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ORIGINAL ARTICLE

Taxonomic identification of dry and carbonized archaeobotanical remains of *Cucurbita* species through seed coat micromorphology

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Abstract *Cucurbita* seeds are difficult to identify to species level using only their external morphology. In this contribution, we discuss anatomical features of fresh, dehydrated and experimentally carbonised specimens that are useful for the identification of archaeological *Cucurbita* seeds. Qualitative and quantitative differences in the seed coat micromorphology were found to be the most helpful diagnostic characteristics of South American *Cucurbita* species.

Keywords Seed coat · Taxonomic identification · *Cucurbita*

Introduction

Cucurbita seeds, including archaeological specimens, are typically identified to species from the qualitative characteristics of their external morphology, particularly colour, margin and funicular attachment (Cutler and Whitaker 1961; Lira Saade 1995). However, these features are not always appropriate for the identification of dried or charred archaeobotanical remains (Smith 2000). To address this problem, several authors have made statistical analyses on measurements of seeds, rinds and peduncles of North American *Cucurbita* (eg. *Cucurbita pepo* L.) to identify useful diagnostic features (Decker and Wilson 1986; Newsom et al. 1993; Smith 2000, 2006). However, the diagnostic criteria of North American *Cucurbita* seeds are not always appropriate for the identification of South American species, which include many closely related taxa, such as *C. maxima* Duchesne ssp. *maxima*, *C. maxima* Duchesne ssp. *andreana* (Naudin) Filov. and *C. moschata* (Lam.) Poir.

Several papers have been written about the testa tissues of *Cucurbita* seeds, most of them providing qualitative descriptions (Lott 1973; Stuart and Loy 1983) but only a few (Singh and Dathan 1972; Teppner 2004) provide data that is useful for differentiating species. The present study is the first archaeobotanical analysis of the characteristics of South American *Cucurbita* seed testae. In this paper, we discuss our results, describing the microscopical features of fresh, dehydrated and experimentally carbonised *Cucurbita* seed coats that are useful for the identification of archaeological *Cucurbita* seeds.

General features of *Cucurbita* seeds

Cucurbita seeds are flattened and ellipsoid, with a relatively more or less pronounced marginal bulge. According to Singh and Dathan (1972) the seed coat is comprised of four layers of tissue: epidermis, hypodermis, sclerenchyma, and aerenchyma, which surround an "inner zone". The epidermis consists of radially elongated cells, which have more or less thickened non-lignified walls (Esau 1977). These cells vary in their length, with longer ones typically occurring around the marginal bulge (Singh and Dathan

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1972; Teppner 2004). The hypodermis consists of small cells with slightly thickened lignified walls where thickening forms a reticule (Esau 1977; Lott 1973). The sclerenchyma layer gives the seed coat its firmness (Hayward 1953) through sclereids with festooned lignified walls (Esau 1977; Singh and Dathan 1972). The aerenchyma is comprised of cells with arm-like extensions, which are interspersed with enlarged intercellular spaces (Esau 1977; Hather 2000; Singh and Dathan 1972). Lott (1973) describes the tissue between the sclerenchymatous layer and the cotyledons as a spongy parenchyma formed by dead irregular-shaped cells with numerous outgrowths.

Methodology

Microscopy was used to study 63 seeds of *Cucurbita ficifolia* Hubber, *C. moschata, C. maxima* Duchesne ssp. *andreana*, *C. maxima* ssp. *maxima* cv. *zapallito*; *C. maxima* ssp. *maxima* cv. *hubbard*; *C. maxima* ssp. *maxima* cv. *ingles* and *C. maxima* ssp. *maxima* cv. *criollo*. Samples of cultivars were provided by the Faculty of Agronomy, La Plata National University (U.N.L.P.) and *C. maxima* ssp. *andreana* samples were collected from several localities in Cordoba province, representing three kinds of pollination (free, autogamic and crossed). Voucher specimens were deposited in the Laboratory of Ethnobotany and Applied Botany (Faculty of Natural Sciences, U.N.L.P.) and in a personal collection at the Archaeological Scientific Department of La Plata Museum of Natural Sciences (U.N.L.P.).

Samples were divided into three groups for different types of analysis. Seeds in Group 1 ($n = 35$) were hydrated and the testae cut into thin sections that were observed employing light microscopy (LM). Only the testa tissue layers were studied.

The Group 1 seeds were subjected to three types of thin sectioning (see Fig. 1):

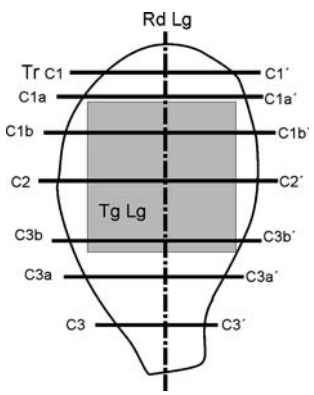

Fig. 1 Sections of the studied *Cucurbita* seeds

1. *Transverse Sections* (*Tr*) were obtained from micropyle (C1), middle (C2), and chalaza or basal (C3) portions of the seed. Successive sections of C1 and C3 were labelled as "a" and "b" as they bordered C2. Between 5 and 11 seeds of each taxon were analysed which resulted in the analysis of 103 thin sections from all the taxa analysed.
2. *Tangential Longitudinal sections* (*TgLg*) were taken from the middle zone (C2). The testa was removed from the seed. For the purposes of separating the sclerenchyma tissue, but maintaining its integrity, tangential sections were immersed in a chemical maceration solution (50% hydrogen peroxide 100 volumes and 50% acetic acid) and boiled for 10–15 min. To achieve a better understanding of sclerenchyma morphology this type of sectioning was performed on two specimens: a single *C. maxima* ssp. *maxima* cv. *criollo* seed and a single *C. maxima* ssp. *andreana*
3. *Radial Longitudinal section* (*RdLg*) The same procedure described above for the tangential longitudinal section was used to obtain a radial view of the sclerenchyma cells of a single *C. maxima* ssp. *maxima* cv. *criollo* seed.

