

Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico

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Questions that still surround the origin and early dispersals of maize (*Zea mays* L.) result in large part from the absence of information on its early history from the Balsas River Valley of tropical southwestern Mexico, where its wild ancestor is native. We report starch grain and phytolith data from the Xihuatoxtla shelter, located in the Central Balsas Valley, that indicate that maize was present by 8,700 calendrical years ago (cal. B.P.). Phytolith data also indicate an early preceramic presence of a domesticated species of squash, possibly *Cucurbita argyrosperma*. The starch and phytolith data also allow an evaluation of current hypotheses about how early maize was used, and provide evidence as to the tempo and timing of human selection pressure on 2 major domestication genes in *Zea* and *Cucurbita*. Our data confirm an early Holocene chronology for maize domestication that has been previously indicated by archaeological and paleoecological phytolith, starch grain, and pollen data from south of Mexico, and reshift the focus back to an origin in the seasonal tropical forest rather than in the semiarid highlands.

early Holocene | maize domestication | phytoliths | starch grains

Investigations at the Xihuatoxtla shelter in Guerrero, Mexico have uncovered a long sequence of human occupation beginning during the early Holocene (1). The stratigraphy, chronology, and other characteristics of this site have been described previously (1). To study the history of plant exploitation and cultivation, we carried out phytolith and starch grain studies of sediments and stone tools recovered from preceramic and ceramic levels that clearly represent an undisturbed sequence of deposition (1). This research is the first to examine early plant use in the deciduous or seasonal tropical forests of the Central Balsas watershed of Mexico, where the wild progenitors of maize (*Zea mays* ssp. *parviglumis* Iltis and Doebley or Balsas teosinte) and *Cucurbita argyrosperma* Huber, the important “silver seeded” squash [*C. argyrosperma* Huber ssp. *sororia* (L.H. Bailey) Merrick and Bates], along with important tree crops, such as *Leucaena* spp. and *Spondias* spp., are members of the natural flora (1–4).

The vegetation and climate of the study region have been described in detail elsewhere (5; see also ref. 1). In brief, the climate is classified as Köppen Aw (tropical wet and dry). The average annual temperature is 27 °C, and the average annual precipitation is approximately 1,100 mm. As in much of the Central Balsas region, the potential vegetation is tropical deciduous (seasonal) forest, which is still found in remnants in areas removed from human population concentrations. Our vegetation surveys indicate that the floral composition and structure of these forests is typical of low-elevation deciduous forest found in other similar regions of the seasonal tropics in Mexico and Central America (5). Paleoecological research carried out on a series of lakes and swamps near the archeological sites indicates that similar forests have occupied the region since the beginning of the Holocene Period (5). In the present vegetation, a change to

lower montane (low mountain) forest occurs at 1,200 m, when oaks and other cooler-loving elements become a part of the flora (5).

We focused our analyses on starch grains and phytoliths, which are effective indicators of wild and domesticated maize and squash remains in archaeological contexts (6–14). As is common in other tropical regions (6, 7, 10–13), the preservation of macrofossil plant remains was very poor, consisting of wood charcoal and a few unidentified seed fragments. The site’s sediments were similarly barren of pollen, which in any case cannot achieve a confident separation of maize and teosinte, because substantial overlap in both pollen size and morphology occurs between maize and many wild members of the genus *Zea*, including Balsas teosinte (9). This paper focuses on the evidence attesting to the utilization of *Zea* and *Cucurbita*. Information on the other plants represented in the starch grain and phytolith records is provided in [supporting information \(SI\) Materials and Methods](#).

Starch grain analysis was performed on a total of 21 ground stone and 5 chipped stone tools. A total of 21 sediment samples were analyzed for phytoliths. Nine of these samples were obtained in 10-cm increments as a column sample from the north wall of unit 1 of the excavations (see Fig. 3 in ref. 1). Twelve samples were directly associated with ground stone tools from units 1 and 2, occurring immediately beneath and within 5–10 cm of the artifacts (1). Phytoliths also were recovered from the surfaces of these artifacts during starch grain analysis. Procedures for microfossil study followed standard methods (see [Materials and Methods](#)). Identification was based on large modern reference collections of wild and domesticated taxa native to Mexico and elsewhere in tropical America that are housed in DRP’s laboratory (see [SI Materials and Methods](#)).

