

Grapes and vines of the Phoenicians: morphometric analyses of pips from modern varieties and Iron Age archaeological sites in the Western Mediterranean

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- 1 Grapes and vines of the Phoenicians: morphometric analyses of pips from
- 2 modern varieties and Iron Age archaeological sites in the Western
- 3 Mediterranean
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Abstract 31

- The present study aims to contribute to the investigation of the role of Phoenicians in the spreading 32
- and trade of the grapevine through the morphometric analysis of grape pips. Waterlogged and 33
- charred samples were selected from three Iron Age sites in the Western Mediterranean: Motya 34
- (Sicily, Italy), Nuraghe S'Urachi (Sardinia, Italy) and Huelva (Andalusia, Spain). While only 35 Motya is a Phoenician foundation, all three were nevertheless associated with Phoenician 36
- expansion and cultural interaction. Ten cultivars from the "Vivaio Federico Paulsen" in Marsala 37
- (western Sicily) were chosen as modern reference material. 38
- 39 The key challenge was the comparison of archaeological pips preserved through different 40 fossilization processes, which was overcome using two reference datasets of the same modern cultivars, one uncharred and one charred. 41
- Statistical analyses of pip outlines show that archaeological remains from these sites is 42
- 43 morphologically comparable to that of modern varieties, suggesting that the archaeological finds
- represent domesticated grapevines. 44
- PCA analyses allowed an inter-site comparison, showing that samples from the three sites are 45
- clearly distinguishable based on their morphology. This indicates the use of different varieties 46
- which may be due to different factors. 47
- Our analysis represents a first step towards a better understanding of diachronic and synchronic 48
- relationship between vines grown in the ancient West Mediterranean, which could be expanded by 49
- analysing grape pips from more contexts and more sites, compared against a wider selection of 50
- modern cultivars. 51
- Keywords: Phoenicians, viticulture, morphometry, Western Mediterranean, experimental 52 53 charring

1. Introduction 54

- This study aims at better understanding the role of Phoenicians in the spreading and trade of the 55 grapevine in the Western Mediterranean through the morphometric analysis of grape pips from 56 three Western Mediterranean sites dated to between the 9th and the 6th centuries B.C.
- 57
- The grapevine (Vitis vinifera L.) is one of the most important fruit crops of the past and present 58
- 59 world, both economically and culturally. The wild and cultivated forms, respectively Vitis vinifera
- L. subsp. sylvestris (C.C. Gmel.) Hegi and V. vinifera L. subsp. vinifera, differ by an array of traits, 60
- including their reproductive biology. Wild grapevine is dioecious and is cross-pollinated, while 61 domesticated grapes are in most cases hermaphrodite and capable of self-pollination (Negi and 62
- Olmo, 1966; This et al., 2006). Domestication has also resulted in an increase in berry size and 63
- sugar content, which are both factors that play a key role in fermentation (Miller, 2008; Bouby et 64
- al., 2013). Differences can moreover be observed in the shape of its seeds, with wild pips being 65
- "small, robust, with a rounded outline, or cordate, with short stalks [...] almost flat ventrally with 66
- 67 sharp angles and a strongly developed chalaza", in contrast with cultivated ones that are large,

elongated, oval or pyriform, with a longer stalk, more rounded ventrally and less sharply sculptured

- 69 (Mangafa and Kotsakis, 1996, p. 409; Levadoux, 1956; Jacquat and Martinoli, 1999).
- 70

The origins of wine production have been traced back to the Caucasus in the 7th-6th millennia 71 72 (McGovern et al., 2017). It is unknown if these first wines were made from wild or cultivated grapes (Bouby et al., 2021). Nonetheless, all evidence indicates that domesticated grapes also 73 originated there (e.g. Myles et al., 2011). Grapevine was cultivated, and probably domesticated, in 74 South West Asia by at least the 4th millennium BC (e.g. Miller, 2008; Fuller and Stevens, 2019). 75 Recent studies based on morphometric analyses of grape pips also suggest an early local 76 domestication in Greece that goes back to the Neolithic (Pagnoux et al., 2021). It is believed that 77 78 new domesticated varieties were subsequently introduced in Greece during the Late Bronze Age, 79 possibly through increased exchanges and trade with the Near East. The importance of fruit trees in past societies is greatly correlated to their role as a source of food 80 products with significant economic value, such as dried fruits, oil and wine. At least in some cases 81 their cultivation was initially based on reproduction by seed, which implies a great deal of 82

uncertainty because of the heterozygosity inherent in many fruit species, which means that seedlings often give rise to offspring that are very different from the parents (Bouby and Ruas, 2014). The switch to vegetative propagation through cuttings, marcotting and grafting is accordingly widely seen as a milestone in the domestication of fruit trees, as this allows the propagation of cultivars that are basically clones (Bouby and Ruas, 2014).

88

89 Grassi et al. (2003), Imazio et al. (2004), Arroyo-García et al. (2006) and more recently Riaz et al. 90 (2008) have performed simple sequence repeats (SSR) analyses on wild and cultivated grapevines. Their results suggest the presence of at least two separate grape domestication events: one in 91 Transcaucasia and another one in Western Europe, possibly in Spain and Sardinia. More recently, 92 De Michele et al. (2019) considered the hypothesis of a domestication centre and/or local 93 94 introgression in Sicily, given the close relation between Sicilian wild populations and local cultivated germplasm. Scienza (2008) and Forni (2012) describe several centers of 95 "accumulation", areas near ports located on major commercial routes, where many varieties were 96 gathered over the centuries due to frequent and intensive contacts and interactions. Some of these 97 98 areas have been identified as key centers of diversity. Distinct groups of cultivated grapevines were proposed by Negrul, who identified three *proles*: a *proles pontica* between Georgia and Asia 99 Minor and in the Balkans, a proles occidentalis in Italy, France, the Iberian Peninsula, and 100 Germany, and a proles orientalis in Central Asia, Persia, Armenia and Afganistan (Dalmasso, 101 102 1961). These groups match recent genetic evidence (eg. Bacilieri et al., 2013). Riaz et al. (2018) suggest that cultivars from the proles pontica were introduced to Western 103

Europe, because the wild grapes of Georgia – identified as an ancient centre of grapevine domestication – are closely related to Caucasian cultivated grapes (*proles pontica*) and the Western Mediterranean ones (*proles occidentalis*).

