Analysis of Microbotanical Remains in Camelid Dental Calculus from the Quilcapampa La Antigua Site

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Report Submitted to
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Introduction

Microbotanical remains present opportunities for investigating diets and food-related practices in the ancient past. Analysis of these remains (starch grains, phytoliths, and pollen) found in soils, artifact residues, and dental calculus can be used to answer both social and environmental questions on topics such as domestication processes, food preparation techniques, diets, paleoclimatic conditions, anthropogenic landscape changes, and irrigation strategies (Henry et al. 2011; Li et al. 2010; Madella et al. 2009; Piperno et al. 2009). For example, microbotanical remains from artifact residues and soils can provide insights into exploited foods and their preparation methods during various stages of use, including processing, cooking, and disposal (Barton and Torrence 2015). Dental calculus analysis provides direct evidence of plant consumption, offering the opportunity for precise taxonomic identifications of these plants that can build upon the results of stable isotope or chemical residues analyses (Henry and Piperno 2008; Power et al. 2015; Wesolowski 2010). Importantly, this method can be applied to analyze the diets of not only humans, but also those of wild and domesticated animals (Hardy et al. 2009). Here we report on the identification of starch grains and phytoliths from dental calculus present on camelid teeth recovered from the Quilcapampa La Antigua site, Sihuas Valley, Peru. The teeth are from the Middle Horizon occupation of the site, dating to roughly A.D. 850-900. Nine samples (one from each tooth) were submitted to the University of California, Santa Barbara Integrative Subsistence Laboratory (UCSB ISL) for analysis.

Recovery and Preservation Bias

Microbotanical remains often persist in conditions that are generally unfavorable for archaeological preservation. These remains have been successfully recovered from artifacts with long-term exposure to temperate and tropical wet-dry climates (Dickau et al. 2007), which represent ideal conditions for the degradation of organic remains. These climates are particularly damaging to macrobotanical remains, which often degrade if not carbonized or suffer fragmentation due to taphonomic factors (Dickau 2010); these outcomes are especially likely for ancient (e.g., Paleoindian) contexts. Carbonized macrobotanical assemblages are also subject to certain biases: the fleshy portions of discarded remains often combust during burning and the plant portions that are used to feed fires typically represent inedible rather than consumable refuse (Minnis 1981). Finally, macrobotanical findings offer indirect evidence of diet and thus cannot be used to provide definitive evidence of consumption. Microbotanical remains serve as a valuable dataset for complementing macrobotanical results through providing evidence of environmental composition, the use of plants that are typically consumed in their entirety or are fully combusted during carbonization (e.g., arrowroot, potato), and compelling information regarding the presence of specific plants/plant parts in human and animal diets (Morell-Hart 2019).

Starch grains and phytoliths, though generally hardy, are subject to specific preservation biases that differentially impact their presence and condition. As starch grains are primarily found in the edible portions of plants (e.g., storage organs, mesocarps) and phytoliths are generally found in the inedible portions (e.g., stems, leaves, cobs), the former commonly appears in contexts directly associated with consumption and food preparation, such as cooking residues, while the latter is more typically found in association with economic activities, waste disposal, or decay, such as expected in soils from domestic food processing areas.

Starch grains, due to their organic nature, are also more susceptible to damage than phytoliths. Damage due to grinding, boiling, baking, freezing, fermentation, and other factors results in remarkable morphological changes to starch grains that often are diagnostic to cause (Henry et al. 2009). However, severe damage can also inhibit taxonomic identification of starch.

Wet heat poses a serious threat to starch preservation (Crowther 2012). Exposure to temperatures in excess of 60°C in wet conditions results in starch gelatinization, a process of structural transformation involving disordering of the crystal structures within each granule, the mechanism of which is incompletely understood (Cornejo-Ramírez et al. 2018; Ratnayake and Jackson 2009; Wang et al. 2018). Percentage of gelatinized starch is highly dependent on temperature, the percentage remaining relatively constant after 200 seconds of exposure (Lund and Lorenz 1984). While most starches begin to gelatinize by 60°C, peak gelatinization temperature is determined by genotype (Bean et al. 2019). Physical consequences of gelatinization include swelling of the granule, loss of birefringence, breakdown of the outer border with prolonged heat exposure, and a liquified appearance due to solubilization and crystalline melting (Liu et al. 2009).

