Morphological variability among European populations of *Vestalenula cylindrica* (Straub) (Crustacea, Ostracoda)

Klaus Minati [a,d,*], Maria Cristina Cabral [b], Radovan Pipík [c], Dan L. Danielopol [d], Johann Linhart [e], Walter Neubauer [e]

[a] Institute for Limnology, Austrian Academy of Sciences, Mondseestrasse 9, 5310 Mondsee, Austria
[b] Departamento e Centro de Geologia da FCUL, Campo Grande, Bloco C6, 4° piso, 1749-016 Lisbon, Portugal
[c] Geological Institute, Slovak Academy of Sciences, Severna 5, SK-974 01 Banska Bystrica, Slovakia
[d] Commission for the Palaeontological and Stratigraphical Research of Austria, c/o Institute for Earth Sciences, University Graz, Heinrichstr. 26, 8010 Graz, Austria
[e] Institute of Mathematics, University of Salzburg, Hellbrunner Strasse 34, 5020 Salzburg, Austria

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ABSTRACT

A morphometric valve variability analysis of adults and juveniles of *Vestalenula cylindrica* [Straub, E.W., 1952. Mikropaläontologische Untersuchungen im Tertiär zwischen Ehingen und Ulm a. d. Donau. Geologisches Jahrbuch 66, 433–524.] from Götzendorf–Pischelsdorf (Lower Austria, Upper Miocene) is presented. The data are compared with those from Studienka (Slovakia, Upper Miocene) and from Boliqueime (southern Portugal, Pleistocene). Specimens from Austria and Slovakia are very similar in their morphology. By contrast definite morphological differences between the Central European populations (from Austria and Slovakia) and the Portuguese one are identified, in both the valve shape and size. The valves from Portugal are more elongated and show a higher variability than the Austrian and Slovakian specimens. By considering the occurrence of a new Recent species of *Vestalenula* in southern France, these morphologic differences could be interpreted as micro-evolutionary changes, which could have occurred between the Miocene and the present time within the European populations.

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1. Introduction

Until 10 years ago the post-Palaeozoic Darwinulidae (Brady and Norman) were only recognised through very few Recent and fossil species belonging to two genera, *Darwinula* Brady and Robertson and *Microdarwinula* Danielopol. The interest in ostracods of the family Darwinulidae increased enormously mainly after the taxonomic revision of this family (Rossetti and Martens, 1998). In that revision, the genus *Vestalenula*, which is discussed in this paper, was introduced new to science. Beside the three genera mentioned above, the family Darwinulidae now contains two other genera with living representatives: *Alicenula* Rossetti and Martens and *Penthesilenula* Rossetti and Martens.

We focussed our interest on samples of the fossil species *Vestalenula cylindrica*, formerly known as *Darwinula cylindrica* (Straub, 1952), which occurred from the Lower Miocene to the Holocene (Janz et al., 2001). Only few and rather incomplete descriptions of this species have been published so far due to very rare findings of *V. cylindrica* in the past decades. Therefore the main goal of our work is to check out if there are

morphological differences in valve shape and size of *V. cylindrica* from different localities and different geological times and to correlate these characteristics with (palaeo)ecological aspects.

V. cylindrica was described by Straub (1952) from Miocene material sampled between Ehingen and Ulm an der Donau in southern Germany. In the same region, Lutz (1965) found Miocene valves of *V. cylindrica* at Undorf–Nittendorf. Freels (1980) mentioned *V. cylindrica* from three locations in Turkey: from the Middle Miocene in Mugla, the Upper Miocene in Bursa and the Pliocene in Maras. *V. cylindrica* was also found by Janz (1997), in the Middle Miocene Kleini-beds of the crater lake of Steinheim in southern Germany and in the river bank of Zayandeh-Rud, Isfahan-Sirjan Basin in the Iran from the Holocene (Janz et al., 2001). Witt (1998, 2000) reported the occurrence of *V. cylindrica* in southern Germany from two localities near Munich in lacustrine Lower Miocene and from Sandelzhausen, from the Middle Miocene. The species was detected in south-eastern Austria at Mataschen by Gross (2004), and in Slovakia at Studienka by Pipík et al. (2004), both from Upper Miocene deposits. The latest report about *V. cylindrica* comes from Cabral et al. (2005a) who found it in Boliqueime, Algarve, southern Portugal in probable Pleistocene deposits. In all these publications the morphology of *V. cylindrica* was described only briefly. The present contribution compares the valve outlines from Central European locations in Austria and Slovakia with

those from the south-western European location in Portugal (Fig. 1) and hypothesises that Central and Western European populations are morphologically different due to ecological and/or evolutionary reasons.

2. Materials and methods

Abbreviations

Lv: Left valve; Rv: Right valve; Bla: Boliqueime left adult; Bra: Boliqueime right adult; Gla: Götzendorf–Pischelsdorf left adult; Glj: Götzendorf–Pischelsdorf left juvenile; Gra: Götzendorf–Pischelsdorf right adult; Grj: Götzendorf–Pischelsdorf right juvenile; Tla: Studienka left adult; Tra: Studienka right adult.

2.1. Sampling sites

2.1.1. Götzendorf–Pischelsdorf (Austria)

The ostracod material from Austria was sampled in Götzendorf–Pischelsdorf, eastern Austria, (Fig. 1A) by G. Daxner-Höck (Natural History Museum Vienna). Approximate coordinates: 48°00′ N and

16°33′ E. It was taken from a layer between plates of limestone, which were overlaid by ~50 cm mould. Due to the vicinity to the well known outcrop Götzendorf–Sandberg (Rögl et al., 1993; Harzhauser and Tempfer, 2004), which belongs to the Upper Miocene and due to fossil records of molluscs, which also fit to that palaeontological time assignment, it can be assumed that the location is also belonging to the Upper Miocene. Anyway it has to be noticed that there has been no geological and palaeontological classification of this outcrop so far (G. Daxner-Höck, pers. comm. 2006). The investigated material of *V. cylindrica* consists of 41 valves, separated in 12 adult left valves, 17 adult right valves, 6 left and 6 right juvenile valves, all in well preserved conditions.

