Evidences of chili pepper (*Capsicum frutescens*) seeds in Chimú contexts of the northern coast of Peru

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Abstract

The biometric and scanning electron microscopy (SEM) study of eight seeds from the Túcume site, six seeds from the El Brujo site and one seed from Cerro La Virgen, corresponding to *Capsicum*, whose results at the level of topography and patterns of reticulations were carried out from the surface of the testa, determined the identification that these seeds correspond to *Capsicum frutescens*, finding that the reticulation patterns at the MEB level are determinant to be able to identify the seeds at the species level and correlate with biometric data and seed shapes taking into account information from the sympatric species *Capsicum chinense*, with which it shares close genetic affinities.

Keywords: seed, SEM, *Capsicum frutescens,* Chimú.

Resumen

Se realizó el estudio biométrico y de microscopía electrónica de barrido (MEB) de ocho semillas del sitio Túcume, seis semillas del sitio El Brujo y una semilla del Cerro La Virgen, correspondientes a *Capsicum*, cuyos resultados a nivel de topografía y patrones de las reticulaciones de la superficie de la testa, determinaron la identificación que estas semillas corresponden a *Capsicum frutescens*, encontrándose que los patrones de reticulaciones a nivel MEB, son determinantes para poder identificar las semillas a nivel de especie y correlacionar con datos biométricos y formas de las semillas, teniendo en cuenta la información de la especie simpátrica *Capsicum chinense,* con la cual comparte afinidades genéticas cercanas.

Palabras clave: Semilla, MEB, *Capsicum frutescens,* Chimú.

Introduction

The genus *Capsicum* has been endemic to America since pre-Hispanic times, spreading in the Old World, transforming the diet of the inhabitants until present. The archaeological record indicates that chili peppers have a long history in various parts of Hispanic America. It has been suggested that domestication occurred with bell pepper (*C. annuum*) and may predate the beginnings of agriculture in America (Pickersgill, 1969). Indeed, there is evidence of a starch morphotype specific to this genus. That indicates that domestication of such species dates back at least 6,000 years BP in several locations in the Western Hemisphere (Perry et al, 2007).

Historically, in addition to the morphological characteristics of its flowers, fruits, and seeds, the distinguishing characteristic of the genus *Capsicum* has been the presence of capsaicin, an oleoresin that is the active component of the genus. However, two wild species (*C. lanceolatum* and *C. rhomboideum*) lack this oleoresin (Moscone et al, 2007). Both wild species live in Mexico and Central America. The latter is also found in Peru.

Botanical and molecular studies showed confusion in the taxonomic identification of *Capsicum* species. Pickersgill (1969) and Eshbaugh (1983) showed that the domestication of chili peppers from wild species was the result of selection according to size, shape, and color of the fruit. That reduced a long list of species under discussion to four domesticated ones such as bell pepper, habanero-type pepper (*C. chinense*), bird pepper (*C. frutescens*) and domesticated pepper (*C. baccatum* var. *pendulum*). A fifth domesticated species (the Andean chili-bell pepper) named tree pepper (*C. pubescens*) was added, showing a distinctive morphology.

It has been suggested that *C. annuum, C. frutescens* and *C. chinense* form a closely related group of domesticated species that evolved in the tropical lowlands of Hispanic América, including the Caribbean, dominating territory in Mexico, the Caribbean and the Amazon region, respectively (Eshbaugh et al, 1983). But other authors have questioned their taxonomic status (Pickersgill, 1988). Some taxonomists continue to treat these three domesticated taxa as distinct species. The corresponding wild (ancestral) forms are interspersed to such an extent that they are often given distinct taxonomic names, which is endorsed by Eshbaugh (1993). We will discuss this questioning below, with the new molecular data obtained from these species.

Considering this evolutionary and taxonomic background, archaeobotanical research is needed to resolve the identity of the chili-bell pepper remains in the contexts of the northern coast of Peru. The main objective of this work is to identify their seeds from Chimú archaeological contexts (Fig. 1). They are the most common remains of chili peppers recovered from the excavations of these sites, which are mostly identified as *Capsicum* spp.

Figure N° 1. Geographical location of the three sampled sites on the northern coast of Perú.

Background

The first systematic studies of *Capsicum* remain on the northern coast of Peru were carried out by Pickersgill (1969) (Table 1). Chili-bell pepper remains at Huaca Prieta were present from the earliest, being considered as probably cultivated (Towle, 1961). Pickersgill's analyses indicated the presence of red and orange fruits in the earliest levels of the site (H.P.3 Ql). Such variability in fruit color suggests selection under cultivation because orange fruits are not reported among wild *Capsicum* species. One of the fruits at this early level still had the calyx present, implying that the non-deciduous characteristic prevents the fruits from detaching rapidly and, therefore, there is a reduction in seed dispersal. Therefore, this botanical characteristic was already established at that time and is characteristic of cultivated peppers.

