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Geometric morphometric analysis of grain shape and the identification of two-rowed barley (*Hordeum vulgare* subsp. *distichum* L.) in southern France

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ABSTRACT

Hulled barley is one of the most frequently recovered cereals in European archaeological sites from Roman and medieval periods. In southern France this cereal is common in carbonized contexts such as cultural layers, ditches, pits, hearths, etc. The distinction between the two subspecies, two-rowed (*Hordeum vulgare* subsp. *distichum* L.) and six-rowed barley (*H. vulgare* subsp. *vulgare* L.) is usually based on morphological characters. The following criteria can be used to discriminate both subspecies from archaeological remains: the number of fertile spikelets per rachis segments, the linear or horseshoe shape depression of the lemma base, the maximum width of the caryopses and the proportion of twisted grains. The recovery of thousands of caryopses, some clearly twisted, and of rachis segments with sterile spikelets from the site of Petit Clos (Perpignan, Pyrénées-Orientales, France) dating to the Roman period suggests that both subspecies were cultivated during this time in southern Gaul. However evidence for two-rowed barley is usually scarce in archaeobotanical reports from Roman and medieval sites. To confirm the presence of two-rowed barley in the carbonized assemblage from Petit Clos and its cultivation, we developed a new method for analysing caryopses shape using geometric morphometrics with landmarks and sliding semi-landmarks. We compared modern reference specimens to the archaeological grains from several excavations from southern France dating from the 1st to the 11th century AD. Several varieties of both subspecies were correctly identified in the modern reference sample using GMM, both before and after carbonization. Archaeological specimens could then be accurately identified. The results confirm that both subspecies of barley were cultivated in southern France during the Roman period.

1. Introduction

Barley (*Hordeum vulgare* L.) has been a staple food source for large portions of the world population since the advent of agriculture. Nowadays hundreds of varieties are known, grouped together as one species, *H. vulgare* (Zohary et al., 2012) comprising of two subspecies, *H. vulgare* susbp. *distichum* L.(two-rowed barley) and *H. vulgare* subsp. *hexastichum* L. (six-rowed barley). The domestication of two-rowed barley (*H. vulgare* subsp. *spontaneum* (C. Koch) Thell.) occurred between 9000 and 8000 cal BC, during the PPNB, in a region between Israel and Syria (Fuller et al., 2012; Tanno and Willcox, 2012; Zohary et al., 2012). Six-rowed forms appeared soon after during the 8th millennia cal BC, a period that shows evidence of increasing intensification of agriculture and domestication (Zohary et al., 2012). Cultivated forms could have been spread from this area by human migration to the Near East, Middle East and Europe (Colledge and Conolly, 2007a). Two-rowed and six-rowed barley are both frequently found on the same sites in Greece from the Neolithic to the Bronze Age (Sarpaki, 1992). Two-rowed barley has also been identified in Neolithic sites in southern Italy (Constantini and Stancanelli, 1994), in three Roman sites in southern France, Le Villard (Puy-de-Dôme, 2nd c. A.D., Bouby, 2001), Bourbousson III (Drôme, 3rd c. A.D., Bouby, 2001) and Petit Clos (Pyrénées-Orientales, 1st c. A.D., Ros, 2010) and is suspected in an early medieval site in the same area of southern France, Manresa (Pyrénées-Orientales, 7th c. A.D., Ruas, 2007 unpublished).

Several criteria are used to discriminate the subspecies in archaeological remains (literature compiled by Jacomet, 2006)

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based on external morphological features. Two-rowed barley is characterized by a single fertile spikelet per rachis segment (the two others being sterile), a horseshoe-shaped depression in the lemma base, and straight shaped grains whose maximum width is below the centre of the grain. By contrast, six-rowed barley develops three fertile spikelets per rachis segment, the depression in lemma base can be either linear or horseshoe shaped and the maximum width of all grains is at the centre.

Another important feature to consider is the straightness of the grain, although this criteria should be used with caution. At each rachis node of six-rowed barley the grains on lateral spikelets are usually twisted while the central grain is straight. The expected ratio of twisted versus straight grains in an assemblage is therefore two to one (literature compiled by Jacomet, 2006). However a small proportion of slightly twisted grains may be present in two-rowed barley, as observed on fresh material (Bouby, 2001). The presence of twisted lateral grains in six-rowed barley is probably due to the presence of three spikelets per rachis segment but a similar reason cannot account for the presence of twisted grains in two-rowed barley and no alternative explanation has been proposed to date.

