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Study of Grapevine (*Vitis vinifera* L.) Seed Morphometry and Comparison with Archaeological Remains in Central Apennines

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Abstract: Studying the evolution of seed morphology and, in turn, the evolution of cultivars across time and space is of fundamental importance to agriculture and archaeology. The identification of ancient and modern grapevine (*Vitis vinifera* L.) cultivars is essential for understanding the historical evolution of grape cultivation. Grape seed morphology provides valuable information to explore the evolution of grape cultivars over time and space. The main aim of our study was to build a comprehensive regional database of grape seed morphological traits from modern and archaeological wine cultivars and wild grape species. We aimed to identify which seeds of modern grape cultivars exhibited morphological similarities to archaeological cultivars. This study focused on fifteen distinct modern types of seeds and two archaeological samples from the Byzantine-to-Early Medieval period. We acquired digital images of seeds using a flatbed scanner. For each sample, 100 seeds were randomly selected, and morphometric data on each seed were gathered using ImageJ. Differences among the seed cultivars were investigated using linear discriminant analysis. Archaeological seeds were found to be more similar to cultivated *V. vinifera* cultivars rather than *V. sylvestris* populations. Among the cultivated cultivars, Sangiovese and Tosta antica resulted to be cultivars most similar cultivars to the archaeological ones. The morphometric analysis of grape seeds proved to be a valuable resource for investigating the evolution of vine cultivars throughout history. Combining image analysis techniques with genetic data will open new perspectives for studying the origins of and variations in grape cultivars, contributing to the conservation and enhancement of viticultural heritage.

Keywords: seed morphology; seed image analysis; grapevine; archaeological grape pips; *Vitis sylvestris*

1. Introduction

One of the Old World's traditional fruits—the grapevine—is a vital component of the earliest genus of fruit trees that shaped the development of horticulture in the Mediterranean region [1]. In Eurasia and North Africa, two forms of *Vitis vinifera* co-exist: the cultivated form, *Vitis vinifera* L., and the wild form, *Vitis vinifera* var. *sylvestris* Willd. (*V. sylvestris*) [2]. The primary difference between wild and cultivated *Vitis* lies in their reproductive systems: wild grapes are dioecious, whereas most cultivated varieties have hermaphroditic flowers. This species includes thousands of known cultivars, grown in a wide array of climatic conditions [3]. According to Martin-Gómez et al. [4], the domestication process, which most likely involved the vegetative propagation of clones by growing

cuttings, caused significant changes in the reproductive biology of grape cultivars. These changes led to different properties, including physical (size, colour), nutritional (sugar, carbohydrate, and phenolic compounds), and seed-shape diversification. The description, classification, phylogenetic relationships, and discrimination of grape cultivars have been traditionally carried out using ampelography, a field of classification which employs the shape and colour of leaves, bunches, and berries [5,6]. Recently, molecular methods based on the detection of both nuclear and chloroplast polymorphisms (DNA fingerprinting) have been widely utilised for the description of samples collected in the field as well as the identification of cultivars [7–9] and molecular markers, and, especially, SSRs (simple sequence repeats) have become an essential tool for the identification of grape cultivars and clonal discrimination. Microsatellite markers are multiallelic, highly polymorphic, and accurate means of detecting genetic polymorphisms [10–13]. When there is access to fresh plant material, these methods are exceedingly simple and precise.

Archaeobotany, also known as paleoethnobotany, is the scientific study of plant remains from archaeological sites for the reconstruction and interpretation of human–plant relationships and historical settings [14,15]. *Vitis* grapevines are propagated vegetatively, so that the individual vines of a cultivar are genetically identical to one another and to the single original seedling from which the cultivar originated. While some cultivars may have originated in the regions with which they are now associated, others are thought to have been introduced by traders or conquerors, most notably the Romans [16,17]. Even these seeds underwent important changes due to domestication processes. Since 1911, it has been widely accepted that domestication altered the dimensions and other morphological characteristics of seeds, enabling the distinction between cultivated and wild vines [18]. The seeds of wild species are small, robust, and with a rounded or cordate outline, with short peduncles, a flat ventral side with acute angles, and a strongly developed chalaza, while those of the cultivated species are large, elongated, oval, or piriform, with an elongated seed stalk [19]. The stalk is generally considered to be a valuable characteristic, as, under the effects of cultivation, its length increases proportionally faster than the length of the corpus [20]. However, many factors determine the shape of seeds, such as, for example, the number of seeds in each berry, the size of the berry, and its maturity [21]. Studies on grape seeds, in many archaeological sites, provide the opportunity to understand the distribution and domestication of wild vines [1,22].

