

## **Rooting ancient plant use: the development of a modern reference collection**

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**Yahaira Núñez Cortés, Ph.D.  
Department of Anthropology  
College of Social Sciences and Interdisciplinary Studies**

### **Introduction**

Past human groups maintained complex relationships with the environments they inhabited. Understanding these interactions is key to addressing a wide range of anthropological questions related to the domestication of plants and animals, settlement patterns, adaptation to specific environmental and climatic conditions, and the use of natural resources for food, housing, ritual, and medicine.

This project focuses on human–plant interactions in the tropical Americas by analyzing starch grains from economically important plants that were potentially used in the past. The close relationship between humans and their natural surroundings is reflected in the abundant representations of animals and plants found in the archaeological record of the tropical Americas (Linares 1977), as well as in the mythological traditions and origin stories of many Indigenous groups throughout Latin America (Bozzoli 1983).

Methodological approaches for investigating past human–plant interactions include both macrobotanical remains (e.g., carbonized seeds and wood) and microbotanical remains (e.g., starch grains, phytoliths, and pollen). More recently, the analysis of starch grains recovered from archaeological tools and dental calculus has opened new avenues for investigating the consumption of specific starchy foods.

Starch grains are semicrystalline structures composed of the six-carbon sugar polymer D-glucose. These granules are formed through the accretion of layers (lamellae) consisting of two polysaccharides: amylose and amylopectin. The relative proportions of these polymers influence starch grain morphology, resulting in variation in size, shape, and other diagnostic characteristics (Pearsall 2016). This morphological variation makes starch grain analysis a powerful tool for identifying the specific plant taxa that were processed and consumed in the past.

### **Project context and objectives**

Tropical environments were long considered unsuitable for the development of complex social structures (Roosevelt 1999). However, a growing body of research and the application of new methodologies has demonstrated that these areas were home to thriving human groups, from the Amazon to the Peten forests. Moreover, it was long sustained that tropical areas were not suitable for studying the interaction of humans and plants, due to the limited preservation of

macro-botanical remains. Nonetheless, the application of microbotanical analyses has been demonstrated successfully at sites located in tropical environments (Davis 2007).

While the study of past human-environment interactions and ancient plant remains has been a long-standing interest for the scholarly community, there are very few reference collections published or easily accessible (but see Pagán Jiménez 2015). Moreover, published images do not capture the full three-dimensional morphology of starch grains. Proper identification of diagnostic starches requires the rotation of the granule under a polarized microscope, and the description and measurement of their characteristics (Maltese cross, lamellae, fissures, pressure facets, etc.).

Large and detailed reference collections permit not only the identification of plant resources but contribute to answering research questions related to the origins of domestication, the utilization of wild resources, the differential exploitation of the environment, the impact of human populations over the forest, types of plant foods processing (i.e. grinding, cutting, boiling, etc.), the identification of usable parts of the plant, and more.

The main objectives of this project were: (1) to create a comprehensive modern reference collection of starch grains from economically important plants likely utilized during pre-Columbian times in the tropical Americas; (2) to provide training and learning experiences for students on the extraction and identification of starch grains, as well as the curation of a reference collection.

Skills acquired by the students included microscopy, data analysis, and curation. The creation of modern reference collections is essential for the accurate analysis and identification of archaeological samples, including those obtained from sediments, grinding tools, ceramics, and dental calculus from human and animal remains.

## **Methodology**

This project was structured into three main activities: (1) obtaining plant tissue, (2) extraction and processing of plant material, and (3) documentation of starch grains. The methods for each of these phases are described in detail below. The laboratory had to be cleaned with recently boiled water before starting the plant processing and in between each of the plants tested to prevent cross-contamination between samples.

The ultimate goal is to start building an in-house reference collection, stored in the form of photographs, flours, and living specimens, that allows us to observe the characteristics of starch grains and verify our identifications when analyzing archaeological plant materials. Digital collections allow for proper curation and storage of the data, as well as to facilitate sharing with other researchers and students working on similar topics.

### **(1) Obtention of plant tissue**

In order to obtain and observe starch grains from a diversity of plants we tested seeds, fruits, and underground storage organs (roots, rhizomes and tubers). We obtained plant materials for a reference collection from three sources: (1) the current living collection at Sacramento State

greenhouses, (2) the University of Minnesota, CBS Conservatory & Botanical Collection, and (3) produce purchased at local grocery stores whose origins are known to be in the tropical areas of the Americas.

Living specimens will remain at the greenhouses of Sacramento State for future analysis and to serve the large Sacramento State faculty and student community.