2.1.2. Studienka (Slovakia)

The locality Studienka is situated on the right bank of the river Rudava, on the left hand side of the road No. 590 leading from Studienka to Malacky (Fig. 1B). Coordinates: 48°30′39″ N and 17°07′23″ E. The outcrop of 6 m height exposes the clastic deposits. The sequence starts with psammitic and aleuritic deposits free of fossils and is followed by
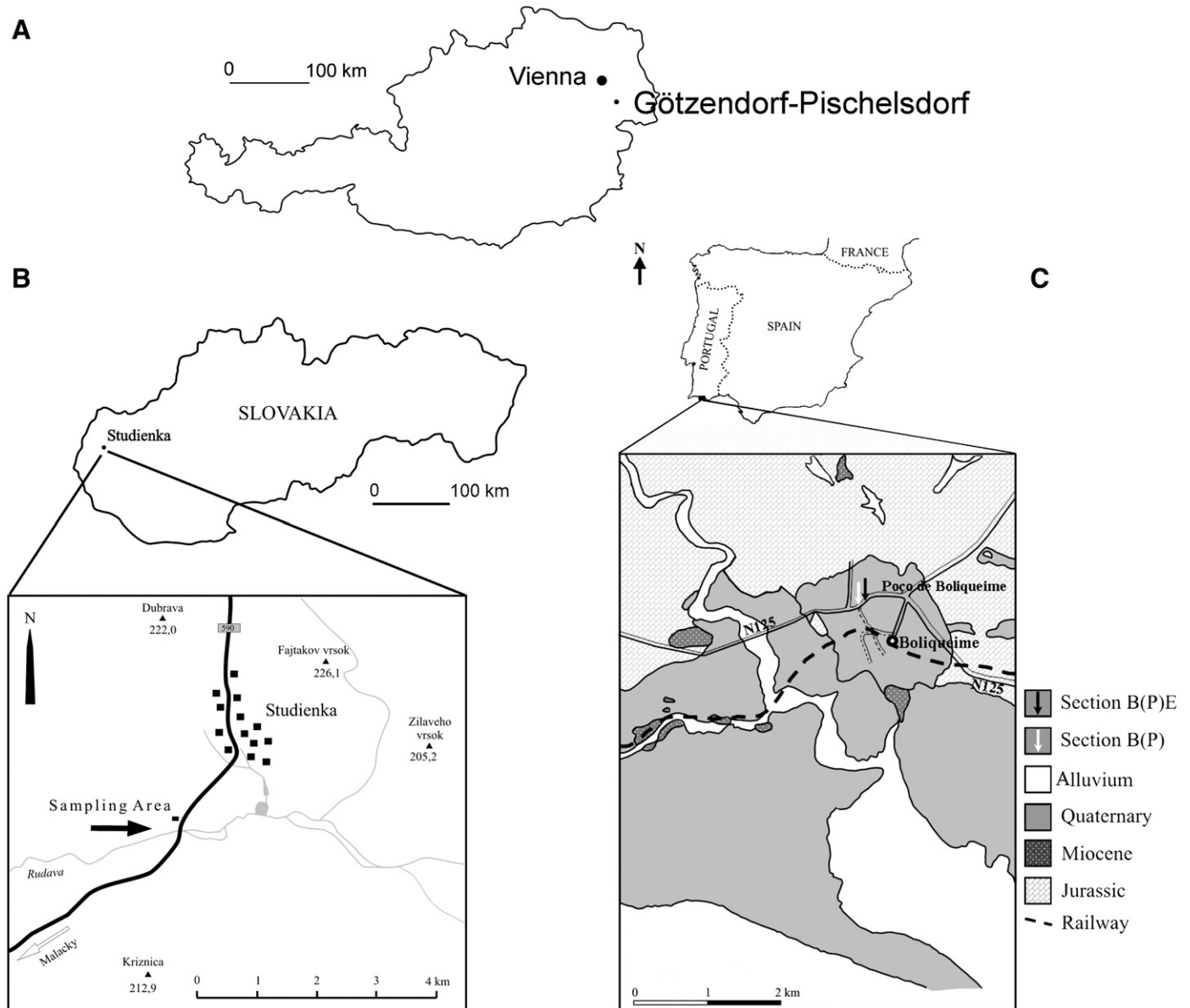

Fig. 1. Location of Götzendorf–Pischelsdorf (A), Studienka (B) and Boliqueime (C). 1 A. Location of Götzendorf–Pischelsdorf in Eastern Austria. 1 B. Position of the sample area situated near Studienka (Slovakia). (after Pipík et al., 2004, modified) 1 C. Geological map of Boliqueime area, Algarve, Portugal. (after Rocha, 1981; Manuppella, 1992, modified).

marshy sedimentation (lignite) with predominant coniferous pollen assemblages (Pipík et al., 2004). The sedimentation continues with bioturbated clay and grey and brown silt. The ostracod community, which contains 5 left and 3 right valves of *V. cylindrica*, appears in clayey layers. The overlaying laminated clays preserve rare freshwater and brackish ostracods, like *Amplocypris recta* (Reuss, 1850), *Darwinula stevensoni* (Brady and Robertson, 1870), *Vestalenula pagliolii* (Pinto and Kotzian, 1961), *Fabaeformiscandona balatonica* (Daday, 1894) and *Cyprideis heterostigma* (Reuss, 1850), which are indicators for the Upper Miocene in the Vienna Basin (Pipík et al., 2004). Generally, grain size decreases upwards and changes from sand to silt and clay. This fining-upward could be considered as a progressive loss of velocity of the water environment and calmer sedimentation (Pipík et al., 2004).

2.1.3. Boliqueime (Portugal)

The Portuguese material of *V. cylindrica* was first described by Cabral et al. (2005a). It was collected in Boliqueime, near Poço de Boliqueime, Algarve, southern Portugal (Fig. 1C), in two small field sections, B(P) – 1 m and B(P)E – 2 m, separated by a gap (Fig. 2). Coordinates: 37°07′30″ N and 08°09′25″ W. Both sections are represented by irregularly bounded, wavy, thin-bedded red-brownish sandstones and pelites, exhibiting clear grain-size variations. They are alternating with less common, weakly developed whitish calcrete levels and slightly ferruginous crusts (2–3 mm thick). A few
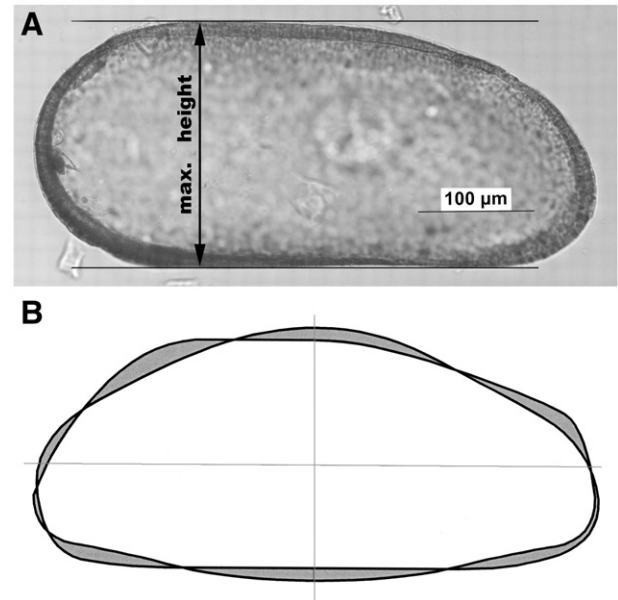
Fig. 3. Demonstration for how to measure the height of a valve (3 A) and area deviation of two valve outlines of *V. cylindrica* (3 B).

