Early millet use in northern China

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It is generally understood that foxtail millet and broomcorn millet were initially domesticated in Northern China where they eventually became the dominant plant food crops. The rarity of older archaeological sites and archaeobotanical work in the region, however, renders both the origins of these plants and their processes of domestication poorly understood. Here we present ancient starch grain assemblages recovered from cultural deposits, including carbonized residues adhering to an early pottery sherd as well as grinding stone tools excavated from the sites of Nanzhuangtou (11.5–11.0 cal kyBP) and Donghulin (11.0–9.5 cal kyBP) in the North China Plain. Our data extend the record of millet use in China by nearly 1,000 y, and the record of foxtail millet in the region by at least two millennia. The patterning of starch residues within the samples allow for the formulation of the hypothesis that foxtail millets were cultivated for an extended period of two millennia, during which this crop plant appears to have been undergoing domestication. Future research in the region will help clarify the processes in place.

starch grain analysis | agriculture origins | early Neolithic | millet domestication | East Asia

The archaeobotanical remains of millets, here defined as grasses classified within the genera *Setaria* and *Panicum*, have been recovered from archaeological sites worldwide (1). As minor grain crops, millets have received particular attention in China because both foxtail millet (*Setaria italica* L. Beauv.) and broomcorn millet (*Panicum miliaceum* L.) were domesticated in northern China where they became the dominant traditional grain crops (2–7). Recent phytolith evidence from the Cishan site in the North China Plain places the domestication of broomcorn millet as early as 10 kyBP, whereas foxtail millet appears to have been exploited later at ~8.7 kyBP. (7). This date is slightly earlier than that of charred grains of foxtail millet from the sites of Xinglonggou (~8–7.5 kyBP) (8) and Yuezhuang (~7.87 kyBP) (9).

The near absence of both very early sites and sustained archaeobotanical research in North China has resulted in large gaps in the archaeobotanical record for millet use during the Neolithic when these crop plants are believed to have initially come into cultivation. Here we present archaeobotanical evidence for millet exploitation during this key time period between 11 and 9.5 kyBP. Ancient starch grains have been recovered from cultural deposits, from carbonized residues adhering to an early ceramic sherd, and from ground stone tools excavated from the sites of Nanzhuangtou and Donghulin, two of the most important sites in the North China Plain that date to the Neolithic.

Site Descriptions

Nanzhuangtou (39°6′40′′N, 115°39′25′′E) is located at 21 m above sea level (ASL) on the piedmont of the Taihang Mountains, 35 km west of Baiyangdian Lake at the western border of the North China Plain (Fig. 1). The cultural deposits at Nanzhuangtou were sealed by both fluvial and lacustrine sediments. During three excavation seasons in 1986, 1987, and 1997 (10, 11), a number of stone tools, including five slabs and four mullers, were recovered from the site along with numerous pottery sherds. Pits, hearths, and ditches were the major feature types at the site. Large numbers of faunal bones were recovered, and archaeological wood, leaves, and seeds were found scattered throughout the cultural deposits. Seven samples from cultural deposits, including organic silt, wood, and charcoal were radiocarbon dated and yielded uncalibrated dates ranging from 10.5 to 9.7 kyBP (10) (calibrated dates in Table S1). Because the organic silt may possibly contain old carbon, dates derived from these samples were excluded in this paper. Five of the remaining six dates fall between 11.5 and 11 kyBP after calibration. We believe that the occupational phase of the Nanzhuangtou site was during this range of dates, earlier than 11 kyBP but later than 11.5 kyBP. No systematic archaeobotanical work on macroremains was carried out at Nanzhuangtou.

The Donghulin site (N39°58′48′′, E115°43′36′′) is situated in the Zhaitang Basin of the Qingshui River, ~78 km west of Beijing City. The site proper lies at 390–400 m ASL atop a piedmont overlooking the northern end of the Taihang Mountains just to the west of the North China Plain (Fig. 1). Four seasons of excavations were completed in 2001, 2003, 2005, and 2006, during which more than 200 m^2 of the entire 3,000 m^2 of the site were exposed. AMS dates derived from charcoal, carbonized seeds, and bone place the occupations at ~11–9.5 kyBP (12–14), and strata representing two distinct and sequential cultural occupations, the early phase (11.15–10.5 kyBP) and the late phase (10.5–9.45 kyBP) (14), were discovered. Artifact assemblages from the site include pottery sherds and a very large number of stone tools, including ground stone slabs and mullers (~144 pieces). Numerous intact hearths and two human burials were also excavated. Faunal analysis revealed a rich assemblage including more than 10,000 bone fragments derived mainly from mammals such as red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), and black bear (*Ursus thibetanus*) (14).

