**ORIGINAL ARTICLE** 



# Identification of archaeobotanical *Pistacia* L. fruit remains: implications for our knowledge on past distribution and use in prehistoric Cyprus

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#### Abstract

*Pistacia* spp. remains are common finds among archaeobotanical assemblages in prehistoric sites in Southwest Asia, both in the form of endocarps and charcoal remains. However, in the absence of a systematic study of the fruit morphology, the archaeobotanical remains of *Pistacia* cannot be identified to the species level and this deprives us of important information on their past geographical distribution and uses. This paper presents a comprehensive study of morphological and traditional morphometric characteristics of modern endocarps of six *Pistacia* species indigenous to Southwest Asia: *P. atlantica* Desf., *P. eurycarpa* Yalt., *P. khinjuk* Stocks, *P. lentiscus* L., *P. terebinthus* L. and *P. vera* L. The observation of recurring morphological features that were shown to remain stable during experimental carbonisation allowed us to set up an identification key whose efficiency was tested through a blind test. The application of established identification criteria to archaeobotanical material from the Late Aceramic Neolithic site of Khirokitia-*Vounoi* in Cyprus, enables an in-depth discussion on the biogeography and use of *Pistacia* species in prehistoric Cyprus. Finally, the paper identifies directions for future research on a major plant resource of the past.

Keywords Pistacia spp. · Identification key · Endocarp morphology · Khirokitia · Aceramic Neolithic · Cyprus

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# Introduction

At least seven species and variable numbers of subspecies of *Pistacia* L. are reported to grow in Southwest Asia and in the Mediterranean basin, where they often constitute major components of local plant communities (Zohary 1973; Al-Saghir and Porter 2012). *Pistacia* spp. are common together with other tree and shrub species in the Mediterranean maquis and they are also conspicuous elements of the semiarid open woodlands, or steppe forests, of the Irano-Turanian flora. Only one species, *P. vera*, is a domesticate and is at present cultivated on a large scale with Iran and the United States as the main producers (source: ©FAOStat 2019).

While the seeds of *P. vera* are well known worldwide as a snack or an ingredient in food preparations, various products from wild *Pistacia* spp. play an important role locally in the regions where they grow. Thus, the fruits of *P. atlantica*, *P. eurycarpa* and *P. khinjuk* are traditionally collected and consumed in different forms throughout the Middle East (Al-Saghir and Porter 2012; Willcox 2016; Ahmed 2017). The resins extracted from several species have multiple uses (medicinal, ritual, as a varnish, in food preparations), while

turpentine and mastic, obtained from *P. terebinthus* and *P. lentiscus* respectively, have been important products of the eastern Mediterranean for millennia (Mills and White 1989; Stern et al. 2008). Oil extracted from *Pistacia* spp. kernels is used in traditional medicine to heal various ailments (Loi 2013); galls and leaves are used for tanning and dyeing; leaves are also given to livestock for fodder and wood from several species is used as fuel, directly or in the form of charcoal.

Abundant remains of seeds/fruits and charred wood of *Pistacia* on archaeological sites in Southwest Asia indicate the importance of these trees and shrubs as major plant resources (Fig. 1). Despite their omnipresence, these remains have attracted surprisingly little attention from archaeobotanists and their potential in terms of information on the ethnobotany, biogeography and ecology during prehistoric and later periods has clearly been underexploited. The main obstacle in interpreting *Pistacia* fruit remains more precisely is the difficulty of identifying them to the

species level. So far few attempts have been made to do so through the systematic study of morphological criteria. In a more general paper on the archaeobotanical results from the site of Timna in the Negev desert of southern Israel, Kislev (1988) discusses the possible distinction between *P. atlan*tica and P. khinjuk as well as P. palaestina (now considered as a synonym of P. terebinthus) on the basis of hilum morphology. Still, this limited study remains an exception and other authors generally rely on modern biogeographical data to infer the most probable species present on archaeological sites or prefer to restrict the identification of fruit remains to genus level (Pistacia sp.). Yet the past biogeography of Pistacia spp. in Southwest Asia is poorly known and is likely to have changed considerably during the Holocene due to human activities and climatic change. It should be noted too that on archaeological sites located in regions where none or only one species of Pistacia grows today, the presence in archaeobotanical assemblages of several fruit morphotypes, potentially corresponding to distinct species, is not rare and

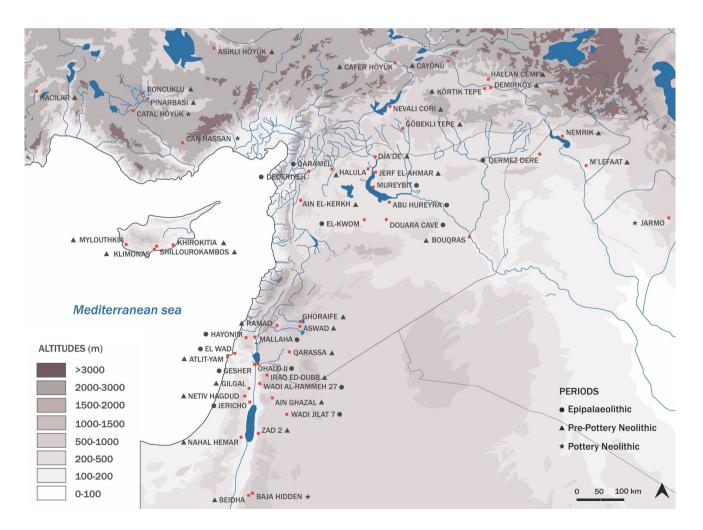


Fig. 1 Map showing selected Epipalaeolithic and Neolithic archaeological sites where *Pistacia* sp. fruit/seed and/or charcoal remains have been recovered (Fig. generated by C. Douché)

suggests different and perhaps wider distribution patterns for some species in the past.

After having encountered numerous *Pistacia* fruit remains on archaeological sites from Cyprus to Iran, the authors of this paper decided to tackle the problem of species identification by establishing an identification key based on the morphological and traditional morphometric study of well identified modern endocarps from six species occurring in Southwest Asia and in the eastern Mediterranean: *P. atlantica* Desf., *P. eurycarpa* Yalt., *P. khinjuk* Stocks, *P. lentiscus* L., *P. terebinthus* L. and *P. vera* L. This paper proposes a detailed and illustrated description of the morphological features that allow the distinction between these species on the basis of the lignified endocarp (or interior fruit wall) of their fruits.

In order to show the importance of species identification in terms of biogeography and ancient uses we have applied the identification criteria to *Pistacia* fruit remains from the Late Aceramic Neolithic (7000–5500 cal BC) site of Khirokitia-*Vounoi* in Cyprus.