Phytoliths, composed of hydrated silica, typically exhibit a pinkish tint unless they have been subject to burning, which results in distortion of the overall shape and pronounced darkening (Piperno 2006; but see Parr 2006 for examples of species producing unburned phytoliths that naturally appear dark in color). Burned phytoliths have also been identified based on higher refractive index values when compared to those of their unburnt counterparts (Elbaum et al. 2003). Prevalence of burned phytoliths can be used to assess changes to landscape management strategies, cooking practices, and use of certain plant species as fuel (Raviele 2011).

Laboratory Procedures

Camelid dental calculus samples from the Quilcapampa site were collected by Aleksa Alaica using sterile dental picks cleaned with methanol. Nine microcentrifuge tubes (one per sample) containing recovered calculus were sent to the UCSB ISL. Each tube was subject to extraction of starch grains and phytoliths according to UCSB ISL standard laboratory procedures developed based on successful protocols by experts in the isolation of microbotanical remains from dental calculus (Henry and Piperno 2008; Mickleburgh and Pagán-Jiménez 2012).

Sample numbers, weights, and volumes were recorded prior to pretreatment. To deflocculate the calculus and ease dispersal, ~1mL of 0.1% alconox solution was added to each sample; alconox solution was substituted for 10% sodium hexametaphosphate solution (Henry and Piperno 2008) as it would have required extra time and funds to obtain and the former surfactant is commonly used in the UCSB ISL to deflocculate sediment samples prior to microbotanical analysis. Tubes were capped and stored in the fumehood for 24 hours. Samples were then sonicated in distilled water for five minutes and centrifuged for three minutes, after which each supernatant was pipetted off using a separate glass Pasteur pipette that had been sterilized in the UCSB ISL prior to use according to standard protocols (pressure cooking in distilled water for two hours and then drying under the fume hood in a sterile environment; see Crowther et al. 2014).

Tube contents were then rinsed in distilled water in two phases: ~1mL of distilled water was added, tubes were vortexed briefly and then centrifuged for three minutes, the supernatant was removed with a sterile pipette, and the entire process was repeated. Chemical pretreatment consisted of the addition of ~1mL of 10% hydrochloric acid to each tube, after which they were stored in the fumehood for 24 hours. The hydrochloric acid was removed by a series of two rinses in distilled water, identical in procedure to the first set of water rinses and ending with the removal of the final supernatant.

Viewing extracted residues on slides requires the addition of a mounting medium to allow for the rotation of microbotanicals so that all perspectives can be considered during identification. One to two drops of 1:1 glycerin to distilled water solution were added to each microcentrifuge tube and the entire contents was mixed using a metal toothpick (sterilized using the lab procedures outlined above). Each extract was examined in its entirety. The contents of each tube were drawn up into a glass Pasteur pipette and the liquid was deposited on a series of sterilized slides, each of which was covered with a sterilized glass cover slip. Edges of the cover slip were sealed with clear nail polish and left to dry for a few minutes.

A Brunel SP-400 Metallurgical Microscope (x50-x600) equipped with transmitted and incident illumination systems and polarization filters for each system. Slides were scanned for starch grains under transmitted polarized light at 100x magnification followed by the removal of the polarization filter and a separate scan for phytoliths at 200x. All microbotanicals were photographed at 400x under nonpolarized, transmitted light, with starch grains also photographed with cross-polarized, transmitted light to record the presence/appearance of extinction crosses (typically visible in undamaged and mildly damaged starch grains).