Table 1.- *Capsicum* seed samples from three archaeological sites

N° Muestra	Sitio	Contexto	Cantidad
1 (5)	El Brujo: Cao Viejo Temporada 1993	Sector SE U: S 0-10 E 80-90 Coord: S 9-10 E 87-90 Capa B Nivel 1	1
2 (6)	Túcume: Huaca 1 Temporada 2011	Sub Sector 1A Unidad IV i Cuadrícula 17 Capa 1	2
3 (7)	Túcume: Plaza Central Temporada 2011	Unidad XXXZ Cuadrícula 3 Capa 4	3
4 (8)	Cerro La Virgen Casma Temporada 1993	Corte 1 Capa A Nivel 1	1
5 (9)	Túcume: Sector I Temporada 2011	Sub Sector 1A Unidad IV i Cuadrícula 9 Capa 5	3
6 (10)	El Brujo: Cao Viejo Temporada 1993	Sector SE U: S 0-10 E 80-90 Coord: S 9-10 E 87-90 Capa B Nivel 1	3
7 (11)	El Brujo: Cao Viejo Temporada 1993	Corte 1	2
8 (12)	El Brujo: Cao Viejo Temporada 1993	Sector NE Corte 2 (Bolsa 46)	1

At the next level (H.P.3 PI), there were several loose seeds along with some intact dehydrated fruits. The mean seed width was 2.93 mm (range from 2.6 to 3.1 mm). These values are within the range of cultivated plants, but not wild ones. Comparative data from Eshbaugh (1964) indicated that the semidomesticated *C. baccatum* var. *baccatum* had a mean seed width of 2.4 mm, whereas the cultivated Peruvian pepper (*C. baccatum* var. *pendulum*) had a mean of 3.0 mm.

Identifying with certainty all four species of *Capsicum* fruits in archaeological material require a preserved calyx. Yet, that is not common. In *C. chinense* and *C. frutescens*, the calyx is shorter in relation to the length of the fruit. In the case of peppers from Huaca Prieta (level H.P.5 B2), preserved calyces with well-developed teeth have been found. However, the other characters of the calyx of

the fruits of the early levels suggest that they are *C. baccatum* var. *pendulum*. The presence of this variety would indicate the existence of its wild ancestor, (*C. baccatum* var. *baccatum*). It has a rather restricted distribution at present and is confined almost entirely to southern Peru and Bolivia (Pickersgill, 1969).

Archaeological chili-bell pepper-seed samples (no whole or fragmented fruits) were found by Dillehay and Bonavía in Huaca Prieta and Paredones (seasons 2007 to 2010), being systematically studied by Chiou and Hastorf (2012). Such studies showed significant differences in seeds from phases 2 and 4 at the Huaca Prieta and Paredones sites (Chicama valley). That contrasts with the rigid homogeneity found in seeds of phase 5. After rigorous discriminant analyses, including modern seeds, involving measurements of the six strongest quantitative values and attributes, seeds of *C. pubescens* were identified in phase 2. In phase 4, seeds of *C. frutescens, C. baccatum* and *C. chinense* were identified. Phase 5 only contained seeds of *C. baccatum* (Chiou and Hastorf, 2012).

Other analyzed archaeological materials of *Capsicum* came from excavations at Punta Grande (1.810 BC) on the Central Coast. That allowed recovery of chili-bell pepper remains. They contained calyxes, therefore being from cultivated plants. The average width of 18 seeds from cut 2, level 2 was 3.07 mm. This falls within the range of cultivated peppers, but since preservation is not optimal, it is also possible that they belong to *C. baccatum* var *pendulum*.

In Los Gavilanes (Huarmey valley), for epochs 2 and 3, chili-bell pepper seeds were identified. Their widths of 3 to 3.5 mm suggest that they could be cultivated forms, and probably correspond to *C. baccatum* (Bonavía, 1982). Other reports for this time (having references to *Capsicum* only), are those indicated for Áspero (Feldman, 1980), Alto Salaverry (Pozorski, 1976), Guitarrero Cave (Lynch, 1978) and in the Asia site, where *C. frutescens* is mentioned, albeit without providing morphological details supporting such identification (Engel, 1964).

In Guitarrero Cave, habanero pepper (*C.* cf. *chinense*) is mentioned (Pearsall, 1978). However, Pickersgill (1980) when analyzing the remains, indicated that these would be cultivated *C. chinense*, which come from Complex II of the cave, and therefore with an antiquity of 8.500 years BC (Bonavía, 1982). This would imply that *C. chinense*, would be older than *C. baccatum* var. *pendulum*, with the domestication process of the chili-bell pepper in earlier contexts than those found in Huaca Prieta.

For the initial period (2.100-1.400 years BC) of the site El Tanque (Ancón-Chillón), remains assigned to *C. chinense* are mentioned (Cohen, 1978). During the early horizon (1.200 years BC), this second species of chili-bell pepper (*C. chinense*), appears again. Nevertheless, later excavations showed that this species was found in preceramic and Initial period sites.

The center of diversity of *C. chinense* is found in the Amazon basin. From there, passing through the Andes, it may have reached coastal sites. At the time of the Chavín influence extending along the northern coast, the appearance of the *C.*

chinense and *C. frutescens* complex has been reported. Although *C. frutescens* was cited for Huaca Prieta, it seems that its history is parallel to that of *C. chinense*. These species had small seeds and deciduous fruits, and persisted as crops on the coast, without replacing each other, until the present (Pickersgill, 1969).