Previous archaeobotanical investigations at Donghulin recovered the charred seeds of hackberry (*Celtis bungeana* Bl.) (12), millets, and Chenopod, as well as seeds from genera *Vitis* and *Vigna* (15). Starch grain analyses were completed on two lithic tools, and tentative identifications of acorns (*Quercus* spp.) were made by Liu et al. (16). Phytolith analyses were completed on residues collected from lithic tools, ceramic sherds, and cultural deposits, but only two fragments of phytoliths from broomcorn millet were recovered from a single sample collected

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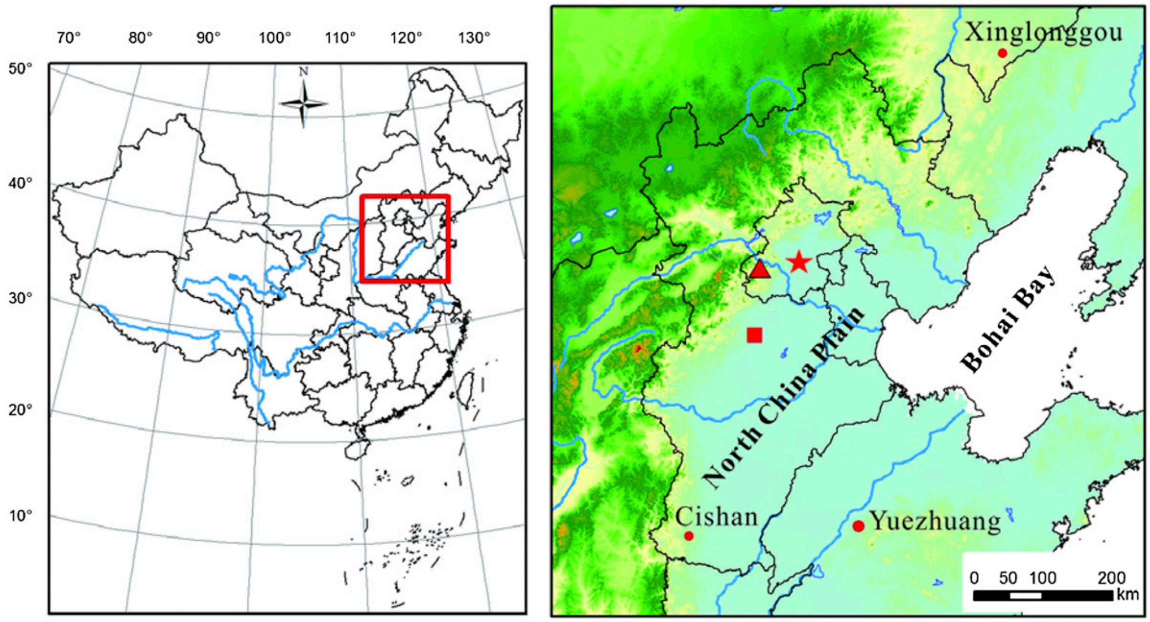

Fig. 1. Location of study region and archaeological sites. The Nanzhuangtou and Donghulin sites are indicated by the red rectangle and triangle, respectively (*Right*). The red star is the locality of Beijing, and the red dots are the localities of other sites mentioned in the text. The light-to-dark green shading indicates low-to-high elevation.

from the ashes of a hearth (field no. T4:HD8) belonging to the late occupational phase (Fig. S1).

Modern Comparative Studies

In China, there are 14 species of grasses that are classified within the genus *Setaria*. Seven species occur in North China, and one of them, green foxtail grass [*Setaria viridis* (L.) Beauv.], has been determined to be the wild progenitor of domesticated foxtail millet. There are 20 known species in the genus *Panicum* in China, among which only broomcorn millet and the wild *Panicum bisulcatum* occur in North China (see http://www.efloras.org/ for more information). Unlike foxtail millet, the wild ancestor of broomcorn millet has not yet been conclusively identified.

