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Early Neolithic human exploitation and processing of plant foods in the Lower Yangtze River, China

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ABSTRACT

The Xiaohuangshan site is an early Neolithic settlement in the lower Yangzi River dating to 9000–7000 BP. In this paper, starch grains extracted from the surfaces of 9 pottery sherds and 6 grinding slabs unearthed from the early Xiaohuangshan phase (9000–8500 cal. BP) are identified from *Oryza*, Triticeae Tribe, *Coix lacryma-jobi*, *Nelumbo* sp., *Dioscorea opposita Trunb*, *Vigna* and *Quercus* spp. Based on the statistical analysis of percentage presence and the minimum numbers of plants identified by starch grains on the grinding slabs and potteries, it can be found that rice has become one of the major staple foods together with Triticeae grasses, Job's tears and acorns, which indicates rice should have been cultivated intentionally at Xiaohuangshan. The results also suggest that grinding slabs from Xiaohuangshan were used mainly for processing wild plants other than rice, and plant residues extracted from their surfaces cannot be utilized independently to explore human's subsistence strategies in that time. In addition, acorns probably were cooked whole in the pottery vessels before they were shelled or ground on the grinding slabs in the Xiaohuangshan site. This paper provides new evidence for the beginning of rice cultivation during the early Neolithic in the Lower Yangtze River.

1. Introduction

The Lower Yangtze River was one of the major centres for the origin of rice agriculture in the world. In recent years, along with excavations of several early and middle Neolithic sites such as Shangshan (c. 11,000–9000 cal. BP), Xiaohuangshan (c. 9000–7000 cal. BP), Kuahuqiao (c. 8000–7000 cal. BP) and Tianluoshan (c. 7000–5500 cal. BP), a large quantity of plant remains have been recovered including abundant rice remains in this region. These findings have caused great concern in the academic community and provided new material for investigating the origin of rice exploitation and domestication in the Lower Yangtze River (Jiang and Liu, 2006; Liu, 2006; Fuller et al., 2007; Liu et al., 2007; Zong et al., 2007; Fuller et al., 2009; Zheng, 2009; Nakamura, 2010; Wang et al., 2010; Zhao, 2010; Crawford, 2012). Evidence from morphological trend analysis of rice phytolith show that rice domestication occurred as early as in the Shangshan site 11,000 BP (Wu et al., 2014), and some scholars have speculated that rice consumption must have been very high and was a primary diet resource at the time based on the studies of rice husk impressions remaining in pottery bodies (Zheng and Jiang, 2007). To investigate human subsistence strategies in the early Neolithic Lower Yangtze River, Liu and her colleagues selected six grinding stones and one flaked tool from the Shangshan and Xiaohuangshan sites for plant residue analysis. However, apart from starches from acorns, tubers, Job's tears and possible water caltrop, no starch grains were identified from rice. Therefore, their paper argued that rice in Shangshan and Xiaohuangshan, most likely wild, was unlikely to have been a primary focus of human subsistence activities (Liu et al., 2010). It is worth noting that grinding stones were regarded as important stone tools used mainly for processing wild plant food resources rather than cultivated rice or millet in the early and middle Neolithic China (Liu et al., 2010; Yang et al., 2009a, b). In this situation, plant residues from grinding stones cannot be used to determine the extent of rice exploitation in the early and middle Neolithic China. To explore the dietary importance of rice and other wild plant foods during the early Neolithic in the Lower Yangtze River, in this paper, 9 pottery bottom sherds and another 6 grinding slabs from the Xiaohuangshan site were examined for starch residue analysis, statistical analysis of percentage presence and the minimum numbers of plants identified by starch grains were also adopted to quantitatively analyse the experimental results.