In many archaeobotanical studies, the taxonomic attribution of diaspores has been achieved by simple morphological observation and comparison with ex situ collections of the seeds [14]. Using digital images, Bacchetta et al. [23] characterised the seeds of wild plants typical of the Mediterranean basin, implementing statistical classifiers able to discriminate seeds belonging to different genera and species and achieving promising results. Currently, this method is fully accepted and utilised not only for archaeological studies [24,25] but also to study cultivated plants and compare different cultivars, contributing to their characterisation and conservation in seed banks [26,27]. Seed image analysis has gained relevance in morphometrics and colour evaluation for its utility in the identification of diasporas of wild plant species, proving a useful tool for taxonomic studies, where very close taxa need to be discriminated between [3,28–32]. Indeed, morphometric analyses can distinguish cultivated grape seeds from wild ones and even some cultivars [19,32–35]. Also, a significant correlation between similarity in pip morphology and the taxonomic relationship was demonstrated [36–38].

This article proposes an accurate identification approach for the recognition of archaeological seeds belonging to the genus *Vitis* in the Abruzzo region, in Central Italy, (Figure 1) based on characterisation by image analysis. In particular, the objectives of this study were the following:

(1) Create a comprehensive database of the morphological characteristics of grape seeds from archaeological, ancient, and contemporary grape cultivars as well as from wild grape species (*V. sylvestris*) found in the Abruzzo region;

(2)   Determine whether and which seeds of modern grapes are morphologically compara-
ble to the archaeological cultivars based on seed morphology.
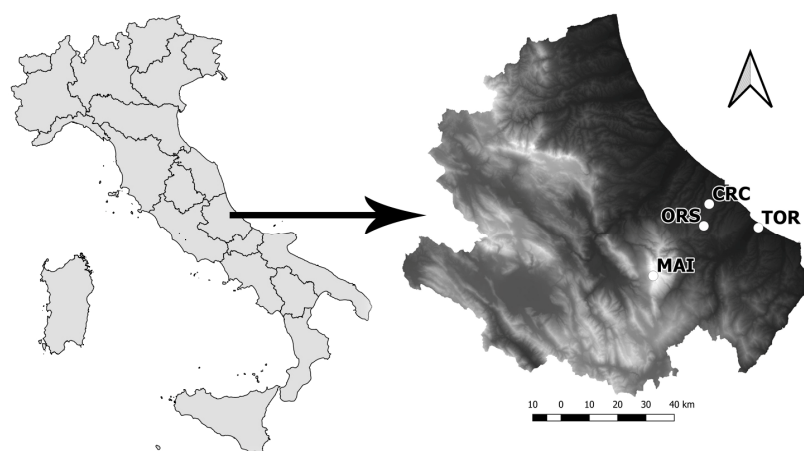
Figure 1. Geographical distribution. The work was carried out in Italy, in the Abruzzo administrative
region. The materials were collected in the following locations: Crecchio (CRC) archaeological
samples, Orsogna (ORS) modern cultivars, Torino di Sangro (TOR) *V. sylvestris,* and Maiella (MAI),
where the ancient cultivars were collected. The Abruzzo region is represented above using a digital
terrain model.

In summary, this article integrates archaeological data in the use of advanced imaging
techniques, filling significant gaps in our understanding of the historical evolution of grape
cultivation, and offers a replicable framework for similar studies in other regions and with
other species.

2. Materials and Methods

Archaeological seeds, wild vine seeds, and seeds of modern cultivars were collected
from four different locations in the Abruzzo region of central Italy, as shown in Figure 1.

2.1. Archaeological Background

The Byzantine settlement of Vassarella-Casino Vezzani was located in the municipality
of Crecchio, in a rural area where there had been a rustic villa dated between the sixth
and early seventh centuries [39]. At Vassarella-Casino Vezzani, there is an abundance
of particular ceramic pieces with shapes clearly intended for the conservation of liquids,
in particular wine, and foods and for their use on the table. This was evidenced by the
sediments we analysed, which notably contained grapes and fragments of fig fruits. The
presence of people and the exploitation of vast areas around the villa is also evidenced
by the presence of peach seeds (*Prunus persica*), cherries (*Prunus avium*), and, above all,
grapes, present both in the ground and in the archaeological sediments in some of the
"Crecchio-type" jugs, attesting to a flourishing viticulture [40]. Another site from which we
analysed archaeological seeds was the tomb of Santa Maria da Piedi, in the historic centre
of Crecchio, dated between the sixth and ninth centuries. The data from this site have not
yet been published. The archaeobotanical remains of grape seeds from both sites are kept
in the archaeological museum of Crecchio.

2.2. Archaeological Seed Collection

Due to their high conservation value, two archaeological sites in Abruzzo were selected
for the collection of seeds. Both sites are in the southern Abruzzo area, within the Crecchio
municipality (CH).

