been few attempts to use a population morphometric analyses for inferring environmental parameters. There have been proposals for different groups, whose morphological variability has the poten-

tial for the biomonitoring of environmental changes. Hallock et al. (2003) proposed the use of population morphometric characteristics in *Foraminifera* for monitoring coral reefs, including heavy metal pollution assessment. Smol (1995) suggested that morphological variation in the silica scales of synurophyte species could be used in biomonitoring studies. The relation between temperature and morphology of silica structures in *Synurophyceae* was studied in experimental populations of *Mallomonas crassisquama* (Siver & Skogstad 1988) and *M. tonsurata* (Gutowski 1996). Similarly, Wanner (1999) proposed that testate amoebae could be used for biomonitoring purposes, because of the well documented effect of temperature on their morphological characteristics. Subsequently, Booth (2001) demonstrated that there is a correlation between population morphometric characteristics of the shells of *Nebela tincta-parvula-collaris* group in two North American Lake Superior wetlands and the pH of the environment. Likewise, Roberts et al. (2002) documented the correlation between morphological variables and salinity levels in a palaeoecological study of an ostracod species, *Limnocythere inopinata*.

So far in phycology, the modern morphometric methods have been used mainly in taxonomic investigations (Pappas et al. 2001, Neustupa 2004, Beszteri et al. 2005, Verbruggen et al. 2005a, 2005b). However, in applied ecology the potential of such studies at the species level of organisms with a high bioindicative potential is obvious (Smol 1995). We believe that if modern morphometric methods are used, members of the genus *Pediastrum* could be convenient model organisms for investigating the interactions between environment and morphology in palaeolimnology and freshwater biomonitoring.

Acknowledgements

Souhrn

Článek přináší výsledky výzkumu tvarové dynamiky okrajových cenobiálních buněk experimentální populace taxonu *Pedistrum duplex* var. *duplex* pomocí metod landmarkové geometrické morfometriky. Klonální populace byly kultivovány v 11 úrovních pH a tvarová dynamika související s úrovní pH analyzována mnohorozměrnou regresí originálních dat a – vzhledem k průkaznému vlivu pH na velikost populací – také mnohorozměrnou regresí dat standardizovaných na průměrnou velikost buněk. Výsledky analýzy relativních warpů (PCA) ukázaly na osách popisujících největší část variability morfologické trendy spojené se změnou velikosti i pH. V článku jsou diskutovány možnosti aplikací geometrické morfometriky u rodu *Pediastrum* pro bioindikační a paleoekologické studie.

References

Arancibia-Avila P., Coleman J. R., Russin W. A., Wilcox L. W., Graham J. M. & Graham L. E. (2000): Effects of pH on cell morphology and carbonic anhydrase activity and localization in bloom-forming *Mougeotia* (*Chlorophyta*, *Charophyceae*). – Can. J. Bot. 78: 1206–1214.

Alles E., Norpelschempp M. & Lange-Bertalot H. (1991): Taxonomy and ecology of characteristic *Eunotia* species in headwaters with low electric conductivity. – Nova Hedwigia 53: 171–213.

Beszteri B., Ács E. & Medlin L. (2005): Conventional and geometric morphometric studies of valve ultrastructural variation in two closely related *Cyclotella* species (*Bacillariophyta*). – Eur. J. Phycol. 40: 89–103.

Bookstein F. L. (1991): Morphometric tools for landmark data: geometry and biology. – Cambridge University Press, Cambridge.

Bookstein F. L. (1997): Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. – Med. Image Anal. 1: 225–243.

Booth R. K. (2001): Ecology of testate amoebae (*Protozoa*) in two lake superior coastal wetlands: Implications for paleoecology and environmental monitoring. – Wetlands 21: 564–576.

Dryden I. L. & Mardia K. V. (1998): Statistical shape analysis. – John Wiley & Sons, New York.

Eilers J. M., Kann J., Cornett J., Moser K. & St. Amand A. (2004): Paleolimnological evidence of change in a shallow, hypereutrophic lake: Upper Klamath Lake, Oregon, USA. – Hydrobiologia 520: 7–18.

Gutowski A. (1996): Temperature dependent variability of scales and bristles of *Mallomonas tonsurata* Teiling emend. Krieger (*Synurophyceae*). – Nova Hedwigia Beih. 114: 125–146.

Hahn A., Gutowski A. & Geissler U. (1996): Scale and bristle morphology of *Mallomonas tonsurata* (*Synurophyceae*) in cultures with varied nutrient supply. – Bot. Acta 109: 239–247.

Hallock P, Lidz B. H., Cockey-Burkhard E. M. & Donnelly K. B. (2003): *Foraminifera* as bioindicators in coral reef assessment and monitoring: The FORAM Index. – Environ. Monit. Assess. 81: 221–238.

Hammer Ø., Harper D. A. T. & Ryan P. D. (2001): PAST: Paleontological Statistics Software Package for Education and Data Analysis. – Palaeontologia Electronica 4 (1): 9 pp.

