Author's Accepted Manuscript

Identifying crop plants with phytoliths (and starch grains) in Central and South America: A review and an update of the evidence
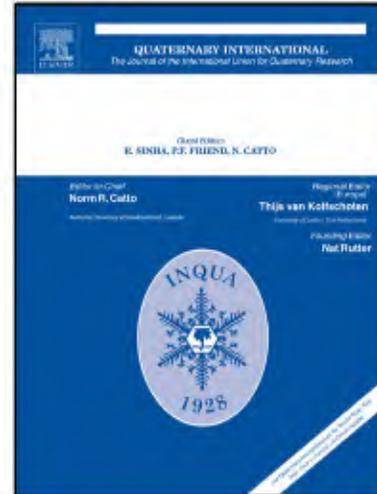
Dolores R. Piperno

Identifying Crop Plants with Phytoliths (and Starch Grains) in Central and South America: A Review and an Update of the Evidence

Dolores R. Piperno

Archaeobiology Program, Department of Anthropology, National Museum of Natural History, Washington, DC; and

Smithsonian Tropical Research Institute, Balboa, Panama.

Abstract

Research carried out during the past 25 years by a number of investigators has demonstrated that phytoliths from some important domesticated plants native to Central and South America can be identified. These plants include major seed crops such as maize (*Zea mays*), important vegetables such as squashes of *Cucurbita* spp., and now-minor root crops (e.g., *Calathea allouia* and *Maranta arundinacea*) that were probably more important in early pre-Columbian economies than they are today. In some cases, phytoliths can identify the wild ancestors of these plants. The New World patterns follow those demonstrated in other areas of the world--out of all of the domesticated species indigenous to a region, a few important ones (e.g., rice, bananas, wheat and barley) can be identified with their phytoliths. In this paper, I review identification criteria developed for New World plants, summarize the underlying biological mechanisms that are now understood to account for phytolith formation and morphology, and discuss recent archaeological applications and new data from the Central and South American tropical forest. I will also mention the growing importance of starch grain analysis in archaeology with regard to recovering and identifying the remains of wild and domesticated species, including maize and teosinte. Phytoliths and starch grains are turning out to be highly complementary types of studies, and are increasingly being applied together.

Key words: Phytoliths, crop plants, agricultural origins and dispersals, lowland tropical forest

1. Why Plants Make Phytoliths

Not long ago, phytoliths were considered by many scientists to be little more than waste products, solid secretions of the soluble silica that was taken up without purpose by plants along with other, more essential elements for plant growth that occurred in ground water. This view was overturned when purposeful, active uptake of silica was demonstrated in a number of different kinds of plants, and when direct genetic control over phytolith formation was demonstrated in major genera of monocotyledons and dicotyledons. Scientists now believe that silica in plants has a number of important functions, from giving plants structural support, to mitigating the toxic effects of aluminum and other heavy soil minerals, to providing protection from herbivores and pathogens (for reviews see Sangster et al. 2001 and Piperno, 2006a:9-15, see also below).

A number of papers published recently have shed more light on these issues. Massey and Hartley (2006) demonstrated that silica in grasses not only deterred feeding by field voles, but also inhibited the growth rates of the voles by decreasing nitrogen absorption from foliage. Ma et al. (2006), working with rice, identified for the first time in a higher plant a silicon transporter gene, called *Lsi1*, that controls silica uptake. Work of this kind is the final link in establishing a molecular basis for silica uptake and for the significant variability in the amounts of phytoliths that different taxa ultimately make. We may expect that genes homologous to *Lsi1* will be found in other plants. Finally, Fauteux et al. (2006) showed that in the model organism *Arabidopsis*, a member of the mustard family, applications of silicon mitigated the stress imposed by pathogens. Thus, the most recent work has confirmed previous views and amplified understanding about the metabolic mechanisms involved in silicon accumulation and the various important roles that silica plays in plants.

2. Phytoliths in Neotropical Crop Plants

2.1 Introduction

A variety of morphological and size markers have been explored by phytolith investigators in the

Americas and elsewhere to define identification criteria. They include: 1) surface and three-dimensional attributes, 2) size (e.g., maximum and mean length and width), and 3) multivariate statistical analysis of combined size and shape attributes. Phytoliths will typically vary morphologically within a single species, depending on which part of the plant produces them. This factor both increases the amount of comparative reference work required and leads to greater possibilities for identifying more than one structure of a consumed or utilized species in varying contexts from the same site, such as in sediments, on stone tools, in pottery, and even on human teeth.

Sometimes, as in the cases of fruits from squash (*Cucurbita* spp.) and bottle gourd (*Lagenaria siceraria*), maize cobs, and rhizomes of root crops, phytoliths are unique to a genus or species and all that is required to make an identification is to recover these diagnostic forms. For each crop discussed below, dozens to hundreds of wild species, including wild ancestors of crops when known, were studied by a number of investigators in independent analyses. This makes it possible to discuss consensus identification criteria developed for the crops.

2.2 Cucurbita and Other Cucurbitaceae

2.2.1 Cucurbita

Phytoliths from the genus *Cucurbita* are highly useful for documenting the earliest history of domesticated squashes and gourds in the Americas. Six different species of *Cucurbita* ranging from eastern North America to southern South America were domesticated before the European Contact, and some of these were among the earliest plants taken under cultivation and domesticated in the New World (Smith 1997; Piperno and Stothert 2003). All parts of the *Cucurbita* plant make high amounts of phytoliths, but the most useful ones, which are diagnostic to the genus and sometimes species, originate in the fruit rind (Fig. 1) (Piperno 2006a). Bozarth was the first to observe and describe these large, spherical phytoliths, noting how they had "scalloped surfaces of deeply contiguous concavities" (Bozarth 1987: 608). Hereafter, they are called scalloped phytoliths.

−3−

Scalloped phytoliths are formed exclusively near the surface of the fruit rind at the interface between the epidermis and underlying mesocarp tissues, a process directed by a gene called *hard rind (Hr)* that codes for both lignin and phytolith production in *Cucurbita* (Piperno et al. 2002). Indeed, *Cucurbita* is one of three genera, all containing major crop plants, where phytolith formation has been recently shown to be under genetic control. The other two are maize and rice (Dorweiler and Doebley 1997; Zheng et al. 2003). In wild species of *Cucurbita*, the dominant *Hr* gene causes the fruit to deposit a heavy layer of scalloped phytoliths and lignified (stone) cells in an interlocking manner within the above-mentioned tissues. This arrangement protects the fruit from herbivores and pathogenic fungi and it is the natural condition in all wild members of the genus. The number of phytoliths coded for by *hard rind* is numerous. In one cm^2 of lignified rind there are thousands of them (Piperno et al. 2002), making their visibility in archaeological sediments very good.

It used to be a mystery when phytolith researchers first studied *Cucurbita* why they couldn't find scalloped phytoliths in many modern domesticated fruits, or even in archaeological sites dating to late time periods, when use of the plant was known or strongly inferred (Piperno 1988; et al. 2000a). With the effects on phytolith production of the *hard rind* gene elucidated, we now understand that when prehistoric humans domesticated *Cucurbita*, they exerted selection pressure for softer, non-lignified, and thus phytolith-free rinds (these would be homozygous for *hr*). These changes made fruits easier to cut and cook. Lignified and silicified rinds are still common in varieties from all domesticated species, and this kind of genetic diversity was likely maintained by some later prehistoric populations as well by the preferential planting of fruits with the dominant *Hr* gene. Harder rinds provide fruits with a longer storage life.

Importantly, the distinctive surface and other features of scalloped phytoliths are determined by their placement in the rind. There are two clearly defined hemispheres of unequal size and sometimes shape, each with different surface features (Fig. 2). The small, regular, and deeply impressed scalloped decorations on the hemisphere of the phytolith that extends into the hypodermal

(sub-epidermal) tissue are made by the hard edges of the small, isodiametric hypodermal cells. The larger, deep impressions on the hemisphere that extends into the mesocarp are made by the wide edges of the lignified stone cells. Also importantly, the tissue and cell arrangements of the fruit rind described above that create these features are the same ones that provide the basis for identifying macroscopic archaeological rind remains from *Cucurbita*, and the differentiation of *Cucurbita* and *Lagenaria*. It appears that the identification of wild and domesticated species may sometimes be made on a morphological basis because in *Cucurbita maxima*, its wild ancestor *C. andreana*, and some land races of *C. moschata*, scalloped phytoliths appear to be unique (Piperno 2006a:66-67; et al. 2002). It should also be mentioned that scalloped phytoliths from *Cucurbita* and other members of the family, described below, are easily separable from superficially similar "facetate" phytoliths produced in tree leaves, which have very different shape and surface features (Figs. 3a and 3b).

