Article

Grapevine in the Ancient Upper Euphrates: Horticultural Implications of a Bayesian Morphometric Study of Archaeological Seeds

Javier Valera [1,*] **, Gonzalo Matilla-Seiquer** [1] **, Concepción Obón** [2] **, Francisco Alcaraz** [3] **and Diego Rivera** [3,*]

[1] Departamento de Prehistoria, Arqueología, Historia Antigua, Historia Medieval y Ciencias y Técnicas Historiográficas, Facultad de Letras, Campus de la Merced, Universidad de Murcia, C/Santo Cristo, 1, 30001 Murcia, Spain; gmatilla@um.es

[2] Departamento de Biología Aplicada, EPSO, Universidad Miguel Hernández de Elche, Carretera de Beniel Km 3.2, 03312 Orihuela, Spain; cobon@umh.es

[3] Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia, 30100 Murcia, Spain; falcaraz@um.es

[*] Correspondence: javier.valera@um.es (J.V.); drivera@um.es (D.R.); Tel.: +34-670973968 (J.V.); +34-868884994 (D.R.)

Abstract: The origins of the main cultivar groups of *Vitis vinifera*, their relationships with wild grapevine populations, and the use of other *Vitaceae* are relevant issues for the improvement and conservation of *Vitis* diversity. Morphometric studies, domestication indices, multivariate analyses, and Bayesian hypothesis testing have been used. Eight different seed types have been identified in the 24 samples analyzed from materials from the Upper Euphrates sites of Tell Khâmis and Tell Qara Quzaq (Early Bronze Age to Hellenistic), ranging from highly domesticated to purely wild. We have been able to establish the predominance among the domesticated of *Proles orientalis* Negrul (three samples, Domestication Index = 1), the existence of and extinct *Proles euphratica* (six samples, Domestication Index = 0.67–0.83) and numerous intermediates and hybrids (eight samples). We have determined the continued presence throughout the period studied of wild grapevines related to *Vitis sylvestris* C.C.Gmelin and *V. caucasica* Vavilov (5 samples, with Domestication Indices = 0.17–0.5). The existence of *Ampelopsis* seeds was established for three samples. We determined that the oldest *Vitaceae* seed linked to human presence, in the Acheulense (780 myr), also belongs to *Ampelopsis*. Finally, "stenosperms" appear associated with *Ampelopsis* seeds (three samples), suggesting anomalies in seed formation due to intergeneric cross-pollination. Moreover, if isolated, they suggest the presence of "stenospermocarpic" *Vitis vinifera* raisins of the *Sultanina* type. Finally, we must reflect on the role that *Ampelopsis* species may have played and their possible cultivation and domestication almost 4000 years ago.

Keywords: ampelography; archaeobotany; oenology; viticulture; plant genetic resources

1. Introduction

1.1. Grapevine Relevance and Diversity

The grapevine, olive, date palm, fig, and pomegranate constitute the core of domesticated fruit species in Western Asia and the Mediterranean [1]. The fruits of the grapevine, *Vitis vinifera* L. (*Vitaceae*), can be consumed directly as table grapes, dried as raisins, or pressed into a must that can be fermented into wine, which contains 12–17% alcohol. The consumption of alcoholic beverages, and not only grape wine, was an important element of the nutrition, ritual and economy of early societies in Mesopotamia, Egypt, Syria, and the Levant [2–4].

Domesticated grapevine belongs to the Genus *Vitis* (*Vitaceae*), which comprises two subgenera and over 60 species. Grapevine (*Vitis vinifera* L.) is widely cultivated, especially in Mediterranean-type climates. More than 40.000 grapevine cultivar names exist worldwide,

corresponding to a little more than 15.000 grapevine genotypes [5–7]. Grapevine is a glyco-phyte, so with low salt tolerance (ClNa up to 40 mM, EC close to 4 dS/m), concentrations of 80 mM (EC close to 8 dS/m) produce significant damage [8].

Wild (*Vitis sylvestris* C.C.Gmelin) and cultivated (*V. vinifera*) grapevines mainly differ in their reproductive biology. Wild grapevines are dioecious, with males producing great quantities of pollen; on the other hand, most cultivated grapevines are self-pollinated hermaphrodites, producing small pollen amounts [9]. Negrul [10,11] argued in 1946 that hermaphrodite-cultivated grapes result from the selection of hermaphrodite branches accidentally appearing in male *V. sylvestris*. According to Sosnovszky [12,13], the ancestors of *Vitis* had bisexual flowers, and unisexual development is the result of reduction through evolution. Some cultivars, such as *Ohanes* and *Bicane*, are functionally female and may require assisted pollination.

Four main theories on the origin of cultivated grapevine have been published [14] with their variants. They are summarized as follows:

1. Monophyletic and Monospecific: Local populations of cultivated grapevine descend from local wild populations. Both are conspecific. This theory was proposed between 1882 and 1946 by De Candolle [15], Hegi [16], Planchon [17], Baranov et al. [18], and Negrul [10] and has been clearly supported by Levadoux [19], who also refers to some Pliocene *Vitis* fossils from Europe, known as *V. parasylvestris* Kirch., *V. tokayensis* St., or *V. ausoniae* Gaud. et Str, as conspecific.

2. Monophyletic and Bispecific: Cultivated grapevine descend from an extinct ancestor that is also presumably an ancestor of wild grapevine, being both two distinct separate species. Occasional hybridization may have produced some cultivars or cultivar groups [20]. Sosnovszky [12,13] stated that the Eurasian cultivated grapevine did not directly derive from *V. sylvestris*, which is morphologically well distinct from *V. vinifera* and extremely polymorphic, with its own history, geographical area, and natural habitat. This author [12,13] suggests that *V. sylvestris* and *V. vinifera* developed independently from a bisexual extinct ancestor who gave place to diverse types of cultivated grapevines; it is quite possible that the cultivated grapevine consists of an anthropogenic hybrid swarm involving crossing with *V. sylvestris* of several extinct *Vitis*.

3. Polyphyletic and Multispecific: Regional populations of cultivated grapevine descend from different wild ancestors extinct or not. Cultivated grapevine is divided into species with their corresponding wild relatives. The primary species hybridized, producing new cultivar groups. In 1925, Andrasovzsky [21] recognized five fundamental species, organized geographically, and the offspring of bispecific crosses between them, as well as pedigrees involving three species.

4. Hybrid Hypothesis: Cultivated grapevine descend through hybridization from wild European and Asiatic grapevines. Terpó [22] attributes the origin of cultivated grapevines to the domestication and crossing among populations of at least two species: *Vitis sylvestris* Gmel. (dioecious) and *Vitis nuristanica* Vassilcz. (hermaphrodite).

In 1807, Clemente [23–26] proposed the first systematic approach to grapevine diversity. Kolenati [27] first discussed in 1846 the origins of cultivated grapevines and proposed a classification of grapevines, wild and cultivated, in Georgia. Different authors followed Clemente's point of view; however, it was not possible to acquire a better view of grapevine diversity patterns until the beginning of the 20th century, when Russian agronomists carried out an in-depth study on the wild and cultivated grapevines of Western and Central Asia, especially in the *Ampelographia USSR* [18,28]. In this framework, the Russian agronomist Negrul proposed the recognition of three groups of cultivars, or *Proles*, namely: *Occidentalis*, *Pontica*, and *Orientalis* [10,19].

1.2. Grapevine in the near East Origins and Domestication

The Near East includes the eastern Mediterranean regions, the territories along the Euphrates and Tigris rivers, and the nearby regions of Central Asia, with the boundary to

the north in the southern Caucasus and to the south in the Arabian and Sahara deserts. *Vitis* traces from the area are derived from pollen, wine residues, grapes (especially seeds), and wood remains [1,9,29]. The archaeobotanical remains that provide the most information are the seeds, which have been preserved by being charred, dried, or waterlogged. The carbonization process, with many variables (exposure time, temperature, humidity, and chemical composition), or conservation in an aquatic environment, can affect the morphology of grape seeds and hinder their taxonomic identification, i.e., their ascription to wild or domesticated populations [29–32].

The pollen record from cores in the present range of wild grape within this area shows low but consistent *Vitis* counts, at least from the beginning of the Holocene, e.g., Ghab Valley (Syria), Lake Van (Turkey), and Lake Urmia (Iran) [33].

The oldest wild grape (*Vitis sylvestris*) seeds (8400 B.C.) associated with human activity, about 3 mm long, were excavated in Turkey at Nevali Çori, near the city of Urfa, on the slope of a tributary valley of the Euphrates (Hilvan province, Turkey). Domestication and cultivation of the grapevine seems to have occurred between the seventh and fourth millennium B.C., and between the Black Sea and Iran, including the Caucasus and the Upper Euphrates [9,34]. Slightly to the east of Lake Urmia, Lake Zeribar (Zagros Mountains, Iran), *Vitis* pollen first occurred in the core just before c. 4300 cal BC. This evidence was interpreted as grape cultivation spreading to the south-east, but there is no indication of substantial plantings. At present, the earliest evidence for grape used in wine production comes from the sixth millennium BC (Neolithic) site of Hajji Firuz Tepe (Lake Urmia basin, Iran) in the form of a tartaric acid residue [33]. The first convincing evidence of grapevine (*Vitis vinifera*) seeds, with indications of grape cultivation, was uncovered in Turkey at Kurban Höyük (5700–5200 B.P. non-calibrated radiocarbon date) [35].

Grapevine cultivation seems to have spread westward from western Asia. In Crete and Greece, the beginning of grapevine cultivation may have started around the fifth millennium B.C. [36]; however, archaeobotany in Greece suggests that there was a transitional period when grapevine seeds were neither domesticated nor wild [37]. This could have a connection between the seeds and the wine pressings found at Dikili Tash, suggesting that the use of grapes to produce wine may have begun independently of the domestication process [38]. In Spain, Phoenician influence during the first millennium B.C. seems to have played an important role in the development of viticulture and wine production, although the grapevine was exploited by local populations in the Neolithic before contact with Mediterranean cultures [29,39]. This would support the theory of an independent domestication center in Western Europe [40].

The analysis of archaeological grapevine seed remains from West Asia and nearby areas and their comparison with modern cultivars may provide interesting data to reconstruct the history of grapevine domestication and cultivation [41]. In West Asia, numerous archaeological grape seeds have been recovered, notably from Chalcolithic and Bronze Age levels, and are attributed to cultivated grapevine [42,43].