For the early intermediate period (400 BC - 450 AD), there is a vessel from the Nasca culture with a representation of a string of *C. pubescens* held in the hands of a man. In this same period, at the Estaquería site in the Nasca valley, *C. chinense* fruits are mentioned, but there is no specific data (Ugent and Ochoa, 2006).

In the late intermediate period (900-1.450 AD), there are numerous representations on Chimú ceramics of *C. annuum* fruits, and other fruits identified as *C. frutescens* (Yacovleff and Herrera, 1934). Dehydrated fruits of *C. pubescens* were discovered at the Necropolis of Ancon site (1.300 years AD), which implies that the four species of chili described above were present in the pre-Hispanic Peruvian coast. Thus, scanning-electron microscopy (SEM) of well-preserved seeds was carried out.

Materials and Methods

Samples

Samples of *Capsicum* seeds were chosen from three archaeological sites of the late intermediate period (900-1.450 years AD): Túcume (La Leche valley), El Brujo (Chicama valley) and Cerro La Virgen (Casma valley) (Fig. 1). These seeds were chosen because of their good preservation and association with cultural elements from that period. Samples were carefully treated with surface cleaning, before being submitted to scanning-electron microscopy.

Conditioning and preliminary classification of remains

Collected samples were classified according to sites. The seeds were conditioned and compressed air, was used to remove dust from them (Fig. 2). It is important to clean the surface topography of the seeds because it contains the fingerprint for their taxonomic characterization by microscopic techniques. Once the remains were removed, seeds were subjected to microscopic observation with a stereoscopic microscope at 50X. Seeds were sorted in relation to their shape and texture of the testa (appearance of the seed coat, which allows to differentiate between species). The five species domesticated in pre-Hispanic times showed different shapes (Fig. 2) and patterns of reticulation or smooth surfaces. This preliminary classification represents the preliminary phase for further analyses using scanning-electron microscopy. Seeds were integrated into a SEM database of different domesticated *Capsicum* species.
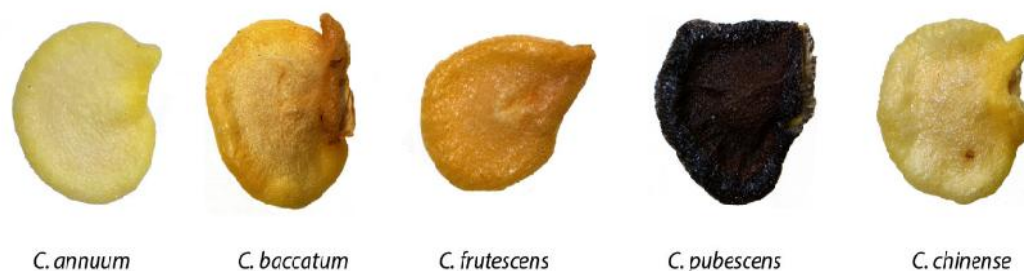
Figure 2. Morphology of the seeds of the five species of *Capsicum* cultivated in Peru. They represent the basis for the preliminary classification of the archaeological remains, according to Chiou and Hastorf (2012).

Figure 3. Archaeological *Capsicum* spp. seeds, before their cleaning treatment for preliminary classification.

Optical microscopy, biometry, and statistical analyses

Once the preliminary classification was done, seeds were subjected to biometric measurements, recording their length and width. That was were carried out using the SEM detector/probe. Studied qualitative/nominal attributes include shape of seed and beak prominence, as well as testa texture. *Capsicum* seed shape is relatively distinct from species to species, with some overlap. Testa texture refers to the appearance of the seed coat. Seed-coat texture is a useful diagnostic feature, especially in the case of *C. pubescens*, which shows an exaggerated reticulation pattern on the outer margins of the seed. On the other hand, beak angle is defined as the area protruding from the seed, allowing to differentiate *Capsicum* seeds from the ones of other species of the Solanaceae family with otherwise similar appearance. A high beak angle, for example, is one of the differentiating attributes of *C. frutescens* seeds (Chiou and Hastorf, 2012). Length and width are two basic measurements (Fig. 3). All seeds were measured in the same way.
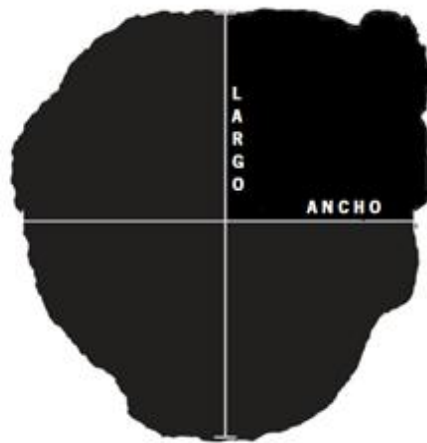
Figure 4. Measurements of length and width of *Capsicum* seeds.

In summary, morphometric attributes revolve around two measurements of all seeds, seed surface topography and seed beak-angle shape. Seed measurements were subjected to descriptive statistics, to obtain the mean and standard deviation of samples from each site.