## (2) Extraction and processing of plant material

The method for extracting starches was modified according to the type of plant material we were working with. To obtain flour from tubers, rhizomes and fruits (squash) we cut and grated the plant material and let it sit in distilled water overnight. The following day, we strained it through a fine mesh (cheese cloth) to allow only the starch material to pass through. This solution of starch and water was centrifuged to concentrate the starch at the bottom; the excess water was decanted. The sample was washed with distilled water and centrifuged two more times to prevent the growth of fungus. The remaining material was left to dry at room temperature. This material was then ready to place on the slide and observe under the microscope.

Starches from fruits were extracted by cutting the plant material and placing a small part of the tissue on the slide with distilled water. A soft pounding on a tray or a mortar with distilled water was required in some cases to extract the starch from the tissue. Seeds were left to soften on distilled water for a couple of hours before cutting or pounding. A little bit of the water solution was placed on the slide for observation.

## (3) Documentation of starch grains

Slides with material, distilled water and glycerin were placed under the microscope for observation, photography with the required polarizations, and measurement. We used a Nikon Eclipse Ci polarizing microscope, and photographed the samples with a Nikon Digital Sight 100 camera and the NIS-Elements D software. Starch grains are three-dimensional objects and, as such, they require rotation to allow us to capture the full surface characteristics across from which the variability of each plant can be observed.

An Excel spreadsheet was created to capture the characteristics of each of the granules observed, including: plant's common name, scientific name, number of grains, 2D form, 3D forms, measurements (microns), presence and type of pressure facets, location of the hilum, lamellae, presence and type of fissures, extinction (Maltese) cross, and any additional comment.

Our descriptions of the observed characteristics were based primarily on those reported in the literature for the construction of starch grain reference collections. Descriptions of two-dimensional (2D) and three-dimensional (3D) morphology followed the examples provided by Ahituv and Henry (2022) and Mercader et al. (2018). Descriptions of hilum position and lamellae were based on the criteria proposed by Louderback et al. (2022), while descriptions of fissures and extinction crosses followed the terminology of Pagán Jiménez (2015). In cases where the existing literature did not provide sufficient descriptive detail for our reference collection, we developed and defined additional attributes to better characterize the observed starch grains.

## Results

To introduce the students to the observation of starch grains under the microscope we initially worked with potato (*Solanum tuberosum*), corn (*Zea mays*), and beans (*Phaseolus vulgaris*). All of these plants present starches that can be distinguished in comparison, and allow for the initial observation of the features that needed to be described. Following this phase, we began processing plant material for either flour or direct extraction on the microscope slides.

### a. Living plant collection

We obtained plant material from the University of Minnesota CBS Conservatory & Botanical Collection, most of which could be propagated at the Sacramento State greenhouses (Figure 1). In collaboration with Daniel Pfarr (Department of Biological Sciences), we established the living collection and grew several chili pepper varieties from seed (Table 1).

Table 1. Species obtained for the living collection at the Sac State green houses.

| Common name                  | Scientific name            | Source                  |
|------------------------------|----------------------------|-------------------------|
| Arrowroot                    | <i>Maranta arundinacea</i> | University of Minnesota |
| Achira                       | <i>Canna indica</i>        | University of Minnesota |
| Air potato (old world)       | <i>Dioscorea bulbifera</i> | University of Minnesota |
| Zamia                        | <i>Zamia nesophila</i>     | University of Minnesota |
| Jalapeño pepper              | <i>Capsicum annuum</i>     | Seeds                   |
| Poblano pepper               | <i>Capsicum annuum</i>     | Seeds                   |
| Cayenne pepper               | <i>Capsicum annuum</i>     | Seeds                   |
| Serrano Tampiqueño pepper    | <i>Capsicum annuum</i>     | Seeds                   |
| Shishito pepper (East Asian) | <i>Capsicum annuum</i>     | Seeds                   |
| Hungarian Hot Wax pepper     | <i>Capsicum annuum</i>     | Seeds                   |
| Cal Wonder pepper            | <i>Capsicum annuum</i>     | Seeds                   |
| Red Habanero pepper          | <i>Capsicum chinense</i>   | Seeds                   |
| Banana pepper                | <i>Capsicum annuum</i>     | Seeds                   |
| Anaheim pepper               | <i>Capsicum annuum</i>     | Seeds                   |

The University of Minnesota, CBS Conservatory & Botanical Collection curators provided the records of each one of the specimens to trace their provenience:

- *Maranta arundinacea*, accession 2019-5-75 received as a plant from Dylan Hannon, Huntington Botanical Gardens, San Marino, California.
- *Canna indica*, accession 2019-7-4 received as seed from Rare Palm Seeds, Munich, Germany.
- *Zamia nesophila*, accession 2019-6-19 received as seed from a private collector.
- *Dioscorea bulbifera*, accession 2022-1-74 unknown provenance, has been a part of our collection for 10+ years.