While these criteria to distinguish the two barley subspecies have been extensively used, most archaeobotanists have expressed reservation towards their use and prefer using "hulled barley" to refer to either subspecies. In the Roman site of Petit Clos, numerous charred hulled barley caryopses were found ($N = 3041$) with only a few chaff remains (lemma bases ($N = 91$) and rachis segment ($N = 6$) (Ros, 2010)). Based on lemma bases and rachis segment criteria, both two- and six-rowed barley were identified. Nonetheless the very small proportion of twisted grains (2%, $N = 61$) favoured the hypothesis of the predominant presence of two-rowed barley. As traditional morphological criteria cannot reliably identify the caryopses, other criteria were explored.

Alternative criteria used to discriminate the caryopses are univariate measurement such as their length, width, and thickness. These were first tested at the Roman site of Bourbousson III (Bouby, 2001) and archaeological charred two- and six-rowed barley grains were both successfully identified and discriminated, confirming potential biometric differences between the two subspecies (Bouby, 2001). In addition the central and lateral grains in six-rowed barley have shape differences, the latter being often narrower than the former. Therefore, six-rowed barley should display greater variation in grain measurements than the two-rowed subspecies that only have central grains. The grains sampled from Bourbousson III and Petit Clos were found to be biometrically similar, indicating the predominant presence of two-rowed barley grains at Petit Clos. Further comparisons with the site of Castlar (Tarn, France, Ruas, 2002) where only six-rowed barley was identified show a large difference in the biometrics of seeds between the two sites (Ros, 2010). The large differences between the six-rowed grains of Castlar and the unidentified caryopses of Petit Clos provide more evidence that the grains of Petit Clos were mainly composed of two-rowed barley (Ros, 2010). Two-rowed barley was therefore most likely present in southern France during the Roman period. Its persistence in this region into the Middle Ages still remains to be investigated.

The comparison of grains measurement is shown to be a useful approach. Nonetheless, this method does not allow identification of individual caryopses. To address this, a precise quantification of the proportion of subspecies in archaeobotanical samples is required. We developed a new method of analysing the shape of caryopses using Geometric morphometrics (GMM). GMM (Adams et al., 2004; Rohlf and Marcus, 1993) allows a more precise quantification of shape than traditional morphometric approaches.

The application of these methods on archaeobotanical remains is increasing, especially on fruits such as olive (Terral et al., 2004), grape (Bouby et al., 2005–2006; Terral et al., 2010), cherry (Burger et al., 2011) and dates (Terral et al., 2012). Nonetheless, these methods have seldom been applied to cereal remains (Apuan et al., 2011) and to our knowledge never to archaeological cereal remains. We chose to apply GMM to archaeological caryopses, as compared to other archaeological cereal remains, grains have the greatest rate of survival (Boardman and Jones, 1990) and are the most common remains in European post-Neolithic archaeological sites (Jacomet and Kreuz, 1999; Colledge and Conolly, 2007b).

We used ten modern cultivated varieties belonging unambiguously to the two subspecies in order to determine if modern caryopses of two-rowed and six-rowed barley can be identified using GMM. This modern reference data was then used to identify archaeological caryopses. Since carbonization can have a strong effect on grain shape (Boardman and Jones, 1990), the fresh referential was analysed before and after experimental carbonization of the dehulled grains (without the lemma and palea).

The archaeological material comes from the sites of Petit Clos and Bourbousson III where the presence of two-rowed barley has been claimed in previous studies (Bouby, 2001; Ros, 2010). To explore the possibility of a continuous presence of two-rowed barley after the Roman period, two Mediterranean medieval sites were also included: Manresa (Pyrénées-Orientales, 7th A.D., Ruas, 2011) and Dassargues (Hérault, 10th A.D., Ros, 2009). The results obtained will provide important information on the role and status of the two barleys during Roman and medieval periods in southern France and show the potential for using GMM in cereal grains identification.

2. Material: archaeological and reference

2.1. Modern reference material

A total of 300 modern dehulled caryopses were analysed. The modern reference sample included five varieties of two-rowed barley (Clarine, Mascara, Mystic, Nectaria, Pastoral) and five varieties of dense-eared six-rowed barley (Actuel, Atenon, Cartel, Esterel, Marcorel) (Table 1). Isolated spikelets were obtained from the Secobra society and grown in Maule's fields (Yvelines, France). For each of the 10 varieties, 30 grains were randomly selected regardless of their central or lateral position on six-rowed barley grains, dehulled and analysed.

The hulls were removed to permit measurement of the grains. After being photographed the 300 caryopses were carbonized in a muffle furnace oven (Nabertherm) to simulate as accurately as possible the effect of carbonization. The grains were charred in reducing conditions, separately wrapped in aluminium and buried in the sand at 250° Celsius for 50 min. This temperature was

Table 1
Hordeum subspecies, varietal denomination and number of modern caryopses analysed as reference material.