#### Taxonomy and biogeography of *Pistacia* spp.

The genus *Pistacia* L. (family Anacardiaceae, order Sapindales) was first established in the 18th century by Linnaeus who attributed six then known species to this group (Linnaeus 1753). The members of the genus are dioecious trees or shrubs bearing alternate, deciduous or persistent leaves, often pinnately compound but sometimes trifoliate or unifoliate. Inflorescences are terminal or axillary, composed of unisexual apetalous and radial flowers of small size. The fruit, stemming from one fertile carpel, is an asymmetrical one-seeded drupe (Al-Saghir and Porter 2012). Despite a relatively limited number of taxa the relationship between the different species and subspecies of *Pistacia* is still problematic and there is no definite consensus either on the number of species or on their phylogenetic relationships. The difficulties are primarily due to the high interfertility between species causing hybridisation and intermediate forms that are difficult to recognise and sometimes misidentified to species level. Distribution patterns that are sometimes overlapping between species and sometimes strongly disjunct within one species further complicate the task (M. Zohary 1952, 1973; D. Zohary 1996).

Since Linnaeus, there have been several revisions of the genus taxonomy and species phylogenetic relations based on morphological (Zohary 1952; Kafkas and Perl-Treves 2001; Karimi et al. 2009; Al-Saghir 2010) and molecular criteria (nuclear and chloroplast genes) (Parfitt and Badenes 1997; Kafkas and Perl-Treves 2001, 2002; Katsiotis et al. 2003; Golan-Goldhirsh et al. 2004; Al-Saghir and Porter 2006; Kafkas 2006; Yi et al. 2008; Shanjani et al. 2009; Arabnezhad et al. 2011; Karimi and Kafkas 2011; Talebi et al. 2012). In his extensive revision of the genus M. Zohary (1952) recognised 11 species and 7 subspecies that he divided into four sections. On the basis of molecular data Yi et al. (2008) recognised 11 species. Somewhat later, a thorough study of the morphological characteristics of a large number of specimens has resulted in a classification with nine species and five subspecies, divided into two sections (Al-Saghir and Porter 2012). For our study we have chosen to use this last classification that takes into account the results of previous phylogenetic studies based on both morphological and molecular data and constitutes the most complete and up to date taxonomic revision of the genus (summarised in Table 1).

Species	Subspecies	Vernacular name		
Section Pistacia				
Pistacia atlantica Desf.	_	Butum		
Pistacia chinensis Bunge	chinensis	-		
	falcata (Becc. ex Martelli) Rech. f.	-		
	integerrima (J.L. Stewart ex Brandis) Rech. f.	-		
Pistacia eurycarpa Yalt.	_	Butum		
Pistacia khinjuk Stocks	_	-		
Pistacia terebinthus L.	_	Turpentine tree		
Pistacia vera L.	_	Pistachio		
Section Lentiscella				
Pistacia lentiscus L.	lentiscus	Mastic, Chios mastic tree		
	emarginata (Engl.) Al-Saghir	_		
Pistacia mexicana Kunth	_	Mexican pistachio		
Pistacia weinmannifolia J. Poiss. ex Franch.	-	-		

Table 1Revised taxonomy ofthe genus Pistacia L. accordingto Al-Saghir and Porter (2012)

The genus Pistacia has a wide modern geographical distribution in the Old World, from North Africa to Southeast Asia, with two main centres of diversity, one in the Mediterranean basin and the Near East and the other in West and Central Asia. One species (P. mexicana) is native to the New World where it grows in southern Texas, Mexico, Guatemala and Honduras (Al-Saghir and Porter 2012).

The distribution patterns for P. terebinthus and P. lentiscus are essentially Mediterranean (Fig. 2a, b) except for the subspecies P. lentiscus ssp. emarginata that grows in eastern Africa. *Pistacia*  $\times$  *saportae*, a hybrid between *P. lentiscus* and P. terebinthus, is common wherever the two grow together (Zohary 1996; Yi et al. 2008; Al-Saghir and Porter 2012).

*Pistacia atlantica* is widely distributed from the Canary Islands in the west across North Africa and the Middle East including the Arabian Peninsula and southern Caucasus (Fig. 2a). Pistacia khinjuk and P. eurycarpa are found from Turkey to Afghanistan and Pakistan (Fig. 2b). Wild P. vera grows in the eastern part of this range and in particular in southern Central Asia (Zohary 1973) (Fig. 2a).

Finally, P. weinmannifolia and P. chinensis have a more eastern distribution even though two subspecies of the latter grow further to the west: P. chinensis ssp. falcata is reported from a rather restricted zone on both sides of the Red Sea (Saudi Arabia, Yemen, Horn of Africa) and P. chinensis ssp. integerrima occurs in the mountains of the Caucasus, Afghanistan, Pakistan and Nepal (Al-Saghir and Porter 2012).

# Materials and methods

#### **Establishing a reference collection**

The modern reference collection of Pistacia endocarps was constituted by sampling from the extensive herbarium collections held in the Muséum national d'Histoire naturelle in Paris (P) and the Roval Botanic Garden of Edinburgh (E) (information on the herbarium specimens and their geographical origin is provided in ESM 1). The collection used in this study comprises a total of 197 individuals (endocarps) from 109 different herbarium specimens representing the six above-mentioned species (Table 2). The species were chosen on the grounds of their modern geographical distribution corresponding to the broader geographical region under study, i.e. Southwest Asia and the eastern Mediterranean basin. Due to their very limited presence (P. chinensis), or complete absence from our study region (P. weinmannifolia, P. mexicana), the three remaining species were excluded. This was also the case for the hybrid form, Pistacia × saportae, present in the herbarium collections but with morphologies too variable to be of immediate use for the identification key. Concerning P. vera, we chose to give priority to herbarium specimens from regions where wild forms of this species can still be found (Iran and Central Asia). Still, while the wild status could be confirmed for the fruits collected by G. Willcox in Tajikistan (personal communication), the

Table 2 Number of sampled herbarium specimens and number of individuals (endocarps) studied per species

Species	No. of sampled herbarium specimens	No. of individuals		
P. atlantica	25	32		
P. eurycarpa	22	36		
P. khinjuk	16	32		
P. lentiscus	19	44		
P. terebinthus	15	30		
P. vera	12	23		
Total	109	197		

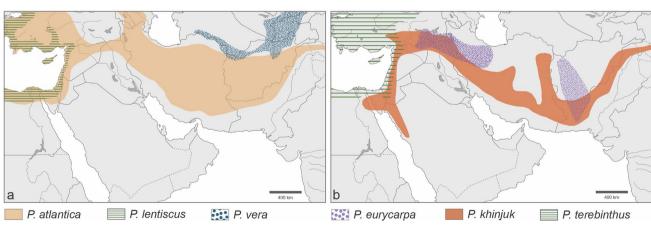
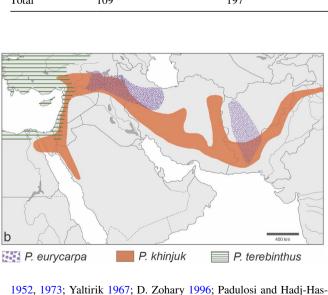


Fig. 2 Modern geographical distribution of Pistacia spp. discussed in the text: a P. atlantica, P. lentiscus, P. vera, b P. eurycarpa, P. khinjuk, P. terebinthus (Figures generated by C. Douché, after M. Zohary



san 2001; Al-Saghir 2010; Al-Saghir and Porter 2012)

wild or domesticated status of herbarium specimens was not clearly indicated (ESM 1).