The differentiation of wild and domesticated plants using size measurements such as maximum and average length has long been a standard approach in archaeobotany. For example, the seeds and peduncles of *Cucurbita* can often be assigned wild or domesticated status when found in archaeological contexts based on these simple size attributes (e.g., Smith 1997, 2006). Studies of all domesticated and most known wild species of *Cucurbita* show that measuring the long diameter and thickness of scalloped phytoliths often can discriminate wild from domesticated species (Piperno and Stothert 2003). In wild varieties, phytoliths seldom come near a maximum size of 100 μm, whereas in domesticated plants phytoliths often well-exceed 100 μm. Mean length and measurements of phytolith thickness are also effective discriminators.

2.2.2 Other Diagnostic Phytoliths from the Cucurbitaceae

Bottle gourd and a little understood but probably once-important crop native to the Neotropics called cassabanana (*Sicana odorifera*) can also be identified through their scalloped phytoliths. As with *Cucurbita*, the tissue and cellular features of the fruit rind that are used to identify macroscopic remains are used to identify their phytoliths. For example, stone cells in bottle gourd are irregular

and loosely organized, with some being large and elongated and others quite small. These characteristics are impressed into the phytolith surface (Fig. 4). Also in contrast to *Cucurbita,* phytoliths in bottle gourd are hemispherical because solid silica is not deposited in the hypodermis of the rind. Bottle gourd and cassabanana phytoliths are very large (often > 100 µm long) and it is likely that discrimination from their wild ancestors will be achievable on the basis of size (Piperno et al. 2000, Piperno 2006a). Recent analysis of wild bottle gourds from Africa in the author's laboratory, for example, indicates that the phytoliths are much smaller, with a mean of about 60 µm in length, than is typical of domesticated fruits from the Americas (author, laboratory notes). Further research may reveal that phytoliths can play an important role in following the domestication and dispersals of bottle gourd in Africa and Asia.

2.3 Phytoliths and Root Crop Identification

The major vehicle for the identification of most root crops in archaeological sites will probably become starch grain analysis. Starch grain studies are increasingly being carried out in tandem with phytolith analysis and the techniques are highly complementary (Dickau et al. 2007; Pearsall et al. 2004; Perry et al. 2006; Piperno 2006b). While phytoliths in underground organs generally haven't been studied as intensively as in above-ground structures, it is clear that phytolith content in many root crops is not good. However, manioc, yams, and other root crops make copious numbers of identifiable starch grains in their underground structures (e.g., Perry 2004; Piperno 2006b). Present information does indicate that the rhizomes, tubers and corms of certain families such as the Marantaceae and Heliconiaceae can have a high phytolith content (Piperno 2006a). Recently, using a large comparative collection of phytoliths from above- and underground plant organs, Chandler-Edzell et al. (2006) described diagnostic phytoliths from the rhizomes of *lerén* (*Calathea allouia*). The phytoliths were not observed in other plants in Chandler-Edzell et al's study or previous research by these and other investigators, and the phytoliths may be species-specific. The Chandler-Edzell study also reported diagnostic phytoliths in roots and other parts of manioc.

Previous work has shown that arrowroot (*Maranta arundinacea*) and *lerén* have diagnostic seed phytoliths, which are distinguishable from closely related congenerics in their respective families (Piperno 1989, 2006a). Seeds are not the plant structure of choice with which to identify root crop cultivation because they would not be expected to have been commonly deposited into human occupations even if the plants were grown. But the phytoliths have nevertheless been found in some early prehistoric sites (below). Rhizome phytoliths occur in arrowroot in high numbers but are less useful because they are not diagnostic at even the genus level (Piperno 2006a). More work has to be done with underground plant organs, but it appears that phytoliths can provide data on a few important economic species.

2.4 Maize

Maize has seen the most work of any New World domesticated plant and a considerable amount of information is available on phytoliths in its wild ancestor, teosinte, as well. Investigators such as Muholland (1993), Bozarth (1993a,b), Bozarth and Guderjan (2004), Iriarte (2003a, b ; et al., 2004), Pearsall (1978; et al. 2003), the author (Piperno 1988, 2006a; Piperno and Pearsall 1993, 1998a), and others have studied maize leaf and cob phytoliths and compared them against phytoliths from large collections of wild grasses. As near as the author can count, over 500 wild species that could conceivably contribute phytolith types used to identify maize and more than 40 races of maize have been analyzed. The accumulated data show that a number of different criteria can be used to identify maize leaves and cobs in archaeological contexts; often multiple markers are used in tandem, providing a considerable degree of robustness to identifications. Two main types of phytoliths are used to identify maize and distinguish it from its wild ancestor teosinte; cross-shaped, which occur mostly in leaves, and rondels, which occur mainly in the glumes and cupules of cobs. The following features have been demonstrated to be important for discriminating maize leaves. First, cross-shapes from maize are often significantly larger than in wild grasses (here width is measured, not length), and they possess different three-dimensional attributes. Variant 1 cross-

bodies from maize usually have an average width of from 12.7 and 15 μm, whereas the preponderance of wild grasses contribute smaller cross-bodies of between 10 and 12.5 μm in average width. Also, maize leaves possess dominant proportions of mirror-image, Variant 1, cross-shapes, whereas many wild grasses, including Balsas and other varieties of teosinte, possess dominant proportions of other types of three-dimensional structures (see Piperno, 2006a, Tables 3.3-3.5).

Figure 5 illustrates some of the different three-dimensional forms of cross-shaped phytoliths recognized in maize and wild grasses. Their patterns of occurrence mirror taxonomic affiliations of plants. For example, Variant 3 cross-shapes and various "blocky" forms are confined to bamboos; Variant 2–the tent-shaped form–is particularly characteristic of Balsas teosinte and a few other wild grasses; "scooped" cross-shapes (and bilobates) are found in the Ehrhartoideae sub-family (rice and wild relatives). It should be mentioned in order to avoid possible confusion that Variant 5 and 6 types were merged into one category in Piperno (1988). In Piperno (1984), when the 3-D features were first described, Variants 5 and 6 were separated, and it was noted that they were sometimes difficult to distinguish and may eventually be subsumed into one group. They were then combined for the formulation of the original discriminant function in Piperno (1988). Piperno, Pearsall (including in Pearsall and Piperno, 1990) and others using the technique (e.g., Iriarte, 2003b, et al. 2004) have consistently applied this procedure since then[1].

_____

[1]Piperno (1988, pg. 71) inadvertently copied the statement from her 1984 paper on possibly combining Variant 5 and 6 in the future into a footnote in her 1988 book. By referring to other discussions in the 1988 phytolith book and presentation/discussions of the same data in Piperno

The reasons behind these size and shape differences documented in cross-shaped phytoliths from maize, teosinte, and wild grass phytoliths can be substantially explained by taking into consideration where in the leaf epidermis the phytoliths form. For example, Variant 1 types, which usually are larger than Variant 6s and 2s, are located predominantly over the veins, whereas Variant 2 and 6 occur in between the veins (see Piperno, 2006a:60 for a detailed explanation and illustrations). Therefore, the kinds of three-dimensional forms that dominate leaf phytolith assemblages in grass species are determined by how many cross-shaped phytoliths are produced in these respective areas of the leaf. Such attributes of the grass epidermis were recognized long ago by plant anatomists like Metcalfe (1960) to be fundamental characteristics of grass genera and species.

The size and shape criteria just discussed can be analyzed formally with multivariate statistics. Between 1988 and the present, three discriminant function studies were carried out on three different data sets (Piperno 1988:82-84; Pearsall 2000; Piperno 2006a:55). The newest one (Piperno 2006a) incorporated all the data the author accumulated since 1988 on Neotropical, Amazonian, and Andean grasses representing different species than those studied in the 1988 formula, as well as Iriarte's (2003b) extensive data set from southern South American. Pearsall's 2000 study used many dozens of previously unstudied grass species from the Neotropics. The data, compiled by a number of different operators in the investigators' respective laboratories, were given to biostaticians for analysis. The results of the discriminant functions are the same with each analysis; using 3 variables—mean size of Variant 1 and Variant 6 cross-shapes and percentage of Variant 1, cross-

---

(2006a, Table 3.3, pp. 50-51) it can be seen that Variant 5 and 6 were combined into a single category in the 1988 volume.

shaped phytoliths have a strong tendency for polarization and separation of the major proportion of the taxa into the two defined groups, maize and wild species. All the discriminant functions (hereafter *df*) classify the great majority, at least 85%, of wild taxa as wild and maize races as maize, which are robust results. Prediction formulas derived from the functions can then be used to identify unknown phytoliths from archaeological samples with a high degree of accuracy.

