1 The *Phytoliths in the Flora of Ecuador* Project: Perspectives on Phytolith Classification, 2 Identification, and Establishing Regional Phytolith Databases

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6 Abstract: The *Phytoliths in the Flora of Ecuador* project was undertaken to establish
7 diagnostic phytolith types and phytolith vegetation signatures to enhance archaeological
8 and paleoenvironmental phytolith applications in the region. In this paper I discuss the
9 "products" of this research, and provide links to on-line resources. Among the lessons
10 learned from this project: the importance of systematic phytolith naming and
11 classification, documentation, and having a strong regional research focus.

12 Keywords: phytolith, classification, on-line databases, curation

13 1. Introduction

14 From 1997 to 2008, with periodic work through 2012, graduate assistants and I
15 studied phytolith production in the flora of Ecuador. The goal of the *Phytoliths in the*
16 *Flora of Ecuador* (PFE) project was to establish diagnostic phytolith types and phytolith
17 vegetation signatures for the region to enhance archaeological and paleoenvironmental
18 phytolith applications. Specifically, phytolith production in vegetation dominants, largely
19 arboreal, were studied for plant communities along two idealized transects that sampled a
20 significant part of the floristic diversity of this tropical country: (1) coastal to Amazon
21 sample, encompassing the outer coastal plain, inner coastal plain, western slopes,
22 interandean zone, eastern slopes, and eastern lowlands between approximately 1° north
23 and 1° south latitude, and (2) coastal plain sample, encompassing the north to south shift

from evergreen forest to open woodland/steppe from 1° north to 4° south latitude, at roughly 80° longitude. We tested plant groups (families, genera, and species) not previously studied for phytolith production, examined Ecuadorian representatives of previously studied groups, and examined replicate samples of important economic and indicator species. Phytolith assemblages of soil samples from four distinctive vegetation formations (xerophytic coastal forest, mixed deciduous/evergreen forest, coastal evergreen forest, and Amazonian lowland forest) were also characterized.

Three outcomes or products of this research were envisioned. The first was publication of phytolith production in tested species, including descriptions and images of diagnostic phytoliths in a searchable, on-line database. Phytolith production/non-production patterns would be presented in tables. The second outcome was to compare phytolith production/non-production in plant taxa studied during the project to the published literature, with the goal of investigating the level at which distinctive phytoliths are “universally” diagnostic (Pearsall 2000). The third outcome was to establish phytolith analogs for four forest formations of lowland Ecuador through multivariate analyses of phytolith assemblages from soil samples.

As I neared retirement and considered how to bring this research to a close, I realized that the PFE project was best understood as the final phase of phytolith classification and identification at the University of Missouri (MU) Paleoethnobotany Laboratory. From this perspective there were two additional products of potential interest to the research community: the MU phytolith classification system and the comparative collection itself.

The goal of this paper is to provide a perspective on phytolith classification, identification, and the process of establishing a regional phytolith database gained through the PFE project. I first discuss three interrelated products of the project, the MU phytolith classification system, the data tables, and the diagnostic database, and provide links to the on-line versions of these resources. I then give an example of how project results can be applied to the goal of drawing inter-regional comparisons in phytolith production patterns. I end by discussing long-term curation of comparative plant specimens and slides. Results of the comparative soil study will be published separately.

2. Phytolith Classification and Identification at MU: Building a Regional Comparative Collection

2.1 The MU Phytolith Classification System

I began paleoethnobotanical research at MU in 1978. Comparative collections from my dissertation research in SW coastal Ecuador formed the core of the phytolith comparative collection. Through the 1980s collections grew as I participated in research projects. Study of comparative plant specimens and soils and archaeological samples often went hand-in-hand (e.g., Pearsall and Trimble 1984), and unknowns were plentiful. When it became difficult for student assistants and I to keep track of increasing numbers of discrete kinds of phytoliths, we developed a hierarchical classification system to name them (Pearsall and Dinan 1992).

The MU phytolith classification system was developed as a desktop identification aid to provide consistency in classifying phytoliths, and to keeping track of unknowns. It is not a key to identifying plant taxa. There were initially 11 major categories in the system (Pearsall and Dinan 1992), expanded to 25 categories by the final update in 2012

(Table 1). Major categories are defined primarily based on cellular origin of phytoliths, with lower order divisions based on morphological details. Second order divisions are given Roman numerals, third order divisions capital letters, fourth order divisions lower-case levels, and so on, as illustrated in Figure 1. Within each category, phytoliths are assigned unique number codes, which indicate how similar each is to other phytoliths in the category.