Scanning-electron microscopy and taxonomic identification

To consolidate the morphological and biometric attributes described above, each seed studied was subjected to SEM. The seed surface shows patterns and reticulations, being therefore appropriate for such kind of surface-scanning study (Fig. 5). That allowed to differentiate the five domesticated species. This part was carried out in the Scanning-Electron Microscopy laboratory of the National Museum of Natural Sciences in Madrid (Spain). The corresponding qualitative contrast comparison was performed, and seed sizes were compared with available data.
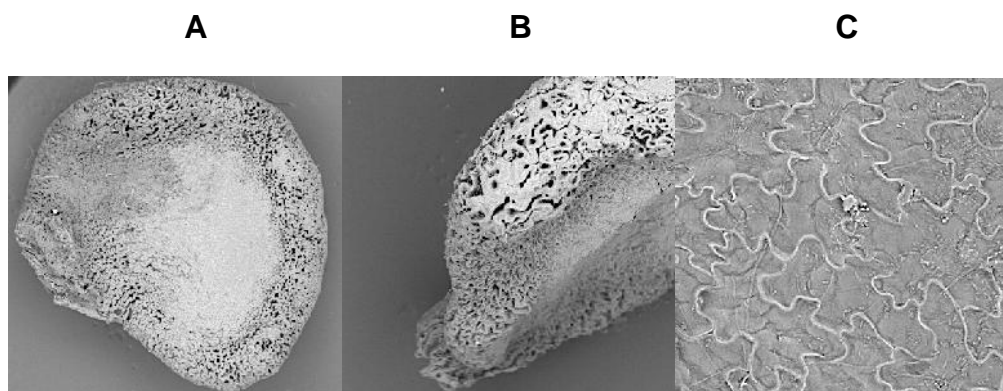
A   **B**   **C**

Figure 5. Pattern of reticulations on the surface of the seed of *C. pubescens*, by scanning-electron microscopy. (A) Complete seed at 50X; (B) Deep reticulation on the margin of the seed at 60X; and (C) Smooth, sinuous crosslinking of the testa surface at 300X. Photos property of ARQUEOBIOS, all rights reserved.
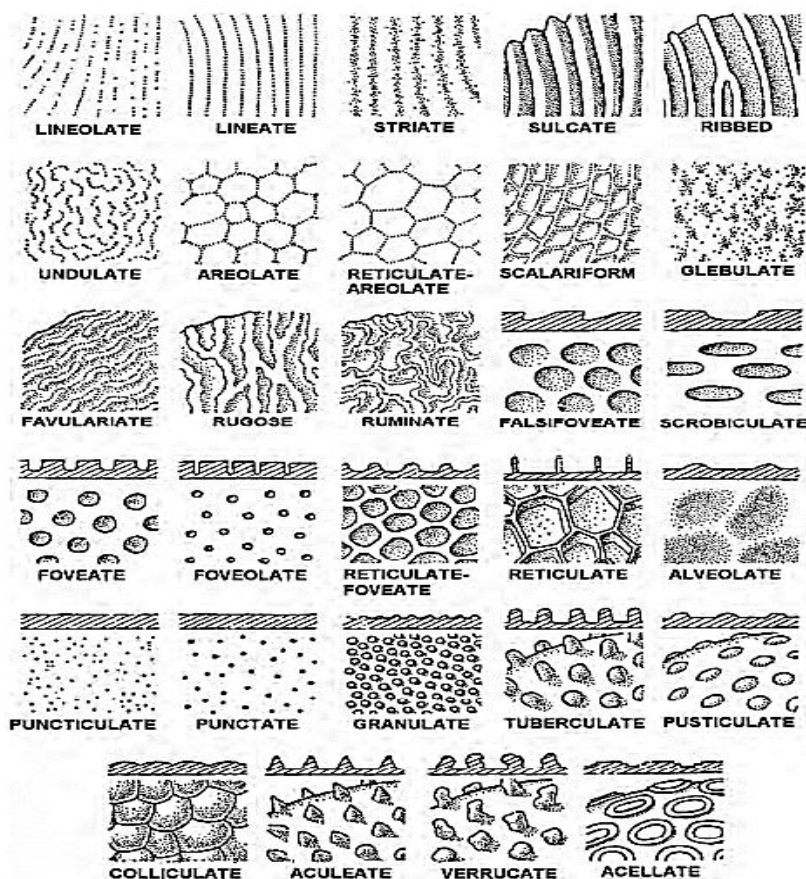
Figure 6. Types of surfaces of the testa of the seeds. Source: Murley, 1951.

Results
Biometrics
The measurements of eight seeds from the Túcume site, six seeds from the El Brujo site and one seed from Cerro La Virgen, yielded the following values:

Table 2. Length and width of *Capsicum* seeds. Samples were obtained from three archaeological sites on the northern coast of Peru.

Sites	Measurements (mm)	
	Length	Width
Tucume	4.42	4.1
	4.1	3.63
	3.96	3.78
	4.05	3.19
	3.48	3.26
	3.45	3.36
	3.09	2.94
	3.57	3.11
El Brujo	3.72	3.38
	4.67	4.38
	3.96	3.81
	5.23	4.3
	4.45	4.18
	3.67	3.45
C° La Virgen	4.18	3.83

Table 3. Length and width of seeds identified as *Capsicum frutescens*.