Today, domesticated grape diversity is the result of millennia of human selection and diffusion. 107 Ancient civilizations such as the Assyrians, Phoenicians, Greeks, Etruscans and Romans spread 108 viticulture first across the Mediterranean basin, and later also into more temperate regions 109 (McGovern, 2003). In the Western Mediterranean, there is an increasingly clear link between the 110 111 Phoenician presence and the development of viticulture (Botto, 2013), as may be seen from both the archaeobotanical record (Buxó 2008; Pérez-Jordà et al., 2017; 2021; Ucchesu et al., 2015), and 112 other elements in the archaeological record such as wine presses (Gómez Bellard et al., 1993) or 113 evidence of trench agricultural systems (Vera Rodríguez and Echevarría Sánchez 2013). Evidence 114 of Vitis vinifera in Phoenician sites is also found in association with animal bones (e.g. Moricca et 115 al., 2020; Portas et al., 2015), as it is believed that the waste from winemaking was used by 116 Phoenicians for meat preservation thanks to the antioxidant capacity of grapes (Sabato et al., 2019). 117 Morphometry, the statistical analysis of form and its (co)variation (Rohlf and Bookstein, 1990), 118 has played a key role in the study of grape pips from archaeological contexts since the early 20th 119 120 century to distinguish between wild and domesticated seeds (Stummer, 1911; Mangafa and Kotsakis, 1996). The approach was initially mainly based on measuring the length and breadth of 121 the whole pip but it has developed to include multiple parameters, such as the length of the stalk 122 or the position of the chalaza (Rivera Núñez et al., 2007); more recently, it has embraced geometric 123 124 morphometrics and, in particular, outline analysis (Terral et al., 2010). Although only genetic analyses can demonstrate a direct connection between modern cultivars and archaeobotanical 125 specimens (Guasch-Jané, 2019), geometric morphometric analyses are useful in archaeobotany, 126 where shape is often the only remaining datum (Portillo et al., 2020). Since most of the surviving 127 plant remains are usually seeds, outline analyses that do not require landmarks are particularly 128 129 suitable and have been successfully used in numerous studies (eg. Ekhvaia and Akhalkatsi, 2010; Terral et al., 2010; Orrù et al., 2013; Ros et al., 2014; Pagnoux et al., 2015; Sabato et al., 2015a; 130 Bonhomme et al., 2017; Bourgeon et al., 2018; Boso et al., 2020). Desiccated remains, whose 131 shape was not modified by (sub)fossilization, have proven to be the most suitable material for 132 morphometric analysis, followed by waterlogged ones (Bouby et al., 2013). An additional 133 advantage that these categories carry is the possibility to perform DNA analyses, which can serve 134 as a complementary tool (Bacilieri et al., 2017). 135

The study of charred grape pips is more challenging, as the (sub)fossilization results in a notable 136 137 degree of deformation, in particular a swelling of the seed, which mostly occurs on the ventral side 138 and which is best observed on the lateral outline (Smith and Jones, 1990; Bouby et al., 2018). This kind of deformation skews the identification of pips, moving them towards the wild morphotype. 139 Despite the distortion of charred botanical remains, differences in seed shape may remain 140 141 informative. Attempts to perform morphometric analyses on charred grape pips have been recently undertaken by Ucchesu et al. (2016) and Bouby et al. (2018), who both concluded that it is possible 142 to distinguish between wild and domesticated pips, even if they are charred. Bouby et al. (2018) 143 have moreover shown that if charring occurred at a temperature of 250°C or les, wild and 144 domesticated pips may not only be correctly distinguished from each other, but it is also possible 145

increases the classification error, which means that cultivar classification may only use modern 147 pips charred at lower temperatures and archaeological remains exposed to charring at low 148 temperatures. Since it is hard to reconstruct the temperature that formed the archaeobotanical 149 material, it is necessary to consider only large groups of very well preserved pips. In contrast, 150 151 inflated specimens (indicative of higher temperatures) should only be considered for distinguishing wild from domesticated pips (Bouby et al., 2018). This paper compares, for the first time, grape 152 pips collected from different sites with Phoenician connections to explore Phoenician interactions 153 with plants and food and the role of commercial routes and trade goods. Were Phoenicians 154 responsible for the spread of viticulture to the western Mediterranean? Or did they exploit and 155 possibly domesticate wild grapes? Are modern cultivars a result of Phoenician influence? 156

157 158

1.1. Study sites

The present study focuses on the analysis of finds from three sites related to the Phoenician-Punicdiaspora: Motya, Huelva and Nuraghe S'Urachi, which are respectively situated in Sicily (Italy),

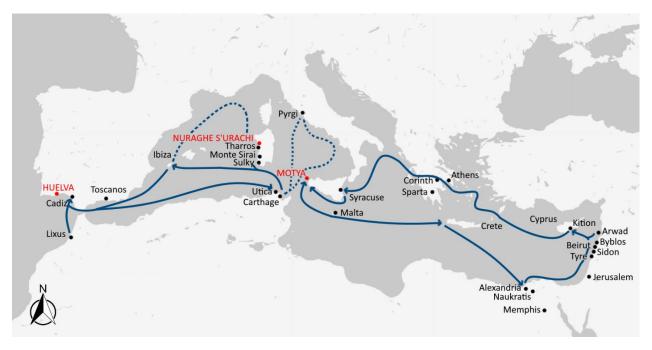
the Iberian Peninsula and Sardinia (Italy; Fig. 1). Motya was a Phoenician foundation, but both
 S'Urachi and Huelva were indigenous settlements already in existence before Phoenicians arrived

in Sardinia and southern Spain.