The objectives of the present study are, therefore:

- To study the morphology of archaeological and modern seeds from the Upper Euphrates.
- To compare them in order to establish a classification that will allow us to distinguish wild from domesticated seeds in the archaeological repertoire.
- To determine, as far as possible, the major groups or *Proles* of *Vitis vinifera* present in the archaeological repertoire and in the modern cultivars analyzed.
- To evaluate to what extent archaeobotanical data can contribute to the understanding of the origin of cultivated grapevines.

2. Materials and Methods

2.1. Archaeological Sites Sampled

In the present paper, we study grapevine seed samples from two sites that our group has excavated, both located in Syria near the Euphrates, Tell Qara Qûzâq [44,45] and Tell

Khamîs [46–50] (Figure 1), and compare them with a wide range of published archaeological seeds from the area and with local modern cultivars.
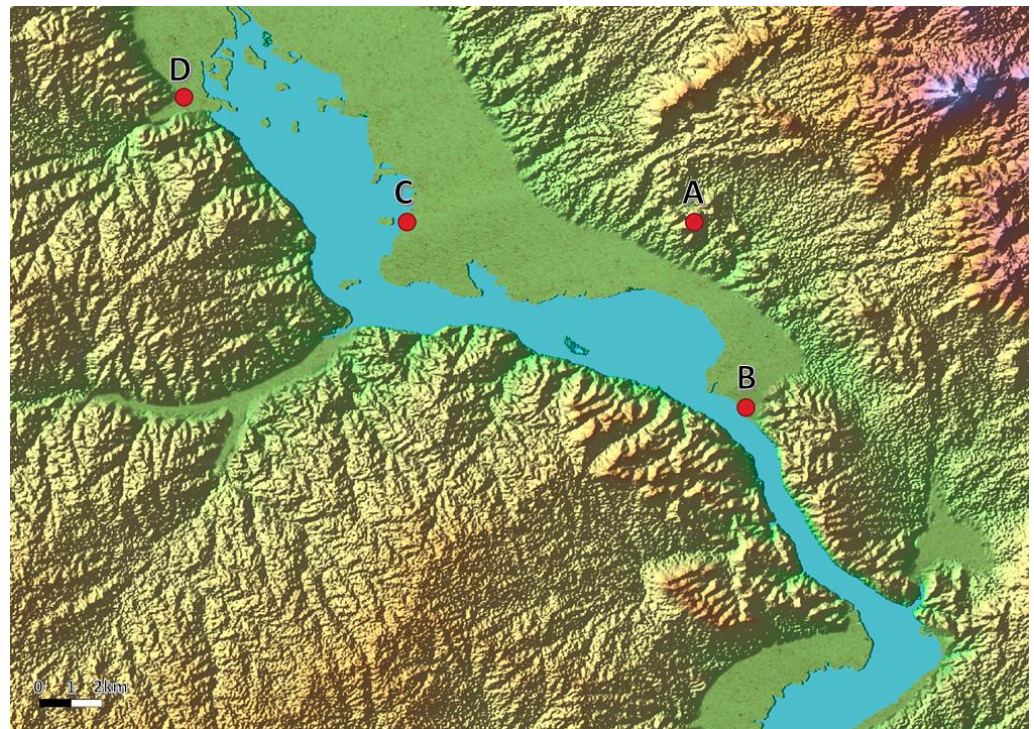

Figure 1. Geographical situation of the sites analyzed in the Syrian Upper Euphrates. A: Tell Khâmis (Ar Raqqah governorate). B Tell Quara Quzaq (Ar Raqqah governorate). Other relevant sites: C, Tell Ahmar (Ar Raqqah governorate), and D, Tell Amarna (Aleppo governorate). Note: the Euphrates waters fill here the Lake Assad since the construction of Tabqa dam.

2.1.1. Tell Qara Qûzâq

Tell Qara Qûzâq is located about 30 km from the Turkish border (36°37′57.80″ N, 38°12′52.92″ W) on the banks of the Euphrates River and about 325 m above sea level. Excavations continued uninterrupted from 1989 to 1999, when the waters of the reservoir rose and flooded the village of Qara Qûzâq, turning the site into an island. Five archaeological levels of occupation have been defined from the Early Bronze Age II (ca. 2080 BC) to the Roman period, with a very marked temporal hiatus between the Middle Bronze Age and the Roman level, signifying the inoccupation of the Tell for about 2000 years [51]. Several archaeobotanical studies have been carried out with the samples collected from the site in a systematic way [44]. The archaeological samples of grape seeds analyzed here from this site are six, for a total of eleven seeds.

2.1.2. Tell Khamîs

Tell Khamîs is located in the Upper Jazira region of Syria (36°43′56.36″ N, 38°07′09.81″ W), 3 km from the eastern edge of the Euphrates River, and at 330 m above sea level. Excavations at this small site began in 1992 and were completed in 2000 by the Institute of the Near East and Antiquity (IPOA/University of Murcia). The chronological period covered by the site dates back to the first half of the third millennium BC. After several temporary hiatuses over 11 archaeological levels, Tell Khamîs was abandoned in the middle of the 2nd century BC [46], with a long period of time in which there was no further occupation until the conversion of Syria into Islamic territory, when the site became a regular burial place [48]. A large number of seeds in a carbonized state belonging to different chronological horizons have been identified, highlighting those of barley and grapevine for their number and

preservation [50]. The archaeological samples of grape seeds analyzed here from this site are 18, for a total of 22 seeds.

2.2. Seed Samples

The study involves the analysis of 782 grape seed samples, including the 24 archaeological samples from the Euphrates Valley that we intend to identify within a range of probability. Comparison seed samples were provided by Erika Maul (The Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany) (cultivars from the Near East), the U.S. National Plant Germplasm System (United States Department of Agriculture, Agricultural Research Service), Rancho Santa Ana, Istituto ed Orto Botanico di Palermo, Botanisches Garten Johanes Gutenberg—Universität Mainz, Berlin Botanisches Garten, Smithsonian Institution, and Giardino Botanico di Padova (seeds of American and Asian species of *Vitis* and related genera). Seeds of other cultivars came from the vine collections of Rioja (Spain) in Mendavia, the La Casa de las Vides nursery in Agullent (Valencia, Spain), the Rojas Clemente vine collection of the Real Jardín Botánico de Madrid (Spain), and the Istituto Agrario di San Michele all'Adige (Trentino, Italy). Wild populations were sampled by the authors, Rafael Ocete, Emilio Laguna, and Encarna Carreño. Anna Nebish and David Maghradze supplied seeds of wild, feral, and cultivated vines from the southern Caucasus. Seeds from other archaeological sites were also included in the main analysis.

The primary raw data matrix used for this study consists of 4028 single rows of analyzed seeds, belonging to 782 seed samples, and 20 columns with observed variables, 11 quantitative, 6 allometric indices and 3 qualitative. The modern reference material used aims to give a global image of the diversity within *Vitis* species and cultivars using a wide range of samples that represent a large number of cultivars, wild and subspontaneous grapevine, in order to compare the morphological differences between them. Of the total number of seeds analyzed, 3483 are modern (481 samples), 398 are archaeological (194 samples), where part of the material is carbonized (251 seeds, 87 samples), and 147 are dry or waterlogged (107 samples).
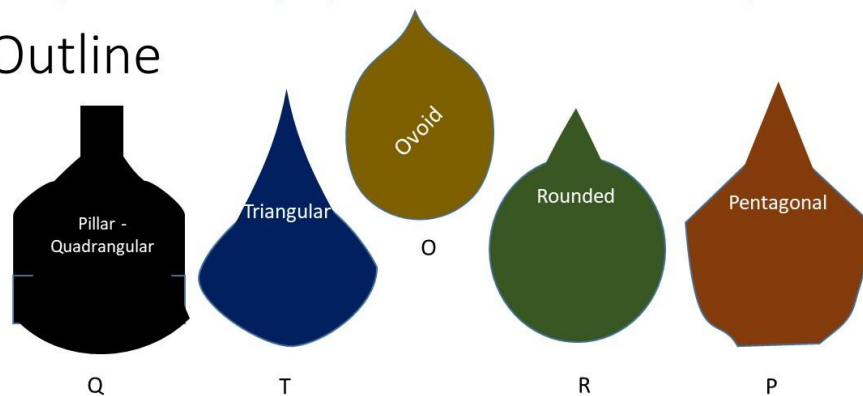
2.3. Characters

Each seed was individually described according to 20 characters. Of these, 11 are quantitative: total length, breadth, and thickness of the seed, breadth of the beak at the junction with the body and at the seed base, length of the beak in dorsal and in ventral view, thickness of beak at the junction, length and breadth of the chalaza scutellum, and distance from the chalaza apex to the seed apex (Figure 2) [52]. Six are allometric: width/length index, width/thickness index, prism volume index, beak length/seed length index, beak width/beak length index, and chalaza width/length index. The qualitative characters are three: Contour type (assessment of shape), with five states (ovoid, quadrangular, triangular, rounded, and pentagonal), arrangement of the fossettes, with four states (parallel, furcate, convergent and divergent) (Figure 2) and presence/absence of radial furrows.

The quantitative and qualitative characters were measured and analyzed using digitally scaled images. In total, 10 seeds of each sample were individually placed, except when the number of seeds available was inferior, on a plasticine support with a built-in scale to be photographed in dorsal, ventral, and lateral views with a Samsung A40 camera and measured using open-source Fiji software [53]. All photographs were taken under the same zoom conditions. Additionally, scale images of fossilized and archaeological seeds from specialized literature were used for measurements. The characters were recorded in an Excel spreadsheet, where the allometric relationships were automatically calculated using algorithms.