Figure 1. *Maranta arundinacea* growing at the Sac State greenhouses; left: leaves and stem; right: rhizome where starches are abundant.

### b. Starch reference collection

We processed and analyzed 17 plant species (Table 2). Although starch grains were not recovered from every species, other plant tissues may prove more suitable for future analyses. For each species, we documented diagnostic starch grain characteristics and recorded maximum grain length (Figure 2). A limited number of grains were measured for white sweet potato, achira, and arrowroot; therefore, additional measurements will be needed to improve statistical reliability.

Table 2. Species processed for starch grain analysis.

| Common name        | Scientific name            | Plant material | Starch present | Starches described | Starches measured |
|--------------------|----------------------------|----------------|----------------|--------------------|-------------------|
| Manioc             | <i>Manihot esculenta</i>   | tuber          | Yes (abundant) | 9                  | 116               |
| Red sweet potato   | <i>Ipomoea batatas</i>     | tuber          | Yes (abundant) | -                  | 51                |
| White sweet potato | <i>Ipomoea batatas</i>     | tuber          | Yes (abundant) | 6                  | 6                 |
| Arrowroot          | <i>Maranta arundinacea</i> | rhizome        | Yes            | 5                  | 5                 |
| Achira             | <i>Canna indica</i>        | rhizome        | Yes            | 6                  | 6                 |

|                        |                              |                   |                |    |    |
|------------------------|------------------------------|-------------------|----------------|----|----|
| Jicama                 | <i>Pachyrhizus erosus</i>    | tuber             | Yes (abundant) | 13 | 13 |
| Zamia                  | <i>Zamia nesophila</i>       | roots             | No             | -  | -  |
| Jaracatiá              | <i>Jacaratia corumbensis</i> | fruit             | No             | -  | -  |
| Cherimoya              | <i>Annona cherimola</i>      | fruit             | Yes (few)      | 18 | 18 |
| Dragon fruit           | <i>Selenicereus undatus</i>  | fruit             | Yes (few)      | 14 | 14 |
| Delicata squash        | <i>Cucurbita pepo</i>        | fruit             | Yes (few)      | 12 | 49 |
| Summer squash          | <i>Cucurbita pepo</i>        | fruit             | Yes (few)      | 13 | 83 |
| Butternut squash       | <i>Cucurbita moschata</i>    | fruit             | Yes (few)      | -  | -  |
| Chayote                | <i>Sechium edule</i>         | fruit             | Yes (few)      | 10 | 82 |
| Pineapple              | <i>Ananas comosus</i>        | fruit             | No             | -  | -  |
| Serrano pepper         | <i>Capsicum annum</i>        | fruit             | No             | -  | -  |
| Air potato (old world) | <i>Dioscorea bulbifera</i>   | Fruit-like growth | Yes (abundant) | 7  | 7  |