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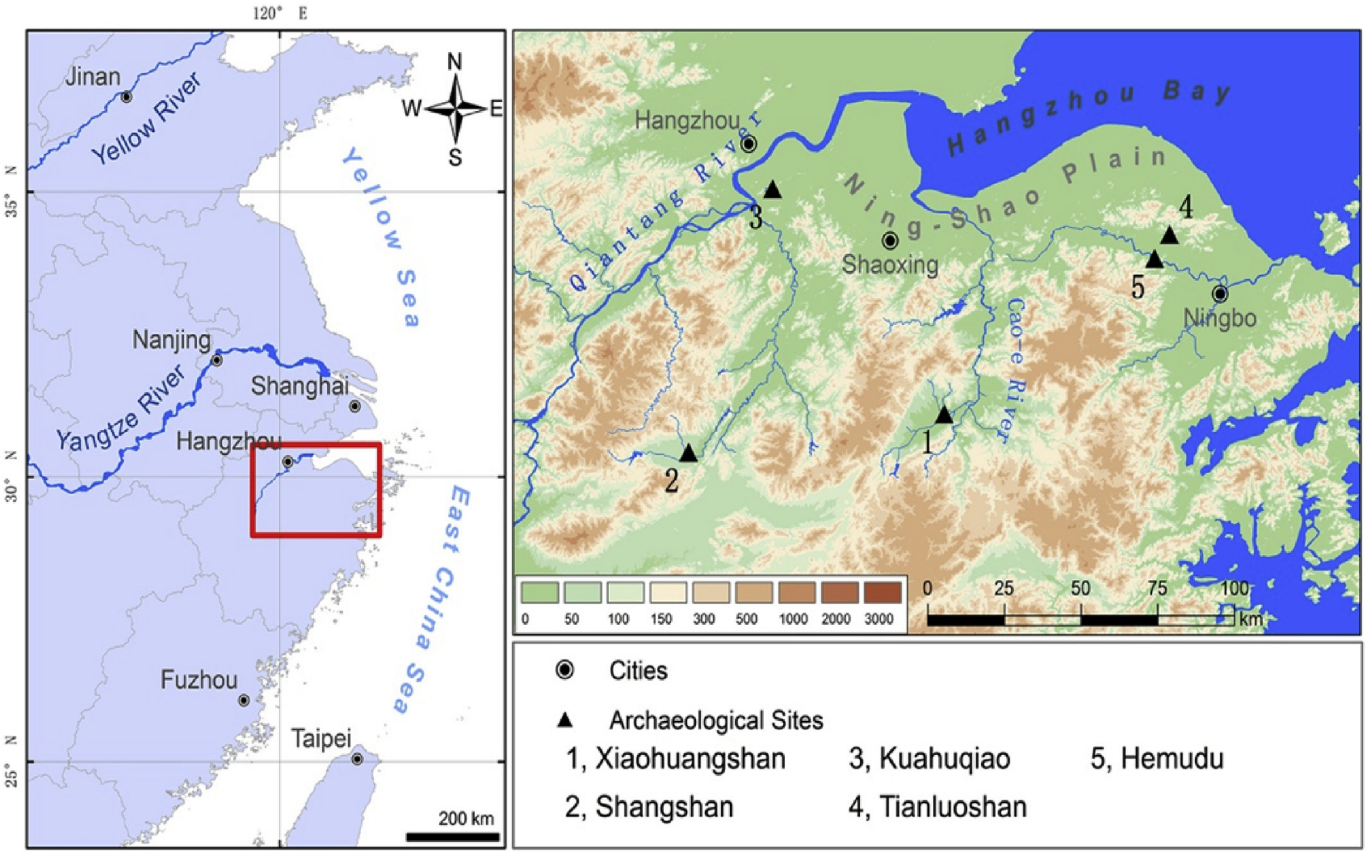

Fig. 1. Geographical location of Xiaohuangshan and other related sites in this paper.

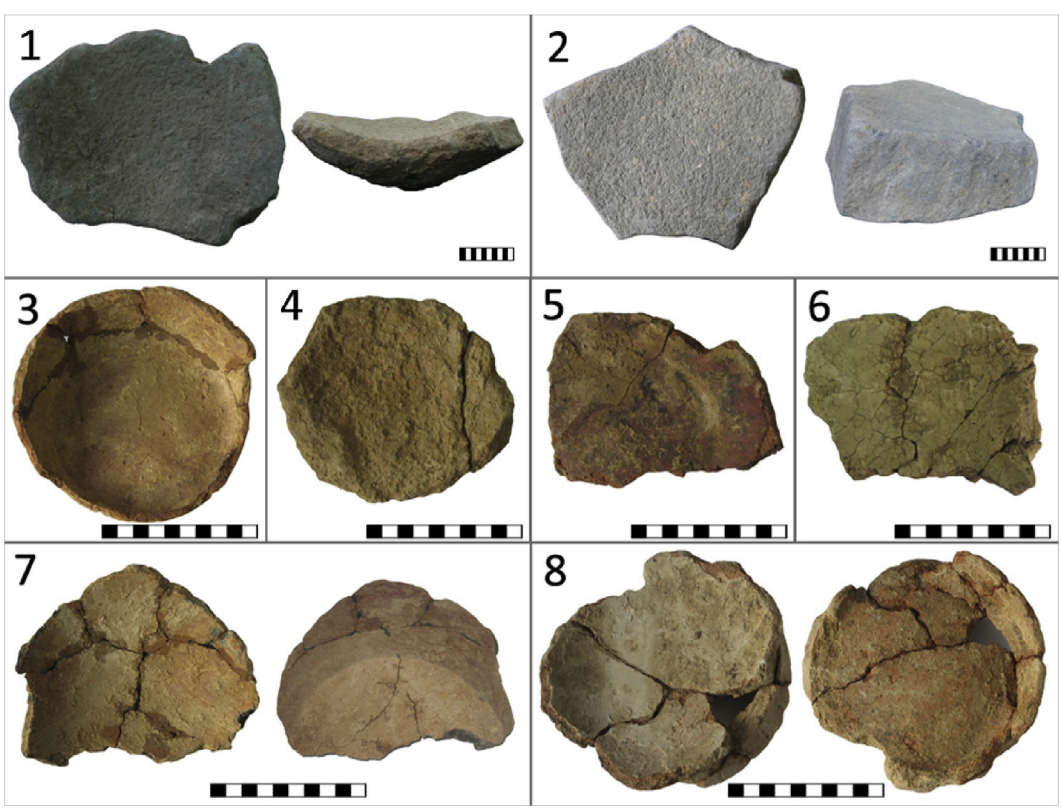

Fig. 2. Part of unearthed artifacts from Xiaohuangshan site for the residue analysis 1. 05XHSAG6①:17; 2. 05XHSAH81:4; 3. 05XHSAT0805⑧:1; 4. XHSAT0402G6:2 5. XHSAT0402G6:4; 6. XHSAT0402G6:5; 7. 05XHSAT0805⑧:2; 8. XHSBT8⑧:1 (Scale bar: 10 cm).