Taxa	Cultivated varieties	Number of caryopses
H. vulgare subsp. *distichum* (two-rowed)	Clarine	30
	Mascara	30
	Mystic	30
	Nectaria	30
	Pastoral	30
H. vulgare subsp. *hexastichum* (six-rowed)	Actuel	30
	Atenon	30
	Cartel	30
	Esterel	30
	Marcorel	30

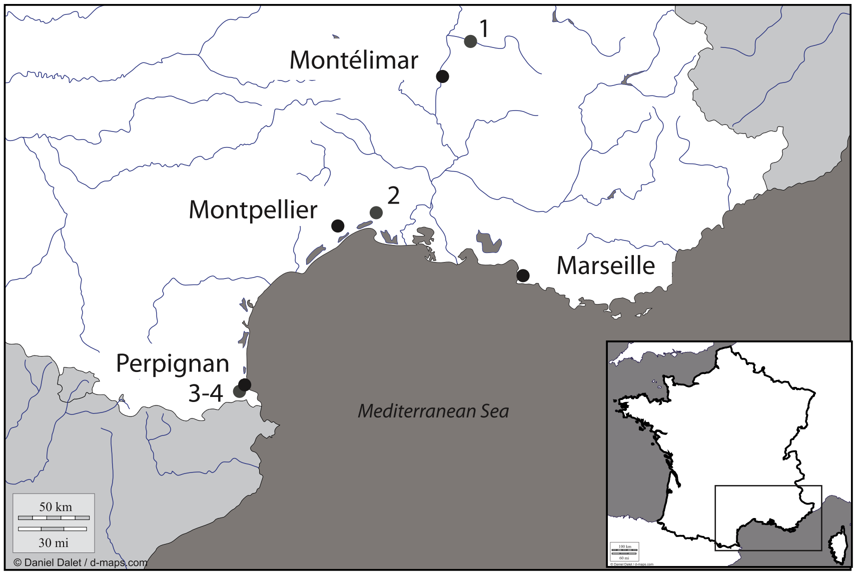

Fig. 1. Geographical location of the studied archaeological sites (1 – Bourbousson III; 2 – Dassargues; 3 – Petit Clos; 4 – Manresa).

selected as it is the lowest temperature at which charring occurs within a practical length of time (Bowman, 1966).

2.2. Archaeological material

330 archaeological specimens were analysed, composed of 100 grains from each of three sites (Petit Clos, Bourbousson III and Dassargues) and 30 grains from a fourth site (Manresa) (Fig. 1, Table 2).

The site of Petit Clos was a great Roman villa in which residues of carbonized storage were discovered and provided numerous seed remains ($N = 4346$) (Kotarba, 1999). The majority of these seeds has been identified as hulled barley (84%) as well as a few remains of naked wheat (*Triticum aestivum*/*durum*/*turgidum*, 1%), fruits (*Vitis vinifera*, *Prunus dulcis*, *Juglans regia*, 5%) and weeds (10%) (Ros, 2010). Both two- and six-rowed barley were identified using lemma bases and rachis segment. The grains are mainly straight and the very small proportion of twisted grains (2%, $N = 61$) favours the hypothesis of the predominant presence of two-rowed barley.

The site of Bourbousson III is a Roman settlement in which two buildings have been excavated. In one of these buildings burned cereal stores were discovered in primary position (Bouby, 2001). The first (containing 96,049 remains) is mainly composed of hulled barley grains (89%) with fewer remains of naked wheat (8%) and weeds (3%) (Bouby, 2001). The grains were stored separately from large amounts of naked wheat grains found in the same building, probably in a wooden trunk. In this site, two-rowed barley identification was based on grains shape and size. No remains of chaff are mentioned.

Dassargues is a rural medieval settlement, characterized by several buildings and a large storage area (silos) (Raynaud et al., 1995). In one of the silos many seed remains ($N = 20{,}665$) were recorded: cereals (99,2%), composed of hulled barley and naked wheat in equal proportion, accompanied by weeds (0.8%) (Ros, 2009). In this site, only six-rowed barley was identified in past studies, by a fragment of ear with central and lateral spikelets still in connections with the rachis. The preservation of the remains of chaff does not allow their subspecies identification.

The site of Manresa contained a large storage area (silos) and a possible pen (Kotarba et al., 2011). In one of the silos a secondary deposit was found ($N = 7499$), composed of wild Poaceae (63%), grains and chaff of cereals (30%) and weeds (7%). This deposit could represent litter remains or processing by-products. Cereals found in this site were mainly composed of hulled barley (13%) (Ruas, 2007 unpublished) but chaff and grain preservation prevented any clear identification using traditional methods, even though the straightness of the caryopses suggested the presence of two-rowed barley. Because of the poor preservation of the grains, the GMM approach could only be applied to thirty specimens.