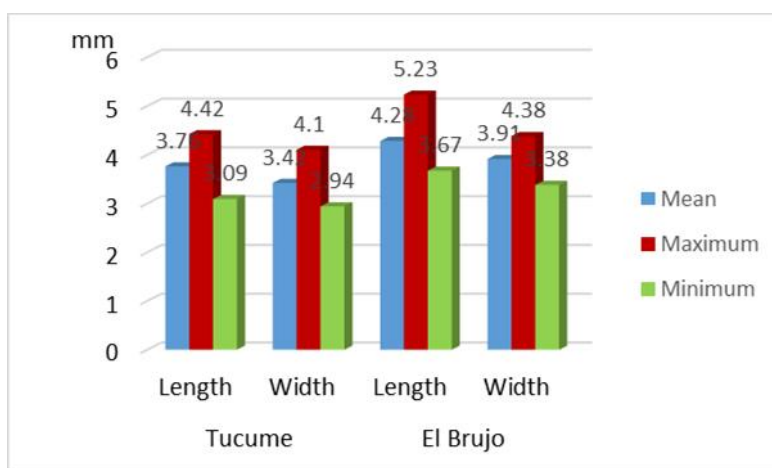| DE	Sites			
	Tucume		El Brujo	
	Length	Width	Length	Width
Mean	3.76	3.42	4.28	3.91
Maximum	4.42	4.1	5.23	4.38
Minimum	3.09	2.94	3.67	3.38
DS	0.43	0.38	0.61	0.43
N	8		6	

Figure 7. Average, maximum, and minimum length and width values of *C. frutescens* seeds from Túcume and El Brujo sites.

Modern C. chinense and C. frutescens seed-surface topography using SEM
SEM photographs are shown for the seed-surface topography of *C. chinense* and *C. frutescens*. They are closely related species, but with significant differences in morphological details of the seed surface, allowing to differentiate them.
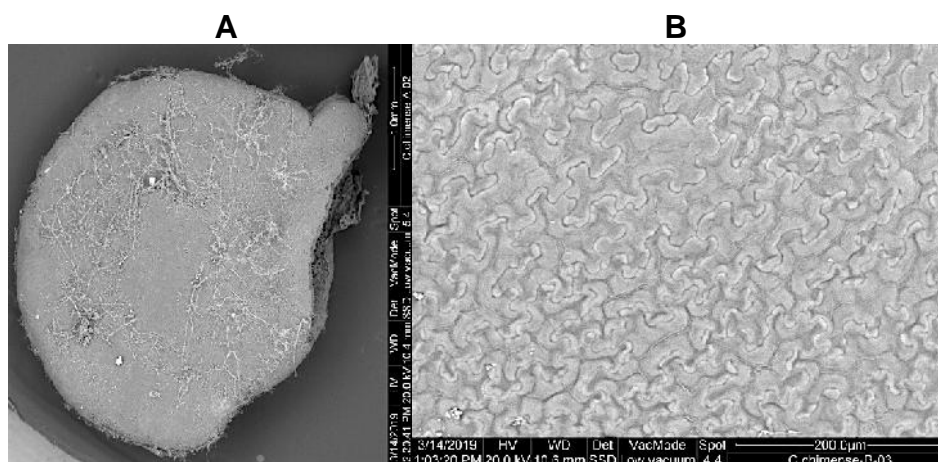
A	B

Figure 8. Modern seed of *Capsicum chinense*. (A) Generally, they are circular, with protruding beak at a great angle, and a small "lip" below it. Captured with SEM at 50X; (B) Smooth surface texture with little indication of reticulation at the epidermal level, sinuous, ruminated. Captured with SEM at 300X. Photos property of ARQUEOBIOS, all rights reserved.
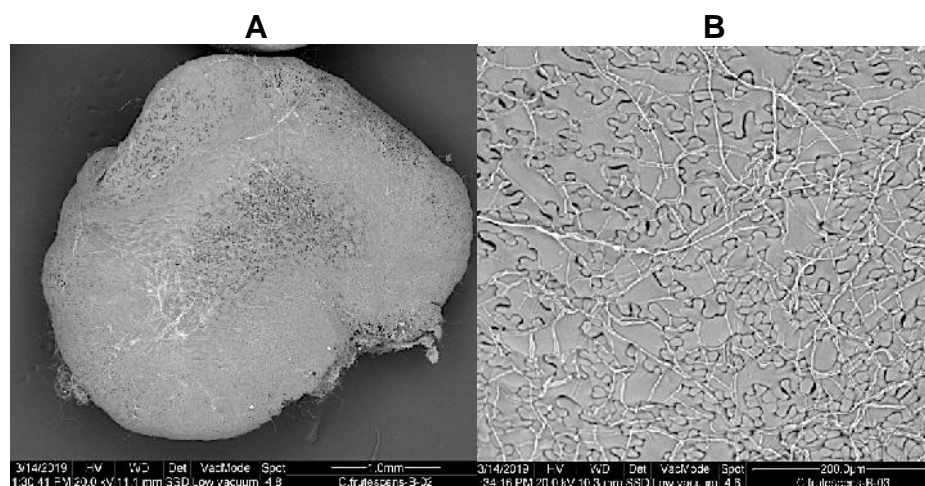
Figure 9. Modern seed of *Capsicum frutescens.* (A) It shows a spherical shape. Capture with SEM at 50X; (B) Smooth-surface texture with wavy-dendritic shapes, capture with SEM at 300X. Photos property of ARQUEOBIOS, all rights reserved.