The Group 2 seeds ($n = 7$) were dehydrated by air-drying and manually fractured in the middle (C2) for studying with a stereoscopic microscope as well as SEM (at magnifications 80, 370 and 700×).

At first, we only considered the qualitative features of the seed coat. As the study progressed, we recognised that there was a need for quantitative analyses because there were so many qualitative similarities among closely related taxa, for example *C. maxima* subspecies and cultivars. Therefore, measurements were taken of seed coat cells from images recorded with a Motic Image Plus 2.0 web camera and its measuring software. To check the accuracy of the Motic Image Plus 2.0 measurements, the results were compared with those made with a drawing tube attached to a Leica microscope (DM/LM). Measurements were also calculated from SEM images using the programme Image Tool 3.0. From each measurement, we calculated means and range of width, length and height of cells. Between 20 and 50 measurements on each taxon were taken. Data on *C. maxima* ssp. *maxima* were produced by combining the four cultivars.

The seeds in Group 3 ($n = 21$) were charred to observe how carbonisation alters seed shape and size due to expansion or shrinkage of the internal tissue. The samples comprised dried seeds of *C. maxima* ssp. *maxima* cv. *criollo*, *C. maxima* ssp. *andreana*, *C. moschata* and *C. ficifolia*, which were charred in both an aerobic and anaerobic atmosphere in an electric muffle oven at 300°C

for 120 minutes. We measured seed dimensions (length, width and height) before and after charring using digital calipers. The best preserved specimens were those charred in the aerobic atmosphere. They were manually fractured in their C2 region and their testae were observed under SEM at magnifications of 80, 370 and 700×.

Results and discussion

Seed areas useful for taxonomic identification

Cutting transverse sections from different parts of the seed (C1, C2 and C3) allowed us to examine the tissue in detail and to conclude that C2 has the most homogeneous tissue morphology. Therefore, it was decided that further taxonomic analysis should be made in this region. It was also decided that both the flattened area of the seed corresponding to the major faces and the marginal bulge should be examined.

Marginal bulge diagnostic characters

The marginal bulge morphology was observed in transverse sections at low magnifications. *C. ficifolia* has the most distinctive marginal bulge since it is rectangular to trapezoidal (Fig. 2a; Teppner 2004). *C. moschata* (Fig. 2b, c) and *C. maxima* ssp. *andreana* (Fig. 2d) have broadly rounded marginal bulges and *C. maxima* ssp. *maxima* has a rounded to flattened one (Fig. 2e). Another diagnostic character is that submarginal bulges are present in *C. ficifolia* (Teppner 2004) and *C. moschata* (Fig. 2a–c), but absent in *C. maxima* ssp. *maxima* and *C. maxima* ssp. *andreana* (Fig. 2d, e) (see dichotomous key below).

Micromorphology: the analysis of seed coat tissues

Epidermis Epidermal cells can be divided into those, which are rectangular, thick walled and arranged in a palisade, such as those of *C. moschata* and *C. maxima* ssp. *maxima* cv. *criollo* (Fig. 3a, b, respectively), and those which are probably rectangular and arranged in a palisade too, but are difficult to characterise because they have such thin walls that after sectioning they were observed to be highly disrupted and disordered. The latter were observed in the other five taxa (Fig. 3c–e). In *C. ficifolia* (Fig. 2a; Singh and Dathan 1972) and *C. moschata* (Fig. 2b), long cells always surrounds short cells encompassing completely the marginal bulge in C2. It is usually mentioned in the bibliography that *Cucurbita* seeds have hair associated with their margins, but this is an erroneous identification of the long epidermal cells. However, we observed that in other areas of the margins of the *C. moschata* seeds (C1b and C3b) there are shorter elongated cells, which appear like "horns", and do not surround the short cells (Fig. 2c). This observation has not been reported previously in the literature although this pattern is described for *C. maxima* ssp. *maxima* (Fig. 2e). This observation highlights the importance of selecting the proper cross section location to make correct identifications. In both *C. ficifolia* and *C. moschata* the bases of the long cells are situated in the submarginal bulges, respectively (Fig. 2a, b; Teppner 2004).

Our observations of the C2 region of *C. maxima* ssp. *maxima* showed that most of the specimens of the four cultivars have a few long cells, which are not much longer than the short ones, forming a protuberance (Fig. 2e). However, some specimens of cultivars criollo and inglés had long cells surrounding short cells encompassing one-third (or less) of the marginal bulge. In some specimens of landraces from the Argentinean northwest, seed coat type 3 with a narrow submarginal bulge and long cells encompassing the marginal bulge as proposed by Teppner (2004) was recognized, therefore it is thought that more specimens of this subspecies should be investigated for this feature.

Contrary to Teppner's (2004) report that in *C. maxima* ssp. *andreana* long cells comprise one-third of the seed margin, we did not observe the presence of long cells in this subspecies when viewed with SEM and light microscopy (Fig. 2d).

