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Rings without a lord? Enigmatic fossils from the lower Palaeozoic of Bohemia and the Carnic Alps

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Fossilized ring-like structures with enigmatic function and taxonomic affiliation were recovered for the first time from the Upper Ordovician of the Carnic Alps and the Silurian of Bohemia. These rings, already mentioned as minor constituents in previous conodont studies (e.g. Webers 1966, p. 1; Bischoff 1973, p. 147), were reported from the Palaeozoic of several regions in Europe and North America. Originally considered as inwardly accreted adhering discs of a benthic hyolithelminth worm with a phosphatic tubular projection, they were later reinterpreted in relation to a putative crinoid epibiont or even as possible scyphozoans. Despite a long debate, neither the function of the enigmatic Palaeozoic rings nor their taxonomic affiliation has been fully clarified. The studied material, extracted by a standard technique in use for conodonts, consists of 235 elements from 16 stratigraphic levels in the Plöcken Formation (Carnic Alps, Cellon Section; *Amorphognathus ordovicicus* Biozone, Hirnantian, Ordovician) and in the Kopanina Formation (Bohemia, Mušlovka Quarry; *Polygnathoides siluricus* Biozone, Ludfordian, Silurian). To explore whether ring size and shape changed over time, we employed a novel combination of geometric morphometric approaches for outlines with no 'homologous' landmarks and showed that only size appreciably varied with an increase of ca. 20%. The emerging data from this study are consistent with the interpretation of the rings as an adhering structure of a benthic organism living on a relatively uniform hard substrate. ☐ *Phosphatic rings, Problematica, Palaeozoic, morphometric analysis.*

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Whilst picking heavy residues in search for conodont elements, special care is taken to search for other organisms or mineralogical phases that can contribute new knowledge on the palaeoecology, biosedimentology or living conditions in general of the analysed strata. During a study for a precise calibration of conodont and graptolite biozonations, specific intervals of the Silurian from Bohemia yielded numerous phosphatic plates of uncertain affinity described as *Eurytholia bohemica* by Ferretti *et al.* (2006). Equally enigmatic phosphatic ring-like structures were also recovered. With the recent recovery of similar material from the Ordovician of the Carnic Alps, we decided to focus our attention on the study of these peculiar elements.

Rings similar to the ones described in this study were mentioned initially as minor constituents in conodont studies. Webers (1966) described 'small lamellar, phosphatic, circular forms' from the Ordovician of Minnesota (p. 72, pl. 14, figs 3, 6). Bischoff (1973) analysed a collection of about 120 circular, more rarely oval-shaped, elements ranging in diameter between 0.29 and 1.08 mm. He interpreted these structures as 'fixation-discs' of conulariids.

Müller *et al.* (1974) were the first authors to provide a specific study dealing only with these enigmatic rings. They described in detail a rich collection of over 1300 elements ranging in age from the Late Cambrian to the Late Devonian from several regions (U.S.A., Canada, Iran, Sweden, Germany, Austria and Belgium). The dimensions of the rings ranged between 0.13 and 0.8 mm and their composition, detected by EDAX analysis, was determined to be

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Dahllite. No presence of fluorine was revealed. The authors tentatively considered the rings, characterized by a phosphatic tubular projection, as inwardly accreted adhering discs built by a benthic worm (Order Hyolithelminthes Fisher, 1962). Owing to the putative extreme fragility of the tubular extension, with a thinner wall and a decreasing diameter moving away from the base, and possibly as a result also of the lab processing techniques, the recovery of the single rings broken on their upper surface (where the membranous structure had been detached) seemed likely. According to these authors, the animals attached to the substratum at a larval stage with the basal ring, which subsequently grew inwards, with the outer dimension of the ring preserving the original maximum diameter. Rings having a small inner diameter, therefore, represent mature organisms, whereas rings with a large inner diameter possibly represent juvenile forms. The enigmatic structures were assigned by Müller *et al.* (1974) to the new genus *Phosphannulus*, and the single new species *Phosphannulus universalis* was described, although these authors recognized that this may in fact encompass several species due to its wide geographical and stratigraphical range (Müller *et al.* 1974, p. 89).

Phosphannulus was subsequently reinterpreted as part of a putative epibiont living attached to crinoidal stems (Welch 1976). This author reported Carboniferous and Permian phosphatic tubes, up to 3-cm long and having an outer mean diameter of 0.65 mm and a basal circular–ellipsoidal expansion (1.67 × 1.91 mm), which he interpreted as the attachment structure to the crinoids. With this living strategy, *Phosphannulus* was able to live increased from the substrate and thus enhance its filter-feeding activity.

Since 1976, despite a long debate that included a further reinterpretation as scyphozoans (e.g. Bischoff 1989) or euconodonts (Buryi & Kasatkina 2005), neither the function of the enigmatic Palaeozoic rings nor their taxonomic affiliation have been clarified. In this study, for the first time, we applied a combination of scanning electron microscopy, and chemical, mineralogical and morphometric techniques to provide a quantitative assessment of the rings variability in composition, dimension and shape, in order to hopefully help solve the mystery of their origin in the future.

Geological setting

The Carnic Alps and Bohemia (Fig. 1) represent two key areas to better unravel the lower Palaeozoic faunal and palaeogeographic evolution of the Peri-Gondwanan Europe. The continuity of the successions exposed in these regions, and their relatively low-grade metamorphic overprint, has preserved a unique range of palaeontological information.

In the Central and Western Carnic Alps, the Middle to Upper Ordovician is represented by a tripartite sequence of rocks with clastics to volcanoclastics at the base, overlain by a limestone-dominated succession with a few metres of sandstones at the top. Two major facies associations occur in the Upper Ordovician of the Central Carnic Alps: shallow-water environments are characterized by quartz arenites and greywackes together with massive cystoid-rich limestones (Wolayer Limestone Formation), whereas more basinal settings are represented by shales and bedded wackestones (Uggwa Limestone Formation). In deeper water settings, the Hirnantian Plöcken Formation, belonging to the *Normalograptus persculptus* graptolite Zone, succeeds the latter and provides unequivocal evidence of the Hirnantian glaciation in this region, as indicated by the presence of diamictite deposits (Schönlaub *et al.* 2011).

The Cellon section (Fig. 2) is exposed in the Cellon avalanche gully near the Plöcken Pass, at an altitude of 1500 m, ca. 1 km from the Austrian–Italian border. Bed-by-bed conodont sampling has been undertaken in the Cellon area for many years. Fourteen stratigraphic levels have been investigated through the Uggwa Limestone Formation and the Plöcken Formation. No enigmatic rings were detected within the residues of the Uggwa Limestone Formation. A few enigmatic rings, however, have been recovered from the Plöcken Formation, either from the basal impure bioclastic limestone lenses, intercalated with siltstones and also containing fossils of the *Hirnantia* brachiopod fauna (level 5), or from impure pyritiferous limestones intercalated in sandstones (levels 6 and 7) higher up in the unit (Table 1). These horizons are represented by bioclastic limestones, rich in ostracodes, echinoderms, trilobites, brachiopods, sponge spicules and gastropod biodebris, commonly pyritized, that clearly show signs of redeposition.

