



Identification of 10,000-year-old rice beer at Shangshan in the Lower Yangzi River valley of China

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The origins of rice domestication and the beginnings of alcoholic fermentation in China are intriguing research topics, with the Shangshan culture in the Lower Yangzi River region being a focal point of archaeological investigations. This study employs a multi-proxy approach (phytolith, starch, and fungi) to analyze microfossil remains associated with pottery vessels from the earliest phase of the Shangshan site (ca. 10,000 to 9,000 cal. BP). The results indicate that rice was consumed as a dietary staple and used for brewing fermented beverages with a *qu* starter containing *Monascus* mold and yeast as fermentation agents. The fermentation ingredients included rice, supplemented with other cereals (Job's tears, Panicoideae, and Triticeae), acorn, and lily. This rice-fungi-based multiplant brewing method marked the earliest-known alcoholic fermentation technique in East Asia. The emergence of this fermentation technology is attributable to the early development of rice domestication and the arrival of the wet-warm Holocene climate, which was favorable for fungal growth. These alcoholic beverages likely played a pivotal role in ceremonial feasting, highlighting their ritual function as a driving factor that may have stimulated the intensive utilization and widespread cultivation of rice in Neolithic China.

fermented beverages | rice | qu starter | Early pottery | fungi

The origins of rice domestication have undergone extensive archaeological scrutiny and discussion in recent years. Archaeologists generally agree, based on existing data, that the earliest stages of rice domestication occurred in the Lower and Middle Yangzi River region. The Shangshan culture in Zhejiang (ca. 10,000 to 8,000 cal. BP) represents a region where early rice domestication emerged. Although the degree of rice domestication is still under investigation, recent studies suggest that this process began early (1–7). Analysis of morphotypes of rice bulliform phytoliths from soil has indicated traits of early domestication around 11,000 y ago (1, 3, 4, 8). A micro-CT analysis of rice spikelet impressions found in the Shangshan pottery clay dating to the 10th millennium BP revealed that 12% of rachis impressions were nonshattering types, a key domestication trait—significantly higher than those observed in modern wild rice populations (7). Additionally, usewear and phytolith residue analyses of stone tools suggest that rice was harvested using flaked tools throughout the Shangshan culture period (9).

While numerous studies have explored spatiotemporal aspects in the origins of rice domestication, few researchers delve into the social mechanisms driving these processes. Hayden (10) suggested that the original domestication of rice in East Asia might be linked to its use as a luxury feasting food, based on suggestively analogous modern ethnographic observations. Liu and colleagues (11, 12) have proposed a hypothesis that links rice domestication and dispersal to ritual feasting activities, such as the emerging mortuary practice of ancestor worship rituals, where rice-based alcoholic beverages were served as offerings. Conversely, Wang (13) argues that the need for rice chaff, an essential component in plant-tempered pottery, played a crucial role in rice cultivation, potentially initiating the domestication process.

These three perspectives employed diverse theoretical and methodological approaches. While Hayden and Liu et al. highlight the role of ritual activities as a driving force, Wang argues for an unintentional process of rice cultivation. Studies by Liu et al. and by Wang (11, 13) not only utilized microfossil analysis but also focused on the Shangshan culture. However, they did not explore the earliest phase of the Shangshan materials (the early part of Early Shangshan Phase; ca. 10,000 to 9,500 cal. BP), nor did they benefit from the latest studies of rice bulliforms and rachilla impressions, which indicate a long history of rice utilization from wild to domesticated in the Shangshan culture area (4, 7). Intending to reassess the several published interpretations based on current data, this study is designed to specifically target the microfossil remains (phytoliths, starches, and fungi) associated with

Significance

The Shangshan culture in ancient China's Lower Yangzi region is central to understanding the origins of rice domestication and early alcohol fermentation. To address these issues, we used various methods to study artifacts from the early phase of the Shangshan site, dating back to ca. 10,000 to 9,000 y ago. By analyzing microscopic remains, including phytoliths, starch granules, and fungi, associated with pottery vessels, we found evidence suggesting that the Shangshan people not only used rice as a staple food but also as a raw material for brewing fermented beverages, marking the earliest known alcohol fermentation technique in East Asia. These alcoholic drinks likely played a significant role in ceremonies, possibly encouraging the widespread cultivation of rice in ancient China.

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pottery vessels dated to the entire Early Shangshan Phase (ca. 10,000 to 9,000 cal. BP). We will address three issues related to the material and social mechanisms that may have played an important role in the early Shangshan rice exploitation and alcohol brewing: 1) whether the harvested rice was wild or was undergoing an early phase of domestication; 2) how rice exploitation was integrated with the procurement and utilization of plant resources in general; and 3) the origin of fermented alcoholic beverages in relation to rice production and dispersal, as well as the beverages' social implications.

1. Archaeological Background and Materials

The Shangshan culture comprises 21 sites primarily located along the Qiantang River valley (Fig. 1A) (14). These sites show conspicuous material advances, such as construction of residential dwellings and storage pits, production of both flaked and polished stone tools, and crafting of pottery vessels. Most Shangshan ceramics are plant-tempered, utilizing a mix of clay with grasses, including leaves, rice chaff, and other plants, indicating intensive utilization of rice. Pottery vessels for culinary use predominantly consist of basins in various sizes, while globular jars, small cups, ring-based plates, and bowls are smaller fractions of the total. Some culinary vessels were stored in pits (*SI Appendix*, Fig. S1:1–4). The diversity of pottery vessel types increases over time, suggesting a rise in functional variation (2, 15).

The Shangshan inhabitants employed a broad-spectrum subsistence strategy that utilized both hunting of animals and exploitation

of diverse plant resources, including rice, acorn, barnyard grass, and various tubers and roots (16–18). Previous studies have uncovered evidence of rice-based fermented beverages or food associated with globular jars and ring-foot plates in ritual contexts at Xiaohuangshan and Qiaotou from the middle Shangshan culture (ca. 9,000 to 8,600 cal. BP) (11, 19). The globular jar, a vessel commonly used for alcoholic fermentation in early Neolithic China (20, 21), first appeared in the early Shangshan culture. Microfossil analysis also indicates that cups were used for drinking fermented beverages at Dawenkou culture sites (6,200 to 4,600 cal. BP) on the east coast (22). These lines of information suggest that production and consumption of fermented foods and beverages using various pottery vessels may have begun during the early domestication of rice, around 10,000 y ago.

To examine this hypothesis, we chose 12 pottery sherds excavated from the early deposits at the Shangshan site (ca. 10,000 to 9,000 cal. BP). The sampling processes were carried out at the Puijiang Museum in 2022 and 2023. These sherds originated from eight globular jars, a cup, a ring-foot bowl, a large basin, and a cooking jar. We also analyzed two soil control samples to compare them with the pottery samples. CONT1 is sediment collected from an unused surface of a stone slab in Layer 7 of the Early Shangshan Phase, and CONT2 is a soil sample from Layer 5 of the Middle Shangshan Phase (Fig. 1B and C and *SI Appendix*, Table S1).