Seven species of *Setaria* and two species of *Panicum* that occur in China were studied for comparative purposes (17). Morphological studies included observations of variation in size, shape, fissuring, and surface features occurring in cultivated millets and their wild relatives collected from different regions in China. We found that the millet starch grains are easily distinguished from those of other economically significant families, such as Fagaceae, Nymphaeaceae, Alismataceae, Araceae, Cyperaceae, Polygonaceae, and Juglandaceae, and from other genera within the grass family Poaceae (17) (Fig. S2).

Our comparative work with modern specimens indicates that to distinguish domesticated millet starch grains from wild, and foxtail millet from broomcorn millet, there are two key factors to be considered. One factor is morphological and applies to single-starch grains, and the second is an assemblage-based approach. The basic morphological form of all studied wild and domesticated millet starches is a polyhedral shape with occasional spherical forms present (Fig. 2). Starch grains from wild millets within the genus *Setaria* are characterized by wrinkled surfaces and rough edges, and the mean size is typically <10 μm, much smaller than both domesticated foxtail millet and green foxtail grass (17). The starch grains from green foxtail grass are the largest among the wild millets, and their range of sizes (<14 μm) and morphology overlap somewhat with foxtail millet.

When both size classes and fissuring patterns are taken into consideration, starch assemblages with characteristics typical of millets can be partitioned into taxonomic groups using a previously published dichotomous key (17) that we developed and then used to determine the species distribution of the millet starch grain assemblages from the two studied sites.

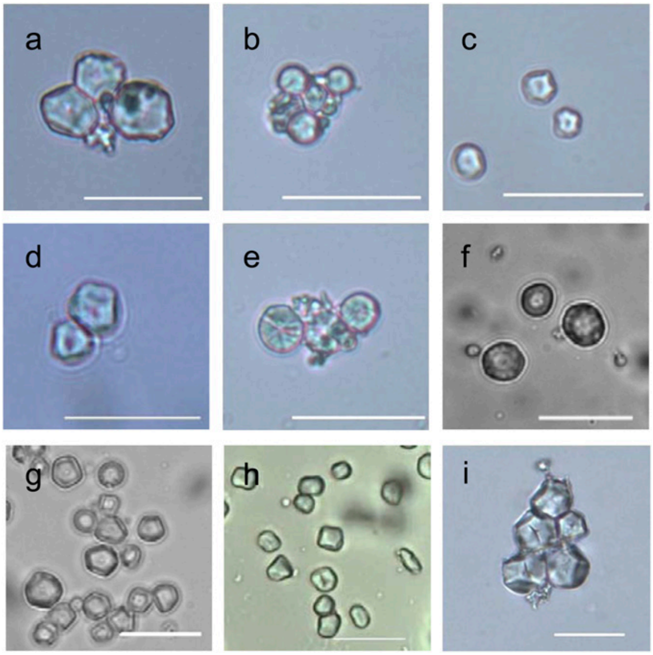

Fig. 2. Characteristic starch grains from seeds of wild and domesticated millets. (*A*) *Setaria plicata* from Guangdong Province. (*B*) *Setaria parviflora* from unknown region in South China. (*C*) *Setaria faberil* from Anhui Province. (*D*) *Setaria chondrachne* from Jiangsu Province. (*E*) *Setaria pumila* from Shandong Province. (*F*) *S. viridis* from Inner Mongolia. (*G*) *Panicum miliaceum* from Shaanxi Provinces. (*H*) *P. bisulcatum* from Beijing. (*I*) *S. italica* from Hebei Province. (Scale bar: 20 μm.)

Results

A fragmentary sandstone slab (field no. G3:110) and an intact sandstone muller (field no. G3:367) from the Nanzhuangtou site were examined. These two artifacts were recovered from the floor of a ditch (field no. G3, zone 5) that had been filled with a midden deposit that included pottery sherds, bone tools, fragments of animal bones, and plant remains. Three sediment samples were analyzed as control specimens, including one from dust of the storeroom where the artifacts were curated, one from lacustrine sediment overlying the cultural deposits, and one from the underwater loess, underlayer of the cultural deposits (Fig. S3).

No starches were recovered from the three control specimens. More than 400 starch grains were recovered from the tools (Table 1), and 255 were identified as starch grains from grasses. Some 205 of the 255 grass starch grains have the polyhedral or spherical shape (Fig. 3) characteristic of millet starches. When the assemblage is viewed as a whole, starch grains with wrinkled surfaces and rough edges, wild characteristics, comprise at least 38% of the millet starches, and starch grains measuring >14 μm in size, characteristic of domesticated seeds, make up about 46.8% of the millet starches (Fig. 4). Of the remaining starch grains in the assemblage, the remaining 50 grass starch grains are derived from grasses in the tribe Triticeae, several species of which occur locally, and 13 starch grains are characteristic of geophytes. Studies to identify and interpret these other residues are ongoing.