In the first site (church of Santa Maria da Piedi, Crecchio, 4,683,200 m N–444,520 m E,
WGS84), 435 seeds were collected on 25 July 1995. The archaeobotanical material from the

necropolis of Santa Maria da Piedi was recovered inside a box burial from the Byzantine era (VI-VII century AD); for this reason, the seeds analysed are charred and have the characteristic appearance of finds preserved in a very dry place. The burial, which is comparable with remains of similar chronology and typology documented in other locations in Byzantine Abruzzo [39], is located beneath the modern flooring of the Church of S. Maria and was found intact during restoration work; therefore, risks of possible contamination of the organic remains were excluded. Originally, the grave was sealed with a limestone slab, and the deceased was without grave goods and had been placed on a thin sandy layer, which constituted the bed of deposition. On the other hand, in the second archaeological site, only 94 seeds were found inside an amphora in the cistern of the Roman-Byzantine villa in the locality of Vassarella di Casino Vezzani (4,684,180 m N–446,210 m E, WGS84), excavated on 13 September 1991. At the time of discovery, the large concrete cistern was almost completely full of water and slime [39]; for this reason, the seeds analysed have the characteristic appearance of finds preserved in a de-oxygenated environment impregnated with water. The Vassarella-Casino Vezzani villa was probably the object of exploitation aimed at supplying the nearby port of Ortona, perhaps initiated by the Byzantines themselves, who had assumed control of it in 538, during the Greek-Gothic war (535–553) [39]. The archaeological filling of the cistern, which probably took place between the sixth and seventh centuries, included numerous examples of fine ceramic tableware shapes, imported mainly from Africa and Asia Minor, and numerous examples of amphorae, some of which were whole, although most were fragments, used for the transport of oil and wine, originating from Africa and the East and thrown in the cistern after having been emptied of their contents.

Plant remains from the sediments of the Villa Vassarella-Casino Vezzani cistern and Tomb 4 of the Santa Maria da Piedi necropolis were recovered using the flotation technique. The other grape seed samples found in these excavations were not taken into consideration as they consisted of a low number of seeds and, therefore, would not have been able to give statistically valid results in the analyses. The seeds were stored away from light in 15 mL plastic vials in a dry, cold environment. Before image collection, the seeds were cleaned to remove contaminants and photographed under a Nikon SMZ 745T stereomicroscope with a DS-Fi3 camera (Figure 2). After image analysis, the sampled seeds were placed back in the original tubes and returned to the Byzantine and Early Medieval Museum of Abruzzo.

2.3. Modern Seed Material

We used a modern pip reference collection consisting of fifteen cultivars. Seeds were collected from twelve different cultivars of vines obtained from a collaboration with Cantina Sociale Orsogna, including eight white grape cultivars (*Cocacciola*, *Falanghina*, *Malvasia*, *Montonico*, *Moscato*, *Passerina*, *Pecorino*, *Trebbiano*) and four red grape cultivars (*Merlot*, *Montepulciano d'Abruzzo*, *Primitivo*, *Sangiovese*). These grape cultivars are among the most cultivated in the Abruzzo region. Furthermore, we collected seeds from three local ecotypes (*Nero Antico*, *Tosta Antica*, and *Vedovella*) that are no longer cultivated because they have been replaced by more productive cultivars of *V. sylvestris* populations conserved in the Abruzzo region [41]. Grapes were harvested when the sugar content in the pulp was at its highest, indicating maturity and coinciding with the point at which the morphological development of the seed was complete [34,42]. The seeds were randomly taken from berries that came from randomly chosen bunches of grapes to maximise the representativeness of the morphometric variability in the batches. We only focused on cultivars from the Abruzzo region. All the seeds were scanned at a high resolution using the same equipment and procedures, and they were all collected in the same year. Previous databases are inconsistent in terms of the number of scanned seeds and their quality [43,44], so we decided to select only our data for further analysis.
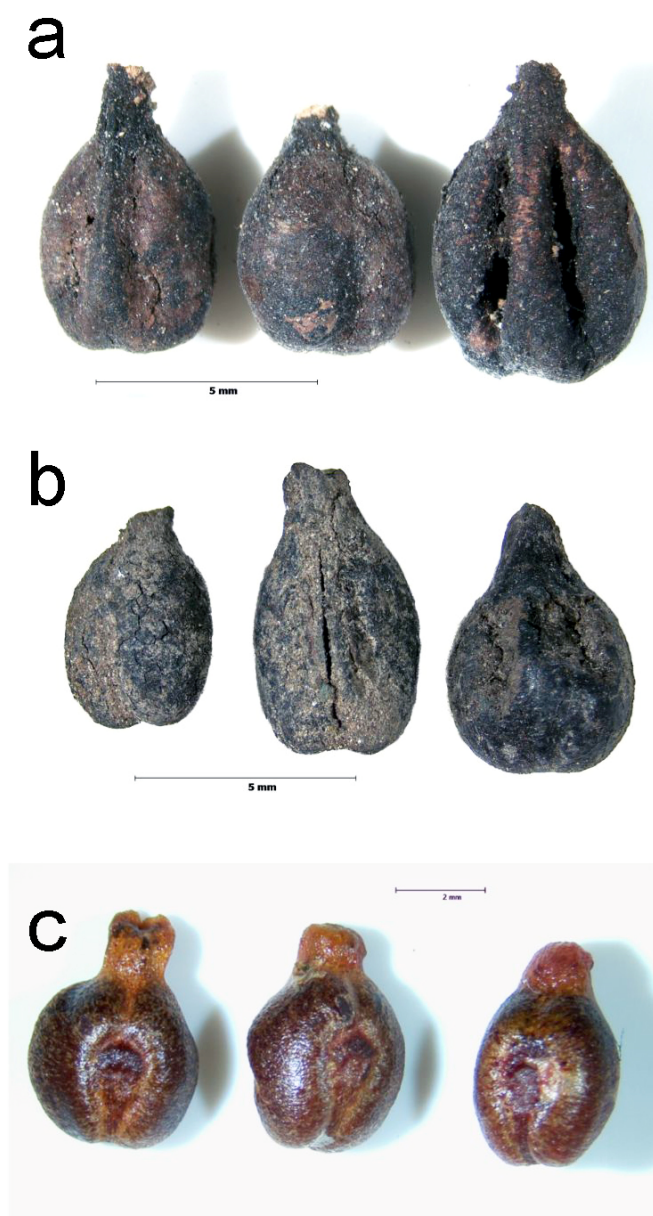
Figure 2. Stereoscopic photographs of archaeological seeds and wild vines. (**a**) Grape seeds from Tomb 4 in Santa Maria da Piedi. (**b**) Grape seeds from Vassarella-Casino Vezzani. (**c**) Grape seeds of Vitis sylvestris harvested in 2021 in the locality of Torino di Sangro (TOR).