The Upper Silurian succession preserved in the Barrandian area of central Bohemia comprises biodetrital limestone-dominated shelf facies in the northern, northeastern and central part of the Prague Synform, and shale-dominated hemipelagic successions in the southern and southwestern part of the Synform. Ludlow sediments, including the limestones, shales and local volcaniclastics, comprise a single lithostratigraphic unit – the Kopanina Formation – that is overlain conformably by the Požáry

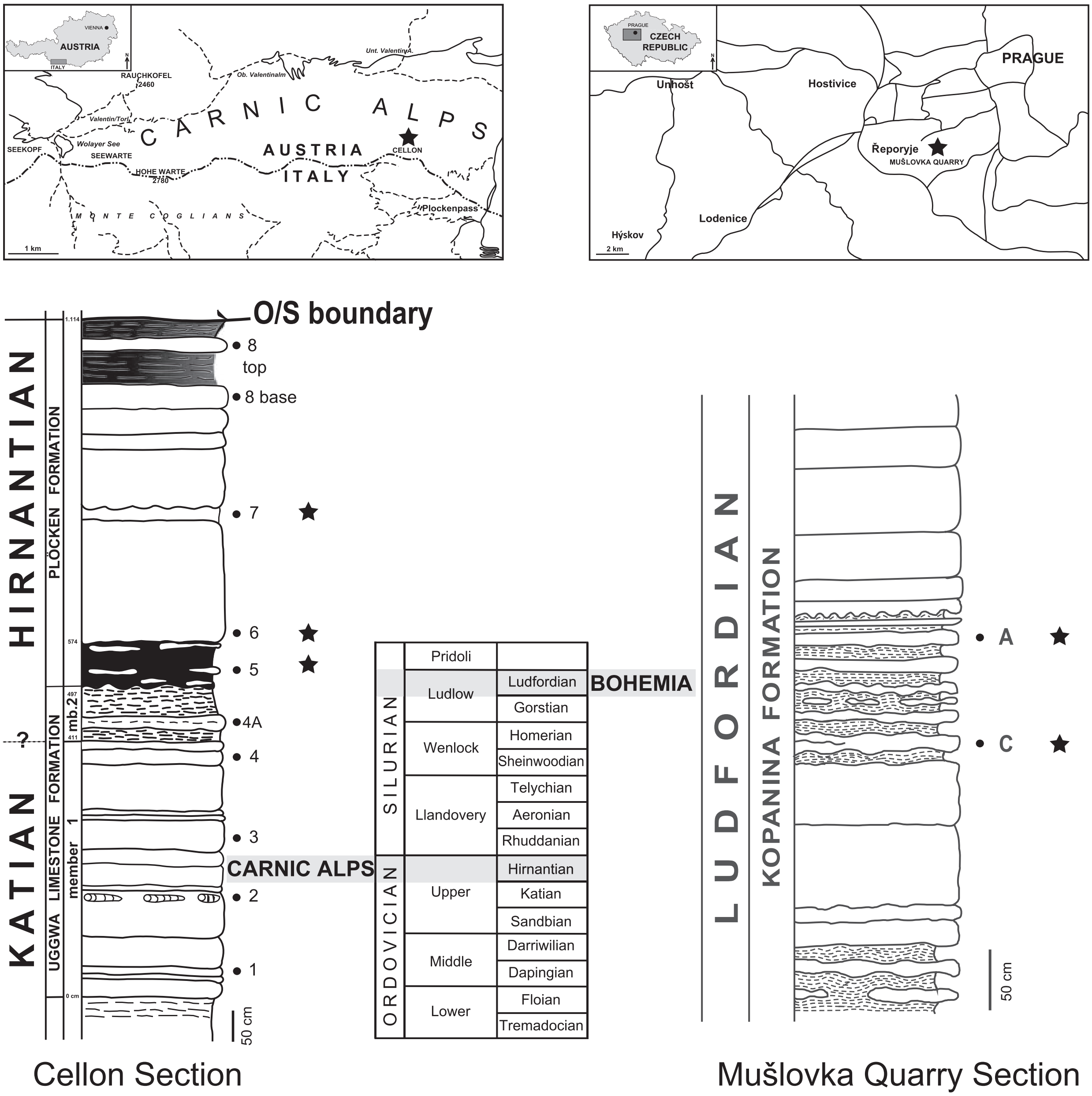

Fig. 1. Location map of the two investigated localities. Stars indicate position of sampled localities (topmost boxed areas) and productive levels in the Cellon and Mušlovka Quarry sections. Simplified stratigraphical section from Cellon modified after Schönlaub *et al.* (2011).

Formation of Pridoli age (see Kříž *in* Chlupáč *et al.* 1998 for review).

Richly fossiliferous biodetrital limestones of the upper Kopanina Formation, exposed in the face of the abandoned Mušlovka Quarry near Praha-Řeporyje (Kříž 1991, 1992), have been sampled repeatedly for conodonts. Biosparitic brachiopod and nautiloid limestones with conodont elements indicative of the lower Ludfordian *Polygnathoides siluricus* Biozone yielded numerous phosphatic plates attributed to *Eurytholia bohemica* by Ferretti *et al.* (2006). Apart from conodont elements and phosphatic plates of *Eurytholia*, enigmatic phosphatic ring-like structures have been recovered from levels A and C of the study by Ferretti *et al.* (2006; Table 1). Also, graptolite zonal index *Saetograptus linearis* was reported by Kříž and Schönlaub *in* the study by Chlupáč & Schönlaub (1980) and Kříž (1992). Platy and slightly lenticular beds of biodetrital limestone interbedded with shale (Fig. 3) form a prominent, thin-bedded, 120-cm thick interval between thick-bedded nautiloid limestone below and thick- and cross-bedded brachiopod dominated limestone above. This particular interval lies between

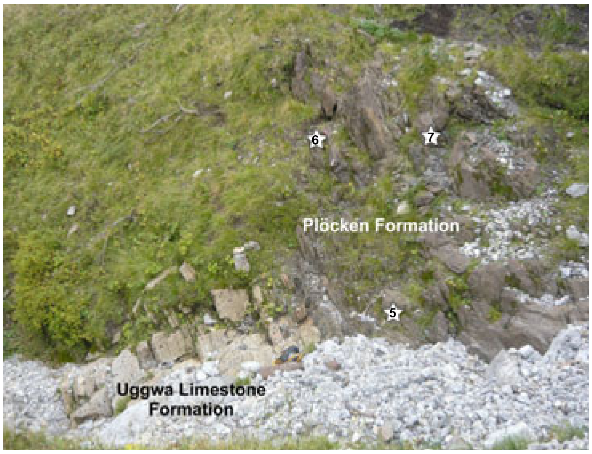

Fig. 2. Close view of the Ordovician exposed in the Cellon Section, Carnic Alps (see also Fig. 1 for details and relative thickness of the exposed formations). Numbers indicate productive levels.