Two intact mullers, one of limestone [field no. T4(10):65] and the other crafted from sandstone [T8(8):2], both of which were recovered from the early phase at the Donghulin site, and a fragmentary andesite slab [field no. T9(3):799], an andesite muller [field no. T9(3):511], a limestone pestle [field no. TG2 (4):2], an early sherd [field no. T9(5):1268], and a sample of sediment that originally surrounded the sherd collected from the late phase were also chosen for study. The sherd was the base of an earthenware vessel and had thick (2–3 cm) carbonized residues adhering to the interior surface. This residue was sampled for starches. Four samples, three sediments and one lithic, were analyzed as control specimens. These controls included one sediment sample collected from the dust of the storeroom where the artifacts were curated, one from the surface soil at the site, one from the loess on which the site stands, and one stone block recovered from a late phase hearth.

No starches were recovered from the four control specimens. More than 793 starch grains were recovered from the tools, carbonized residue, and cultural deposits (Table 1), 742 of which were identified as starch grains from grass seeds. Subsequent analyses and comparative studies have led to the identification of 525 of the 742 grass starches as derived from millets, and the remaining 217 from grasses classified within the tribe Triticeae (Fig. 3). Additionally, 50 starch grains characteristic of geophytes were identified. During the early occupational phase, the starch grains with wrinkled surfaces and rough edges, characteristics of

Table 1. Starch grain frequencies from the lithic tools, sherd, and sediments

Samples	Field no.	Subsamples	Millets (W)	Triticeae	Geophytes	UD	Total
Nanzhuangtou site							
Slab	G3:110	Used facet	>109	25	10	82	>226
		Unused facet	44	8	2	24	78
		Total	>153 (52)	31	12	106	>304
Muller	G3:367	Used facet	32	9	1	23	65
		Unused facet	20	10	0	>9	>39
		Total	52 (26)	19	1	>32	>104
		Grand Total	>205 (78)	50	13	>138	>408
Donghulin site							
		Early occupational phase					
Muller 1	T4(10):65	Used facet	76	28	0	6	110
		Unused facet	28	6	0	7	41
		Total	104 (22)	34	0	13	151
Muller 2	T8(8):2	Facet A	16	9	2	1	28
		Facet B	29	22	1	3	55
		Facet C	12	24	1	2	39
		Facet D	96	51	3	5	155
		Total	153 (61)	103	7	11	277
		Grand total	257 (83)	137	7	24	428
		Late occupational phase					
Muller 3	T8(3):99	Used facet	3	4	0	3	10
		Unused facet	0	0	0	1	1
		Total	3 (1)	4	0	4	11
Pestle	TG2(4):2		6 (1)	10	1	3	20
Slab	T9(3):799	Soil*	73	7	20	2	102
		Unused facet	44	11	4	2	61
		Used facet	85	9	4	1	99
		Total	202 (29)	27	28	5	262
Sherd	T9(5):1268		40 (9)	6	0	4	50
Cultural deposits	T9(5)		17	3	1	1	22
		Grand total	268 (40)	50	30	17	365

*Soil adhering to the tool, including used facet and unused facet.
UD, undiagnosed starch grains; W, starch grains similar to wild millet grasses.

Fig. 3. Starch grains recovered from the sites of Nanzhuangtou and Donghulin. (*A* and *B*) Polyhedral and spherical starch grains recovered from the stone tools excavated from the Nanzhuangtou site. (*C*–*E*) Starch grains recovered from the stone tools excavated from the Donghulin site. Note wrinkled surface and rough edges in *E*. (*F*) Starch grain recovered from the carbonized residues adhering to the sherd, Donghulin site. (*G*) Starch grain recovered from the cultural deposits, Donghulin site. (*H*) Starch grain from the tribe Triticeae. (*I*) Starch grain from a geophyte source. (Scale bars: *A*–*G*, 10 μm, *H* and *I*, 20 μm.)