Results

Starch Grain Studies. Starch grains were recovered from 19 of the grinding stones and 3 of the chipped stone tools. Maize was the dominant starch type on every tool, accounting for 90% of all grains recovered (Table 1; Fig. 1). Previous research has demonstrated that starch grain size and morphology are of significant utility for differentiating maize from wild grasses native to North, Central, and South America (6–8, 10, 13) (see also [SI Materials and Methods](#) and [Table S1](#)). Our work in Mexico required a good understanding of starch grain characteristics in wild *Zea*, represented by 4 species and 3 subspecies of teosinte

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Table 1. *Cucurbita* and maize cob phytoliths and maize starch grains from Xihuatoxtla

Provenience, cm below surface/ layer	Length of <i>Cucurbita</i> phytoliths, μm , mean (range)	Thickness of <i>Cucurbita</i> phytoliths, μm , mean (range)	Number of <i>Cucurbita</i> phytoliths measured	Length of maize starch grains, μm , mean (range)	Number of maize starch grains measured	Maize cob phytoliths
Column sample						
Unit 1, 325a, 0–20/A/B	50		1		NA	WT + Cob-type
Unit 1, 325b, 20–40/B			0		NA	WT + RT + Cob-type
Unit 1, 325c, 40–48/B			0		NA	Cob-type
Unit 1, 325d, 49–54/C	65 (59–72)	53 (45–58)	7, 3		NA	Cob-type
Unit 1, 325e, 54–60/C	61 (47–87)	47 (40–54)	4, 2		NA	WT + Cob-type
Unit 1, 325f, 60–65/D	80 (54–106)	59	12, 1		NA	WT + Cob-type
Unit 1, 325 g, 65–75/D/E	73 (36–108)	55 (30–84)	62, 27		NA	RT + Cob-type
Unit 1, 325 h, 80–90/E	51 (42–60)		2		NA	None (phytoliths uncommon)
Grinding stones and associated sediments						
Unit 1, 310a, 30–35/B			NA	15 (12–20)	6	Stone, 0; sediments, NA
Unit 1, 312a, 40–45/B			NA	16	1	Stone, 0; sediments, NA
Unit 1, 314c, 50–55/C	54		1	14 (10–20)	11	Stone, cob-type; sediments, cob-type
Unit 1, 315c, 57/C	56 (47–61)	48	3, 1	17 (12–24)	18	Stone, cob-type; sediments, cob-type
Unit 1, 316c, 60–65/D	59 (45–87)	48 (39–61)	29, 16	15 (14–16)	2	Stone, WT + cob-type; sediments, 0
Unit 1, 316d, 60–67 cm/D			NA	16 (6–24)	68	
Unit 1, 318d, 70–75/E	53 (41–75)	41 (32–64)	28, 10	17 (12–26)	22	Stone, RT + cob-type; sediments, RT + cob-type
Unit 1, 318e, 70–75/E	69 (48–100)	53 (40–84)	29, 15	16 (8–24)	80	Stone, cob-type; sediments, 0
Unit 1, 319d, 78/E	59 (36–120)	36 (27–48)	37, 8	16 (12–24)	8	Stone, cob-type; sediments, RT + cob-type
Unit 1, 322c, 85–90/E	53 (41–75)	29	7, 1	18 (12–24)	11	Stone, cob-type; sediments, cob-type
Unit 2, 361a, 10–20/B			NA	15 (12–20)	3	Stone, cob-type; sediments, NA
Unit 2, 362a, 20–30/B			NA	17 (10–22)	8	Stone, cob-type; sediments, NA
Unit 2, 364a, 45/B			NA	19 (12–24)	3	Stone, 0; sediments, NA
Unit 2, 365, 45–50/B			NA	16 (10–22)	2	Stone, RT; sediments, NA
Unit 2, 365a, 49/C			0	16 (10–28)	24	Stone, cob-type; sediments, 0
Unit 2, 365c, 51/C			NA		0	Stone, 0; sediments, 0
Unit 2, 365b, 54/C			0	14 (10–18)	5	Stone, 0; sediments, 0
Unit 2, 366a, 57/C	67		1	15 (12–18)	2	Stone, 0; sediments, 0
Unit 2, 367, 55–60/C			0		0	Stone, 0; sediments, NA
Unit 2, 367a, 63/D			0	12 (10–14)	9	Stone, 0; sediments, RT + cob-type
Unit 2, 368a, 63/D			0	20 (12–28)	3	Stone, 0; sediments, 0
Chipped stone						
Unit 1, 308a, 20–25/B			NA	14	1	NA
Unit 1, 322a, 85–90/E			NA	17 (16–18)	4	NA
Unit 2, 370, 70–75/E			NA	17 (12–24)	8	NA