Starch grains and phytoliths were identified with reference to photographs of modern plants native to Ecuador (Pagán-Jiménez 2015) and the paleoethnobotanical comparative collection at the UCSB ISL. Interpretations of damage patterns were developed based on the results of modern experimental studies (Babot 2003; Henry et al. 2009) and my research on the impacts of chuño production practices on starch morphology and metrics (Melton et al. in prep). Consultation with Dr. Ruth Dickau (Stantec, Inc.) helped to refine preliminary identifications.

Taxonomic identification was not always possible—some plant specimens were too severely damaged or lacked diagnostic features altogether. As a result, these specimens were classified as “unidentified starch grain” or “unidentified phytolith.” In other cases, probable identifications were made—for example, if a specimen closely resembled a potato starch grain, but a clear taxonomic distinction was not possible (e.g., the specimen was heavily damaged), then the specimen was identified as a probable potato starch and recorded as “potato cf., *Solanum tuberosum* cf.” Names and descriptions of phytolith morphotypes adhere to the guidelines of the International Code for Phytolith Nomenclature 2.0 (International Committee for Phytolith Taxonomy 2019).

Quantification Methods

Basic counting and presence/absence measures are used to report the results of starch grain and phytolith analysis. Each starch grain or phytolith is counted individually, measured, and qualitative observations related to morphology are recorded. Counts are then summed by sample (e.g., artifact residue, tooth, soil sample). Ubiquity (presence/absence) of plant taxa in each sample is also observed. Counts of starch grains and phytoliths on archaeological specimens cannot reliably be used to ascertain exactly how much plant material initially came into contact with the sample (Raviele 2011). Common methods of evaluating phytolith concentration involve quantification of silica microspheres (Aleman et al. 2013) and the addition of *Lycopodium clavatum* tablets, each containing a known quantity of spores, prior to flotation (Bozarth et al. 2009). The low counts of phytoliths expected for a dental calculus assemblage did not necessitate the application of these measures. Thus, we rely on raw counts and taxonomic identifications to derive inter-sample comparisons and broadly comment on dietary diversity among camelids who inhabited the Quilcapampa site.

Results

Microbotanical remains recovered from the analysis of calculus on camelid teeth at the Quilcapampa site include four starch grains and one phytolith (Table 1). Four samples, ACL-10424, 10498, 10500, and 10501, yielded starch grains or phytoliths; the remaining five samples

were devoid of these remains. Morphological attributes of recovered starch grains and phytoliths are recorded in Table 2.
Results are discussed according to sample:

ACL-10424 yielded one maize starch grain (Figure 1). This angular and irregular grain is characteristic of maize starch in terms of size and morphological attributes. The extinction cross is obscured in the lower left corner of the starch in Figure 1c, likely due to damage but the type is unclear due to its mild presentation.

ACL-10498 yielded one unidentified phytolith (Figure 2). This phytolith roughly fits the shape expectations for the spheroid echinate morphotype (commonly found in the palm family), but it is much larger than comparative phytoliths and thus remains unidentified.

ACL-10500 yielded one potato starch grain (Figure 3). The size, shape, and hilum location fit expectations, but its internal structure is clearly damaged (signified by "bubbled" appearance). Its extinction cross is obscured on the left side due to damage. The softened appearance of the border and "bubbled" internal presentation fit expectations for chuño production. This process of freeze-drying potatoes by alternating time in the sun and storage in a prepared earthen pit commonly results in a subtle and fragmented border, flattened appearance, and damage to the internal structure of starches that produces a "bubbled" presentation. Furthermore, the size of the starch is within the range of length to width ratios expected for modern chuño negro comparative specimens; an argument for qualitative and quantitative differentiation of chuño negro and chuño blanco starches will be presented in a forthcoming publication (Melton et al. in prep).