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2. Materials and methods

The Xiaohuangshan site (29°35′15′Ń, 120°43′18′É) is located at Shangdushan village, approximately 100 km southeast of Hangzhou City, Zhejiang Province (Fig. 1). The site was excavated from March 2005 through January 2007 by the Zhejiang Provincial Institute of Cultural Relics and Archaeology, and nearly 5000 m^2 village occupations were uncovered. During the excavations, many ditches, house foundations, pits, burials and a large amount of stone tools and pottery sherds were recovered. Eight ^{14}C dates from different strata place the site occupation to 9000–7700 cal. BP (Zhang et al., 2005). The deposits of the Xiaohuangshan site can be divided into three strata: lower, middle and upper (Liu et al., 2010). In this paper, nine sand-tempered pottery fragments and six grinding slabs unearthed from lower stratum were selected for starch grain analysis (Fig. 2).

Sampling procedure and protocol: (i) Wash the surface of artifacts by application of pure water twice in order to eliminate the contamination during burial and excavation (Hart, 2011). (ii) Use "Ultrasonex" ultrasonic toothbrush with 1.6 MHz to brush area of interest. (iii) Collect residue samples into a clean plastic tube by pipette tip. To prevent contamination, soil and samples from unused surface are collected. All supplies were disposable and boiled in water at 100 °C for 30 min.

Extract starch from samples: (i) Use 6% H_2O_2 to remove organic impurities followed by anti-flocculation processing in 5% Calgon. (ii) dissolve minerals with 10% HCl solution. (iii) The starch grains were extracted by heavy liquid (CsCl) with a specific gravity of 1.9. (iv) The supernatant is transferred and diluted with pure water, centrifuged at 2500 rpm for 10 min to remove heavy liquid. Starch samples were mounted on microscope slides with25% Glycerol. In order to prevent pollution, blank control trial was conducted in the experiment.

3. Results

3.1. Identification of starch grains

A total of 779 starch grains were recovered. According to grain size, morphological characteristics and extinction cross shapes, all these starch grains could be classified into 7 types (A—F) (Table 1). No starch grains were found in the samples of soil and unused surface, indicating that ancient starches were associated with tool use.

Table 1
Ancient starch grains extracted from the surface of unearthed artifacts in Xiaohuangshan site

Serial number	Category										
	Artifacts	Type A		Type B	Type C	Type D			Type E	Type F	Total
		AI	AII			DI	DII	DIII			
05XHSA0904H51:1	slab			3	8						11
05XHSA0904H51:2	slab	4		7					1	3	15
05XHSAG4①:16	slab			12	16	2					30
05XHSAG6①:17	slab			2	8					3	13
05XHSAH81:4	slab			153	56			1		6	216
05XHSAT1005⑧:2	slab			3	15	1				4	23
05XHSAT0805⑧:1	jar		10	26	80	4	1			1	122
05XHSAT0805⑧:2	jar		5	11	44						60
XHSBT8⑤:1	circle foot basin		14	14	12				1		41
XHSAT0402G6:1	flat-bottomed vessel			1	4			1			6
XHSAT0402G6:2	round-bottomed vessel		20	37	59			6			122
XHSAT0402G6:3	circle foot basin			1	1						2
XHSAT0402G6:4	flat-bottomed vessel		10		6						16
XHSAT0402G6:5	flat-bottomed vessel		13	25	47	1		3			89
XHSAT0402G6:6	flat-bottomed vessel			6	5			2			13
Total		4	72	301	361	8	1	13	2	17	779

Type A (Fig. 3: a, a′, b, b′, c, c′) can be divided into two groups. Group AI is polyhedral or round polyhedral shape with centric hilum closed, lamellae invisible and the extinction cross is shaped as "X". Group AII is a kind of compound starch grain which is typical morphological characteristic of Oryzeae Tribe (Wei et al., 2008a, b, c) (Fig. 4: a, a′, b, b′). In rice endosperm cells, well developed starch grains are polyhedral with compact arrangement in amyloplast, and these starch grains kept the outline of amyloplast after amyloplast envelope degraded. Poorly developed starch grains are round polyhedral with loose arrangement in amyloplast, and these starch grains could not keep the outline of amyloplast after amyloplast envelope degraded (Wei et al., 2008a, b, c). Starch of Type AI and AII are derived from these two cases. In the result of micro measurement, the size of these starches (3.58–8.43 μm) is in ranging of *Oryza sativa and Oryz. rufipogon* Griff., the mean value of the grain size was slightly higher than that of the modern rice (Fig. 5: a). Evidence of spikelet and phytolith indicates that the domestication of rice had began from early Neolithic in the Xiaohuangshan site (Zheng, 2009; Wu et al., 2014). We conclude that Type A starch grains may be from the seeds of *Oryza* sp..

Type B (Fig. 3: d, d′, e, e′, f, f′) are a lenticular or discoid shape, with flat surface and centric hilum, lamellae is visible on large grains, and the extinction cross is shaped as either "x" and "+". Under the microscope, a lot of small starch grains can be observed attaching with the larger ones, this phenomenon is very common in Triticeae Tribe starch (Yang and Perry, 2013) (Fig. 4: c, c′). The ultrastructural observation shows that the large starch grains formed at the young stage of endosperm development by amyloplast constricting, and many small starch grains could form and develop in one amyloplast (Wei et al., 2008a, b, c). The starch grains of Type B (4.06–39.77 μm) and *Triticum aestivum* L. have a large overlapping in particle size, but the mean value (23.98 ± 7.99 μm) is higher (Fig. 5: b), some wild species such as *Aegilops* will be matched (Piperno et al., 2004). Furthermore, although no macrobotanic wheat remains dated to 4500 years ago have been recovered in China, starches from *Aegilops* and *Agropyron* have been reported in many Paleolithic sites (Liu et al., 2011; Guan et al., 2014).