Table 1. Ring abundance in the productive levels.

Locality	Section	Level	Rings
BOHEMIA	Mušlovka Quarry	A	166
		A (resampled in 2012)	24
		C	11
CARNIC ALPS	Cellon	7	12
		6	20
		5	2
		Total	235

Fig. 3. Detail of the two Silurian levels sampled at Mušlovka Quarry, Bohemia (hammer for scale).

samples 2 and 4 of Kříž & Schönlaub (1980). Level A of the present study is the next limestone bed above sample 3 of the latter authors and level C is the next limestone bed above the massive nautiloid limestone of sample 2 (Fig. 3).

Material and methods

Study material

The material studied here was recovered from 16 stratigraphical levels (two in Bohemia and 14 in the Carnic Alps). Collected samples were processed with the standard technique in use for conodonts. Samples were treated with an up to 10% solution of formic acid, and the insoluble residue passing through a 2-mm sieve was washed through a 100-μm sieve every time the acid was changed (i.e. to obtain a 100-μm to 2 mm residual fraction). Material from the Mušlovka Quarry Section that was sampled in 2012, which was prepared specifically for the investigated rings, was washed only through the 100-μm sieve (to obtain the >100 μm fraction). Residues were later concentrated, if abundant, with sodium polytungstate. The enigmatic rings were handpicked from the residue, and the recovered specimens were first characterized by optical examination with a transmitted and reflected optical light microscope, a Zeiss Stemi SV 11 microscope.

Some specimens were also prepared to expose a sectioned view of their internal micro-structure. Isolated rings were first attached (under the microscope) to an adhesive tape mounted on the bottom of a small plastic cylindrical container. Remet Hardrock 554 glue (hardened by Hardrock 554 hardener), from which air had been removed by vacuum, was introduced to the cylinder drop by drop with a disposable syringe. When the glue was hardened, the resulting cylinder was polished first with sand paper and later with abrasive to remove the adhesive tape and obtain a polished surface exposing the internal structure of the rings. This was then mounted on a glass slide by a two-component Bindulin glue, and the exposed face was ground using progressively finer abrasive grit until the sample was only about 90-μm thick.

Isolated and thin-sectioned specimens were mounted on aluminium stubs previously covered with carbon conductive adhesive tape. Au-coated specimens and C-coated thin-sections were observed with an Environmental Scanning Electron Microscope FEI ESEM-Quanta 200, equipped with an Oxford EDX INCA 300 energy-dispersive X-ray spectrometer system. ESEM observations were conducted in high vacuum. The operating conditions of the scanning electron microscopes were 5–25 keV accelerating voltage for imaging and 5–15 keV for elemental analyses.

X-ray diffraction measurements were performed on selected samples using a Gandolfi Camera with a diameter of 114.6 mm, and by using an X-ray beam operating at 40 keV and 30 mA.

Conodont processing, optical microscopy and XRD analyses were performed at the Dipartimento di Scienze Chimiche e Geologiche of the University of Modena and Reggio Emilia (Modena, Italy), whereas ESEM-EDX analyses were carried out at the Centro Interdipartimentale Grandi Strumenti (C.I.G.S.) of the same university.

The material examined in this study consists of 235 elements (Table 1). The best preserved and most abundant material was represented by the Bohemian samples. Specifically, the two investigated levels from the Kopanina Formation in the Mušlovka Quarry (levels A and C, *Polygnathoides siluricus* Biozone, Ludfordian; Ferretti *et al.* 2006) produced about 86% of the entire collection.

In the Cellon Section of the Carnic Alps, only some levels within the Plöcken Formation were productive (Cellon levels 5, 6 and 7; *Amorphognathus ordovicicus* Biozone, Hirnantian; Ferretti & Schönlaub 2001; Schönlaub *et al.* 2011).

General morphology

Rings are approximately circular and mostly shaped like a small, truncated cone, with walls of variable thickness surrounding a central cavity. The width of the cavity varies widely relative to the size of the ring (e.g. Fig. 4A,F), which has a longest axis ranging from 30 to 535 μm. The height of the rings, measured at two different positions, is extremely variable even within the same specimen (Table 2). One side, that we conventionally regarded as the lower ('aboral') part of the structure, is generally relatively flat and wide (e.g. Fig. 4K). The opposite side (upper or 'oral' side) had a more disparate appearance, which might take the form of a narrow thin ridge (e.g. Fig. 4F,N), a large flat surface (e.g. Fig. 4A,B) or something intermediate between these two extremes (all other rings in Fig. 4). Thus, rings resemble respectively either 'dog bowls' or, in other cases, thinner and sometimes thicker 'do-nuts'.

The dog bowl-like rings are in some instances characterized by an expansion of the lower inner side into a flat platform (e.g. Fig. 4C,D). In others (Fig. 4L,P), a small lateral indentation can be observed on the outer side of the rings.

Remarkably, both the border of the upper ridge (Fig. 4M2,P) and its central outline (Fig. 4D,E,M2, P1) are intact (i.e. showed no signs of fracture), an observation which is consistent in both upper and lower views.

Mineralogical and chemical analyses

Mineralogical and chemical analyses (XRD and ESEM-EDX) showed that the main constituent of the rings is a carbonate–fluoroapatite. Ca, P, O, C and F are the main elements, with minor amounts of Fe, Si, Al and Mg. This appears to be a primary phosphatization because no other phosphatized fossils have been found with the fauna. Most of the material from the Carnic Alps revealed a secondary enrichment in Si. It is noteworthy that any calcareous layer that may have been present would have been destroyed by lab processing.

To reveal any significant variation in elemental composition through the rings, ESEM-EDX spectra were collected at equally spaced points on multiple transects across oral, aboral and interior (longitudinal thin-sectioned) surfaces of seven different specimens selected from the best preserved material within level Mušlovka Quarry A. No regular trends of variation in major element composition across the whole ring thickness from outside to inside were detected, an observation confirmed by elemental mapping (Fig. 5). The outer ring margin of a single-fractured specimen, which was otherwise similar in compositional patterns to the other rings (Fig. 6), showed a non-uniform distribution of phosphorous (Fig. 7).