Seed dimensions
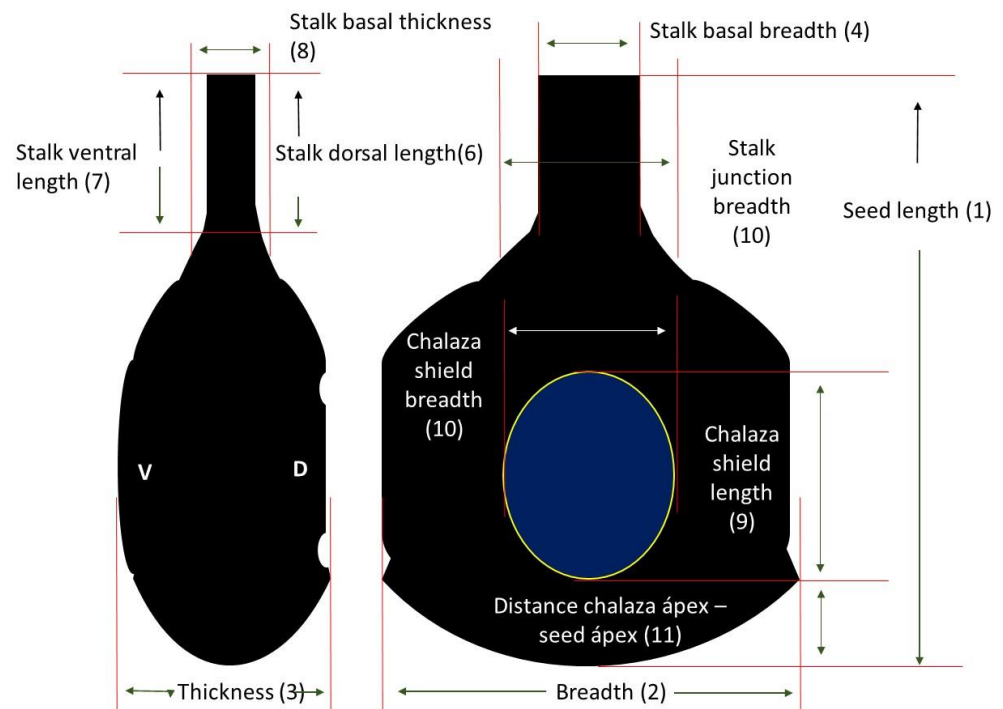
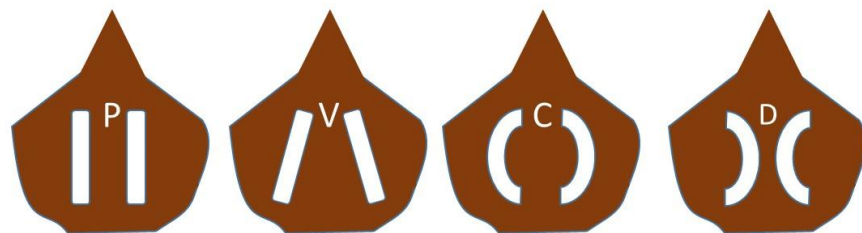
Outline

Fossettes

Figure 2. Qualitative and quantitative characters analyzed in the grapevine seeds. Abbreviations for Fossettes types: P, parallel. V, furcate. C, convergent and D, divergent.

The SEM images were made at the Scientific and Technical Research Area of the University of Murcia. The microstructure of the archaeological seeds was investigated by means of field emission scanning electron microscopy (FE-SEM) (ApreoS Lovac IML, Thermofisher, Waltham, MA, USA). Specimens were mounted on aluminum stubs and platinum sputter-coated with a 5.0 nm thin layer (Leica EM ACE 600). Samples were examined using a FE-SEM (ApreoS Lovac IML, Thermofisher, Waltham, MA, USA) with a selected voltage of 10 kV and 0.20 nA for imaging.

2.4. Morphometric Indices

2.4.1. Stummer's Index

In 1911, Stummer [54] proposed an index based on the allometric relationship between seed width and seed length. This index makes it possible to quite effectively differentiate the extreme forms, but intermediate values are found in both wild and cultivated populations (Table 1). Stummer's index values ranging from 0.44 to 0.53 would be exclusive to cultivars, while 0.76 to 0.83 would be unique to Austrian wild/ferals. Values between 0.53 and 0.76 were found in both cultivars and wild vines. In 1956, Levadoux [19] demonstrated that this index has limited validity and is not useful for distinguishing wild vines from cultivated vines.

Table 1. Stummer's index for wild and domesticated grapevine seeds [1].

Range of Values	Taxa
44–53	*V. vinifera*
54–75	Intermediate or hybrids
76–83	*V. sylvestris*

[1] Formula: B/L × 100.

2.4.2. Facsar–Perret's Index

In 1997, Perret [55] proposed a new index based on the allometric relationship between the length of the beak or column and the total length of the seed. Apparently, this index makes it possible to quite effectively differentiate between wild and cultivated populations, with the border situated between 18 and 19 (Table 2). Although it was attributed to Perret, this index was previously proposed by Facsar in 1970 and Facsar and Jerem in 1986 [56,57].

Table 2. Facsar–Perret's index for wild and domesticated grapevine seeds [2].

Range of Values	Taxa
12–18	*V. sylvestris*
19–30 (35)	*V. vinifera*

[2] Formula: LS/L × 100. LS: stalk lengt, L: seed length.

2.4.3. Mangafa and Kotsaki's Indices

The formulae proposed by Mangafa and Kotsakis in 1996 [58] were successfully applied to local Greek samples of both modern seeds and archaeological remains. The four formulae (Table 3) are based on the combined use of relationships and constants involving variables such as seed length (L), stalk length (LS), and chalaza position (PCH).

Table 3. Mangafa and Kotsakis's indices for wild and domesticated grapevine seeds [3].

Range of Values	Taxonomic Information
Range of values (Formula (1))	Seed classification
<−0.2	Wild grapes
−0.2 < x < 0.2	Wild grapes (64.7% probability to be wild)
0.2 < x < 0.8	Domesticated grapes (76.2% probability to be cultivated)
>0.8	Domesticated grapes
Range of values (Formula (2))	Seed classification
<−0.2	Wild grapes
−0.2 < x < 0.4	Wild grapes (64.7% probability to be wild)
0.4 < x < 0.9	Domesticated grapes (76.2% probability to be cultivated)
>0.9	Domesticated grapes

Table 3. *Cont.*

Range of Values	Taxonomic Information
Range of values (Formula (3))	Seed classification
<0	Wild grapes
0 < x < 0.5	Wild grapes (90.1% probability to be wild)
0.5 < x < 0.9	Domesticated grapes (63.3% probability to be cultivated)
>0.9	Domesticated grapes
Range of values (Formula (4))	Seed classification
<−0.9	Wild grapes
−0.9 < x < 0.2	Wild grapes (90.1% probability to be wild)
0.2 < x < 1.4	Domesticated grapes (63.3% probability to be cultivated)
>1.4	Domesticated grapes

[3] Formula 1: $-0.3801 + (-30.2 \, LS/L) + 0.4564 \, PCH - 1.386 \, L + 2.88 \, PCH/L + 9.4239 \, LS$. Formula 2: $0.2951 + (-12.64 \, PCH/L - 1.6416 \, L + 4.5131 \, PCH + 9.63 \, LS/L)$. Formula 3: $-7.491 + (1.7715 \, PCH + 0.49 \, PCH/L + 9.56 \, LS/L)$. Formula 4: $0.7509 + (-1.5748 \, L + 5.297 \, PCH - 14.47 \, PCH/L)$. LS, stalk length; L, seed length; PCH, chalaza position.

2.4.4. Domestication Index

Although the above indices serve the same purpose, to separate wild from domesticated forms, their results differ from case to case. The combined use of the six indices may be able to better discriminate seeds from wild or cultivated grapevines. The combined domestication/wild index is calculated individually for each seed using the following Formula: (1), where *NIT* means indices exceeding, above or below, the threshold value, and *NI* means the indices considered:

$$DW_i = \sum_{i=1}^{n} NIT_i / \sum_{i=1}^{n} NI_i \tag{1}$$

Threshold values for recognizing a seed as wild: Stummer > 75, Facsar-Perret < 19, Mangafa and Kotsakis F1 < −0.2, Mangafa and Kotsakis F2 < −0.2, Mangafa and Kotsakis F3 < 0 and Mangafa and Kotsakis F4 < −0.9.

The sum of the wild index (*WI*) and the domestication index (*DI*) values, which is complementary to the previous one, will always be equal to one.

Values of the *DI* (domestication index) range from 0 to 1, with intermediate values, based on six indices: 0.17, 0.33, 0.5, 0.67, and 0.83. Seeds with index values between 0.67 and 1, both included, would undoubtedly be domesticated seeds and present the "domestication syndrome", and those with values between 0 and 0.33 would be truly wild and present the "wild syndrome". In the present work, the value 0.5 is tentatively interpreted as wild.

We usually work with samples consisting of several seeds, which, in the case of modern populations, wild or cultivated, usually come from the same cluster, although not always. There are three relevant parameters when inferring from the results of individual seeds the characteristics of the whole sample, both for *DI* and *WI*:

1. The mean of the *WI*, wild index values for individual seeds, ranging from 0 to 1.
2. The standard deviation of the *WI* index values.
3. The proportion of seeds within each sample exceeding the wild threshold, *PW*, proportion wild, ranging from 0 to 1.

2.4.5. Hybridization Index

The standard deviation of the *WI* index values has shown to be useful to distinguish hybrids and hybrid swarms from pure wild and pure domesticated individual populations. Values above 0.2 of the standard deviation of the WI index points to the hybrid or mixed nature of the sample.

2.5. Multivariate Analysis

2.5.1. Variables

The data matrix consists of 782 samples (rows) and 227 columns of variables resulting from the segmentation in mutually excluding states or classes of the 20 primary variables above described, in the form of a spectrum of frequencies expressed in percentages with the following structure, from left to right: length (25 classes), width (21), thickness (9), width/length ratio (29), width/thickness ratio (10), volume (12), beak length in dorsal view (9) and in ventral view (9), beak length/seed length ratio (16), beak width at base (11), beak width (11) and beak thickness at junction with body (6), beak width/length ratio (9), chalaza shield length (18), chalaza shield width (6), chalaza width/chalaza length ratio (9), chalaza apex to seed apex distance (10), outline (5), dorsal radial grooves (2).

2.5.2. Data Analyses

The chi square dissimilarity index was calculated based on the above data matrix using the tool Darwin 6.0 [59,60]. This measure expresses a value x_{ik} as its contribution to the sum x_i on all variables and is a comparison of unit profiles (2).

$$d_{ij}^2 = \sum_{k=1}^{K} \left(\frac{x_{ik}}{x_i} - \frac{x_{jk}}{x_j} \right)^2 \left(\frac{x}{x_k} \right) \tag{2}$$

For $j \neq i$.

where d_{ij}: dissimilarity between units i and j; i, j = 1, 2, ... , N (samples, rows), N = 782; k = 1, 2, ... , K (variables, columns).

where d_{ij} = 1 means varieties i and j differ in all variables, and d_{ij} = 0 means varieties i and j are identical.