Long cells of the major faces were also analysed (Fig. 3). Our measurements were found to contrast with those published by Singh and Dathan (1972) since *C. moschata* was observed to have the widest epidermal cells [65.6 (44.3–84.4) μm] while *C. maxima* ssp. *maxima* cv. *criollo* was found to have the longest cells. A problem that we found in the published literature is that particular traits of this last cultivar are regarded as being representative of the whole sub-species. In the case of epidermal cell width, our results demonstrate that, taking into account data from all the cultivars of *C. maxima* ssp. *maxima*, this trait is similar to that of *C. maxima* ssp. *andreana*. Therefore, we argue that the width of the epidermal cells cannot be used as a diagnostic feature. For example, the width of epidermal cells of *C. maxima* ssp. *maxima* cv. *criollo* [43.8 (28–67.7) μm] is significantly different from that of *C. maxima* ssp. *andreana* [30.3 (22–40) μm]. However, when other cultivars are considered in the analysis such as *C. maxima* ssp. *maxima* cv. *ingles*, with a mean of 34.2 and a range of 24.4–40.2 μm, the range of values overlaps with those of *C. maxima* ssp. *andreana*. There were no differences between measurements of different tissues obtained from fresh seeds for thin section analysis and desiccated seeds for SEM analysis. Finally, we argue that from *C. maxima* ssp. *maxima* values [40.25 (24.4–67.7) μm] only those higher than 40 μm are diagnostic for taxonomic recognition of this domesticated subspecies. *C. ficifolia* is in an intermediate position among

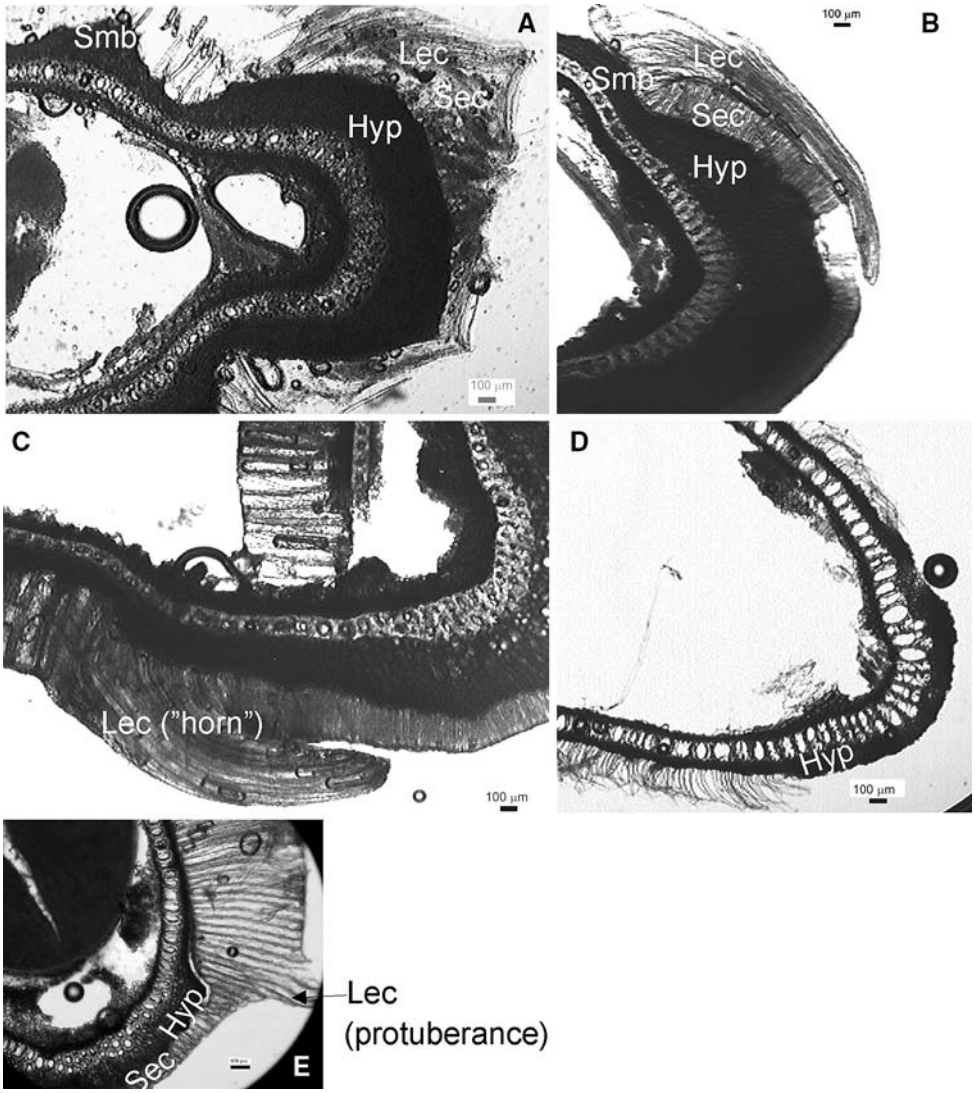

Fig. 2 Marginal bulges. **a** *C. ficifolia*; **b** *C. moschata* (cross sectioned in C2); **c** *C. moschata* (cross sectioned in C1b); **d** *C. maxima* ssp. *andreana*; **e** *C. maxima* ssp. *maxima* cv. *criollo*. *Smb* submarginal bulge, *Lec* long epidermal cells, *Sec* short epidermal cells, *Hyp* hypodermis

the other species with a mean of 51.9 and a range of 29.9–80 µm.

The same can be said about epidermal cell length. For this feature, the range of variation of *C. maxima* ssp. *andreana* [270.07 (241–315) µm] falls within that of *C. maxima* ssp. *maxima* at 147.43–743.7 µm, corresponding the higher values with *C. maxima* ssp. *maxima* cv. *criollo* and the lower ones with *C. maxima* ssp. *maxima* cv. *zapallito*. This pattern is not surprising if we consider that the former is a wild variety of the latter, and that usually variations in the domestic forms include those of the wild forms (Galvan 2006). Finally, we can recognize the domesticated subspecies by epidermal cell lengths greater than 315 µm or less than 213 µm.