ACL-10501 yielded a potato starch grain and an unidentified starch grain (Figures 4 and 5). Damage on the potato starch is evidenced by missing areas along the upper portion of the border in Figure 4a and obscuring of the upper right quadrant of the extinction cross in Figure 4b. Based on the broken nature of the border, the damage was likely caused by grinding activities. Grinding damage is expected for dental calculus, particularly those of camelids, as mastication is involved in deposition. There is also a possibility for exposure to boiling or baking based on uneven internal texture (Henry et al. 2009:Figure 3), which could have occurred prior to consumption. The unidentified starch grain could not be identified to any comparative taxon and did not meet expectations for plants that commonly produce angular starches, such as maize.

Conclusions

The Quilcapampa La Antigua assemblage revealed evidence of maize and potato, two domesticates widely represented in macrobotanical samples collected from this site (Biwer 2019). The discovery of these remains importantly supplements our knowledge of ninth-century camelid diets and foddering practices, topics which are poorly understood. Microbotanical results presented in this report suggest that camelids either obtained maize and potato in field settings, from raiding human trash, or through being fed these crops as part of foddering practices. The damage patterns on the potato starches from ACL-10500, corresponding to chuño negro preparation, and ACL-10501, matching expectations for boiling or baking, support the second and third hypotheses. The unidentified phytolith indicates that these remains can be recovered from camelid teeth, but further phytolith research is needed to gain insights into grazing practices.

Table 1. Taxonomic Identifications of Starch Grains and Phytoliths in Camelid Dental Calculus from Quilcapampa.

Common Name	**Scientific Name**	**Sample No.**	**Starch/Phytolith**	**Size (Length x Width)**
Maize	*Zea mays*	ACL-10424	Starch	18.5 x 15 µm
Potato	*Solanum tuberosum*	ACL-10500	Starch	45.5 x 39 µm
Potato	*Solanum tuberosum*	ACL-10501	Starch	26.5 x 25.6 µm
Unidentified Starch	-	ACL-10501	Starch	16.5 x 22.2 µm
Unidentified Phytolith	-	ACL-10498	Phytolith	27 x 35 µm

Table 2. Morphological Attributes of Starch Grains and Phytoliths in Camelid Dental Calculus from Quilcapampa.

Common Name	Starch/ Phytolith	Sample No.	Hilum	Shape/ Morphotype	Lamellae	Extinction Cross	Fissure	Surface Topography	Border	Margin
Maize	Starch	ACL-10424	Visible, Centric, Closed	Angular, Irregular	Not visible	Centric, Visible, Obscured in bottom half, possibly due to damage (type uncertain)	Y-Shaped, Present	Bumpy; Possible Pressure Facet	Visible, Single	Angular (with some undulation on top side)
Potato	Starch	ACL-10500	Open, Visible, Eccentric	Slightly Ovate (possibly due to damage)	Not visible	Eccentric, Visible, Slightly obscured on left side (likely due to damage)	N/A	Holes throughout; Relatively flattened appearance (but slightly raised laterally)	Low visibility, Possibly not intact in upper left corner	Undulating, Irregular
Potato	Starch	ACL-10501	Visible, Open	Slightly Ovate	Not visible	Centric, Visible, Extremely obscured on right side due to damage	N/A	Fractured appearance, particularly along border, Uneven internal texture	Low visibility, Not intact along upper and lower sides	Undulating
UID Starch	Starch	ACL-10501	Centric, Open, Visible	Angular (roughly hexagonal), Irregular	Not visible	Centric, Visible, Obscured in bottom right quadrant	X-Shaped (barely)	N/A	Mostly intact, damaged along right side	Irregular, Undulating on top right corner
UID Phytolith	Phytolith	ACL-10498	-	Spheroid echinate	-	-	-	"Bumpy" surface	-	-