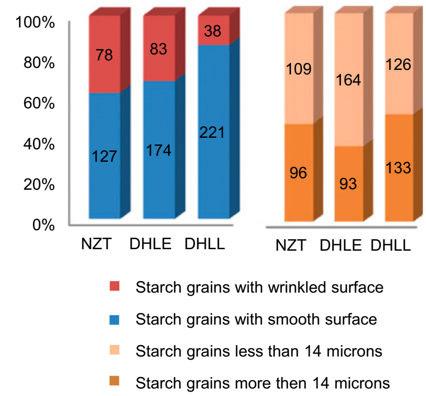

Fig. 4. Site assemblage compositions of diagnostic characteristics of millet starch grains. From left to right, Nanzhuangtou (NZT), the early occupation of Donghulin (DHLE), and the late occupation at Donghulin (DHLL). The blue and red graph shows the ratio of starch grains with wrinkled surfaces to those that are smooth. The yellow graph shows the ratio of starch grains that are >14 μm to those <14 μm.

wild millets, make up 32.3% of the millet starches. In contrast, about 14.9% of millet starch grains during the late occupational phase have these features. Starch grains measuring >4 μm increased from 36.2% in the early occupation to 51.4% during the late occupation (Fig. 4).

All millet starch grains >14 μm bear morphological features consistent with those of domesticated foxtail millet. The remaining starch grains, which measure <14 μm, vary in morphologies. Some have wrinkled surfaces like those from wild millet grass, and some have smooth surfaces that are the same as starch grains from both wild and domesticated foxtail and broomcorn millets (Fig. 3). Because the broomcorn millet phytoliths were recovered from the late phase occupation at Donghulin, this assemblage of starch grains (<14 μm) bearing smooth surfaces is very likely derived from broomcorn millet. We are not yet comfortable making a secure identification of these starch grains because they also occurred in the early phase occupations at Donghulin and Nanzhuangtou when there is no supporting phytolith evidence, and the starch grains from both wild and domesticated foxtail and broomcorn millets overlap with one another in this size zone (17).

Discussion

Before these studies, the earliest evidence for millet exploitation in China dated to ~10 kyBP at the Cishan site in North China (7). The millet starches from Nanzhuangtou and Donghulin extend the record of millet exploitation in China by at least 1,000 y and foxtail millet by at least 2,000 y. The starch remains also indicate processing of millets using ground stone tools as well as the cooking of the grains in ceramic vessels.

The millet starch assemblage from these two sites also changes between the early and late occupations. During the period between 11 and 9.5 cal kyBP, the proportion of starch grains with wrinkled surfaces and rough edges, which are features diagnostic of wild foxtail grasses, decreases from 38.0% (the Nanzhuangtou site) and 32.3% (the Donghulin site) of the total population to 14.7% at both sites. Meanwhile the starch grains measuring >14 μm, a size class only recorded in domesticated foxtail millet, increased from 46.2% (the Nanzhuangtou site) and 36.2% (the Donghulin site) to 52.6% (Fig. 4).

It is well documented that the basic morphologies of starch grains are under genetic control (18, 19). These finds, therefore, indicate that some genotypes of domesticated foxtail millet may have occurred as early as 11 kya, perhaps resulting from the management of wild millet grasses, or possibly cultivation.

Though we are aware that the numbers of samples thus far analyzed from the two sites is small, we believe that the starch data may indicate a pattern that deserves further study. During the domestication of cereal crops in the southwestern Asia and East Asia, there are two changes that are most commonly documented in the archaeobotanical record: increase in seed size and loss of natural dispersal mechanisms via development of a nonshattering rachis (20–22). In macrobotanical assemblages, the proportion of shattering to nonshattering rachises can be studied, and changes in this proportion over time allow for an understanding of the temporal span of the processes of domestication (20–22). In these microbotanical assemblages, wild and domesticated characteristics of the millet starches overlap somewhat; thus, the proportion of wild-type starches in the assemblage cannot be assumed to represent an exact fraction of wild seeds. Further complicating the interpretation of domestication is the possibility that immature seeds were harvested with mature seeds. All polyhedral starch grains in millets are produced from mature or near-mature seeds, whereas the starch grains from immature seeds are very small, olivary, or spherical in morphology, not unlike those from wild millets. However, over a period of about 1,500 y, the relative proportions of starches with wild vs. domesticated traits can be documented as shifting toward domesticated characteristics, more so than would be seen if random processes of deposition were at work.