On the other hand, *C. moschata* seeds have longer epidermal cells [366.4 (351.1–389.5) µm] than those of *C. ficifolia* [128.37 (105.71–157.14) µm], although, as mentioned before, among all taxa the longest epidermal cells are found in the seeds of *C. maxima* ssp. *maxima* cv. *criollo* [725.72 (705.39–743.7) µm].

Hypodermis Presence or absence of sub-marginal bulges is a diagnostic feature, particularly in the hypodermal layer. The position of the border between the epidermal and hypodermal layers differs between taxa. In the seeds of *C. maxima* ssp. *maxima* (Fig. 3b) and *C. maxima* ssp. *andreana* (Fig. 4a), the hypodermis-epidermis divide appears as a regular line, while in the seeds of *C. moschata* it varies from epidermal cell to epidermal cell in each specimen, because the line of epidermal cells is placed at different heights (Fig. 3a). In *C. ficifolia* this hypodermis-epidermis divide is also irregular, occurring in "waves" that coincide with the border of the epidermal cells (Fig. 4b).

Hypodermal thickness was evaluated by counting the number of layers present or by measuring tissue thickness (in µm). Despite Singh and Dathan (1972) stating that the quantity of cell layers of this tissue is diagnostic, our

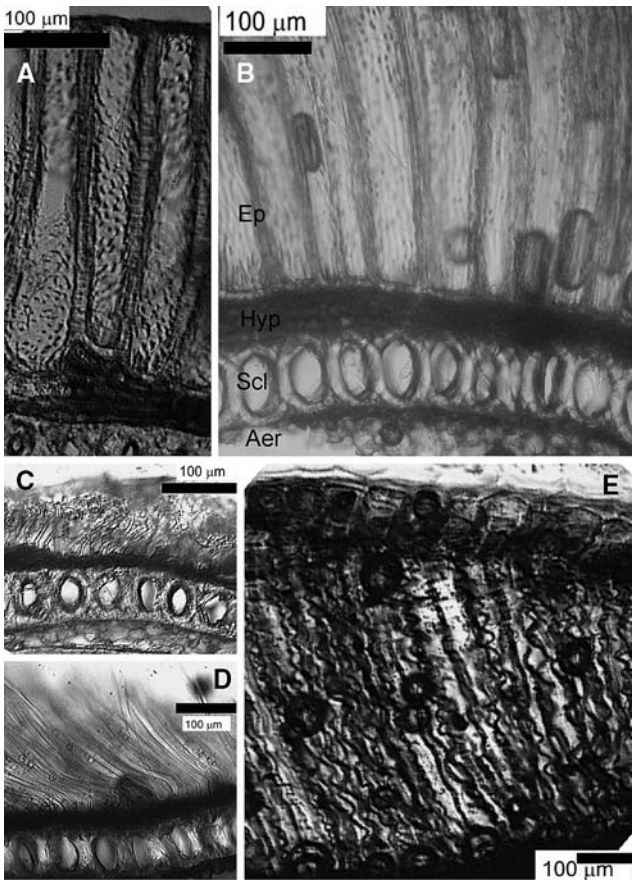

Fig. 3 Epidermis. **a** *C. moschata*; **b** *C. maxima* ssp. *maxima* cv. *criollo* (cells with thick walls arranged in a palisade); **c** *C. maxima* ssp. *andreana*; *Ep* epidermis, *Hyp* hypodermis, *Scl* sclereids, *Aer* aerenchyma; **d** *C. maxima* ssp. *maxima* (cells with thin walls showing a loose arrangement after thin sectioning); **e** *C. ficifolia* (sinuate walls are an effect of microtomy) *Ep* epidermis, *Hyp* hypodermis, *Scl* sclereids, *Aer* aerenchyma

observations revealed, however, that there is no regular patterning in this tissue, and that the quantity of layers is highly variable, even in the same seed. For this reason, the number of cell layers in the hypodermis cannot be used as a diagnostic feature for archaeobotanical identification. While variations in the numbers of layers present in the hypodermal tissue in the same seed may not be a problem for botanical studies, for archaeobotanical studies, which usually deal with fragmentary material, the use of this feature as diagnostic could lead to misidentification.

The number of layers in the marginal bulge area usually increases towards the marginal areas (see dichotomous key below). Singh and Dathan (1972) state that this phenomenon does not happen in *C. maxima* ssp. *maxima*. However, our observations show that an increase in the number of layers occurs even in this taxon (Fig. 2e), although less obviously than in the other taxa analysed (Fig. 2a–c). In *C. maxima* ssp. *andreana* this difference is even minor (see dichotomous key below).

Despite the fact that the number of layers in major faces and in the marginal bulge of *C. maxima* ssp. *andreana* is usually less than that in *C. maxima* ssp. *maxima*, this feature is not enough for differentiations between both species. Therefore, other quantitative traits should be assessed to better support identifications. Tissue thickness is not diagnostic for differentiation between *C. maxima* ssp. *maxima* [44.07 (26.5–75.1) µm] and *C. maxima* ssp. *andreana* [34.29 (23.6–46.6) µm]. While it is accurate to say that samples with a hypodermis thicker than 46 µm belong to *C. maxima* ssp. *maxima*, it is not possible to identify either of the two sub-species from a hypodermis thinner than that amount. The same can be said about cell size (length and width) because the range of *C. maxima* ssp. *maxima* [9.68 (4.7–15.3) µm] overlaps that of *C. maxima* ssp. *andreana* [7.25 (4.93–9.76) µm], and *C. maxima* ssp. *maxima* can only be confidently affirmed by the presence of hypodermal cells size more than 10 µm.