Table 1. Categories in the MU Phytolith Classification System

10	Epidermal quadrilaterals (large cells of the epidermis, quadrilateral, square to elongated, often very two-dimensional in appearance, variable surface and edge characteristics)
11	Epidermal quadrilaterals, three-dimensional (variable surface and edge characteristics)
12	Epidermal quadrilaterals, carbon-occluded (not further divided; includes all shapes and sizes of cells in 10 and 11)
20	Epidermal, nonquadrilateral (originally all nonquadrilateral cells of the epidermis, later restricted to leaf epidermis; irregular in shape, usually two-dimensional in appearance)
21	Epidermal nonquadrilaterals, leaf epidermis, fragments (variable surface characteristics)
22	Epidermal nonquadrilaterals, seed or fruit epidermis (variable surface and edge characteristics; irregularly shaped, polyhedral in top view, cylindrical; includes Poaceae fruitcase and glume bodies)
23	Epidermal nonquadrilaterals, carbon-occluded (not further divided; includes plates and cell walls of other tissue origins, such as parenchyma)
24	Epidermal nonquadrilaterals, three-dimensional/blocky (rounded and angled edges)
25	Mesophyll (not further divided)
26	Epidermal nonquadrilaterals, rhizome, tuber, or root epidermis (cylindrical and flat)
30	Short cells (Poaceae, produced in the leaf vein area)

94	31	Intercostal cells (quadrilateral, rounded)
95	40	Dermal appendages (prickles, hairs, edge spines, including base cells)
96	50	Bulliform cells (Poaceae, located in the mesophyll or epidermis, shapes variable)
97	60	Anatomical origin unknown (a category for plant silica of unknown origin in the plant)
98	70	Honeycombed material (amorphous, lightly silicified tissues)
99	80	Stegmata and other spheres (spherical-spheroidal mesophyll cells; troughed bodies,
100		conical bodies)
101	90	Other biogenic silica (diatoms, sponge spicules, and the like)
102	100	Crystalline bodies (cystoliths [silicified outgrowths of cell walls, occurring in the
103		epidermis and subepidermis, variable shape, usually nodular surfaces], raphids, other crystals)
104	110	Sclereids (sclerenchyma cells, varied in form, but often elongated and angled; not
105		subdivided)
106	120	Stomata
107	130	Silica casts of parenchyma (for artifact residue applications)
108	140	Silica casts of transport tissues (xylem, phloem, for artifact residue applications)
109	150	Fibers (for artifact residue applications)
110	160	Secretory cells

111

112 [insert Figure 1. Caption: A mock-up of a section of Category 22, with images of
113 cards and photographs inserted]

114

115 The classification system was contained in two card file boxes. For each type
116 there was an index card with a sketch and description of the type, and photographs of
117 representative examples. As new phytoliths were added, numbers of existing types were
118 changed as needed. A common change was to add a lower classification level: for

example, 80IE (original irregularly angled/folded sphere) became 80IEa (original type) and 80IEb (angled/folded sphere with nodules). For phytoliths that were very similar, notes were added on how to distinguish between types. Diagnostic phytoliths were flagged. Consistency in descriptions was maintained by an in-house dictionary of standard terms. The classification was eventually compiled as a Word document. Beginning after the 2012 update, all original cards and photographs were digitally imaged and inserted into the text portion of the classification by volunteers Howard and Laurel Wilson, and pdf files of each category produced (see phytolith.missouri.edu, MU Phytolith Classification System).

Over the 20 years of its use, the MU classification system fulfilled its primary purpose, to maintain consistency in how phytoliths were classified from one project to the next. Type numbers appear cumbersome, but with use become familiar. Having to check off a number on a counting form takes the researcher through a series of decisions. Moving back and forth between comparative phytolith slides and type descriptions reinforces criteria critical for correct classification. The classification system provides context for the comparative collection, and helps insure that tallies of phytoliths observed in new plant taxa are comparable to those studied previously. The system proved quite flexible. It was easy to expand, and different levels could be used during scanning (for example, epidermal quadrilaterals could be tallied all together [10], or by length [10I, 10II]). Whatever form descriptions and names take, they are an essential part of phytolith research. See Bowdery et al. 2001 and Madella et al. 2005 for discussion of phytolith naming and description, and standard terms and approaches.

A digital version of the system was created for several reasons. The system is part of the record of phytolith research at MU, and is most usefully archived digitally. Photographs and descriptions document the morphologies of types tallied in phytolith production tables (see 2.2), allowing researchers to draw comparisons to other data sources. The desktop version of the system helped students and visitors learn about phytoliths, and the digital version may do the same. There are a few cautionary notes/disclaimers. The classification system is not a key to plant species. While the system contains diagnostic phytoliths (for example, the types shown in Figure 1), most of the photographs are of representative examples. No corrections were made to cards or photographs as they were digitally imaged; the system is presented in its unedited, working form, spelling errors and all. Finally, this tool is no substitute for a comparative collection. See phytolith.missouri.edu for a detailed guide to the digital version of the classification system.

2.2 Phytolith Production Tables

Phytolith studies at MU centered primarily on Neotropical and economic taxa. During the 1990s Dolores R. Piperno and I collaborated on two projects focused on developing phytolith applications in archaeology (Piperno and Pearsall 1993a). We characterized phytoliths in the reproductive structures of maize and teosinte (Piperno and Pearsall 1993b), expanded the geographic range of a discriminant function for identifying maize using cross bodies (Piperno and Starczak 1985) by studying 100 species of wild panicoid grasses (formulas in Pearsall 2000:391-392), completed the first major survey of silica bodies in New World tropical grasses (Piperno and Pearsall 1998), and developed a protocol for identifying rice (Pearsall et al. 1995; Zhao et al. 1998). At MU we worked on

classifying the non-regular or complex short cells that were often abundant in tropical grasses and added these to the MU classification system.