Topographies of archaeological seeds of Capsicum frutescens

Three seeds from the Túcume site were analyzed by SEM, showing characteristics of seed shape and topography of the preserved surface of the testa. Images that best represent the characteristics of this species are shown.

Sample 7a (Túcume)

Figure 10. SEM of *Capsicum frutescens* seed from the Túcume site. (A) Complete seed at 50X; (B) Wavy-dendritic reticulations of the seed surface (sample 7a of Table 1), which typifies the species, captured at 300X. Photos property of ARQUEOBIOS, all rights reserved.

Sample 9b (Túcume)

Figure 11. SEM of *Capsicum frutescens* seed from the Túcume site. (A) Complete seed at 50X; (B) Wavy-dendritic reticulations on the surface of the seed that has lost the epidermis but maintains the trabeculae and edges of the sinuous and dendritic reticulations (sample 9b of Table 1), which typifies the species, captured at 300X. Photos property of ARQUEOBIOS, all rights reserved.

The seed samples showing the characteristics of shape and surface (testa) from El Brujo site are shown below.
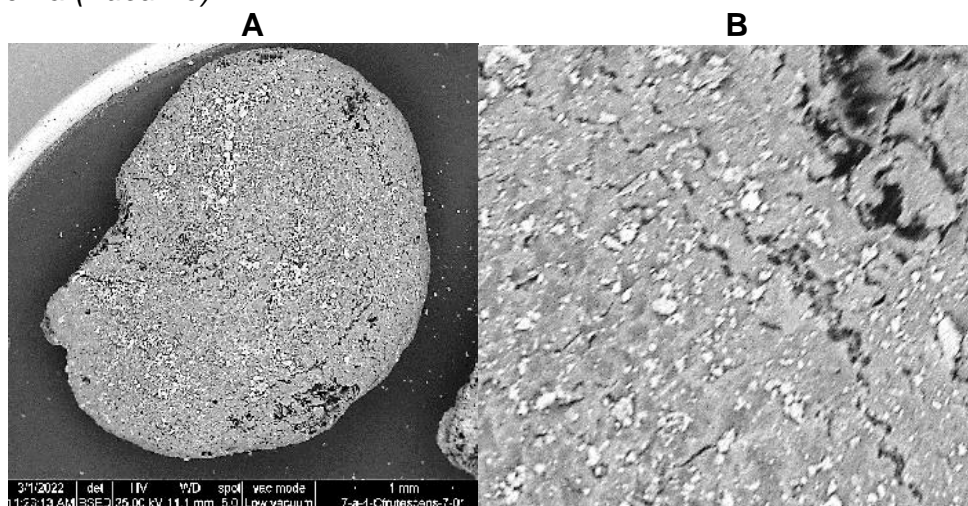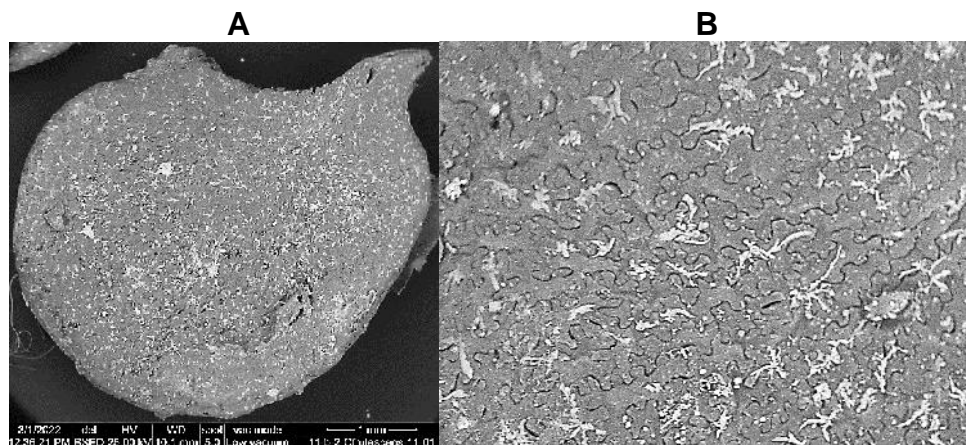
Sample 10b (El Brujo)

Figure 12. SEM of *Capsicum frutescens* seed from El Brujo site. (A) Complete seed at 50X; (B) Wavy-dendritic reticulations of the seed surface (sample 10b of Table 1), which typifies the species, captured at 300X; (C) Enlarged view of the wavy-dendritic reticulations of seed testa, captured at 500X. Photos property of ARQUEOBIOS, all rights reserved.

Sample 11b (El Brujo)

Figure 13. SEM of *Capsicum frutescens* seed from El Brujo site. (A) Complete seed at 50X; (B) Wavy-dendritic reticulations of the seed surface (sample 11b of Table 1), which typifies the species, captured at 300X. Photos property of ARQUEOBIOS, all rights reserved.