Sclerenchymatous tissue On the major faces, sclerenchyma tissue is composed of one cell layer. In the marginal bulge area, this layer can have irregularities in cell morphology or the number of cell layers may increase from one to three. For these reasons, we will consider only major faces for our analysis.

Sclereids typically have thick walls and a thin lumen (Lott 1973) but the proportions vary among species. *C. moschata* (Fig. 5a) has thicker walls compared with

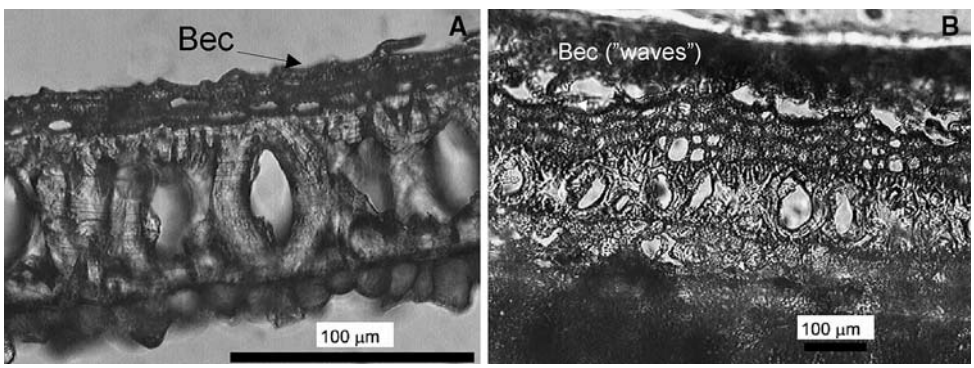

Fig. 4 Hypodermis. **a** *C. maxima* ssp. *andreana*, **b** *C. ficifolia*. *Bec* base of epidermal cells

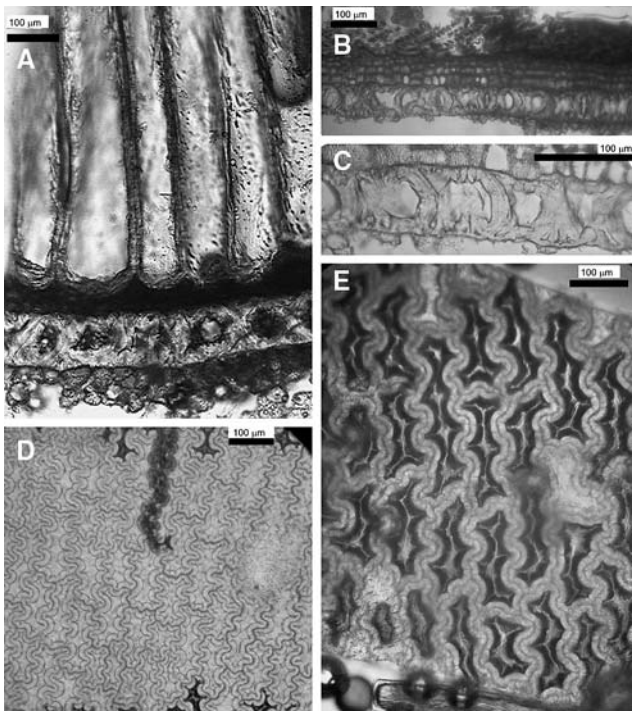

Fig. 5 Sclerenchymatous layer. **a** *C. moschata* (transverse section), **b** *C. ficifolia* (transverse section), **c** *C. ficifolia* (transverse section) detail, **d** *C. maxima* ssp. *andreana* (tangential-longitudinal section), **e** *C. maxima* ssp. *maxima* cv. *criollo* (tangential-longitudinal section)

those of the other species. A similar morphology was also observed in samples corresponding to *C. maxima* ssp. *andreana* that were produced from cross-pollination. This was the only difference in diagnostic characters noted among *C. maxima* ssp. *andreana* seeds resulting from a different kind of pollination. *C. ficifolia* has thinner walls and highly irregular sclereid morphology in cross section (Fig. 5b). The opposite pattern occurs in *C. maxima* ssp. *maxima* (Fig. 3b) and *C. maxima* ssp. *andreana* (Fig. 3c) which have very regular circular cells in this section.

Quantitative analysis showed that *C. moschata* has the shortest sclereids [58.32 (50.4–67.3) µm]. These sclereids are even shorter than those of non-domesticated taxa such as *C. maxima* ssp. *andreana* [81.25 (65.4–95.4) µm]. In cross section, *C. maxima* ssp. *maxima* has sclereids ranging from 66.8 µm (*C. maxima* ssp. *maxima* cv. *zapallito*) to 125.4 µm (*C. maxima* ssp. *maxima* cv. *criollo*) and a mean of 91.16 µm. These results demonstrate that the range of values of *C. maxima* ssp. *maxima* sclereid heights overlaps and includes those of *C. maxima* ssp. *andreana*. Therefore, only extreme values of the range can be considered diagnostic, for example only sclereids with heights of more than 95 µm correspond with *C. maxima* ssp. *maxima*. The opposite happens with sclereid width in cross section, since *C. maxima* ssp. *andreana* values [62.88 (48.8–89.2) µm] include those of *C. maxima* ssp. *maxima* [68.88 (52.8–85.9) µm]. In this case, the wild subspecies can be identified when sclereid width is between 49 and 53 µm in cross section.