Phytoliths from comparative specimens were described, classified, and potential diagnostics photographed. After review, raw data were compiled into spreadsheets. This was the case, for example, for panicoid grasses studied to expand the geographic focus of the cross-body discriminant function. In 2004 I examined these leaf and inflorescence data to determine which complex short cells could be classified to grass subfamily, and which were redundant, and revised the MU short cell counting form (see phytolith.missouri.edu, Counting forms, Guide to the classification system). Removing redundant forms from subfamily counts increased the precision of ratios used for drawing inferences about past environments or contributions of crop plants to assemblages.

As described earlier, phytolith production was studied in a wide range of taxa during the PFE project. Data were first compiled in 2005, and posted on the website (see phytolith.missouri.edu, Flora of Ecuador Data Table). A second compilation was begun in 2008 to include plant taxa and tissues studied later in the project (e.g., phytoliths from roots and fruits, Chandler-Ezell et al. 2006, Pearsall et al. 2003), as well as data from plants studied prior to the PFE project, and not included in the 2005 compilation. Table 2 illustrates a portion one of these updated tables, which are organized by botanical subclass and order; several are posted on the website (Malvales, Magnoliidae, Zingiberidae). I plan to post all updated tables in the near future.

[insert Table 2, which is a separate Word file]

Unlike the example in Table 2, not all specimens in the PFE subclass and order data tables have been studied, although all were processed to extract phytoliths. The goals of the project proved too ambitious for the resources available. To study patterning of phytolith production at the family, genus, and species levels, it is necessary to examine multiple genera per family, more than one species in a genus, and multiple examples of species. With the help of MU herbarium director Dr. Robin Kennedy, genera were selected to provide representative coverage of the major floristic divisions of coastal and Amazonian Ecuador, with reduced coverage of the Andes. We selected two species per genus whenever possible. Replicates of species were largely confined to economic taxa, but we also processed new examples of plants for which old slide-mounts existed (since these could no longer be rotated to examine phytolith three dimensional morphologies). Since we also elected to process leaf and inflorescence tissues separately, numbers of specimens processed outstripped the pace of scanning, which proceeded by botanical family.

Even with these limitations, PFE results provide insights into Neotropical taxa that are phytolith producers, and those that are not (all processed specimens are included in the tables, whether or not extract was present). Patterns of production can be examined at the level of genus, family, order, and subclass. The tables provide taxonomic context for diagnostics. A few observations from the tables: We examined seven genera of Asteraceae, and observed distinctive Asteraceae epidermal phytoliths in only four. Examination of four species of *Cordia* (Boraginaceae) showed strong similarities in silicification of tissues within the genus. A second genus in this family was also a phytolith producer, and productive taxa were found in the Verbenaceae, a related family

of the Lamiales. We studied 15 genera in the Euphorbiaceae (Euphorbiales) and found that half were non-producers. Diagnostic phytoliths were observed in four productive genera, however, suggesting that this large family is worth studying. The same probably cannot be said of the Fabaceae (Fabales). We sampled 39 genera, 31 of which were scanned, and extracted phytoliths from 16. Unicellular hairs, previously identified in *Phaseolus*, were observed in *Dialium*; the latter differ in tip curvature. Most phytoliths are not diagnostic. A number of families tested in the Magnoliales, including the Lauraceae, were phytolith producers, with some promising diagnostics. The large Rosidae subclass included three phytolith-producing families (Dichapetalaceae, Chrysobalanaceae, Burseraceae) but appears dominated by non-producers. The Zingiberales are abundant phytolith producers, with strong genus and family-level patterning, as observed by Piperno (1989, 2006), Chen and Smith (2013), and our earlier research (Chandler-Ezell et al. 2006). Our large sample confirms the usefulness of seed (22) and root/tuber/rhizome (26) epidermal phytoliths for subfamily, genus- and some species-level identifications.

2.3 The MU Diagnostic Database

A *diagnostic phytolith* is one that can be used to distinguish among plant taxa in a given flora (Pearsall 2000:376-8). The most useful diagnostics are those that can be used to distinguish among taxa at the level of family or below. As more plant taxa are studied, there are increasing numbers of one-to-one matches between phytoliths and plant taxa (Piperno 2006). Distinctive phytoliths are produced in the inflorescences of many plants, for example, permitting genus- and species-level identifications. Phytoliths with similar

morphologies may be distinguishable on the basis of size. In some cases combinations of types may be characteristic of different plant taxa.

A primary goal of the PFE project was to discover and describe diagnostic phytoliths in previously unstudied plants, document the occurrence of known diagnostics in Ecuadorian taxa, and share these results through an on-line database (Figure 2). The database was set up in 2000 by then graduate student Neil A. Duncan, and expanded in 2005 (currently 310 images), with further additions planned in the near future (see phytolith.missouri.edu, Image database). Potential diagnostics were evaluated on the basis of: (1) distinctiveness, (2) estimated abundance in tissues, and (3) distribution within the study population. Since diverse morphological features are useful for describing and distinguishing among phytoliths (e.g., see features discussed in Bowdery et al. 2001 and Madella et al. 2005), our approach was to describe the diagnostic using the appropriate characteristics in one field in the database. Our evaluation of the diagnostic level of the type was entered in the *Comments* field. Each record also includes the MU classification number and the family, genus and species of the specimen. Lab specimen numbers and sources of specimens are found in the appropriate phytolith production table. Tables also show what other kinds of phytoliths occur in the taxon and how common the diagnostic is.