These pairwise dissimilarities can be represented in a multidimensional space, but in order to obtain a meaningful graphic representation of these relationships in a two-dimensional plane, we used cluster analysis.

Cluster analysis is a term used to name a set of numerical techniques whose main purpose is to divide the objects of study into discrete groups. These groups are based on the characteristics of the objects. We used minimum variance clustering (Ward's method), which focuses on determining how much variation is within each cluster. In this way, the clusters will tend to be as distinct as possible since the criterion for clustering is to have the least amount of variation [61]. Ward's method produces a single tree. For the graphic representation, we opted for the software Figtree version 1.4.4 [62].

The use of distance-based trees to allocate archaeological seed samples is not new; in 2015, Pagnoux et al. [63] assigned archaeological grape seeds to the groups defined by UPGMA cluster analysis; their tree is based on Mahalanobis distances among comparison grapevine wild individuals and cultivars. Rivera et al. in 2014 [64] tentatively allocated archaeological *Phoenix* seed samples using a method based on the minimum variance Ward's principle.

2.6. Allocation of Archaeological Samples to Categories and Taxa

2.6.1. Bayes–Laplace Theorem

For the interpretation of archaeological seed samples, we adopted a Bayesian approach. We try to answer the question: What is the conditional probability that an archaeological seed or seed sample belongs to a determined *Vitis* taxon Θ_i given that it presents the domestication index value x_j and/or it belongs to the cluster y_j? The framework is based on the knowledge provided by hundreds of comparison samples (c. 700), whose taxonomic identity we "a priori" know in each case not only from the morphology of the seeds but also from the study of the grapevine plant from which the sample was collected. Identification is based on ampelographic characters. So far, the most relevant ampelographic data included in the Vine Descriptors (IPGRI-UPOV-OIV 1997) [65] have been collected, especially those

related to the hairs covering the leaves and the characteristics of the grape berry, either recorded directly in the field, since more than two hundred of these vines were grown on a farm in Molina de Segura (Spain), or from the databases (FNGR 2023; VIVC 2023) [66].

This allows us to construct a discrete joint probability function $p(X,\Theta)$ that assigns a posterior probability value to each particular combination of a *Vitis* taxon and a domestication index value, or of a *Vitis* taxon and a Ward's tree cluster.

The Bayes' rule (3) makes it possible to approximate the answer:

$$p(\theta|x) = p(x|\theta)p(\theta)/p(x) \tag{3}$$

where $p(\theta \,|\, x)$ is the posterior probability distribution for the parameter θ given a single observed value of the variable $X = x_j$, in our case the degree of domestication, which is represented by the domestication index value, which ranges from 0 (clearly wild) to 1 (cultivar with fully domesticated traits).

When considering the Bayes' rule in terms of individual probabilities, (3) can be read as (4).

$$Posterior\ probability = \frac{likelihood \times prior\ probability}{marginal\ likelihood} \tag{4}$$

Given a value for the data, for instance $X = x_4$, and a specific value for the parameter θ (*Vitis* taxa), such as, *theta* $= \theta_3$, we can obtain (5)

$$p(\theta_3|x_4) = p(x_4|\theta_3)p(\theta_3)/p(x_4) \tag{5}$$

In (5), both likelihood $p(x_4|\theta_3)$ and marginal likelihood $p(x_4)$ are values that can be calculated on the basis of the joint distribution generated from the comparison samples. The prior probability $p(\theta_3)$ can also be calculated as the sum of probabilities for this taxon given the distribution of all x values on the sample data alone. However, the very nature of the prior allows the inclusion of data on the regional prevalence of the different taxa from other well-established sources of evidence (catalogues of local varieties, germplasm collections, field studies). In this study, we have paid attention, in the case of domesticated vines, to the geographical variation in the proportions of the different *Vitis vinifera* "*Proles*" and, in the case of the rest, to the ratio, *V. sylvestris*/*V. caucasica*. *Vitis caucasica* Vavilov is still unclear as a taxon, but we use this to name the ensemble of wild grapevines in the Southern Caucasus that present a very low domestication index value of <0.5. Furthermore, we pay attention to the low relevance of the fossil grapevine species, introduced as an outgroup and treated as such.

2.6.2. Application to the near East Seeds Question

Following the Bayesian approach, we can advance in our evaluation of the probabilities of the different hypotheses by considering a set of the available "a priori" evidence on the relative frequencies of the hypotheses, especially considering time range and geographic constraints. For example, it is much less likely to find seeds of an American grapevine species in a European Neolithic site than those of *Vitis sylvestris*. A careful elaboration of the "a priori" distribution of probabilities based on solid and logically coherent evidence is as fundamental as a clear definition of the different hypotheses and of the variable(s) to be considered.

Among the cultivars, we assume "a priori" a proportion of *Proles* and *Subproles* in the sense of Negrul (1946) [11], similar to the present one in West and Central Asia [66–68], which implies: *Orientalis antasiatica* ($p = 0.43$), *Orientalis caspica* ($p = 0.25$), *Pontica* ($p = 0.17$), *Occidentalis* ($p = 0.07$), although most of the stages of introduction and translocation of varieties had not yet taken place. For hybrids, using the same sources ($p = 0.07$), this leads us to the following "a priori" scenario (Table 4). The "a priori" or "prior" probabilities used are also based on the assumption that at that time, 3rd mill. BC, 30% of the vines in the territory were wild and 70% cultivated. If we were to assume different proportions, the following probabilities would differ.

Table 4. Alternative hypotheses and their respective priors and likelihoods [1].

Groups	Prior	L1	L2
Vitis vinifera Cultivars			
Oriental cultivars. *Proles orientalis* Negrul *Subproles antasiatica*.	0.303	0.178	0.042
Oriental cultivars. *Proles orientalis* Negrul *Subproles caspica*.	0.177	0.086	0.041
Western and Mediterranean cultivars in a broad sense. *Proles Pontica* Negrul.	0.118	0.103	0.035
Western and Mediterranean cultivars in a broad sense. *Proles Occidentalis* Negrul.	0.051	0.059	0.056
Varieties with intermediate characteristics resulting from hybridization between the previous groups.	0.049	0.082	0.077
Wild grapevines in natural habitats			
Vitis sylvestris, we include the variability inherent to western wild vines that do not descend from cultivated plants.	0.024	0.070	0.104
Feral grapevines, which descended from cultivated plants and although they show partial reversion to ancestral characters, they conserve traits derived from domestication.	0.028	0.037	0.068
Vines related to wild vines from the Caucasus (*Vitis caucasica* Vavilov *sensu auct*.) or other eastern regions, their probability is small but we do not rule them out. They are divided into:			
• Direct hybrids of wild Caucasian grapevines with cultivars.	0.060	0.099	0.038
• Purely Caucasian feral.	0.060	0.052	0.041
• Wild Caucasian grapevines.	0.043	0.001	0.068
Unlikely hypotheses			
American grapevine species: we should rule out the possibility that an American vine could have been present in Western Asia in such early times, but what we do assume is a very low probability.	0.0005	0.076	0.074
Eastern Asian grapevine species: These are unlikely, but given the ancient connection facilitated by the Silk Road, their presence is not impossible.	0.084	0.156	0.101
Finally, fossils: These are extremely unlikely, but we do not rule out the survival of a living fossil.	2.5×10^{-5}	6×10^{-5}	0.255

[1] L1, Posterior probability combining prior and the Likelihood based on DI values. L2, Normalized Marginal Likelihood considering the allocation to clusters 1 to 13 as in Figure 3.

In the Bayesian method, we can combine the evidence resulting from the study of several variables by concatenating results in which the posterior probability distribution function of the first analysis will be used as the a priori distribution for the second and so on. In the present study, we combine the results of the morphometric indices summarized in the domestication index for each of the seeds as a starting analysis and then use the results of the multivariate analysis in terms of the assignment of each of the samples to one or another of the 13 clusters.
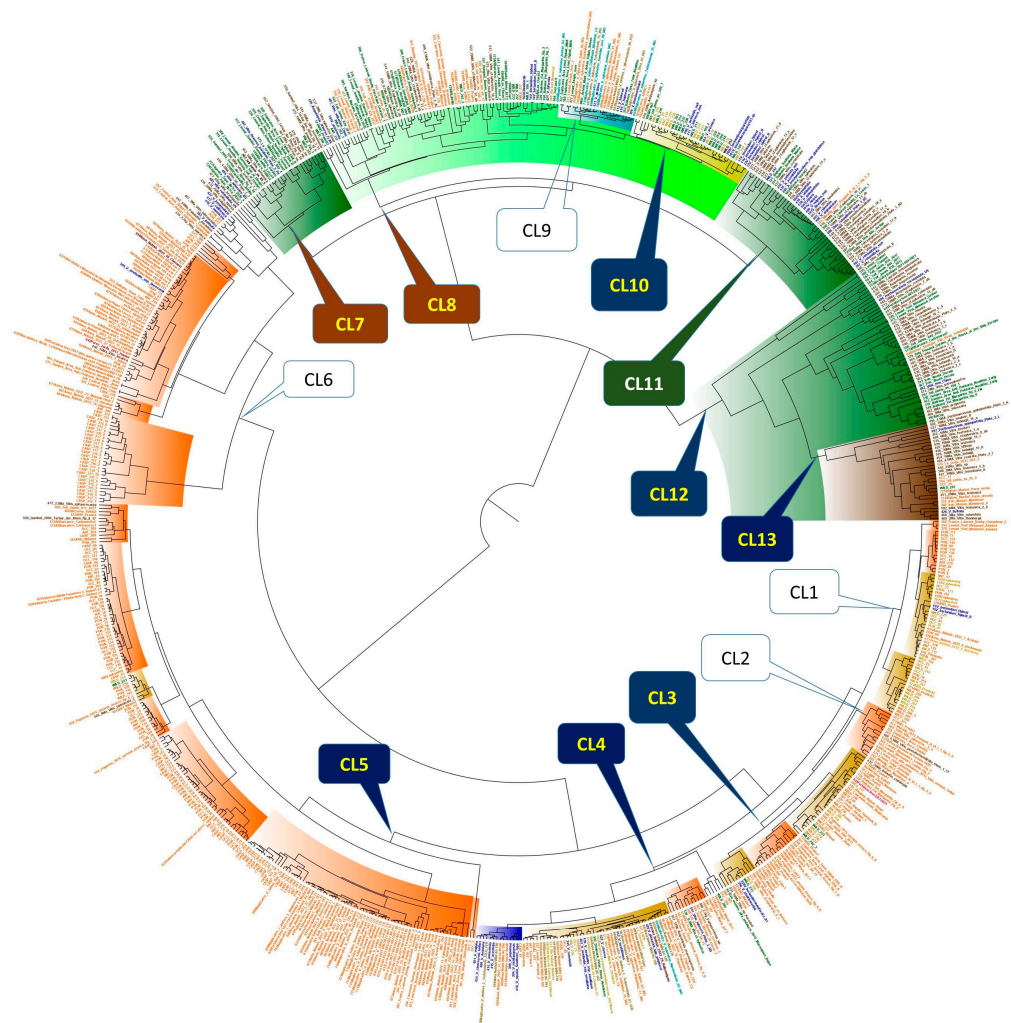
Figure 3. Minimum variance Ward's tree. Cluster labels: Deep blue filled and yellow characters for clusters with Tell Khâmis seed samples. Dark green filled and white characters for clusters with those from Tell Quara Quzaq. Dark brown for those with both. White filled and black characters for the rest. Color codes (RGB system) for sample labels: *Vitis caucasica*: 0-153-153; *V. sylvestris*: 0-102-0; *V. vinifera*: 255-102-0; *V. vinifera × V. sylvestris*: 204-153-0; other wild *Vitis* species: 0-0-204; *Vitis* seeds fossil: 102-51-0; *V. vinifera × V. caucasica*: 153-102-0; *V. vinifera × V. amurensis*: 102-0-0.