3. Methods

3.1. Morphometrics

Each grain was first photographed in two dimensions in ventral view, using a stereoscopic binocular microscope (Leica DFC420). Grain shape was assessed by digitizing three landmarks and thirty-eight sliding semi-landmark coordinates on the digital images

Table 2
Archaeological material analysed.

Site name, town (department), context	Number of caryopses	Dating century A.D.	Excavation supervisor	Reference
Petit Clos, Perpignan (Pyrénées-Orientales), ditch-pit	100	1st	J. Kotarba	Ros, 2010
Bourbousson III, Crest (Drôme), storage	100	3rd	V. Bastard	Bouby, 2001
Manresa, Canohès (Pyrénées-Orientales), ditch-pit	30	7th	C. Jandot/J. Kotarba/A. Vignaud	Ruas, 2011
Dassargues, Lunel (Hérault), pit	100	10th-11th	Cl. Raynaud	Ros, 2009

using TpsDig2 v2.16 (Rohlf, 2010a) (Fig. 2-1, 2). In order to capture the specific shape of barley grains (spindle-shaped body, tapering at each end, with a shallow furrow running along the ventral side), two landmarks were positioned at the apical part of the grain, while a third one marked the basal end of the furrow. 32 sliding semi-landmarks were placed at equal distances along the outline of the grains (Fig. 2), 15 on each side of the caryopsis and 2 between the two apical landmarks.

Landmark coordinates were superimposed using a Generalized Procrustes Analysis (GPA) (Goodall, 1995; Rohlf and Slice, 1990) and the semi-landmarks were allowed to slide along the chord drawn between adjacent points to minimize the sum of Procrustes distances between each individual and the mean shape (Perez et al., 2006; Sampson et al., 1996; Sheets et al., 2004; Zelditch et al., 2004). The GPA provides shape data (new coordinates after superimposition) for each grain. The superimposition using sliding semi-landmarks was performed in TPS Relw v1.49 (Rohlf, 2010b).

3.2. Statistical analyses

3.2.1. Modern reference material

Differences in shape between varieties and subspecies were tested using one-way MANOVA, with shape as the dependent variable and the variety or subspecies as a factor. Canonical Variate Analyses (CVAs) were also used, coupled with leave-one-out cross validation percentage (CVP) that measures the accuracy of the CVA to correctly assigned unidentified specimens to the subspecies or variety level. Owing to the relatively small sample size and the large number of variables, dimensionality reduction methods were used (Baylac and Friess, 2005; Evin et al., 2013). To visualize differences in shape, deformations along the factorial axes were calculated by multivariate regressions (Monteiro, 1999). We analysed the effect of carbonization on shape using MANOVAs (testing for differences between carbonized versus non-carbonized grains) and CVAs for each subspecies separately. MANOVA results were provided as follows: degree of Freedom of the effect (Df), the *F*-statistic with the numerator and denominator degrees of freedom in lower index ($F_{\mathrm{numDf,denDf}}$), and the *p* value (*p*) of the test. The homogeneity of the carbonization effect among subspecies was tested using a two-way MANOVA with shape as the variable, subspecies as the main classifier and carbonized versus non-carbonized as a subclassifier. The overall phenotypic similarities between varieties were depicted using a Neighbour-joining network computed from the Mahalanobis's D2 distances.

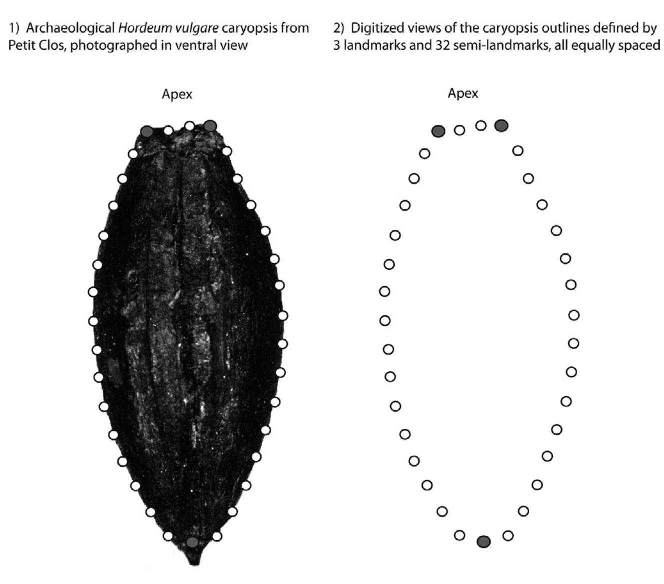

Fig. 2. Position of landmarks (in grey) and sliding semi-landmarks (in white) used to quantify the morphology of the grains: 1 – Archaeological charred *Hordeum vulgare* caryopsis from Petit Clos, photographed in ventral view, 2 – Landmarks and sliding semi-landmarks configuration.