All analyzed sherds are plant-tempered, but one of them also contains visible sand-temper. To comprehend the functions of

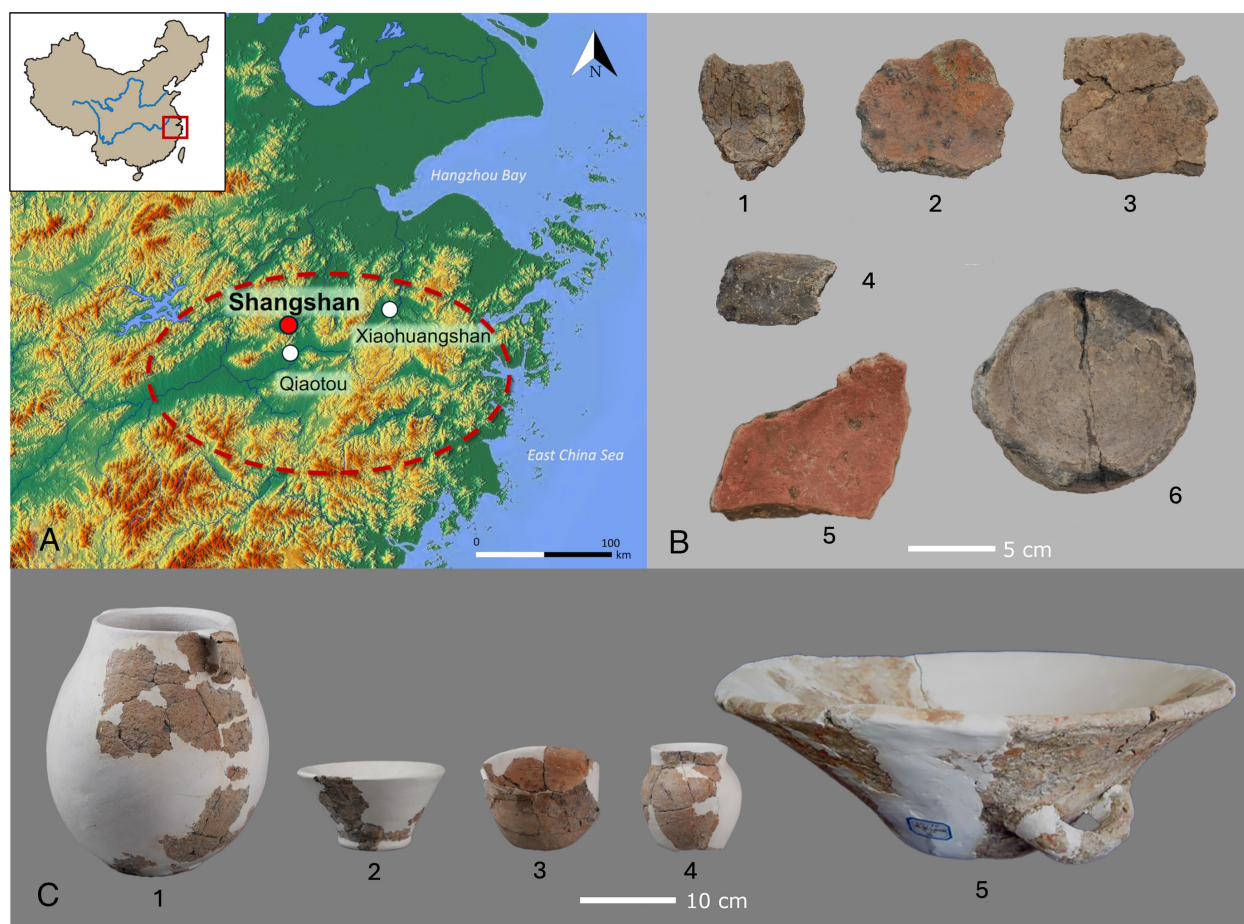


Fig. 1. Site locations and artifacts. (A) Locations of the Shangshan, Qiaotou, and Xiaohuangshan sites (dots) and the distribution area of the Shangshan culture (red circle). (B) Selected pottery sherds analyzed. 1: cup sherd (CUP); 2: jar sherd (JAR3); 3: jar sherd (JAR5); 4: rim sherd from sand-tempered cooking pot (POT); 5: large basin sherd (LBAS); 6: base of the ring foot bowl (BWL). (C) Corresponding complete vessels. 1: globular jar; 2: ring foot bowl; 3: cup; 4: flat-based jar; 5: large basin.

different vessel types in connection with culinary practices, we tentatively categorized the pottery samples into four groups based on their presumed functions: 1) eight globular jars potentially designated as fermentation vessels (JAR1-8); 2) two serving vessels, comprising a cup and a bowl (CUP, BOWL); 3) a large basin used as general processing vessel (LBAS); and 4) a sand-plant-tempered pot designated as a cooking vessel (POT). During the early Shangshan phase, large basins are the most numerous vessel type, accounting for 70.6% of the pottery assemblage, followed by globular jars (28.2%), and the cups and bowls, together comprising about 1% (15) (*SI Appendix, Fig. S1:5*).

Two approaches were utilized for sherd analysis. The first approach entails extracting phytoliths from 1) the interior surface of each sherd to examine plant remains and 2) plant-tempered pottery clay to analyze plant material inclusions, with a specific focus on rice. The presence of rice bulliform phytoliths in the clay provides a potential avenue for evaluating the extent of rice domestication. Moreover, scrutinizing phytoliths found in the vessel temperers can offer insights into the types of plants collected from various vegetation environments, including rice, for pottery production.

The second approach involves extracting residues from the interior surface of each sherd to examine food remains, including starch and fungi. The brewing method for making rice-based beer involved preparing a *jiuqu* or *qu* starter compound containing primarily rice, molds, and yeasts. Subsequently, the *qu* was mixed with steamed rice and other starchy plants for fermentation (11, 19). This brewing tradition has been practiced for thousands of years and is still the most commonly employed fermentation method today in China (23). Unlike use of enzymes drawn from malt in western brewing practices, in Chinese fermentation, the needed enzymes are produced from a diverse community of microorganisms that make up the *qu* starter. These key microorganisms include molds (such as *Monascus*, *Aspergillus*, and *Rhizopus*), which are essential for producing amylases, proteases, and other enzymes that break down starches and proteins into fermentable sugars and amino acids, yeasts, lactic acid bacteria, and other bacteria and fungi (24–26). Based on our fermentation experiments and ethnographic observations, *qu* is typically crafted from raw or semicooked grains. This method results in numerous starch granules remaining recognizable in form but often displaying damage from enzymatic digestion and mild gelatinization. Conversely, the grains employed in subsequent brewing undergo cooking, leading to the extensive gelatinization of starch, often to the point of being unrecognizable and challenging to identify (27).

To overcome this challenge, we applied the trypan blue stain, as described by Barton (28), to identify damaged and gelatinized starches. Consequently, the confirmed identification of damaged and gelatinized starch, coupled with the presence of fungal particles on ceramic vessels, serves as valuable evidence for the remnants of fermented foods.

2. Results

2.1. Phytolith Remains. In total, 10,532 phytoliths were found in 21 distinct morphotypes, identifiable to the subfamily level. Those primarily include: bulliform with fish-scale facets from rice leaves and double-peaked glume cells from rice husk (Fig. 2 *A, B* and *G*), *Phragmites* bulliform (Fig. 2*C*), square saddle of Arundinoideae and tall saddle of Bambusoideae (Fig. 2 *H* and *I*), squat saddle of Eragrostoideae (Fig. 2*J*), rondel and trapeziform sinuate of Pooideae (Fig. 2 *L* and *N*), bilobate of Panicoideae (Fig. 2*K*), and phytoliths from woody plants (Fig. 2 *M, O* and *V*).

2.1.1. Phytoliths in pottery residues. In total, 4248 phytoliths were recovered from residues on 11 out of 12 sherds, except for the cooking vessel (POT). Of these phytoliths, 2704 (63.7% of the total) were classified into seven taxonomic types. The majority of phytoliths in the residues are spheroid ornate, constituting a percentage of 53.8% with a ubiquity of 91.7%. In particular, fermentation vessels and serving vessels generally exhibit a higher percentage of spheroid ornate phytolith compared to the large basins and cooking pot, reaching up to 100% and 93.8% in JAR3 and JAR5, respectively. The second phytolith type, derived from rice husks (3.7%), was present in nearly half of the pottery residue samples (ubiquity of 41.7%), but in minor proportions (0.2 to 2.10%) across most samples, except for JAR7 (33.5%) and BWL (23.1%), which were used specifically for fermentation and serving. Bulliform phytoliths from rice leaves have a relatively high ubiquity of 41.7% but are present in low quantities (0.3%). The mean percentage of rice bulliforms with ≥ 9 fish-scale facets in the CUP, JAR6, and JAR2 is 45.5% (56 out of 123) (Fig. 3 and *SI Appendix, Table S2*; bulliform identification method, *SI Appendix*).