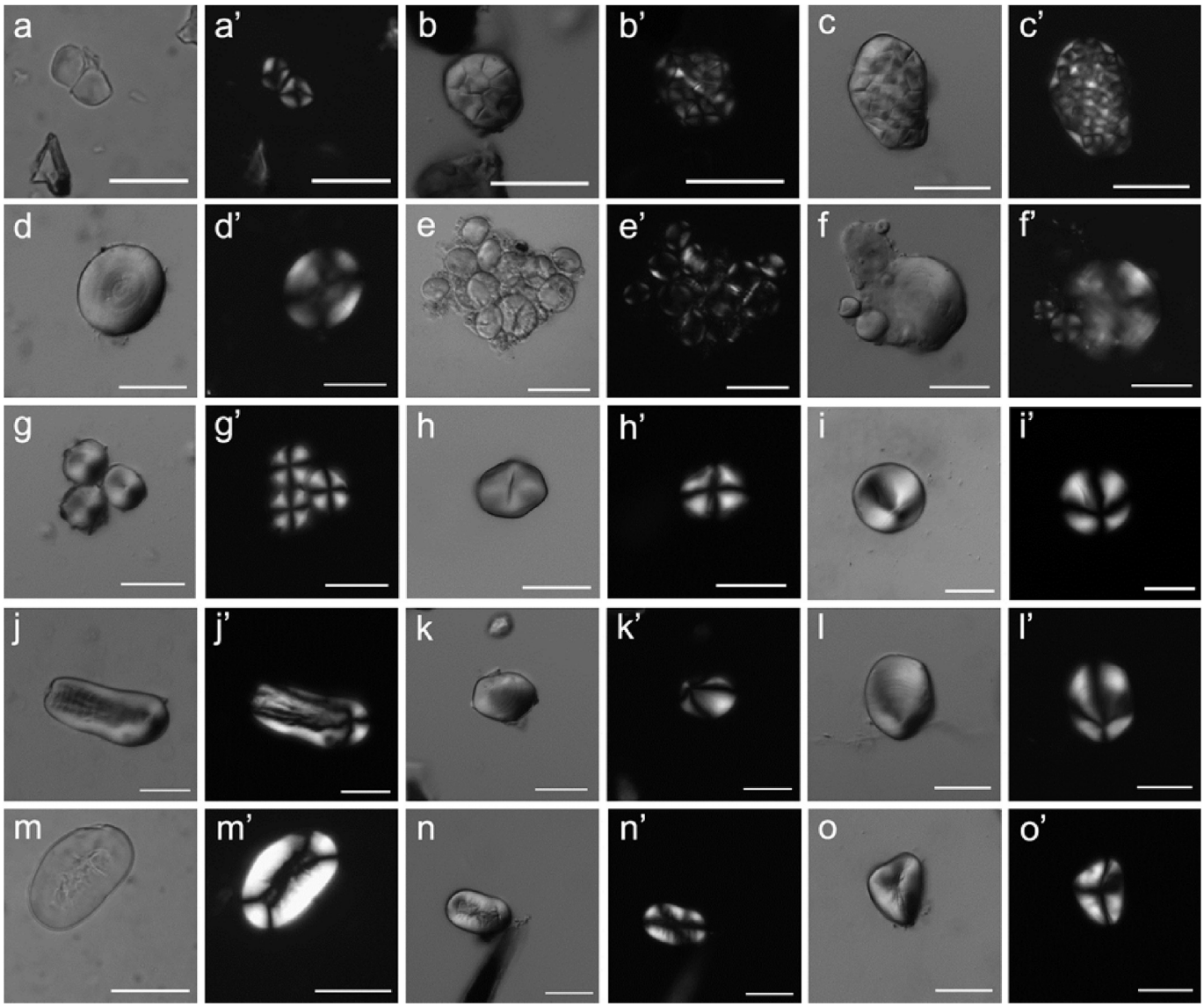

Fig. 3. Starch grains from residue samples in Xiaohuangshan Site(scale bar: 20 μm) Type AI(a, a′). Type AII(b, b′, c, c′). Type B(d, d′, e, e′, f, f′). Type C(g, g′, h, h′). Type DI(i, i′) Type DII(j, j′). Type DIII(k, k′, l, l′). Type E(m, m′, n, n′). Type F(o, o′).

It is concluded that starch of Type B may come from a wild species of Triticeae Tribe.

Type C (Fig. 3: g, g′, h, h′) are spherical or oval-spherical in shape, more or less polyhedral with two or three flat facets, centric hilum that normally have T- or linear-shaped fissures, Z-shaped arm on the extinction cross is the typical morphological characteristic of these starch grains. Compared to modern references, starch grains of Type C reveal a great similarity to *Coix lacryma-jobi* (Liu et al., 2014a, b) (Fig. 4: d, d′), which also shows considerable overlapping in size (Fig. 5: c). Starch grains of Job's tears are also found in the Shangshan site (11,400–8600 BP) (Liu et al., 2010) and the Kuahuqiao site (8000–7000 BP) (Yang and Jiang, 2010), which dates covered the Xiaohuangshan period. It is indicated that Type C starch grains may come from Job's tears.