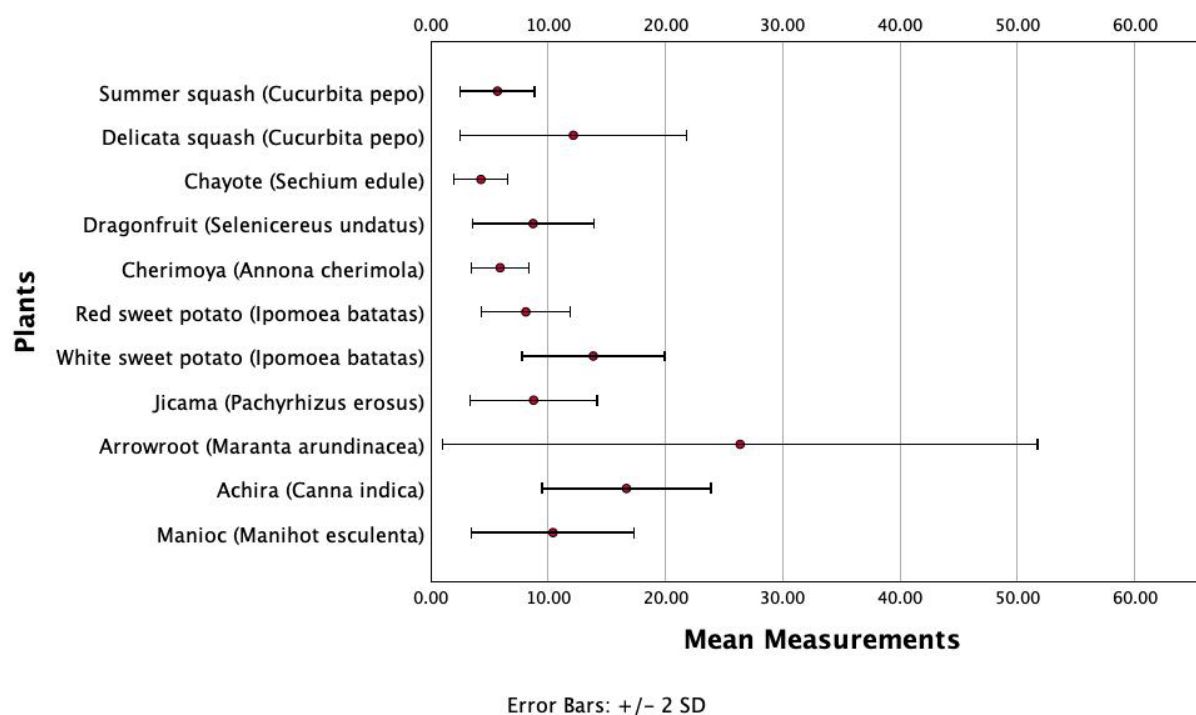


Figure 2. Mean and standard deviation of the starches from analyzed economic plants.

### Manioc (*Manihot esculenta*)

Manioc starch grains displayed dome, sphere, hemisphere, and polyhedral-shaped 3D forms. The hilum was consistently centric, whereas the lamellae were generally indistinct. Dome-shaped grains commonly exhibited five or more pressure facets on the distal surface.

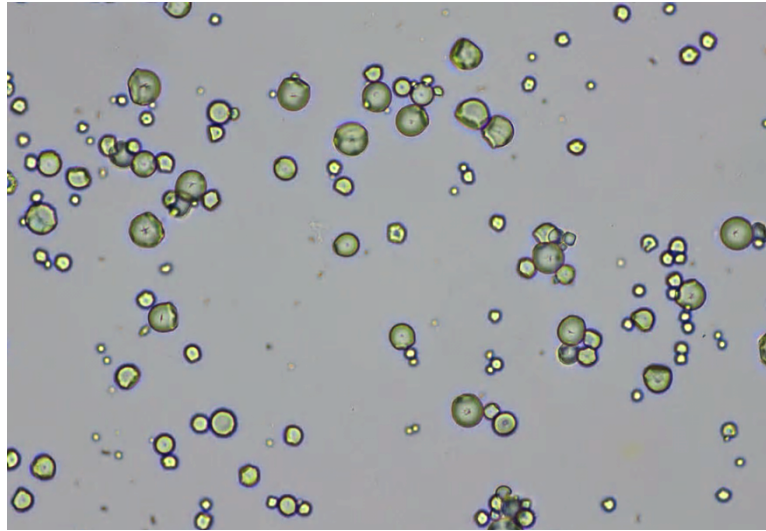


Figure 3. Starch grains of manioc (*Manihot esculenta*) under the microscope.

### White sweet potato (*Ipomoea batatas*)

Sweet potato starches displayed a variety of 3D forms, from cones, domes, spheres and hemispheres. Hilum positioning varied between centric, slightly eccentric and eccentric. We were unable to observe the lamellae. Depending on the 3D form, we were able to describe from 1 to 4 facets.

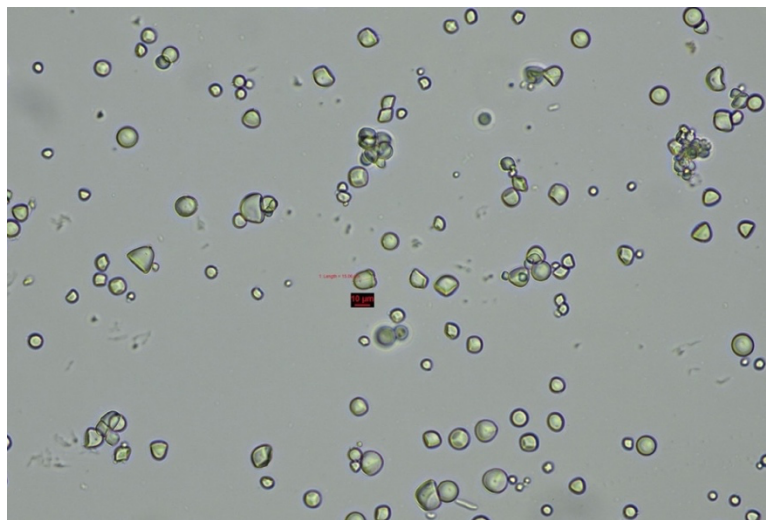


Figure 4. Starch grains of white sweet potato (*Ipomoea batatas*) under the microscope.