- 164 Phoenicians and their material culture travelled the length and breadth of the Mediterranean in the
- 165 first half of the 1st millennium BC (Aubet Semmler, 2001; López-Ruiz and Doak, 2019). Their
- 166 expansion from modern Lebanon towards the Western Mediterranean involved the major islands
- and many mainland coastal areas of the Mediterranean, such as Crete, Sicily, Sardinia, Andalusia
- and coastal North Africa. In all these areas, commercial and agricultural settlements were newly
- 169 established or created in association with existing indigenous ones, and the relations between
- 170 Phoenicians and local communities became a key aspect of the economic and social transformation
- 171 of these regions.
- While only Motya is a Phoenician foundation, all the three sites studied were nevertheless firmlyassociated with Phoenician expansion and cultural interaction.
- 174 Motya is a small island of about 45 ha situated in a sheltered lagoon on the Sicilian west coast,
- 175 where it had offered an ideal "stop-over point" on trade routes into the West Mediterranean,
- 176 including Sardinia, since the second millennium BC. The establishment of a permanent settlement
- 177 in the 8th century BC enabled the Phoenicians to consolidate their trade with the indigenous
- inhabitants of western Sicily (Nigro and Spagnoli, 2017; Nigro, 2018). The island had previously
- 179 been inhabited by local Sicilian communities (Nigro, 2018).
- 180 Nuraghe S'Urachi is situated on the central west coast of Sardinia, on the northern shores of the
- 181 Gulf of Oristano. The nuraghe itself was first built sometime in the Middle Bronze Age or mid- 2^{nd}
- millennium BC and remained continuously occupied well into the 1st millennium BC. From around
- the turn of the 8th-7th century, Phoenician material culture was imported in increasingly abundant
- 184 quantities and subsequently locally produced, suggesting a stable presence of Phoenicians (Stiglitz,
- 185 2007; van Dommelen et al., 2020).

Situated in the coastal wetlands created by two rivers flowing into the Atlantic, the site of Huelva was likewise an indigenous settlement, where Phoenician material culture was imported as early

- as the 9th century BC. It offered access to an important mining district in the interior, which enabled
- 189 its inhabitants to establish connections across both the Atlantic and the Mediterranean (Ruiz-
- its inhabitants to establish connections across both the Atlantic and the Mediterranean (Ruiz Gálvez Priego 1986; 2014). A small quantity of Sardinian pottery amidst a rich variety of
- 191 Phoenician and other imports shows the involvement of both indigenous communities in
- 192 Phoenician commercial networks Gonzales de Canales et al., 2006).



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Figure 1. Map of the Mediterranean with the sites of Motya (37°52'05"N, 12°28'10"E), S'Urachi (40°00'56"N, 8°34'57"E), and Huelva (37°14'53"N, 6°56'58"W) along the Phoenician trade routes.

196 2. Materials and Methods

197 **2.1. Modern material**

Ten cultivars collected from the "Vivaio Federico Paulsen: Centro Regionale per la Conservazione 198 199 della Biodiversità Agraria" in Marsala (TP, western Sicily) were selected as modern reference material (http://vivaiopaulsen.it). The choice of cultivars was based on their geographic origin, 200 with three cultivars regarded as native to Georgia ("Chichvi", "Ogialesci" and "Zerdagi"), five to 201 Sicily ("Albanello", "Catarratto", "Inzolia", "Perricone" and "Vitrarolo") and one to mainland 202 Italy ("Coda di Volpe" - Tab. 1; Ansaldi et al., 2014, Galet, 2000). Sicilian varieties were chosen 203 because they are assumed to be local, as they are attested in Sicilian writings since the 16th century 204 (Ansaldi et al., 2014). They may therefore be related to archaeological samples. The present study 205 is moreover the first one to use a reference collection focused on Sicilian cultivars. The origin of 206 207 "Zibibbo", also known as "Muscat of Alexandria", is debated. It has a long history of cultivation in Sicily, presumably starting with the Arabic domination of the Island (ca. 9th century AD), even 208 if it only first described as a cultivar in 1696 by Francesco Cupani (De Lorenzis et al., 2015). 209

Although many cultivars have both black and white mutants (e.g. "Pinot"), we have maintained a

balance in our sample between black and white grapes. 30 grape pips of each cultivar have been

sampled for the present study, and each pip was given a unique identification code in order to

compare its morphology before and after charring.

Abbreviation	Cultivar	Origin	Berry color	Number of pips
Alban	"Albanello"	Sicily	White	30
Catar	"Catarratto"	Sicily	White	30
Chicv	"Chichvi"	Georgia	White	30
Codad	"Coda di Volpe"	Italy	White	30
Inzol	"Inzolia"	Sicily	Black	30
Ogial	"Ogialesci"	Georgia	Black	30
Perri	"Perricone"	Sicily	Black	30
Vitra	"Vitrarolo"	Sicily	Black	30
Zerda	"Zerdagi"	Georgia	Black	30
Zibib	"Zibibbo"	Uncertain	White	30

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Table 1. Modern cultivars selected for the reference collection

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217 **2.2. Charring conditions**