Associated phosphatic elements

Other phosphatic organisms were recovered in the same samples that produced the rings under investigation. Together with conodonts and brachiopods, some problematic plates occurred in the Silurian residues from Bohemia. In particular, level A of the Mušlovka Quarry produced a remarkably rich assemblage of phosphatic plates of uncertain affinity described as *Eurytholia bohemica* by Ferretti *et al.* (2006). In contrast, no plates were recovered from the Ordovician horizons of the Carnic Alps, where these enigmatic plates were reported only from younger sediments in the Rauchkofel Boden Section (Ferretti & Serpagli 2008). A combination of X-rays and EDAX analysis had revealed for the plates a shell composition of calcium phosphate, with iron detected in the outer massive layer and fluorine enrichment in the inner reticulated layer (Ferretti *et al.* 2006). Additional phosphatic plates recovered in the newly

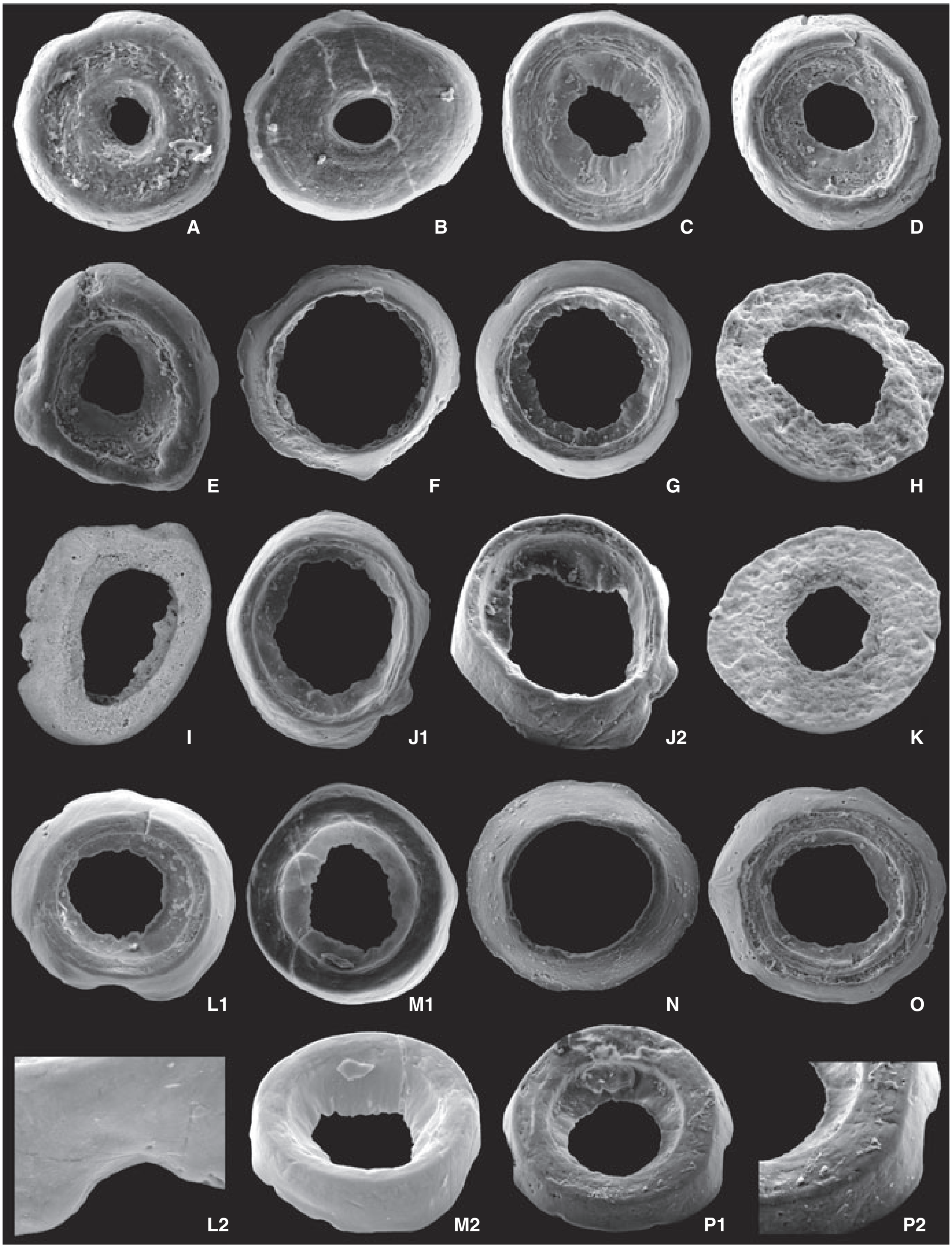

Fig. 4. Scanning electron micrographs of enigmatic phosphatic rings from Bohemia and the Carnic Alps. The material described in this study has been deposited at the Palaeontological Collections of the University of Modena and Reggio Emilia (under catalogue number IPUM 28235). A, upper view of ring n. 3, Mušlovka Quarry A, ×130. B, upper view of ring n. 64, Mušlovka Quarry A, ×130. C, upper view of ring n. 7, Mušlovka Quarry A, ×125. D, upper view of ring n. 4, Mušlovka Quarry A, ×120. E, upper view of ring n. 2, Mušlovka Quarry A, ×115. F, upper view of ring n. 28, Mušlovka Quarry C, ×100. G, upper view of ring n. 15, Mušlovka Quarry A, ×95. H, lower-lateral view of ring n. 21, Mušlovka Quarry A, ×115. I, upper-lateral view of ring n. 38, Cellon 6, ×115. J, upper (1) and upper-lateral (2) views of ring n. 1, Mušlovka Quarry A, ×125 and ×140, respectively. K, lower-lateral view of ring n. 22, Mušlovka Quarry A, ×135. L, upper view (1) of ring n. 11, Mušlovka Quarry A, ×130; detail (2) of the small lateral indentation observed on the outer lateral side of the same ring, ×415. M, upper (1) and upper-lateral (2) views of ring n. 60, Mušlovka Quarry A, ×145 and ×160, respectively. N, upper view of ring n. 57, Mušlovka Quarry C, ×110. O, upper view of ring n. 5, Mušlovka Quarry A, ×85. P, upper-lateral view (1) of ring n. 63, Mušlovka Quarry A, ×125; detail (2) of the same specimen showing the non-fragmented upper ridge of the ring and the presence of a small outer lateral indentation, ×195.

Table 2. Ring thickness measured in some rings (in two different parts of the specimen).