conglomeratic layers occur, which include rounded to subrounded greywacke and quartz clasts (length from 4 mm to 7 cm) and rare micas. The sample material from Portugal consists of 19 adult valves (8 left valves, 11 right valves). Based on the occurrence of ostracods such as *Candona neglecta* Sars, 1887, *Fabaeformiscandona* cf. *fabaeformis* (Fischer, 1851), *V. pagliolii*, *Vestalenula* cf. sp. B Danielopol, 1980, *Penthesilenula brasiliensis* (Pinto and Kotzian, 1961), *D. stevensoni*, *Ilyocypris bradyi* Sars, 1890 and *Ilyocypris gibba* (Ramdohr, 1808), a Pleistocene age is the most probable one (Cabral et al., 2005a).

2.2. Data analyses

All valves were photographed in external view using a Nikon light microscope and a Nikon digital camera and processed with the TPS-dig software, version 1.37 (Rohlf, 2003). With this TPS-programme (TPS stands for thin plate spline) it is possible to digitise (valve-) outlines as preparation for morphometric analyses (Zelditch et al., 2004), which are described later. Valve lengths and heights were determined with the software EclipseNET, version 1.20, measuring the maximum distance between two parallel lines, one tangential to the lowest point of the ventral margin and the other one tangential to the highest point of the dorsal margin (as shown in Fig. 3A). The morphometric analysis of the outlines was conducted with the software Morphomatica, version 1.6 (Brauneis et al., 2006a). A detailed description of this programme is presented in Brauneis et al. (2006b). It has to be noticed that the outlines of right valves were mirrored in that working process in order to get just one valve orientation. This allows the Morphomatica software to compare the outlines of left and right valves (Fig. 4).

For the reconstruction of valve outlines we used the B-splines approach adapted to ostracods and known as Linhart's algorithm (*cf.* Baltanás et al., 2003; Brauneis et al., 2006b). The Linhart's algorithm assumes that each outline is given by a sequence of (digitised) points $p_1, \ldots, p_n$. To compare the shape of two outlines, they first have to be superimposed. Ideally, this should be done so that the "difference" between them is as small as possible. But since this would be a very difficult task, the two outlines are instead positioned such that the centroids and the main axes of inertia coincide. To avoid differences between valves caused only by the valve size, the outlines have to be scaled appropriately. It seems to
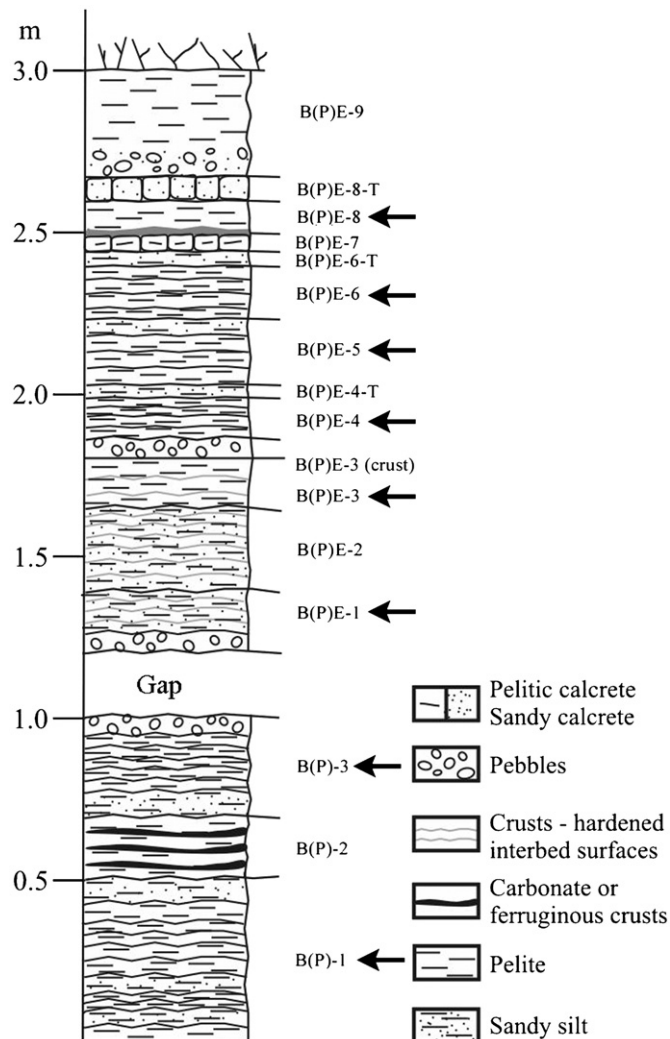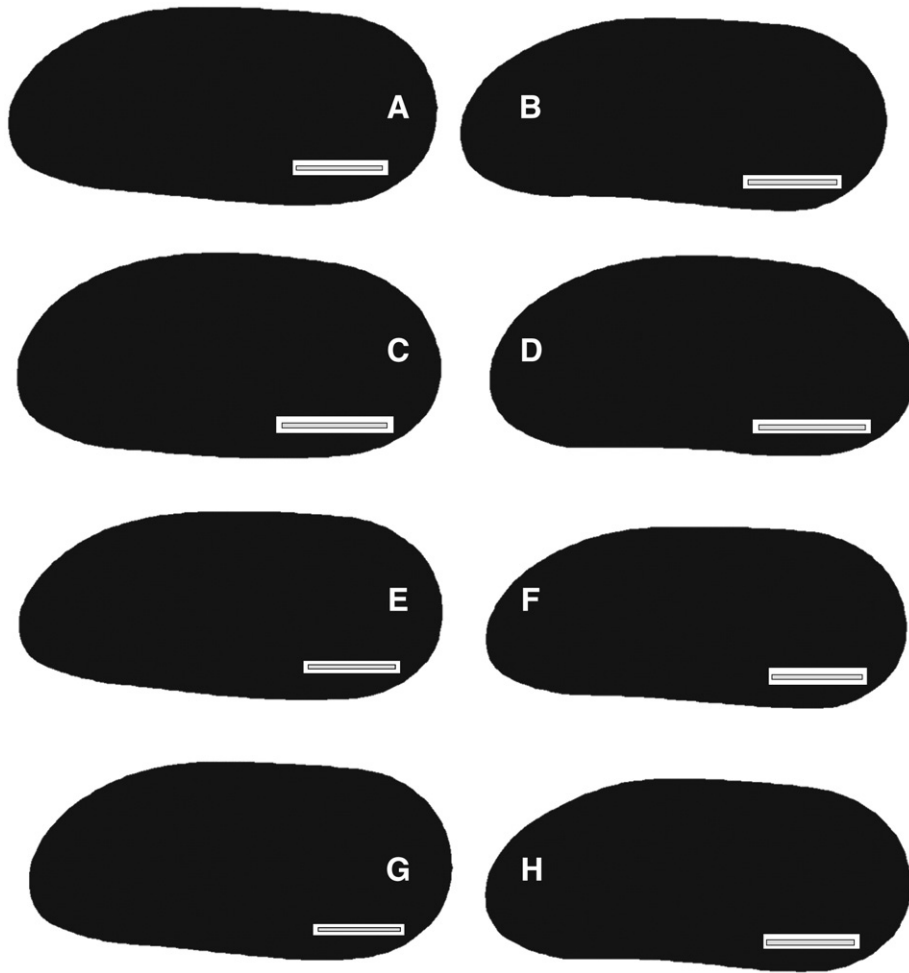