Experimental charring was performed on the selected pips in order to train identification models 218 with pips representative of well-preserved archaeobotanical material. Charring conditions were 219 established following the studies realized by Ucchesu et al. (2016) and Bouby et al. (2018), which 220 evaluated the most suitable conditions of experimental charring for morphometric studies. They 221 222 respectively defined temperature ranges of 240-310°C and 250-450°C, below which charring resulted in heterogeneous carbonization and above which the pips disintegrated. Oxygen 223 availability and duration of heating had a smaller impact on pip deformation, although it seemed 224 slightly more accentuated under oxidizing conditions. Complete charring of the reference 225 226 assemblages occurred already after 20 minutes. Bouby et al. (2018) concluded that only pips charred at a low temperature should be used for identification at the cultivar level, as charring at 227 250°C allowed not only to distinguish correctly between wild and domesticated pips, but also to 228 identify accurately specific varieties. 229

Each of the selected pips, which had previously been given a unique identification code, was wrapped in two layers of aluminum foil in order to re-create reducing conditions, and to simulate the taphonomic factors which resulted in the creation of the archaeological assemblage. The pips were placed in a Thermolyne 48000 furnace at ambient temperature, the temperature was set at 250°C and the furnace was turned on. After 90 minutes the sample tray was removed from the oven.

236 **2.3. Archaeological material**

- 237 Archaeological grape pips were collected from the western Mediterranean sites of Motya, Nuraghe
- 238 S'Urachi and Huelva. The archaeological material from Motya consisted of 189 grape pips
- 239 deriving from an 8th-6th century BC refuse pit, which had been preserved by carbonization and
- 240 retrieved using the bucket floatation technique. The grape seeds come from six stratigraphic units
- interpreted as four depositional events (Table 2; Moricca et al., 2021). Few pedicels were found
- compared to the number of grape seeds. This is coherent with wine-making refuse as studied by
- 243 Margaritis and Jones (2006).

Filling layer	Stratigraphic unit (US)	Chronology
(FL)		
I-III	1112	End of 7 th – mid 6 th century BC
	2268	
IV	1406	Mid-7 th century BC
	1407	
V	1492	End of 8 th – first half of the 7 th century BC
VI	7234	Mid-second half of the 8 th century BC

244

Table 2. Stratigraphy of the Motya deposit (modified after Moricca et al., 2021).

246

The material from the sites of S'Urachi and Huelva was by contrast preserved by waterlogging. The 179 grape pips from S'Urachi come from the fill of a trench that was backfilled with rubbish from the early 7th century BC onwards, along with other fruits, cereals as well as large quantities of animal bones and pottery (Pérez-Jordà et al., 2020).

The 253 pips from Huelva were recovered from a fill context that possibly represents waste dumped in a port area. The sediment was extracted mechanically but associated radiometric dates and archaeological evidence allow us to place it in the 9th-8th century BC (Pérez-Jordà et al., 2017).

254 **2.4. Morphometric analysis**

Modern (both pre- and post-charring) and archaeological grape pips were positioned on a blue 255 background and photographed in dorsal and lateral position (Fig. 2) at a fixed magnification using 256 257 an Olympus SZ-ET stereomicroscope and an Olympus DP 12 camera. These images were processed in order to obtain black masks on a white surface. Outlines coordinates (x; y) were 258 extracted and 360 points, equally spaced along the curvilinear abscissa were sampled. 259 Normalization of the outlines was carried out by centering, aligning them along their longest axis, 260 261 scaling them using their centroid size, and by defining the first point right above the centroid (Bouby et al., 2018). Elliptic Fourier transform (EFT) approach (Kuhl and Giardina, 1982) 262 subsequently enabled us to turn the shape into multivariate coefficients. EFT involves the 263 decomposition of x- and y- coordinates as two harmonic sums of trigonometric functions 264 (Bonhomme et al., 2014). The two views were treated separately, and their coefficients were later 265 combined. In our case, seven harmonics were chosen for the two views, enough to gather 99% of 266 the total harmonic power. Each harmonic corresponds to four coefficients, so EFT resulted in 56 267 coefficients (2 views \times 7 harmonics \times 4 coefficients per harmonic), which weretreated as 268

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- quantitative variables. The Momocs package (Bonhomme et al., 2014; version 1.3.2 available at
- 270 https://github.com/MomX/Momocs) was used for morphometric analysis. All analyses were
- performed in the R environment, version 4.0.2 (R Core Team, 2020).



272 273

Figure 2. Pip #4 from stratigraphic unit US 1492 (Motya) in dorsal and lateral views.

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275 **2.5. Statistical analyses**

To begin with, shape variability of the modern charred and uncharred pips and the archaeological 276 seeds were assessed and compared at different levels using Principal Component Analyses (PCA). 277 Preliminary analyses were conducted to quantify any error related to the positioning of pips for 278 photographic documentation and their graphical elaboration. A PCAgeo (Fig. 3) on the matrices of 279 the EFT coefficients of modern uncharred pips was first calculated to characterize seed shape 280 variability at the cultivar level. A mean score was drawn up for each variety. The first two principal 281 components (PC 1 and PC 2) are shown in Figure 3, along with the corresponding morphological 282 space that illustrates the shape components captured by these PCs. Secondly, charred samples were 283 projected as supplementary observations in PCAgeo to visualize the effects of charring on pip shape 284 (PCA_{exper}; Fig. 4). Here again, only average scores per variety are represented. Mean shapes for 285 modern pips were also calculated to visualize these changes (Fig. 5). 286

Regarding the archaeological pips, a PCA_{archeo} (Fig. 6) was carried out in order to compare the pips
from the sites of Motya, Nuraghe S'Urachi and Huelva. We used the first 12 PCs, that gather 95%
of the total variance to test for differences between assemblages using a permutational MANOVA
as implemented in the package 'vegan' (Oksanen et al., 2019). We then carried out two PCAs

291 (PCA_{water} and PCA_{char}; Fig. 7 and 8) on modern material (uncharred and charred). The waterlogged

(Huelva and S'Urachi) and charred (Motya) archaeological pips were reprojected on these PCAsas additional observations.