3.2.2. Identification of the archaeological grains

The quality of preservation of the archaeological caryopses was quite heterogeneous, due to different degrees of carbonization. Thus, archaeological specimens which were the most affected by carbonization more closely resemble the experimentally carbonized caryopses, while better preserved archaeological caryopses were more similar to fresh modern caryopses. For this reason, and to the fact that we could not quantify the degree of carbonization of the caryopses, archaeological specimens were assigned to one of the two subspecies using a predictive discriminant analysis based on combined datasets including the modern caryopses before and after carbonization. The level of confidence in a predictive discriminant analysis is estimated by *a posteriori* probabilities of classification (50%–100% in the case of two groups). To assess the confidence of the identification, we compared the proportion of caryopses assigned to each form using 50% and 95% thresholds. Morphometric and statistical analyses were performed in R v2.13.1 (R Development Core Team, 2011) using "Rmorph" (Baylac, 2012), "ape" (Paradis et al., 2004).

4. Results

4.1. Variability of subspecies and of varieties

The two subspecies, *H. vulgare* subsp. *hexastichum* and *H. vulgare* subsp. *distichum*, differed significantly in grain shape (Df = 1, $F_{9, 290} = 60.104$, $p < 2.2\text{e-}16$). The six rowed barley has proportionally more rounded grains than the two-rowed (Fig. 3A). The specimens can be correctly assigned to the proper subspecies with a probability of 91%.

Altogether the ten varieties exhibit significant differences in shape (Df = 9, $F_{252, 2439} = 4.1689$, $p < 2.2\text{e-}16$). The cross-validation procedure correctly assigned only 53.7% of the grains to the correct variety. According to the neighbour-joining network, the varieties are grouped together within the two subspecies (Fig. 3B). The analyses reveal significant differences within each of the two subspecies, among the two-rowed barley varieties (Df = 4,

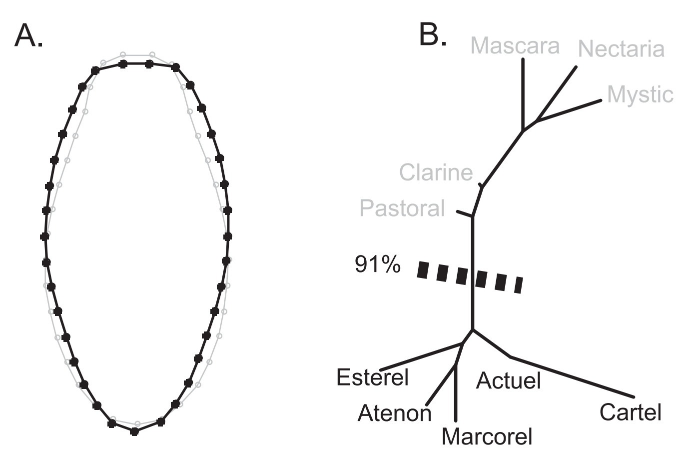

Fig. 3. A – Shape differences between the two-rowed (grey) and six-rowed (black) barley subspecies, B – Neighbour-joining network of the Mahalanobis distances, with mention of the cross-validation percentage (91%) among the subspecies.

$F80, 516 = 4.3528$, $p < 2.2e\text{-}16$) and among the six-rowed barley varieties (Df = 4, $F72, 524 = 4.4018$, $p < 2.2e\text{-}16$).

4.2. Effects of carbonization

The carbonization had an effect on both two-rowed (Df = 1, $F24, 295 = 154.87$, $p < 2.2e\text{-}16$) and six-rowed (Df = 1, $F5, 294 = 261.61$, $p < 2.2e\text{-}16$) grain shape, and did not appear to be homogeneous between subspecies (interaction term of the MANOVA: Df = 1, $F26, 571 = 13.823$, $p < 2.2e\text{-}16$). Despite significant differences in the effect of carbonization, the shape differences between non-carbonized and carbonized caryopses appeared to be overall relatively similar within each subspecies (Fig. 4). After carbonization, caryopses show a proportionally more rounded shape and are proportionally shorter and wider (Fig. 4). Differences in carbonization between subspecies can be noticed: in some six-rowed barley grains, the twisted furrow became straighter or even completely straight because of the distension of the grain. On the contrary, no twisting was observed on two-rowed barley caryopses once carbonized.

The shape of carbonized grains differed between the two subspecies (Df = 1, $F14, 285 = 22.975$, $p < 2.2e\text{-}16$) and 86% of the specimens could be correctly assigned to the subspecies level. When carbonized and non-carbonized grains are pooled, 87% of the specimens can be correctly assigned.