2.1.2. Phytoliths in pottery clay. In total, 4672 phytoliths were recovered from pottery clay, with 1309 (28.0%) identifiable into specific taxonomic categories. The most prevalent phytolith types are those from Panicoideae, constituting a percentage of 7.8%, with ubiquity of 75.0%, followed by rice husk (6.7% in percentage), common bulliform (6.4%), woody (2.0%), and Pooideae (2.0%). Notably, the content of spheroid ornate, which dominates in pottery residues, is absent in pottery clay. Among rice phytoliths of bulliform and husk types, the ubiquity level is as high as 66.7% and 41.7%, respectively. The proportion of husk type is also extremely high in JAR3 (61.8%), but lower in BWL (14.3%), and trivial in the rest of pottery clays (only 1.1%). By contrast, rice bulliforms show extremely low content in all clay samples analyzed, present only at 0.3% (Fig. 3 and *SI Appendix, Table S2*).

In the two soil control samples (CONT1 and CONT2), 1612 phytoliths were recovered. These phytolith assemblages exhibit significant differences from those of pottery residues but show similarities with pottery clay samples (Fig. 3), being characterized predominantly by common bulliforms, with rice phytoliths as secondary constituents, while spheroid ornate is absent (Fig. 3). Specifically, the percentage of rice bulliforms is 4.2% in CONT1 and 2.8% in CONT2, while rice husks account for 0.7% and 3.3%, respectively. The percentages of rice bulliforms with ≥ 9 fish-scale facets are 46.0% (23 out of 50) in CONT1 from the early Shangshan and 53.8% (28 out of 52) in CONT2 from the middle Shangshan.

2.1.3. Spheroid ornate. We compared spheroid ornate with modern species from the *Quercus* and Cyperaceae, using similar morphological types from the phytolith database (<https://www.phytocore.org>). We found significant morphological and dimensional similarities between the spheroid ornate in our samples and those associated with *Quercus* bark (Fig. 2 *O* and *V* and *SI Appendix, Fig. S4*). Both exhibited spherical or near-spherical shapes with ornate surface, and the diameters were consistent: 10.7 ± 3.1 ($n = 103$) in archaeological samples and 11.0 ± 3.5 ($n = 50$) in modern *Quercus* plants.

2.2. Starch Remains. All residue samples exhibited varying levels of starch content ($n = 59$ to 130). A total of 1,063 grains were recovered, with 649 (61.1% of the total) identified in six types corresponding to specific taxonomy (*SI Appendix, Table S3*). Identification criteria were based on our experimental study and prior research (27, 29–31).

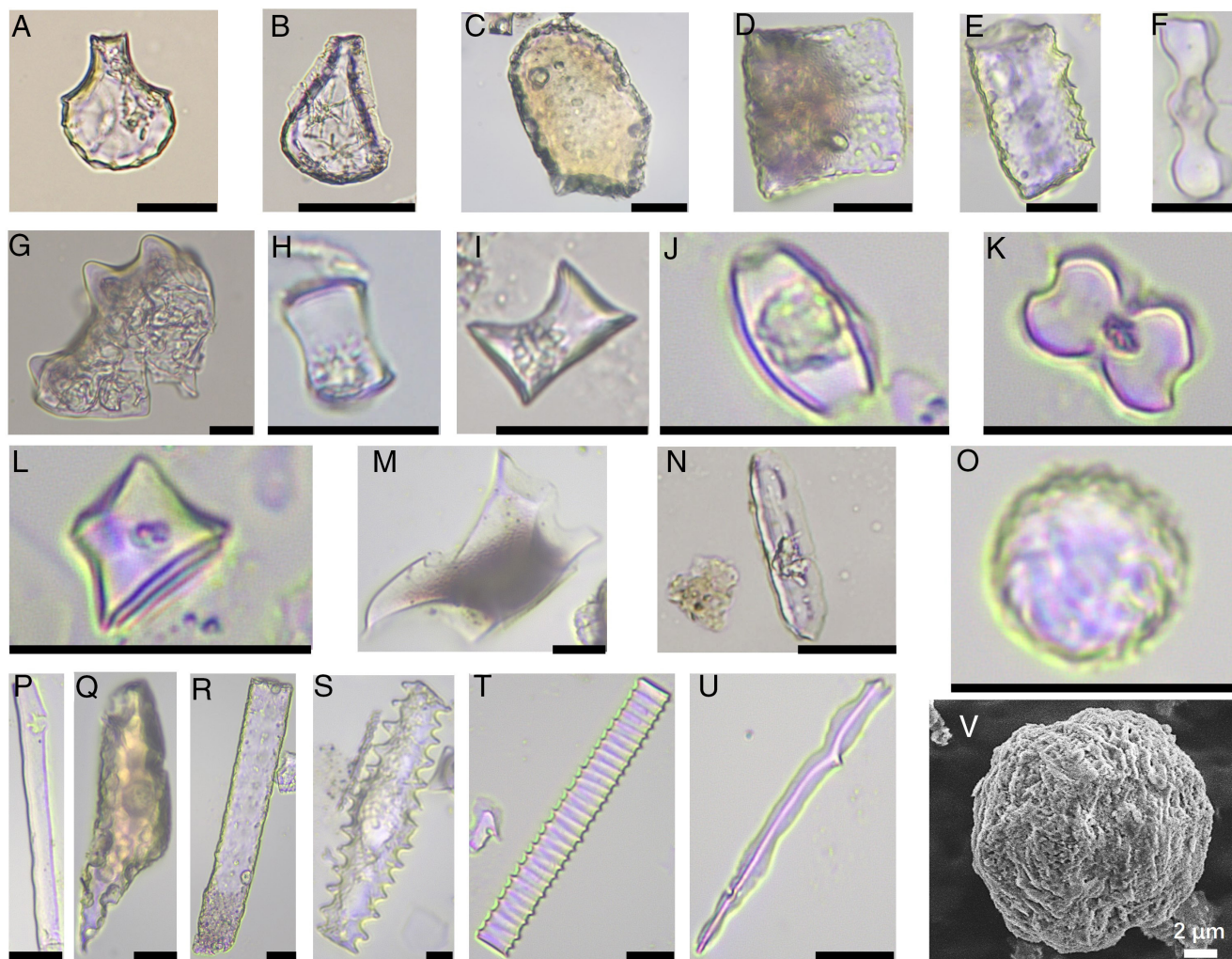


Fig. 2. Common phytolith types identified from samples of the Shangshan Culture. (A) Domesticated rice bulliform. (B) Wild rice bulliform. (C) *Phragmites* bulliform. (D) Square (bulliform). (E) Rectangular (bulliform). (F) Polylobate. (G) Rice husk. (H and I) Tall saddle. (J) Squat saddle. (K) Bilobate. (L) Rondel. (M) Gymnosperm. (N) Trapeziform sinuate. (O) Spheroid ornate. (P) Elongate cavate. (Q) Unciform hair cell. (R) Elongate smooth. (S) Elongate echinate. (T) Vascular tissue. (U) Pteridophyte. Scale. (V) Scanning electron microscope of Spheroid ornate. The black scale bar, 20 μ m.

Most starches ($n = 927$; 87.2% of the total) exhibit various forms of damage, classified into two categories. 1) Damage attributed to enzymatic digestion ($n = 596$; 56.1% of the total), showing random pitting, deep channels, broken edges, central depression, and lacking extinction cross, but remaining weakly birefringent (Fig. 4:3,6,7,10). Such altered forms can result from various factors, including fermentation. Our experiment indicates that during the brewing process, most rice granules become blurry or disappear, and only a few remain recognizable (*SI Appendix, Fig. S2:1-4*). 2) Gelatinization due to high-heat cooking (steaming or boiling; $n = 331$; 31.1% of the total), characterized by evenly expanded surfaces, with starch grains tending to fuse into large masses (Fig. 4:13). Additionally, some starches ($n = 414$; 38.9% of the total) that are too damaged or gelatinized, or too lacking in any diagnostic features, are classified as unidentifiable (UNID) and gelatinized mass (*SI Appendix, Table S3*).