The taxonomic identification of herbarium specimens was systematically verified by the use of descriptions in floras and other studies, mainly taking into account the foliar morphology (M. Zohary 1952, 1986; Yaltirik 1967; D. Zohary 1996; Rechinger 1969; Meikle 1977; Kafkas and Perl-Treves 2001; Tsintides et al. 2002; Ghaemmaghami et al. 2009; Karimi et al. 2009; Al-Saghir and Porter 2012).

Securely identified herbarium specimens associated with a certain quantity of mature fruits were selected for sampling. Despite the destructive sampling regulations of herbarium collections, we could in most cases attain 30 individuals (fruits) per species by sampling one fruit from each specimen. In a few cases, additional fruits could be obtained thus allowing a larger sample size (Table 2). In addition several wild *P. vera* endocarps, collected by G. Willcox in Tajikistan in 1990 and now held in the reference collection of the archaeobotanical laboratory of the UMR 7209 in Paris, were added to the modern reference collection (ESM 1).

# Preparation of the modern reference collection, observation methods and documentation

After soaking in water for 3 h, the fleshy parts of the fruits (mesocarp and epicarp) were removed manually and the endocarps were dried at room temperature for 24 h. The study of the endocarps was carried out with the help of a binocular stereomicroscope (Nikon SMZ1270, magnifications

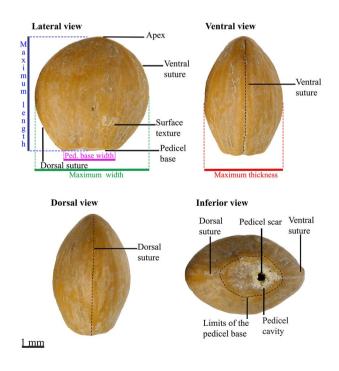


Fig. 3 Morphological characteristics and linear measurements of *Pistacia* endocarps under study (abbreviation: *Ped.* Pedicel)

 $6.3 \times -80 \times$ ) and the morphology of each individual was observed and described from four different views (lateral, ventral, dorsal and inferior view) (Fig. 3).

Linear measurements (length, width and thickness) were systematically taken with the use of NIS-Elements Imaging software. In addition, the width of the pedicel base of *P. khinjuk* and *P. terebinthus* endocarps was measured in order to further investigate possible distinction between these two species (Fig. 3).

Drawings were made by hand with the help of a binocular stereomicroscope (Nikon SMZ800, magnifications  $10\times-63\times$ ) and a Nikon zoom loupe drawing tube at the Atelier Iconographie Scientifique (UMS 2700, MNHM, Paris). Photographs were taken with a Canon EOS 6D camera (Canon Macro Photo Lens MP-E 65 mm 1:2.8,  $1\times-5\times$ ), using the Helicon program at the imaging station of the collection management unit of Zoology-Comparative Anatomy (Direction des Collections, MNHN, Paris).

#### Statistical analysis of linear measurements

The statistical analysis was conducted including all modern endocarps, using the packages dplyr (Wickham et al. 2019) and car (Fox and Weisberg 2019) of R software (R Core team 2018). The significance level ( $\alpha$ ) was set at 0.05 for all statistical tests. The normality of the data and homoscedasticity of the variances were tested by the Shapiro–Wilk (function shapiro.test) and Levene's test (function levene.test) respectively. The Kruskal Wallis test (function kruskal.test) was performed in order to compare the medians of the linear measurements of the species (length, width, thickness). Finally, in order to compare the distribution and the means among the species, a Wilcoxon signed-rank test with Bonferroni correction was run on the data to adjust the significance level (function pairwise. wilcox.test) for each size variable.

The normality of the data and homoscedasticity of the variances of the width measurements of pedicel bases of *P. khinjuk* and *P. terebinthus* were tested by the Shapiro–Wilk test (function shapiro.test) and Fisher's F-test (function var.test) respectively. An independent samples t-test (function t.test) was applied to the dataset to compare the means of the two groups.

#### **Experimental carbonisation**

In order to evaluate the effects of charring on endocarp morphology and to make certain that the morphological characteristics observed on modern uncharred endocarps are not substantially altered by the carbonisation process, we experimentally carbonised modern endocarps from four wild species (*P. atlantica*, *P. eurycarpa*, *P. lentiscus*, *P. terebinthus*). Ten individuals from each were exposed to three different temperatures (400, 450 and 500 °C) for 60 min, in anoxic conditions in a preheated muffle furnace (Nabertherm L15/11). Charred endocarps were then observed with a binocular stereomicroscope (Nikon SMZ1270, magnifications 6.3×–80×). This preliminary study showed that while the carbonisation process generally resulted in reduction in size, important morphological features were not altered in a significant way. The general shape of endocarps in lateral view as well as the morphology of the pedicel base remained clearly visible and can thus be considered as diagnostic criteria to be used also when working with carbonised material. The wrinkled surface of *P. atlantica* sometimes became smoother near the apex but this diagnostic feature was still discernible near the pedicel base.

### Results

Detailed and exhaustive descriptions, as well as size measurements and size ratios of the six *Pistacia* spp. endocarps are presented in Table 3. Morphological as well as traditional morphometric criteria contribute to the identification of *Pistacia* spp. (Table 3, Figs. 4, 5, 6, 7, 8) and are combined in the identification key below.

Our observations on modern uncharred and experimentally charred specimens have shown that three criteria are particularly useful for differentiating the *Pistacia* spp. included in our study: the size (length, width, thickness, and size ratios), the shape of the endocarps and the morphology of the pedicel base.

The morphology of the pedicel base appears to be an important criterion and was also recognised as such by Kislev (1988). This characteristic allows the discrimination of two main groups and hence is the starting point of the identification key. Three species, *P. atlantica*, *P. eurycarpa*, and *P. khinjuk*, present a clearly circumscribed, well-defined pedicel base, with conspicuous limits, forming a concave cavity (crater-like), while in *P. lentiscus*, *P. terebinthus*, and *P. vera* endocarps, the limits of the pedicel base are hardly visible or absent and the pedicel base is convex to slightly convex in lateral view (Fig. 4a–f).

Measurements and size ratios proved useful for distinguishing between certain species but in other cases the endocarp dimensions overlap, and we must rely on qualitative criteria. Some general observations can be noted. Thus, the endocarps of *P. vera* and *P. atlantica* are always longer than broad (oval to obovate shape), while *P. eurycarpa* and *P. lentiscus* endocarps are systematically broader than long (elliptical shape). *P. terebinthus* and *P. khinjuk* are generally slightly longer than broad but sometimes they are almost as broad as long (oval to orbicular shape; see size ratios, Table 3).