Locality	Section	Level	Ring	Thickness 1 (micron)	Thickness 2 (micron)
BOHEMIA	Mušlovka Quarry	A	1	82	120
			2	55	72
			3	55	48
			4	59	34
			5	97	82
			6	64	84
			7	54	55
			8	77	78
			9	106	65
			11	66	78
			12	62	90
			13	55	84
			14	55	53
			15	62	97
			16	62	54
			17	112	95
			18	103	83
			58	94	65
			59	59	50
			60	24	78
			61	120	82
			62	63	61
			63	60	41
			64	43	57
			65	86	75
			66	50	54
			67	94	116
			68	72	83
			69	63	52
	Mušlovka Quarry	C	23	77	59
			24	30	61
			25	81	76
			26	63	70
			28	81	89
			54	82	145
			55	79	32
			56	73	71
			57	92	104

prepared material were analysed under ESEM-EDX analysis (Fig. 8A,B). No diagnostic compositional differences or similarities with the rings have been identified that allow us to infer the affinity of either group. We note, however, that the net-like structure that is always present on the lower surface of the plates (Fig. 8B) has never been observed on any parts of the rings.

Special attention was reserved to the possible recovery of phosphatic tubes in the newly prepared material from Bohemia. Only broken pieces of possible cylindrical structures were in fact reported in the already available 100 μm–2 mm conodont heavy fractions. Three tubular but again fragmented elements with a cylindrical appearance were recovered in the new material collected at Mušlovka (heavy fraction >100 μm). They all appear broken at the extremities (Fig. 8C,D), and no indication of possible interlocking or affinity with the rings has emerged. ESEM-EDX analysis revealed a carbonate–apatite, with fluorine mostly absent apart from within small patches in the outermost layer, and a significant decrease in phosphorus moving from the outside to the inside of the tube wall.

Geometric morphometrics

To explore whether the size and shape of the rings changed over time, we employed a novel combination of geometric morphometric approaches (Adams *et al.* 2004; Viscosi & Cardini 2011) for the quantitative description of outlines with no 'homologous' landmarks (see Supporting Information for more information and detailed results). To this aim, we used the best preserved samples in the data set, which are from stratigraphic levels A ($N = 29$) and C ($N = 9$) of Mušlovka Quarry (Table 2). The outline of the inferior external border of the rings was digitized automatically from high-resolution computerized images (Fig. 9) and an improved algorithm (Haines & Crampton 2000) for Fourier analysis was used, which normalizes for the starting point of outlines by rotating specimens to minimize root-mean-square differences between curves of the entire sample under study. The accuracy of the method was first verified by using, for each ring, two replicas with different random starting points: all replica pairs were correctly identified as identical. This process allowed us to implement 'mathematical homologization' of the outline points, which was necessary because rings have no known biologically homologous landmark to 'anchor' the analysis of form; therefore, the conservative approach is to minimize differences between the outlines that result simply from rotation and use these rotated outlines as the starting configurations for a conventional, landmark-based, geometric morphometric analysis using Procrustes methods. In this way, results from our analysis are comparable to other geometric morphometric approaches (e.g. the use of Fourier coefficients as shape variables), but exploit the robust statistical framework and interpretive tools that have made landmark methods mainstream in biology (O'Higgins 1997; Adams *et al.* 2004 and references therein). Thus, using this procedure, all steps of the data collection (including repositioning and rescanning specimens, redigitizing outlines) were repeated on 20 specimens and used to test measurement error, which was negligible in both size and shape in a permutational ANOVA (Anderson 2001, 2005; Viscosi & Cardini 2011; Franklin *et al.* 2012). Finally, differences between stratigraphical levels were tested using permutation tests on all 38 specimens. These were significant ($P = 0.0007$, 29.8% of

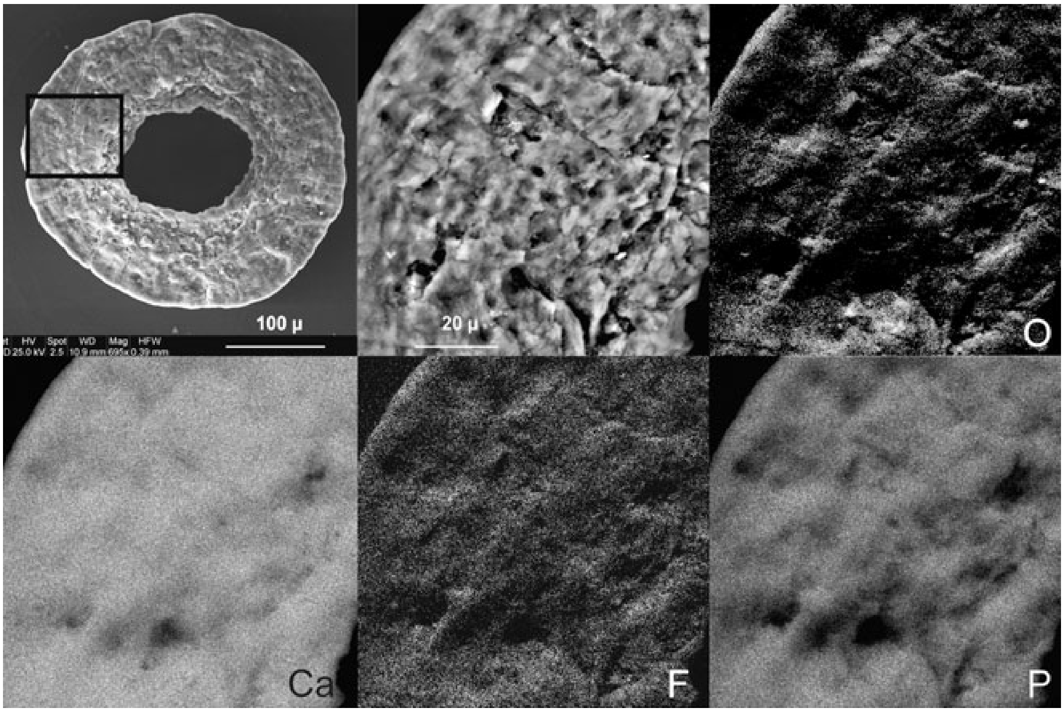

Fig. 5. EDX elemental map distribution of main elements (O, Ca, F and P) of the aboral surface of ring n. 22; Mušlovka Quarry A.