When the trypan blue staining method was applied to subsamples, more gelatinized starches were identified, while some displayed recognizable granules, resembling those found in rice-compound granules (Fig. 4:14,15). These alterations align with our reference database (*SI Appendix, Fig. S2:15,16*), as well as with samples from other experimental fermentation and cooking (27, 30, 31). Each starch type, along with detailed starch

counts, measurements, and morphological features are documented in *SI Appendix, Tables S3 and S4*.

Type I rice starches (*Oryza* sp.) were present in all 12 vessels, typically appearing in a compound form comprising multiple small granules, with some isolated single granules. A total of 436 rice compounds and single granules were recorded, and 812 granules were measured for size. Most individual granules exhibited blurry birefringence without an extinction cross, although a few displayed a clear extinction cross, indicative of fermented rice (Fig. 4:1-4, compared with *SI Appendix, Fig. S2:1-4*). The quantity of Type I starch is calculated from rice compounds and isolated individual granules, resulting in the highest count of all starch Types (41.0% of the total), with 100% ubiquity through the assemblage.

Type II Job's tears starches (*Coix lacryma-jobi*) were identified in eight vessels ($n = 59$; 5.6% of the total; 66.7% ubiquity). The size range and starch morphologies of Type II starches align with Job's tears (Fig. 4:5, compared with *SI Appendix, Fig. S2:5 and SI Appendix, Fig. S3*).

Type III Panicoideae starches were present in 11 vessels ($n = 28$; 2.6% of the total; 91.7% ubiquity). These granules, resembling barnyard grass (*Echinochloa* sp.), are small, circular, or polygonal, particularly consistent with *Echinochloa colona* in shape and size range (Fig. 4:6, compared with *SI Appendix, Fig. S2:6 and*

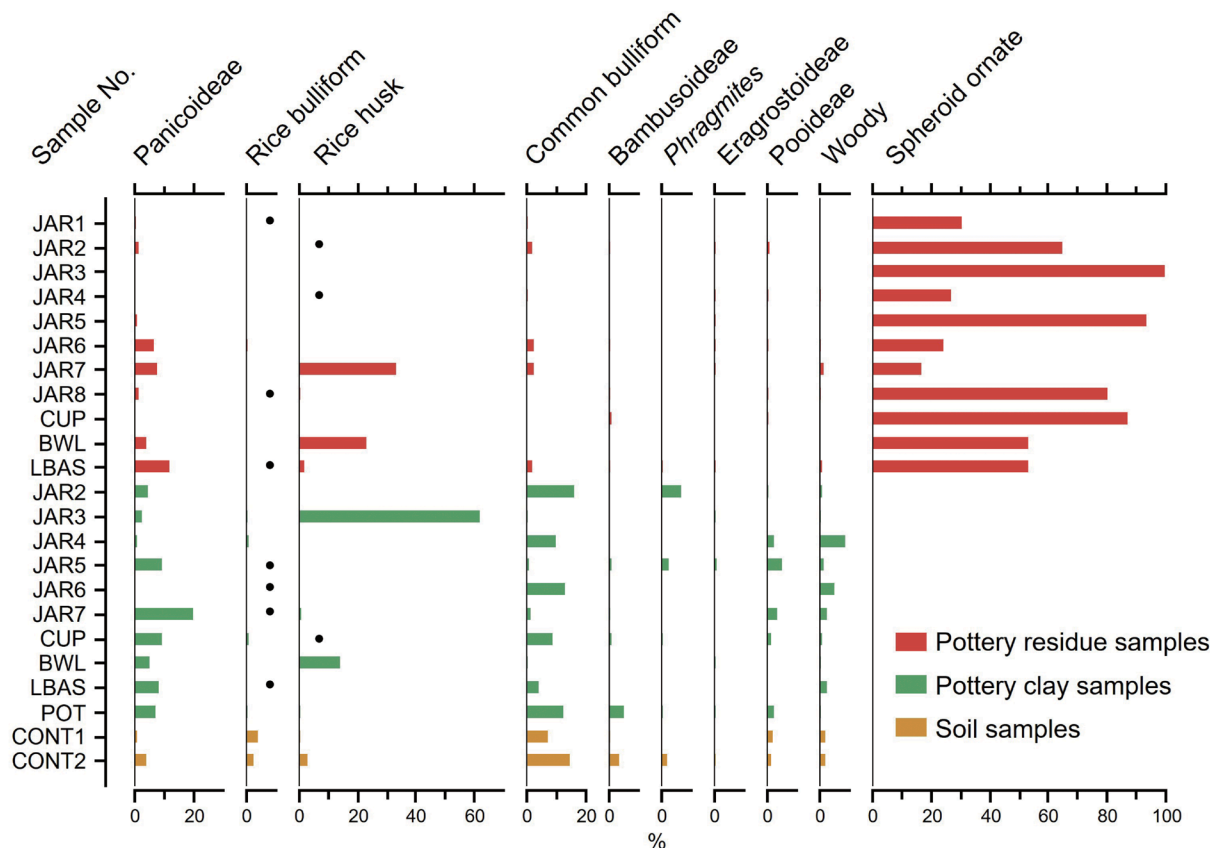


Fig. 3. Assemblages of major phytolith types identified from Shangshan samples. The black circle indicates that the percentage of phytoliths is 0.1 to 0.2%.

SI Appendix, Fig. S3). However, some Type III starches may potentially be small Job's tears granules, similar to barnyard grass in form. *E. colona*, a weed found in damp places, is widely distributed in warm regions throughout China (32:515-518).

Type IV, Triticeae wild grass starches were found in six vessels ($n = 10$; 0.9% of the total; 50.0% ubiquity), with some displaying enzymatic digestion (Fig. 4:7; compared with *SI Appendix, Fig. S2:7*).

Type V acorn starches, identified in 10 vessels (Fagaceae; $n = 111$; 10.4% of the total; 83.3% ubiquity), exhibit diverse forms and sizes comparable to various acorn species in our reference database, including *Cyclobalanopsis fleuryi*, *Lithocarpus brevicaudatus*, *Lithocarpus glaber*, and *Quercus fabri* (Fig. 4:8-11, compared with *SI Appendix, Fig. S2:8-11* and *SI Appendix, Fig. S3*). These oak species are prevalent in southern China, including Zhejiang (33).

Type VI lily starches were identified in three vessels (*Lilium* sp.; $n = 5$; 0.5% of the total; 25.0% ubiquity), and their shapes resemble starches from many lily species (Fig. 4:12 compared with *SI Appendix, Fig. S2:12*).

In summary, rice emerges as the most prevalent starch type in both piece count and ubiquity, followed by acorn, Job's tears, Panicoideae (mainly barnyard grass), Triticeae grass, and lily. The presence of diverse plants aligns with the broad-spectrum subsistence revealed by previous studies. The prevalence of rice suggests its incorporation into the regular human diet during the earliest phase at Shangshan. Additionally, rice starches in the globular jars and serving vessels exhibit high levels of enzymatic damage and gelatinization, consistent with the effects of alcohol fermentation.

2.3. Fungal Remains. All residue samples exhibited varying quantities of fungal particles ($n = 9$ to 103), with a total of 486 recorded, including hyphae, mycelia, cleistothecia, sporangia, and yeast cells (*SI Appendix, Table S5*). We identified fungal remains based on the morphological structures of these elements documented in

published materials (23:166-169, 34-38:154-156) and observed in our reference data (more detail in *SI Appendix*).