*Arrowroot (Maranta arundinacea)*

Arrowroot starch grains are characterized by lumpy, lenticular three-dimensional (3D) morphology. They possess an eccentric hilum, and their lamellae are clearly visible. One smaller starch grain lacked visible lamellae.

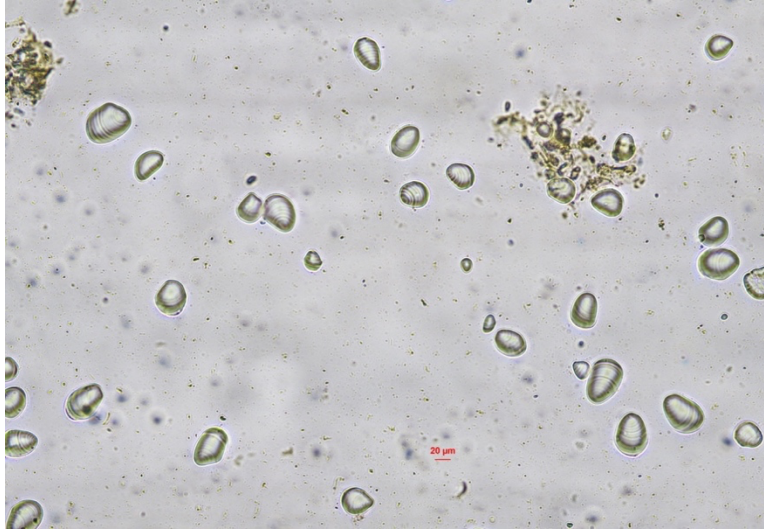


Figure 5. Starch grains of arrowroot (*Maranta arundinacea*) under the microscope.

*Achira (Canna indica)*

Achira starch grains exhibited lumpy lenticular, oval lenticular, and lenticular biconvex three-dimensional (3D) morphologies. Most grains were lamellated and possessed an eccentric hilum. Some grains also exhibited undulating margins.

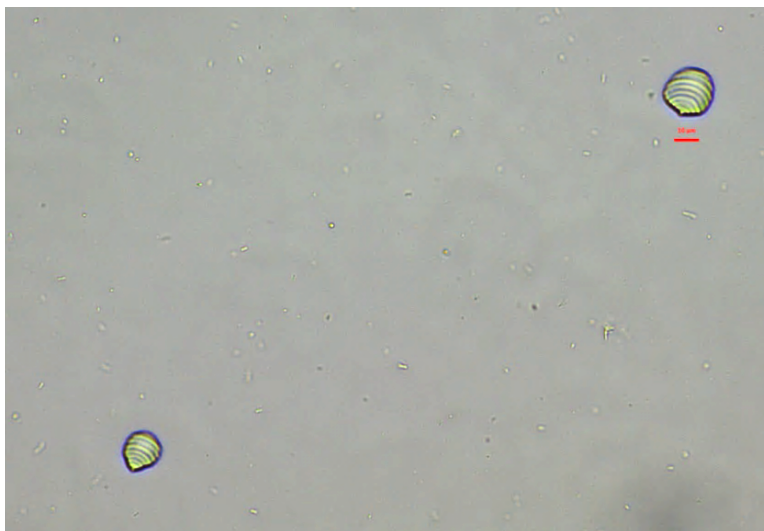


Figure 6. Starch grains of achira (*Canna indica*) under the microscope.



*Jicama (Pachyrhizus erosus)*

Jicama starch grains ranged from cylindroid and dome-shaped to cone-shaped, spherical, and irregular forms. These are non-lamellated with centric and slightly eccentric hilum. We observed one to two facets in a couple of cases.