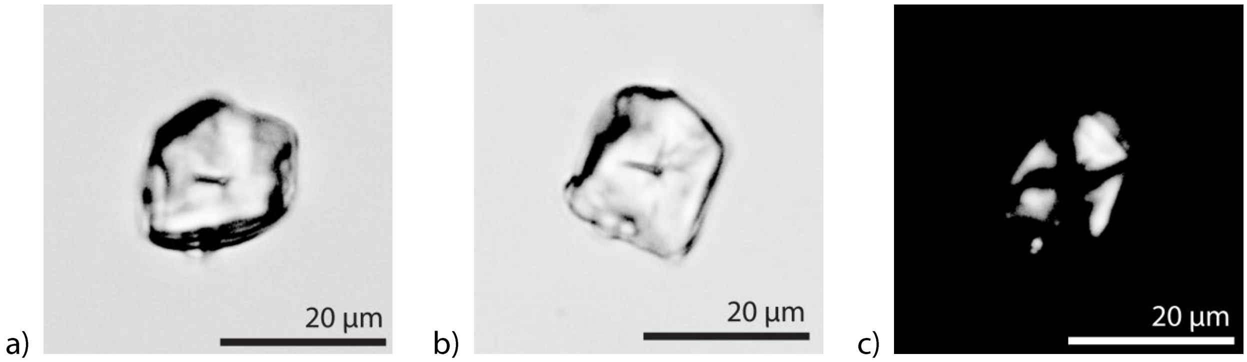

Figure 1. Maize starch grain visualized under non-polarized (a, b) and polarized (c) light, ACL-10424.

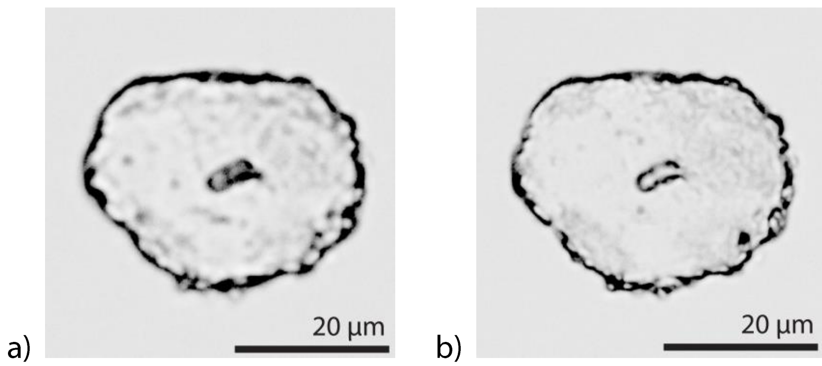

Figure 2. Unidentified phytolith visualized under non-polarized light (a, b), ACL-10498.

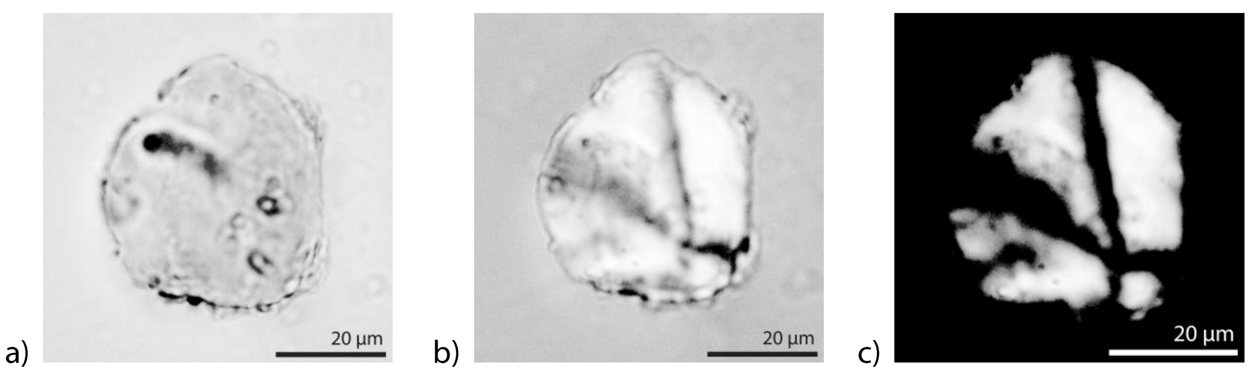

Figure 3. Potato starch grain visualized under non-polarized (a), polarized (b), and strongly polarized light (c), ACL-10500.