[insert Figure 2. Caption: An example of a screen in the MU Phytolith Database.]

The database proved to be a useful resource at the lab. Students and visitors used it to help them recognize diagnostics on comparative slides, and to learn the features that

distinguished diagnostics from related forms. Database photos provided a quick review of types during diagnostic scanning. I used it in teaching to illustrate phytolith diversity, and as an identification aid during lab exercises. Comments from colleagues suggest the database has been a useful on-line resource as well.

I made this database freely available on-line to share the results of our research in a timely fashion. This mode of data sharing was part of the project proposal, and was the first product of the research. In a rapidly developing field like phytolith analysis, sharing basic data on types is critical. Whether print publication or on-line format is appropriate for presenting new types or new data on type distributions may be subject to debate. The MU database is not peer-reviewed (although a number of types are published), but each potential diagnostic was discussed at lab meetings, and compared to existing types. This is a regionally-focused database, not a universal phytolith key. The electronic format facilitates posting of multiple images of types, to show range of variation in form, something difficult to accomplish in print format. While nothing substitutes for a comparative collection, on-line resources and print publications each facilitate research.

3. Inter-regional Comparisons in Phytolith Production: Malvales Example

One goal of the PFE project was to compare phytolith production/non-production in plant taxa studied during the project to the published literature, to investigate the level at which distinctive phytoliths were “universally” diagnostic. I explored this approach in 2008 by examining the Malvales (see phytolith.missouri.edu, Malvales chapter, for data tables and photographs of types). Most taxa tested in the Bombacaceae were silica-accumulators, and a number produced diagnostic phytoliths.

There are 15 genera and 47 species of Bombacaceae in Ecuador (Jørgensen and León-Yáñez 1999). It is a tropical family of large trees (Mabberley 1997). Seven genera were studied: one species each of *Ceiba*, *Huberodendron*, *Ochroma*, *Pachira*, *Pseudobombax*, and *Quarariba*, and four species of *Matisia*. Prior to this project, four genera that occur in Ecuador had been tested (*Bombacopsis*, *Cavanillesia*, *Ceiba*, and *Ochroma*) (Piperno 1988; Runge 1996; Kealhofer and Piperno 1998).

Ceiba

Four species of *Ceiba* occur in Ecuador; *C. pentandra* (L.) Gaertn., the common coastal species that also occurs in the Amazon (Jørgensen and León-Yáñez 1999), was studied. It is likely that the wood specimen (2866) is also this species.

Neither the leaf nor inflorescence of *Ceiba pentandra* produced silica. Piperno (1988) also found *Ceiba pentandra* to be a non-producer, but Runge (1996) recovered calcium oxalate druses and silicified epidermis (polyhedral-shaped) with thickened cell walls and a stippled surface from an African specimen of this species.

The *Ceiba* wood specimen produced tabular crystals with granular surfaces (100Va, VA), probably composed of calcium carbonate, and also spherical cystoliths (100IA, VA) of various sizes. Cystoliths are considered generalized arboreal indicators (Piperno 1988); the status of tabular crystals remains to be determined.

Huberodendron

H. patinoi Cuatrec. is the single representative of the genus in Ecuador (Jørgensen and León-Yáñez 1999). *Huberodendron* had not been studied prior to this project. No diagnostic phytoliths were produced in the inflorescence except cystoliths (100Cb, elongated, VR), which are generalized arboreal indicators. Silicification was higher in the

leaf, in which a genus-level diagnostic phytolith was identified, a hair base composed of rounded cells with a large, rounded central cell (40IVBa201c, M). Sclereids (110), which are generalized arboreal indicators, were also present.

Matisia

Matisia had not been studied prior to this project. There are 18 tree species in Ecuador (Jørgensen and León-Yáñez 1999).

Matisia cf. *alata* Little produced only non-diagnostic phytoliths in the leaf. Spherical yellowish bodies that appeared to be oil droplets were very abundant. Small nodular spheres (80ICa1, M, 8-16 microns) occurred in the inflorescence. Nodular spheres were first described in the Marantaceae by Piperno (1988, e.g., *Maranta arundinacea*, *Stomatanthe lutea*) and later by Kealhofer and Piperno (1998) and Runge (1999, *Halopegia azurea*).

Matisia coloradum Benoist produced no diagnostic phytoliths in the leaf, except for polyhedral non-quadrilateral epidermis (20IBa, R), which is a generalized dicot indicator (e.g., Piperno 1988). The inflorescence produced no diagnostic phytoliths.

A family-level diagnostic was observed in *Matisia cordata* Bonpl., 120IIA (R), stomata with four large, turgid subsidiary cells. A generalized dicot indicator (polyhedral non-quadrilateral epidermis, 20IBa, M) and an arboreal indicator (sclereid, 110, R-A) are also present. A second phytolith type first identified in the Marantaceae (Piperno 1988) was found in *M. cordata*, nodular conical bodies (80IIIB, R). Small nodular spheres were also present. Irregular rugulose epidermis (22IBd, R) was documented.

Matisia longipes Little produced only a trace of extract in the inflorescence.