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3. Results

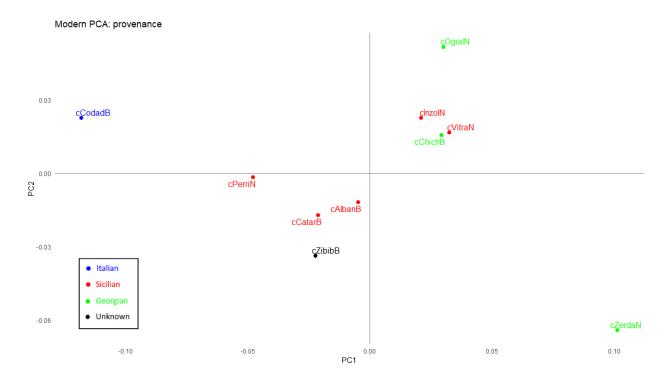
The first two principal components (PCs) of the PCA performed on uncharred modern samples (PCA_{geo}) explain 59.2% of the total variance (Fig. 3). PC1 (38.2%) distinguishes between roundish pips with a short stalk, characterizing shapes close to the wild morphotype, and more elongated pips with a longer stalk, typically associated with cultivated grapes. PC2 (21.0%) mostly captures the straightness/curviness of the pip outline in lateral view.

301 3.1. Provenance of cultivars

302 The application of PCA_{geo} to modern materials makes it possible to highlight the differences in

so2 The application of PCA_{geo} to modern materials makes it possible to highlight the differences in seed shape between cultivars, which can be compared to the geographical origin of each cultivar

304 (Fig. 3).



305 306

Figure 3. PCA on reference cultivars in association with their geographical origin (PCA_{geo}).

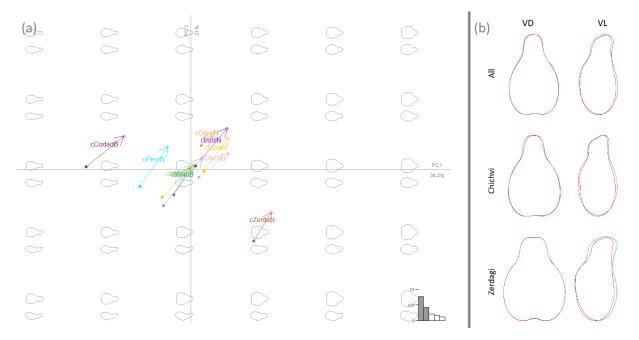
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The center of the plot is occupied by the Sicilian cultivars, while the Georgian ones are shifted towards the righthand side of the graph. Pips of the Georgian cultivars are broader and more laterally curved than the Sicilian ones, which present a shape closer to that of wild plants. "Coda

di Volpe", which is the only cultivar from the Italian mainland in the analysis, presents by contrastpips that are on average more elongated and straighter in the lateral outline.

313 **3.2. Charring effect**

PCA on reference materials has enabled us to describe the effects of experimental charring in terms of changes in pip shape (PCA_{exper}; Fig. 4). This is reflected in a general inflation of the pip bodies, resulting in an overall plumpness (PC1) and a straightening of the lateral outline (PC2). All cultivars follow a similar pattern. The calculated mean shapes associated with the charring conditions (Fig. 5) show that charring mostly affects the lateral section, particularly on the ventral side with an inflation of the body and a change in inclination of the beak. The changes in the dorsal view are nevertheless much lower.



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Figure 4. The effects of charring on modern cultivars: a) PCA describing the effects of charring on modern cultivars
 (PCA_{exper}); b) mean shapes of all pips, Chichvi pips and Zerdagi, dorsal (left) and lateral (right) outlines. Blue line
 for uncharred pips, red for charred ones.

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326 3.3. Comparison of archaeological samples

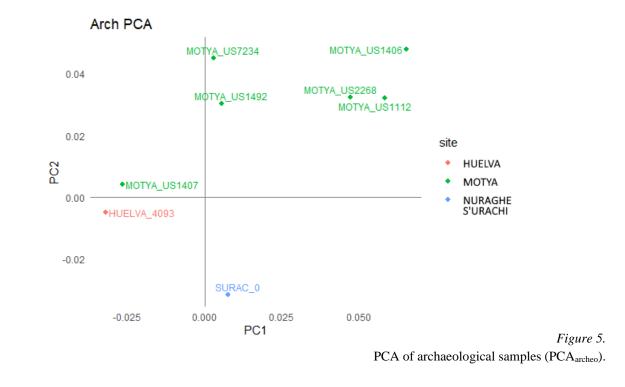
A PCA of all the archaeological samples was also carried out (PCA_{archeo}; Fig. 6). In PCA_{archeo}, the first two PCs explain 59.6% of the total variance. PC1 (40.9% of variance) distinguishes roundish pips with a short stalk from the more elongated ones, with a longer stalk, while PC2 (18.7% of variance) is correlated with the curvature of the lateral outline.

331 While some differences can be seen along PC1, with pips from Motya's stratigraphic units 1112,

1406 and 2268 being rounder than the others, most differences can be observed along PC2.

333 Waterlogged pips, particularly from S'Urachi are characterized by a more curved lateral outline,

while pips from the Motya deposit have a straighter lateral outline. This is coherent with the results of the previous analyses, as PC2 is mostly explained by charring. Pips from Huelva and Motya's US 1407 are very similar, as they are the most elongated ones among the studied pips; they also have a similar curvature in the lateral outline. While looking specifically at the waterlogged assemblages, it is possible to see that pips from S'Urachi are the broadest. This analysis does not, however, take into consideration the effect of charring, which our experiments have shown to cause an inflation of the pip body.