3. Results

3.1. Probability-Based Allocation of Grapevine Seeds to Major Types

The Bayesian sequential combination of domestication indices and cluster allocation (Figure 3) has allowed us to assign the archaeological samples with varying degrees of probability (Supplementary Table S1) to the different groups of wild, domesticated, and hybrid grapevines. It should be emphasized that the method followed gives priority in its results to the information obtained from the multivariate analysis of the morphology of the seeds and the assignment of the seeds to each of the 13 clusters identified, previous data on the characteristics of the cultivated and wild vines in the area, and, finally, the information provided by the six domestication indices combined into a single one that we abbreviate as DI and whose values range from 0 to 1 for each of the seeds.

3.1.1. Domesticated Grapevines of the *Proles orientalis* Negrul

Domesticated grapevines are related to modern cultivars belonging to *Proles orientalis Subproles antasiatica* (probability, *p* = 0.55–0.87) and associated with high domestication

values (*DI* = 1). Additionally, they are related to a lesser extent with *Proles orientalis Subproles caspica* (*p* = 0–0.15), *Proles Pontica* (*p* = 0–0.16), and *Proles Occidentalis* (*p* = 0.1–0.12), but clearly unrelated to wild grapevines. Three samples of Tell Khâmis presented this high domestication syndrome. The group is otherwise highly heterogeneous as the seeds have been assigned separately to clusters 4, 5, and 13 (Figure 3).

- Middle Bronze Age: 268 TK bm 68 11;
- Assyrian: 264 TK as 95 6B;
- Persian–Hellenistic: 565 TK 011 2017.

Modern seeds that presented similar profiles in the study include numerous Eastern cultivars, such as an Uzbekistani cultivar, several Afghan raisin cultivars, Chaouch Blanc, Chaouch Rose, Shiradzouli violet, Beylerce, Konya Bozkir; Rasheh, Besni, Hasand-ede Beyazi, Rumi Ahmar, Razakisi Antep, Haci Tespihi, Zonguldak, Dabouki, Mecka, Nehelescol, and Chasselas.

Similar seeds were found in the Urartian levels of Yoncatepe (Van, Eastern Turkey) [69].

3.1.2. Domesticated Grapevines Belonging to a Particular Euphrates *Proles*

Domesticated grapevine cultivars are unrelated to modern cultivars but related to Caucasian and Asian wild grapevines. Numerous groups of samples present the paradox of having high values for the domestication index (*DI* = 0.67–0.83) but a very low probability of belonging to any of the known domesticated *Proles* (at least those included in our collection of comparison samples) and at the same time a high probability (*p* = 0.36 to 0.42), of being an Asian *Vitaceae* species, or, to a lesser extent, being *V. caucasica* (*p* = 0.26 to 0.32), or a hybrid (*p* = 0.21 to 0.22). Furthermore, all of them cluster together in cluster 8 of the multivariate analysis (Figure 3). This suggests the existence in the ancient Upper Euphrates of a peculiar set of domesticated varieties, possibly derived from hybridization between Asian and South Caucasian vines. At present, these varieties seem to have disappeared, although it would be very useful to prospect the tributary valleys of the Euphrates in Syria and Iraq in search of them, either cultivated or feral. It should be noted that no relationship has been found between this group of archaeological samples and the numerous current varieties from Turkey, Georgia, or Armenia that have been introduced to the comparison matrix. It would also be very interesting to be able to include in further studies the wild and feral vines recently studied in Israel.

- Early Bronze Age: 180 AR QQ 1 14-92.
- Middle Bronze Age: 580AR QQ MBA W78b.
- Assyrian: 263 TK as 95 6; 266 TK as 108 1, (Figure 4E).
- Aramean: 268 TK bm 68 12, (Figure 4B).
- Persian-Hellenistic: 559 TK 028 2017.

No single modern seed was found close to this group, and only one archaeological seed sample was allocated to this group from the coastal plain of Sidon, dated from the Late Iron Age at Phoenician Tell el-Burak (Lebanon) [70].

3.1.3. Domesticated Grapevines Related with Asian Wild Grapevines

Unrelated to modern cultivars but related to Asian *Vitaceae* species (*p* = 0.5–1) and to a lesser extent with European wild (*p* = 0–0.37) and hybrid (*p* = 0–0.29) grapevines, this group also presents the paradox of associating high values of the domestication index (DI = (0.5) 0.67 to 1) with a very low probability of belonging to any known domesticated grapevine *Proles* and, at the same time, a high probability of being an Asian wild species. This group could be related to the previous one and draws our attention, once again, to the influence of the Asiatic species of *Vitis* or *Ampelopsis* on the ancient grapevines of the Euphrates and the Near East. All of them were allocated together in clusters 7, 12, and 13 of the multivariate analysis (Figure 3).
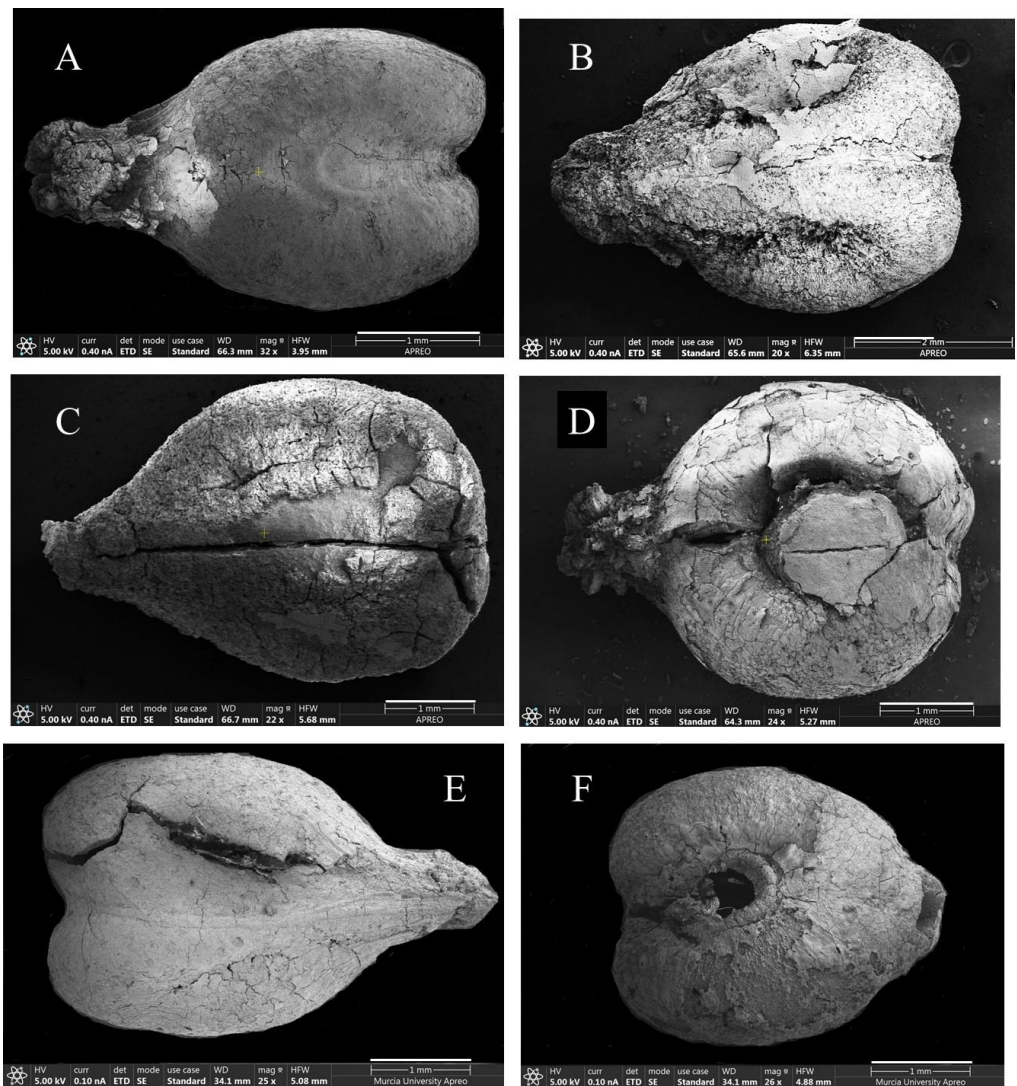
Figure 4. Archaeological *Vitis* seeds. SEM images. (**A**) Domesticated grapevines related with Asian wild grapevines, Assyrian: 264 TK as 95 6A. (**B**) Domesticated grapevines belonging to a particular Euphrates *Proles*, Aramean: 268 TK bm 68 12. (**C**) Domesticated grapevines related with Asian wild grapevines. Early Bronze Age: 180AR QQ 2 14-92. (**D**) Wild Eurasian-Caucasian grapevines, Middle Bronze Age: 185AR QQ 1 137-92. (**E**) Domesticated grapevines belonging to a particular Euphrates *Proles*, Assyrian: 266 TK as 108 1. (**F**) Domesticated grapevines related with Asian wild grapevines. Assyrian: Assyrian: 264 TK as 95 6A; 262 TK as 85 5 (the beak was broken during the handling for SEM). Scale bars: above 1 mm, below 1 mm, except B, where is 2 mm.