2.4. Seed Image Analysis

Digital images of the modern and archaeological seeds were acquired using an Epson GT 15,000 flatbed scanner with a digital resolution of 600 dpi and a colour depth of 24 bit. Seed samples consisting of 100 seeds were randomly chosen from the original seed batches [45]. Image acquisition was performed after seed drying at 15 °C and 15% R.H. to overcome differences in dimension and shape due to seed hydration.

To characterise seed shape heterogeneity, each batch of seeds was scanned three times, with the seeds randomly arranged on the flatbed scanner each time [34]. The obtained digital images were stored in TIFF (Target Image File Format) [46]. Before image acquisition, the scanner was calibrated for colour matching using a colour calibrator (Datacolor Spydercheckr). The digital images of the seeds were analysed using the open-source software ImageJ 1.50i (Figure 3), considering ten descriptive parameters of shape and size measured for each seed (Table 1): area, perimeter, width, height, the primary axis

of the best-fitting ellipse, the secondary axis of the best-fitting ellipse, circularity, aspect ratio, roundness, and solidity (Figure S1).
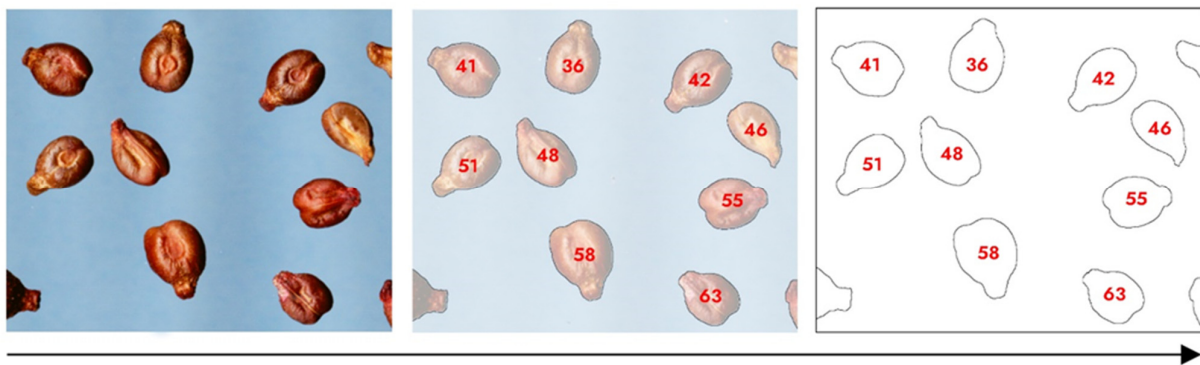
Figure 3. A portion of the scan analysed by the software. In particular, we have a small portion of the scan of the Sangiovese cultivar. The software assigns a number to each pip, to which all the shape and size indicators are associated.