In tangential longitudinal section, *C. maxima* ssp. *andreana* sclereids (Fig. 5d) are shorter [233.55 (146.12–365.64) µm] than those of *C. maxima* ssp. *maxima* [349.01 (208.69–587.88) µm] (Fig. 5e). Thus, the wild form can be distinguished in tangential longitudinal section if sclereid height is between 146 and 209 µm and that of the domestic taxa if it is between 366 and 587 µm in height.

Aerenchyma Characterisation of this tissue must be carried out on the major faces since aerenchyma is not well developed in or absent from the marginal bulge area. Singh and Dathan (1972) state that wild species have one or two layers of aerenchyma along major faces and that these layers are formed by small cells, while domesticated species have bigger cells and several layers.

The quantification of cell layers was not possible due to the heterogeneous disposition of cells among seeds in most of the taxa considered. Therefore, instead of cell layers, total aerenchyma thickness was recorded (see dichotomous key below). However, in *C. maxima* ssp. *andreana* (Fig. 6a) alone, it was possible to observe always two layers (in agreement with Singh and Dathan 1972).

We did not carry out a morphological analysis because aerenchyma cells have highly heterogeneous shapes (Fig 6b). However, from our observations we can confirm

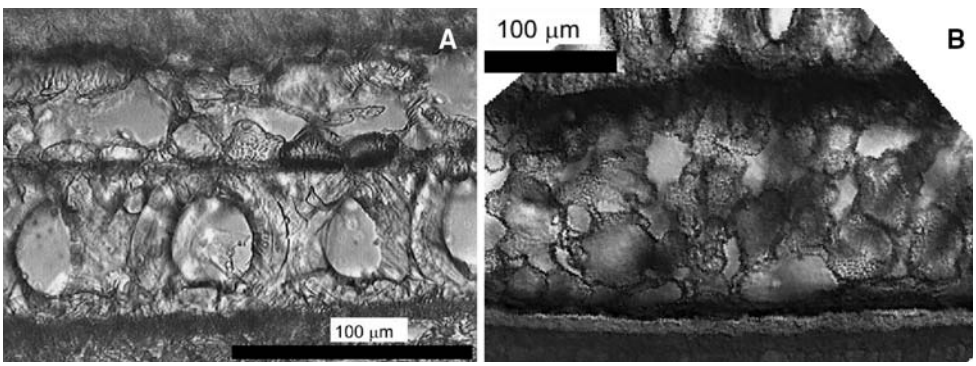

Fig. 6 Aerenchyma. **a** *C. maxima* ssp. *andreana*, **b** *C. maxima* ssp. *maxima* cv. *hubbard*

that these cells are usually smaller in the zone closer to the sclerenchymatous layer, as reported by Lott (1973) and Esau (1977).

Given the high variability of this tissue, both in the number and positioning of cell layers and shapes, we concluded that these criteria alone are not useful for analysing archaeobotanical samples, since they could lead to misidentifications.

Dichotomous key for species differentiation

This analysis of the internal morphology of seed coats of the main South American *Cucurbita* taxa resulted in the identification of a number of useful characteristics for distinguishing between different species:

A. With submarginal bulges. Long epidermal cells always surround short ones, completely enclosing the marginal bulge. The hypodermis is a sinuous layer in major faces increasing its height (7–9 layers) in marginal bulges.

B. Rectangular to trapezoidal marginal bulge. The epidermal cells have thin walls [width: 51.9 (29.9–80) μm; length: 128.37 (105.71–157.14) μm] and acquire a disrupted and disordered arrangement after thin sectioning. The hypodermis appears as "waves" in areas where it borders epidermal cells, and has 3–5 layers in major faces. Thin walled sclereids with wide lumen, heterogeneous morphology and irregular walls. Aerenchyma thickness between 55 and 100 μm *C. ficifolia*

BB. Broadly rounded marginal bulge. Epidermal cells rectangular in shape arranged in a palisade [width: 65.6 (44.3–84.4) μm; length: 366.4 (351.1–389.5) μm]. Basis of epidermal cells at different heights. Hypodermis with 2–4 layers in major faces. Short sclereids [58.32 (50.4–67.3) μm] with thickened walls and narrow lumen in cross section. Aerenchyma thickness between 100 and 150 μm *C. moschata*

AA. Narrow or absent submarginal bulges. Long epidermal shape a protuberance or encloses one-third of or the entire marginal bulge. Hypodermis is not a sinuous layer. Sclereids with thin walls and wide lumens of homogeneous morphology and uniform walls.

C. Rounded to flattened marginal bulge. Epidermal cells rectangular in shape arranged in a palisade, thin to thick walled, those thin walled acquire a loose arrangement after thin sectioning, length more than 315 or less than 213 μm, width greater than 40 μm. Hypodermis with 4–5 layers in major faces, thicker than 47 μm and with cells bigger than 10 μm, 7–8 layers in the margin. Sclereid more than 95 μm in cross section; in tangential longitudinal section between 366 and 587 μm. Aerenchyma thickness between 150 and 200 μm *C. maxima* ssp. *maxima*

CC. Broadly rounded marginal bulge. Epidermal cells with thin walls which acquire a disrupted and disordered arrangement after thin sectioning. Hypodermis with 2–3 layers in major faces, 5–7 layers in margin. Range of sclereid height in tangential longitudinal section between 146 and 209 μm. Aerenchyma thickness less than 55 μm *C. maxima* ssp. *andreana*

Changes after carbonisation

Both macro (see Table 1) and micromorphological *Cucurbita* seed testa characteristics survived charring. In an aerobic atmosphere, the general morphology of the testa tissues and of the margin persists except in cases where the epidermis is comprised of thin-walled cells, such as that of *C. maxima* ssp. *andreana* (Fig. 7a) and *C. ficifolia* (Fig. 7b). In these cases only the bases of epidermal cells remain, from which their width can be used as a diagnostic character. It is worth mentioning that the width of *C. ficifolia* cells does not change with carbonisation, while *C. maxima* ssp. *andreana* instead shows an overall size reduction of 58% (carbonised sample mean: 13 ± 3 μm; range: 9–16 μm). In *C. moschata* (Fig. 7c) and *C. maxima* ssp. *maxima* (Fig. 7d) both the length and width of epidermal cells are reduced, mostly in the former (Table 2). Nevertheless, the epidermal cells of *C. maxima* ssp. *maxima* are still the longest and those of *C. moschata* the widest.