- Early Bronze Age: 180AR QQ 2 14-92, (Figure 4C).
- Assyrian: 264 TK as 95 6A, (Figure 4A); 262 TK as 85 5, (Figure 4F).

Archeological seeds with similar features were found in Bronze Age levels of Konar Sandal, Halil Rud basin, southeastern Iran (but see below) [71] and in Late Bronze levels of Tall al-'Umayri, Jordan [72]. Finally, from the Petra Garden and Pool Complex in Jordan, a Hellenistic/Roman pleasure garden [73], and in medieval Tashbulak, on the Silk Road [74].

With a similar pattern of likely relationships but with lower domestication index values (*DI* = 0.17–0.33), seeds of the wild type were identified from Tuzusai, Central Asian mountains, Kazakhstan (410–150 BC) [75], in the Euphrates zone of Urartian levels in Yoncatepe (Van), eastern Turkey, mixed with other *Vitaceae* seed types [76], and in Bronze Age levels (2480–2290 calibrated BC) of Konar Sandal, Halil Rud basin, southeastern Iran [71] mixed with the domesticated type. In addition, also related to the wild syndrome

are two seed samples from Nepal from the Late Pleistocene Besigaon section of the Gokarna Formation, Kathmandu Valley, in central Nepal: 53 kyr [77] and 45 kyr [78].

The few grape seeds found at Tuzusai (Kazakhstan, 410–150 BC). These are assumed to be cultivated and likely domesticated [75] however our results may question this view. Miller [33] states that northern Central Asia lies outside the range of wild *Vitis sylvestris* indicating that the pips probably came from domesticated grapes. These pips may represent exotic exchange goods or a locally grown horticultural product. The presence of a small number of grape pips does not prove viticulture occurred at Tuzusai. Grapes may have been imported from other regions in the form of raisins [75].

3.1.4. Eurasian Hybrid Wild Grapevines

Hybrid wild (DI = 0.17–0.5) grapevines are related to Eurasian hybrid modern grapevines (p = 0.19–0.64) (Supplementary Table S1) and to European wild (p = 0.30–0.81) grapevines, but are unrelated to Caucasian or Asian wild grapevines. Only two samples follow this anomalous pattern. The samples are allocated to clusters 3 and 10 (Figure 3).

- Assyrian: 265 TK as 100 1.
- Persian-Hellenistic: 560 TK 009 2017 A.

No modern samples of wild or cultivated vines from the Near East have been found that could be included in the group. The single archaeological grapevine seed sample that approximates this group is that found at Petra, Jordan; 150 B.C.–A.D 40 by Jacquat and Martinoli [79].

3.1.5. Eurasian Domesticated Hybrid and Feral Grapevines

Domesticated grapevines (DI = 0.67–0.83) are related to Eurasian feral modern grapevines (p = 0.42–0.57) and, to a similar extent, to European hybrid grapevines (p = 0.43–0.55), but are unrelated to wild grapevines (Supplementary Table S1). All the samples are allocated to cluster 3 (Figure 3). Three samples of Tell Khâmis present this clearly transitional syndrome:

- Middle Bronze Age: 186 AR TK.
- Aramean: 268 TK bm 68 13.
- Persian-Hellenistic: 560 TK 009 2017 B.

Similar grapevine seeds were found in Middle Bronze Age levels of Mezraa Höyük, Upper Euphrates, Turkey [69], and mixed with hybrid wild types in the same sample of charred grape pips from the Nabataean period, Petra, Jordan, 50 B.C–A.D 100 [79], also at the Early Bronze Age settlement of Ḥirbet ez-Zeraqōn in Northern Jordan [80] and in Dayr al-Barshā Middle Egypt during the late Antique/early Islamic period [81].

3.1.6. Wild Eurasian—Caucasian Grapevines

Wild grapevines are unrelated to modern cultivars, directly related to Caucasian wild grapevine with a medium probability (p = 0.3 to 0.5), and, to a lesser extent, are related to Asian wild grapevines (p = 0.23 to 0.33) and Eurasian wild grapevines (*V. sylvestris*) (p = 0.13–0.3). These are typical wild grapevines with low domestication index values (p = 0.17 to 0.33 (0.5)). Furthermore, all of them cluster together in cluster 8 of the multivariate analysis and with the samples allocated above to the *Proles Euphratica* (Figure 3), from which they differ in their relatively low domestication index (DI = 0.17–0.5).

- Early Bronze Age: 577AR QQ EBA H84; 578AR QQ EBA W80.
- Middle Bronze Age: 579AR QQ MBA W27; 185AR QQ 1 137-92, (Figure 2D).
- Persian-Hellenistic: 562 TK 029 2017. S

Similar grapevine seeds of the wild type were found in pigeon dung samples from a Byzantine (6th century AD) dovecote near Shivta, Israel [82]. This suggests the presence of this type of wild grapevine in the Eastern Mediterranean, whose seeds were transported by doves and other birds. Relatively similar to this group but with an even lesser relation to Eurasian wild and a relatively high relation to Caucasian hybrids were found in samples

from the Euphrates and Levant areas: in Middle Bronze Age levels of Mezraa Höyük, Upper Euphrates, Turkey [69] and Early Bronze Age levels of Tell Lachish, Israel [83].

More striking is the similarity with the analyzed samples of *Vitis heyneana* Schult, a species that currently ranges from Afghanistan to China and Japan [84].

3.1.7. Wild Asian Grapevines Allocated to Genus Ampelopsis

Wild grapevines are unrelated to Caucasian wild grapevines and related to Asian wild grapevines belonging to the genus *Ampelopsis* with an extremely high probability ($p = 0.99$). These are typical wild grapevines with low domestication index values ($DI = 0$ to 0.5) and a very low probability of belonging to any of the known domesticated *Proles*. The multivariate analysis allocates them all to cluster 12 (Figure 3), except for the QaraQuzaq Early Bronze Age sample, which is allocated to cluster 11.

- Early Bronze Age: 185AR QQ 2.
- Assyrian: 267 TK as 108 1.
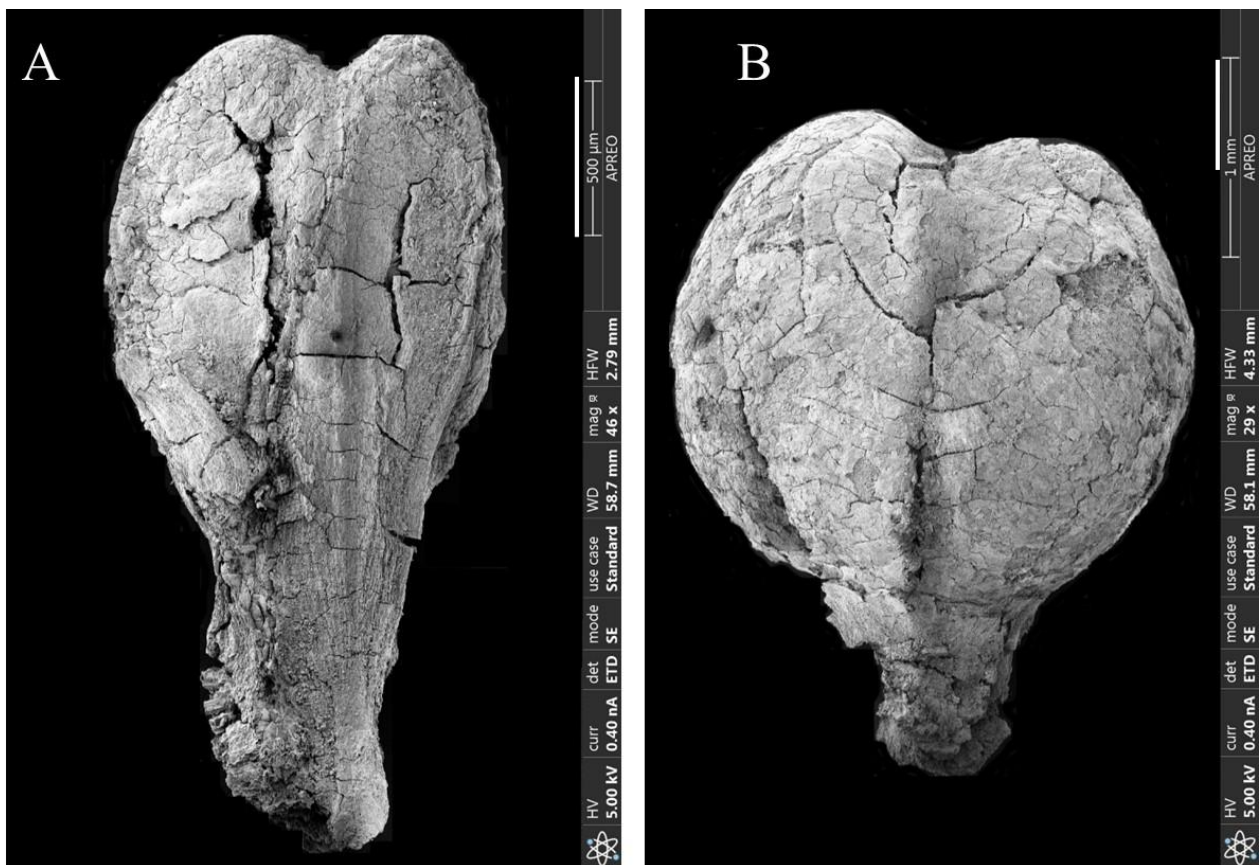- Persian-Hellenistic: 566 TK 040 2017 (Figure 5B).

Figure 5. Archaeological *Vitaceae* seeds of the Upper Euphrates. Raisin type seeds with low domestication syndrome remotely linked to genus *Ampelopsis*, SEM images. (**A**) "Stenosperm" type. Wild Asian grapevines allocated to genus *Ampelopsis*; Persian-Hellenistic: 561 TK 064 2017. (**B**) *Ampelopsis* seed type, Persian-Hellenistic: 566 TK 040 2017. Scale bars: 0.5 mm.

