To determine the origin of the perforations in the Petersfels Glycymeris shells, whether they were caused by abrasion or occurred naturally, we employed a rigorous analytical approach. We conducted a comprehensive 2D shape analysis complemented by metric measurements and multivariate statistical methods. Our study utilized a Hirox HRX-01 3D digital microscope set at a consistent 20X magnification. This enabled us to capture both experimentally abraded perforations and intact or nearly complete archaeological ones. Additionally, we included a set of shells with naturally occurring perforations to compare and analyze metric and shape variations among the three groups.

For the shape analysis, we applied Elliptic Fourier Analysis (EFA) (Rohlf, 1990) to the 2D outlines of the perforations, extracted from high-resolution images using *DiaOutline* software (Wishkerman & Hamilton, 2018). The raw 2D coordinates were processed and analyzed in R (Posit team, 2023; R Core team, 2023) using the *Momocs* package (Bonhomme *et al.*, 2014), following standard procedures (Falcucci *et al.*, 2024; Leplongeon *et al.*, 2020; Matzig *et al.*, 2021). Before EFA, we standardized the outlines by centering, scaling, and rotating them. EFA was performed with harmonics capturing 99.9% of cumulative harmonic power (n = 56). Subsequently, we conducted Principal Component Analysis (PCA) to explore shape variability across the dataset, categorizing perforations into archaeological, experimental, and natural groups.

To investigate the relationship between size and shape, we conducted another PCA using the main Principal Components (PCs) derived from the 2DGM analysis (n = 3), along with area (in mm²) and diameter (in mm) measurements obtained from the 3D digital microscope. We further assessed inter-group variability through non-parametric MANOVA (PERMANOVA) with 10,000 permutations, using the vegan package (Oksanen *et al.*, 2022) and *pairwiseAdonis* for Euclidean distance calculations (Martinez Arbizu, 2017). Finally, to quantify morphological and metric variations across archaeological, experimental, and natural perforations, we conducted disparity tests (Guillerme, 2018), bootstrapping the PCA data 1,000 times as per Matzig *et al.* (2021).

Bonhomme V., Picq S., Gaucherel C. & Claude J. (2014) Momocs: Outline analysis using R. Journal of Statistical Software, 56: 1-24. doi:https://doi.org/10.18637/jss.v056.i13

Falcucci A., Arrighi S., Spagnolo V., Rossini M., Higgins O.A., Muttillo B., Martini I., Crezzini J., Boschin F., Ronchitelli A. & Moroni A. (2024) A pre-Campanian Ignimbrite techno-cultural shift in the Aurignacian sequence of Grotta di Castelcivita, southern Italy. Scientific Reports, 14: 12783. doi:10.1038/s41598-024-59896-6

Guillerme T. (2018) dispRity: A modular R package for measuring disparity. Methods in Ecology and Evolution, 9: 1755-1763. doi:https://doi.org/10.1111/2041-210X.13022

Leplongeon A., Ménard C., Bonhomme V. & Bortolini E. (2020) Backed Pieces and Their Variability in the Later Stone Age of the Horn of Africa. African Archaeological Review, 37: 437-468. doi:10.1007/s10437-020-09401-x

Martinez Arbizu P. (2017) pairwiseAdonis: Pairwise Multilevel Comparison using Adonis. R package version 0.4.

Matzig D.N., Hussain S.T. & Riede F. (2021) Design Space Constraints and the Cultural Taxonomy of European Final Palaeolithic Large Tanged Points: A Comparison of Typological, Landmark-Based and Whole-Outline Geometric Morphometric Approaches. Journal of Paleolithic Archaeology, 4: 27. doi:10.1007/s41982-021-00097-2

Oksanen J., Simpson G., Blanchet F., Kindt R., Legendre P., Minchin P., O'Hara R., Solymos P., Stevens M., Szoecs E., Wagner H., Barbour M., Bedward M., Bolker B., Borcard D., Carvalho G., Chirico M., De Caceres M., Durand S., Evangelista H., FitzJohn R., Friendly M., Furneaux B., Hannigan G., Hill M., Lahti L., McGlinn D., Ouellette M., Ribeiro Cunha E., Smith T., Stier A., Ter Braak C. & Weedon J. (2022) vegan: Community Ecology Package. R package version 2.6-2. doi:https://CRAN.R-project.org/package=vegan

Posit team (2023) RStudio: Integrated Development Environment for R. Posit Software. PBC, Boston, MA

R Core team (2023) R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna. doi:https://www.R-project.org/

Rohlf F.J. (1990) Morphometrics. Annual Review of Ecology and Systematics, 21: 299-316. doi:10.1146/annurev.es.21.110190.001503

Wishkerman A. & Hamilton P.B. (2018) Shape outline extraction software (DiaOutline) for elliptic Fourier analysis application in morphometric studies. Applications in plant sciences, 6: e01204-e01204. doi:10.1002/aps3.1204

RESULTS

The morphometric analysis of the perforations reveals distinct patterns. The first three PCs from the PCA of the perforation outlines explain 89.2% of the variance in the dataset (Fig. S#). PC1 primarily captures the transition from oval to more elliptical shapes, reflecting the relationship between the maximum length and width of the perforations. PC2 and PC3 describe the symmetry of the perforations: PC2 captures distal-proximal asymmetry, while PC3 captures lateral left-right asymmetry. Spearman's rank correlation analyses were performed to evaluate potential allometric signals for PC1, PC2, and PC3 using circumference measurements. The correlations with PC1 (rho = 0.0202, p = 0.8) and PC3 (rho = 0.1125, p = 0.2) are weak and non-significant, while the correlation with PC2 shows a moderate, statistically significant negative allometric signal (rho = -0.252, p < 0.01).

Mean shape comparisons reveal that archaeological perforations tend to have a more elliptical shape compared to experimental perforations (Fig. S#). However, the PCA plot shows significant overlap among the three groups, with a notable increase in morphological similarity observed in the archaeological specimens (Fig. S#). Since shape data alone did not fully capture the variability within the dataset, we performed a second PCA combining the first three PCs from the outline analysis with metric data. Interestingly, both the circumference and area exhibit higher values and greater dispersion among the natural perforations (Fig. S#). The first four PCs from this second PCA explain 98.8% of the variance, with PC1 being strongly correlated with the circumference and area of the perforations (Fig. #a). The morphological parameters derived from the previous 2DGM analysis are correlated with PC2. Specifically, the elongation of the perforations (represented by PC1 in the 2DGM) is negatively correlated with PC2, while the values associated with proximal-distal and lateral asymmetries (PC2 and PC3 in the 2DGM analysis) show positive correlations with PC2, contributing significantly to this dimension.

The PCA biplot demonstrates marked differences between natural perforations and archaeological/experimental perforations. The mean and confidence intervals of the latter two groups fall on the negative axis of PC1 and the positive axis of PC2, contrasting sharply with the natural perforations (Fig. #c). The confidence ellipse and high dispersion of data points in the natural perforation group further emphasize the increased variability in both size and shape for these specimens. Disparity analysis, based on the first four PCs of the combined shape and size PCA, effectively illustrates within-group variances. Natural perforations show a substantially higher sum of variance compared to both archaeological and experimental specimens (Fig. #b). Overall, these combined results support the interpretation that the perforations in the Glycymeris shells recovered at the site were the result of anthropic abrasion.