Discriminant function is not the only method used to identify archaeological cross-shaped phytoliths. A broad array of qualitative and other phytolith characteristics are assessed independently of the *df*'s, an especially useful exercise because *df*'s are not expected to accurately classify 100% of all species considered. A few quick and effective identification rules have been derived from the accumulated data. One is that if phytolith assemblages contain more than 50% of cross-bodies that are not Variant 1 in 3-D morphology, they should probably be discounted as representative of maize decay. Use of this criterion alone eliminates the many wild grasses that produce high proportions of Variant 5 and 6 cross-bodies, including the genus *Tripsacum* and the few other wild species where cross-bodies are as large as in maize (e.g., *Cenhrus echinatus*; *Oplismenus hirtellus*). A second effective identification rule is: if large-sized (greater than 16 μm wide) cross-bodies account for more than about 10% of all the cross-bodies measured, and if they are mostly (> 50 to 60%) Variant 1, maize is very likely present. Research by a number of investigators has demonstrated that wild grasses may contribute some cross-shaped phytoliths in the large-sized class (greater than 16 microns wide). However, in virtually all of them, only a small proportion are Variant 1, whereas in maize, they are almost always Variant 1 (see Piperno 2006a:56 for more details on this kind of analysis).

The effectiveness of these rules is demonstrated by a blind analysis the author carried out of teosinte and *Tripsacum* samples provided to her by John Doebley. For example, in four different specimens of *Zea luxurians*, a teosinte endemic to Guatemala, cross-bodies were as large in mean size as in maize, resulting in *df* values that placed it within the 95% confidence intervals for maize

in Piperno's 1988 *df*. However, because the great proportion of the phytoliths in every specimen were Variants 2 and 6, the author correctly classified every one as a wild grass in the blind study (see Piperno 2006a: Table 3.5, pg. 57). In the same blind study, six out of six and seven out of seven leaf specimens of Balsas teosinte and *Tripsacum*, respectively, were accurately classified as a wild grass based on phytolith three-dimensional morphology. Every specimen of Balsas teosinte, maize's wild ancestor, examined in the blind and other studies has dominant proportions of the tent-shaped, Variant 2 phytoliths, and on this basis alone are distinguished from maize (Fig. 5) (Piperno 2006a, Table 3.5, pg. 57). Balsas teosinte cross-bodies are also significantly smaller than in maize (Piperno 2006a, Table 3.5, pg. 57). These results importantly indicate that in archaeological sites located in Mexico where Balsas teosinte is native, phytolith criteria can distinguish between the decay of maize and teosinte leaves.

In summary, a variety of size and morphological criteria can be used to identify phytoliths from maize leaves. The use of multiple criteria is an important aspect of maize identification using phytoliths that is applicable to other crop plants. The diversity of phytoliths occurring within a single species reduces reliance on a single type of phytolith or methodological approach to identification. It allows definition of mutually exclusive phytolith assemblages that clearly indicate to the investigator whether potential confuser taxa are present or not.

Moving to the identification of maize cob using phytoliths, the focus shifts to different kinds of phytoliths that predominate in cobs. It is established through studies carried out by John Doebley and colleagues that phytolith formation in maize cobs and equivalent structures in teosinte, called fruitcases, is regulated by a genetic locus called *teosinte glume architecture 1*, or *tga1* (Dorweiler and Doebley 1997). This gene directs the teosinte fruitcase, the structure that surrounds the kernel, to be highly lignified (very hard) and to silicify both its long and short epidermal cells. *tga1* also causes the glume and rachid (homologous to the maize cupule) to completely envelop the kernel. The alternative copy of the *tga1* allele that resulted when fruitcases were transformed into cobs by

humans during the domestication process shuts off most of the lignin production and limits silicification to just the short cells of maize cupules and glumes, so that cobs produce mostly rondel phytoliths and lack many of the other types of epidermal phytoliths found in teosinte (Figs. 6-8). Because maize glumes are lightly lignified, the rondel phytoliths they contain also have lost the characteristic decorations that typify those in teosinte. The result is the formation of different and distinguishable phytolith assemblages in teosinte fruitcases and maize cobs (Figs. 6 and 7). Some phytolith types are diagnostic of maize and teosinte. Another effect of artificial selection on *tga1* was that maize glumes became smaller and less completely enveloped the kernel. Thus, a genetic locus that accounted for a number of crucial phenotypic changes during maize domestication also coded for different types of phytoliths in maize and its wild ancestor, and created the basis for archaeobotanical identifications of the phytoliths.

In North America and in lowland Central and South America there have been, as mentioned, extensive comparisons of maize cob phytoliths with those from wild grasses by different researchers and a consensus has developed regarding maize identification (e.g., Bozarth 1993; Bozarth and Guderjan 2004; Boyd et al. 2006; Iriarte 2003, et al. 2004; Pearsall et al. 2003; Piperno and Pearsall 199a). What are called wavy-top rondels (Bozarth 1993) appear to be diagnostic of maize (Fig. 8). What are called ruffle-top rondels (Pearsall et al. 2003a; Piperno and Pearsall 1993) are found in teosinte and less commonly in corn, and identify the genus *Zea* (Fig. 8). Other types of phytoliths found in teosinte fruitcases and harder-glumed maize varieties can also identify *Zea* at the genus level (Iriarte 2003a,b, et al. 2004; Pearsall et al. 2003; Piperno 2006a; Piperno and Pearsall 1993). Phytoliths Recovered from Ancient Maize Cobs and Squash Fruits

Before moving to a discussion of archaeological phytolith records, it is worthwhile to consider some data recently accumulated by the author on phytoliths in archaeological macro-fossil specimens of maize and *Cucurbita*. A considerable amount of work goes into the construction of modern reference collections with the expectation that they will serve as suitable comparisons for

specimens retrieved from the archaeological record. The passage of thousands of years of time creates some uncertainty as to whether size and morphological features in prehistoric cultivars were like those in plants today, given that ancient germ plasm has probably been lost and modern cultivars studied are sometimes products of recent improvement efforts and may not be closely comparable to traditional land races. The macro-fossil specimens studied here come from coastal Peru and Tabasco, Mexico. At the famous site of Huaca Prieta, Peru excavated by Junious Byrd (Byrd et al. 1985), macrobotanical remains of squashes dating from about 4300 B.P. to 3000 B.P. were common. I analyzed three different fruit rinds from the site identified by Thomas Andres as *Cucurbita moschata*, a domesticated species native to South America. The scalloped phytoliths in the rinds were closely comparable in size and shape to those typically found in modern specimens of the plant. For example, they had markedly elongated mesocarpal hemispheres, a type that may be diagnostic to this species (Fig. 9; see also Piperno 2006a:65-71). No phytoliths like the singular forms that occur in modern specimens of *Cucurbita andreana* and its domesticated product *C. maxima* were noted, supporting previous observations that those phytoliths may also be diagnostic to species (Piperno et al. 2002; Piperno 2006a:65-71).

Interestingly, in one of the Huaca Prieta rinds there was a distinctive scalloped phytolith that has not been observed in modern squash varieties (Fig. 10). It is similar to other phytoliths in modern *C. moschata* but possesses some singular characteristics, such as a flat and grainy proximal end (Fig. 10). It is possible the phytolith represents a now-extinct land race, although more work of this nature is obviously necessary. Two of the rinds had few scalloped phytoliths, far fewer than would be found in highly lignified wild and domesticated fruits, a finding that supports data obtained from modern plants that domesticated fruits heterozygous for the *Hr* locus (*Hr hr*) often have a depauperate phytolith content. Phytoliths from fruits such as this would be few and far between in sediments into which the rinds decayed. As explained above, soft rinds from domesticated fruits homozygous at the *hr* locus would leave no phytoliths at all. It is clear that visible phytolith records

will not always be obtainable from sites where domesticated *Cucurbita* was present, especially during later prehistoric periods when non-lignified fruits were probably widespread.