The endocarps of *P. vera* are clearly distinguishable from all other species both in respect to size and morphology. The endocarps of wild *P. vera* are generally smaller than domestic forms (Zohary and Hopf 2004) but even in these cases they are invariably bigger than those of all other studied *Pistacia* spp., with lengths attaining 10–20 mm (see ESM 2).

Moreover, their asymmetrical shape, oblique pedicel base in lateral view and slightly curved dorsal suture are highly diagnostic criteria (Table 3, Figs. 4f, 5f), allowing the distinction of them from other species. Finally, this is the only species whose endocarps split longitudinally into two (they bisect) when ripe, a characteristic selected for in the domestic species as it greatly facilitates the extraction of the edible seed.

According to their size, wild *Pistacia* spp. can be divided into two groups. *P. eurycarpa* and *P. atlantica* form bigger endocarps than *P. khinjuk*, *P. terebinthus* or *P. lentiscus* (Table 3, Figs. 6, 7, 8, ESM 2–3).

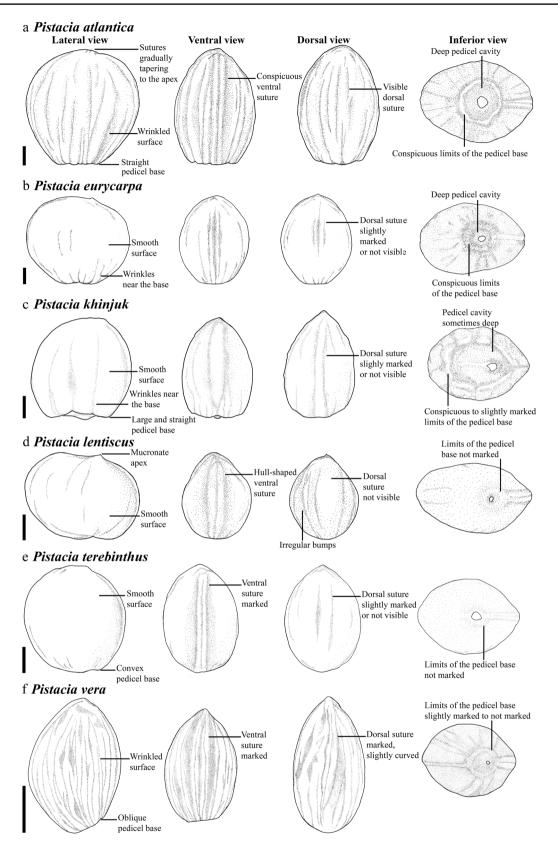
Both *P. eurycarpa* and *P. lentiscus* have broadly elliptic endocarps (Fig. 5b, d; see also size ratios in Table 3) but they can be separated thanks to their statistically significant difference in size (Wilcoxon signed-rank test p-values  $< \alpha$ ; ESM 4). Indeed, none of their linear measurements overlap. While *P. eurycarpa* forms the largest endocarps of all wild species, *P. lentiscus* always represents the lowest values (Figs. 6, 7, 8). The limits of the pedicel base constitute a supplementary criterion for the discrimination of the two species. Moreover, *P. lentiscus* is also characterised by two unique morphological characteristics: a mucronate apex and a hull-shaped ventral suture (Figs. 4d, 5d). These features were both well preserved after the experimental carbonisation of modern endocarps.

While the length measurements of *P. atlantica* and *P. eurycarpa* may sometimes overlap (Table 3; Fig. 6), their width and thickness measurements are statistically different (Figs. 7, 8; Wilcoxon signed-rank test p-values  $< \alpha$ ; ESM 4). The oval shape of *P. atlantica* sometimes resembles that of *P. terebinthus* and *P. khinjuk*, but *P. atlantica* can be distinguished from the two others by qualitative criteria (form of the pedicel base, surface texture, dorsal and ventral sutures) (Table 3; Fig. 4a, c and e).

The endocarps of *P. khinjuk* and *P. terebinthus* may appear similar both in shape and morphological characteristics. Moreover, their size differences are not statistically significant (Table 3; Figs. 6, 7, 8; Wilcoxon signed-rank test p-values >  $\alpha$ ; ESM 4). However, P. terebinthus generally represents more orbicular forms in lateral view and its pedicel base is narrower than P. khinjuk (Fig. 9; Maximum width/Pedicel base width size ratios in Table 3). Another difference is that P. khinjuk has a conspicuous pedicel base, forming a crater-like cavity, while P. terebinthus generally has a convex pedicel base whose limits are not clearly visible (Fig. 4c and e). Still, in some observed cases the pedicel base of P. khinjuk is less pronounced and the distinction between the two can then be ambiguous. According to Kislev (1988) the presence of a small radial ridge stretching from the pedicel scar to the rim of the pedicel base is characteristic of *P. khinjuk*. This criterion has also been observed in our reference collection, but it is not systematically present and therefore cannot be considered as an entirely reliable criterion for specific identification.

#### Table 3 Morphological descriptions, size measurements and ratios of the *Pistacia* spp. endocarps (for details see ESM 2)