Starch grains of Type D are extremely eccentric hilum and well-defined lamellae, which are generally considered from underground storage organs of geophytes. The major difference among these three types is the shape of grains. Type DI (Fig. 3: i, i′) is spherical with smaller size (11.85–28.39 μm, N = 8), while the Type DII (Fig. 3: j, j′) is elongated ovate with relatively large size (50 μm, N = 1). The combination of DI and DII is the typical characteristics of starch grains from the root of *Nelumbo nucifera* (4.38–65.66 μm, N = 144) (Wan et al., 2011) (Fig. 4: e, e′). The shape of Type DIII (Fig. 3: k, k′, l, l') starch is quadrilateral oval with the size (14.67–33.28 μm N = 13) matching to *Dioscorea opposita Trunb* (12.53–53.36 μm, N = 186) (Wan et al., 2011) (Fig. 4: f, f′). These starch grains have been found in early Shangshan phase (Liu et al., 2010), so it is reasonable to conclude that Type DIII starches originate from the Chinese yam.

Type E (Fig. 3: m, m′, n, n′) is kidney-shaped, radiated fissures and lamellae are visible. And only two starch grains of this type have been recovered. The morphological characteristics match with starch from Phaseoleae (Liu et al., 2011). Comparing with modern reference of *Vigna* spp (Wang et al., 2013) (Fig. 4: g, g′), type E starch grains (28.22–31.82 μm, N = 2) are similar to *Vigna radiata* (7.09–35.01 μm, N = 187) in size. Starch of *Vigna* spp. has been found from charred residues adhering to pottery sherds at Kuahuqiao (Yang and Jiang, 2010). Due to the limited number of starch grains, Type E may come from the species of Phaseoleae, among which *Vigna* spp. presents the best possibilities.

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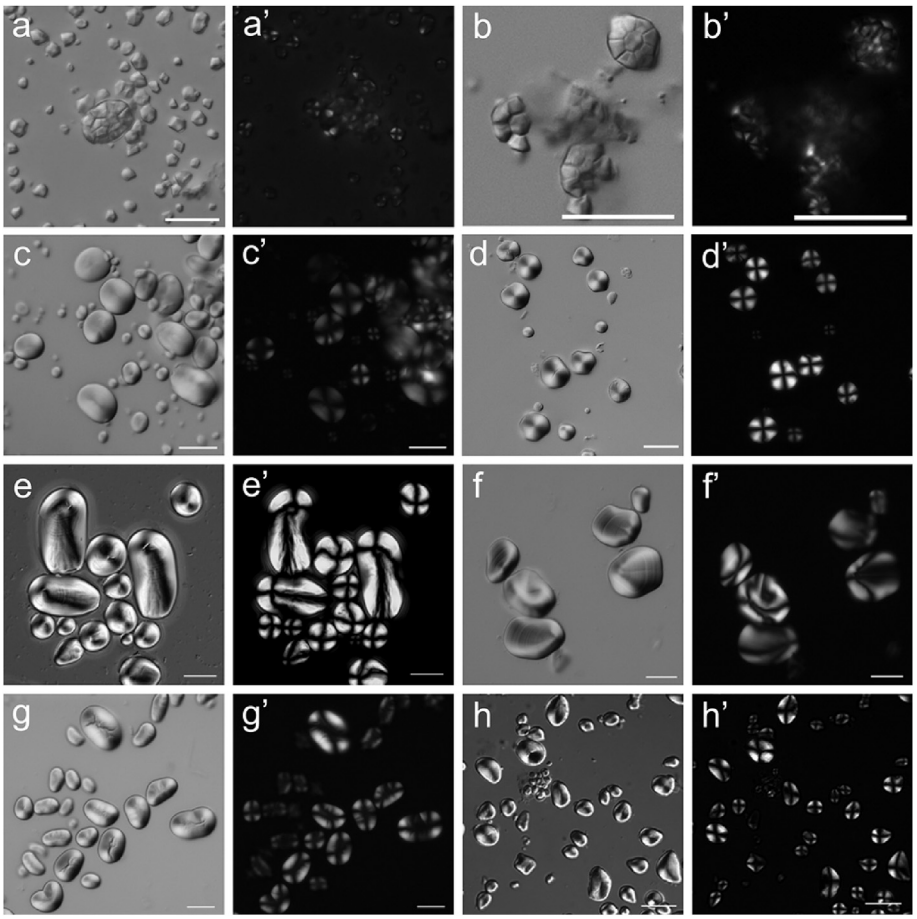

Fig. 4. Comparative starch samples from modern plants (scale bar: 20 μm) a, a′: *O. sativa* (Hunan), b, b′: *O. rufipogon* Griff. (Dongxiang, Jiangxi), c, c′: *Triticum aestivum* L. (Bengbu, Anhui), d, d′: *Coix lacroyma-jobi* L. (Bengbu, Anhui), e, e′: *Nelumbo nucifera* (Wuhu, Anhui), f, f′: *Dioscorea opposita* (Hefei, Anhui), g, g′: *Vigna radiata* (Nanyang, Henan), h, h′: *Quercus acutissima* (Hefei, Anhui).