Rugulose non-quadrilateral epidermis (22IBd, C) and a family-level diagnostic, stomata

120IIA (VR), were present. The leaf was highly silicified. One interesting form observed was a facetate hemisphere (80IFb202, R), first identified in *Pseudobombax millei* (see below), and now determined to be a family-level diagnostic. Similar, but distinguishable hemispherical to elliptical facetate phytoliths occur in the New World Burseraceae and Ebenaceae (MU Phytolith Database; also Piperno 1988, *Protium panamense*, Burseraceae), and more irregular forms are documented in the Annonaceae (e.g., Piperno 1988). Two smooth hairs appear to be species-level diagnostics: short conical hair (40IIIAa202Ba2, R) and long, thin hair (40IIIAb200Ab2, C). Other distinctive forms include sclereids (110, VR).

Ochroma

Ochroma pyramidalis (Cav. ex Lam.) Urb. is the single Ecuadorian species in the genus (Jørgensen and León-Yáñez 1999). It was studied by Kealhofer and Piperno (1998), and found to be a non-producer of phytoliths.

The inflorescence produced very rare small nodular spheres (80ICa1), as well as non-diagnostic phytoliths. The leaf produced sclereids (110, R, generalized arboreal indicators).

Pachira

Pachira aquatica Aubl. is one of three Ecuadorian species in this genus (Jørgensen and León-Yáñez 1999). *Pachira* had not been studied prior to this project. The inflorescence produced only non-diagnostic phytoliths. The leaf produced some interesting forms. Hair base 40IVAA300 (R) is a genus-level diagnostic. It has short, regular appendages from a solid, angular rim. The center is a smooth rounded concavity. Distinctive stomata with four subsidiary cells outside the guard cells (120IIA, M-A) were

silicified in the leaf. These were also observed in *Matisia longipes*, and are a family-level diagnostic. Broken examples (20VD, C, pieces of 120IIA) were also present. Sclereids (110, C) occur in the leaf.

Pseudobombax

Pseudobombax had not been studied prior to this project. There are three Ecuadorian species (Jørgensen and León-Yáñez 1999). *Pseudobombax millei* (Standl.) A. Robyns was studied. Leaf and inflorescence produce similar phytoliths, but silicification is higher in the leaf. Facetate hemispheres (80IFb202) are common in the leaf and moderate in the inflorescence. These are diagnostic at the family level since they also occur, rarely, in *Matisia longipes*. Ruminant nodular spheres (80ICc) are very abundant in the leaf and abundant in the inflorescence. The same kinds of spheres are produced in *Costus guaniensis*, in the Zingiberaceae. Cystoliths, including bulbous (100ID) are very abundant in the leaf and rare in the inflorescence. These are generalized arboreal indicators, as are sclereids (110). In addition, the leaf only produces single-outline trichomes with an enclosed verrucose cystolith (40IIBc3, C), which is a genus level diagnostic, and small smooth nodular spheres (80ICa1, VA; mixed Marantaceae/Bombacaceae type).

Quararibea

Four species of *Quararibea* are native to Ecuador (Jørgensen and León-Yáñez 1999). The specimen studied was collected by Pearsall in Manabí Province, Ecuador (E1079) and identified by P. Bech and B. Ollgaard as *Quararibea* cf. *grandifolia* Little. *Q. grandifolia* is considered synonymous to *Matisia grandifolia* Little by Jørgensen and León-Yáñez (1999), and is not one of the four *Quararibea* species listed for the country.

370 Whether considered *Quararibea* sp. or *Matisia grandifolia*, specimen E1079
 371 produced a diagnostic hair base (40IVAa201Aa, M), as well as a diagnostic segmented
 372 hair (40IIIBa203A, M). Sclereids (110, C) and spherical cystoliths (100IA, VA), both
 373 generalized arboreal indicators, were present, along with epidermal non-quadrilateral
 374 20IAa (M) or anticlinal epidermis, a dicot indicator.

375 In summary, the Bombacaceae, a tropical family of large trees, produces the
 376 following diagnostic phytoliths:

377 **40IIBc3** non-armed trichome with spherical verrucose cystolith as base. *Pseudobombax*
 378 *millei* leaf. Genus-level diagnostic
 379 **40IIIAa202Ba2** medium unicellular hair, curved, with smooth surface and bulbous base.
 380 *Matisia longipes* leaf. Species-level diagnostic
 381 **40IIIAb200Ab2** long unicellular hair, non-armed, no interior space. *Matisia longipes*
 382 leaf. Species-level diagnostic
 383 **40IIIBa203A** segmented hair, non-armed, with a broad tip. *Quariba* cf. *grandifolia*/M.
 384 *grandifolia* leaf. Genus- or species-level diagnostic
 385 **40IVAa201Aa** hair base with discernable center and long radiating appendages. *Quariba*
 386 cf. *grandifolia*/M. *grandifolia* leaf. Genus- or species-level diagnostic
 387 **40IVAa300** hair base with discernable center and short radiating appendages. *Pachira*
 388 *aquatica* leaf. Genus-level diagnostic
 389 **40IVBa201c** hair base comprised of rounded cells, with a single row of attached cells.
 390 *Huberodendron patinoi* leaf. Genus-level diagnostic