Lateral view	Ventral and dorsal views	Inferior view
Pistacia atlantica		
- Endocarp always longer than broad	- Bilaterally symmetrical	- Bilaterally symmetrical
<ul> <li>Mostly bilaterally symmetrical</li> </ul>	<ul> <li>Shape ovate</li> </ul>	- Shape elliptic
- Shape oval to slightly obovate	- Conspicuous ventral suture, often in the	- Limits of the pedicel base circular
- Apex: ventral and dorsal sutures gradually tapering to the apex;	form of double ridges	to elliptic, conspicuous (crater-like
rounded	- Dorsal suture clearly visible	- Deep, concave pedicel cavity
- Pedicel base straight	- Thickness range (mm): 3.16-4.48	- Pedicel scar circular to elliptic
		- redicer scar circular to emplic
- Wrinkled surface with numerous conspicuous longitudinal lines,	- Width/Thickness ratio: 1.17-1.70	
sometimes continuous from the apex to the pedicel base	- (Width/Length) x 100 ratio: 76.56-	
- Length range (mm): 4.23-7.13	111.26	
- Width range (mm): 4.18-6.46		
- Length/Width ratio: 0.90-1.31		
- Length/Thickness ratio: 1.20-2.06		
-		
Pistacia eurycarpa	Dilata alla anno stale d	Bileter ller en et die 1
- Endocarp always broader than long	- Bilaterally symmetrical	- Bilaterally symmetrical
- Generally, bilaterally symmetrical	- Shape ovate	- Shape elliptic, sometimes with a
<ul> <li>Shape broadly elliptical to reniform</li> </ul>	- Ventral suture marked, often in the form	slightly narrower ventral part
<ul> <li>Apex: ventral and dorsal sutures gradually tapering to the apex;</li> </ul>	of double ridges	- Limits of the pedicel base circular
rounded	<ul> <li>Dorsal suture slightly marked or not</li> </ul>	to elliptic, conspicuous (crater-like
<ul> <li>Pedicel base concave</li> </ul>	visible	- Deep, concave pedicel cavity
- Surface generally smooth, with wrinkles appearing near the	- Thickness range (mm): 3.75-5.60	- Pedicel scar circular to elliptic
pedicel base	- Width/Thickness ratio: 1.36-1.72	realeer sear encatar to emptie
	- (Width/Length) x 100 ratio: 100.30-	
- Length range (mm): 4.89-7.28 Width range (mm): 5.10.8.84		
- Width range (mm): 5.10-8.84	140.69	
- Length/Width ratio: 0.71-1.00		
- Length/Thickness ratio: 1.06-1.61		
Pistacia khinjuk		
- Mostly bilaterally symmetrical	- Bilaterally symmetrical	- Bilaterally symmetrical
- Shape variable from orbicular to oval and ovate	- Shape ovate	- Shape elliptic, sometimes with a
- Apex: ventral and dorsal sutures gradually tapering to the apex;	- Ventral suture marked to very well	slightly narrower ventral part
rounded	marked, often in the form of double	- Limits of the pedicel base elliptic,
<ul> <li>Pedicel base straight and large</li> </ul>	ridges	conspicuous to slightly marked. If
- Surface generally smooth, with wrinkles appearing near the	<ul> <li>Dorsal suture slightly marked or not</li> </ul>	conspicuous, in the form of a crate
pedicel base	visible	-Pedicel cavity sometimes deep and
- Length range (mm): 3.74-6.33	- Thickness range (mm): 2.68-4.30	concave (if marked limits of the
- Width range (mm): 3.81-6.23	- Width/Thickness ratio: 1.21-1.72	pedicel base)
- Pedicel base width range (mm): 2.13-3.95	- (Width/Length) x 100 ratio: 80.50-	- Pedicel scar circular to elliptic
		- I euleer sear circular to emptie
- Length/Width ratio: 0.85-1.24	117.55	
<ul> <li>Length/Thickness ratio: 1.19-1.86</li> </ul>		
<ul> <li>Maximum width/Pedicel base width ratio: 1.32-2.07</li> </ul>		
Pistacia lentiscus		
- Endocarp always broader than long	- Generally, bilaterally symmetrical but	- Bilaterally asymmetrical
- Bilaterally asymmetrical	sometimes asymmetrical (due to bumps)	
- Shape elliptic to orbicular	- Shape ovate	bumps)
- Apex: mucronate	<ul> <li>Ventral suture very well marked, hull-</li> </ul>	- Limits of the pedicel base not
<ul> <li>Pedicel base convex, slightly narrow</li> </ul>	shaped	marked; pedicel base slightly
<ul> <li>Surface generally smooth but with irregular bumps</li> </ul>	<ul> <li>Dorsal suture not visible</li> </ul>	convex
- Length range (mm): 2.86-4.56	- Thickness range (mm): 1.71-3.00	<ul> <li>Pedicel cavity absent</li> </ul>
- Width range (mm): 3.24-4.91	- Width/Thickness ratio: 1.33-2.26	- Pedicel scar circular to elliptic
- Length/Width ratio: 0.67-1.15	- (Width/Length) x 100 ratio: 86.89-	_ sales sea encatar to emptie
- Length/Thickness ratio: 1.14-2.28	148.43	
Pistacia terebinthus		
- Bilaterally symmetrical to slightly asymmetrical	- Bilaterally symmetrical	- Bilaterally symmetrical
- Shape oval to orbicular	- Shape oval to ovate	- Shape elliptic to orbicular, ventral
- Apex: ventral and dorsal sutures gradually tapering to the apex;	- Ventral suture marked, often in the form	
	- ventral suture marked, often in the form	part sometimes narrower than the
rounded to occasionally pointed	of double ridges	dorsal
- Pedicel base straight to slightly convex	- Dorsal suture slightly marked or not	- Limits of the pedicel base not
- Surface smooth, very rarely wrinkled near the pedicel base	visible	marked; pedicel base convex
- Length range (mm): 3.65-5.71	- Thickness range (mm): 2.29-3.79	- Pedicel cavity absent
- Width range (mm): 3.26-5.53	- Width/Thickness ratio: 1.09-1.87	- Pedicel scar circular to elliptic
- Pedicel base width range (mm): 1.84-3.07	- (Width/Length) x 100 ratio: 74.03-	1
- Length/Width ratio: 0.84-1.35	119.18	
	117.10	
- Length/Thickness ratio: 1.17-2.03		
- Maximum width/Pedicel base width ratio: 1.54-2.35		
Pistacia vera		
- Endocarp always longer than broad	- Bilaterally symmetrical	- Bilaterally symmetrical
- Always bilaterally asymmetrical	- Shape generally ovate	- Shape elliptic to orbicular, ventral
- Shape irregularly oval	- Ventral suture marked, often in the form	part sometimes narrower than the
- Apex: ventral and dorsal sutures gradually tapering to the apex	of double ridges	dorsal part
- Pedicel base oblique	<ul> <li>Dorsal suture marked, slightly curved</li> </ul>	- Limits of the pedicel base slightly
- Surface wrinkled with dense and conspicuous longitudinal	- Endocarp splits in two valves when	marked to absent; pedicel base
continuous lines, extending from the apex to the pedicel base	ripening	slightly convex
- Length range (mm): 9.91-19.15	- Thickness range (mm): 5.88-10.14	- Pedicel cavity absent to slightly
- Width range (mm): 6.54-11.65	- Width/Thickness ratio: 1.05-1.38	marked
	- (Width/Length) x 100 ratio: 53.20-77.94	- Pedicel scar circular to elliptic
		- realcel scar circillar to elliptic
- Length/Width ratio: 1.28-1.88 - Length/Thickness ratio: 1.42-2.43	- (widu/Lengui) x 100 lauo. 55.20-77.94	· · · · · · · · · · · · · · · · · · ·



**Fig. 4** Main morphological characteristics of **a** *P. atlantica*, **b** *P. eurycarpa*, **c** *P. khinjuk*, **d** *P. lentiscus*, **e** *P. terebinthus*, and **f** *P. vera* endocarps in the four observation views (lateral, ventral, dorsal and inferior views) (Drawings generated by M. Rousou); scale bars = 1 mm and 5 mm (*P. vera*)