Fig. 2. Boliqueime, lithological sections, B(P) and B(P)E. Arrows mark layers with the studied *V. cylindrica*. (after Cabral et al., 2005a, modified).

Fig. 4. A–H: Calculated mean outlines of sampled *V. cylindrica* valves from Götzendorf–Pischelsdorf, Boliqueime and Studienka. All scale bars: 100 μm. A–D: Götzendorf–Pischelsdorf. E–F: Boliqueime. G–H: Studienka. All valves in external view (B, D, F, H are mirror images of original right valves). A: Adult, Lv; B: Adult, Rv; C: Juvenile, Lv; D: Juvenile, Rv; E: Adult, Lv; F: Adult, Rv; G: Adult, Lv; H: Adult, Rv.

be most natural to determine the scaling factor such that the outlines get equal area ("normalised for area").

B-splines are piecewise polynomial curves which may be used to approximate outlines. The shape of a B-spline is determined by relatively few control points, which together form the so-called control polygon. In the case of quadratic B-splines, the sides of this polygon are tangential to the B-spline curve. Thus the control points provide a good and intuitively appealing means to describe an outline by a few parameters, namely the coordinates of the control points. More details may be found in Baltanás et al. (2003).

A very appropriate measure for the difference of two superimposed (and scaled) outlines is the so-called area deviation. This is the area of the part of the plane, which is inside the first outline and outside the second or vice versa. Thus it may be viewed as the area "between" the outlines (the shaded region in Fig. 3B). To compute it, we first approximate the outlines by B-spline curves. Then we compute a large number (say 100) of points on each curve corresponding to equally spaced parameter values. These points define two closed polygons. Their area deviation may be considered as a good approximation of that of the original outlines. By computing mean shape outlines of original TPS data using the Morphomatica 1.6 software, the valve outlines can be displayed in a suitable way for visualising the shape differences (*cf.* Fig. 4).

One of the central problems in the reconstruction of the outlines is the computation of the centroid and the main axis of inertia. We present here information concerning the approach used for the ostracod material.

The simplest way would be to compute the arithmetic mean of the coordinate vectors of the points $p_1,\ldots,p_n$, that is

$$c = \frac{1}{n}\sum_{i=1}^{n} p_i.$$

This gives the centroid of these points, not of the real outline. Consequently, it only makes sense if the points are distributed very uniformly. If parts of the outline are rugged or jagged, there will be relatively many points p_i concentrated in these parts and c tends to move towards them. So the centroids of two very similar outlines may be rather different, if only one has some rugged parts. This is why the above centroid should be replaced by the center of gravity of the domain A surrounded by the given outline. This center, s, is defined by

$$s = (x_s, y_s) = \frac{1}{a}\int_A (x,y)\, \mathrm{d}x\mathrm{d}y,$$

where x_s and y_s denote the coordinates of s, and a is the area of A. It turns out that s can be computed in the following rather simple way (x_i and y_i denote the coordinates of p_i, and the points are given in counterclockwise order):

$$x_s = \frac{1}{6a}\sum_{i=1}^{n} (y_{i+1} - y_i)(x_i^2 + x_i x_{i+1} + x_{i+1}^2),$$
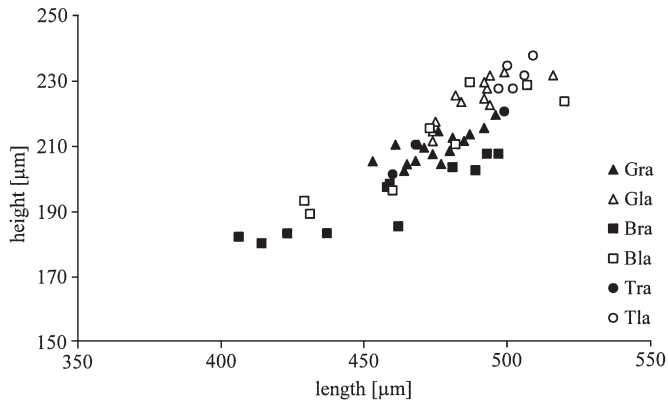
Fig. 5. Valve size dispersion diagram for *V. cylindrica* from Götzendorf–Pischelsdorf, Boliqueime and Studienka. Bla: Boliqueime left adult; Bra: Boliqueime right adult; Gla: Götzendorf–Pischelsdorf left adult; Gra: Götzendorf–Pischelsdorf right adult; Tla: Studienka left adult; Tra: Studienka right adult.

and similarly y_s (with x and y interchanged and the whole expression multiplied by −1). Of course, (x_{n+1}, y_{n+1}) is understood to be equal to (x_1, y_1).

Similarly to the centroid, the axes of inertia should also be calculated not only for the points, but for the whole outline or, more precisely, for the domain surrounded by the outline. The moment of inertia to an axis (passing through the origin) is defined as the integral of the squared distance from this axis, taken over the considered domain. It may be computed in a similar, but somewhat more complicated way as the center of gravity above. If the moment of inertia is not equal for all axes directions, there is a unique direction, which yields the minimum moment, and this is taken to be the new x-axis. This direction is given by an eigenvector of a certain 2×2 matrix and thus is not difficult to compute. The new y-axis is, of course, perpendicular to the new x-axis and corresponds to the maximum moment of inertia.

Multivariate statistic analyses of the data were performed with the computer programmes of the Primer 6 (Plymouth Routines In Multivariate Ecological Research) package (Clarke and Gorley, 2006). We used non-metric Multidimensional Scaling (n-MDS) and the randomisation test for difference of groups, ANOSIM (analysis of similarities), for 1-way layout (Clarke and Warwick, 2001).

