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Quantitative variation of morphological 3D-traits in ostracod shells *Eucypris virens* – a case study

Kai Pfennings[1,2*], Finn Viehberg[3]

[1]Marine Research Department, Senckenberg am Meer, Wilhelmshaven, Germany (kai.pfennings@senckenberg.de)
[2]Institute of Geology and Mineralogy, University of Cologne, Cologne, Germany
[3]Institute for Geography and Geology, University of Greifswald, Greifswald, Germany

Background

Recent molecular studies suggest that we have a higher species diversity than identification efforts relying on 2D morphometric methods indicate. Thus, the fossil morphospecies identified in geo-archives hold a higher species diversity, too. However, quantifying valve shape in its 3D entirety will be a powerful tool to investigate both taxonomy and speciation, and could put morphological descriptions on a par with advanced molecular methods.

The aim of this study is **1)** to adopt 3D geometric morphometric curve- and surface-sliding semilandmark workflows used in paleoanthropology to feature-poor non-marine ostracod valves of the morphologically-variable species *Eucypris virens* (Jurine, 1820); and **2)** to compare morphological findings to genetic clades, some of which are considered cryptic species.

Material

The 82 (♀ right) valves used for this study were provided by the research group of Koen Martens from the Royal Belgian Institute of Natural Sciences. Individuals are from the work of Bode et al. (2010) (Europe and North Africa), and Koenders et al. (2016) (Australia). In total, 24 genetically-distinct groups were identified in previous studies (Fig. 1).

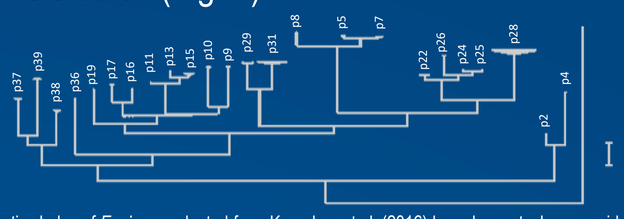


Fig. 1 Genetic clades of *E. virens;* adapted from Koenders et al. (2016) based on cytochrome oxidase I [COI]