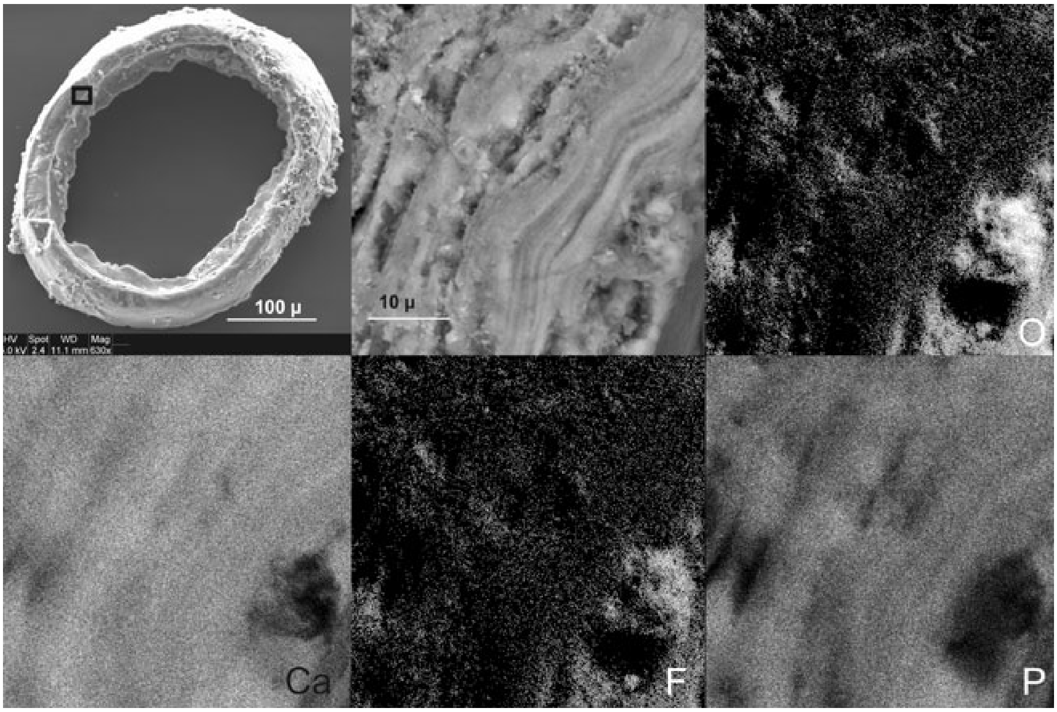

Fig. 6. EDX elemental map distribution of main elements (O, Ca, F and P) of the inner part of ring n. 13, exposed along a broken surface; Mušlovka Quarry A.

variance explained) in mean size, as suggested by the minimal overlap of the main range of variation in size in the two levels (Fig. 10). Indeed, size increased about 20% in C compared with A. In contrast, mean shape differences were not significant (P = 0.4391, 2.5% of variance explained). This is summarized in Figure 11 using a between-group principal component analysis (BG-PCA; Seetah *et al.* 2012).

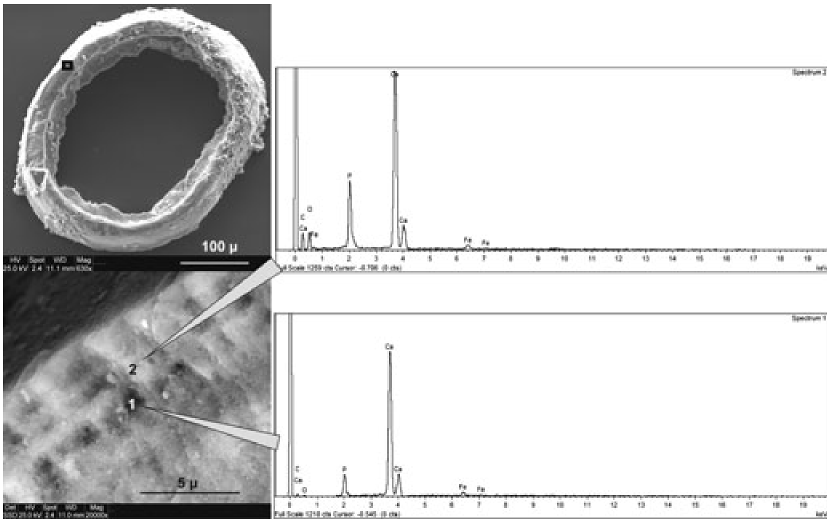

Fig. 7. EDX spectra showing the non-uniform distribution of phosphorous in the outermost part of ring n. 13, exposed along a broken surface; Mušlovka Quarry A.

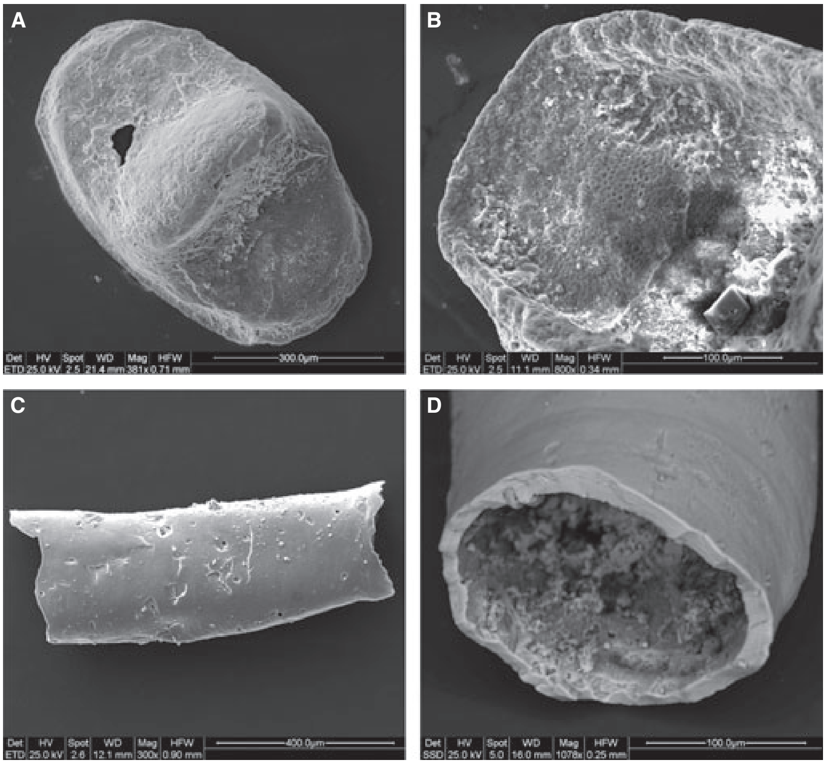

Fig. 8. Upper (A) and detail in lower view (B) of *Eurytholia bohemica*, Mušlovka Quarry A. Note the net-like pattern of the inner layer. (C, D) lateral views of phosphatic tubes, Mušlovka Quarry A.