The n-MDS used is a non-parametric ordination method, which constructs a "map" of the distances between the various outline-shapes. Because these distances are expressed as the ranks of their degree of dissimilarity and not of their absolute differences no axes are
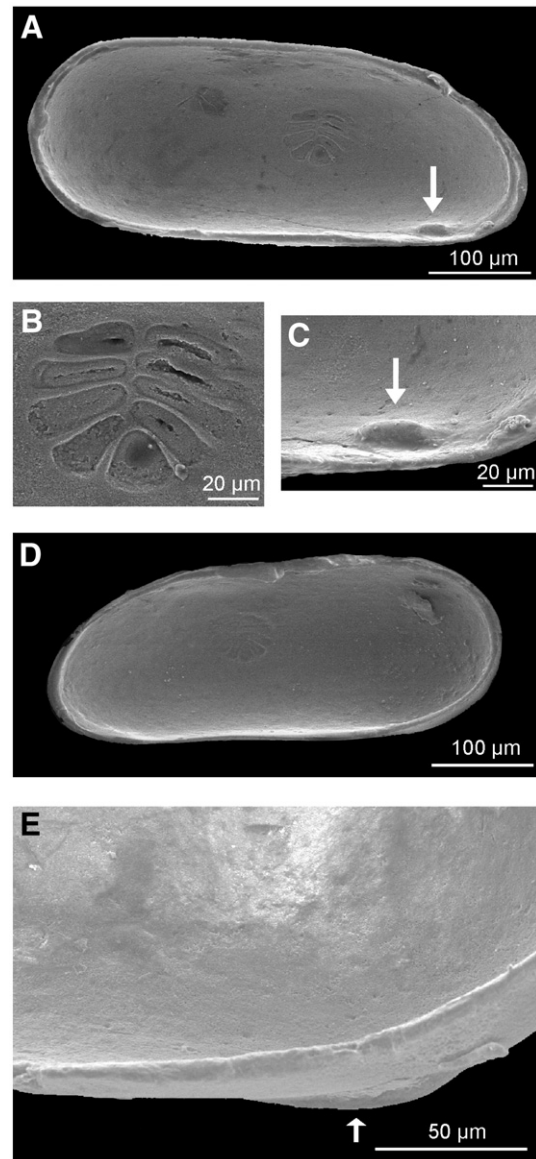

Fig. 6. SEM pictures showing valve details of *V. cylindrica* from Götzendorf–Pischelsdorf. A: Lv, internal view, B: Lv, detail of muscle scar, C: Lv, detail of anterior ventral tooth, D: Rv, internal view, E: Rv, detail of posterior keel. Arrows mark valve features mentioned in the text.

Table 1
Mean length, height and h/l ratio of all *V. cylindrica* valves from Götzendorf–Pischelsdorf, Boliqueime and Studienka

Götzendorf–Pischelsdorf (N=41)	Mean length (±SD)	Mean height (±SD)	Mean H/L [%] (±SD)
Lv adults (n=12)	489.08 (±12.18)	223.83 (±6.87)	45.76 (±0.79)
Rv adults (n=17)	474.94 (±12.4)	209.25 (±5.1)	44.07 (±0.72)
Lv juveniles (n=6)	399.00 (±8.94)	191.83 (±2.71)	48.08 (±0.75)
Rv juveniles (n=6)	395.83 (±10.55)	183.00 (±4.62)	46.45 (±0.78)
Studienka (N=8)			
Lv adults (n=5)	502.00 (±4.76)	231.20 (±4.38)	45.98 (±0.67)
Rv adults (n=3)	475.67 (±20.60)	210.33 (±9.50)	44.22 (±0.60)
Boliqueime (N=19)			
Lv adults (n=8)	473.63 (±32.75)	210.38 (±15.58)	44.32 (±1.28)
Rv adults (n=11)	459.60 (±32.44)	194.40 (±10.80)	42.44 (±1.29)

N=total number of specimens at one location. n=part of all specimens at one location; e.g. just left or right valves or juveniles.

displayed in a n-MDS plot. We used this method to demonstrate dissimilarity differences within and among samples. The nonparametric test ANOSIM is a permutation/randomisation method, analogue to univariate ANOVA, which tests for significant differences between groups of samples. The 1-way layout ANOSIM is based on the corresponding rank similarities between samples using the following statistic test:

$$R = (\bar{r}_B - \bar{r}_W)/1/2M$$

$\bar{r}_B$ is defined as the average of all rank similarities among items within one sample; $\bar{r}_W$ is the average of rank similarities arising from all pairs of items between different samples; $M = n(n-1)/2$, where n is the total number of items under consideration. The value of R varies between 1, when samples are completely separated, and 0, if the null hypothesis, that there is no separation on the averages between and among samples, works. As a rule of thumb, Clarke and Gorley (2001, p. 60) consider that
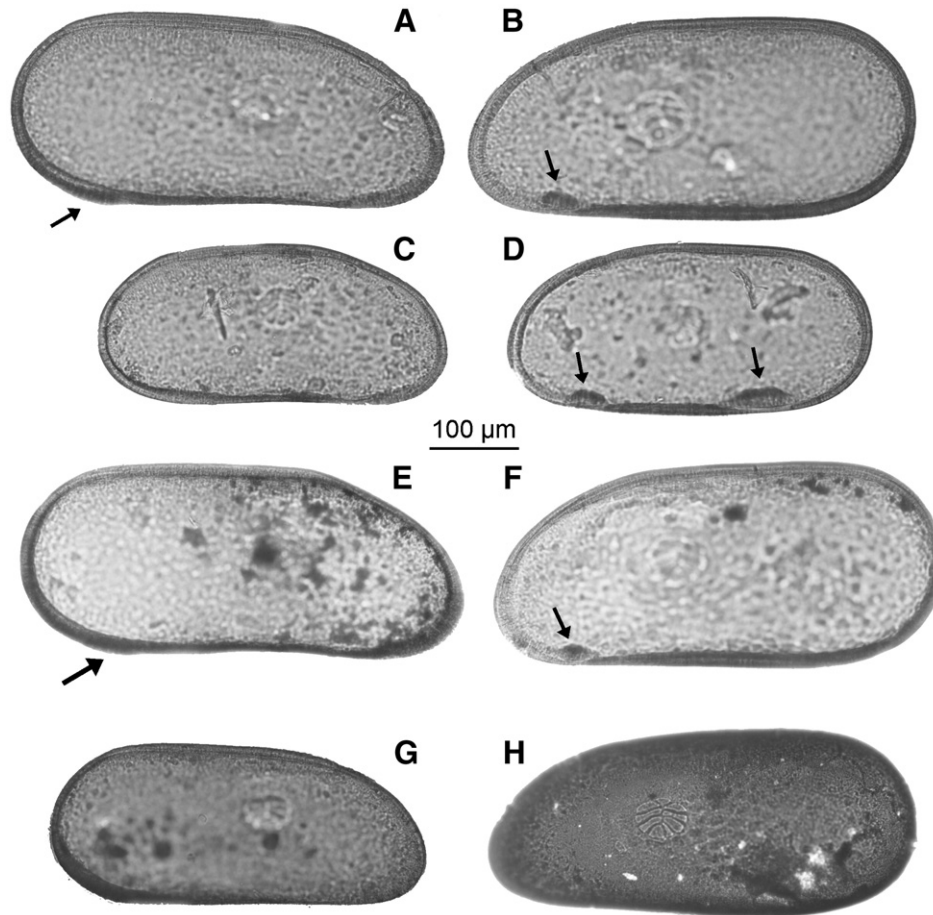
Fig. 7. *Vestalenula cylindrica* from Götzendorf–Pischelsdorf (A–D), *V. cylindrica* from Studienka (E–F), *V. cylindrica* from Boliqueime (G–H). All valves were photographed in external view using a transmitted light microscope. A: Adult, Rv; B: Adult, Lv; C: Juvenile, Rv; D: Juvenile, Lv; E: Adult, Rv; F: Adult, Lv; G: Adult, Rv; H: Adult, Lv. Arrows mark valve features mentioned in the text.