The most numerous elements are cleistothecia, consistent with genus *Monascus* mold ($n = 347$; 71.4 % of the total). *Monascus* produces a bright reddish color and has been used as fermentation starter, food pigment, and an ingredient in traditional Chinese medicine. *M. purpureus*, in particular, is known to have been included in the red rice *qu* starter used for brewing traditional rice-based red beer, often referred to as red rice wine or red wine (23:166-169, 39:192-203). In the Shangshan samples, *Monascus* cleistothecia are often fragmentary, but when their structures are intact, they exhibit various developmental phases. Some show the growth of ascogenous hyphae (Fig. 5:1), while others manifest the ascogenous development phase (Fig. 5:2); in some cases, red cleistothecia connect with hyphae (Fig. 5:3), while other cases contain asci and ascospores in mature phases (Fig. 5:4,5). Mycelia and septate hyphae, consistent with those from *Monascus*, were also found (Fig. 5:6,7). A few fungal sporangia ($n = 16$; 3.3% of the total) are present, but they are mostly unidentifiable taxonomically. Yeast cells are round or oval, some showing small protrusions on the parent cell, which resemble the budding process of yeast. These fungal elements match very well in morphology with those from *Monascus* mold and *Saccharomyces cerevisiae* yeast in our reference database (Fig. 5:11-17) and in abovementioned, published information.

In summary, fungal elements were found in all samples, but they were generally more numerous in the fermentation and serving vessels (jars, cup, and bowl) ($n = 25$ to 103) than in the large basin ($n = 21$) and the cooking pot ($n = 9$). The coexistence of *Monascus* mold elements and yeast cells, along with rice starch in the globular jars, indicates that a *qu* starter involving *Monascus* mold was most likely used for making rice-based beer.

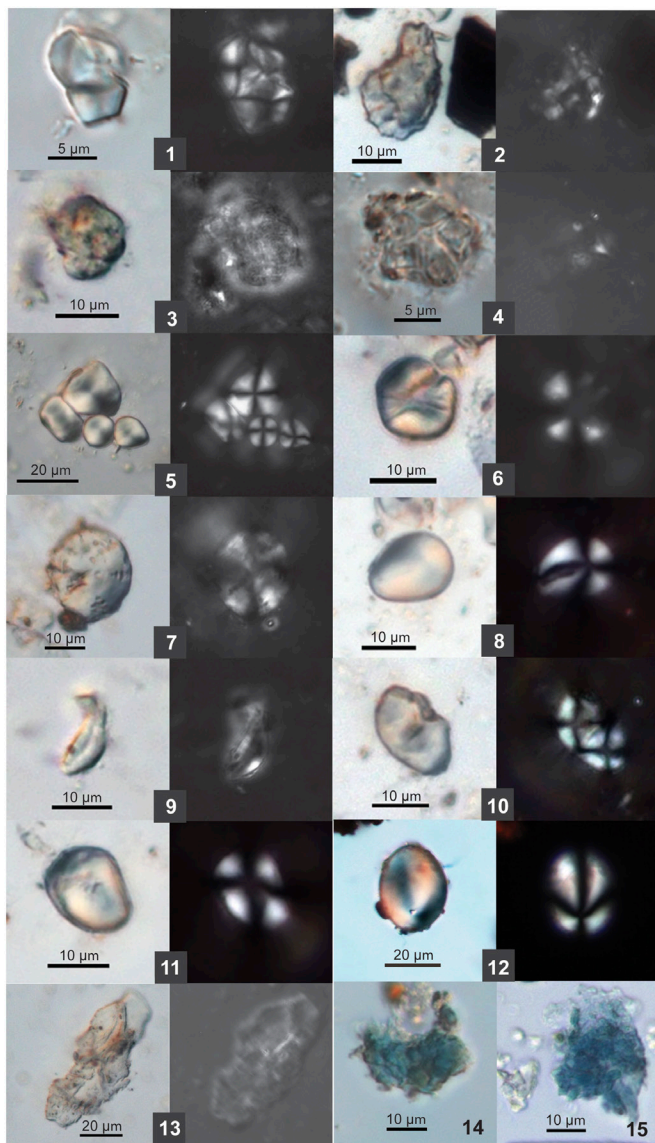


Fig. 4. Examples of starch types and damaged forms from Shangshan pottery. 1: Type I rice compound showing extinction crosses; 2: Type I rice compound exhibiting birefringence without crosses; 3: Type I rice compound showing weak birefringence; 4: Type I rice compound, granules becoming separated; 5: Type II Job's tears, showing various shapes; 6: Type III Panicoidae (likely barnyard), showing deep channels due to enzymatic digestion; 7: Type IV Triticeae, showing deep channels and micropitting due to enzymatic digestion; 8: Type V acorn, similar to *Cyclobalanopsis fleuryi*; 9: Type V acorn, similar to *Quercus fabri*; 10: Type V acorn compound, similar to *Lithocarpus glaber* or *C. fleuryi*, also showing missing part due to enzymatic digestion; 11: Type V acorn, similar to *Lithocarpus brevicaudatus*; 12: Type VI lily; 13: gelatinized starch mass; 14,15: gelatinized starches resembling rice compound granules, stained with trypan blue.

2.4. Control soil samples. CONT1 revealed two damaged starches and one mold, none of which could be unidentified taxonomically, while CONT2 contained two acorn starch granules. These findings differ significantly from those of the pottery residue samples, supporting the conclusion that starch contamination of pottery residues from the surrounding ancient soil deposits can be excluded.

3. Discussion

By integrating multiple lines of evidence from microfossil analysis, we can summarize the following observations and address the questions posed at the beginning of this paper.

3.1. The Rice Utilization in Early Shangshan Culture. Evidence of wild rice collection has been found at the Upper Paleolithic cave site at Diaotonghuan in the middle Yangzi River region, approximately 300 km west of Shangshan and dating to ca. 12,000/11,000 BP (40). In the Shangshan culture area, the distribution of wild rice can be traced back to 100,000 BP, with predomestication cultivation starting ca. 13,000 BP, leading to the domestication phase by 11,000 BP (4). Our evidence indicates that in the earliest stages of the Shangshan culture, the proportion of rice bulliform with ≥ 9 fish-scale facets had already reached $44.5 \pm 3.9\%$ on average (CUP, JAR2, JAR6, CONT1), surpassing the threshold for domestication (40%), and it further reached 53.8% (CONT2) by the middle Shangshan culture (Fig. 6).

The use of 40% as a threshold for rice domestication is based on comparative studies of modern domesticated and wild rice (3, 41). Domesticated rice typically exhibits a proportion of bulliform phytoliths with ≥ 9 fish-scale facets ranging from 40% to 67%, while wild rice shows a range of 4% to 33% (4) (Fig. 6). This threshold highlights a key morphological change associated with the leaf-rolling trait, which enhances photosynthesis and improves rice yield (43, 44). The transition from natural permanent wetlands to human-managed intermittent wetlands or paddy fields favored rice with this trait, increasing its competitiveness and likelihood of selection and inheritance (45).

The increased rate of rice domesticity observed in this study is consistent with previous analysis of sediment deposits and micro-CT observations of spikelet bases at Shangshan and Hehuashan (4, 7), together supporting the scenario that the Upper Paleolithic population in the middle-lower reaches of the Yangtze River had been gathering wild rice and then cultivating rice for several thousand years before the Shangshan culture, when rice had already become one of the well-established plant resources.

3.2. Implications of Rice Phytoliths in Pottery Clays. Through comparative analysis of phytolith assemblages between pottery clay, pottery interior-surface residues, and soil samples, significant differences were observed, thereby precluding the possibility of cross-contamination of phytoliths (Fig. 3). Furthermore, the presence of rice phytoliths in the pottery clay suggests that the incorporation of rice leaves and husks as admixtures into clay was a prevalent practice during that period.

It has long been recognized that the deliberate selection of traits, such as nonshattering spikelets, during the sowing of harvested seed stock, played a crucial role in the domestication of cereals. This practice, particularly evident in the Near East, has been supported by the analysis of usewear on stone sickles (46, 47). Similarly, the examination of usewear and residue on flaked stone tools from Shangshan has also provided evidence of the harvesting of rice stalks (9). In the present study, the finding of a substantial concentration of rice bulliform phytoliths, displaying domestication traits within pottery clay, not only hints at their supplementary use as a by-product in pottery vessel production but also strengthens the idea of rice stalk harvesting contributing to domestication.