The maize cobs studied (n = 4) come from the site of San Andrés, Mexico (Pope et al. 2001) and date to about 2500 B.P. The phytoliths removed from them are closely comparable in size and morphology to those found in modern Latin American and other maize races, and are unlike those in teosinte (e.g., rondels with undecorated edges dominate the assemblages; there are few to no elongate phytoliths from long epidermal cells) (Fig. 11). This is expected given the age of the cobs, by which date the genetic loci responsible for the transformation of teosinte into maize, including the gene controlling phytolith formation in each, *tga1*, had long been chosen by human selection. The continued building of a library on phytoliths in ancient crop plants and, whenever possible, their wild ancestors will allow better identifications of archaeological phytoliths and add to the increasing resolution of phytolith data.

3. Archaeological Results

A considerable amount of archaeological phytolith work has taken place in Central and South America the past 25 years, and most crop plants with identifiable phytoliths discussed above were identified in pre-Columbian occupations. Following the literature from the early days of phytolith activity in paleoethnobotany to the present, it can be seen how identification criteria have developed and evolved--from Pearsall's (1978) original use of broad size categories (e.g., small, large, extra-large) to identify maize leaves with cross-shaped phytoliths; to the development of more precise size and shape criteria for these cross-body forms (Piperno 1984; Pearsall and Piperno 1990); to use of robust statistical approaches in cross-shaped phytolith identification (e.g., Iriarte 2003; Mulholland 2003; Piperno 1988, Pearsall 2000); to the widespread recognition of maize cob phytoliths (e.g., Bozarth 1993a, Bozarth and Guderjan 2004; Iriarte 2003b, et al. 2004; Mulholland 2003), and

finally, incorporation of starch grain data. The latter allows the generation of two independent lines of empirical data, providing evidence–often from the same plant processing stone tools or ceramic cooking vessels-- for the presence of two different parts of the same, consumed structure of the plant, its kernels and chaff (glumes/cupules) (see Dickau et al., 2007; Iriarte et al. 2004; Perry et al. 2006, 2007; Pearsall et al. 2003, 2004, Piperno et al. 2000). During the course of this research, a great deal of data have been generated from Panama and Ecuador.

In central Panama, a continuous 11,000 year-long sequence of human occupation and plant exploitation has been retrieved during the past 25 years from excavations of more than 15 rock shelters and open air sites led by Richard Cooke of the Smithsonian Institution and Tony Ranere of Temple Unviversity (Cooke and Ranere 1984; Ranere and Cooke 2003). The sites contain rich stone tool, faunal, and ceramic records that are well-studied and dated. Several sites, largely rock shelters and caves, have human occupations dating from 11,000 to 5000 B.P.

In southwestern Ecuador, Las Vegas is a well-known and important preceramic (early to early-middle Holocene; 10,000 - 7000 B.P.) occupation of the coastal region by tropical foragers and horticulturists that has been studied by Karen Stothert and colleagues, including the author (Stothert 1985; Stothert et al. 2003). A long series of internally consistent ^{14}C determinations on charcoal, shell, and human skeletons make the chronology of this early human presence among the best understood in northern South America. The well-accepted radiocarbon sequence for the Vegas culture provided a good opportunity for a detailed, comparative carbon-14 study of the cytoplasmic carbon occurring within phytoliths that is derived from the plant cells in which phytolith formation took place. The Valdivia culture of southwestern Ecuador represents the initial appearance of ceramics and the early Formative period in the region (see Pearsall 2003 for a review). Valdivia sites

are located less than 50 km from the Vegas occupations and they have been well-studied by a number of archaeologists. Pearsall's long-term, multiproxy archaeobotanical research on Valdivia sites is a model for reconstructing economic plant usage, providing a large amount of data through the years.

3.1 Crop Plant Phytolith Records

3.1.1 Ecuador

Crop plant phytoliths (and starch grains) are common in sediments and on stone tools from these sites. Table 2 contains a list of the taxa present. Currently, the earliest cultivar identified is *Cucurbita*, whose scalloped fruit rind phytoliths are abundant at the Vegas sites (Piperno and Stothert 2003). The phytoliths become larger through time and by 10,000 - 9300 B.P. they have lengths and thicknesses found only in domesticated species (Piperno and Stothert 2003). The species represented is probably *C. ecuadorensis*, native to this part of South America, although especially during later periods at Vegas (ca. 9000 - 7000 B.P.), the presence of *Cucurbita moschata*, also native to South America, cannot be ruled out. A detailed carbon-14 study was carried out on 15 phytolith samples from the two Vegas occupations examined, Sites 80 and 67. The phytolith dates were in good stratigraphic order and consistent with the ^{14}C determinations on associated shell, human bone, and charcoal (Stothert et al. 2003; see Piperno 2006a for an extended discussion). The phytolith dates confirmed that *Cucurbita* rind phytoliths recovered from Vegas sediments and assigned to the early Holocene period were indeed deposited at that time. The stratigraphic integrity of the dates further indicated that any movement and mixing of phytoliths downward through the soil columns was inconsequential.

3.1.2 Roots for Vegas

Adding to the evidence for early Holocene plant cultivation and domestication at the Vegas sites is the recent identification of phytoliths from the rhizomes of *Calathea allouia* (*lerén*) (author, laboratory notes). The phytoliths are the diagnostic forms described by Chandler-Ezell et al. (2006) (Fig. 12). They first occur in the same phytolith samples from Site 80 containing the earliest evidence for domesticated *Cucurbita ecuadorensis*, where the phytoliths are directed dated to 9300 B.P. The *lerén* phytoliths continue to be present throughout the remainder of the occupation. This constitutes definitive evidence that early Vegas food production included at least one root crop. It is possible that future starch grain work planned by the author and Stothert on human teeth from Sites 80 and 67 will reveal others.

3.1.3 Squash and Root Crops in Panama

In central Panama, *Cucurbita* phytoliths occur by 8000 B.P. (Piperno 2006a). The morphology of the phytoliths indicates that the species present is probably *C. moschata*, which was domesticated in northern South America and was probably the only squash species grown in Panama in the pre-Columbian era. Around the same time as *Cucurbita* or somewhat earlier, the first indications of arrowroot and *lerén* appear. Both of these root crops are evidenced by their seed phytoliths, and starch grains from arrowroot were also recovered from the earliest grinding stones identified in the region that date from 7000 to 6000 B.P. (Piperno et al. 2000b). In addition, the author has recently found the distinctive rhizome phytoliths from *lerén* just described for Vegas occurring at the Aguadulce Rock Shelter in some of the same stratigraphic levels as the seed phytoliths, and dating to ca. 8000 B.P. (author, laboratory notes). As in Ecuador, this is the first empirical evidence in Central America that *lerén* was grown for its underground organs. Sauer (1950) postulated that both of these now-minor root crops may have been among the earliest cultivars in the lowland Neotropics, a

suggestion being borne out with the increasing accumulation of empirical data.

Interestingly, cassabanana (*Sicana odorifera*), another domesticated member of the Cucurbitaceae with high amounts of diagnostic phytoliths, has not been recovered thus far from pre-Columbian contexts. Cultivated now throughout Central and South America, its area of origin is thought to be Brazil. Considering the phytolith record, it is possible that the plant was domesticated and/or dispersed out of its native area rather late in prehistory.

3.1.4 Maize in Panama and Ecuador

There is considerable evidence that maize arrived in Central Panama by 7000 B.P. Cross-shaped phytoliths from maize leaves and/or wavy top and ruffle top rondels from cobs routinely occur in sediments and on stone artifacts dating to the 7000 B.P. - 5000 B.P. period (Table 3) (see Piperno 2006a:140-149). At the Aguadulce Shelter, Panama, one of the best studied preceramic (11,000-5000 B.P.) occupations, sediment phytolith assemblages that contained maize leaf and/or cob phytoliths provided internally consistent radiocarbon ages of 4250 ± 60, 6207 ± 60, 6910 ± 60 and 7061 ± 81 B.P. (Piperno et al. 2000a). At this site, maize starch grains were present on grinding stones from the same contexts that the sediment maize phytoliths came from, and phytoliths from the glumes and cupules of maize cobs occurred on the grinding stones that yielded starch grains from maize kernels (Piperno 2006a, b, et al. 2000a). From these and other stone tools dated from 7000 B.P. to 5000 B.P., starch grains from manioc, arrowroot, and yams were recovered, expanding the list of root crops present at that time and providing another line of evidence for arrowroot cultivation (Piperno et al. 2000b; Piperno 2006b).