pairs of samples, which display an $R \geq 0.75$ are well separated from each other, those with $0.5 \leq R \leq 0.75$ as overlapping but clearly different and those with $R \leq 0.5$ barely separable at all.

The pattern of valve disparity, respectively the degree of shape dispersion (or variation) in the morphospace within populations was estimated using the mean pair-wise distance index (MPD) of the area deviation. It is similar to the solution proposed by Ciampaglio et al. (2001), who computes Euclidian distances. For that, the non-parametric bootstrap mean and the 95% confidence limits with 5000 permutations were computed, using the computer programme "Ecological Methodology" which accompanies the similarly titled text-book of Krebs (1999).

Basic descriptive statistics of mean, minimum, maximum, range, standard deviation and confidence limits as well as a Kolmogorov–Smirnov test to check for goodness of fit of the data to a normal distribution were executed with the BIOMstat 3.3 software (Rohlf, 2002).

3. Results

3.1. Götzendorf–Pischelsdorf

The ostracod material of Götzendorf–Pischelsdorf consists of left adult valves with a minimum of 474 µm and a maximum of 516 µm in length and a minimum of 211 µm and a maximum of 232 µm in height. Right adult valves differ from 461–496 µm in length and from 202–219 µm in height. The left juveniles show a range from 387–410 µm in length and 188–195 µm in height, right ones from 385–407 µm in length and 177–190 µm in height. As expected, the right valves show a

slightly more elongated shape than the overlapping left valves, which can be seen in the valve height/length ratio (Fig. 5, Table 1). In contrast to the adult Rv, on the adult Lv an internal anterior tooth is visible (Fig. 6A, C and D), which is typical for the genera *Vestalenula*, *Microdarwinula* and *Penthesilenula* (it lacks in genus *Darwinula*). As characteristic for darwinulid ostracods, the central muscle scars are arranged in form of a rosette (Fig. 6B). A posterior, elongated keel on the right valve of the adults (Figs. 6E and 7A) can clearly be seen,
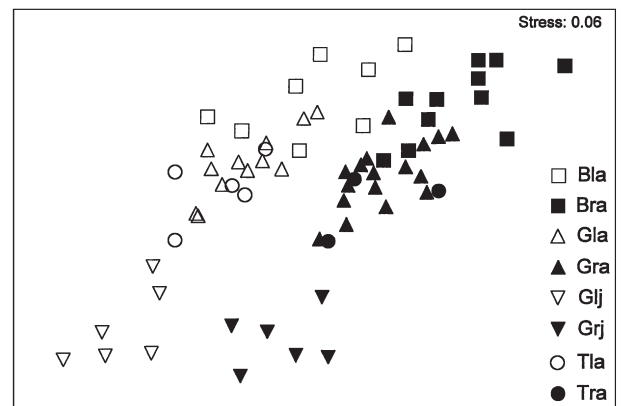

Fig. 8. n-MDS (non-metric Multidimensional Scaling) plot for *V. cylindrica* from Götzendorf–Pischelsdorf, Boliqueime and Studienka. Glj: Götzendorf–Pischelsdorf left juvenile; Grj: Götzendorf–Pischelsdorf right juvenile; Other abbreviations see Fig. 5.

Table 2
ANOSIM test

Groups	R statistic	P Significance level
Bra–Grj	0.996	0.001
Bla–Glj	0.985	0.001
Gra–Grj	0.970	0.001
Gla–Glj	0.941	0.001
Glj–Grj	0.933	0.002
Glj–Tla	0.915	0.002
Tla–Tra	0.908	0.018
Grj–Tra	0.883	0.012
Gla–Gra	0.856	0.001
Bra–Tra	0.561	0.008
Bra–Gra	0.507	0.001
Bla–Tla	0.481	0.007
Bla–Gla	0.449	0.001
Gra–Tra	0.145	0.190
Gla–Tla	0.001	0.410

Pairwise tests among groups of *V. cylindrica* from Götzendorf–Pischelsdorf, Boliqueime and Studienka.

which points to the *danielopoli* group of the genus *Vestalenula* (Rossetti and Martens, 1998). Besides the latter character, which is missing in left adult valves (Fig. 7B), juveniles additionally have a posterior tooth on their left valves, whereas there are no teeth on right juvenile valves (Fig. 7C and D). The separation of left and right juvenile valves can be visualized in a very apparent way with a n-MDS-plot and a table displaying ANOSIM data (Figs. 7 and 8; Table 2). As one can see in Table 2, all groups containing juvenile valve data have values of R>0.75 and are therefore separated clearly. A higher variability in the left valves is reflected by larger confidence limits for the bootstrapped mean estimates than in the right valves (Table 3). Compared to the adults, the juvenile valves show more symmetric and less elongated outlines; a fact which is obvious by calculating the mean outlines from adult and juvenile left and right valves (Fig. 4A–D).

3.2. Studienka

Left adult valves from Studienka vary between 497–509 μm in length and 227–237 μm in height, whereas right ones feature a range between 460–509 μm in length and 201–220 μm in height (Table 1). The specimens show the typical characters of the *Vestalenula danielopoli* group like the posterior, elongated keel on the Rv (Fig. 7E) and the anterior tooth on the Lv (Fig. 7F). It has to be noticed that the valve variability as well in the height as in the length is much higher in the right valves than in the left valves (*cf.* the higher standard deviation in the height and length distribution of the right valves, Table 1).