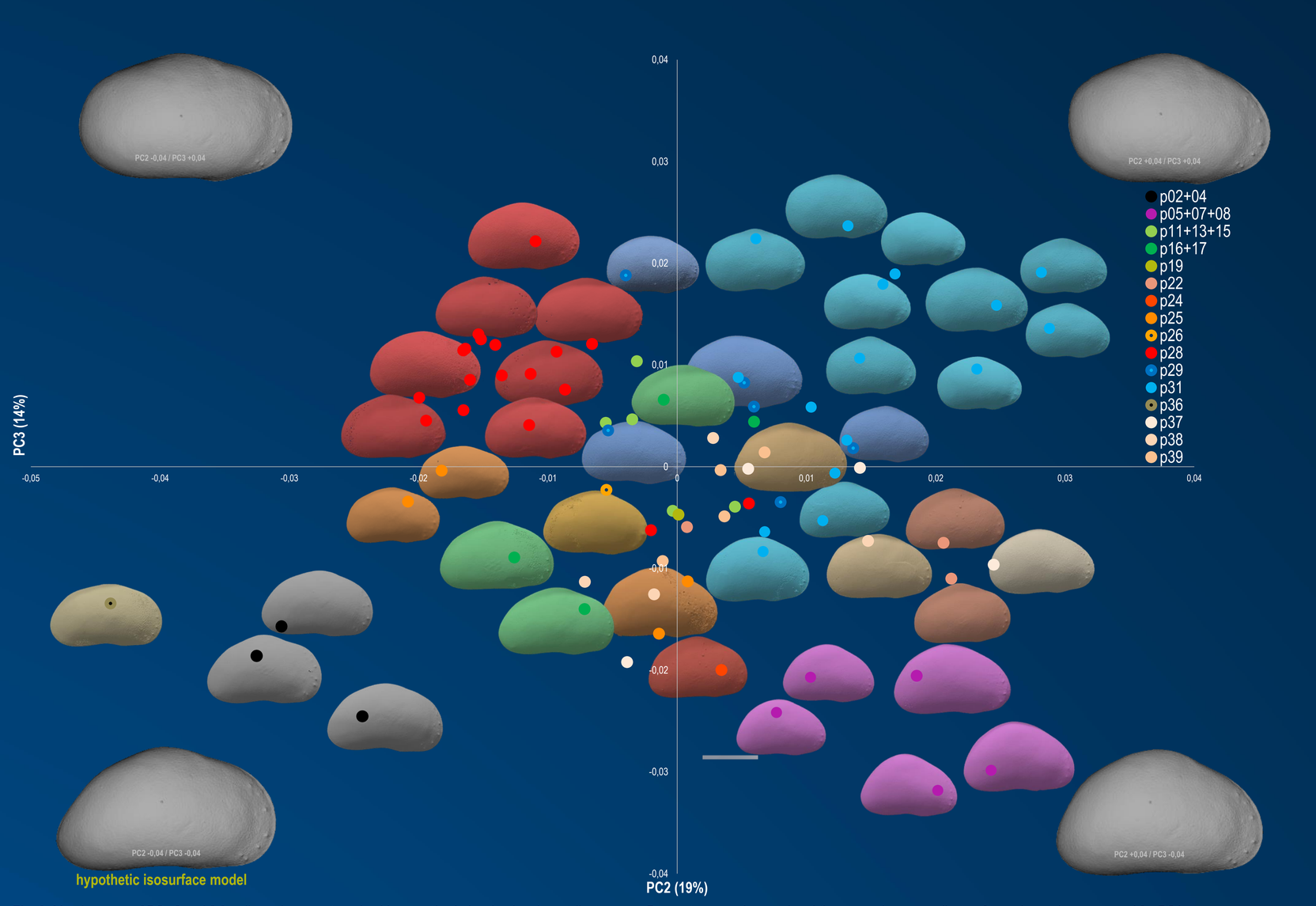


Fig. 2 Morphospace of PCs 2 and 3 for all specimens categorized by genetic clades; landmark coordinates were Procrustes-superimposed and the mean shape coordinates for each species were used for PCA

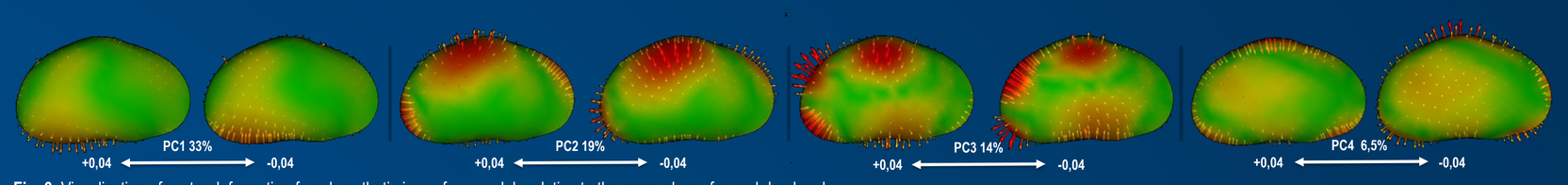
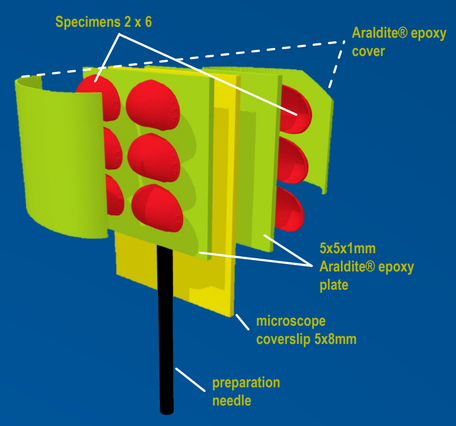