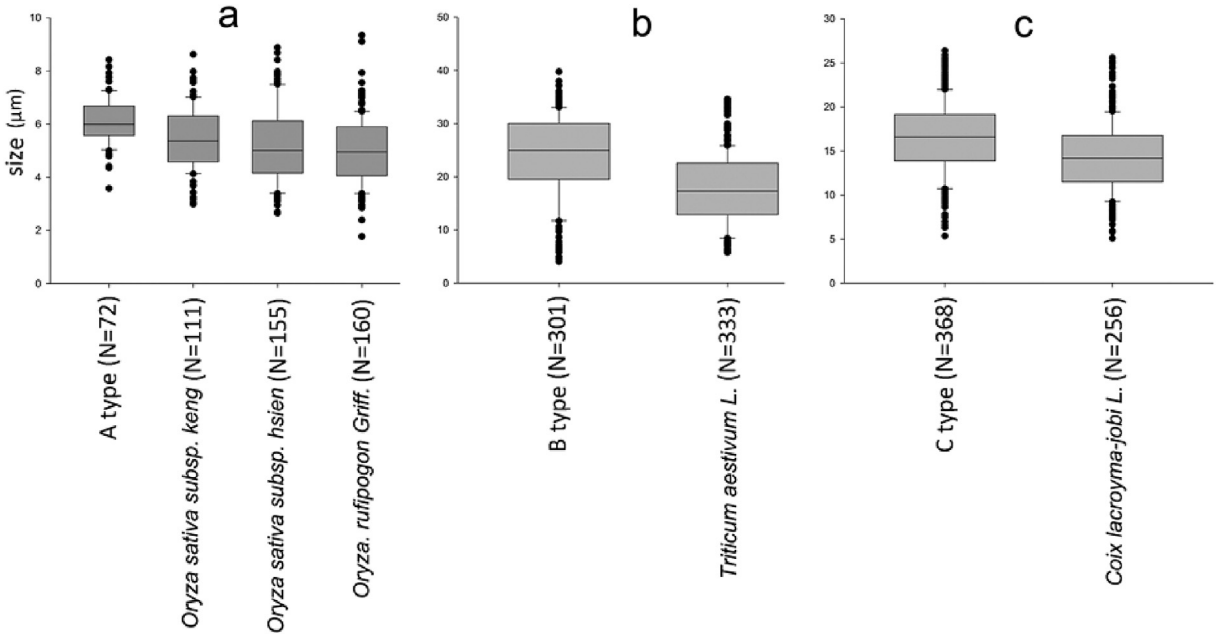

Fig. 5. Comparison of size ranges between ancient starch grains and modern reference. a. Type A and modern cultivated\wild rice; b. Type B and modern cultivated wheat; c. Type C and Job's tears.

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Type F (Fig. 3: o, o′) starch grain is triangular ovate or water drop-shaped, centric hilum and the extinction cross is shaped as "x" and "+". This morphotype resembles starch from fruits of Fagaceae plants (Yang et al., 2009a, b) (Fig. 4: h, h′). Maximum length measurements reveal that the size of Type F starch (9.73–25 μm, N = 10) is in range of *Quercus acutissima* (5.4–26.1 μm, N = 132), and higher than *Castanea mollissima* (3.39–21.85 μm, N = 172). Furthermore, starch grains of *Quercus* plants have also been found in early Shangshan phase (Liu et al., 2010). Based on the dates above, Type F are identified as *Quercus* spp..

3.2. Quantitative analysis

Compared with the total amount of starch grains contained in a plant, ancient starch grains extracted from prehistoric artifacts are very few in number. Also, because of the absence of research on ancient starch preservation mechanism, absolute numerical analysis of ancient starches is inappropriate for assessing economic or dietary importance of plant.

This paper tries to explore the processing methods of different plants by analysing the percentage presence (Pearsall, 2013) of starch grains on the artifacts. Percentage presence is the proportion of samples including a certain starch grain in all examined samples. Considering "presence" and "absence" as statistical standard, error in the quantity caused by non-quantifiable factors can be eliminated as much as possible, so percentage presence of starch grains can reflect the situation of plant processing more accurately along with the increasing of inspected samples.

Given that starch grains of a particular plant come from at least one instance of the plant, the minimum number of plants (Pearsall, 2013) in contact with artifacts can be calculated. Based on this hypothesis, the composition proportion of each plant species on the artifacts could be deduced by minimum number, which reflects the exploitation degree of different plants. Accuracy of this proportion depends on the sampling numbers.

The distribution situation of starch grains from different species on the artifact surfaces is closely related to the implements function, so the statistical analysis of different starch grains on grinding slabs and potteries should be conducted separately (Table 2).

situation of *Oryza* sp. and *Quercus* spp. is significantly different. Percentage presence and composition proportion of *Oryza* sp. on the surfaces of potteries are obviously higher than that of grinding slabs. However, Percentage presence and composition proportion of *Q.* spp. on the grinding slabs are much higher than that of potteries.

4. Discussion

The identification of starch grains from 15 unearthed artifacts indicates that the plant foods human exploited in the early Xiaohuangshan phase include rice, Triticeae grasses, Job's tears, Chinese yam, lotus root, acorns and beans. Quantitatively, the percentage presence and composition proportion of cereal including Job's tears, Triticeae grasses and rice are significantly higher than that of other plants, which suggests that cereal was the main plant food during the early Xiaohuangshan phase in the Lower Yangtze River. Job's tears has a wide geographic distribution in China and is generally used for foods and traditional medicine. Although starches from Job's tears have been recovered as early as 11.4 ka cal. BP (Liu et al., 2010). and through the whole Neolithic period in eastern China (Yang and Jiang, 2010; Zhang et al., 2011; Wan et al., 2012a, b; Liu et al., 2013; Dong et al., 2014; Li et al., 2014; Tao et al., 2015), the macrobotanic remains of Job's tears so far have been found only at the Hemudu site in Zhejiang Province c. 7000 BP in China (Yu and Xu, 2000). Today, no evidence shows whether Job's tears were domesticated in Neolithic China.