3.3. Boliqueime

11 right adult valves (406–497 μm length; 180–207 μm height; Fig. 7G) and 8 left adult valves (429–520 μm length; 189–229 μm height; Fig. 7H) feature a very high variability among each other (Table 1), as well in valve size (Fig. 5) as also in the shape of the valve outline (Fig. 8). This can also be seen in the higher MPD of the

Portuguese material compared to those from Austria and Slovakia (Table 3). Although the right and left valves present high size variability, this variation is more considerable in the left valve, especially for the measurements of valve height (Table 1). By creating calculated mean outlines of the left and right valves, the more elongated shape of the right valve can be seen clearly (Fig. 4E and F).

4. Discussion

The analysis presented here is based on a high number of specimens of *V. cylindrica* from Götzendorf–Pischelsdorf. Therefore it offers an excellent possibility to compare populations faraway in space and time, like those from Central Europe (Götzendorf–Pischelsdorf and Studienka of the Upper Miocene) and those from south-western Europe (Boliqueime of a most likely Pleistocene age). If one compares the juveniles which were found in the material from Götzendorf–Pischelsdorf in a pairwise test among each other and with valves from the other populations, they all are clearly separated (Fig. 7, Table 2). The variability for both the shape (Table 3) and the size (Table 1) of the adult valves is higher in the Portuguese population as compared to the Götzendorf–Pischelsdorf one.

4.1. Relationships between populations at the European scale

The *V. cylindrica* at Götzendorf–Pischelsdorf apparently lived under sub-tropical climate conditions in a shallow, stagnant or slowly flowing floodplain area, in which carbonate rich sediments accumulated. Rögl et al. (1993) and Harzhauser and Tempfer (2004) suggested that the Götzendorf–Pischelsdorf area was a wetland and as such one should be comparable to the present-day wetland of the Lobau at Vienna (Danielopol et al., 2000). In the recent oxbow systems of the Danube like the Eberschüttwasser, we observed rich populations of Darwinulidae (*D. stevensoni* Brady and Robertson, 1870) living in shallow water between the roots of aquatic macrophytes or even colonising the superficial layer of the alluvial sediments below the bottom of the oxbow lakes.

The population of Studienka which is time-coeval with the Götzendorf–Pischelsdorf population apparently lived in the littoral area of a shallow eutrophic lake (Pipík and Bodergat, 2003; Pipík et al., 2004). It is interesting that the morphology of the few investigated specimens resembles more those of Götzendorf–Pischelsdorf than those of Boliqueime. The contrasting degree of morphologic variability of Boliqueime and Götzendorf–Pischelsdorf populations suggests that the environmental conditions at the latter site were less fluctuating.

4.2. The high morphological variability of the Portuguese population

The Portuguese *Vestalenula* shells were sampled from red pelite and sandstone layers which alternate with indurated calcrete sediments. This lithology suggests that the habitat of *Vestalenula* was a body of shallow water within a wetland area (either a fluvial-palustrine or an alluvial-palustrine one) with strong water level fluctuations or astatic conditions (Cabral et al., 2005a). The succession of non-indurated and indurated soil layers as well as the interbedded strata of fine grained sediments (pelites) with coarser sediments

Table 3
MPD with lower and upper confidence limits of left and right valves from *V. cylindrica* from Götzendorf–Pischelsdorf, Boliqueime and Studienka

	Götzendorf–Pischelsdorf Lv (n′=66)	Götzendorf–Pischelsdorf Rv (n′=136)	Boliqueime Lv (n′=28)	Boliqueime Rv (n′=55)	Studienka Lv (n′=10)	Studienka Rv (n′=3)
MPD (mean pairwise distance)	19.326	19.091	27.577	22.985	19.033	23.385
Lower confidence limit (95%)	17.500	18.012	24.490	20.720	15.401	18.100
Upper confidence limit (95%)	21.341	20.188	30.890	25.229	23.013	28.570

n′=(n*(n−1))/2. (n is effective number of specimens, whereas n′ is a multiple of n, caused by the matrix calculated through pairwise comparison).

(conglomerates) indicates an environment with variable conditions of depositional energy. The red colour of the sediment and the carbonated-ferrigineous crusts as well as the co-occurrence of ostracods which are known to live in warm climate areas with *V. cylindrica* (this is the case with *Zonocypris* and *Sclerocypris* species) suggests that the *Vestalenula* population at Boliqueime developed under subtropical warm climate with contrasting seasons (Cabral et al., 2004, 2005b). The ostracod assemblage at Boliqueime is well diversified. Besides *V. cylindrica* there are three other darwinulids, *V. pagliolii*, *P. brasiliensis* and *D. stevensoni*. This suggests that the aquatic environment offered good conditions for benthic creeping meiofauna as the darwinulids (Cabral et al., 2005a).

In our opinion, the high morphological variability observed in the Portuguese material is not caused by the low sample size, especially if it is noticed that the variability in Studienka is not as high as in Boliqueime but the number of valves is lower. One should expect an increase of the phenotypic variability with the increase of the number of valves found in the samples, hence a higher degree of disparity for the Götzendorf–Pischelsdorf population. Another possibility could be the difference in the origin of the studied valves. While those from Götzendorf–Pischelsdorf originate from the same sampled horizon, those from Boliqueime come from different layers (Fig. 2).

4.3. Variability of European populations

The morphologic variability of the Portuguese valves could be the reflection of the environmental variability at Boliqueime during the time compared to the two sites in Central Europe. Considering a similar study dealing with the limnic ostracod *Eucypris virens* (Jurine), Baltanás et al. (2002) made the following remark about this latter species which could apply to the *V. cylindrica* population from Boliqueime too: " … the genotype maybe one of the … important driving forces in determining shape and shape variability in ostracod populations." Hence the interest to insist during future investigations on darwinulid palaeogenetics is at hand.