Some vessels also exhibit notably high proportions of rice husk phytoliths (double-peaked glume cells) in the clay (up to 61.8% in JAR3), significantly exceeding the rice husk phytolith content found in the soil samples (0.7% and 3.3% in CONT1 and CONT2, respectively). The substantial presence of rice husks in pottery clay suggests that rice was extensively utilized as a botanical resource and that the chaffs leftover from rice consumption were also employed in ceramic production during the Early Shangshan Phase.

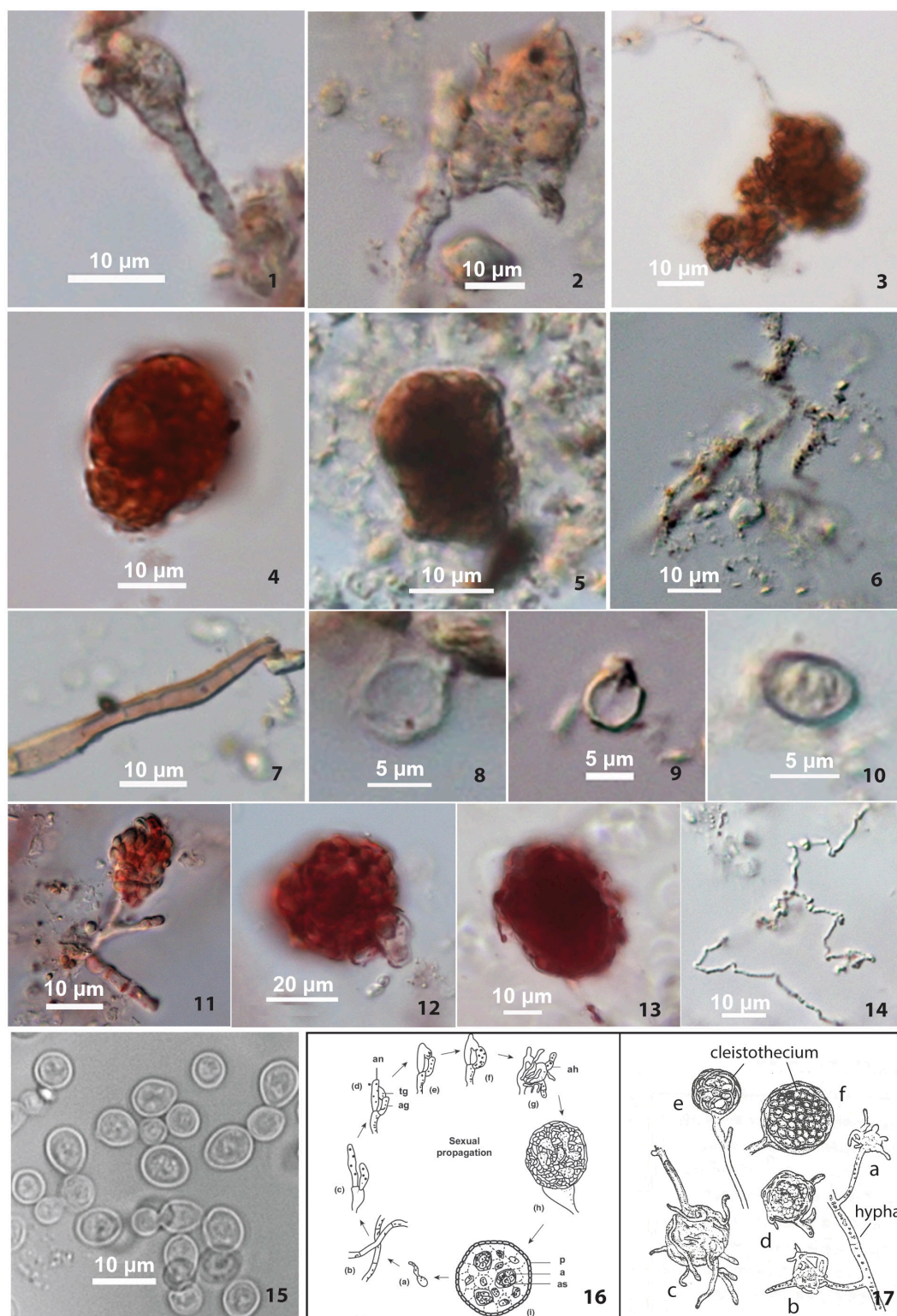


Fig. 5. Fungal elements from Shangshan pottery compared with modern *Monascus* mold. (1 to 10 Shangshan material). 1: Development of ascogenous hyphae, comparable with 16:d-f; 2: ascogenous development phase, comparable with 11 and 16:a; 3: cleistothecium connected with a hypha; 4,5: round and oval mature cleistothecia, showing numerous ascospores inside, compared with 12,13; 6: mycelium, compared with 14; 7: hypha, septate; 8,9: yeast cells with protrusions, probably in budding process; 10: oval shape yeast cell. (11 to 15 modern *Monascus* and yeast). 11: ascogenous development phase; 12,13: round and oval mature cleistothecia; 14: mycelium; 15: *Saccharomyces cerevisiae* yeast; 16: developmental stages of *Monascus* from ascospore forming vegetative hyphae to matured ascogonium (36: figure 2); 17: *M. purpureus* in various developmental phases, a-c: ascogenous; d, e: immature; f: mature (23: figure 4-6).

Notably, among the 12 pottery clay samples analyzed, three exhibit rice bulliforms and two show prominent rice double-peaked glume cells. Rice phytoliths in these six samples ($n = 328$) account for only 7.0% of total number of phytoliths ($n = 4,672$). In fact, diverse phytolith types were found in the pottery clays and residues, originating

from Panicoideae, Pooideae, reeds, bamboo, and bark of *Quercus*. Some of these phytoliths corresponded to identified starch types, such as Panicoideae and Triticeae. Based on current morphological observations, measurements, cross-referenced starch granule evidence, and previous studies confirming acorn use at the Shangshan site (17, 48),

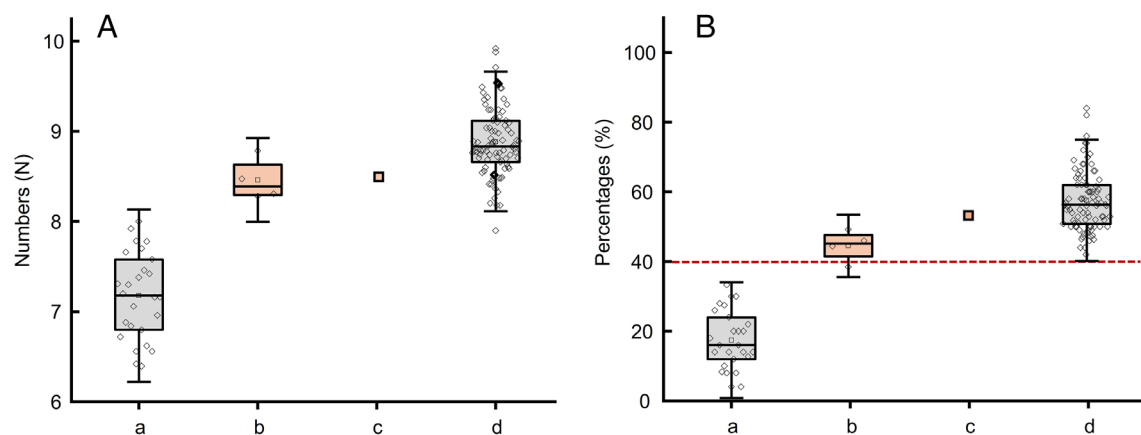


Fig. 6. Comparative analysis of rice bulliform fish-scale facet numbers among Shangshan and modern rice samples. (A) Comparisons of the numbers of rice bulliform fish-scale facets. (B) Comparisons of the percentages of rice bulliforms with ≥ 9 fish-scale facets. a: Modern wild rice field; b: early Shangshan samples (CUP, JAR2, JAR6, CONT1); c: middle Shangshan sample (CONT2); d: modern domesticated rice paddy. The red dashed line represents the domestication threshold as evaluated by rice phytoliths. The data for modern references are cited from refs. 41, 42.

it is likely that the spherical phytoliths are derived from the *Quercus* genus, though the specific use of its bark remains unclear. Together, these microfossil remains illustrate the extensive range of plants utilized by the Shangshan inhabitants for both food procurement and pottery production, reflecting a broad-spectrum subsistence economy that included rice cultivation.