Table 1. Morphometric features of grape seeds obtained by image analysis (software ImageJ 1.50i). UOM is the unit of measure.

| Feature	Description	UOM
Area	Area of selection as square millimetres	mm^2
Perimeter	The length of the outside boundary of the selection	mm
Major ellipse	The primary axis of the best-fitting ellipse	mm
Minor ellipse	The secondary axis of the best-fitting ellipse	mm
Circularity	$4\pi \times$ area/perimeter^2; a value of 1.0 indicates a perfect circle	DN
Aspect Ratio	major axis/minor axis of the best-fitting ellipse	DN
Roundness	4area$/(\pi \times$ major_axis$^2)$ or the inverse of the aspect ratio	DN
Solidity	area/convex area	DN
Feret's diameter	The longest distance between any two points along the selection boundary (maximum calliper)	mm
Feret's minimum	The minimum calliper diameter	mm

2.5. Statistical Analysis

The data obtained from the collection of modern, wild, cultivated, and archaeological grape pips were used to establish a database of morphometric grape pip descriptive parameters. Intraspecific diversity among the four populations was investigated using linear discriminant analysis [47]. Additionally, to evaluate the quality of the discriminant functions for each statistical comparison, Wilks' lambda tests were conducted [27]. All the morphometric data were normalised (between 0 and 1) before statistical elaborations, and both the normal distribution and homoscedasticity of each variable was tested. We conducted two LDAs. The first one reduced the high number of cultivars by excluding those cultivars which were less similar to the archaeological ones. Indeed, only cultivars with a predicted archaeological batch overlap higher than 5% were retained for the second LDA. The second LDA was performed to identify the most similar cultivars in terms of grape pip morphology. This method is frequently used to categorise and pinpoint unidentified groups that exhibit certain quantitative and qualitative characteristics [23,24,46,48]. To achieve maximum discrimination, this is carried out by choosing the combination of predictor variables while simultaneously minimising the within-class distance and maximising the distance between classes [49].

To analyse the differences between the different cultivars considered, a one-way ANOVA was performed (followed by Tukey's test for multiple comparisons). Differences between the samples were considered significant at $p < 0.05$. All statistical analyses were performed using R statistical Software (version 4.4.1).