A total of 13 genera and 175 species within the tribe Triticeae were distributed throughout China, including 99 endemic and 8 introduced species. More than 50% of these species are members of the genus *Elymus*, and they are typically used in modern times as forage plants. Chinese genera of known economic value include *Triticum*, *Hordeum*, *Agropyron*, *Elymus*, *Elytrigia* and *Leymus* (Yang and Perry, 2013). Today the Triticeae starches have been found on scrapers, flakes, grinding stones and other artificial implements in many sites both in north and south China through the Upper Paleolithic to the late Neolithic (Liu et al., 2011; Zhang et al., 2011; Wan et al., 2012a, b; Li et al., 2013; Liu et al., 2013; Dong et al., 2014; Guan et al., 2014; Li et al., 2014; Xiaoyan, 2014; Liu et al., 2014a, b),

Table 2
Starchy plants presentation on slabs and potteries in Xiaohuangshan

Artifacts	Species							
	Series no.	*Oryza*	Triticeae	*Coix lacryma-jobi*	*Nelumbo nucifera*	*Dioscorea opposita*	*Vigna*	*Quercus*
Slabs	05XHSA0904H51:1	−	+	+	−	−	−	−
	05XHSA0904H51:2	+	+	−	−	−	+	+
	05XHSAG4①:16	−	+	+	+	−	−	−
	05XHSAG6①:17	−	+	+	−	−	−	+
	05XHSAH81:4	−	+	+	−	+	−	+
	05XHSAT1005⑥:2	−	+	+	+	−	−	+
Potteries	05XHSAT0805⑧:1	+	+	+	+	−	−	+
	05XHSAT0805⑧:2	+	+	+	−	−	−	−
	XHSBT8⑤:1	+	+	+	−	−	+	−
	XHSAT0402G6:1	−	+	+	−	+	−	−
	XHSAT0402G6:2	+	+	+	−	+	−	−
	XHSAT0402G6:3	−	+	+	−	−	−	−
	XHSAT0402G6:4	+	−	+	−	−	−	−
	XHSAT0402G6:5	+	+	+	+	+	−	−
	XHSAT0402G6:6	−	+	+	−	+	−	−

Notes:"+"means presence of starch, "−"means absence of starch.

The quantitative results (Fig. 6: a, b, a′, b′) show that the percentage presence of *C. lacryma-jobi*, Triticeae, *N. nucifera*, *Dioscoreae oppsita* and *Vigna* are consistent with the composition proportion of these plants either on potteries or on grinding slabs, while the indicating a wide exploitation of this plant in the prehistoric China. Although a long history of exploitation of Triticeae grasses occurred in prehistoric China, it is generally accepted that these plants were not domesticated.