3.3. Starch Taphonomy. Starch granules have frequently been recovered from deep archaeological sediments and ancient artifacts, with their age, preservation mechanisms, and links to human activity being subjects of research (29, 49–51). Composed of the glucose-based polysaccharides, amylose and amylopectin, starches are susceptible to degradation by fungal and bacterial enzymes. However, several environmental factors, such as very dry or cold conditions, compact sediment, encapsulation, and burial, contribute to their resistance to enzymatic digestion and long-term preservation (49, 52, 53).

In our study of food residues on pottery vessels, the protective effect of embedding in organic or inorganic material is particularly relevant, as it can shield starch from enzymatic degradation. Fermentation vessels often display compact residue layers on their interior surfaces due to repeated instances of brewing activity (54–56). This is consistent with ethnographic records showing that in many African communities, beer jars are not fully washed after each use, so as to preserve beneficial microorganisms, like yeasts, for future brewing (57, 58). This taphonomic process helps preserve microorganisms in fermentation vessels.

3.4. Pottery Vessels and Foodways. The distribution of microfossil remains in the vessels, combined with the information about vessel types, sheds light on the vessel functions and culinary patterns as follows.

Three types of microfossil remains coexist in all 12 vessels. These include starches exhibiting characteristics of enzymatic damage, gelatinized starch masses caused by cooking, and fungal particles consisting mostly of *Monascus* mold and yeast. These observations indicate that all vessels were in contact with cooked and fermented foods, likely involving various processing methods.

When comparing the microfossil remains among the vessels, we find that fermentation vessels and serving vessels (globular jars, cup, and bowl) generally exhibit a higher quantity of rice starch, phytoliths from rice husks, and fungal particles than the large basin and cooking pot (Fig. 7), suggesting that these vessels were used for processing or serving rice-based food, among other

culinary materials. Many globular jars were likely used as fermentation containers, while the cups and bowls may have been used for serving fermented beverages. Notably, although simple-formed pottery vessels first appeared in southern China around 20,000 y ago (59), these three Shangshan types represent newly emerged pottery forms in early Holocene of East Asia, and their association with fermented beverages highlights their importance in functions likely related to communal feasting activities.

The large basin revealed an elevated number of acorn starches, along with rice, other cereals, *Monascus* mold, and gelatinized starches (Fig. 7). Previous studies have proposed several possible functions for these vessels, including processing acorn meal (16) and stone boiling cooking (60). Our analysis indicates that the examined basin was likely multifunctional; it was used for processing acorn meals as well as serving cooked foods, possibly including solid food associated with fermented rice. This scenario finds its parallel at the Xiaohuangshan site, where a plate has revealed evidence of fermented foods, likely in solid form (11). Interestingly, cooking meat with the dregs of red rice beer has been a long culinary tradition in China, as recorded in ancient texts (61). Therefore, not only brewing rice-based beer but also cooking solid foods with the rice beer dregs may have been practiced 10,000 y ago at Shangshan. Nevertheless, whether some large basins at Shangshan also functioned as stone-boiling containers remains to be investigated in the future.

The cooking pot revealed an elevated level of gelatinized starches and diverse starchy foods (rice, *Panicoideae*, and acorn), along with *Monascus* molds, suggesting its use for cooking a variety of foods, including fermented rice. The low quantity of recognizable starch grains is likely due to severe gelatinization caused by boiling. Additionally, the absence of phytoliths in the cooking pot indicates that the contents within the vessel underwent a thorough dehulling treatment, further substantiating its use for culinary purposes (Fig. 7).

To summarize, at the beginning of the Shangshan culture, rice was not only consumed as a daily dietary food but also used for brewing fermented beverages, involving the preparation of a *qu* starter containing *Monascus* mold and yeast as fermentation agents. The fermentation ingredients consisted mainly of rice and were supplemented with other cereals (Job's tears, *Panicoideae* such as barnyard grass, and *Triticeae*), acorn nuts, and lily. This rice-fungi-based multiplant brewing method marked the first known alcoholic fermentation technique in East Asia.

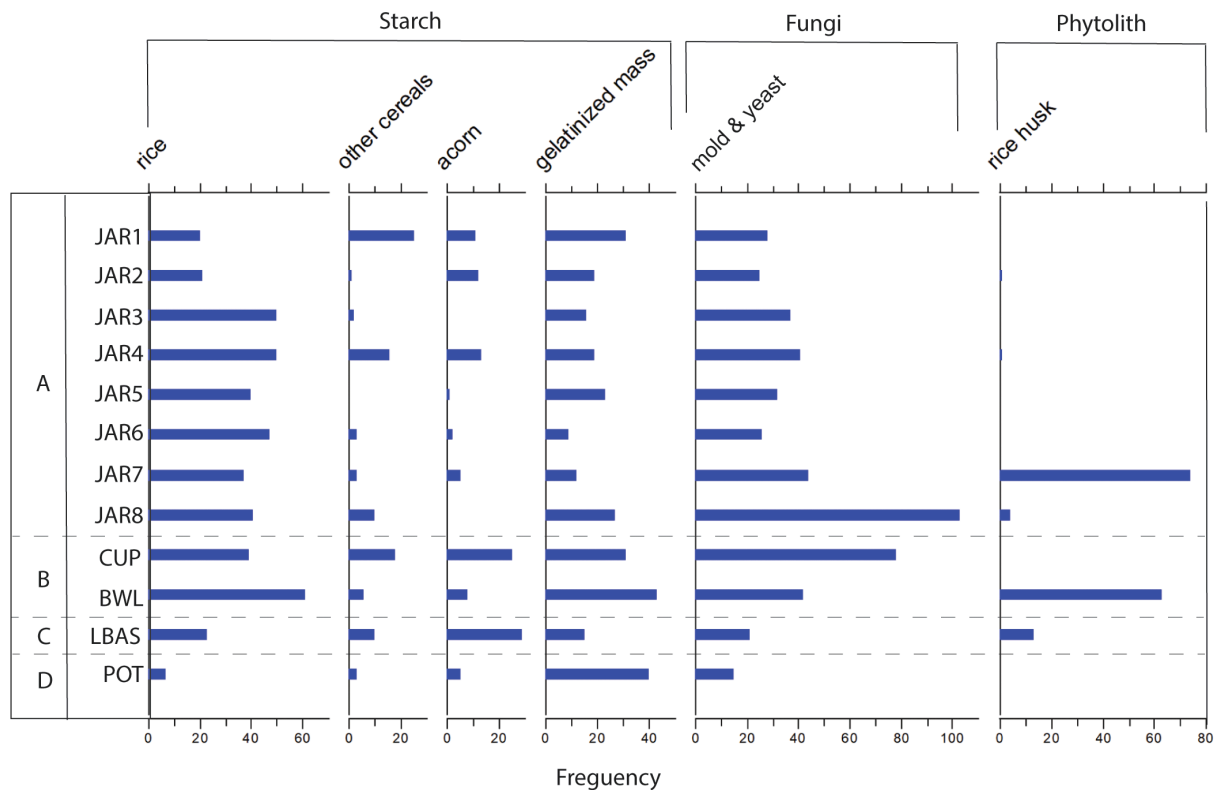


Fig. 7. Comparison of starch, fungi, and rice husk phytoliths among different pottery types. (A) Fermentation vessels. (B) serving vessels. (C) food processing vessel. (D) cooking vessel.

3.5. Rice Beer Making. Pottery clay samples manifesting a notable number of rice bulliforms do not always coincide with other pottery clay samples that show significant presence of rice husk phytoliths (Fig. 3), suggesting that the vessels concerned may have been crafted during different stages of the process involving rice—one type to utilize harvested rice leaves and another type to employ rice chaffs. The act of harvesting rice stalks evidently played a central role in domestication, suggesting that humans deliberately selected specific types of rice or rice cultivated in certain areas. Moreover, rice served as the primary ingredient for brewing beer, indicating a close association between cultivation of rice and production of rice-based alcoholic beverages.