Whereas the first axis of the CVA (75.38% of total variance) tends to separate carbonized versus non carbonized specimens, the second axis (23.88% of variance) tends to separate two- from six-rowed barley (Fig. 5).

4.3. Identification of the archaeological grains

Archaeological grains were identified using modern specimens, including fresh and charred remains (Figs. 6 and 7). Considering the identification of all the specimens without taking into account the *a posteriori* probabilities of identification, both subspecies were identified at all four sites in different proportions (Fig. 7). Two-rowed barley was the main cereal in only one site (Bourbousson III), reaching 53% of the total number of the studied grains. In the other three sites (Petit Clos, Manresa, Dassargues) it barely reaches

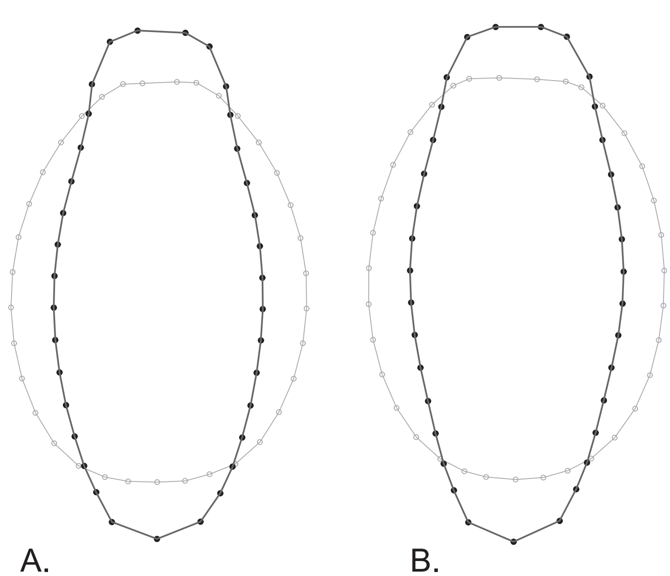

Fig. 4. Shape differences between the two-rowed (A) and six-rowed (B) barley subspecies, before (black) and after (grey) carbonization.

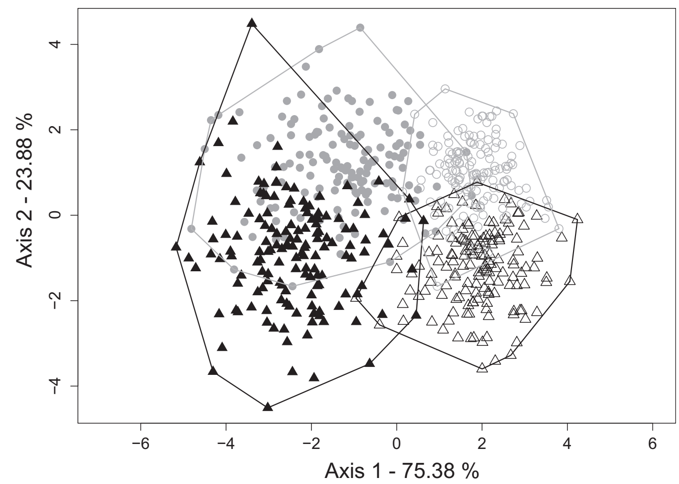

Fig. 5. Two first axes of the Canonical discriminant analysis performed on the shape of two-rowed (grey) and six-rowed (black) barley subspecies, before (open symbols) and after carbonization (solid symbols).