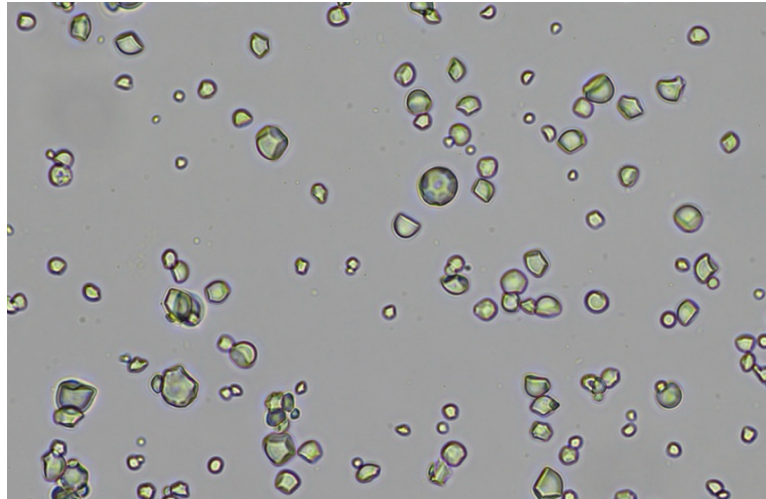


Figure 7. Starch grains of jicama (*Pachyrhizus erosus*) under the microscope.

*Cherimoya (Annona cherimola)*

Rotation of the grains was limited because they remained embedded within the surrounding cells. Consequently, only one spherical grain could be fully characterized. 2D shapes include lumpy circular, triangular swollen, D-shaped, and irregular polyhedral. Hilum positioning was described as centric, slightly eccentric and eccentric. Lamellae were not clearly visible.

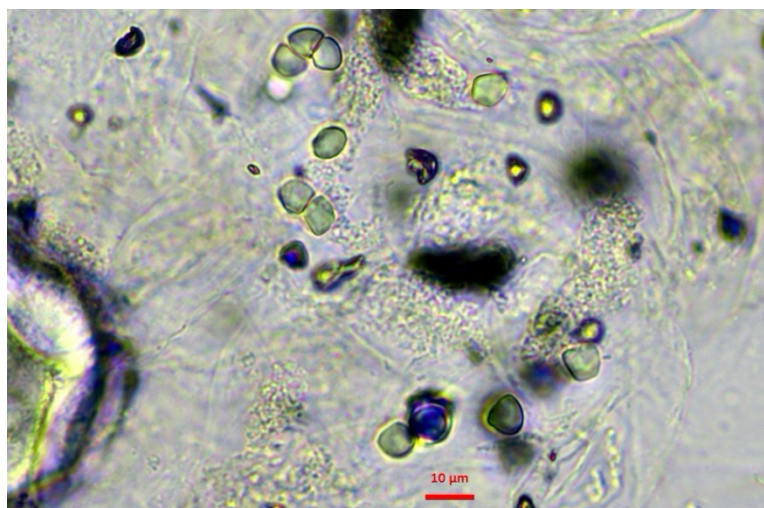


Figure 8. Starch grains of cherimoya (*Annona cherimola*) under the microscope.

### Dragon fruit (*Selenicereus undatus*)

Starches of dragon fruit include spherical, lumpy spherical, hemispherical and dome 3D forms, with one to two sunken facets. Hilum positioning varies from centric to slightly eccentric. The lamellae were not clearly visible.

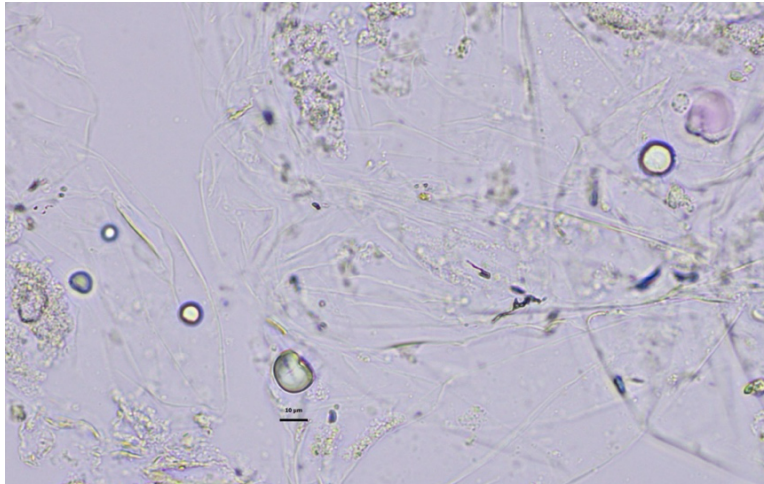


Figure 9. Starch grains of dragon fruit (*Selenicereus undatus*) under the microscope.

### Delicata squash (*Cucurbita pepo*)

The 3D shapes of starches in delicata squash are variable, from hemispherical, cone, dome, pear shape, lumpy spherical and lobate. Most of the grains observed were lamellated, with an eccentric or slightly eccentric hilum. Particularly distinctive were dome and cone-shaped grains with a corrugated distal surface. Some sunken facets were observed.