Head M. J., Seidenkrantz M. S., Janczyk-Kopikowa Z., Marks L. & Gibbard P. L. (2005): Last interglacial (Eemian) hydrographic conditions in the southeastern Baltic Sea, NE Europe, based on dinoflagellate cysts. – Quaternary International 130: 3–30.

Jankovská V. & Komárek J. (2000): Indicative value of *Pediastrum* and other coccal green algae in palaeoecology. – Folia Geobot. 35: 59–82.

Jankovská V. & Pokorný J. (2002): Palaeoecology of a medieval fishpond system (Vajgar, Czech Republic). – Folia Geobot. 37: 253–273.

Klingenberg C. P., Barluenga M. & Meyer A. (2002): Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. – Evolution 56: 1909–1920.

Komárek J. & Jankovská V. (2001): Review of the green algal genus *Pediastrum*; implication for pollen-analytical research. – Bibliotheca Phycologica 108: 1–127.

Lezine A. M., Duplessy J. C. & Cazet J. P. (2005): West African monsoon variability during the last deglaciation and the Holocene: Evidence from fresh water algae, pollen and isotope data from core KW31, Gulf of Guinea. – Palaeogeography, Palaeoclimatology, Palaeoecology 219: 225–237.

Milecka K., Szeroczynska K. (2005): Changes in macrophytic flora and planktonic organisms in Lake Ostrowite, Poland, as a response to climatic and trophic fluctuations. – Holocene 15: 74–84.

Neustupa J. (2004): Two new aerophytic species of the genus *Podohedra* Düringer (*Chlorophyceae*) – Arch. Hydrobiol./Algol. Stud. 112: 1–16.

Nitardy E. (1914): Zur Synonymie von *Pediastrum*. – Beih. Bot. Centralbl. 32: 111–194.

Pappas J. L., Fowler G. W. & Stoermer E. F. (2001): Calculating shape descriptors from Fourier analysis: shape analysis of *Asterionella* (*Heterokontophyta*, *Bacillariophyceae*). – Phycologia 40: 440–456.

Parra B. O. O. (1979): Revision der Gattung *Pediastrum* Meyen (*Chlorophyta*). – Bibliotheca Phycologica 48: 1–186.

Roberts J. H., Holmes J. A. & Swan A. R. H. (2002): Ecophenotypy in *Limnocythere inopinata* (*Ostracoda*) from the late Holocene of Kajemarum Oasis (north-eastern Nigeria). – Palaeogeography, Palaeoclimatology, Palaeoecology 185: 41–52.

Rohlf F. J. (1993): Relative warp analysis and an example of its application to mosquito wings. – In: Marcus L. F., Bello E. & Garcia-Valdecasas A. (eds.), Contributions to morphometrics, p. 134–159, Museo Nacional de Ciencias Naturales, Madrid.

Rohlf F. J. (2004a): TpsDig Version 1.40. – Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

Rohlf F. J. (2004b): TpsRelw Version 1.40. – Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

Rohlf F. J. (2004c): TpsRegr Version 1.29. – Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

Rohlf F. J. (2004d): TpsSuper Version 1.13. – Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

Rohlf F. J. (2005): TpsDig Version 2.04. – Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

Rohlf F. J. & Corti M. (2000): Use of two-block partial least-squares to study covariation in shape. – Syst. Biol. 49: 740–753.

Sheets D. H. (2002): IMP-Integrated Morphometrics Package. – Department of Physics, Canisius College, Buffalo.

Sheets D. H., Kim K. & Mitchell C. E. (2004): A combined landmark and outline-based approach to ontogenetic shape change in the Ordovician trilobite *Triarthrus becki*. – In: Elewa A. M. T. (ed.), Morphometrics. Applications in biology and paleontology, p. 67–82, Springer Verlag, Berlin.

Siver P. A. & Skogstad A. (1988): Morphological variation and ecology of *Mallomonas crassisquama* (*Chrysophyceae*). – Nord. J. Bot. 8: 99–107.

Smol J. P. (1995): Application of chrysophytes to problems in paleoecology. – In: Sandgren C. D., Smol J. P. & Kristiansen J. (eds.), Chrysophyte algae. Ecology, phylogeny and development, p. 303–330, Cambridge University Press, Cambridge.

Verbruggen H., De Clerck O., Cocquyt E., Kooistra W. H. C. F. & Coppejans E. (2005): Morphometric taxonomy of siphonous green algae: A methodological study within the genus *Halimeda* (*Bryopsidales*). – J. Phycol. 41: 126–139.

Verbruggen H., De Clerck O., Kooistra W. H. C. F. & Coppejans E. (2005): Molecular and morphometric data pinpoint species boundaries in *Halimeda* section *Rhipsalis* (*Bryopsidales*, *Chlorophyta*). – J. Phycol. 41: 606–621.

Wanner M. (1999): A review on the variability of testate amoebae: Methodological approaches, environmental influences and taxonomical implications. – Acta Protozool. 38: 15–29

Zelditch M. L., Swiderski D. L., Sheets D. H. & Fink W. L. (2004): Geometric morphometrics for biologists: a primer. – Elsevier Academic Press, London.