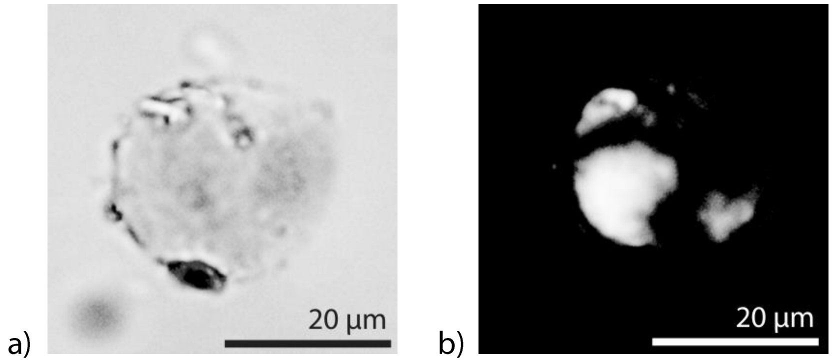

Figure 4. Potato starch grain visualized under non-polarized (a) and polarized light (b), ACL-10501.

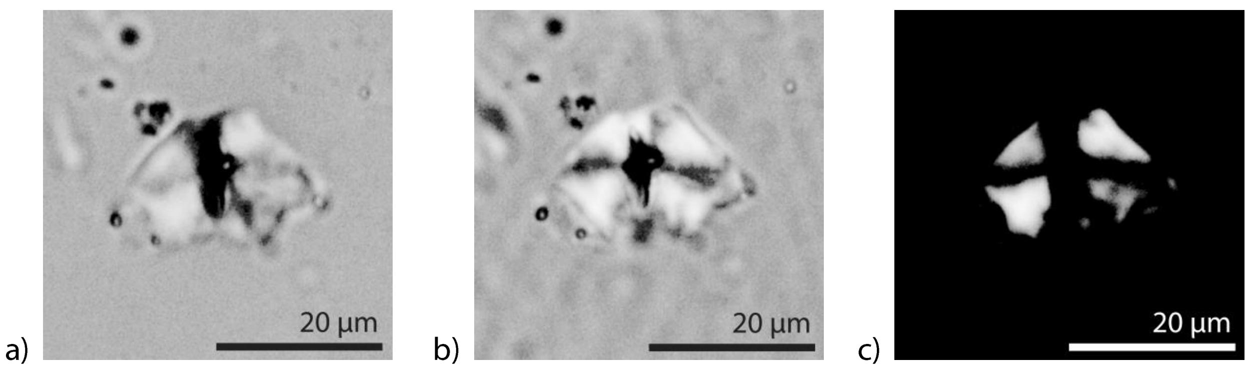

Figure 5. Unidentified starch grain visualized under non-polarized (a), polarized (b), and strongly polarized light (c), ACL-10501.

References Cited

Aleman, Julie C., Audrey Sant-Jean, Bérangère Leys, Christopher Carcaillet, Charly Favier, and Laurent Bremond
2013 Estimating Phytolith Influx in Lake Sediments. *Quaternary Research* 80(2):341-347.

Babot, M. del P.
2003 Starch Grain Damage as an Indicator of Food Processing. In *Phytolith and Starch Research in the Australian-Pacific-Asian Regions: The State of the Art*, edited by D.M. Hart and L.A. Wallis, pp. 69-81. Pandanus Books, Canberra, Australia.

Barton, Huw, and Robin Torrence
2015 Cooking Up Recipes for Ancient Starch: Assessing Current Methodologies and Looking to the Future. *Journal of Archaeological Science* 56:194-201.

Bean, Scott R., Lijia Zhu, Brennan M. Smith, Jeff D. Wilson, Brian P. Ioerger, and Michael Tilley
2019 Starch and Protein Chemistry and Functional Properties. In *Sorghum and Millets: Chemistry, Technology and Nutritional Attributes*, edited by John R. N. Taylor and Kwaku G. Duodu, pp. 131-170. 2nd ed. Elsevier, New York.