3. Results

The average pip size ranged from 13.487 ± 1.244 mm^2 in *V. sylvestris* to 21.149 ± 2.818 mm^2 in the Nero Antico cultivar, which, along with Falanghina, had the largest pips among all the vines (Figure 4a). Compared to modern seeds, the two archaeological samples had medium-sized seeds, with an area of 15.091 ± 2.431 mm^2 for Vassarella and 16.408 ± 2.236 mm^2 for Santa Maria da Piedi (Table 2).
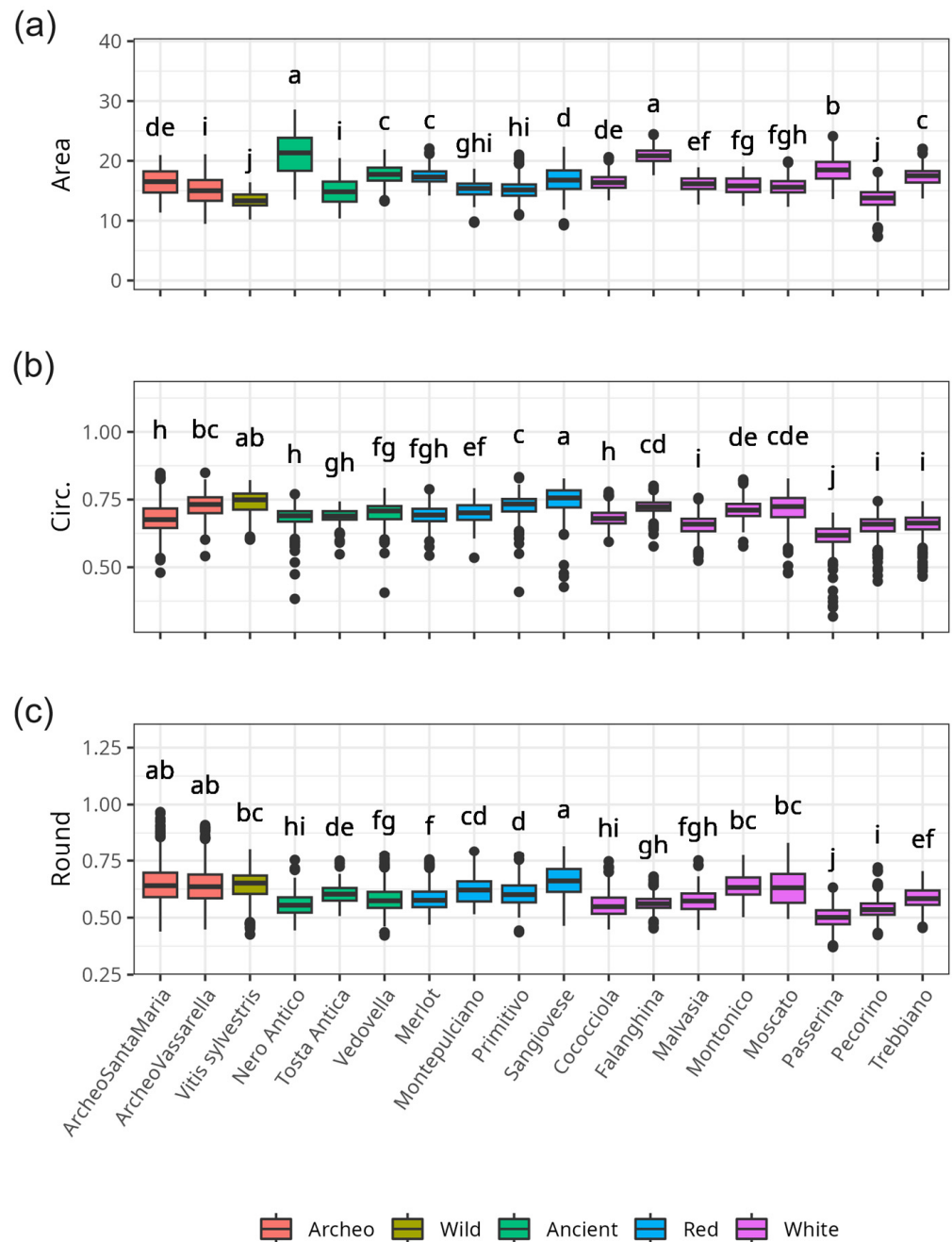
Figure 4. (**a**) Boxplot of the area for the single grape cultivars. Values with different letters are significantly different (one-way ANOVA, Tukey tests, $p < 0.05$). (**b**) Boxplot of the circularity for the single grape cultivars. Values with different letters are significantly different (one-way ANOVA, Tukey's tests, $p < 0.05$). (**c**) Boxplot of the roundness for the single grape cultivars. Values with different letters are significantly different (one-way ANOVA, Tukey's tests, $p < 0.05$).

Table 2. Mean and standard deviation of the morphometric features studied. In the first column, we have the different vine cultivars studied. Perim. is the perimeter of the seed; Major is the major ellipse; Minor is the minor ellipse; Circ. is the circularity; Feret is Feret's diameter; MinFeret is Feret's minimum; AR is the aspect ratio; and Round is the roundness.