The *R* statistic of the ANOSIM test for the couples of Portuguese–Austrian populations and respectively the Slovakian one, approach a value of ~0.5 which points out to partial overlapping populations (Table 2), which is also visible in the n-MDS-plot (Fig. 8). If Slovakian valves are compared with Austrian ones, the values for *R* statistic lie between 0.001 and 0.145 and a *P* value of 0.19 and 0.41 which points to a lack of separation of the two populations (Table 2). Valves from Götzendorf–Pischelsdorf and Studienka cluster together in a better way than Boliqueime-Götzendorf–Pischelsdorf or Boliqueime-Studienka (ANOSIM, Table 2). That can also be shown by comparing area deviations between computed mean valve outlines from Götzendorf–Pischelsdorf versus Boliqueime (Fig. 9A and B), Götzendorf–Pischelsdorf versus Studienka (Fig. 9C and D) and Boliqueime versus Studienka (Fig. 9E and F). By doing so, the more elongated shape of all right valves is clearly visible. Furthermore the nearly equal overlap of Austrian and Slovakian outlines is very remarkable (Fig. 9C and D). Left valves of Studienka do not overlap completely with valves from Götzendorf–Pischelsdorf, whereas right valves overlap very well with those valves (Fig. 8). While the MPD of the left valves are nearly the same as of the left valves from Götzendorf–Pischelsdorf, they differ clearly in the right valves which can be explained by the small sample size (3 specimens) of right Slovakian valves (Table 3). Outlines from Boliqueime compared to those from Austria and Slovakia show a strong trend for morphological differentiation (Fig. 9A, B, E and F). As a result the sample from Boliqueime is separated well from those from Götzendorf–Pischelsdorf and Studienka, whereas the latter one is not significantly different from Götzendorf–Pischelsdorf. All these observations point to a closer connection among the Central European populations than to the south-western European population of Portugal. It is important to emphasise that the divergence of the valve morphology occurs among populations, which are geographically widely separated. Valves from Boliqueime show a trend towards
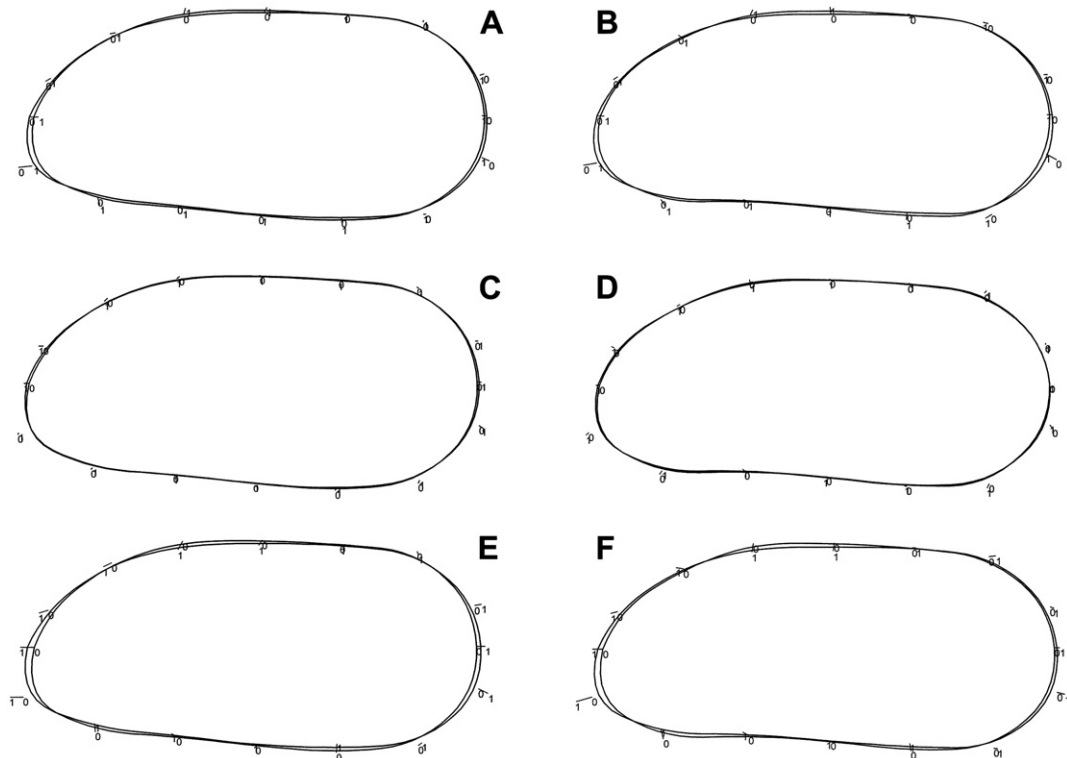
Fig. 9. Comparison of calculated mean outlines of valves of *V. cylindrica* from Götzendorf–Pischelsdorf, Boliqueime and Studienka. A: Gla (1)–Bla (0), B: Gra (1)–Bra (0), C: Gla (1)–Tla (0), D: Gra (1)–Tra (0), E: Bla (1)–Tla (0), F: Bra (1)–Tra (0). Abbreviations see Fig. 5. 0 and 1 in brackets show from which locations compared valves come from.

a decrease in the valve size, both in length and height (Fig. 5) and towards a general elongation of the valve shape.

4.4. Comparison with other Darwinulidae

The elongation of the valve is also characteristic for a newly discovered Recent subterranean species of the genus *Vestalenula* in southern France (Artheau, 2006). The *V. cylindrica* from Portugal shows more morphological affinities to this new species than to the *V. cylindrica* from Götzendorf–Pischelsdorf and Studienka. For that reason we consider that the Boliqueime morphotype is the derived state compared to the Götzendorf–Pischelsdorf-Studienka type. This points to a possible existence of a geographical micro-evolutionary pattern as well in Central- as also in South-West Europe. A similar pattern of geographical differentiation of the valve morphology was noticed by Pinto et al. (2004) for *P. brasiliensis*. This latter species occurs in South America (Brazil), Africa and Europe. Pinto et al. (2004) distinguished morphologically four main lineages, two in Brazil, one in South Africa and one in Ireland.

Without knowing the size variability and without looking to the inner morphological details, it is difficult to separate the *Vestalenula* populations optically just using the stereo-microscope. Therefore we want to emphasise that the B-spline algorithm and the area deviation method, used here and implemented in the new version of the "Morphomatica" programme (Brauneis et al., 2006a,b) combined with multivariate statistical techniques like the non-metric dimensional scaling offer excellent possibilities to describe the subtle morphological differences in the valve outlines.

5. Conclusion

An Upper Miocene population of adult and juvenile valves of *V. cylindrica* from Götzendorf–Pischelsdorf (Austria) was morphologically compared among each other and with adult specimens from a second Upper Miocene location in Studienka (Slovakia) and one probable Pleistocene location in Boliqueime (southern Portugal). By comparing length, height and h/l ratio, the valves from Central Europe (Austria and Slovakia) cluster together, while those from South-West Europe show clear morphologic differences. The Portuguese ostracods are more elongated and show a higher morphologic variability than the populations from Central Europe. By taking into consideration the new Recent hyporheic species from southern France (Artheau, 2007), which morphologically resembles the Portuguese type, this trend of valve elongation could be explained as some kind of microevolution within the genus *Vestalenula*.

In conclusion, we consider that our comparative approach of the morphological studies of fossil darwinulids should be extended. It is expected that similar microevolutionary patterns will occur within other widely distributed species such as *P. brasiliensis*, discussed above (*cf.* Rossetti and Martens, 1998; Pinto et al., 2004; Cabral et al., 2005a).

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