Modern specimens of wild *Vitaceae* with similar characteristics have been identified (Supplementary Table S1) as: *Ampelopsis orientalis* (Lam.) Planch., which currently grows in Syria and Turkey [85]; and *Ampelopsis vitifolia* (Boiss.) Planch., which currently extends through Central Asia, from Iran to the Himalayas. Along with the above are included modern seeds determined as *Vitis* sp. by the US gene bank (S518_DVIT_1445) from Afghanistan, which are possibly *Ampelopsis*, and a fossil seed, 45 myr, from Late Pleistocene plant

macro-fossils from the Gokarna Formation of the Kathmandu Valley, Central Nepal [78], determined as *Vitis* sp., but which may possibly also be ascribed to the genus *Ampelopsis*. The seed from Acheulian levels at Gesher Benot Ya'aqov, Israel [86], very rounded, is allocated to this group. Carbonized remains from lron Age Raja-Nal-ka-Tila (1st millennium BC) of Ganga Plain, India [87] are included in this group. Kohneh Pasgah Tepesi (eastern Azerbaijan, Iran) in levels of the Late Chalcolithic and the Early Bronze Age [88] yielded *Vitaceae* seeds that also fall within this group and in 7th to 5th cent. BC levels of Deir'Alla ,Jordan. In this period, Deir 'Alla was a temple tell, as in Late Bronze Age times. A small group of priests would have lived there permanently, while seminomadic tribes stayed in the vicinity of the Tell only during the winter months [89].

3.1.8. Raisin Type Seeds with Low Domestication Syndrome Remotely Linked to Genus Ampelopsis

Thin sterile-like types with intermediate domestication index values $DI = 0.5$–0.67. Three seeds were found conforming to this type in Tell Khâmis.

- Persian-Hellenistic: 561 TK 064 2017; 563 TK 059 2017; 564 TK 038 2017 (Figure 5A).

It is relevant to mention that this group includes apparently sterile, tiny, elongated seed samples, such as those from Early Bronze Age Ras an-Numayra, Jordan [90]. This abnormal "stenosperm" seed type was relatively common in the study of modern grapevine tables and raisin seedless-like varieties of the "Sultanina" type from Afghanistan [67] and in some of Afghan comparison samples within this study (Figure 6).

Figure 6. Modern *Vitis* seeds of a modern Afghan Raisin cultivar "Stenosperm" type. Digital microscopy. Scale bars: 1 mm.

4. Discussion

4.1. The Horticultural Relevance of of the Seed Types Identified

4.1.1. Domesticated Grapevines of the *Proles orientalis* Negrul

A group of seeds presents clearly domesticated characteristics and is assigned in the present work to the set of the three *Proles* of Negrul [11] (Table 5) with a probability = 1. Among the recognized *Proles*, assignment to the *Proles orientalis* is in the majority, with

values of p = 0.7–0.87, predominating in all cases the *Subproles antasiatica*. The results confirm our "prior" hypothesis about the existence of this set of table and raisin varieties, but also for wine, known as *Proles orientalis*, in the Near East, at least since the Middle Bronze Age.

Table 5. Negrul's *Proles* of *Vitis vinifera* [5].

Taxa	Leaf	Cluster Size and Shape	Berry	Area and Typical Cultivars
Pontica Negr.	Mature leaf below with mixed hairy cover: cobwebby and hairy, irregularly curved leaf edge	Medium, compact, table, raisins and wine	Spherical, rarely ovoid, bittersweet, medium size, acidity 0.6–1%; seeds small to large. some seedless cultivars	West Caucasus, Balkans, Anatolia, Mediterranean. Frost hardy (Saperavi, Rkatsiteli, Cinuri).
Occidentalis Negr.	Mature leaf below with hairy cover: cobwebby, leaf edge folded downward	Small, compact, raisins and wine	Spherical; rarely ovate, small or medium, acidity 0.6–1%; seeds small shortly stalked, no seedless cultivars	Western and Central Europe. Frost hardy (Pinot, Gamay, Semillon, Riesling, Mourvedre, Muscat of Alexandria).
Orientalis Negr	Leaf above green, glabrescent, below glabrous to hairy-pubescent, leaf edge folded upwards	Large, conical, often branched, table	Ovate or oblong, medium to large; acidity 0.3–0.6%, seeds medium to large, long stalked, frequently seedless	Central Asia, East Caucasus. West Asia, frost tender (Baian Shirey, Muscat, Chasselas, and Kismisci).

[5] Data elaborated and synthesized by the authors based on different bibliographic sources, particularly [11,91].

De Lorenzis et al. [92], by genotyping germplasm from Central Europe, Armenia, Azerbaijan, Georgia, and Moldova by SSR markers, investigated the genetic relationships among samples along an East-to-West gradient. The identification of three different groups was explained based on geographical origin and human uses and it was in agreement with the *Proles* classification proposed by Negrul [11]:

1. Wine varieties from the West (Central European Cultivars); cf. *Proles occidentalis* (Table 5).
2. Wine varieties from the East (Armenia, Georgia, Moldova); cf. *Proles pontica* (Table 5).
3. Table varieties from the East (Azerbaijan). cf. *Proles orientalis* (Table 5).

Ampelographic studies of selected Syrian grapevine cultivars show their relationships with *Proles orientalis*, especially with regard to five highly discriminating traits (shoot internode length, berry weight, berry elongation, 100-seed weight, and titratable juice acidity) [93], and ampelographic analysis of Israeli grapevine shows clear differences between the *V. vinifera* and *V. sylvestris* groups in terms of flower, leaf and bunch parameters, and that most of *V. vinifera* in the area belongs to *Proles orientalis*, which is consistent with our results based on archaeological seed samples [94].

We must highlight the relevance of the *Proles orientalis*, in front of the *Proles pontica*, at least 4000 years ago, which extended the original area of the former from Central Asia (Negrul 1946) to Western Asia.

Among the grapevine cultivars described by Galet in 1970 [67] from Afghanistan, 29 belong to *Proles orientalis Subproles caspica*, 16 to *Proles orientalis Subproles antasiatica*, 6 to *Proles pontica*, and only two to *Proles occidentalis*. It is unclear to what extent the *Proles occidentalis* and *Pontica* varieties were recently introduced into Afghanistan or are due to a hypothetical introduction by the Greeks in the 4th century B.C. as a consequence of the campaigns of Alexander the Great. One of the open questions, especially concerning the ancient varieties of *Proles antasiatica*, is what the prioritized destination of their fruits was: table grapes or raisins? The available evidence deserves to be analyzed. In the stated

Near Eastern sites, it is common to interpret grape remains as raisins in preference to table grapes. While their use for winemaking is interpreted only when specialized containers and contexts are also found, along with the presence of marker chemical substances such as tartrates and characteristic anthocyanins, the fragmentary nature of our Upper Euphrates archaeological samples prevents us from going further in determining whether the grapes were actually consumed fresh or in the form of raisins. However, their preservation in the archaeological register of raisins' pips seems more likely.

4.1.2. Domesticated Grapevines Belonging to a Particular Euphrates *Proles*

While there is clearly an existence of a group of vines with domesticated features (DI = 0.67–0.83) in their seeds of hybrid origin, the fact they would have intervened with wild vines of the Caucasus and Asia leads us to propose the existence of a fourth singular *Proles euphratica* with more rounded seeds than in *Proles orientalis* and with Stummer [54] index values from 0.73 to 0.82 instead of 0.49 to 0.7, similar to the characteristics of wild vines. *Proles euphratica* is the name we propose here for this group of domesticated grapevines with characteristic seeds (4.7–5.5 × 3.5–4.1 mm) found in Middle Bronze, Assyrian, Aramaic, Persian, and Hellenistic levels from Upper Euphrates sites. These varieties appear to be unrelated to modern cultivars from the area or elsewhere but are related to Caucasian and Asian wild vines. This suggests that this peculiar set of domesticated varieties probably derived from hybridization between Asian and South Caucasian vines. The non-existence of similar grape vines in Turkey or other neighboring areas today may be explained by the abandonment of their cultivation due to their low productivity or the fact that they were exclusively wine grapes in a context where, since the 8th century, wine consumption was excluded for religious reasons.

To verify the persistence, or, if applicable, the date of extinction and further ampelographic characteristics of the here proposed *Proles euphratica*, it would be necessary to extend the ampelographic sampling of wild and feral vines, especially in the rivers and streams tributaries of the Euphrates in northern Syria and Iraqi Kurdistan, to expand the study of their seeds and archaeological remains of vines from the Near East with the methodology proposed here.

However, similar DI values were found in our study in samples also allocated to cluster 8 (Figure 2) from archaeological Chalcolithic to Medieval sites of Spain, Italy, Armenia, and the Balkans, and modern samples of wild and feral grapevines from Georgia, and Spain, and *Vitis piasezkii* Maxim. from China.

Vitis vinifera was probably cultivated in Emar (Syria). Apart from a fragment of wood from a Late Bronze Age stratum, many grape pips have been identified in levels of this period [95]. Carbonized seeds and charcoal of *Vitis sylvestris* and *V. vinifera* were repeatedly recovered from mid-third millennium BC contexts at Tilbeshar, Horum, and Jerablus Tahtani in the Middle Euphrates Valley; tartaric acid associated with gypsum basins found in houses at Titris Höyük suggests that grape processing was widespread there. The area continued to be a recognized center of wine production and export into the early second millennium [96]. Vine seeds, fruits, and pedicels have been recovered from archaeobotanical samples from the Middle Bronze Age site of Tell Tweini, Lattakia (Syria), probably the ancient city of Gibala [97]. It would be particularly useful to be able to study samples from these deposits in detail in order to establish the possible extent of *Proles euphratica* and the other types detected in this work.

4.1.3. Domesticated Grapevines Related with Asian Wild Grapevines

This group establishes a bridge between the cultivated vines of the *Proles euphratica* with various Central Asian vines and, possibly, with *Ampelopsis* species.

A stem was discovered in the Yanghai Tombs, Turpan District in Xinjiang, China. Anatomical features showed it to be of grape (*Vitis vinifera* L.). Radiocarbon dating indicates it to be nearly 2300 years old, which would suggest that there was grape cultivation at least from that time. To date, this is the earliest physical evidence of *V. vinifera* cul-

tivation in China [98]. Two grape (*V. vinifera*) pips were discovered in the gut contents (665–770 cal. years AD) of a person unearthed at tomb 75TAM601 in Astana Cemetery, the public graveyards of the ancient Gaochang people in Turpan, Xinjiang. Grapevine (*V. vinifera*) cultivation was introduced into Turpan c.300 BCE, and Xinjiang is considered to be the earliest place to cultivate grapevine in China [99]. We cannot rule out the existence of other *Vitis* species or even *Ampelopsis* in these *Vitaceae* materials.