Grape Cultivars	Area [mm²]	Perim. [mm]	Major [mm]	Minor [mm]	Circ.	Feret [mm]	MinFeret [mm]	AR	Round	Solidity
Nero_Antico	21.15 ± 3.29	19.67 ± 1.69	6.95 ± 0.60	3.86 ± 0.36	0.68 ± 0.04	7.26 ± 0.62	4.13 ± 0.38	1.81 ± 0.16	0.56 ± 0.05	0.94 ± 0.01
Tosta_Antica	14.84 ± 2.10	16.42 ± 1.17	5.58 ± 0.43	3.37 ± 0.27	0.69 ± 0.03	5.96 ± 0.42	3.59 ± 0.26	1.66 ± 0.11	0.60 ± 0.04	0.93 ± 0.01
Vedovella	17.75 ± 1.75	17.87 ± 1.00	6.25 ± 0.35	3.62 ± 0.31	0.70 ± 0.04	6.59 ± 0.33	3.87 ± 0.35	1.74 ± 0.18	0.58 ± 0.06	0.95 ± 0.01
ArcheoSantaMaria	16.41 ± 2.24	17.38 ± 1.44	5.70 ± 0.50	3.66 ± 0.35	0.68 ± 0.06	6.21 ± 0.58	3.90 ± 0.34	1.57 ± 0.19	0.65 ± 0.08	0.94 ± 0.02
ArcheoVassarella	15.09 ± 2.43	16.11 ± 1.33	5.46 ± 0.52	3.51 ± 0.40	0.73 ± 0.04	5.81 ± 0.53	3.68 ± 0.40	1.57 ± 0.20	0.65 ± 0.09	0.95 ± 0.01
Merlot	17.44 ± 1.35	17.78 ± 0.83	6.17 ± 0.40	3.60 ± 0.21	0.69 ± 0.03	6.69 ± 0.41	3.90 ± 0.21	1.72 ± 0.16	0.59 ± 0.06	0.94 ± 0.01
Montepulciano	15.29 ± 1.39	16.54 ± 0.95	5.61 ± 0.40	3.47 ± 0.22	0.70 ± 0.04	6.10 ± 0.46	3.74 ± 0.22	1.62 ± 0.16	0.62 ± 0.06	0.94 ± 0.01
Primitivo	15.23 ± 1.59	16.23 ± 0.90	5.66 ± 0.36	3.42 ± 0.27	0.73 ± 0.04	5.97 ± 0.35	3.69 ± 0.27	1.66 ± 0.16	0.61 ± 0.06	0.95 ± 0.01
Sangiovese	16.80 ± 2.23	16.81 ± 1.35	5.70 ± 0.49	3.75 ± 0.33	0.75 ± 0.05	6.01 ± 0.51	3.92 ± 0.33	1.53 ± 0.18	0.66 ± 0.07	0.96 ± 0.01
Passerina	18.44 ± 2.00	19.50 ± 1.43	6.87 ± 0.50	3.42 ± 0.24	0.61 ± 0.05	7.30 ± 0.49	3.83 ± 0.28	2.02 ± 0.20	0.50 ± 0.05	0.91 ± 0.01
Pecorino	13.67 ± 1.76	16.24 ± 1.27	5.68 ± 0.43	3.06 ± 0.23	0.65 ± 0.04	6.07 ± 0.48	3.34 ± 0.26	1.86 ± 0.15	0.54 ± 0.04	0.93 ± 0.02
Trebbiano	17.35 ± 1.45	18.26 ± 1.06	6.15 ± 0.39	3.59 ± 0.18	0.66 ± 0.05	6.71 ± 0.40	3.91 ± 0.19	1.71 ± 0.13	0.59 ± 0.05	0.93 ± 0.01
Malvasia	16.11 ± 1.24	17.58 ± 0.85	5.99 ± 0.35	3.43 ± 0.21	0.66 ± 0.04	6.51 ± 0.37	3.78 ± 0.22	1.76 ± 0.16	0.57 ± 0.05	0.92 ± 0.01
Cococciola	16.43 ± 1.39	17.39 ± 0.78	6.14 ± 0.38	3.41 ± 0.24	0.68 ± 0.03	6.58 ± 0.34	3.67 ± 0.27	1.81 ± 0.18	0.56 ± 0.06	0.94 ± 0.01
Montonico	15.84 ± 1.56	16.70 ± 0.85	5.64 ± 0.35	3.58 ± 0.26	0.71 ± 0.04	6.03 ± 0.36	3.83 ± 0.27	1.58 ± 0.15	0.64 ± 0.06	0.94 ± 0.01
Moscato	15.67 ± 1.38	16.61 ± 0.76	5.62 ± 0.26	3.55 ± 0.34	0.72 ± 0.06	5.93 ± 0.26	3.78 ± 0.36	1.60 ± 0.20	0.63 ± 0.08	0.95 ± 0.02
Falanghina	20.86 ± 1.31	19.05 ± 0.66	6.87 ± 0.31	3.87 ± 0.17	0.72 ± 0.03	7.17 ± 0.28	4.13 ± 0.18	1.78 ± 0.11	0.56 ± 0.04	0.96 ± 0.01
Vitis_sylvestris	13.49 ± 1.24	15.14 ± 0.87	5.23 ± 0.39	3.29 ± 0.25	0.74 ± 0.04	5.48 ± 0.38	3.49 ± 0.25	1.60 ± 0.21	0.63 ± 0.07	0.95 ± 0.01

Circularity is a factor that accurately describes the shape of a grape seed. This value ranges from 1 for a perfect circle to 0 for an extremely elongated shape. *Vitis vinifera* seeds recorded higher circularity values (0.740 ± 0.045), indicating more rounded shapes. The circularity values for the two archaeological samples were as follows: 0.728 ± 0.044 for Vassarella and 0.683 ± 0.059 for Santa Maria da Piedi (Figure 4b).

Circularity is calculated as $4\pi A/P^2$, while roundness is calculated as $4A/(\pi L^2)$. Circularity values decrease with surface irregularities, whereas roundness values are not affected by them.

The data show that Passerina (0.500 ± 0.047) and Pecorino (0.540 ± 0.044) had the lowest average roundness values, while Sangiovese (0.661 ± 0.072) and the two archaeological sites of Santa Maria da Piedi (0.648 ± 0.081) and Vassarella (0.646 ± 0.087) had the greatest average roundness values (Figure 4c). The aspect ratio (AR) values showed an inverse relationship to the circularity and roundness values.

Linear discriminant analysis (LDA) identified four different wine cultivars and the wild relative Vitis vinifera as being closely related to the archaeological samples (Figure 5). Among the cultivated grape seeds, those that most closely resembled the archaeobotanical finds were the Sangiovese vine and the local vine known as Tosta Antica (Figure 5). The linear discriminant analysis graph indicated that not all populations were well differentiated, suggesting close relationships and the possibility of gene hybridization and introgression events.