From these results, we concluded that, contrary to the case of fresh samples, the width of epidermal cells can be used to distinguish *C. maxima* ssp. *maxima* from *C. maxima* ssp *andreana* in carbonised samples since their means do not overlap.

The hypodermis does not show significant alterations in cell morphology or quantity of cell layers. Neither in the sclerenchymatous layer are the morphological characteristics modified, but heights are reduced (Table 2). As in the case of epidermal cells, differences between sclereid length of *C. maxima* ssp. *maxima* and *C. maxima* ssp. *andreana* are increased with carbonisation and overlapping disappears.

The aerenchyma is well preserved after carbonisation; however, this tissue shows alterations in cell arrangement, becoming more tightly grouped and having less intercellular space, promoting an extreme reduction of the tissue (Table 2).

Table 1 Changes in seed dimensions (mm) after charring

	n	Length			Width			Thickness		
		X	Range	*S*	*X*	Range	*S*	*X*	Range	*S*
C. maxima ssp. *maxima* cv. *criollo*										
Fresh	98	18	13.3–21.8	2.2	10.5	7.3–13.9	1.5	3.6	2.2–5.3	0.8
Ae	3	12.2 (–21%)	11–13.5	1.3	7.1 (–19%)	6.3–7.7	0.7	3.9 (–2.5%)	3.3–5.0	10
An	3	13.1 (–17%)	12.8–13.5	0.4	7 (–18%)	6.9–7.2	0.2	5.2 (+22%)	4.9–5.5	0.3
C. maxima ssp. *andreana*										
Fresh	6	8.3	6.6– 9.6	0.8	5.3	4.4– 6.5	0.5	1.9	1.2– 2.6	0.5
Ae	3	6.47 (–19%)	6.3–6.8	0.3	4.3 (–25%)	4.1–4.4	0.2	1.2 (+9%)	1.2–1.2	0
An	3	6.6 (–18%)	6.1–6.9	0.5	4.2 (–10%)	4.2–5.1	0.5	1.5 (+17%)	1.2–1.5	0.2
C. moschata										
Fresh	20	15.3	13.6–17.2	0.9	8.5	7.4– 9.4	0.5	2.8	1.8–3.3	0.3
Ae	2	14.3 (–11%)	13.8–14.8	0.7	7.6 (–16%)	7.4–7.8	0.3	3.5 (+25%)	3.2–3.9	0.5
An	3	14.2 (–13%)	14–14.5	0.3	7.2 (–19%)	7.0–7.5	0.3	3.6 (+24%)	3.1–4.0	0.5
C. ficifolia										
Fresh	60	17.9	15.5–20.4	1	11	9.3–13.8	1.2	2.6	1.8–3.3	0.5
Ae	2	17.7 (–10%)	17.3–18.1	0.6	9.9 (–16%)	9.8–10.0	0	3.6 (+24%)	3.0–4.2	0.9
An	2	16.3 (–12%)	16.2–16.3	0.1	10 (–14%)	8.9–11.0	1.5	2.7 (0%)	2.5–2.9	0.3

X average, *S* standard deviation, *Ae* aerobic, *An* anaerobic charring conditions. Sign + or – in front of percentages indicates increase or decrease of dimensions, respectively, compared to fresh seeds

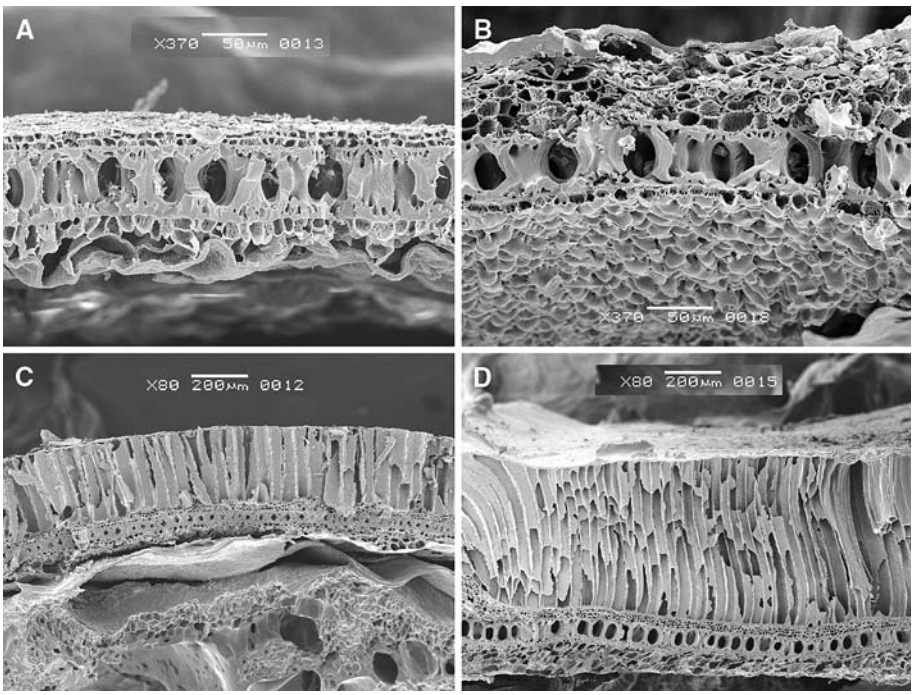

Fig. 7 Experimentally carbonised samples at 300°C in an aerobic atmosphere. **a** *C. maxima* ssp. *andreana*, **b** *C. ficifolia*; **c** *C. moschata*, **d** *C. maxima* ssp. *maxima*