- 391 **80ICa1** small smooth nodular sphere. *Matisia cf. alata* (inflor), *M. cordata* (leaf),
 392 *Ochroma pyramidalis* (inflor), *Pseudobombax millei* (leaf). Mixed
 393 Marantaceae/Bombacaceae type.
- 394 **80ICc** ruminant nodular sphere. *Pseudobombax millei*, leaf and inflorescence. Mixed
 395 Zingiberaceae/Bombacaceae type.
- 396 **80IFb202** faceted hemisphere with irregular concavities. *Matisia longipes* leaf,
 397 *Pseudobombax millei* leaf, inflorescence. Family-level diagnostic.
- 398 **80IIIB** nodular conical. *Matisia cordata*, leaf. Mixed Marantaceae/Bombacaceae type.
- 399 **120IIA** stomata with four large turgid subsidiary cells. *Matisia cordata* (leaf), *M.*
 400 *longipes* (inflor), *Pachira aquatica* (leaf). Family-level diagnostic. Broken (20VD):
 401 *Pachira aquatica* (leaf).

402 In addition, the following more generalized indicators are present:

- 403 **20IAa** epidermal non-quadrilateral, anticlinal cells. *Quararibea cf. grandifolia* (leaf).
 404 **20IBa** epidermal non-quadrilateral, polyhedral cells. *Matisia coloradum* (leaf), *M.*
 405 *cordata* (leaf).
- 406 **100IA** spherical cystoliths.
- 407 **110** sclereids.

408 As Piperno (1988, 2006) early observed, it is often hairs and hair bases that are
 409 silicified in dicotyledon taxa that silicify tissues. This is the case in the Bombacaceae. For
 410 the most part hairs and hair bases were lightly silicified in the specimens studied, and
 411 unlikely to survive in soils. This limits their usefulness in archaeological and
 412 paleoenvironmental applications. 80IFb202, facetate hemisphere with irregular

413 concavities, is well-silicified, however, and potentially useful as a Bombacaceae family
414 indicator.

415 A striking feature of phytolith production in the Bombacaceae is the fact that
416 three kinds of well-silicified phytoliths produced in this family also occur in families in
417 the Zingiberales. Smooth nodular spheres (80ICa1) and conical bodies (80IIIB) were
418 originally described by Piperno (1988) for the Marantaceae; ruminant nodular spheres
419 (80ICc) were identified at MU in *Costus* in the Zingiberaceae as well as in the
420 Bombacaceae. These forms are so similar to Marantaceae and Zingiberaceae examples
421 that we have classified them under the same numbers. There are some subtle differences
422 in the number and arrangement of nodules, however, which may allow separation if a
423 large enough sample was present. The spheres are also produced more abundantly in the
424 Marantaceae.

425 In addition to the taxa studied during this project, two other genera with
426 Ecuadorian representatives were studied by Piperno (1988): *Bombacopsis quinata*, *B.*
427 *sessiles*, and *Cavanillesia platanifolia*. Only *B. sessiles* commonly silicified tissues
428 (epidermis, stomata, and tracheids). *Bombax* and *Durio*, which do not occur in Ecuador,
429 were studied by Kealhofer and Piperno (1998), who found that *Bombax* was not a
430 phytolith producer (two species studied), while *Durio* (two species) silicified hair bases,
431 epidermal cells, and small spheres, none of which were considered diagnostic.

432 Other Malvales families studied at MU, the Bixaceae, Malvaceae, Sterculiaceae,
433 and Tiliaceae, are characterized by low phytolith production, varying from non-
434 production to production of non-diagnostic silica or generalized woody indicators such as
435 cystoliths and sclereids. There are a few exceptions in which distinctive hair bases or

hairs are silicified, for example, in *Gossypium* leaf. *G. barbadense* fruit also contained silicified secretory cells (160II, R-M) similar, but distinguishable from, secretory cells produced by manioc, *Manihot esculenta* (Chandler-Ezell et al. 2006). While these lightly silicified forms are of limited use in soil or sediment work, they demonstrate that even in low silica-accumulating families distinctive phytoliths may be produced. Overall, study of Ecuadorian taxa in these families produced results consistent with prior research. There are a number of cases in the Malvales in which the same species was studied more than once. In some cases there are observable differences in the extent of cell wall silicification, a common silica accumulation mechanism in dicotyledons. In *Ceiba pentandra* leaf and inflorescence, for example, New World specimens were non-producers while an African specimen exhibited silicification in some polyhedral-shaped epidermal cells. In *Theobroma cacao*, all replicates produced silica, but the extent of cell wall silicification varied. Some variation is likely due to differences in the quantity of tissue from which silica was extracted and the manner and success of the extraction; other differences may relate to the maturity of tissues or environmental conditions.

4. Curation of Phytolith Reference Collections

Botanical reference collections are modern, identified plant tissues to which unknown archaeological materials are compared (Pearsall 2000). Such collections are essential for achieving accurate identifications, and are valuable research resources. Pearsall (2014) and Balick and Herrera (2014) recently reviewed the nature, organization, and curation of botanical collections. Here I would like to make two points concerning phytolith collections arising from the PFE project.