#### Identification key to Pistacia spp.

1 a. Limits of the pedicel base conspicuous to very conspicuous; deep pedicel base; pedicel cavity present (concave)	2
b. Limits of the pedicel base slightly marked to not visible; pedicel base narrow to convex; pedicel cavity not present	3
<ul> <li>2 a. Endocarp broader than long (length/width ratio between 0.71 and 1.00; width usually</li> <li>&gt; 5 mm); broadly elliptical to reniform shape in lateral view; very broad form in lateral, ventral/dorsal and inferior views; surface generally smooth, with wrinkles appearing near the pedicel base</li> </ul>	P. eurycarpa
b. Endocarp longer than broad (length/width ratio 0.90–1.31); oval to obovate shape in lateral view; densely wrinkled surface; dorsal suture conspicuous	P. atlantica
c. Oval to orbicular shape in lateral view; generally smooth surface; dorsal suture not visible	P. khinjuk
3 a. Endocarp always longer than broad (length/width ratio: 1.28–1.88); very large endocarp (length: 9.91–19.15 mm; width: 6.54–11.65 mm; thickness: 5.88–10.14 mm); oblique pedicel base in lateral view; marked and slightly curved dorsal suture; dense, wrinkled surface	P. vera
b. Mucronate apex; hull-shaped ventral suture; broad form in lateral view (length/width ratio 0.67–1.15; asymmetrical endocarp (presence of bumps on the surface)	P. lentiscus
c. Symmetrical endocarp; oval to orbicular shape in lateral view, smooth surface	P. terebinthus

#### Blind test of the identification key

In order to evaluate the reliability of the morphological and morphometric criteria and the identification key, an additional experiment (blind test) was set up. 40 modern endocarps including all the six *Pistacia* spp. concerned were randomly selected from the modern reference collection. They were then randomly picked from a pot and given a serial number (1–40). Five persons, all with reasonable experience in seed/fruit identification (two postdoctoral researchers and three PhD candidates in archaeobotany), but with little or no previous experience in *Pistacia* identification, participated in the blind-test. They were asked to identify independently the series of modern specimens by using the identification criteria and identification key. Their determinations were then compared to the known taxonomic identification.

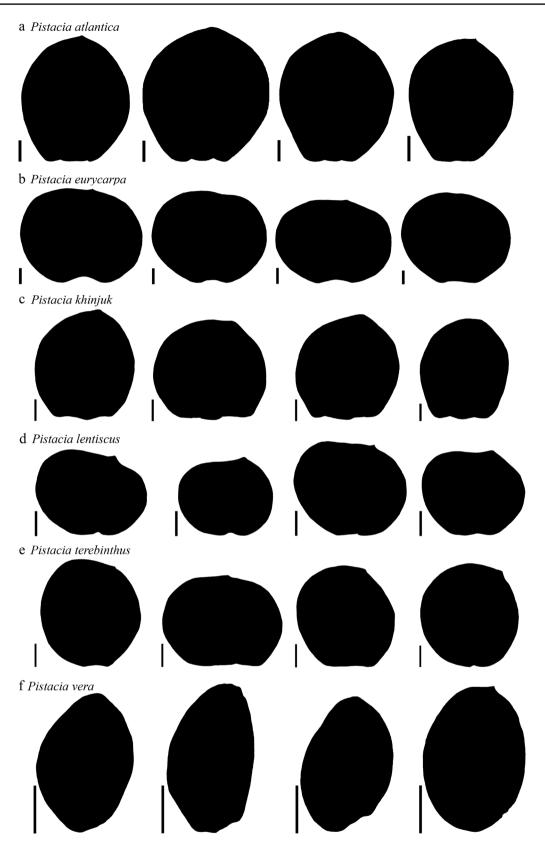
In some cases, the persons doing the blind test hesitated between two species (for example *P. khinjuk/terebinthus*) but only accurate attributions to a single species were considered as a correct answer.

The degree of success was calculated according to the number of correct attributions. A Shapiro-Wilks normality

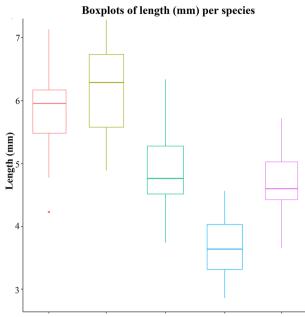
test (function shapiro.test;  $\alpha = 0.05$ ) (R core team 2018) followed by a Spearman's rank correlation (function cor) was calculated to evaluate the statistical dependence of the experience (in years of practicing archaeobotany) of the participants and the number of correctly attributed identifications (ESM 5).

The general degree of success of the blind test was of 90% with individual results varying from 87.5 to 92% of correct attributions. 7% of the attributions corresponded to difficulties in distinguishing between *P. khinjuk* and *P. terebinthus*. Three specimens in particular (3, 21, 37) appeared to be problematic (ESM 5). The possible overlap already observed between these two species was thus confirmed and should be kept in mind while working on sites where the two may have grown sympatrically (e.g. in the Levantine region).

The remaining 3% of the attributions correspond to inconsistent confusions among other species (ESM 5), that might be related to various factors, such as the time dedicated to observation or overall experience in seed and fruit analysis. Indeed, the number of correctly attributed identifications showed to be strongly correlated to the number of years of experience (Spearman's rho = 0.7254763; ESM 5).



**Fig. 5** Most typical shapes attested in lateral view among **a** *P. atlantica*, **b** *P. eurycarpa*, **c** *P. khinjuk*, **d** *P. lentiscus*, **e** *P. terebinthus*, and **f** *P. vera* endocarps (Drawings generated by M. Rousou); scale bars = 1 mm and 5 mm (*P. vera*)



P. atlantica P. eurycarpa P. khinjuk P. lentiscus P. terebinthus

Fig. 6 Boxplots of length measurements of wild *Pistacia* spp. (*P. atlantica, P. eurycarpa, P. khinjuk, P. lentiscus, P. terebinthus*) (for details see ESM 3)

# Application of the identification key to the *Pistacia* remains from Khirokitia-*Vounoi*

In order to illustrate the benefits of identifying *Pistacia* fruit remains to the species level, we have applied the results of the morphological study and the identification key to *Pistacia* fruit remains from Neolithic Khirokitia-*Vounoi* in Cyprus. The site is located on a hillside surrounded by a meander of the Maroni river approximately 6 km from the present southern coastline (Fig. 10).

Khirokitia was first discovered and excavated between 1936 and 1946 by P. Dikaios (Department of Antiquities of the Republic of Cyprus), then in 1972 by D. Christou and N. P. Stanley-Price (Department of Antiquities of the Republic of Cyprus), and from 1976 to 2009 by the French Archaeological Mission, conducted by A. Le Brun and O. Daune-Le Brun.

More than 40 years of excavations have revealed 11 successive architectural phases of a large village composed of circular stone-built or stone-and-mudbrick buildings organised into two sectors (eastern and western) and surrounded by an enclosure wall (Le Brun 1984, 1989, 1994). A specific Late Aceramic Neolithic culture—the so-called *Khirokitia culture*—developed at the site from the early seventh to mid-sixth millennia BC and was characterised by the apparent interruption of contacts with the continent and the appearance of specific insular traits in the material culture and social organisation. In the mid-sixth millennium cal BC

the village was abandoned and after a short hiatus it was re-occupied by a Ceramic Neolithic settlement dated to the fifth millennium cal BC. Khirokitia remains hitherto exceptional for Cypriot prehistory, in terms of preservation and site dimensions, and was inscribed on the UNESCO World Heritage list in 1998.