NA, phytolith or starch grain studies were not carried out; WT, wavy-top rondel; RT, ruffle-top rondel. Sample numbers in the "Grinding stones and associated sediments" section include grinding stones, all *Cucurbita* phytoliths retrieved from the sediments associated with each stone, and all starch grains derived from the stones themselves. All samples in the "Column sample" section are sediments. In the "Number of *Cucurbita* phytoliths measured" column, the first number is the number of samples measured for length, and the second number is the number of samples measured for thickness. In the "Maize cob phytoliths" column, sediments refer to those sampled from the immediate vicinity of the grinding stones. All starch grains exhibited the characteristic extinction crosses under cross-polarized light. Cross-shaped phytoliths typical of maize leaves occurred in low numbers (e.g., < 10 observed on scans of entire slides) in some preceramic and ceramic levels. Sample sizes were not sufficient for robust size and statistical analysis.

occurring from Mexico to Nicaragua (3). Recent research by I.H. and D.R.P. has demonstrated that size and morphological criteria can be effectively used to separate the teosintes, including *ssp. parviglumis*, from maize (9); see *SI Materials and Methods* and *Table S2* for a summary. These data, combined with the results for non-*Zea* wild grasses, provide a secure method for identifying maize in archaeological starch grain records from the Central Balsas region.

Tables 1 and 2 summarize size and morphological characteristics of the grass starch grains isolated from the artifacts. For

both the preceramic- and ceramic-phase artifacts, average grain size was commonly 12–17 μm , which is characteristic of maize and outside the mean for any species or subspecies of teosinte (9; *Table S2*). The largest average size recorded in Balsas teosinte, the wild progenitor of maize, is 9.5 μm (9). The maximum size of individual grains in the Xihuatoxtla samples is commonly 20–26 μm , which also indicates maize, because it greatly exceeds that recorded in all types of teosinte except Race Chalco (*Zea mays ssp. mexicana*), which is known to frequently hybridize with maize. Starch grain morphological characteristics also indicate

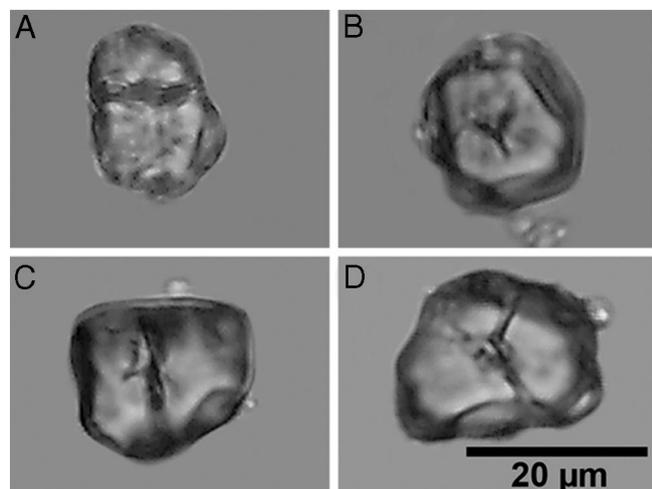


Fig. 1. Starch grains from maize recovered from early preceramic grinding stones 318d (A and B) and 318e (C and D). The grains have irregular shapes and surface contours, along with defined compression facets and transverse fissures (A) or y-shaped and other fissures (B–D).

the presence of maize (Table 2; Fig. 1); for comparison, Fig. S1A–C shows modern teosinte and maize starch grains. For example, the grains isolated from the preceramic- and ceramic-

period artifacts are predominantly irregularly shaped, and most have defined compression facets, unlike those of teosinte. Conversely, oval and bell-shaped grains, which are common in teosinte, were rare to absent on the tools, as they are in maize (9; Table S2).