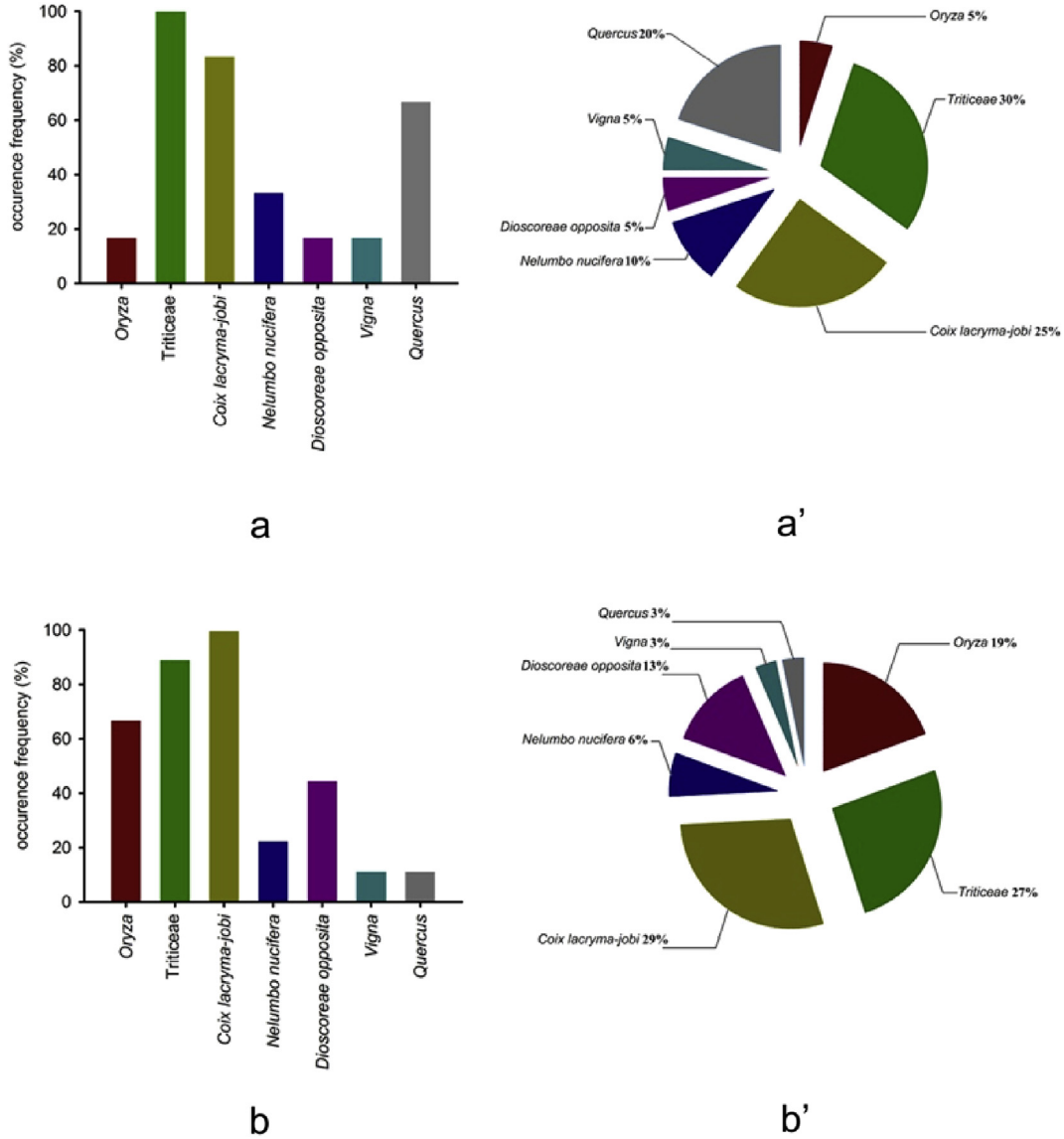

Fig. 6. Percentage presence and proportion of plant species a, Percentage presence of each plant on slabs a′, Proportion of plant species contact with slabs b, Percentage presence of each plant in potteries b′, Proportion of plant species contact with potteries.

Lotus and Chinese yams today are widely distributed and used for food in China. Starch granules from lotus root and Chinese yam have been recovered both on grinding slabs and in pottery from the Xiaohuangshan site with a lower percentage presence and proportion of the minimum number. Although these tubers rarely survive identifiably in macrobotanic remains, evidences from starch analysis demonstrate that the exploitation history of lotus and yams in China can be dated back to the Upper Paleolithic and early Neolithic (Liu et al., 2010; Liu et al., 2013). The statistical analysis results in this paper indicate that the exploitation degree of lotus and yams in the early Xiaohuangshan phase is not high.

Vigna plant starches have been recovered from the prehistoric sites both in north and south China (Liu et al., 2010; Yang and Jiang, 2010; Wan et al., 2012a, b; Liu et al., 2013; Dong et al., 2014; Liu et al., 2014a, b;). The lower percentage presence and proportion of the minimum number of this plant starches on grinding slabs and potteries show that although it has been used for food in the early Xiaohuangshan phase, Vigna plant was still a supplement food in the subsistence strategies of Xiaohuangshan people.

The middle and Lower Yangtze River is one of the major centres for the origin of rice agriculture in the world, phytolith analysis indicates that rice domestication began from cal. 11,000 BP in the Lower Yangtze River (Wu et al., 2014). However, no rice starches were obtained when 7 stone implements, including 6 grinding stones from the Shangshan and Xiaohuangshan sites were selected for the starch analysis (Liu et al., 2010). To date, the extent of the rice consumption in human's diets during the early Neolithic is unclear. In this paper, 9 potteries were examined for starch analysis, of which 6 examples were found to have been used for rice processing, indicating rice was a major staple for humans during the early Xiaohuangshan phase. It is worth noting that only a few rice starches were recovered on the grinding slabs in this experiments. This result is consistent with Liu and her colleges studies (Liu et al., 2010), which indicates grinding stones unearthed from Shangshan and Xiaohuangshan site were used mainly to process wild plant food resources other than rice.

Acorns today are distributed widely throughout China, and their history of exploitation can be dated back to the upper Paleolithic

(Guan et al., 2014). Macrobotanic remains and starches from acorns have been recovered in many early and middle Neolithic sites in northern and southern China (Liu et al., 2010; Liu et al., 2011; Liu et al., 2013; Xiaoyan, 2014; Liu et al., 2014a, b; Yang et al., 2015), which indicates this nut was an important plant food during that time. It is interesting that the percentage presence and proportion of the minimum number of acorn starches on grinding slabs are much higher than that on potteries in our studies. The traditional method of processing and cooking acorns has been studied. Acorns were dried, shelled, broken into pieces, and soaked in 0.5% alkaline water for 2 d, with the water changed each 20 m. The acorns were then dried and ground with tools to produce fine flour which could be used for food directly. Alternatively, during the processing cycle, hot water (70–80 °C) could also be used to soak acorns to remove the tannic acid and other soluble impurities that are harmful to human health (We et al., 2007). In this study, we found large quantities of acorn starches were found on the grinding slabs but few on potteries. Therefore, we speculate that acorns were likely cooked with shells in the pottery vessels to remove the harmful ingredients and then processed with grinding stones to shell or grind into flour for food directly. This would explain why, much more acorn starch remained on the surface of grinding stones, whereas very little remained on potteries.

5. Conclusion

In this study, 779 starch grains from 9 pottery fragments and 6 grinding slabs belonging to the early Xiaohuangshan phase (9000–8500 cal. BP) are identified from *Oryza*, Triticeae Tribe, *C. lacryma-jobi*, *Q.* spp., *Nelumbo* sp., *D. opposita Trunb* and *Vigna*, which indicates that people exploited many types of plants as their plant food during the early Neolithic in the Lower Yangtze River. The common recovery of rice starch on pottery surfaces suggests that rice had become one of the major staples at the Xiaohuangshan site. This result provides important evidence for the beginning of rice cultivation in the early Neolithic Lower Yangtze River. The differences in the percentage presence and composition proportion of starches from acorns and rice recovered respectively on grinding stones and potteries suggest that grinding slabs were not used mainly to process rice in the Xiaohuangshan site, and plant residues extracted from the surfaces of grinding stones cannot be used independently to investigate the origin and exploitation degree of rice in that time.

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