Most recently, Dickau (2005, et al. 2007) carried out a large analysis of stone tools from archaeological sites in central Panama that had not been investigated with starch grain study. She

recovered maize starch from implements at the site of Cueva de los Ladrones dating from 7000 B.P., onwards, adding another line of evidence for the early presence of maize in the region. Previous phytolith work by the author and colleagues had documented 7000 B.P. - 5000 B.P. maize leaf phytoliths and pollen at the site (Table 3) (Piperno et al. 1985). Recent studies by the author indicate that cob phytoliths are present in the same sediments that contained the earliest maize leaf phytoliths and pollen (author, laboratory notes).

It is worthwhile to examine how the identification criteria for maize leaf phytoliths developed from modern plants that were outlined earlier were operationalized for evaluating archaeological phytolith assemblages. Table 3 shows that sample size is good for cross-shaped phytoliths from a number of sites occupied in central Panama between 8600 B.P. and A.D. 500. Once determined, phytolith size values and three-dimensional characteristics were plugged into the discriminant function prediction formula. In the earliest contexts from Cueva de los Vampiros dated to 8600 B.P., cross-shaped phytoliths have characteristics of, and are identified as wild grasses. In contrast, maize leaf phytoliths are consistently identified in contexts dating from ca. 7000 B.P. to much later periods, showing similar size and morphological patterns throughout. The same results were obtained when the data values were used with the prediction formula from the author's earlier discriminant function (Piperno 1988), indicating the robustness of the results. As previously discussed, grasses often contribute multiple diagnostic phytolith characteristics as well as mutually exclusive phytolith assemblages, and employing a fuller assortment of phytolith attributes provides a particularly robust avenue to identification. Using the other identification criteria, also discussed above, that are applied independently of the discriminant function analysis (such as how many cross-shapes of all sizes are Variant 1 and how many large-sized cross-shapes (> 16 microns wide) are Variant 1s, and other

features), phytoliths identified as maize contain all the attributes of maize. For example, the great majority of cross-shapes are Variant 1 in three-dimensional structure and they have large average widths (13.3 to 14 μm). The average size of the cross-shapes is entirely consistent with the size in modern maize races. Furthermore, like in maize but not in wild grasses, individual cross-bodies in the large size category (>16 μm wide) occur in proportions of greater than 10% of all the cross-bodies present and virtually all of these are Variant 1 types; these are important diagnostic characters. Moreover, the cross-body and other phytolith types used to assess maize presence do not conform with any of the hundreds of cross-shaped producing wild, non-*Zea* grasses that phytoliths from modern maize races were compared to (see Piperno 2006a). Thus, all the phytolith patterns strongly indicate late preceramic through early ceramic period maize presence.

Another interesting feature of the Aguadulce Rock Shelter phytolith record should be discussed with regard to maize presence. In a deep, preceramic, 5cm-thick level from a column sample dating to shortly before 7000 B.P., there is a unique maize cob phytolith assemblage. This assemblage is the oldest maize at the site, and the phytoliths are closer in overall morphology to those from modern teosinte fruitcases than they are to modern maize. For example, phytoliths common in teosinte fruitcases but absent in most modern maize cobs occur, and there are significant percentages of elongated and ornamented epidermal and other glume phytoliths that are characteristic of teosinte but not of modern maize (see Piperno 2006a, Table 7.2, pg. 147). Are they the remains of teosinte, which today does not grow further south than northern Nicaragua? Almost certainly not. Rather, because the production of these phytoliths is primarily controlled by the *tga1* gene, which makes the glume harder and causes it to more completely encase the kernel, they should in this region far removed from the natural distribution of teosinte be interpreted to indicate the presence of maize

cobs with the *tga1* allele that caused them to have harder and/or larger glumes than we find today.

Walton Galinat (2001) discusses extant maize races that still have the teosinte allele for *tga 1*, a primitive trait chosen by modern cultivators because, by hardening cobs the allele confers resistance to fungi and other pathogens that would otherwise cause them to rot. This feature is particularly advantageous in humid environments. Therefore, there seem to be two possibilities that would account for these phytoliths at Aguadulce. Maize may have left Mexico and arrived in Panama before the maize allele for *tga1* was fixed by human selection. Alternatively, humans maintained variability at the *tga1* locus, selecting for the allele that was most useful under the prevailing ecological circumstances. Further studies will determine which is the better explanation in preceramic sites in Panama. Also noteworthy about the Aguadulce phytolith record is that after ca. 7000 B.P. at Aguadulce, cob phytoliths become much more similar to those in modern maize (Piperno 2006a, Table 7.2). Presumably, the phytoliths were derived from varieties with softer glumes.

As in Panama, investigations of maize in Ecuador using phytoliths have a long history, one also recently complemented by starch grain studies. The author's research at the Vegas sites, which identifies maize leaf phytoliths by ca. 6600 B.P., is discussed in detail in Piperno (2006a:147-148) and will not be reviewed here. It should be noted that additional studies are underway to search for cob phytoliths at Vegas occupations and determine more radiocarbon ages for phytolith assemblages containing cob and leaf phytoliths. Pearsall's work addresses maize presence at early ceramic Valdivia occupations in the region beginning at ca. 5500 B.P. Starting with her original study (Pearsall 1978) that established size as a robust criterion to identify cross-shaped phytoliths from maize, Pearsall has identified maize leaf and cob phytoliths in a variety of contexts from Valdivia I-

III periods (ca. 5500- 4300 B.P.). As in Panama, identification methods have evolved and been strengthened through time, so that multivariate statistical analyses combined with a number of other independent criteria are used to assess whether archaeological cross-shaped phytoliths are from maize. In addition, extensive comparative studies of rondel and other cob-derived phytoliths are employed to identify maize cob decay (e.g., Pearsall et al. 2003; Pearsall 2000). As in Panama, size and morphological attributes of cross-shaped phytoliths in Valdivia sites are closely comparable to those found in modern races of maize, as also demonstrated by the predictions of Pearsall's discriminant function formulae and application of other discriminating criteria. Finally, Pearsall et al's (2004) analyses of starch grain residues removed from dozens of plant grinding stones from intact house floors dating to ca. 4300 B.P. at Real Alto are concordant with the phytolith results; maize starch grains are present in high numbers on most of the stones examined. Maize cob phytoliths were also recovered from the artifacts.

4. Some Comments on Phytolith ^{14}C studies

It has been conclusively shown that retained within phytoliths is some of the carbon that was present in the plant cells where phytolith formation took place. Some of the first botanists who carried out dedicated phytolith research during the last century noticed these often-visible occlusions and suggested they were remnants of cellular organic material. These beliefs were later confirmed by a number of investigators, who also analyzed in detail the composition of the occluded carbon (see Piperno, 2006a:125-132 for a detailed discussion). The ability to directly date this organic material using AMS techniques is obviously a step forward in our efforts to provide sound chronologies of plant use. As investigators increasingly employ phytoliths for carbon-14 study they will need to

recognize some important aspects inherent to phytolith dating.

First, in contrast to most other materials submitted for carbon-14 study, the phytolith analyst must carry out the laboratory procedures necessary for isolating phytoliths from their sedimentary or other matrices. It cannot be stressed enough that before phytoliths are sent to the radiocarbon facility, a rigorous pretreatment is necessary to remove potentially contaminant organic materials still clinging to them. In the author's laboratory, a strong oxidizer made from concentrated nitric acid and potassium chlorate is used, and phytoliths are treated twice with these chemicals, before and after they are isolated from sediments. Weaker oxidizers, such as hydrogen peroxide, do not work as well in removing adherent material and their use may result in erroneous or inconsistent dates. An example of this kind of error is discussed by Boyd et al. (2001), who processed phytolith samples from Garua Island, West New Britain using a weak and strong (chromic acid) oxidizer and then dated each extract. The weak reagent failed to remove organic materials adhered to phytoliths and resulted in erroneously young dates, whereas the same samples treated with chromic acid yielded older ages that agreed well with dates that were determined on other kinds of associated materials.

Obviously, pretreatment methods must not introduce carbon (e.g., by using reagents containing sources of it), and phytoliths should be floated from sediments using methods that provide the cleanest separations possible (in this regard, the author exclusively uses a heavy liquid made from cadmium and potassium iodide). As with any other dating substrate, pretreatment must yield very clean samples, especially as very small quantities of carbon will be analyzed.