The starch grain assemblages also contain higher frequencies of a type of fissure called a “transverse” fissure (a natural crack on the grain formed at the grain hilum or center) than are seen in modern teosinte samples (Fig. 1A); for example, the highest percentage of transverse fissures recorded in teosinte is 20% in the race Central Plateau. The archaeological frequencies are characteristic of Mexican popcorns (9; Table S2). Four of the preceramic grinding stones (316d, 318d, 318e, and 365a) have particularly good sample sizes, clearly demonstrating that every aspect of the starch grain assemblages indicates the presence of maize throughout the Xihuatoxtla preceramic sequence. Starch from few other taxa was present, indicating that maize was a focus of early food processing strategies. Grain morphology suggests the presence of popcorns or other hard-endosperm maize types (6, 9).

Eight of the grinding stones from which maize starch was recovered were securely stratified deep in preceramic levels (layers D and E), originating from 60–90 cm of units 1 and 2, well below a date of 4730 ± 40 B.P. (5590–5320 cal. B.P.) on charcoal from 49 cm b.s. (layer C) of unit 2 (Table 1) (1). A large piece of charcoal from 65 cm b.s. of unit 1 (layer D) yielded an age of 7920 ± 40 B.P. (8990–8610 cal. B.P.; intercept, 8710 cal. B.P.) (1). Four of the grinding stones and 2 of the chipped stone tools with maize starch occurred below this dated sample.

Table 2. Morphology of maize starch grains recovered from the Xihuatoxtla stone tools

Provenience	Tool Cat. no.	Shape				Hilum cavity	Compression Facets		Fissures		<i>n</i>
		Round	Oval	Bell	Irregular		Slight	Defined	Transverse	Total with fissures	
Unit 1											
Ceramic											
Layer B	310a	0	17	0	83	0	33	67	17	67	6
	312a	0	0	0	100	0	100	0	100	100	1
Layer C	314c	46	0	0	54	46	73	27	0	18	11
	315c	11	0	0	89	11	44	56	33	77	18
Preceramic											
Layer D	316c	0	0	0	100	100	50	50	0	50	2
	316d	9	0	3	88	19	26	74	23	46	68
Layer E	318e	13	1	0	86	35	19	81	25	61	80
	318d	5	0	0	90	18	9	91	41	74	22
	319d	12	0	0	88	12	38	62	0	50	8
	322c	9	0	0	91	9	27	73	36	72	11
Unit 2											
Ceramic											
Layer B	361a	33	0	0	67	33	33	67	0	67	3
	362a	25	0	0	75	5	38	62	12	37	8
	364a	0	0	0	100	0	33	67	64	64	3
	365	50	0	0	50	0	50	50	50	50	2
Layer C	365a	4	0	0	96	42	33	67	32	64	25
	365b	0	0	0	100	0	20	80	40	60	5
	366a	50	0	0	50	0	50	50	50	100	2
Preceramic											
Layer D	367a	44	0	0	56	11	78	22	0	33	9
	368a	0	0	0	100	33	0	100	33	99	3
Scrapers											
Unit 1											
Ceramic (B)	308	0	0	0	100	0	0	100	100	100	1
Preceramic	322	0	0	0	100	25	25	75	0	25	4
Unit 2											
Preceramic	370	0	0	0	100	25	12	88	25	75	8

n, total number of grains present on each tool. The numbers represent the percentages of each attribute present in the starch grain assemblages. See Table S2 for the same analysis of modern varieties of teosinte and Mexican maize.

human selection for reduced lignification and silicification of fruits was underway by 8700 cal. B.P.*

We cannot say with certainty which domesticated taxon was used, because the types of phytoliths present occur in both of the domesticated squashes native to Mexico, *C. pepo* and *C. argyrosperma* (11, 14). The former occurs at 10,000 cal. B.P. in the central Mexican highlands (18), and it conceivably could have been dispersed at an early date to the study region. Because the wild ancestor of *C. argyrosperma* is native to the central Balsas watershed (4), and its early domesticated product would have been better adapted to the region's climate than *C. pepo*, the preceramic squash may more likely be from *C. argyrosperma*.