Discussion

Capsicum seeds from three archaeological sites of Peru were studied. Biometric data of samples from Túcume and El Brujo showed differences in length and width. Greater values were observed for six seeds from El Brujo site. That may be due to: i) There was a greater fruit selection at El Brujo, with larger fruit sizes implying also larger seeds. This situation would have been different for the seeds from Túcume (Tables 1 and 2 and Fig. 7); ii) Other possibility is taphonomy variables, such as seed dehydration contributing to the decrease in seed size, apart from influencing diagnostic qualitative characteristics.

Comparative data on measurements of archaeological seeds of *Capsicum* have been reported for the preceramic levels of Huaca Prieta. Thus, in H.P.3 P1, several loose seeds presented an average width of 2.93 mm (Pickersgill, 1969), indicating that they are cultivated peppers. At the same site, seeds of *C. baccatum* var. *baccatum* were identified, showing an average width of 2.4 mm. In this case, they are a wild chili-bell pepper. Other seeds, identified as *C. baccatum* var. *pendulum*, had an average width of 3 mm (Eshbaugh, 1964). The latter is the domesticated or cultivated variety of *C. baccatum*.

Chiou and Hastorf (2012) analyses of chili-bell pepper seeds from Huaca Prieta identified four species of cultivated peppers: *C. chinense, C. frutescens, C. baccatum* and *C. pubescens*. The latter species was cultivated in the Andes. It is indicated that the values of *C. baccatum* and *C. pubescens* are well separated by canonical analyses, while the *C. frutescens* and *C. chinense* species exhibit overlapping values.

On the central coast at the Punta Grande site (1810 BC), the mean width of 18 seeds was 3.07 mm. Therefore, they were cultivated peppers, being identified as *C. baccatum* var. *pendulum* (Pickersgill, 1969).

For late sites, systematic studies of chili peppers are very scarce, and the identification of the remains is done at the generic level. Therefore, no biometric data of the seeds or descriptions of dried fruits are available. Remains of chili-bell pepper seeds from contexts at the Pachacamac site were measured and described in shape, for a total six seeds. Five of them were identified as *C. chinense* and one as *C. baccatum* (Chiou and Hastorf, 2015). The average seed length of *C. chinense* seeds was 4.37 mm. That is very close to the average value of *C. frutescens* seeds from the El Brujo site (Table 3).

Seed length and width ranges of *C. chinense* and *C. frutescens* species depend on their evolutionary characteristics and taxonomic backgrounds, including hybridization events. In the case of Huaca Prieta, they show overlapping values, being identified by qualitative characteristics (Chiou and Hastorf, 2012).

The six studied specimens from Pachacamac were relatively poorly preserved. Four seeds were identified as *C. chinense*, one specimen potentially being *C. baccatum* and other considered as *C. frutescens*. Yet, that is open to debate, considering the difficulties to differentiate modern seeds of *C. chinense versus C. frutescens*, being even harder for archaeological samples.

Another key attribute used in our analyses was SEM of testa surfaces of seeds from Túcume and El Brujo. We have a database of images of testa surfaces of modern *C. annuum*, *C. baccatum* var*. pendulum, C. chinense, C. frutescens* and *C, pubescens* seeds. That allowed us to identify archaeological seeds from Túcume and El Brujo sites (Figs. 10 to 12). We have also considered the shape of the seeds. Nevertheless, that may not be convenient, due to the lack of preservation of the beak or radicle in archaeological seeds. Therefore, we have used the scanning of the surface of the seed testa.

The seeds of *C. frutescens* are teardrop-shaped with a protruding beak, forming the tip of the seed. The texture of the surface of the testa is generally smooth, and for *C. chinense*. The seed is generally circular in shape with a "fish-mouth "-shaped attachment opening. It comprises the beak protruding at a large angle, and a small "lip" below it. The seed surface texture is smooth with little hint of reticulation at the epidermal level, as exhibited at 50X, 300X and 500X SEM magnifications (Chiou and Hastorf, 2012). We have also found discriminating details for *C. frutescens* and *C. chinense*. Thus, we have found that *C. chinense* has a smooth-surface texture (Fig. 8), with sinuous and ruminated (intestinal laps-like) reticulations (Fig. 8B). In the case of *C. frutescens*, the surface texture is also smooth, but with wavy forms and dendritic patterns (similar to dendrites) (Fig. 9B). Therefore, seed testes from both archaeological sites were in good preservation. Thus, we identified the seeds from Túcume and El Brujo as *C. frutescens* (Table 3, Figs 7 and 10 to 12).

In the case of the Huaca Prieta samples, taxonomic identifications were carried out using various qualitative attributes and measurements. When subjected to

canonical analyses, allowed the identification of four species, highlighting the non-existence of *C. annuum*, a species that was domesticated in Mexico (Chiou and Hastorf, 2012). In the case of the Pachacamac samples, identifications were based on the shape of the beak or radicle, as well as the transverse cross-section (seed margin). There are no images of the surface topography because the seeds had poor preservation and absence of most part of the testa surface (Chiou and Hastorf, 2015).

On the other hand, it should be noted that the seeds analyzed in this work from both sites (Túcume and El Brujo) were recovered from domestic contexts (living quarters). Previous works have reported that the seeds of *C. pubescens* and *C. frutescens* from Huaca Prieta were also found in domestic contexts, while *C. baccatum* was found mainly in banqueting areas, and *C. chinense* was recovered from all contexts of Paredones (Chiou and Hastorf, 2012).