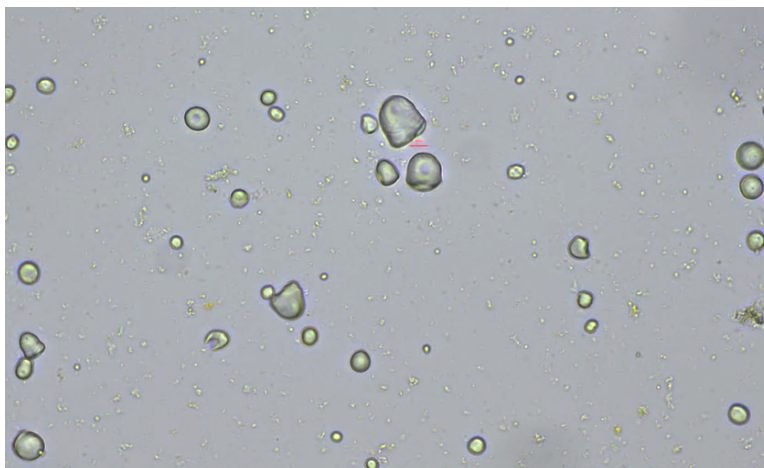


Figure 10. Starch grains of delicata squash (*Cucurbita pepo*) under the microscope.

### Summer squash (*Cucurbita pepo*)

Summer squash starch grains were predominantly dome-shaped or spherical in three-dimensional (3D) morphology. They possess an eccentric hilum, while the lamellae are generally not clearly visible. Some starch grains exhibit concentric rings around the body or a corrugated surface texture.

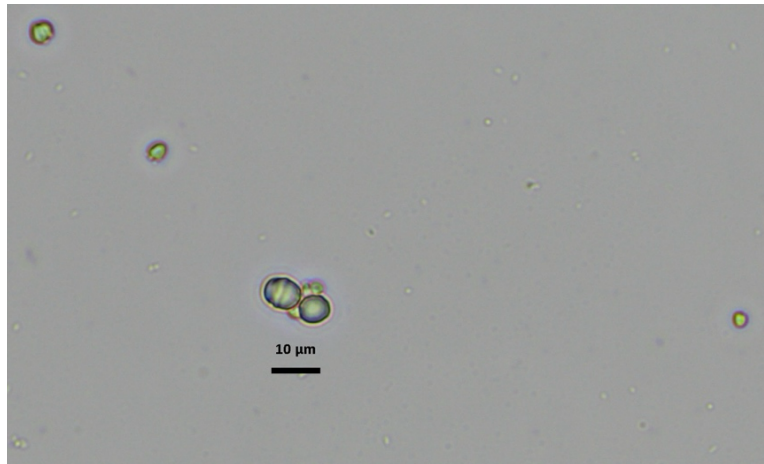


Figure 11. Starch grains of summer squash (*Cucurbita pepo*) under the microscope.

### Chayote (*Sechium edule*)

Chayote starches have hemispherical and spherical 3D shapes, with at least two generally angular facets. The hilum was centric, and the lamellae were generally indistinct.

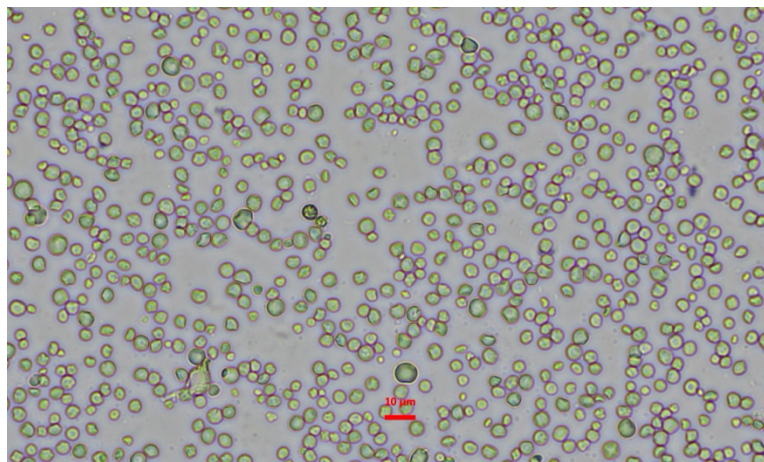


Figure 12. Starch grains of chayote (*Sechium edule*) under the microscope.