Biwer, Matthew E.
2019 Colonialism, Cuisine, and Culture Contact: An Analysis of Provincial Foodways of the Wari Empire (A.D. 600-1000). PhD dissertation, Department of Anthropology, University of California, Santa Barbara.

Bozarth, S.R., K. Price, W.I. Woods, E.G. Neves, and R. Rebellato
2009 Phytoliths and *Terra Preta*: The Hatahara Site Example. In *Amazonian Dark Earths: Wim Sombroek's Vision*, edited by William I. Woods, Wenceslau G. Teixeira, Johannes Lehmann, Christoph Steiner, Antoinette M.G.A. WinklerPrins, and Lilian Rebellato, pp. 85-98. Springer, New York.

Cornejo-Ramírez, Yaeel Isbeth, Oliviert Martínez-Cruz, Carmen Lizette Del Toro-Sánchez, Francisco Javier Wong-Corral, Jesús Borboa-Flores, and Francisco Javier Cinco-Moroyoqui
2018 The Structural Characteristics of Starches and Their Functional Properties. *CyTA – Journal of Food* 16(1):1003-1017.

Crowther, Alison
2012 The Differential Survival of Native Starch During Cooking and Implications for Archaeological Analyses: A Review. *Archaeological and Anthropological Sciences* 4(3):221-235.

Crowther, Alison, Michael Haslam, Nikki Oakden, Dale Walde, and Julio Mercader
2014 Documenting Contamination in Ancient Starch Laboratories. *Journal of Archaeological Science* 49:90-104.

Dickau, Ruth
2010 Microbotanical and Macrobotanical Evidence of Plant Use and the Transition to Agriculture in Panama. In *Integrating Zooarchaeology and Paleoethnobotany*. Springer, New York.

Dickau, Ruth, Anthony J. Ranere, and Richard G. Cooke
2007 Starch Grain Evidence for the Preceramic Dispersals of Maize and Root Crops into Tropical Dry and Humid Forests of Panama. *PNAS* 104(9):3651-3656.

Elbaum, Rivka, Steve Weiner, Rosa M. Albert, and Michael Elbaum
2003 Detection of Burning of Plant Materials in the Archaeological Record by Changes in the Refractive Indices of Siliceous Phytoliths. *Journal of Archaeological Science* 30(2):217-226.

Hardy, Karen, Tony Blakeney, Les Copeland, Jennifer Kirkham, Richard Wrangham, Matthew Collins
2009 Starch Granules, Dental Calculus and New Perspectives on Ancient Diet. *Journal of Archaeological Science* 36(2):248-255.

Henry, Amanda G., and Dolores R. Piperno
2008 Using Plant Microfossils from Dental Calculus to Recover Human Diet: A Case Study from Tell al-Raqā'i, Syria. *Journal of Archaeological Science* 35(7):1943-1950.

Henry, Amanda G., Alison S. Brooks, and Dolores R. Piperno
2011 Microfossils in Calculus Demonstrate Consumption of Plants and Cooked Foods in Neanderthal Diets (Shanidar III, Iraq; Spy I and II, Belgium). *PNAS* 108(2):486-491.

Henry, Amanda G., Holly F. Hudson, and Dolores R. Piperno
2009 Changes in Starch Grain Morphologies from Cooking. *Journal of Archaeological Science* 36(3):915-922.

International Committee for Phytolith Taxonomy
2019 International Code for Phytolith Nomenclature (ICPN) 2.0. *Annals of Botany* 124(2):189-199.

Li, Rencheng, John A. Carter, Shucheng Xie, Shengli Zou, Yansheng Gu, Junying Zhu, and Beisheng Xiong
2010 Phytoliths and Microcharcoal at Jinluojia Archaeological Site in Middle Reaches of Yangtze River Indicative of Paleoclimate and Human Activity During the Last 3000 Years. *Journal of Archaeological Science* 37(1):124-132.