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results coherent with those obtained in PCA_{archeo}.

Archaeological waterlogged samples were later projected on PCA_{geo}, previously obtained for modern uncharred pips (Fig. 7). PCA_{water} allows morphological comparison of the pips from Huelva and S'Urachi with modern cultivars. Although the pips from S'Urachi are closest to those of "Albanello", they do not match the modern cultivars. This is also the case with the ones from Huelva.

The permutational MANOVA carried out to test differences between assemblages was comprised of a pairwise comparison of modern varieties (both charred and uncharred) and archaeological

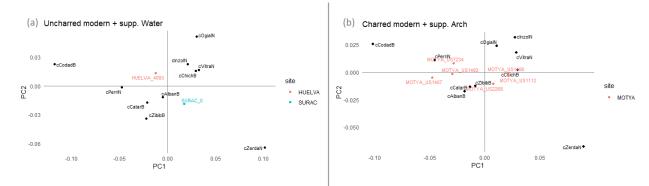
samples. In pairwise comparisons, p-values lower than 0.05, which indicate differences in the

pairwise comparison, were seen for: "Albanello" and Huelva; "Coda di Volpe" and Motya

- US2268; "Ogialesci", S'Urachi and Motya US1407; "Perricone", Motya US1407 and US2268;
- 357 "Zerdagi", Motya US1492 and US2268.

For this reason, separate PCA analyses were also performed on the waterlogged (Supplementary Material 1) and charred archaeological pips (Supplementary Material 2). These analyses showed

- 358 Finally, average scores for samples from Motya were plotted on the PCA previously obtained for
- the experimentally charred modern cultivars (Fig. 8). This shows that the samples from Motya are
- relatively distant from modern cultivars. Even so, the pips from US 1407 and US 7234 are most
- 361 similar to "Perricone", while the mean score for US 1492 falls between "Perricone" and
- 362 "Catarratto", US 2268 is closest to "Zibibbo", and the pips from US 1112 and US 1406 are finally
- 363 closest to "Chichvi".



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Figure 6. a) PCA of waterlogged archaeological seeds and modern uncharred cultivars (PCA_{water}); b)PCA of charred grape pips from Motya and modern reference cultivars experimentally charred (PCA_{char}).

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368 **4. Discussion**

369 The present study represents a first attempt to evaluate differences in assemblages of grape pips from Phoenician sites and sites that underwent Phoenician influence. Here we show that: a) there 370 is a correlation between the geographical origin of modern cultivars and the shape of their pips; b) 371 the results of the charring experiments are coherent with the existing literature; c) a comparison 372 between modern and archaeological pips allows the identification of the sites with the most and 373 374 least "domesticated" pips; d) no direct match with modern cultivars has been found, even if some similarities may be noted. 375 In the first place, we have found a correlation between the pip shape of modern cultivars with their 376

geographical origin, despite a slight overlap of the Sicilian and the Georgian clusters. This
correlation has previously been observed, just as it has also been noted that considering a higher
number of varieties results in less clear-cut differences between geographical groups (Pagnoux et
al., 2015).

- The results concerning the deformation of pips by charring are consistent with the experiments conducted by Smith and Jones (1990) and Bouby et al. (2018) and show that the grapevine seeds become rounder when charred. The stalk is less affected by deformations, at least in its length.
- 384 Charring also affects the lateral side by decreasing its curvature.
- 385 PCA_{archeo} carried out on archaeological pips allows a first comparison of their morphology.
- 386 Although slight differences were seen in the roundness of the pips, with those from Huelva and
- 387 Motya's US 1407 being the slimmest, and those from three stratigraphic units in Motya being the

- roundest, most differences concerned the straightness of the lateral outline and may be ascribed tocharring.
- A more appropriate comparison of archaeobotanical samples was carried out indirectly, by
 projecting waterlogged pips on PCA_{geo} and charred ones on PCA_{exper}.
- 392 The comparison between waterlogged seeds from Huelva and S'Urachi highlighted differences
- rather than similarities. On average, pips from the former site were slimmer and their lateral outline
- 394 was less curved, in comparison to the ones coming from the Sardinian excavation. At the site of
- Sa Osa, which is only 10 km from S'Urachi, *Vitis vinifera* remains were found in levels dating to the 13th century BC (Orrù et al., 2013), suggesting an earlier tradition of vine cultivation. This could have been maintained on the island in the following centuries, as suggested by the high concentrations of *Vitis* pollen in the adjacent Mistras lagoon between the middle Bronze Age and the Punic Period (approx. from 3500 to 2500 cal BP; Di Rita and Melis, 2013). In contrast, in the
- 400 Huelva area grape pips are not present before the $9^{\text{th}}-8^{\text{th}}$ centuries.
- 401 Comparison with modern varieties is not as straightforward. Even if pips from S'Urachi share some features with the modern ones of "Albanello", "Chichvi", "Vitrarolo" and "Zibibbo" grapes, 402 no clear correspondence is evident. Even if this lack of conformity may be due to the limited size 403 of the reference collection, we should consider that these are modern varieties, that were quite 404 405 likely introduced later in history. How closely related can modern and ancient varieties be in a given region? Since grapevines are commonly managed through vegetative propagation it is 406 possible for varieties to remain genetically unchanged for centuries. Nonetheless, new cultivars 407 can be created through sexual crosses or somatic mutations. Ancient DNA studies that may help 408 to trace kinship with modern varieties have been undertaken on archaeological pips from several 409 410 archaeological sites in France (Ramos-Madrigal et al., 2019). Although a clear match ("Savagnin blanc") was only found at a medieval site, several pips from Roman sites show first-degree 411 relationships with modern French and Swiss cultivars. This suggests that at least some varieties 412 may remain virtually unchanged since Roman times, and possibly for a longer time. 413
- 414 Misidentification can, nonetheless, be influenced by taphonomic factors, including the fact that 415 remains may become slightly swollen because of waterlogging (Pagnoux et al., 2015).
- The samples from Motya, which have been preserved through charring, differ mostly by their 416 roundness. Pips from the most recent stratigraphic units (US 1112 and US 2268), which make up 417 418 a single filling layer (Table 2), are the wider ones, with their shape appearing to be of a "least domesticated" type. The expression "least domesticated" refers to the morphology, describing the 419 rounder pips with shortest stalk, closest to the wild morphotype. Similarly, the "most 420 domesticated" pips are the most elongated ones with long stalks. The presence of different 421 422 morphologies within one filling layer may be related to the fact that these stratigraphic units also included materials from pre-Phoenician layers (such as pottery and other objects), dated back to 423 the 16th-13th century BC. The "least domesticated" pips could therefore have been deposited along 424 with the latter. Pips of units 1407, 1492 and 7234 seem by contrast more elongated and with longer 425 stalks. It is surprising, therefore, that pips from stratigraphic units 1406 and 1407, which also 426
- 427 belong to the same depositional layer, differ quite substantially in shape. This may however be due