The Shangshan site unveiled 183 pits, several of which contained a collection of vessels, including basins and jars arranged in clusters, likely interred as caches (*SI Appendix*, Fig. S1:4). Similar features have been documented at other Shangshan culture sites, such as Qiaotou and Xiaohuangshan, where pottery jars and plates have been determined to be linked with fermented foods and potentially utilized for ceremonial feasting activities, including mortuary rituals near human burials (11, 19). Our analysis encompasses cups, bowls, and large basins within the inventory of vessels used for fermented foods. Considering that the functions of many such pottery vessels are related to fermented beverages, as well as their deliberate placement in caches, it is plausible that these are remnants of materials associated with ceremonial feasting activities.

To summarize, the food practices of the early Shangshan community can be depicted as a complex interplay between natural resources and human activities. Various botanical resources were procured and processed not only for food consumption but also for pottery manufacture. The production of different forms of pottery vessels and the brewing of rice-based fermented beverages emerged simultaneously, forming interdependent activities that supported early Neolithic lifeways for both subsistence requirements and ritual needs (Fig. 8).

3.6. Origin of Alcoholic Fermentation and Spreading of Rice Cultivation. The current evidence suggests that the brewing of rice beer probably first emerged in the Lower Yangzi River region during the early Holocene, a period when rice domestication was already underway. During the early Holocene, the East Asian summer monsoon was significantly strengthened (62), leading to a period of relatively higher temperatures and increased precipitation (63). Precipitation levels are estimated to have been about 30% higher than modern values (64). The warm and humid climate was conducive not only to increase of human populations and emergence of domesticated rice (4) but also to the flourishing of fungi. These cultural and natural conditions set the foundation for development of alcohol brewing. Creating a *qu* fermentation starter requires an environment with high humidity and warm temperatures for molds to grow on cereals (25), and availability of rice appears to be the natural matrix for *Monascus* mold to grow.

These conditions may have led to a natural fermentation process, at first unintentionally resulting from moldy leftover rice, a scenario first suggested by Jiang Tong (fourth century AD) in his book “Jiugao.” A similar method of using moldy leftover rice or millet to make *qu* starter has been observed among the aboriginal people of Taiwan (65). When early Shangshan people noticed the results of this natural fermentation and experienced the psychoactive effects of alcohol, they plausibly replicated the process and increased production by using pottery vessels.

Alcohol-related vessels have frequently been found in ritual contexts, such as burials and communal gathering locations, underscoring the ritual significance of alcoholic beverages. This tradition was not only commonly practiced in the Shangshan culture area but also expanded to the Yellow River regions by 9,000 to 8,000 cal. BP, as exemplified by the burial patterns of the Jiahu-Peiligang early Neolithic culture (12). This phenomenon suggests that the ritual function of alcoholic beverages may have been a driving factor that stimulated the intensive utilization and widespread cultivation of rice in Neolithic China.

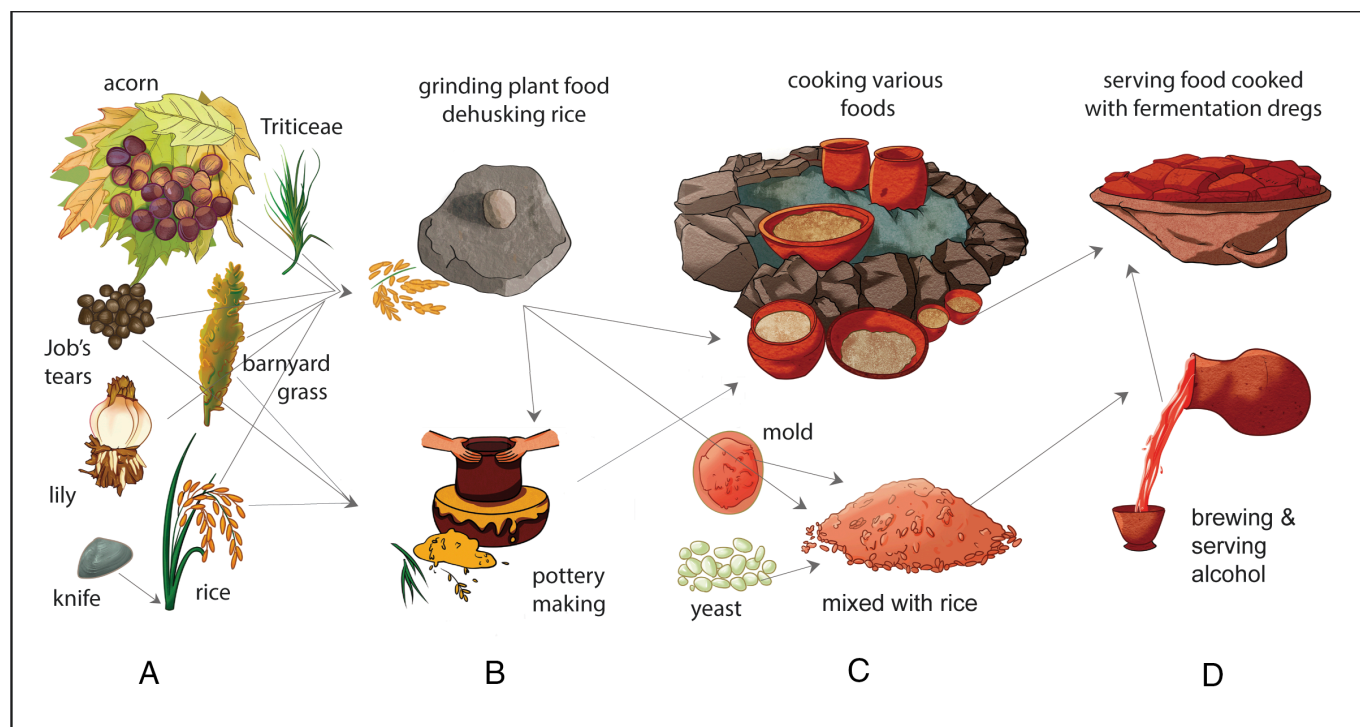


Fig. 8. Reconstruction of the production-consumption process involving pottery and food in early Shangshan culture. (A) Plant procurement: collecting wild plants and harvesting rice. (B) plant processing: for food and pottery making. (C) food processing: cooking food and making *qu* starter with molds, yeast, and rice. (D) food consumption: eating and drinking.

4. Conclusion

Microfossil analyses conducted in this study have unveiled multiple strands of evidence from starch, phytolith, and fungal particles present on pottery vessels, indicating extensive utilization of rice during the early Neolithic era in the Lower Yangtze River region. During the Early Shangshan Phase, ca. 10,000 to 9,000 y ago, while rice underwent in-depth exploitation, diverse types of pottery vessels were produced, facilitating the first experiments with brewing fermented beverages. In addition to being consumed as a staple food source, rice is likely served as the principal ingredient, along with *Monascus* mold, yeast, and other starchy plants, in crafting *qu*-based fermented beverages. These alcoholic beverages probably played a pivotal role in various ritual feasting activities and, along with other technological advances, contributed to the formation of new lifeways, marking the beginning of the Neolithic era in East Asia.

5. Methods

For starch and fungal analyses, the residue samples were processed for microfossil analyses with standard protocols described in previous publications (66). This involved two procedures: 1) EDTA (ethylenediaminetetraacetic acid; 0.1%) dispersion to release microparticles from small sediment microaggregates; and 2) SPT heavy liquid (sodium polytungstate, density 2.35) separation to extract microbotanical remains (more details in *SI Appendix*).

A wet oxidation method was used for extracting phytoliths from residue and pottery clay, as well as from sediment control samples (67) (more details in *SI Appendix*). A median of 300 phytolith grains were identified and counted in

each sample and recorded according to published references and criteria (67, 68). Specific phytolith content levels are expressed as a percentage of all phytoliths counted in each sample (more details in *SI Appendix*).

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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The authors declare no competing interest.

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