Phytoliths, like other sources of carbon, may also have special properties that need to be considered. For example, it is well established that phytoliths are depleted in the heavy isotope ^{13}C compared with whole plant tissue, which means that the most accurate phytolith ages will have been

corrected using $\delta^{13}C$ values derived directly from the same phytoliths. If this isn't possible because not enough carbon was provided by the dated phytoliths to also derive $^{13}C/^{12}C$ ratios, the age may be erroneous by a few hundred years.

5. Summary

In Central and South America, phytoliths from crop plants, including maize, squash, bottle gourd, arrowroot, and *lerén*, routinely occur in archaeological sites of preceramic to early ceramic age. Morphological and size attributes of the phytoliths are closely comparable with those in modern cultivated species and phytoliths recovered directly from ancient macro-specimens of squash and maize. Evidence indicating the dispersals of manioc, arrowroot, *lerén*, squashes, and maize into southern Central America and South America from their domestication cradles well before 5000-4000 B.P. continues to increase, and is now independently supported by a number of starch grain studies (see Dickau et al., 2007 and Pohl et al., 2007 for the most recent evidence and reviews of the subject). The wider implications of the accumulated microfossil data with regard to the origins and development of New World agriculture are well-discussed elsewhere (e.g., Dickau et al. 2007; Piperno and Pearsall 1998b; Pohl et al. 2007; Sandweiss 2007). Essentially, the data raise to their deserved stature the crucial roles that Neotropical people and plants played in creating agricultural economies and the myriad, important social developments that resulted. Because of preservation biases introduced by a previous dependence on macro-fossil plant remains, and prejudicial views in a part of the scholarly community about the potential of tropical forest to support prehistoric complexity, these roles had long been in the shadows.

References

Boyd, B., Torrence, R., Lentfer, C., and Jacobsen G. (2001). Dating fossil opal phytoliths from Garua Island, West New Britain for determination of phytolith assemblage chronology. AINSE Progress Report for 01/014.

Bozarth, S. (l986) Morphologically distinctive *Phaseolus*, *Cucurbita*, and *Helianthus Annuus* Phytoliths. In Rovner, I. ed. *Occasional Papers No.l of The Phytolitharian*, pp. 56-66. North Carolina: North Carolina State University.

Bozarth, S.R. (1987) Diagnostic opal phytoliths from rinds of selected *Cucurbita* species. *American Antiquity* 52, 607-615.

Bozarth S.R., 1993a. Maize (*Zea mays*) Cob phytoliths from a Central Kansas Great Bend Aspect archaeological site. *Plains Anthropologist* 38, 279-286.

Bozarth, S.R., 1993b. Opal phytolith analysis of shell middens at Sites P-309B and P-313N, iHoopes, J. ed. *Prehistoric Human Ecology on the Golfo Dulce, Southwestern Costa Rica*, in preparation.

Bozarth, S. R. and Guderjan, T.H.(2004) Biosilicate analysis of residue in Maya dedicatory cache vessels from Blue Creek, Belize. *Journal of Archaeological Science* 31, 2-5-215.

Byrd, J.B., Hyslop, J., and Skinner, M.D. (1985) The preceramic excavations at the Huaca Prieta, Chicama Valley, Peru. Anthropological papers of the American Museum of Natural History, v. 62, pt. 1.

Chandler-Ezell, C., Pearsall, D.M., and Zeidler, J.A. (2006) Root and tuber phytoliths and starch grains document manioc (*Manihot esculenta*), arrowroot (*Maranta arundinacea*), and lleren (*Calathea sp.*) at the Real Alto site, Ecuador. *Economic Botany* 60, 103-120.

Cooke, R. and Ranere, A.J. (1984) The "Proyecto Santa Maria". A multidisciplinary analysis of Prehistoric adaptations to a tropical watershed in Panama. In Lange, F. ed. *Recent Advances in Isthmian Archaeology*, pp. 3-30. Oxford: Proceedings of the 44th International Congress of the Americanists, British Archaeological Reports, International Series 212.

Dickau, R. (2005) Resource Use, Crop Dispersals, and the Transition to Agriculture in Prehistoric Panama: Evidence from Starch Grains and Macroremains. Unpublished PhD Dissertation, Temple University, Philadephia.

Dickau, R, Ranere, A.J., and Cooke, R.G (2007) Starch Grain Evidence for the Preceramic Dispersal of Maize and Root Crops into Tropical Dry and Humid Forests of Panama. *Proceedings of the National Academy of Sciences USA*, in press.

Dorweiler, J.E. and J. Doebley (1997) Developmental analysis of *Teosinte Glume Architecture1*: A key locus in the evolution of maize (Poaceae). *American Journal of Botany* 84, 1313-1322.

Fauteux, F., Chain, F., Belzile, F., Menzies, J.G., and Bélanger, R.R. (2006) The protective role of silicon in the *Arabidopsis*-powdery mildew pathosystem. *Proceedings of the National Academy of Sciences USA* 103:17554-17559.

Galinat, W. (2001) A reconstruction of a possible role of crucial observations leading to a rapid

domestic transformation of wild teosinte into the first maize. *Economic Botany* 55, 570-574.

Iriarte, J. (2003a) Emergent Cultural Complexity in the Wetlands of Uruguay during the Middle Holocene. Unpublished PhD Dissertation, Department of Anthropology, University of Kentucky, Lexington.

Iriarte, J. (2003b) Assessing the feasibility of identifying maize through the analysis of cross-shape size and three-dimensional morphology of phytoliths in the grasslands of southeastern South America. *Journal of Archaeological Science* 29,1085-1904.

Iriarte, J., Holst, I., Marozzi, O., Listopad, C., Alonso, E., Rinderknecht, A., and Montaña, J. (2004) Evidence for cultivar adoption and emerging complexity during the Mid-Holocene in the La Plata Basin, Uruguay. *Nature* 432, 614-617.

Ma, J.F., Tamai, K., Yamaji, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., and Yano, M. (2006) A silicon transporter in rice. *Nature* 440:688-691.

Massey, F.P. and Hartley, S.E. (2006) Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates. *Proceedings of the Royal Society B* 273:2299-2304.

Metcalfe, C.R., l960. *Anatomy of the Monocotyledons.I. Gramineae.* Oxford University Press, London.

Mulholland, S.C. (1993) A test of phytolith analysis at Big Hidatsa, North Dakota. In Pearsall, D.M. and Piperno, D.R. eds. *Current Research in Phytolith Analysis: Applications in Archaeology and Paleoecology,* pp. 131-145. MASCA Research Papers in Science and Archaeology,Vol. 10. Philadelphia: MASCA, The University Museum of Archaeology and Anthropology, University of Pennsylvania.

Pearsall, D.M. (1978) Phytolith analysis of archaeological soils: Evidence for maize cultivation in Formative Ecuador. *Science* 199, 177-178.

Pearsall, D.M. (2000) *Paleoethnobotany: A Handbook of Procedures*, 2nd edition. Academic Press, San Diego.

Pearsall, D.M. (2003) Plant food resources of the Ecuadorian Formative: An overview and comparison to the Central Andes. In Raymond, J.S. and R.L. Burger, R.L. eds. *Archaeology of Formative Ecuador*, pp. 213-257, Washington DC:Dumbarton Oaks.

Pearsall, D.M. and Piperno, D.R. (1990) Antiquity of Maize Cultivation in Ecuador: Summary and Reevaluation of the Evidence. *American Antiquity* 55, 324-337.

Pearsall, D.M., K. Chandler-Ezell, and A. Chandler-Ezell (2003) Identifying maize in Neotropical sediments and soils using cob phytoliths. *Journal of Archaeological Science* 30,611-627.

Pearsall, D.M., K. Chandler-Ezell, and J.A. Zeidler (2004) Maize in ancient Ecuador: Results of residue analysis of stone tools from the Real Alto site. *Journal of Archaeological Science* 31, 423-442.

Perry, L. (2004) Starch analyses reveal the relationship between tool type and function: An Example from the Orinoco Valley of Venezuela. *Journal of Archaeological Science* 31,1069-1081.

Perry, L., Sandweiss, D.H., Piperno, D.R., Rademaker, K., Malpass, M.A., Umire, A., and de la Vera, P. (2006) Early maize agriculture and interzonal interaction in southern Peru. *Nature* 440, 76-79.