Discussion

Our evidence of maize during the early ninth millennium cal. B.P. confirms an early Holocene time frame for its domestication, as has been indicated by a large corpus of archaeobotanical and paleoecological data bearing on its dispersal into southern Central America and northern South America (6, 7, 10–13, 19). Maize and also possibly *C. argyrosperma* squash join the increasing number of major and now-minor crop plants shown to have been brought under cultivation and domesticated in Mexico and South America between 10,000 and 7500 cal. B.P., about the same time as agriculture emerged in the Old World (6, 13, 17–20).

Neither Balsas nor other teosintes grow near Xihuatoxtla today, but the environment is suitable for Balsas teosinte, which is common just 50 km to the west. Although pollen from teosinte and maize cannot be reliably distinguished, *Zea* pollen grains dating to the Late Pleistocene Period recovered from Lake Ictaxiola, located 20 km west of Xihuatoxtla, may suggest that teosinte occurred in the study region during the early pre-Columbian era (5). In any case, our phytolith and starch evidence indicates that teosinte was not exploited either as a grain or for its stalks at Xihuatoxtla. Some investigators, arguing based on the paucity of evidence of teosinte utilization at other sites in Mexico, have proposed that the hard fruitcase that tightly encloses teosinte kernels made wild maize unsuitable for use as a grain by hunters/gatherers and early cultivators, and that teosinte was first exploited and cultivated for its sugary pith (the inside of the stalk) and/or green ears (15, 16). It is further argued that what particularly attracted people to teosinte and then early maize was the utility of the stalk sugar for making fermented beverages, a product that would have been important in the ceremonial life of communities (16).

The degree to which our data can speak directly to the first issue—whether or not teosinte was used and initially cultivated as a grain plant—is unclear. Even if teosinte was known to Xihuatoxtla's occupants, the early availability of maize likely would have made it of little utility to them. Furthermore, teosinte may have been brought under cultivation and domesticated elsewhere in the Río Balsas watershed, even possibly (as explained below) in areas of this region of tropical southwestern Mexico where it does not grow today. It also is important to note that arguments positing the low utility of mature teosinte seeds as human food, which are based almost entirely on the costs of processing them, may have been overstated. As described by Beadle (21), the seeds can be soaked and ground, and even popped. Today in Guerrero, traditional farmers grind *Z. parviglumis* seeds and use the product for chicken and pig feed, as well as for medicinal purposes (22). Moreover, the costliness of

individual subsistence resources and their perceived utility must be judged by measuring them against the availability, abundance, and ultimately the costs of other potential resources in the local environment (19).

What is clear is that throughout the preceramic sequence at Xihuatoxtla, maize kernels were commonly processed and consumed, indicating that early domesticated maize was a more significant grain crop that some investigators have supposed. If this is true, then the data open questions about the degree of cob evolution under human selection occurring during the ninth millennium cal. B.P. *Teosinte glume architecture 1 (tga1)* is a major domestication gene in *Zea* that controls crucial phenotypic attributes key to the efficient utilization of maize as a grain (23, 24). By controlling both phytolith and lignin formation, *tga1* regulates glume hardness and also determines the degree to which *Zea* seeds are enclosed by the glume and cupule, thus underwriting the production of naked grains with soft glumes when fully transformed from its wild state (23, 24). Morphological analysis of the oldest known macrofossils of cobs from Guilá Naquitz Cave, Oaxaca indicates that the gene was already altered by human selection by 6200 cal. B.P. (25, 26). Molecular research on modern teosinte and maize suggests that human transformation of this locus may have been underway during the early Holocene period (24).

Our phytolith evidence adds more information to this scenario. By directing which cells of the glumes and cupules are silicified and determining the degree of phytolith surface ornamentation, *tga1* underwrites the phytolith traits in maize and teosinte fruits that distinguish them from one another (11, 23; [SI Materials and Methods](#)). Therefore, the presence of phytoliths diagnostic of cobs and the absence of phytoliths that could be from teosinte fruitcases at Xihuatoxtla suggest that this early maize was undergoing human manipulation at the *tga1* locus.