428 to the small sample size obtained from US 1407. There exists in fact overlap between the datasets,

- 429 if we examine the values obtained for single pips. The differences may be explained by the
- 430 presence of different grape varieties within the same depositional layer. A second explanation
- 431 could be uneven charring in the archaeological context. The context of retrieval is a disposal pit
- that is several meters wide (Moricca et al., 2021). This would allow for the possibility that higher
- temperatures were achieved in US 1406 than US 1407, found on the opposite sides of the same
- filling layer. Charring at higher temperatures has been proven to cause an inflation of the pip bodies
 (Bouby et al., 2018). Nonetheless, this explanation seems less likely. Such reasoning may also
- 436 apply to the samples from US 1112 and 2268, even if the values are not as contrasting.
- 437 In terms of their correspondence to modern cultivars, the outlines of pips from units 1112 and 1406
- 438 best resemble pips from "Chichvi" grapes, while the seeds from the other samples present shapes
- noticeably different from the modern reference cultivars. It is interesting in this regard to consider
 the results of the genetic study carried out by Riaz et al. (2018), who analyzed 1378 cultivated and
- 441 wild grape samples collected around the Mediterranean basin and Central Asia. While most Italian
- 442 cultivars clustered with those from France, Spain and Pakistan Turkmenistan, a small subset was
- 443 associated with wild and cultivated grapevines from Georgia. This suggests that the first 444 domesticated cultivars in Central Asia and Caucasus (the *proles pontica*) somehow did leave a 445 genetic footprint in the Western European *proles occidentalis*.
- "Zerdagi" is the modern variety with the broader seeds and least elongated stalks, and which is
 therefore most like wild grapes, but there are no subfossil equivalents in either the waterlogged or
 charred assemblages. The same holds for the "Coda di Volpe" variety, which has the slimmest
 pips and the shortest stalks. These observations may suggest a certain level of domestication of the
- 450 vine from all the sites studied.
- Although this reference collection is smaller than those used in other morphometric studies of
 archaeological grape pips (e.g. Bouby et al., 2021; Pagnoux et al. 2015; Bonhomme et al., 2021),
 it still encompasses the previously observed archaeological variability.
- The results of PCA_{water} and PCA_{char} suggest that archaeological material from the three sites is, morphologically, broadly comparable to the modern varieties, which similarly suggests that the archaeological finds represent, broadly speaking, domesticated grapes. If we take into consideration the effects of charring, we note that the "slimmest" pips come from three stratigraphic units at Motya, which may suggest the presence, or perhaps cultivation, of "more strongly domesticated" varieties at or near the Sicilian site.
- A diversity of cultivated grapes can be observed in the studied sites. This may be ascribed to localadaptation, one hypothesis could be introgression with local grapevines, as suggested by Forni
- 462 (2012). Another option to consider is that the pips of Motya could be related to the *proles pontica*,
- 463 which is believed to have left a genetic footprint in the *proles occidentalis* in the Western
- 464 Mediterranean (Riaz et al., 2018). This might explain differences between the samples from Motya
- and those from the indigenous sites of S'Urachi and Huelva.
- 466 Sicilian viticulture is structured by a group of varieties of regional interest, and a bigger group of
- 467 minor varieties present with a certain frequency only in specific viticultural areas or at the level of

468 a few strains. The latter are referred to as "reliquia" and include "Inzolia" and "Vitrarolo" (Scienza 469 and Failla, 2016). Genetic studies have identified the variety "Sangiovese", as the progenitor of