We have not identified any *C. pubescens* seed in Chimú site. That would indicate that this high Andean chili-bell pepper would not have been available in these late coastal sites. It should be noted that *C. pubescens* seeds are characterized by very thick and highly reticulate margins, which would be immediately identifiable by SEM scanning.

According to existing data, *C. chinense* was widespread along the pre-Hispanic Andean coast, just as *C. baccatum* was also present along the coast, from the preceramic to the late intermediate, as mentioned for Huaca Prieta, sites from the early period, early intermediate, to Pachacamac (Chiou and Hastorf, 2012, 2015).

The texture of the seed epidermis from El Brujo correspond to *C. frutescens* (Fig. 12B, 12C and 13B)*,* discriminating them from the other three South American species. Yet, there is a discordance in some cases, since the shape of the seeds and the beaks or radicles have characteristics of *C. chinense* (Fig. 12A and 13A). Why do we have these contradictions between these two characteristics? We will try to explain it from the evolutionary, taxonomic, and genetic point of view, in order to put shed light for further studies.

Pickersgill (1971) points out that *C. chinense* is a closely related species to *C. frutescens*. The latter usually has small deciduous fruits and is found in weedy conditions; and in some cases, escaped from cultivation. This raises the question of whether *C. frutescens* is the progenitor or ancestor of *C. chinense*. Indeed, the differences between *C. chinense* and *C. frutescens* are much smaller than between other pairs of domesticated wild species, such as domesticated *C. annuum* var. *annuum* versus wild *C. annuum* var. *glabriusculum*; and domesticated *C. baccatum* var. *pendulum* versus wild *C. baccatum* var. *baccatum*.

Ecologically, *C. chinense* and *C. frutescens* are sympatric throughout their range (Peru, Brazil, Colombia, and Bolivia. Studies of their distribution have not allowed to decipher which one is the older ancestor. No natural hybrids between *C. frutescens* and weedy forms of *C. baccatum* have been recorded, but there

are hybrids between *C. frutescens* and *C. chinense*, of which there are no specific studies, for example, of their seeds.

Molecular studies by Walsh and Hoot (2001) of the atpB-rbcl region of the chloroplast were used to ascertain the phylogeny of 11 species of *Capsicum*. The results showed that *C. annum* (the species domesticated in Mexico), *C. frutescens* and *C. chinense* are closely related, with the two last ones having a closer genetic relationship. *C. chinense* is somewhat more distant from *C. annuum*. On the other hand, *C. galapagoense* showed between *C. frutescens* and *C. chinense*. That would imply that *C. galapagoense* would have had a continental origin.

Eshbaugh et al, (1983a), using data from isoenzyme studies, proposed that *C. annuum, C. frutescens* and *C. chinense* form a close-knit complex. They would have arisen from an ancestral gene pool, giving rise to the gene pool of *C. frutescens* and *C. chinense*. Taxonomists have a dilemma as to whether to recognize one, two or three species within this complex. On the other hand, five distinct taxonomic species are recognized for commercial purposes. Qualitative seed databases with morphological characteristics of the whole plant should be taken into account, in order to better classify these plants.

The nuclear-ribosomal DNA internal-transcribed spacer (rDNA-ITS) region has been widely used for species identification but has rarely been used in *Capsicum*. Genetic diversity assessments and phylogenetic analyses were carried out using rDNA-ITS of 28 *Capsicum* accessions. They included five domesticated and two wild species. Genetic diversity was evaluated using single-nucleotide polymorphisms (SNP). *C. annuum* had the lowest genetic diversity of all species in this study. The phylogenetic tree formed a species-specific clade for *C. annuum, C. baccatum* and *C. pubescens*. *C. chinense* clade overlapped was within the *C. frutescens* clade, implying that it was a cultivated variant of *C. frutescens*. This study indicated that the rDNA-ITS region can be used for simple identification of domesticated *Capsicum* species. It demonstrated the nearly parallel evolutionary origin of *C. frutescens* and *C. chinense* (Shiragaki et al, 2020).

C. frutescens and *C. chinense* are morphologically similar. Molecular studies further show their genetic similarity. However, the genetic relationship between these two species is not yet conclusive. Archaeobotanical studies of chili-bell pepper seeds of these two species should be carried out to shed light on this topic. Phylogeny studies should consider the evolution, taxonomy and genetics of these two species. Therefore, further work is needed at the archaeological, morphological and molecular levels. For instance, comparing ancient DNA and their modern representatives, to find out the ancient and modern relationships of these species of chili-bell pepper.

Conclusions

The canonical-statistical techniques and the resolutive power of scanning-electron microscopy are currently the best tools for the study of seeds of archaeological origin. In the case of cultivated seeds of the *Capsicum* genus,

these techniques are proving efficient for the identification of cultivated chili-bell pepper seeds. That way, it is possible to obtain information on their evolution and dispersal in different periods and sites on the Peruvian coast. Additionally, it should allow to further discover other aspects of their domestication and ecology. The good conservation of botanical remains in the dry Peruvian coast should continue to allow these studies. That may include new research developments on seeds and fruits of cultivated *Capsicum*. That should allow to compare the genetic information available on the four cultivated species of *Capsicum* in this territory.

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