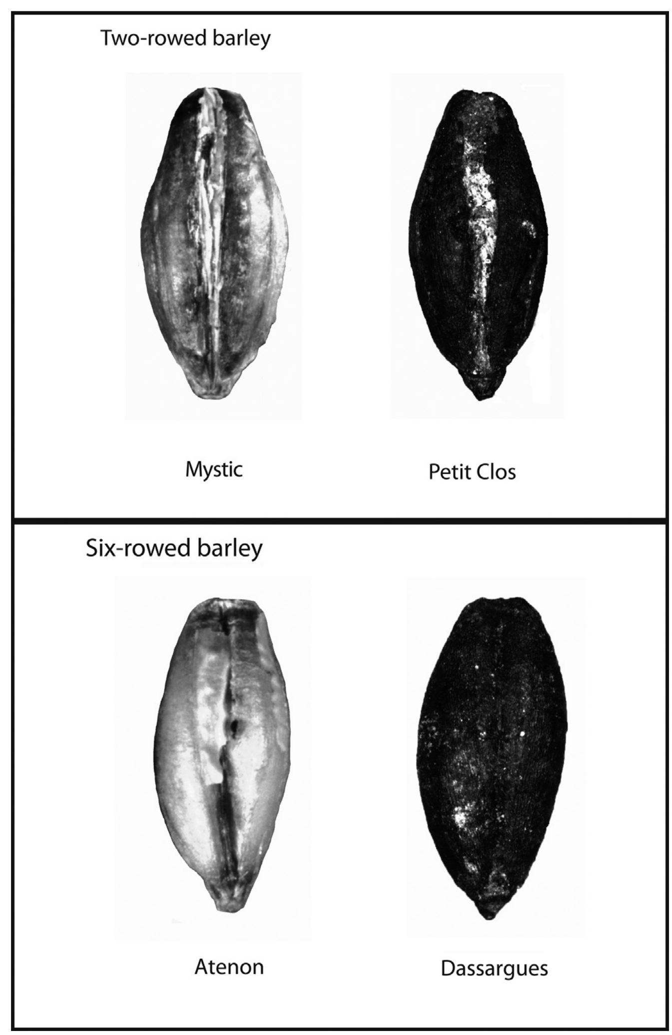

Fig. 6. Photographs of two- (top) and six-rowed (back) grains, modern (left) and archaeological (right).

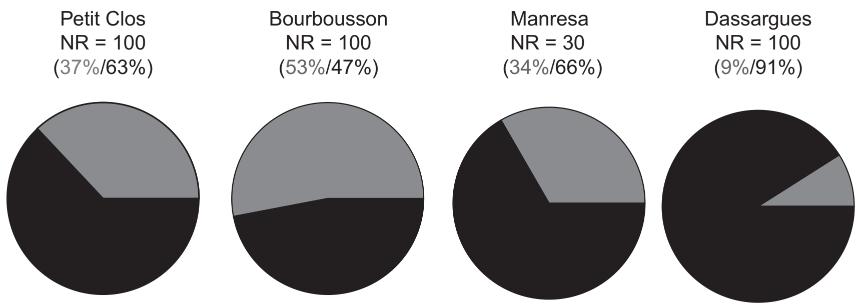

Fig. 7. Frequencies of the two subspecies for each archaeological site (grey = two-rowed barley; black = six-rowed barley).

a third of the studied grains (Fig. 7). However when considering only the 95% confidence identifications, there were no caryopses of two-rowed barley identified at Dassargues, while this subspecies is still identified in the three other sites, but in smaller proportions (one grain out of thirty at Manresa). Six-rowed barley was present at all sites.

5. Discussion

This first attempt to use geometric morphometrics on barley proves that it can efficiently distinguish two-rowed from six-rowed barley on modern dehulled caryopses, whether the grains are carbonized or not. The archaeological charred grains were identified by comparison to a modern source of reference composed by both carbonized and fresh grains. The identification of both subspecies in at least three of the four sites suggests the cultivation and role of these cereals during Roman and medieval periods in southern France.

5.1. Discussion of methods

The geometric morphometric analysis of the barley caryopses shape allows the discrimination of the two modern subspecies. Modern uncharred caryopses of the two-rowed and six-rowed barleys can be correctly attributed to their proper subspecies with a probability of 91%. Since carbonization induces a greater degree of variability in shape, a slightly lower number of specimens (87%) can be correctly identified using the carbonized modern samples.

Six and two-rowed subspecies did not react homogeneously to carbonization. Little is known about the different factors that can affect the way subspecies react to carbonization. We can suggest it might be related to the composition of the grains (properties of shape, water content), linked to the storage conditions (hulled or dehulled), or by grains exposed to different types of carbonization (oxidized or reduced). Unfortunately, it is impossible to determine the effects of all of these factors for the archaeological caryopses. Nonetheless, the presence of partially hulled grains, rachis and lemma bases suggest that barley would have been charred as spikelets (Ros, 2009, 2010; Ruas, 2011) and not as dehulled grains. At the sites of Petit Clos and Dassargues it is suggested that the grains were well preserved because they were charred as spikelets (Ros, 2009, 2010), which may explain why some of the archaeological grains bear a greater similarity in shape to the modern fresh grains than to the experimentally dehulled ones. Other factors could also be involved, such as the charring of surface grains could have represented a protection against carbonization for other grain remains positioned underneath.

In future studies it would be of particular interest to include entire charred spikelets in the reference material and to test different experimental conditions (temperature, humidity, duration) to improve our understanding of the effect of carbonization. It would be also interesting to compare the efficiency of GMM methods with the traditional criteria of identification. For example, it would be interesting to determine if GMM techniques can distinguish the original positions of the caryopses (lateral or central) in six-rowed barley.