The studies of wheat, barley, and rice spikelet bases suggested the domestication process was very slow, occurring over a period of at least 2,000–3,000 y (20–22). The millet starch grains from these two sites allow us to formulate a hypothesis that, during the period of 11–9.5 cal kyBP, foxtail millet was still undergoing domestication, or perhaps interbreeding with nearby wild grasses, because the starch grains with wild traits do not disappear during this time. Nonetheless, though we believe that we may have uncovered an important pattern indicating the trajectory of the domestication of foxtail millet, for the moment, we view this model as a hypothesis to be tested by future research.

Conclusions

The data from these studies extends the archaeobotanical record of millet exploitation to 11 cal kyBP in East Asia. The presence of the starch grains on processing tools is a strong indicator that millet seeds were ground into flour or meal using stone tools, then cooked in earthenware vessels as early as 10 cal kyBP. Other grasses and geophytes were also part of the diet during this time in the North China Plain. We believe these data may indicate that the domestication of foxtail millet occurred over an extended period, perhaps two millennia or more. Future research in this region should help clarify the trajectory of this important crop plant.

Materials and Methods

Modern Reference Collection. Starch grain identifications were based upon one-on-one comparisons between ancient starches and those derived from a modern reference collection of more than 200 Asian species housed at the Institute of Geographic Sciences and Natural Resources Research at the Chinese Academy of Sciences. The following studies of millets were conducted with this reference collection.

Starch grains from 31 modern samples of millets derived from the seeds of seven species within the genus *Setaria* and two species within the genus *Panicum* were analyzed to determine diagnostic morphological characteristics. The modern samples were collected from 14 provinces ranging from north to south in eastern China, including Inner Mongolia, Guangdong, Gansu, and Jiangsu. Two to four seeds were soaked for 1 h, then crushed to release starches. A drop of water/starch slurry was placed onto a clean glass slide, mounted in 10% glycerine and 90% ultrapure water, and sealed with acrylic nail polish. Starch grains were examined using compound light microscopy in both white and cross-polarized light at 400× magnification. A total of 100–150 starch grains were measured for each sample, and morphological features of the starch grains, including basic shape and surface micromorphology, were examined. Details of morphological studies have been published previously (17).

Archaeological Samples. ***Stone tools.*** To allow for future studies of the same artifacts, one-third of the length of each muller was sampled, as was one-quarter of the slab. To dislodge adhering sediment and starch, tools were shaken in an ultrasonic water bath for 5–10 min, and starch was then isolated with heavy liquid flotation using a solution of CsCl at a density of 1.8. The recovered residue was mounted in 10% glycerine and 90% water on a slide and examined with both white and cross-polarized light at a magnification of 400×. Starch grains were counted, analyzed for morphological features, then recorded and compared with those from the modern reference collection. For a detailed protocol, see refs. 24–26.

Carbonized residues. A solution of 6% H_2O_2 was used to break down some of the larger charred particles by oxidation, releasing starch grains that could possibly be trapped within them, as well as to oxidize some of the extraneous organic material from the residues. A heavy liquid solution of CsCl at a density of 1.3 was added to the residue to remove any material with a specific gravity <1.3. Starch grains were then separated from the solution using a CsCl solution at a density of 1.8. The material recovered from the second extraction was mounted in 10% glycerine and 90% water on a clean glass slide. For a detailed protocol, see refs. 27 and 28. In this study, 0.05 g of carbonized residue was subsampled from the larger fragments for analysis.

Sediments. Five grams of sediment was weighed for analysis. The sediment was crushed to a fine powder with a mortar and pestle, and 6% hydrogen peroxide was added to the powder to release the starch grains as well as to oxidize some of the extraneous organic materials. A 5% Calgon solution was added to disperse the sediments and remove clay. A heavy liquid solution of CsCl at a density of 1.8 was used on the prepared sediment sample to extract the starch grains. For detailed methods, see Yang et al. (29).

Identification of Ancient Starch Grains. Our starch keys and classifications emphasize attributes demonstrated by previous studies (17, 23–31) to be useful in identification: overall grain shape; contour and surface features; position and form of the hilum and fissure, if any; number and characteristics of pressure facets; presence or absence of demonstrable lamellae; and mean length averaged from the measurement of 100–150 grains. Each starch grain observed under the microscope was photographed, then grouped based on morphological features. In addition to our comparative work, the numerous previously published studies available on starch grain morphology were also consulted (17, 23–31).

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