The BG-PCA shows an almost complete overlap of the groups along the main axes of between group (bgPC1) and residual variance (resPC1), which explains respectively 29.8% and 36.4% of total shape variance. Mean shape differences, in this figure, are magnified 10 times and shown using Thin Plate Spline (TPS; Viscosi & Cardini 2011) deformation grids and grey-scale coded Jacobian expansion, which measure the degree of local expansion or contraction of the grid (black and dark grey indicate expansions; light grey and white indicate contractions). Sample variances did not change significantly over time in either size or shape ($P > 0.05$), and the covariation of size and shape (i.e. allometry) was

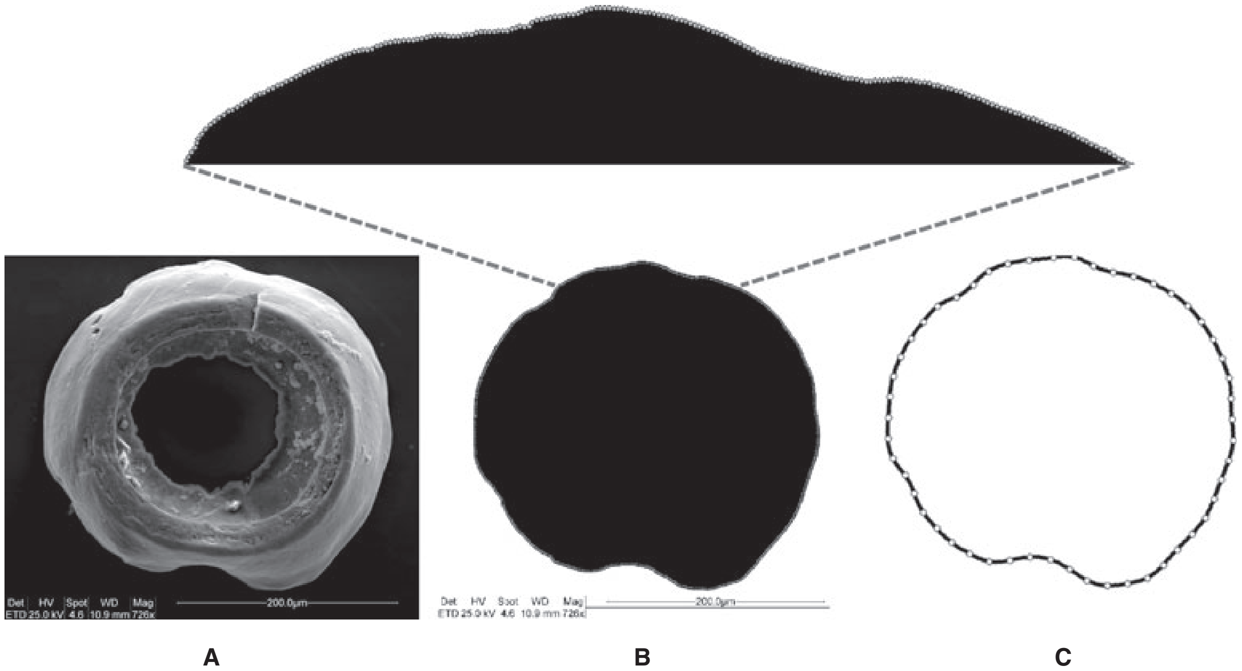

Fig. 9. Example of outline digitization and reconstruction: A, original SEM picture of a ring; B, enhanced image with an automatically digitized outline (points become visible by zooming in as shown in the frame above); and C, reconstructed outline with the 51 landmarks used in the statistical analysis emphasized.

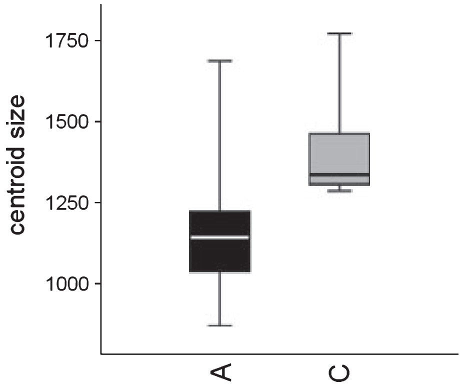

Fig. 10. Box-plot of centroid size (median, 25–75% quartiles, minimum and maximum).

marginally significant in A ($P = 0.04$, Fig. 12) and not significant in C ($P > 0.05$).

Conclusions

This study adds another small piece of information in the complex puzzle of the interpretation of the function of these enigmatic Palaeozoic rings. It also raises some intriguing questions about the modalities of growth, the patterns of taxonomic variation and the evolution of their yet unidentified Palaeozoic 'lord'. Overall, our observations are consistent with the interpretation of the rings as an adhering structure of a benthic organism living on a hard substrate. The flat lower surface might have represented the attachment area, which glued the organism to a relatively uniform and solid substrate. The absence of any sign of fracture or fragmentation of ring borders seems compatible with an adhesion mechanism in which the organism was articulated to the rings using soft tegumental tissues. The occurrence of frequent indentations on the outline may be indicative of a high population density, or a colonial way of life, with individuals living in such close proximity that they could affect the growth of the adhesion ring of neighbouring specimens. Finally, the geometric morphometric analysis provided evidence of constant shape but variable size across levels, which supports empirical observations of morphological conservation through time. Most importantly, it demonstrated the flexibility and heuristic potential of a novel combination of geometric morphometric approaches for the analysis of outlines in the absence of clearly 'homologous' landmarks. This might have broad applications to the study of poorly known structures and both biological and non-biological shapes.

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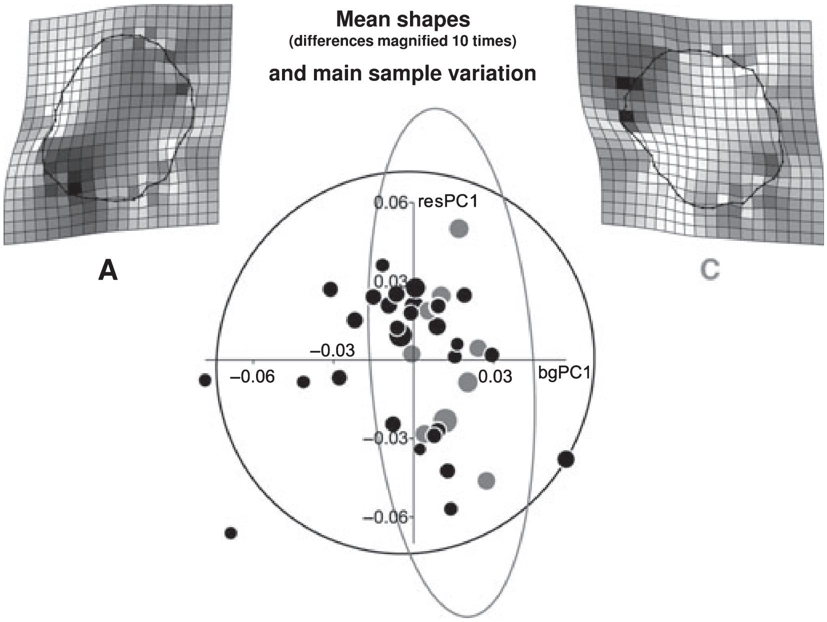

Fig. 11. BG-PCA scatterplot of shape variation. 95% confidence ellipses are shown for each group; symbols are proportional to centroid size.