5.2. Archaeobotany: status and cultivation of two-rowed and six-rowed barley in ancient and medieval southern France

Though hulled barley is commonly discovered in archaeological sites, it appears that the importance of the two-rowed subspecies in Western Europe during the Roman and medieval periods, and possibly also during proto- and prehistorical periods, is probably largely underestimated by archaeobotanists. During the Roman and medieval periods in southern France hulled barley was the second most common cereal along with naked wheats (*T. aestivum/durum/turgidum*) (Bouby, 2010; Ruas, 2005). However, so far only the presence of six-rowed barley has been discussed for protohistorical periods (Bouby, 2001, 2010; Marinval, 1988; Ruas and Marinval, 1991). The rare mentions of two-rowed barley date only from the Roman period (Bouby, 2001; Ros, 2010). No archaeobotanical evidence existed in medieval France (Ruas, 2005), although two-rowed barley is commonly mentioned in historical texts (e.g. Comet, 1992; Diot and Laborie, 1989). The reason row-rowed barely is rarely mentioned is still not clear but could be due to the scarcity of clearly identifiable chaff and grains.

By contrast, both subspecies are described as being cultivated in historical texts since the Roman period. Latin authors, as Columella (1st 17 c. A.D., book 2, 9) and Palladius (5th 18 c. A.D., book II) mention two-rowed barley (named "*distichum*" or "*galaticum*" cultivated as a spring crop) and six-rowed barley (also named "*hexastichum*" or "*cantherinum*" cultivated as a winter crop). The name of "*galaticum*" for two-rowed barley would suggest the exploitation of this cereal in Gaul (Ferdière, 1988). Columella considered both cereals as highly valuable, for bread-making as much as for fodder (book 2, 9). In medieval Languedoc, two-rowed barley was cultivated as a spring crop in mountainous areas (Leroy Ladurie, 1966).

The cultivation and role of these cereals during Roman and medieval periods in southern France can be investigated further using the weed species associated with barley remains and by considering the archaeological contexts. Our study reveals the presence of both subspecies in at least three of the four sites studied. Two-rowed barley appears to be dominant in Bourbousson III (53% of the grains), important in Petit Clos (37%) and residual in Manresa (only one out of thirty). The presence of this subspecies in Dassargues is suspected but not be confirmed using grains identified with the highest probabilities (>95%). The recovery of such high proportions of two-rowed barley clearly proves the sowing and harvesting of this cereal in French Gaul since at least the 1st century A.D.

Nowadays, *Hordeum distichum* varieties (two-rowed barley) are mainly cultivated as spring crops, although they can be negatively affected by the early dryness of Mediterranean climate in coastal plains of southern France. Within the four sites, Bourbousson III is the only one in which two-rowed barley has been identified as a spring crop because of the associated presence of several spring crop weeds such as *Chenopodium album* or *Anagallis arvensis* (Bouby, 2001). In the three other sites, the status of barley subspecies seems a bit more ambiguous. In Petit Clos, both subspecies were mixed and cultivated as a winter crop, as indicated by the presence of associated winter segetal weeds such as *Avena sterilis*, *Galium aparine* or *Raphanus raphanistrum*. In Manresa, the season of cropping is uncertain and two-rowed barley was found in too small a proportion to allow conclusions about its culture. Its presence could also have been tolerated as an edible cereal weed. It is not clear how these cereals were used on these sites. In Manresa, grains might have been used with other Poaceae remains as fodder or litter. In Dassargues, the large concentration of six-rowed barley was stored mixed with naked wheat before carbonization. Both species were cultivated as winter crops, indicated by the presence of associated winter segetal weeds such as *Agrostemma githago*, *Galium spurium*, *Lolium temulentum* and *Sherardia arvensis*.

6. Conclusions

The analysis undertaken in this study is the first archaeobiological application of geometric morphometrics in order to characterize past cereal agro-biodiversity in methodological, taxonomical, archaeobiological and historical perspectives. Previous studies had pointed out the limits of the morphological parameters (dimension, presence of a twist, and surface sculpture) traditionally used to characterize archaeological barley caryopses. Geometric morphometrics appears to accurately quantify, characterize and discriminate the shape of archaeological caryopses from modern barley varieties. This study has also illustrated the need for carefully designed experiments and maximizing the volume of data (number of grains, of sites, periods, etc…) for both reference and archaeological material. Further studies on caryopses originating from burned spikelets, on the variation in caryopses shape in different varieties and of different geographic origins, and of archaeological grains from a greater number of excavation sites would all provide important data.

Our study confirmed that the importance of two-rowed barley in Western Europe during Roman and medieval periods and potentially during proto- and prehistorical periods is largely underestimated by archaeobotanists. It would be of special interest to retrace the geographic origins of the two-rowed barley cultivated during the Roman period in southern France, and more generally in Europe, to determine if the cultivation of the subspecies originated during or prior to this period. In addition, we will have to investigate the possibility of identifying different varieties that could have been used for different purposes such as bread and fodder.

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