First, phytolith collections should include both slide-mounted extracts and the tissues from which those extracts originated. Many slides made and scanned for the PFE project remain usable, while others have deteriorated. In hindsight, it would have useful to make two sets of slide-mounts per specimen: one in a medium that hardened, creating a stable slide for long-term curation, and one in a medium allowing continued phytolith rotation until slides leaked and dried. Images of phytoliths in different rotations can augment later study of specimens in fixed slide-mounts. Collections are more useful long-term, however, if they include "extra" extract to make new slides (or extra tissues to process). While ideal, this may not be possible when sampling from herbarium sheets, and is a limitation of that research design. Plant collecting remains an essential part of building comparative collections.

Second, it is important to consider the future of a comparative collection even while building and studying it. In addition to curating specimens for long-term stability, there are other actions we can take to prepare these valuable resources for our successors: make collections accessible (physically and/or through electronic means) so that their value is appreciated; maintain electronic inventories that include specimen proveniences and link specimens to data sheets, photographs, and compilations; and maintain a strong theme and geographic focus.

5. Conclusion

In this paper I provided perspectives on phytolith classification, identification, and the process of establishing a regional database gained through the *Phytoliths in the Flora of Ecuador* project, and placed that project in the context of phytolith studies at the

University of Missouri. I summarized the goals of the project, discussed the products of that research, and provided links to on-line versions of resources.

I end with insights into the research process gained from the PFE project. These observations are not unique to that project, or necessarily original to me, but "lessons learned." Naming and classifying phytoliths are essential first steps in research, and must be done in a way that insures that types remain stable. The MU hierarchical classification system accomplished this: I can compile data from specimens scanned in 1992 and 2012 and be confident that types are comparable. The MU system is only one approach; establishment of standard names is an important advance in the field. What is essential is that types are documented by slides, images, and descriptions.

Documentation is vital for all research, but especially for long-running projects: individuals' lab notebooks track day to day activities and decisions; paper forms link specimens and data compilations; inventories (of comparative specimens, slides, digital images) provide structure; on-line resources like databases facilitate communication. Documentation is essential, and there is never too much.

A regional focus facilitates phytolith research. The focus of the PFE proved too broad to be completed with the available resources, but having a broad focus structured our efforts, allowed immediate applications to archaeological and paleoenvironmental projects, added value to the comparative collections, and insured systematic coverage of taxa. Stepping back from one's chosen region is also valuable, for example, comparing phytolith production patterns within plant groups across regions contributes to growth of the field as a whole. It was not possible to accomplish many such reviews during the PFE project, but more could be done with the available data and specimens.

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Table 2

Table 2. A mock-up of part of the Zingiberales table, showing phytolith production in *Calathea* and *Maranta* (Marantaceae) for categories 22, 26, and 80. The complete table includes four families and all categories shown in Table 1.

MU#	Species	Part	Extract Wt.	Seed epidermal	Rhizome, tuber, root epidermal		Nodular sphere	Angled sphere	Conical
				22	26 I	26 II	*80 IC	*80 IE	*80 III
274	<i>Calathea allouia</i>	leaf	no extract						
275	<i>Calathea allouia</i>	leaf	no extract						
2346	<i>Calathea</i> aff <i>allouia</i> (Aubl.) Lindl. Det. L.Andersson, 1994	inflor	no extract						
2347	<i>Calathea allouia</i> (Aubl.) Lindl.	inflor	present	*22VIIBc2 M; 22VIIBc3 VA; *22VIIBd VA			80ICa1 C		*80IIIB R
2348	<i>Calathea allouia</i> (Aubl.) Lindl.	inflor	no extract				80ICa1 M, 80ICd C	80IEa M	*80IIIB C
2596	<i>Calathea allouia</i>	leaf	0.0028				80ICa1 R, 80ICa2 VA, 80ICc M	80IEa VA; 80IEb VA	80IIIB A
2597	<i>Calathea allouia</i>	stem	0.2357				80ICa1 M	80IEa A, 80IEb C	80IIIB VA
2598	<i>Calathea allouia</i>	inflorescence	0.0679	*22VIIBc2 A, *22VIIBc3 A, *22VIIBd M, *22VIIC M			80ICa1 M, 80ICa2 R	80IEa C	80IIIB VA
2599	<i>Calathea allouia</i>	tuber	no extract						
2672	<i>Calathea allouia</i>	tuber	0.0753		*26IAa A, *26IAb P, *26IB P	*26IIB A	C	C	M
2458/2	<i>Calathea allouia</i> (Aubl.) Lindl.	leaf					80ICa1 M	80IEb VA	80IIIB VA
2021	<i>Calathea altissima</i> (Poepp. & Endl.) Koern.	leaf	n.a.				VA		VA
2022	<i>Calathea altissima</i> (Poepp. & Endl.) Koern.	inflor	n.a.	*22VIIBc1 VA					
2023	<i>Calathea crotalifera</i> S.Watson	leaf	n.a.				VA*		VA
2024	<i>Calathea crotalifera</i> S.Watson	inflor	n.a.	*22VIIBb VA			VA*		A
2091	<i>Calathea crotalifera</i> S.Watson	leaf	no extract						
2720	<i>Calathea inocephala</i> (O. Ktze) Kennedy & Nicolson	stem			*26IB M		VA	M	VA
2721	<i>Calathea inocephala</i> (O. Ktze) Kennedy & Nicolson	root					A	M	
2722	<i>Calathea inocephala</i> (O. Ktze) Kennedy & Nicolson	root					P		
2025	<i>Calathea insignis</i> Petersen	leaf	n.a.				A		VA
2026	<i>Calathea insignis</i> Petersen	inflor	n.a.				80ICa1 VA, 80ICa2 P		A
2593	<i>Calathea lutea</i>	floret, 2 g	0.0121				80ICd C		80IIIB VA
2594	<i>Calathea lutea</i>	floret, 1g	0.0198				80ICd VA	80IEa R	80IIIB VA
2595	<i>Calathea lutea</i>	leaf 1g	0.0112				80ICa1 VR		80IIIB VA
2723	<i>Calathea macrosepalia</i> K. Schum.	root					P		
2724	<i>Calathea macrosepalia</i> K. Schum.	rhizome					P		
1384	<i>Calathea majestica</i> (Linden) Kennedy	leaf	present						80IIIB VA
1385	<i>Calathea majestica</i> (Linden) Kennedy	fruit	present	22IBc R, *22VIIBc1 VA, *22VIIBd C					
1741	<i>Calathea majestica</i> (Linden) Kennedy	Leaf	lost						
2725	<i>Calathea marantifolia</i> Standl.	stem				*26IIB C	VA	VA	
2027	<i>Calathea marantina</i> (Willd. ex Koern.) K.Koch	leaf	n.a.						A
2028	<i>Calathea marantina</i> (Willd. ex Koern.) K.Koch	inflor	n.a.	*22VIIBb VA					A
2029	<i>Calathea peoppigiana</i> Loesener ex H.Kennedy	leaf	n.a.					VA	VA
2030	<i>Calathea peoppigiana</i> Loesener ex H.Kennedy	inflor	n.a.				C		VA
2349	<i>Calathea</i> sp. - cf. <i>C allenii</i> Woods	Leaf	lost						
2350	<i>Calathea</i> sp. - cf. <i>C allenii</i> Woods	inflor	lost						
2729	<i>Maranta amazonica</i> L. Andersson	root							
2730	<i>Maranta amazonica</i> L. Andersson	rhizome						R	R
32	<i>Maranta arundinacea</i> L.	leaf	0.0019				VA	A	VA
213	<i>Maranta arundinacea</i> L.	seed and bracts	present						
214	<i>Maranta arundinacea</i> L.	root and bracts	present						
2037	<i>Maranta arundinacea</i> L.	leaf	n.a.				VA*		VA*