- 470 numerous Sicilian cultivars, amongst which "Perricone". Furthermore, multivariate statistical
- 471 analyses carried out on 11 SSR loci of 46 Sicilian varieties have shown a clear distinction between
- 472 varieties based on their geographical origin, with Eastern Sicilian cultivars ("Nerello mascalese",
- 473 "Nerello cappuccino", "Frappato", "Perricone" and "Carricante") clustering on one side, and those
- 474 typical of western Sicily ("Inzolia", "Catarratto", "Grecanico", "Nero d'Avola") on the other (De
- 475 Lorenzis et al., 2014).
- 476 A correspondence between our morphometric results and the ampelographic history of Sicily is
- not straightforward, either. Even so, pips from S'Urachi find morphological similarities with those
 of "Albanello", while three samples from Motya are plotted in the space between "Catarratto" and
- 479 "Perricone". It is however unclear how we may interpret such associations.
- The substantial intra-site diversity in pip morphology at Motya should also be noted, as the sample would seem to cover a range of different varieties. Taphonomic factors, such as differential charring, may however have affected pip morphology. At all the sites the morphology of the pips suggests fully domesticated grapes. The clear differences between pip morphology at the three sites goes against the idea that Phoenicians favoured the spread of grape varieties. It appears more likely that their viticulture was based on local grape varieties, and thus varied with the sites where they settled
- they settled.
 The first evidence of viticulture in the Central and Western Mediterranean comes from Northern
 Italy (Cremaschi et al. 2016; Pecci et al. 2020) and Sardinia (Sabato et al. 2015) and has been
- Italy (Cremaschi et al., 2016; Pecci et al., 2020) and Sardinia (Sabato et al., 2015) and has been dated to the Middle and Late Bronze Age (ca. 1650-930 BC). It does not, however, point to the exclusive use of domesticated varieties. There is little or no evidence to assess the continuity, scale and prominence of viticulture in the western Mediterranean during the later 2nd millennium BC,
- 492 but that changes by the beginning of the 1st millennium with new finds from Tunisia, Sicily,
 493 Sardinia and the Iberian Peninsula, which may be associated with a Phoenician presence. The rapid
- 494 growth of vine cultivation and wine production in the first half of the new millennium generated 495 increasingly frequent exchanges of wine amphorae across the Western Mediterranean (Botto,
 - 495 increasingly frequent exchanges of white amphorae across the western includent (Botto,
 2013). Vine cultivation and wine production also became widespread in indigenous areas, where
 amphora production and wine presses have been found (Pérez-Jordà et al., 2013). Thanks to the
 Orientalizing influence, which includes not only Phoenicians, but also Greeks and Etruscans, wine
 - 499 soon became a very successful and widely distributed product with substantial agricultural, 500 economic and cultural impact and significance (Botto, 2013). This underscores the interest of 501 trying to define in precise terms the history, distribution, expansion and cultural appreciation of 502 this crop. The varieties that were introduced and/or created in each of the areas are an essential 503 element of this historical process, which indicates the relevance of the line of research that is 504 opened with this work.

505 **5.** Conclusions

- The present study has first of all allowed us to characterize the pip morphology of grapes cultivated 506
- 507 by Phoenicians and connected indigenous communities in the West Mediterranean and to assess
- the similarities and difference between these ancient seeds and modern varieties using geometric 508
- morphometry. We first of all observed that all the studied archaeological grape pip samples fall 509
- 510 within the range defined by the modern cultivars, which is reason to describe our archaeological
- samples as 'domesticated-looking'. Taking in consideration the effects of charring on pip shape, 511 pips from three stratigraphic units at Motya (1407, 1492 and 1407) appear to be the slimmest. 512
- More specifically, pips from stratigraphic unit 1407 are the most elongated, closely followed by 513
- those from units 1492 and 7234. Although it was not possible to associate them with any specific 514
- modern variety, they broadly resemble the Sicilian cultivars "Perricone" and "Catarratto". In terms 515
- of elongation, these are closely followed by pips from Huelva, which do not resemble any of the 516
- reference cultivars. The remaining pips from S'Urachi and Motya (US 2268, 1406 and 1112) are 517
- rounder. 518
- 519 None of the pips investigated resembles those of the Georgian cultivar "Zerdagi", which has the roundest pips amongst the selected modern cultivars. 520
- The key challenge for the present study was the comparison of archaeological pips preserved 521
- through different fossilization processes (waterlogging and charring). Although it is impossible to 522
- 523 undo the charring conditions that created the archaeobotanical assemblage, experimental charring
- has been used to obtain material that may be compared to well-preserved archaeobotanical 524
- material. The use of two reference datasets of the same modern cultivars, one uncharred and one 525 charred, has enabled us to tackle the complications of differential preservation.
- 526
- Our analysis represents a first step towards a better understanding of diachronic and synchronic 527 528 relationship between vines grown in the ancient West Mediterranean. We are well aware that the small number of reference cultivars used in this study may not adequately represent the diversity 529
- of modern grapevine cultivars. Even so, our selection still offers a general impression of modern 530
- similarities with the archaeological samples, which supports our suggestion that local cultivation 531
- 532 and selection could have produced important changes and long-term consequences.
- We recommend that future studies be carried out with a larger number of modern reference 533 cultivars that better represent contemporary biological diversity. An additional focus could be 534 placed on modern Sardinian and Iberian cultivars. If the number of samples from each 535 536 archaeological site could also be increased, we would also gain a better understanding of grape 537 diversity at each site. Even so, finds from other sites would likely be necessary to take a broader view and to evaluate the role of Phoenicians in the diffusion of the grapevine across the western 538 Mediterranean. 539
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- 551

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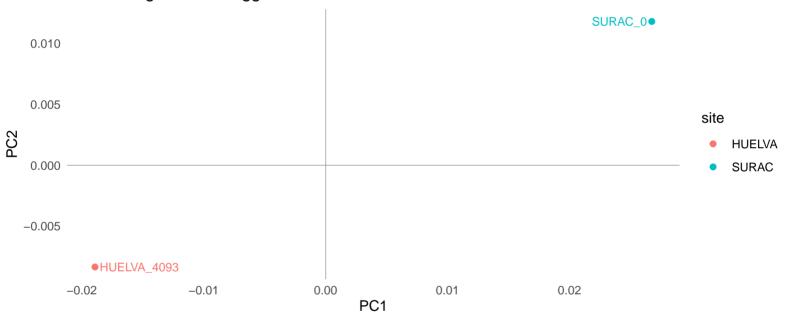
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823 Supplementary materials

- 824 *Supplementary material 1.* PCA of waterlogged archaeological samples.
- 825 *Supplementary material 2.* PCA of charred archaeological samples.

Supplementary Material 1

Archaeological waterlogged PCA



Supplementary Material 2