### c. Future work

At least two *Zamia* species (*Zamia fisheri* and *Zamia pumila*) are currently being cultivated in the Sacramento State greenhouse and will be included in future starch grain analyses. Although pepper plants were successfully grown from seed during this project, the fruits had not matured by the end of the semester, preventing the extraction and analysis of their starch grains. Characterizing the starch grains of these species will therefore be an important objective for upcoming semesters.

Additional plant taxa to be included in the sampling are pejobaye (*Bactris gasipaes*), papaya (*Carica papaya*), mountain papaya (*Jacaratia mexicana*), avocado (*Persea americana*), guapinol (*Hymenaea courbaril*), jocote (*Spondias purpurea*), jobo (*Spondias mombin*), ñampi (*Dioscorea trifida*), achirilla (*Canna jaegeriana*), achira (*Canna tuerckheimii*), bijao (*Calathea latifolia*), broadleaf arrowhead (*Sagittaria latifolia*), arracacha (*Arracacia xanthorrhiza*), passion fruit (*Passiflora adenopoda*), zarzaparrilla (*Smilax spinosa*), arrowleaf elephant's ear (*Xanthosoma sagittifolium*), emperor zamia (*Zamia imperialis*), and Panamanian cycad (*Zamia hamannii*).

The application of starch grain analysis to archaeological contexts has generated important insights into past human diets and plant use in many regions of the world (Morell-Hart 2020; Piperno & Dillehay 2008). This project is part of a broader research program investigating the variability of dietary practices among the human populations that inhabited southern Central America during the last 2,000 years of the pre-Columbian period. In the coming semesters, we will apply starch grain analysis to archaeological dental calculus, whose mineralized structure traps and preserves microscopic plant remains, providing direct evidence of the plants consumed by individuals. Continued expansion of the modern reference collection is essential for the accurate identification of these archaeological starch grains and will remain a central component of this research.

### d. Limitations

The first step of this project was the preparation of the starch laboratory at Sacramento State. The preparation of the lab came with delays related to the arrival of the appropriate equipment for the microscope. We dedicated the first months to preparing flours from various tubers. After the microscope was ready, we were able to proceed with the description and photography of the samples.

We also had challenges obtaining living collections from botanical gardens and conservatories. However, contacts were made with the University of California Botanical Garden at Berkeley, the UC Davis Botanical Conservatory, and the New York Botanical Garden. We expect to continue conversations with these institutions to obtain samples in the future.

### Impact on diversity and inclusion

Research on diet and culture, in both past and present human groups, has demonstrated the close interconnection between food and identity. Food has the capacity to awaken a sense of belonging through ingredients, flavors, memories, and recipes that have been passed down for generations. My experience with students at Sacramento State and elsewhere has shown me that through



talking about food and plants, we can connect with our own identities and the areas where we come from.

Demographic data for students in the Anthropology Department (which closely follow demographics in Sacramento State) encompass a total of 36% of the students who identify as Latinx; most of them coming from Mexico, but also Guatemala, El Salvador, Puerto Rico and Cuba. All of these countries are located within the tropical regions of the Americas, which are the focus of this project. As such, this project allows students to establish close connections with the environments, the ingredients, and the foods that form part of the Latinx identity. Moreover, this project draws on past Indigenous groups' knowledge about the use of resources from these tropical environments. This project and the collections that derive from it, will serve as material to continue exploring the history of these past populations and the deep historical roots of many of the foods that identify us as Latinx.

It was long held that tropical environments could not sustain complex societies in the past, or that their presence may have been the result of contact with more “developed” groups in other areas (Roosevelt 1999). It was also sustained that the tropics could not have provided the conditions for the preservation of botanical remains to answer questions about the human-environment interactions. Today we know that tropical environments not only did sustain complex societies, but that human groups were highly diverse in these regions. As teaching material, this reference collection serves to introduce the students to the richness of the tropical environments and strategies used by human groups to develop and thrive there for centuries. It opens avenues for the study and discussion of diversity in cultural expressions, management of natural resources, responses to climate change, and current cultural management models in tropical regions.

## **Conclusions**

This project successfully established a starch grain laboratory at Sacramento State and initiated a reference collection of starch grains from tropical plants of the Americas. The laboratory provides a foundation for student involvement and fosters interdisciplinary collaboration.

As part of the project, two students were trained in the extraction, identification, and curation methods used to build the reference collection. Their participation throughout all phases of the project allowed them to develop practical skills in starch grain extraction and identification while gaining hands-on research experience.

The next phase of the project will focus on expanding the reference collection through the analysis of additional plant samples and applying these methods to archaeological materials. Future efforts will also include disseminating the project's findings through presentations at national conferences and publications in peer-reviewed journals.

## **Acknowledgements**

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