Piperno, D.R. (1984) A comparison and differentiation of phytoliths from maize and wild grasses: Use of morphological criteria. *American Antiquity* 49,361-383.

Piperno, D.R. (1988) *Phytolith Analysis: An Archaeological and Geological Perspective.* San Diego: Academic Press.

Piperno, D.R., 1989. The occurrence of phytoliths in the reproductive structures of selected tropical angiosperms and their significance in tropical paleoecology, paleoethnobotany, and systematics. *Review of Paleobotany and Palynology* 61,147-173.

Piperno, D.R. (2003) A few kernels short of a cob: On the Staller and Thompson late entry scenario for the introduction of maize into northern South America. *Journal of Archaeological Science* 30, 831-836.

Piperno, D.R. (2006a) *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists.* Lanham, MD: AltaMira Press.

Piperno, D.R. (2006b) Identifying manioc (*Manihot esculenta* Crantz) and other crops in pre-Columbian tropical America through starch grain analysis: A case study from Panama. In Zeder, M., Emschwiller, E., Bradley, D. and Bruce Smith *Documenting Domestication: New Genetic and Archaeological Paradigms*, pp. 46-67, Berkeley:University of California Press.

Piperno, D.R. and Pearsall, D.M. (1993) Phytoliths in the reproductive structures of maize and teosinte: Implications for the Study of Maize Evolution. *Journal of Archaeological Science* 20, 337-362.

Piperno, D. R. and Pearsall, D.M. (1998a) The Silica Bodies of Tropical American Grasses: Morphology, Taxonomy, and Implications for Grass Systematics and Fossil Phytolith Identification. *Smithsonian Contributions to Botany*. Number 85. Smithsonian Institution Press, Washington DC.

Piperno, D.R. and D.M. Pearsall (1998b) *The Origins of Agriculture in the Lowland Neotropics.* San Diego:Academic Press.

Piperno, D.R. and K.E. Stothert (2003) Phytolith evidence for early Holocene *Cucurbita* domestication in Southwest Ecuador. *Science* 299,1054-1057.

Piperno, D.R. I. Holst, T.C. Andres, and K.E. Stothert (2000a) Phytoliths in *Cucurbita* and other Neotropical Cucurbitaceae and their occurrence in early archaeological sites from the lowland American tropics. *Journal of Archaeological Science* 27,193-208.

Piperno, D. R., A. J. Ranere, I. Holst and P. Hansell (2000b) Starch grains reveal early root crop horticulture in the Panamanian tropical forest. *Nature* 407:894-897.

Piperno, D.R., I. Holst, L. Wessel-Beaver and T.C. Andres (2002) Evidence for the control of phytolith formation in *Cucurbita* fruits by the Hard Rind (*Hr*) Genetic Locus: Archaeological and ecological implications. *Proceedings of the National Academy of Sciences USA* 99,10923-10928.

Piperno, D.R., K. Clary, R. G. Cooke, A.J. Ranere and D. Weiland (1985) Preceramic maize in Central Panama: Phytolith and pollen evidence. *American Anthropologist* 87, 871-878.

Pohl, M., Piperno, D.R., Pope, K., and Jones, J. (2007) Pre-Columbian Maize Dispersals in the Neotropics. New Microfossil Evidence from San Andrés, Tabasco, Mexico. *Proceedings of the National Academy of Sciences USA*, in press.

Ranere, A.J. and Cooke, R.G. (2003) Late glacial and early Holocene occupation of Central American tropical forests. In Mercader, J., ed. *Under the Canopy: The Archaeology of Tropical Rain Forests*, pp. 21-248. New Brunswick: Rutgers University Press.

Sandweiss, D.W. (2007) Small is big: the microfossil perspective on human-plant interaction. *Proceedings of the National Academy of Sciences USA*, in press.

Sangster, A.G., M.J. Hodson, and J.J. Tubb (2001) Silicon Deposition in Higher Plants. In Datnoff, I.E., Snyder, G.H., and Korndorfer, G.H., eds. *Silicon in Agriculture*, pp. 85-113. Amsterdam: Elsevier Science.

Sauer, C.O. (1950) Cultivated plants of South and Central America. In Steward, J. ed. *Handbook of South American Indians*, pp. 487-543. Bureau of American Ethnology, Bulletin 143, Vol. 6. Washington, D.C.:U.S. Government Printing Office.

Smith, B. (1997) The initial domestication of *Cucurbita pepo* in the Americas 10,000 years Ago. *Science* 276, 932-934.

Smith, B. (2006) Seed size as a marker of domestication in squash (*Cucurbita pepo*). M. Zeder, E. Emschwiller, and B. Smith. *Documenting Domestication:New Genetic and Archaeological Paradigms*, Berkeley:University of California Press.

Stothert, K. (l985) The preceramic Las Vegas culture of coastal Ecuador. *American Antiquity* 50,613-637.

Stothert, K., D.R. Piperno, and T.C. Andres (2003) Terminal Pleistocene/early Holocene human adaptation in coastal Ecuador: the Las Vegas evidence. *Quaternary International* 109-1 10,23-43.

Zheng, Y., Y. Dong, A. Matsui, T. Udatsu, and H. Fujiwara, 2003. Molecular genetic basis of determining subspecies of ancient rice using the shape of phytoliths. *Journal of Archaeological Science* 30:1215-1221.

Table 1. New World Crops That Can Be Identified With Their Phytoliths in Central and South America

Maize; leaves and cobs

Squashes and Gourds of *Cucurbita* spp.; fruit rinds.

Bottle Gourd (*Lagenaria siceraria*); fruit rinds.

Cassabanana (*Sicana odorifera*); fruit rinds.

Arrowroot (*Maranta arundinacea*); seeds; A now minor-root crop that may have been among the first root crops domesticated and important in early horticultural economies in Central and South America.

Lerén (*Calathea allouia*); seeds and rhizomes; A now-minor crop that, like arrowroot, may have been an early and important domesticate.

Manioc (*Manihot esculenta*); roots and leaves.

Table 2. Crop Plants Identified Through Phytolith Analysis in Panama and Ecuador during the Early and Middle Holocene Periods (10,000 to 4000 B.P.)

Crop	Region	Earliest Appearance
Cucurbita ecuadorensis	Southwest Ecuador	10,000 - 9300 B.P.
Cucurbita moschata	Southwest Ecuador (?)	Before 7000 B.P.
	Central Panama	Ca. 8000 B.P.
Lagenaria siceraria	Southwest Ecuador	9300 B.P.
	Central Panama	Ca. 8000 B.P.
Calathea allouia	Southwest Ecuador	9300 B.P.
	Central Panama	Ca. 8000 B.P.
Maranta arundinacea	Central Panama	Ca. 8000 B.P.
	Southwest Ecuador	Ca. 5500 B.P.[#]
Manihot esculenta	Southwest Ecuador	Ca. 4300 B.P.[#]
Zea mays	Southwest Ecuador	Ca. 6600 B.P.
	Central Panama	Ca. 7100 B.P.[#]

#Starch grains from these cultivars occur on stone tools from associated contexts. In Panama, the earliest grinding stones analyzed date to 7000 B.P. and starch grains from manioc and arrowroot are present on them. In Ecuador, maize phytoliths and starch grains are continually present in Valdivia I-III occupations.

Sources: Chandler Edzell et al. 2006; Pearsall 2000; Pearsall et al. 2003, 2004; Piperno 2006a; Piperno and Pearsall 1998b; Piperno and Stothert 2003

Table 3. Characteristics of Cross-Shaped Phytoliths Isolated from Archaeological Sites in Central Panama and Ecuador

	% Variant		Size μm: Variant		n	% Large/%Large Var 1	df classification* Wild	Maize	df prediction
	1	6	1	6					
Central Panama									
Cueva de los Vampiros									
Preceramic deposits: 8610 B.P.	95	5	10.7	10.3	50	0	0.3933	0.6088	Wild
Cueva de los Ladrones									
x+Early Ceramic Deposits: 4500 B.P. 27-32 and 37-42 cm b.s.	80	15	13.3	11.7	100	15/>80	0.3244	0.6756	Maize
x+Preceramic Deposits: 6910 B.P. 97-102 and 107-113 cm b.s.	70	25	13.5	11.7	100	16/>80	0.6168	0.3832	Maize
#*Aguadulce Rock Shelter* Early Ceramic Deposits x*+2-7 cm a.d. and 0-5 cm b.d.	80	15	13.8	12.4	83	29/>80	0.7377	0.2703	Maize
Sitio Sierra x2300 B.P. - A.D. 500	82	18	14.0	13.2	50	20/>80	0.7759	0.2241	Maize

*The classification equation uses the formulas given below for the maize and wild groups. The group with the highest "score" is the one in which archaeological phytoliths have a high probability of membership.