Application to an archaeological case

A carbonised *Cucurbita* seed (Fig. 8a, b) found at El Shincal Inka site (A.D. 1430–1550, northwest Argentina) was identified using the features analysed in this work:

- Epidermal layer has a spongy appearance similar to that of *C. maxima* ssp. *maxima* cv. *zapallito* charred seeds (see Fig. 8c).
- 4–5 hypodermal layers, the line of contact between epidermis and hypodermis is regular.

Table 2 Values obtained after carbonisation: proportional reduction in epidermal cells measurement, sclereid height (transverse section) and aerenchyma thickness

	Epidermis				Sclereid	Aerenchyma
	Length (μm)	Red (%)	Width (μm)	Red (%)	Height (μm)	Thickness (μm)
Cucurbita moschata	276 ± 8	24.5	43 ± 5	35	46 ± 2	66 ± 13
	(260–288)		(35–50)		(42–50)	(50–85)
C. maxima ssp. *maxima*	573 ± 40	21	37 ± 5	16	80 ± 8	94 ± 22
cv. *criollo*	(506–618)		(24–48)		(68–94)	(59–127)
C. maxima ssp. *andreana*	No data	No data	No data	No data	55 ± 2	36 ± 6
					(50–60)	(26–47)
C. ficifolia	No data	No data	No data	No data	50 ± 3	83 ± 4
					(45–54)	(80–88)

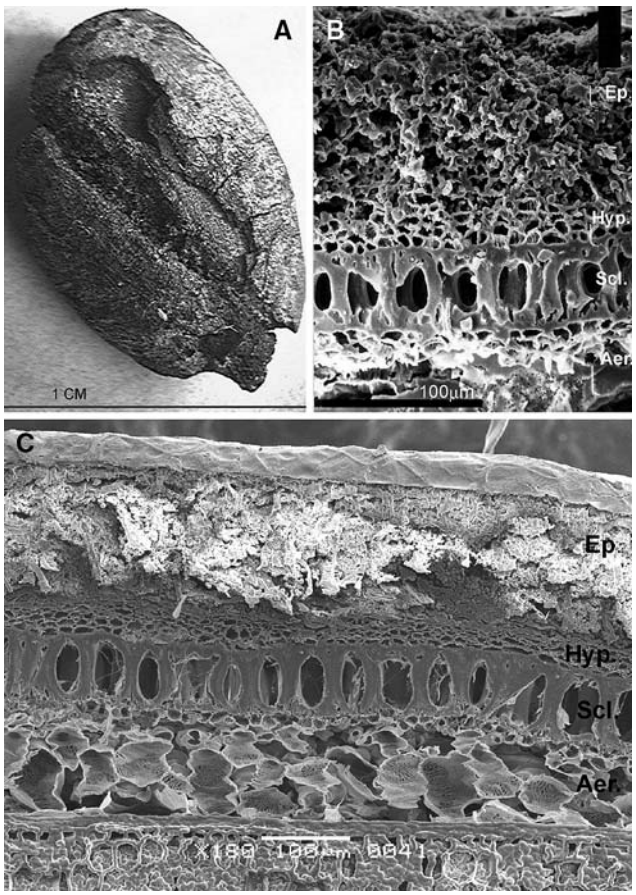

Fig. 8 Archaeological seed from an Inka site in northwest Argentina. **a** carbonised seed, **b** transverse section of the carbonised seed at SEM 350×, **c** modern and fresh reference seed of *C. maxima* ssp. *maxima* cv. *zapallito* with SEM, *Ep* epidermis, *Hyp* hypodermis, *Scl* sclereids, *Aer* aerenchyma

- Regular sclereids, thin and uniform walls, wide lumen, height: 75 μm.
- More than 2 layers of aerenchyma, 105 μm thickness.

Identified taxon: *Cucurbita maxima* ssp. *maxima* (a cultivated form with thin walled epidermal cells).

Conclusions

There is much confusion in the published literature on the criteria for identifying South American *Cucurbita* seeds from testa morphology. In this study, we tested the published data and, based on the results, present new criteria for the identification of *Cucurbita* seeds from testa morphology. For archaeobotanical purposes, we conclude that the transverse view in the middle of the seed (C2) allows a better interspecific comparison. In addition, we note that tissues of the C2 major faces have different anatomical characteristics than at the margins and therefore both areas have distinct useful diagnostic features.

The analysis of seed coat micromorphology is a suitable method for taxonomic identification at a specific level. The position, character and morphology of epidermal cells, the quantity and positioning of cells in the hypodermis layer, the shape and size of sclereids and thickness of the aerenchyma can be combined to identify the main South American *Cucurbita* species. Moreover, these qualitative and quantitative methods can be used to identify to the subspecies level, in this case that of *C. maxima*. We observe that carbonisation does not alter morphology but does modify quantitative traits. The methods of analysis established in this study will contribute to future archaeobotanical investigations of *Cucurbita* species recovered from South American archaeological sites as well as to our knowledge of the domestication history of this genus.

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