Starch grain and phytolith data dating between ca. 7600 and 5300 cal. B.P. from central Pacific Panama and southwestern Ecuador, 2 key and early dispersal route regions of the seasonally dry Neotropics, similarly show an emphasis on maize kernel exploitation and processing in domestic contexts (6, 7, 10). As at Xihuatoxtla, the Panamanian records lack stalk phytoliths (see [SI Materials and Methods](#)). The Ecuadorean records have not yet been checked. Although we cannot rule out the possibility of low-level stalk utilization, which could hinder empirical documentation, or the possibility that other early people not yet studied were routinely exploiting stalk sugar, the theory that the use of stalk sugar for the production of alcoholic beverages or other purposes was the primary motive for the early cultivation and diffusion of maize (16) is not supported by current data.

On the basis of molecular data from modern maize, it has been proposed that the most primitive surviving land races are from the semiarid and cool Mexican highlands, outside of the natural habitat of Balsas teosinte, and that domestication thus occurred first in the highlands and later spread to the lowlands (2). Our archaeological evidence for maize in seasonal tropical forest 2500 years earlier than has been documented in the dry highlands supports instead an opposing, and less conflicting, scenario that maize was domesticated at lower and moister elevations in the Balsas watershed, where *Z. mays* ssp. *parviglumis* is native. Also of relevance to this issue is that despite the excellent preservation of macrobotanical remains at Guilá Naquitz Cave and in the Tehuacan Valley sequences (27) and the more recent generation of phytolith data at Guilá Naquitz (26), a premaize use of teosinte before the appearance of maize, which would be expected if maize was of highland derivation, cannot be detected in either the southern or central Mexican highlands.

Moreover, as a result of significant Late Pleistocene cooling and downslope vegetational movement (5, 28), any significant difference in the geographic distribution of *Z. mays* ssp. *parviglumis* before about 8600 years ago likely would have placed it in

*Note that attempts were made to directly date preceramic phytolith assemblages containing early *Cucurbita*, but were unsuccessful. Two small samples, 318e and 325g, broke during shipment to the radiocarbon facility, and what remained in the tubes could not be used. Another phytolith sample, from 78 cm of unit 1, did not contain sufficient carbon to allow dating. Obtaining very clean phytolith separations from Xihuatoxtla that were suitable for dating was difficult, and we decided not to use any more of the remaining sediments in further dating attempts.

even lower-lying tropical habitats (below 400 m) in the states of Guerrero and Michoacán, where it is largely absent now. For this and other reasons discussed herein, the possibility that teosinte was domesticated outside of the Central Balsas region cannot be ruled out at this time. Nonetheless, our data shift the focus of investigation back to lower elevations. The documentation of an early Holocene antiquity for maize in the Central Balsas River Valley should stimulate research directed toward uncovering the earliest stages of its transformation from teosinte.

Materials and Methods

Stone Tool and Sediment Starch Sampling. The sampling of stone tools involved a series of steps designed to carefully evaluate whether grains recovered from the stones represent plants that were processed with the artifacts (6). Unwashed grinding stones were first placed under a stereoscopic microscope at a power of $\times 100$. The point of a fine needle was inserted deep into cracks and crevices of the surfaces to loosen and remove any residue. The residue was then transferred directly to microscope slides, mounted in water, and examined at a power of $\times 400$. Between 8 and 21 locations covering the used and nonused facets of 9 grinding stones were examined in this manner. Because this step is time-consuming, a focus was put on preceramic-phase artifacts for the needle point studies; those analyzed by this method were 319d, 318d, 318e, 316d, 322c, 315c, 361a, 365a, and 367a (see Table 1 for the proveniences of these stones). For stones not analyzed in this way, starch analysis began with the next step, described below.

The stones were then placed underneath running water and washed with a brush to remove any sediment that was strongly adhered to them when they were removed from the ground. The sediment was saved, and starch was removed from it through the addition of a heavy liquid metal at a density of 1.8 made from cesium chloride (CsCl). Next, the washed stones were shaken in an ultrasound unit for 5–10 min to further dislodge starch grains, which were then isolated from the solution using CsCl as in the previous step. Between 10 and 20 cm³ of sediment sampled from immediately beneath and around (within 10–15 cm) the stones while the excavation was in progress also was analyzed for starch content using this heavy liquid treatment.

Chipped stone tools were analyzed by placing them directly in the ultrasound unit and continuing the analysis as described above.

Phytolith Removal from Sediments. Phytolith extraction from sediments followed standard techniques involving sediment dispersion, removal of carbonates and organic materials, and separation of phytoliths using a heavy liquid solution at a density of 2.3 (11).

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