Maize Prediction: $-1.96669 + 0.1597589$ (_ width Var. 1) $- 0.0126672$ (_ width Var.6) $+ 8.20956^{-3}$ (% Var. 1)

Wild Prediction: $2.96669 - 0.1597589$ (_ width Var. 1) $+ 0.0126672$ (_ width Var.6) $- 8.20956^{-3}$ (% Var. 1)

Notes: The table is from Piperno (2006a). [x]Extra-large Variant 1 cross-bodies are present. [+]Maize-specific wavy top rondel phytoliths are also present. #Preceramic deposits contain few to no cross-shaped phytoliths; wavy top rondel and other cob phytoliths are present, however (see text and Table 7.2). *Phytolith assemblages containing maize leaf phytoliths from 2-7 cm a.d. (above datum) were directly dated to 4250 ± 60 B.P., and phytolith assemblages from underlying preceramic levels containing maize cob phytoliths provided internally consistent dates of 6207 ± 60 B.P., 6910 ± 60 B.P., and 7061 ± 81 B.P. Sitio Sierra phytolith assemblages have not been searched for wavy top rondels.

Figure Legends

Figure 1. Scalloped phytoliths from the fruit rind of the domesticated species *Cucurbita ficifolia*, native to Andean South America. The phytoliths on the bottom is 125 microns long.

Figure 2. Scalloped phytoliths from the domesticated species *Cucurbita moschata*. They have the same morphology as those in 3.6 a, but are larger than in *C. sororia* and other wild species. hh = hypodermal hemisphere, mh = mesocarpal hemisphere. Reprinted from Piperno et al. 2002. Copyright National Academy of Sciences USA.

Figure 3a. Facetate phytoliths from Asian Fagaceae (*Lithocarpus acuminatissima*), left, and Annonaceae (*Goniothalamus marcani*), right. Reprinted from Kealhofer and Piperno 1998.

Figure 3b. Spherical facetate, right (*Unonopsis pittieri*), and irregular facetate phytoliths, left (*Guatteria dumetorum*) from tropical American Annonaceae. Reprinted from Piperno 2006a, Copyright AltaMira Press.

Figure 4. A phytolith from bottle gourd showing how the irregular pattern of the stone cells is discernible on the phytolith surface.

Figure 5. Cross-body three-dimensional structures from different kinds of grasses. Reprinted from Piperno 2006a, Copyright AltaMira Press.

Figure 6. Phytoliths from the fruitcases of teosinte, Race Balsas (*Zea mays* ssp. *parviglumis*). Both the long and short cells (producing rondel phytoliths) are silicified. This process is under the control of the gene *tga1*. Reprinted from Piperno 2006a, Copyright AltaMira Press.

Figure 7. Phytoliths from the glumes and cupules of maize, Race Amarillo. In contrast to teosinte, phytolith assemblages under the control of the maize allele of *Tga1* predominantly contain short cell phytoliths (rondels) with few to no other types present.

Figure 8. Examples of wavy-top rondels from maize. The phytolith on the bottom right is a ruffle-top rondel from maize, for comparison. Reprinted from Piperno 2006a, Copyright AltaMira Press.

Figure 9. A phytolith from a fruit rind of *Cucurbita moschata* recovered from Huaca Prieta, Peru. It is like those found in modern fruits of the crop. The longest dimension of the phytolith measures 66 µm.
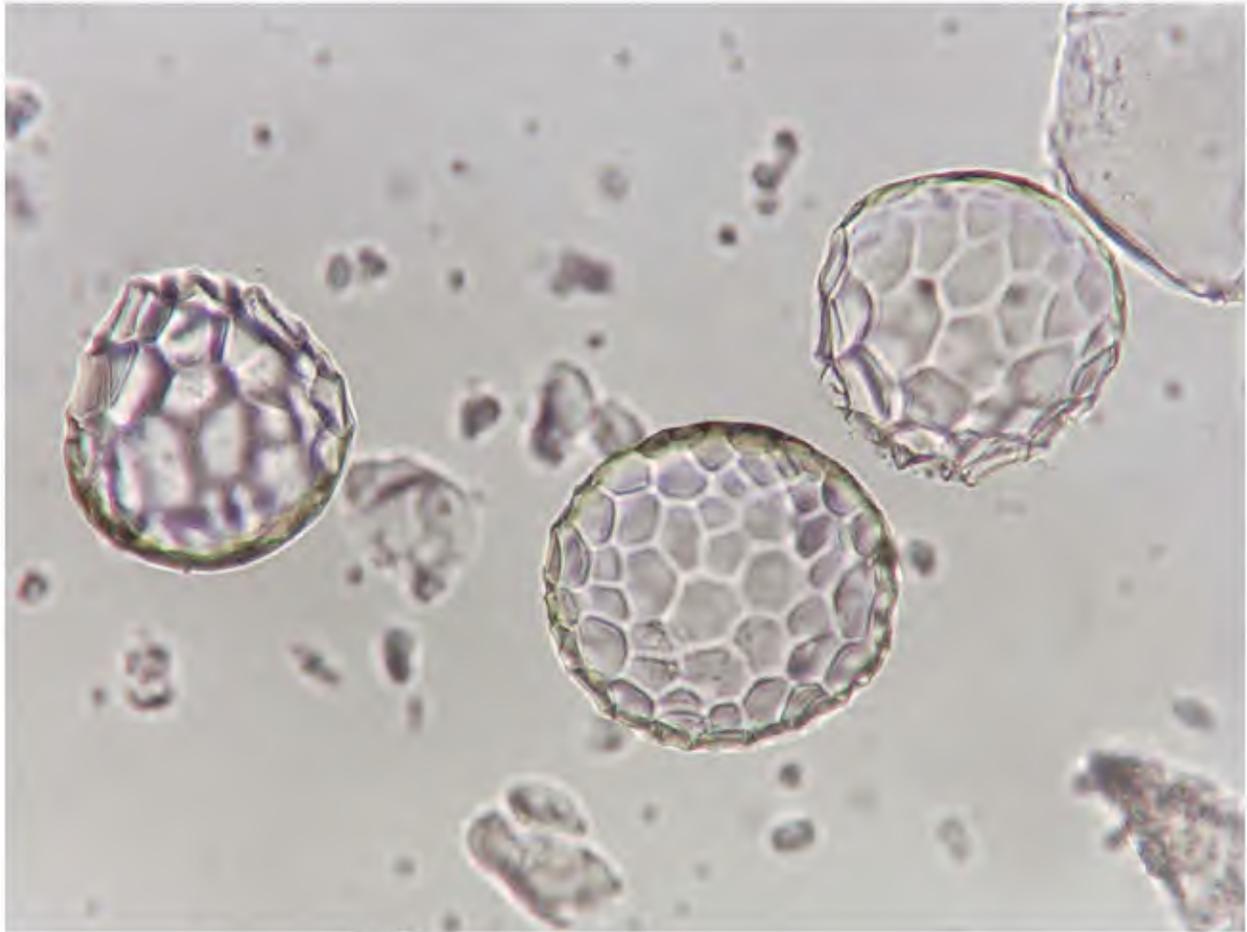
Figure 10. A phytolith from a fruit rind of *Cucurbita moschata* from Huaca Prieta, Peru. It has attributes unlike in phytoliths from modern fruits. The longest dimension is 54 µm.

Figure 11. A "spooled" type of rondel phytolith recovered from a maize cob from San Andrés, Mexico. It is like phytoliths typically found in modern maize races.
Figure 12. Center, a phytolith from the rhizome of *Calathea allouia* recovered from a preceramic level of Vegas Site 80. The phytolith assemblage of which it was a part was directly dated to 9320 ± 250 B.P. The phytolith is 30 µm long.

Figure.1

Figure.2

Figure.3a

Figure.3b

20µm

Figure.4

Figure.5

Leaf Cross-shaped bodies 3-D Variants

Figure.6

Figure.7

Figure.8

Figure.9

Figure.10

Figure.11

Figure.12