Figure 5. Linear discriminant analysis. The points represent the measured seeds with 80% confidence ellipses for each cultivar. The isolines represent the kernel density estimate for each cultivar.

4. Discussion

The seeds of *V. vinifera* had higher AR values, corresponding to lower circularity and roundness, characteristics of an elongated or piriform seed morphology [15,50,51]. The seeds of *V. sylvestris* had intermediate AR values. Smaller differences between circularity and roundness were due to the irregularity of the perimeter of the images [4]. Wild seeds are smaller and robust and have a rounded, heart-like shape (cordate), featuring short stalks, a flat ventral side with sharp angles, and a prominent chalaza. In contrast, cultivated seeds are larger, elongated, oval, or pyriform, with elongated stalks [15]. The process of domestication and cultivation of the vine in Italy was first documented between the Bronze Age and the Iron Age [52], about 3500 years ago, both in the peninsula and in Sicily and Sardinia [53,54]. In Abruzzo, the first evidence of viticulture dates back to the end of the Bronze Age and the beginning of the Iron Age [40,55]. However, the archaeobotanical finds studied in this paper date back to periods between the end of the Roman Empire and the Early Middle Ages, when viticulture was well established and widespread throughout the region. The grape seeds found in Villa Vezzani could also have been of foreign origin. Indeed, as suggested by the presence of imported pottery and the numerous amphorae on-site, there was extensive trade between Abruzzo, Africa, and Asia [56]. The LDA results indicated that the Vassarella seeds were most similar to the archaeological seeds from Santa Maria and the Sangiovese cultivar. This result is also supported by the fact that Sangiovese has been cultivated since the 16th century and, likely, even earlier [57].

The seeds found in the tomb near Santa Maria da Piedi were located near the head of the buried body. This practise, called the funeral banquet, was widespread in the Mediterranean between late antiquity and the Early Middle Ages [58]; therefore, this suggests that these seeds may have belonged to plants of local origin. In particular, the LDA separated the two archaeological seed samples quite distinctly, with Santa Maria being more similar to Tosta Antica. The Tosta Antica cultivar is a recently discovered local cultivar that is currently being studied and is reported only on the eastern slope of the Maiella, in the Aventino river valley (Abruzzo). Another interesting fact is the strong characterisation of the pips of the local Nero Antico cultivar compared to the other vines taken into consideration. This vine was recently discovered in the Aventino valley (Abruzzo) and is undergoing study and characterisation for many different aspects: morphological, phenotypic, and genetic [59].

Lastly, the results of this analysis suggest that the seeds from the archaeological sites belonged to the cultivated form of *V. vinifera* and not the wild form (*V. sylvestris*). In fact, the

classic shape and size of wild grapevine seeds were not recognisable in the archaeological samples. Consequently, it is presumable that viticulture was already practised in the Crecchio area in the Roman-Byzantine period and the Early Middle Ages, which is, to this day, an area strongly suited to viticulture.

Many studies have shown the slight impact of carbonization on the morphology of grape seeds [19,32–34,60]. All these studies concur that charring causes a slight size reduction [35]. However, this small deformation has little effect on grape seeds, and it is still possible to distinguish among grapevine cultivars even after charring, especially, as in our case, when contour analysis is used instead of classical distance measures [32]. Considering other cultivars and enlarging the database could allow for more accurate identification in the future. Furthermore, the integration of genetic testing alongside morphometric analysis could provide a more comprehensive understanding of grapevine evolution and cultivar identification, addressing the need for a multidisciplinary approach to elucidating the intricate relationships between different grape cultivars.

5. Conclusions

The greater resemblance of the archaeological seeds to the cultivars of *V. vinifera* rather than *V. sylvestris* could prove that, in southern Abruzzo, grapes have been cultivated since the Byzantine era.

Four wine cultivars were closely linked to the archaeological samples, the most closely related being the Sangiovese cultivar and the local cultivar, Tosta Antica.

Grape pips can provide useful information to explore the evolution of grape pip morphology and, consequently, the evolution of vine cultivars over space and time. Indeed, similarity with ancient seeds can suggest the ancient origin of some currently cultivated cultivars.

This method can be easily applied to many other species that are interesting from an agronomical and botanical point of view as well as to any other geographical area, extended or isolated, as it has already been widely demonstrated in the literature [14,23,24,48,61,62]. Integrating genetic testing with morphometric analysis could offer a more comprehensive understanding of grapevine evolution and cultivar identification, highlighting the need for a multidisciplinary approach to elucidating the intricate relationships between different grape cultivars.

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