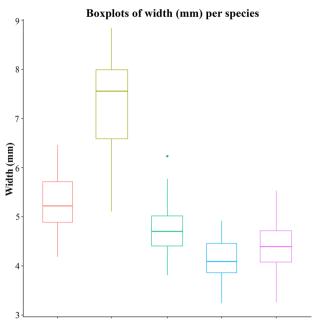
During the French excavations (1976–2009) soil samples were systematically collected and charred plant remains were extracted by manual flotation using a small-size mesh (0.25 mm). A large body of remains was thus obtained and studied successively by several archaeobotanists (Miller 1984; Hansen 1989, 1994) with the most recent and comprehensive study having been conducted as part of a PhD thesis (Parés 2015). In this work the high occurrence of *Pistacia* remains (present in 56.5% of the samples) and their predominance (93%) among the fruit remains in general was confirmed (Parés and Tengberg 2017).

*Pistacia* spp. endocarps were concentrated in outdoor spaces within the village including two exterior pits (13133, 13139) and one floor (10868). These finds have provided the major part of the individuals used in our study (Table 4). The spatial analysis of lithic artefacts, faunal and botanical remains show similarities in composition and differences in density between interior and exterior areas suggesting that the latter were used for various activities and received refuse from interiors and hearths (Le Brun 1984, 1994; Astruc 2002; Legrand 2007).

The endocarps recovered from Khirokitia were well preserved and fragmentation is low. This is in contrast to many other prehistoric sites in the Near East where endocarps are often highly fragmented probably as a result of the crushing of the fruits in order to extract oil from the seeds (Willcox 2016). Moreover, several endocarps, that are not included in the present study, still preserve the remains of the mesocarp or show traces of insects (*Eurytoma plotnikovi, Megastigmus pistaciae*) that develop inside the endocarp and consume the seed (Braham et al. 2010; Parés 2015).

Among the endocarps, at least two different morphotypes were recognised during previous studies but not identified to species level (Parés 2015). 113 endocarps from different levels and contexts were sufficiently well preserved to allow the observation of diagnostic features (Table 4). These were examined under a binocular stereomicroscope (Nikon SMZ 1270, magnifications  $6.3\times-80\times$ ) and linear measurements (length, width and thickness) were taken (NIS-Elements Imaging software; ESM 6).

By using the key based on the criteria described above, three species could be identified at Khirokitia (Fig. 11a–c). Almost 30% of the endocarps were attributed to *P. atlantica* according to several diagnostic features: conspicuous limits of the pedicel base, deep pedicel cavity, elongated endocarp in lateral view, oval to slightly obovate shape in lateral view, wrinkled surface (Fig. 11a). One endocarp was identified



P. atlantica P. eurycarpa P. khinjuk P. lentiscus P. terebinthus

Fig. 7 Boxplots of width measurements of wild *Pistacia* spp. (*P. atlantica, P. eurycarpa, P. khinjuk, P. lentiscus, P. terebinthus*) (for details see ESM 3)

as *P. lentiscus* on the basis of its small size, broad form in lateral view as well as the presence of a mucronate apex (Fig. 11b). Finally, the morphology of a little more than 70% of the endocarps corresponded to that of *P. terebinthus* with an oval to orbicular shape in lateral view, indistinct limits of the pedicel base, a narrow and convex pedicel base and a smooth surface (Fig. 11c).

Besides the morphological criteria used to distinguish *P. terebinthus* from *P. khinjuk*, which may in some cases overlap, biogeographic and ecological data further reinforces the identification of *P. terebinthus* at Khirokitia. Indeed, only the Mediterranean *P. terebinthus* is part of the indigenous Cypriot flora where it often grows in association with *Quercus coccifera* (Zohary 1973; Meikle 1977). *Pistacia khinjuk* is an Irano-Turanian species growing at higher altitudes (1,500–1,800 m above sea level) in the Kurdo-Zagrosian sector (Yaltirik 1967; Rechinger 1969; Zohary 1973).

*P. terebinthus* is the most frequently encountered species (present in 6 samples out of 9) and the one represented by the highest number of remains (82 endocarps) in the Khirokitia archaeobotanical assemblage. *P. atlantica* is also well represented (present in 4 samples, 30 endocarps in total) while *P. lentiscus* appears only once in our samples, in one of the later levels. It seems premature though to draw any conclusions in terms of ecology and use of the different species or their variation through time from these first results.

The results of the identification show us nevertheless that the three species were indeed present on Cyprus during

Boxplots of thickness (mm) per species

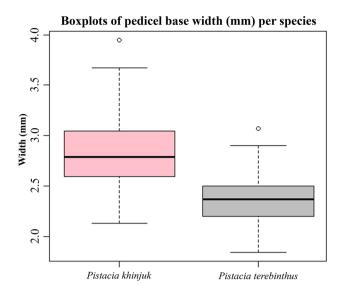
P. atlantica P. eurycarpa P. khinjuk P. lentiscus P. terebinthus

Fig. 8 Boxplots of thickness measurements of wild *Pistacia* spp. (*P. atlantica, P. eurycarpa, P. khinjuk, P. lentiscus, P. terebinthus*) (for details see ESM 3)

the Late Aceramic Neolithic and that *P. terebinthus* and *P. atlantica* had been available and used at least since the early seventh millennium BC.

Today four species and one hybrid of Pistacia are growing on the island (Meikle 1977). P. atlantica (0-1,500 m a.s.l.) is currently common in abandoned fields, along field margins and on rocky slopes, while several trees and tree clusters have been documented in the regions of Akamas, Paphos, Limassol and Kiti. P. terebinthus is a very common species (0–1,500 m a.s.l.) growing on rocky slopes, in pine forests and in maquis formations. P. lentiscus is the most thermophilous species (0-800 m a.s.l.) attested on rocky slopes, among pine forest and on sand dunes (Tsintides et al. 2002). A single  $P \times saportae$  plant has been documented in Paphos but hybrids between P. lentiscus and P. terebinthus may be more common in localities where they grow sympatrically (Tsintides et al. 2002). Finally, P. vera (0-700 m above sea level) was introduced in Cyprus for fruit production (Meikle 1977).