4.1.4. Eurasian Hybrid Wild Grapevines

By hybrids we mean individuals and populations that present, on average, intermediate characteristics between the domesticated vine and the wild vine and, at the same time, a great variability in the diagnostic parameters. With similar DI values, we found in clusters 3 and 10 (Figure 3) samples from hybrid populations (European wild vine x domesticated vine or American vines x domesticated grapevine) in natural habitats in Spain as well as archaeological remains from the Levant and the Balkans.

The above suggests the existence in the Upper Euphrates of hybridization processes in wild grapevines populations produced by the domesticated ones, which may have been due to the presence of vineyards in proximity to the wild populations. The analysis of genetic diversity among grapevine (*Vitis vinifera* L.) cultivars in Tartous province (Syria) using microsatellite markers detected high levels of polymorphism, which may be due to continuous seed propagation by birds, natural hybridization between native and introduced plants, and human selection, and also found that vines classified as table grapes with white to raspberry-colored fruit were included in the same cluster despite their genetic variation [100].

4.1.5. Eurasian Domesticated Hybrid and Feral Grapevines

This group is closely related to the previous one but with a significant degree of domestication and is assigned to the high DI values part of cluster 3 (Figure 3), together with feral grapevines from natural habitats in Spain and France, and archaeological materials from Spain, Italy, Hungary, the Balkans, Egypt, the Near East, and the Caucasus. The above also suggests the existence in the Upper Euphrates and abroad, since at least the Bronze Age, of hybridization processes between wild and domesticated grapevine populations, which could have been intentional or casual, which modified the morphology of cultivated grapevines. This would indicate that hybridization is a phenomenon that may have been relevant in the origin of many modern grapevine varieties.

Drori et al. [94] show the existence in Israel of two distinct populations that cluster closely together, suggesting a common genetic origin, one mostly of *V. vinifera* (together with Central Asian cultivars and some from *Proles orientalis Subproles caspica*), another cluster with the *V. sylvestris* from Northern Israel, and a third mixed. This suggests the possibility of wide hybridization between domesticated and wild grapevines in the Near East, which, according to our study of archaeological seeds, may have occurred over a few centuries.

4.1.6. Wild Eurasian—Caucasian Grapevines

These seeds are typical of wild vines and have similarities with the various types of wild vines that we have recognized "a priori" that witnesses the continued presence of clearly wild vines in the high Euphrates throughout all the periods studied, living, for some of these, with the presence of domesticated grapevines.

The group of vines whose seeds present low DI values allocated to cluster 8 includes Asian vines, such as *Vitis ficifolia* Bunge, which is widespread in China, Korea, and Japan, *Vitis amurensis* Rupr. from China, Korea, Siberia, and Japan, and *Vitis wilsoniae* H.J.Veitch; numerous wild grapevine populations (*Vitis sylvestris*) in Spain, Italy, and the Caucasus, American wild vines, fossil vines, and archaeological materials from Hungary, the Balkans, Spain, and the Near East.

Wan et al. [101] place the origin of the *V. heyneana* / *V. vinifera* / *V. sylvestris* clade at around 6.3 Mya and report an unexpected *V. sylvestris*-derived position that conflicts with *V. sylvestris* being the progenitor of *V. vinifera*, as the phylogenetic position suggests that *V. sylvestris* is derived from *V. vinifera*. They interpret this discrepancy in terms of erroneous inference due to the effects of clonal propagation in the grapevine cultivars analyzed [101]. We detected high variability in the domestication values of *V. heyneana*, DI = 0–0.67.

The phylogenetic structure within the genus *Vitis* was analyzed by Aradhya et al. [102] using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers, resulting in fourteen clusters, most of which contained a moderate frequency of mixed genotypes, suggesting interspecific gene flow within the subgenus *Vitis*. The AFLP-based tree clearly separated two clusters within *V. sylvestris*, assigned *V. vinifera* cultivars predominantly to one of them, and supported the close relatedness of *V. heyneana*. The SSR-based analysis was less conclusive.

The nuclear microsatellite-based study by Doulati-Baneh et al. [103] of wild grapevine populations in the Zagros Mountains of Iran grouped the accessions into three clusters corresponding to the geographical distribution of the populations. Most of the populations had an unbalanced sex ratio, with a modest number of female individuals in most of the Zagros Mountains, reducing the species seed dispersal ability. This was also confirmed by the field assessment during the plant sampling activity; a large proportion of individuals were older than 25–30 years, and only a few young seedlings were detected in only one population. Thus, Iranian wild grape populations have a reduced regeneration capacity, probably due to modest seed production and/or environmental disturbances leading to an inhospitable habitat for young seedlings.

All grape pips analyzed in Greece by Pagnoux et al. [104] from the Late Neolithic are morphologically wild. The change from the wild to the domesticated form occurred during the Middle Bronze Age (1900–1700 BC). The picture we get from the Upper Euphrates area is far more complex because wild types persisted until later periods coexisting with domesticated types.

4.1.7. Wild Asian Grapevines Allocated to Genus Ampelopsis

The presence of endemic *Ampelopsis* species in the area even today makes us think that, logically, they were also present during the periods analyzed. It is pending identification whether that presence is merely accidental from wild populations or if they were cultivated for any purpose. The finding of similar seeds in a garden purely dedicated to enjoyment at Hellenistic levels of Petra in Jordan is particularly informative.

Likely, the wild grapevine, which was a plant component of an Acheulian diet at Gesher Benot Ya'aqov, Israel, in the southern Hula Valley and assigned to the Lower–Middle Pleistocene, dated c. 780,000 BC [86], belongs to the genus *Ampelopsis* [78]. With the DI = 0.5, this seed is neither typically wild nor domesticated; however, we are not aware that this index remains useful for *Ampelopsis*.

Most of the wild vine species, both American and Asian, as well as the fossils studied, were ascribed to cluster 11.

4.1.8. Raisin Type Seeds with Low Domestication Syndrome Remotely Linked to Genus Ampelopsis

Abortive seeds alone do not explain this strange morphology since they are often morphologically similar to normal seeds and only differ in that they are empty instead of containing an embryo and endosperm [105].

Stenospermocarpic grapes, such as the "Sultanina" variety, are heritable, and these embryos can be obtained via normal pollination and fertilization, but seed development ends prematurely, leading to embryo abortion occurring at 2–4 weeks after blooming. Together with parthenocarpic grapes, these conform to two groups of seedless cultivars [106]. *Keshmesh*, meaning sultana, is a very common name for grapes used to make sultanas

in Iran. In this case, similar names imply identical usage but do not necessarily indicate genetic similarity between the cultivars [107].

Proles antasiatica vines are now often used for raisin production and show anomalies in seed formation. Among the 55 Iranian grapevine cultivars investigated by Abiri et al. [108], 20 cultivars formed seeds in a rudimentary form, while seeds were well developed in 34 cultivars and one cultivar was seedless. On average, the seed number per berry is two, with a maximum of four. As expected, larger berries were found in less dense bunches.

Levchenko et al. [109] have shown the close genetic relationship within the *Vitaceae* family between the genera *Vitis* and *Ampelopsis*, which are closer in terms of the evolution of the grape culture as a whole. They obtained artificial intergeneric hybrids that produced c. 25% of plump seeds and 10% of viable embryos. Thus, 75% of the seeds were abnormally thin. It is relevant to mention here that when we found these abnormal types of seeds together with those that were *Ampelopsis*-like, we suspected that this anomaly could be due to hybridization or merely intergeneric cross-pollination.

5. Conclusions

The development of a new domestication index for the seeds of *Vitis vinifera–V. sylvestris*, based on the combination of six pre-existing indices, together with the multivariate analysis of the morphometry of the seed, the hierarchical classification of the samples, based on a very comprehensive collection of comparisons, and the integration of data from both sources using the Bayes–Laplace method, constitutes a very sensitive tool that allows one to successfully analyze alternative hypotheses about the identity of the archaeological seeds of *Vitis*.

As a consequence, in the study of the archaeological seeds of two sites of the Upper Euphrates, we have been able to establish the predominance, among the clearly domesticated ancient varieties, of *Proles orientalis* Negrul, together with the existence of some domesticated vines with peculiar characteristics, which we include in a possible extinct *Proles* that we call *Euphratica*.

Along with the above, it was determined that wild vines related to *Vitis sylvestris* C.C.Gmelin and *V. caucasica* Vavilov continued to be present throughout the period studied, from Early Bronze to Hellenistic.

In parallel, seeds with hybrid characteristics have been recorded, and much more significantly, the existence of seeds of the *Ampelopsis* type has been documented, among which is the oldest vitaceous seed linked to human presence in the Acheulean (780 myr).

Finally, the presence of "stenosperms" that appear to be associated with fully developed seeds of the *Ampelopsis* type has been identified, which suggests the existence of anomalies in the formation of the seeds due to intergeneric cross-pollination. Additionally, in the cases where they appear isolated, they suggest the presence of "stenospermocarpic" *Vitis vinifera* raisins of the Sultana type.

If we consider the relevance of this study for the future of the grapevine, specifically in terms of adaptation to climatic changes, resistance to pests, and new varieties with new characteristics, we can affirm that what is most relevant in our opinion is the detection of the early existence of "stenosperms" and therefore of seedless grapes of the "sultanina" type about four thousand years ago, which indicates the capacity of adaptation of this type of grape as well as those of the *Proles orientalis* Negrul to climatic and salinity changes along millennia. The possible extinction of *Proles euphratica* would suggest that not all local vines were able to overcome climatic crises, changes in use, or the appearance of eventual pests and would point to the value of ancient local varieties for their resilience. Finally, the study of history can help the farmer improve his vineyard through the idea of not despising local cultivars and local wild populations that have survived environmental adversities for centuries and whose communities are also repositories of feral grapevines. Furthermore, farmers can appreciate them as sources of genetic resources for the improvement of their vineyards, with preference for the introduction of exotic varieties whose possibilities of adaptation to local conditions and climate change are uncertain.

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