Figures and figure supplements

‘Palaeoshellomics’ reveals the use of freshwater mother-of-pearl in prehistory

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DOI: https://doi.org/10.7554/eLife.45644.003
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DOI: https://doi.org/10.7554/eLife.45644.004
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DOI: https://doi.org/10.7554/eLife.45644.013
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DOI: https://doi.org/10.7554/eLife.45644.023
Appendix 1—figure 1. (a) Double-button samples from the archaeological sites of Havnø (Denmark), Hornstaad-Hörnle IA (Germany) and Peștera Ungurească (Romania). Findings of double-buttons and of Unio ornaments as reported in the literature, compared to the present occurrence of Unio.
pictorum (b), Margaritifera margaritifera (c), Unio crassus (d), Pseudunio auricularius (e) (data obtained from GBIF, the Global Biodiversity Information Facility, GBIF.org, 2018). The three sets of double-buttons (Doppelknöpfe) analysed here come from the archaeological sites of Havnø (Denmark), Hornstaad-Hornle IA (Germany) and Peștera Ungureasca (Romania) and approximately span the period between 4200 and 3800 BCE.

DOI: https://doi.org/10.7554/eLife.45644.028
Appendix 1—figure 2. The double-buttons from Havno.
DOI: https://doi.org/10.7554/eLife.45644.029
Appendix 1—figure 3. Excavations at Hornstaad D-Hörnle site (a) and the discovery of the double-buttons (b).
DOI: https://doi.org/10.7554/eLife.45644.030
Appendix 1—figure 4. Marine and freshwater shells included in this study for comparative analysis: possible sources of raw material used for the manufacture of the double-buttons.

DOI: https://doi.org/10.7554/eLife.45644.031
Appendix 1—figure 5. SEM microstructural analysis of archaeological double buttons (a–c) and mollusc shells (d,e). Double-buttons: a) Havnø (HavA, HavB, HavC), b) Hornstaad (HorA, HorB, HorC), c) Peștera Ungurească (PesB). Reference shells: d) freshwater unionoid shells (modern *Unio pictorum*, *Margaritifera margaritifera* and sub-fossil *Unio crassus*, *Pseudunio auricularius*), e) marine shells (*Modiolus modiolus* and *Ostrea edulis*). DOI: https://doi.org/10.7554/eLife.45644.032
Appendix 1—figure 6. FTIR-ATR spectra of the double-buttons. Asterisks mark aragonite marker absorption bands. Nacreous and prismatic layers of (a) HavC, (b) PesB, (c) FTIR-ATR spectra comparison between HavC nacre and prismatic layers (black) with calcitic prismatic layer of Pinna nobilis (red) and aragonitic Unio truncatus (blue), confirming the fully aragonitic mineralogy of both layers; (d) FTIR-ATR spectra of all the double-buttons, sampled in ‘bulk’: the presence of doublets (CO in-plane bending mode) at ~712 and ~700 cm$^{-1}$ (dashed line) in all samples indicates absence of recrystallization of the biogenic carbonate.

DOI: https://doi.org/10.7554/eLife.45644.033
Appendix 1—figure 7. Relative THAA composition of double-button samples.
DOI: https://doi.org/10.7554/eLife.45644.037
Appendix 1—figure 8. Principal Component Analysis (PCA) plot showing the similarity or differences between the amino acid composition of double-buttons and a range of shell taxa (reference taxa from Demarchi et al., 2014).

DOI: https://doi.org/10.7554/eLife.45644.038
Appendix 1—figure 9. Total hydrolysable amino acid D/L values for all double-buttons (Glx vs Asx, left; Val vs Ala, right).

DOI: https://doi.org/10.7554/eLife.45644.039
Appendix 1—figure 10. Protein Hic74 identified in *Unio pictorum*: sequence coverage, highlighting in pink the product ion spectra ("Sp") shown below. Sequences reconstructed by assisted de novo on the basis of mono-charged ions mainly (spectra were acquired on the 400-1600 m/z range and multiply-charged ions were detected).
DOI: https://doi.org/10.7554/eLife.45644.041
Appendix 1—figure 11. Product ion spectra [1 - 5] supporting the coverage of protein Hic74 identified in *Unio pictorum*. DOI: https://doi.org/10.7554/eLife.45644.042
Appendix 1—figure 12. Product ion spectra [6 - 10] supporting the coverage of protein Hic74 identified in *Unio pictorum*. DOI: https://doi.org/10.7554/eLife.45644.043
Appendix 1—figure 13. Product ion spectra [11 - 15] supporting the coverage of protein Hic74 identified in *Unio pictorum*.

DOI: https://doi.org/10.7554/eLife.45644.044
Appendix 1—figure 14. Product ion spectra [16 - 20] supporting the coverage of protein Hic74 identified in Unio pictorum. DOI: https://doi.org/10.7554/eLife.45644.045
Appendix 1—figure 15. Product ion spectra [21 - 25] supporting the coverage of protein Hic74 identified in Unio pictorum.
DOI: https://doi.org/10.7554/eLife.45644.046
Appendix 1—figure 16. Protein Hic74 identified in the double-button HavC: sequence coverage, highlighting in pink the product ion spectra (‘Sp’) shown below. Sequences reconstructed by assisted de novo on the basis of mono-charged ions mainly (spectra were acquired on the 400-1600 m/z range and multiply-charged ions were detected).

DOI: https://doi.org/10.7554/eLife.45644.048
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DOI: https://doi.org/10.7554/eLife.45644.049
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DOI: https://doi.org/10.7554/eLife.45644.050
Appendix 1—figure 19. Product ion spectra [11 - 15] supporting the coverage of protein Hic74 identified in the double-button HavC. DOI: https://doi.org/10.7554/eLife.45644.051
Appendix 1—figure 20. Product ion spectra [16 - 20] supporting the coverage of protein Hic74 identified in the double-button HavC.
DOI: https://doi.org/10.7554/eLife.45644.052
Appendix 1—figure 21. Product ion spectra [21 - 26] supporting the coverage of protein Hic74 identified in the double-button HavC.
DOI: https://doi.org/10.7554/eLife.45644.053
Appendix 1—figure 22. Marine shell proteins identified in double-button HavB: (a) MS60-related protein (*Pinctada fucata*); (b) Precollagen D (*Mytilus edulis*). Note that both are supported only by repetitive low complexity (RLC) domains. Sequences reconstructed by assisted de novo on the basis of mono-charged ions mainly (spectra were acquired on the 400–1600 m/z range and multiply-charged ions were detected).

DOI: https://doi.org/10.7554/eLife.45644.054
Appendix 1—figure 23. Circular diagram representing the extent of similarity between the proteomes of six reference mollusc shells based on the identified EST sequences.

DOI: https://doi.org/10.7554/eLife.45644.055
Appendix 1—figure 24. Circular diagram representing the similarity between the proteomes of the seven double-buttons based on the identified EST sequences.
DOI: https://doi.org/10.7554/eLife.45644.056
Appendix 1—figure 25. Pairwise MS/MS comparison of the seven archaeological double-buttons and six reference shells (freshwater and marine): the cluster dendrogram is obtained from a distance matrix from proteome-wide distance calculations of product ion spectra implemented in R using the DISMS2 code.

DOI: https://doi.org/10.7554/eLife.45644.057