*P. terebinthus, P. lentiscus* and *P. atlantica* all appear to have grown in the surroundings of Khirokitia and to have been exploited during the Late Aceramic Neolithic. The three species are still found in the vicinity of the site, with *P. atlantica* trees being less well represented than the two others. Different uses of these three species are possible. Their fruits can be consumed, and oil can be extracted from the kernels of *P. lentiscus* and *P. atlantica* (Lanfranchi et al. 1999; Loi 2013). The resins obtained by making cuts in the



**Fig.9** Boxplots of pedicel base width measurements of *P. khinjuk* and *P. terebinthus* (for details see ESM 3)

trunks of the turpentine and mastic trees, and also *P. atlantica*, could have had medical, ritual or other uses (Axiotis et al. 2018). The absence of evidence for the systematic crushing of fruits for oil extraction together with a correlation between the presence of endocarps, pedicel fragments and charcoal fragments belonging to *Pistacia* spp. suggest that at least part of the remains result from the use of the wood as fuel (Parés 2015; Parés and Tengberg 2017). In that case fruits and other parts of the infructescence would have become carbonised as a result of the burning of fruitbearing branches. A similar explanation has been advanced for the simultaneous presence of *Pistacia* wood and fruits at the Neolithic site of Kissonerga-*Mylouthkia* (Murray 2003).

The anthracological analysis conducted at Khirokitia has revealed the presence of at least two distinct anatomical types of *Pistacia* wood. A first study identified one of these as lentisk (*P. cf. lentiscus*) (Thiébault 2003) and an ongoing

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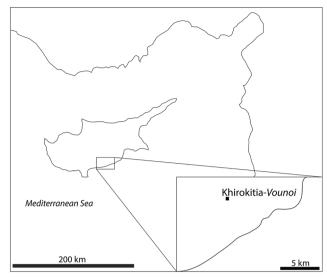


Fig. 10 Location of the Late Aceramic Neolithic site Khirokitia-Vounoi (Fig. generated by M. Rousou)

study (PhD of Maria Rousou) will bring further precision to the determination of *Pistacia* wood from the site.

# **Conclusions and perspectives**

The study of morphological and traditional morphometric criteria of a large number of modern individuals has allowed us to explore the inter- and intra-specific variability of six *Pistacia* spp. Our observations on the modern uncharred as well as on the modern experimentally charred endocarps, have led to the recognition of the most pertinent characteristics and to the creation of an identification key to determine *Pistacia* endocarps to species level, applicable to both modern and archaeobotanical material. According to the identification key, all of the studied *Pistacia* spp. can be distinguished by morphological criteria, including linear measurements and their ratios.

**Table 4** Identifications of the *Pistacia* fruit remains recovered at Khirokitia-Vounoi according to the identification key established by the present study (Abbreviations: *E.* eastern; *W.* western)

Sector	W	W	W	Е	Е	Е	E	Е	Е	Total	Percentage (%)
Stratigr. level	Ic	II	IIIb	A2	A2	A2	D1	D1	E3		
Sample	9196	10868	11674	12436	12405	11909	13139	13133	9122		
P. atlantica		3		1	1		25			30	26.55
P. lentiscus						1				1	0.88
P. terebinthus	1	47	5				13	15	1	82	72.57
Total	1	50	5	1	1	1	38	15	1	113	
Percentage (%)	0.88	44.25	4.42	0.88	0.88	0.88	33.63	13.27	0.88	100	100

E eastern, W western

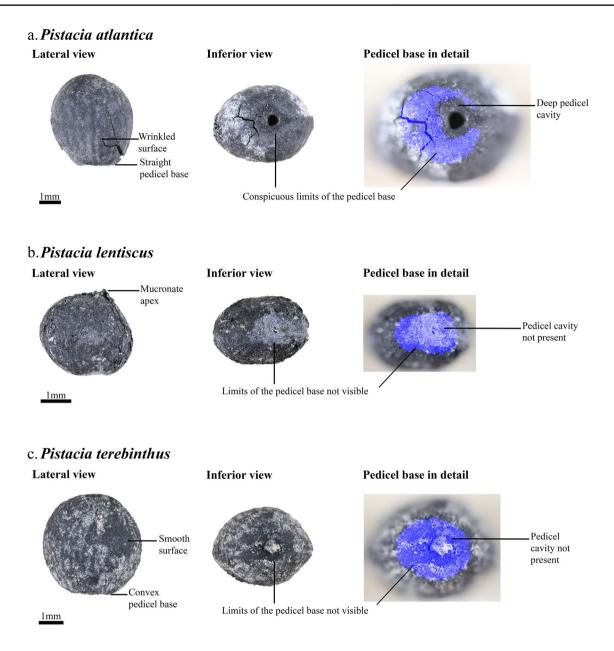


Fig. 11 Photographs of a *P. atlantica* (sample 10868), b *P. lentiscus* (sample 11909) and c *P. terebinthus* (sample 13139) charred endocarps from Khirokitia-*Vounoi* in lateral and inferior views. Detail of

the pedicel base showing (in blue) the parts of the endocarps at the same point of focus

The usefulness of morphological criteria for the identification of *Pistacia* fruit remains has been further demonstrated by the blind test. This test also permitted the drawing of our attention once more to the only major difficulty that may appear: the distinction between *P. terebinthus* and *P. khinjuk* that sometimes show overlapping characteristics. However they have different distribution patterns and ecological requirements, with *P. terebinthus* having mainly Mediterranean affinities while *P. khinjuk* is characteristic of the Irano-Turanian flora with a more oriental distribution pattern (Zohary 1973). Nevertheless, in regions where these species may grow sympatrically, we encourage archaeobotanists to be cautious and not push identifications beyond *P. terebinthus/P. khinjuk* until further criteria have been established.

Parallel to the morphological study a protocol for the acquisition of data for geometric morphometrics (photographs and landmarks) was established and applied in a preliminary study. While a standard approach combining morphology with linear measurements, as applied in this paper, proved to be efficient for the identification of endocarps, geometric morphometrics can be useful in specific cases. Thus, we intend to develop this approach notably for the distinction between *P. terebinthus* and *P. khinjuk*.

A more comprehensive experimental study needs to be developed with the testing of more parameters, in order to evaluate the impact of carbonisation on the size and shape of endocarps. This can be done by including a larger number of individuals from all *Pistacia* spp. and considering variables such as the temperature and duration of carbonisation and the presence/absence of the fleshy parts of the fruit. If the presence of endocarps in the archaeobotanical assemblages results from the use of wood and fruit-bearing branches as fuel, fruits were probably carbonised intact. On the other hand, as demonstrated by ethnobotanical studies, the endocarp of wild fruits is not necessarily removed prior to consumption (Della et al. 2006; Hadjichambis et al. 2008; Ahmed 2017).

In addition to research concerning charred fruits, a thorough wood anatomical study of *Pistacia* spp. would be useful for our understanding of the past distribution and exploitation of a major plant resource. Wood anatomical characteristics, such as ring porosity, the arrangement of pores in early wood, presence/absence of year rings and ray width, may be useful for distinguishing several species or groups of species, such as *P. atlantica/terebinthus* and *P. lentiscus* (Grundwag and Werker 1976; Schweingruber 1987; Neumann et al. 2001).

Finally, future research will develop the identification key to encompass *Pistacia* spp. not investigated in this study. It could also include evaluation of the role of hybrid forms in archaeobotanical assemblages by including modern specimens of known hybrids and explore possible changes in endocarp morphology according to the degree of ripening of fruits.

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