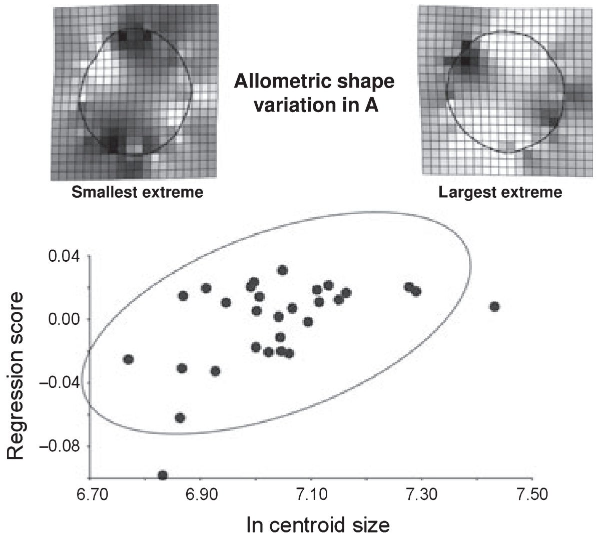

Fig. 12. Significant allometry in A is shown using a scatterplot of regression scores onto the natural logarithm of centroid size together with the corresponding 95% confidence ellipse. Shapes corresponding to opposite extremes of the allometric trajectory are shown using TPS deformation grids and Jacobian expansion factors as in Figure 11.

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References

Adams, D.C., Rohlf, F.J. & Slice, D.E. 2004: Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology 71*, 5–16.

Anderson, M.J. 2001: Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences 58*, 626–639.

Anderson, M.J. 2005: *PERMANOVA: A FORTRAN Computer Program for Permutational Multivariate Analysis of Variance*. Department of Statistics, University of Auckland, New Zealand. <http://www.stat.auckland.ac.nz/~mja/Programs.htm>.

Bischoff, G.C.O. 1973: On the nature of the conodont animal. *Geologica et Paleontologica 7*, 147–174.

Bischoff, G.C.O. 1989: Byroniida new order from early Palaeozoic strata of eastern Australia (Cnidaria, thecate scyphopolyps). *Senckenbergiana lethaea 69*, 467–521.

Buryi, G.I. & Kasatkina, A.P. 2005: A comparative morphological analysis of the rounded phosphatic structures in euconodonts and their functional significance. *Paleontological Journal 39*, 52–56.

Chlupáč, I., Kříž, J. & Schönlaub, H.P. 1980: Field Trip E: Barrandian. *In* Schönlaub, H.P. (ed.): Second European Conodont Symposium (ECOS II), Guidebook, Abstracts. Abhandlungen der Geologischen Bundesanstalt 35, pp. 147–180.

Ferretti, A. & Schönlaub, H.P. 2001: New conodont faunas from the Late Ordovician of the Central Carnic Alps, Austria. *Bollettino della Società Paleontologica Italiana 40*, 3–15.

Ferretti, A. & Serpagli, E. 2008: *Eurytholia* plates (Problematica) from the late Silurian of the Austrian Carnic Alps. *Revue de Micropaleontologie 51*, 183–187.

Ferretti, A., Serpagli, E. & Štorch, P. 2006: Problematic phosphatic plates from the Silurian-Early Devonian of Bohemia, Czech Republic. *Journal of Paleontology 80*, 1026–1031.

Fisher, D.W. 1962: Small conoidal shells of uncertain affinity. *In* Moore, R.V. (ed.): *Treatise on Invertebrate Paleontology, Part W, Miscellanea*, pp. W98–W143. Geological Society of America, Boulder, and University of Kansas Press, Lawrence.

Franklin, D., Flavel, A., Kuliukas, A., Cardini, A., Marks, M.K., Oxnard, C. & O'Higgins, P. 2012: Estimation of sex from sternal measurements in a Western Australian population. *Forensic Science International 217*, 230.e1–230.e5.

Haines, A.J. & Crampton, J.S. 2000: Improvements to the method of Fourier shape analysis as applied in morphometric studies. *Palaeontology 43*, 765–783.

Kříž, J. 1991: The Silurian of the Prague Basin (Bohemia): tectonic, eustatic, and volcanic controls on facies and faunal development. *Special Papers in Palaeontology 44*, 179–204.

Kříž, J. 1992: Silurian field excursions: Prague Basin (Barrandian), Bohemia. *National Museum of Wales, Geological Series 13*, 1–111.

Kříž, J. 1998: Silurian. *In* Chlupáč, I., Havlíček, V., Kříž, J., Kukal, Z. & Štorch, P. *Palaeozoic of the Barrandian*, pp. 79–100. Czech Geological Survey, Praha.

Kříž, J. & Schönlaub, H.P. 1980: Excursion, Stop 1. Daleje Valley, Mušlovka Quarry section. 153–157. *In* Schönlaub, H.P. (ed.): Guidebook and Abstracts, Second European Conodont Symposium – ECOS II. Abhandlungen der Geologischen Bundesanstalt 38, pp. 1–213.

Müller, K.J., Nogami, Y. & Lenz, H. 1974: Phosphatische Ringe als Mikrofossilien im Altpaläozoikum. *Palaeontographica Abteilung A 146*, 79–99.

O'Higgins, P. 1997: Methodological issues in the description of forms. *In* Lestrel, P.E. (ed.): *Fourier Descriptors and their Applications in Biology*, pp. 74–105. Cambridge University Press, Cambridge, UK.

Schönlaub, H.P., Ferretti, A., Gaggero, L., Hammarlund, E., Harper, D.A.T., Histon, K., Priewalder, H., Spötl, C. & Štorch, P. 2011: The Late Ordovician glacial event in the Carnic Alps (Austria). *In* Gutiérrez-Marco, J.C., Rábano, I. & García-Bellido, D. (eds): *Ordovician of the World*, pp. 515–526. Cuadernos del Museo Geominero, Instituto Geológico y Minero de España, Madrid.

Seetah, T.K., Cardini, A. & Miracle, P.T. 2012: Can morphospace shed light on cave bear spatial-temporal variation? Population dynamics of *Ursus spelaeus* from Romualdova pećina and Vindija (Croatia). *Journal of Archaeological Science 39*, 500–510.

Viscosi, V. & Cardini, A. 2011: Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *In* Lalueza-Fox, C. (ed.): *PLoS ONE 6*, e25630.

Webers, G.F. 1966: *The Middle and Upper Ordovician Conodont Faunas of Minnesota*, pp. 1–123. Special Publication Series 4. Minnesota Geological Survey, Minneapolis.

Welch, J.R. 1976: *Phosphannulus* on Paleozoic crinoid stems. *Journal of Paleontology 50*, 218–225.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Detailed Results and Methods.