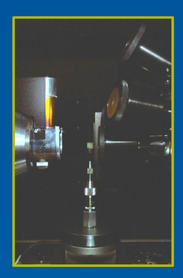
Fig. 3 Visualization of vector deformation from hypothetic isosurface models relative to the mean shape for each landmark

Digitalization

µ-CT Target

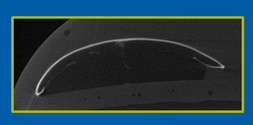


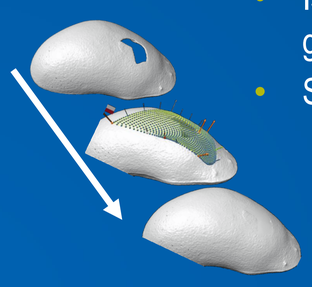
µ-CT Scan



- Zeiss Xradia XCT-200 (60kV,8W)
- Voxel size: 5.4261x5.4261µm
- Peter Michalik, University of Greifswald

Isosurface Models

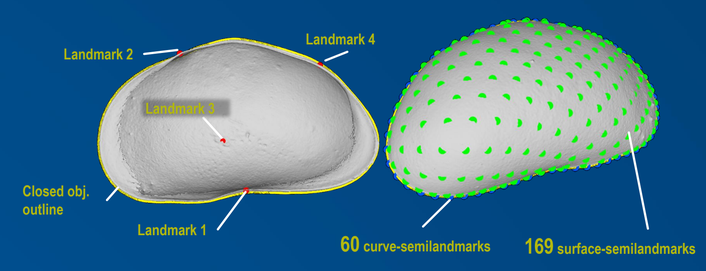




- Segmentation of single valves from µ-CT image stack
- Isosurface rendering based on grey values
- Surface repair if needed

Geometric Morphometrics

Template Landmark Set



Project landmark set to target valves

Based on 4 landmarks and a closed outline curve

Sliding

3D curve- and surface-sliding semilandmarks on each valve are slide to minimize thin-plate spline bending energy to the mean Procrustes shape

Analysis

Generalized Procrustes analysis was used to eliminate size-dependent effects. Principal component analysis (PCA) was used to examine and visualize the main patterns of shape variation. Hypothetical isosurface models were rendered to specific PC scores and vector deformation was calculated to localize deformation. Discriminant analysis was used to test for morphological differences between genetic groups.

References

Bode, S. N., Adolfsson, S., Lamatsch, D. K., et al. 2010. 'Exceptional cryptic diversity and multiple origins of parthenogenesis in a freshwater ostracod', Molecular Phylogenetics and Evolution, 54: 542-52.

Koenders, Annette, Schön, Isa, Halse, Stuart, et al. 2016. 'Valve shape is not linked to genetic species in the Eucypris virens (Ostracoda, Crustacea) species complex', Zoological Journal of the Linnean Society

Results

The combination of PC2 (19% of total variability) and PC3 (14%) provides a suitable overview of genetic clades and their associated shapes

- Genetic cluster p2 + p4 is assigned by a typical anteriorly-broadened, posterodorsal-pointed shape
- Cluster p5 + p7 + p8 is assigned by doming of the posterodorsal area and a trigonal shape (Fig. 3, PC3 (+ PC4))
- Group p28 and p31 can be distinguished from each other by their compactness (PC2); both contain individuals from Australia and Europe

Conclusions & Outlook

- 3D curve- and surface-sliding semilandmarks are useful tools for quantifying small-scale changes on feature-poor ostracod shells
- At least three genetic clades previously considered cryptic are morphologically distinguishable
- Ecophenotypic knowledge is needed for further work and can be proven with hypothetical shapes
- Cost-effective digitization and high throughputs require technical solutions for scan targets
- Advances in computing may allow for landmark-free morphometric methods to replace landmark-based analyses

Acknowledgements

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Marine Research Department, Senckenberg am Meer, Wilhelmshaven, Germany