Liu, Qiang, Elizabeth Donner, Richard Tarn, Jaspreet Singh, and Hyun-Jung Chung
2009 Advanced Analytical Techniques to Evaluate the Quality of Potato and Potato Starch. In *Advances in Potato Chemistry and Technology*, edited by Jaspreet Singh and Lovedeep Kaur, pp. 221-248. Academic Press, Cambridge, Massachusetts.

Lund, Daryl, and Klaus J. Lorenz
1984 Influence of Time, Temperature, Moisture, Ingredients, and Processing Conditions on Starch Gelatinization. *Critical Reviews in Food Science & Nutrition* 20(4):249-273.

Madella, M., M.K. Jones, P. Echlin, A. Powers-Jones, and M. Moore
2009 Plant Water Availability and Analytical Microscopy of Phytoliths: Implications for Ancient Irrigation in Arid Zones. *Quaternary International* 193(1-2):32-40.

Melton, Mallory A., Rita Panjarjian, Matthew E. Biwer, and Amber M. VanDerwarker
In prep A Method of Identifying Chuno Blanco and Chuno Negro in Archaeological Samples Based on Metrics and Morphological Attributes. (article to be submitted to *Journal of Archaeological Science*)

Mickleburgh, Hayley L., Jaime R. Pagán-Jiménez
2012 New Insights into the Consumption of Maize and Other Food Plants in the Pre-Columbian Caribbean from Starch Grains Trapped in Human Dental Calculus. *Journal of Archaeological Science* 39(7):2468-2478.

Minnis, Paul E.
1981 Seeds in Archaeological Sites: Sources and Some Interpretive Problems. *American Antiquity* 46(1):143-152.

Morell-Hart, Shanti
2019 Techniques for Integrating Macrobotanical and Microbotanical Datasets: Examples from Pre-Hispanic Northwestern Honduras. *Journal of Field Archaeology* 44(4):234-249.

Pagán-Jiménez, Jaime R.
2015 *Almidones: Guía de Material Comparativo Moderno del Ecuador para los Estudios Paleoetnobotánicos en el Neotrópico.* Aspha Ediciones, Buenos Aires.

Parr, Jeff F.
2006 Effect of Fire on Phytolith Coloration. *Geoarchaeology* 21(2):171-185.

Piperno, Dolores R.
2006 *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists.* Altamira Press, Lanham, Maryland.

Piperno, Dolores R., Anthony J. Ranere, Irene Holst, Jose Iriarte, and Ruth Dickau
2009 Starch Grain and Phytolith Evidence for Early Ninth Millennium B.P. Maize from the Central Balsas River Valley, Mexico. *PNAS* 106(13):5019-5024.

Power, Robert C., Domingo C. Salazar-García, Lawrence G. Straus, Manuel R. González Morales, and Amanda G. Henry
2015 Microremains from El Mirón Cave Human Dental Calculus Suggest a Mixed Plant-Animal Subsistence Economy During the Magdalenian in Northern Iberia. *Journal of Archaeological Science* 60:39-46.

Ratnayake, Wajira S., and David S. Jackson
2009 Starch Gelatinization. *Advances in Food and Nutrition Research* 55:221-268.

Raviele, Maria E.
2011 Experimental Assessment of Maize Phytolith and Starch Taphonomy in Carbonized Cooking Residues. *Journal of Archaeological Science* 38(10):2708-2713.

Wang, Shujun, Chen Chao, Fengjuan Xiang, Xiu Zhang, Shuo Wang, and Les Copeland
2018 New Insights into Gelatinization Mechanisms of Cereal Endosperm Starches. *Scientific Reports* 8:3011.

Wesolowski, Verônica, Sheila Maria Ferraz Mendonça de Souza, Karl J. Reinhard, Gregório Ceccantini
2010 Evaluating Microfossil Content of Dental Calculus from Brazilian Sambaquis. *Journal of Archaeological Science* 37(6):1326-1338.