2038	<i>Maranta arundinacea</i> L.	inflor	n.a.	*22VIIAb VA				
2670	<i>Maranta arundinacea</i> L.	fleshy rhizome	0.0085	*22VIIBb M, *22VIIBd R	*26IB C			80IIIB A
2671	<i>Maranta arundinacea</i> L.	roots	0.0219	*22VIIBb R		80ICa1 C	80IEa C	80IIIB A
2673	<i>Maranta arundinacea</i> L.	root, root hairs	0.0118		*26IB C	C	M	A-VA
2674	<i>Maranta arundinacea</i> L.	rhizome	0.0708		*26IB C	C	M	A
2035	<i>Maranta gibba</i> J.E. Smith in Pees	leaf	present			80ICa1 VA		80IIIB VA
2036	<i>Maranta gibba</i> J. E. Smith in Pees	inflor	n.a.	*22VIIAa VA		p*		
2732	<i>Maranta gibba</i> J. E. Smith	stem base				80ICd C		C
2733	<i>Maranta gibba</i> J. E. Smith	root				R		
2734	<i>Maranta orbiculata</i> (Koern.) Schum.	stem base	P, indistinctive					
2735	<i>Maranta orbiculata</i> (Koern.) Schum.	root	P, indistinctive					
2736	<i>Maranta sobilifera</i> L. Anderss.	stem base				C		M
2737	<i>Maranta sobilifera</i> L. Anderss.	root						

*=category contains a diagnostic type

P=present, VR= very rare, R=rare, M=moderate, C=common, A=abundant, VA=very abundant

CATEGORY 22

Epidermal Non-Quadrilateral Seed or Fruit Epidermis

22VII. Cylindrical seed bodies (Marantaceae)

22VIIA. smooth/solid cylinder shaft (*Maranta* spp., genus level diagnostic)

- long cylinder, smooth surface (*Maranta* spp., genus level diagnostic)
(surface may have few to many large projections)
- shortened cylinder, grainy surface (*Maranta arundinacea*, species level diagnostic)
- shortened cylinder, smooth surface; robust single and bifurcated angular projections; tip smooth (*Stromanthe*, genus level diagnostic)

22VIIAa card 2

22VIIAa photo 2

22VIIAb card 1

22VIIAb photo 2

Figure 2

MUno 221C

Image N532

Recno 8

Family Burseraceae

Genus Trattinnickia

Species glaziovii

Authority Swart

Comments

Seed epidermis. Projection is not centered and may not be present on all fragments. The projection is often very low. Surface decoration not always evident unless focus is moved up and down. Diagnostic level: family

Description

Seed epidermis; Non-quadrilateral; Often sinous double outline; Prominent rounded, low projection and fine surface decoration.

Entered by Karol Chandler-Ezell

Updated 10/7/2002