Excavation at Hanjing site yields evidence of early rice cultivation in the Huai River more than 8000 years ago

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Abstract: Through the analysis of macro- and micro-plant remains, food residues and the rice-field like features from the mid-Neolithic site of Hanjing in the Huai River region, we propose an early beginning of rice cultivation at Hanjing. The presence of non-shattering rice spikelet bases and the increasing percentages of rice phytoliths confirm the appearance of domesticated rice in the Hanjing archaeobotanical assemblage. However, as indicated by the different prediction rates of rice domestication shown by morphometric of the double-peaked *Oryza*-type glum cells and fish-scale decorations on the *Oryza*-type bulliform cells from different cultural phases before 7,000 BP, rice cultivation was at an early stage of development. Our findings provide new and significant evidence towards the establishment of the Huai River as another important center for early rice cultivation and domestication in prehistoric China.
Keywords: rice field-like features, *Oryza*-type bulliform, double-peaked *Oryza*-type, rice cultivation, rice domestication

1. Introduction

Rice feeds more than half of the world's population (FAO, 2013). It has been central to the economic and social development of ancient cultures, states and empires across East, Southeast and South Asia (Bray, 1994; Fuller et al., 2016). The developmental history of rice farming and landscape modification remains crucial to understand social and economic evolution of our own species. The Lower Yangtze River is long considered one of the most important centers for rice domestication as convincingly attested by the excavation and intensive research first at the Hemudu site in the 1970s and later at the Shangshan and Tianluoshan sites in the early 21st century. Archaeobotanical research of macro- and micro- plant remains has made a particularly important contribution to understand the process of rice domestication and intensification of rice farming, although the exact timing and places of these processes remain open questions. Parallel to the important discoveries from the Lower Yangtze River, evidence of early rice consumption (phytolith remains) in the Middle Yangtze River has been found at several Late-Pleistocene sites, including Yuchanyan, Xianrendong and Diaotonghuan, some of which date back to as early as 20,000 BP (Zhao, 1998; Boaretto et al., 2009; Wu et al., 2012). In their computational modelling, Silva and colleagues have predicted that the Middle Yangtze River is another important center for rice domestication (Silva et al., 2015), in accordance with the results of some pilot archaeogenetic studies (e.g. Allaby et al., 2008; Fuller et al., 2010). However, it remains unclear how and when rice was domesticated there, not least because the archaeological gap between the Late-Pleistocene and Middle-Neolithic rice still needs to be filled.

In addition to the Lower and Middle Yangtze River regions, the Huai River and its neighboring regions have emerged as another important center for early rice domestication in light of several recent discoveries. The discovery of abundant rice remains with domesticated features at the Jiahu site (9000-7500 BP) of the Upper Huai River in Wuyang County, southern Henan Province has stimulated heated scholarly debate on the significance of these groundbreaking finds (e.g. Chen et al., 1995; Crawford and Shen, 1998; Liu et al., 2007; Qin, 2012; Cheng, 2016). The percentage of rice spikelet bases with domesticated trait (non-shattering) reached 68% at the Jiahu site (Jiahu phase 1) (Cheng, 2016) and the morphological features of rice grains and rice husk impressions are similar to cultivated *Oryza japonica* found in burnt earth. However, it is not until the discoveries of more carbonized rice remains at the Shunshanji and Hanjing sites (8500-7000 BP) in Sihong County (Luo et al., 2016; NM and SM, 2016), Jiangsu Province, that it becomes clear that the Huai River valley was a center witnessing not only early exploitation and human management of local wild rice but probable rice domestication and the establishment of rice farming. Indeed, the probable rice fields or modified wild habitats of rice at the Hanjing site (NMC et al., 2018) predate the large-scale construction of paddy fields during the Majiabang or Hemudu (7000-5800 BP) period around the Taihu Lake region and the Ningshao Plain of the Lower Yangtze River.

Morphological characteristics on rice spikelet bases as well as rice grain size are considered a direct scientific index to differentiate wild rice from its domesticated counterparts (Fuller et al., 2007, 2008, 2009; Zheng et al., 2007, 2016). In addition, rice phytolith is increasingly used as another indicator of rice cultivation and domestication, especially when macro-plant remains of rice spikelet bases and grains are absent from archaeological sites due to conservation and sampling issues. The significance of morphometric of double-peaked *Oryza*-type glum cells from rice husks was first proposed by Zhao et al. (1998) and has been subsequently applied to estimate the process of rice domestication at the Diaotonghuan (Zhao, 1998), Shangshan (Wu et al., 2014) and other Late Paleolithic to Early Neolithic archaeological sites. Meanwhile, the size of *Oryza*-type bulliform cells from rice leaves (Fujiwara, 1993) together with fish-scale decorations on the cells (Fujiwara, 1976) has been systematically studied and proposed as anther criterion rice domestication in recent years (Lu et al., 2002; Fuller et al., 2007; Huan et al., 2015, 2020; Luo et al., 2016, 2021; Ma et al., 2016; Qiu et al., 2019). Nevertheless, the morphology of rice phytoliths is affected by environmental, physiological and other factors, including the ratio of the number of leaf midribs, a plant's metabolic function and stage of plant growth as well as various genetic mechanisms (Parry and Smithson, 1958; Piperno, 1988, 2006; Jane and Chiang, 1991; Wang, 2005; Qu et al., 2010; Huan et al., 2015). As a result, it is important to consider morphological features of rice phytoliths as an informative reference rather than decisive evidence for rice domestication from the perspective of quantitative statistics.

Considering the above methodological limitations, we examined both macro- and micro-botanical remains from different archaeological features at the Hanjing site in order to find new clues for early rice cultivation and utilization in the middle and lower reaches of the Huai River..

2. Regional settings and the excavation of the Hanjing site

The Hanjing site (32°35.457' N, 118°13.053' E) is located at Hanjing village, Meihua Town of Sihong County in Jiangsu Province, and four kilometers to the east of the Shunshanji site (Figure 1) (NMC et al., 2018). Both sites are situated on the floodplains of the Huai River, with the Hongze Lake, near the Grant Canal, sitting to their southeast. The Huai River has frequently shifted its river courses throughout the Holocene (TEEB, 2012). The volatile nature of the Huai River imposes significant threats to historical and modern societies, especially during monsoon seasons when ample rainfall quickly fills river channels and floods low-lying areas. Broadly speaking, the Holocene vegetation changes in the Huai River basin correspond to Holocene climate changes as detailed in the supplementary information (Li, 2006; Yin, 2018).

The Hanjing site covers an area of ca. 5 hectares. The elevation is ca. 20 meters above the sea level. Three seasons of field surveys and excavations were carried out by the joint archaeological team of National Museum of China, Nanjing Museum and Sihong County Museum from 2014 to 2016. The excavations covered an area of 1150 square meters and confirmed that Hanjing was a moated site (Figure 1) and occupied during the mid-Neolithic period (NM and SM, 2016). The moat was of an irregular rounded shape and 7-15 meters wide. Inside the enclosed area (29,220 m^2), several house foundations, postholes, ditches, and ash pits, as well as burials were excavated. Numerous pottery shards, stone tools, and abundant organic remains, including animal bones and plant remains were found during the excavations (*See Supplementary material for detail*).

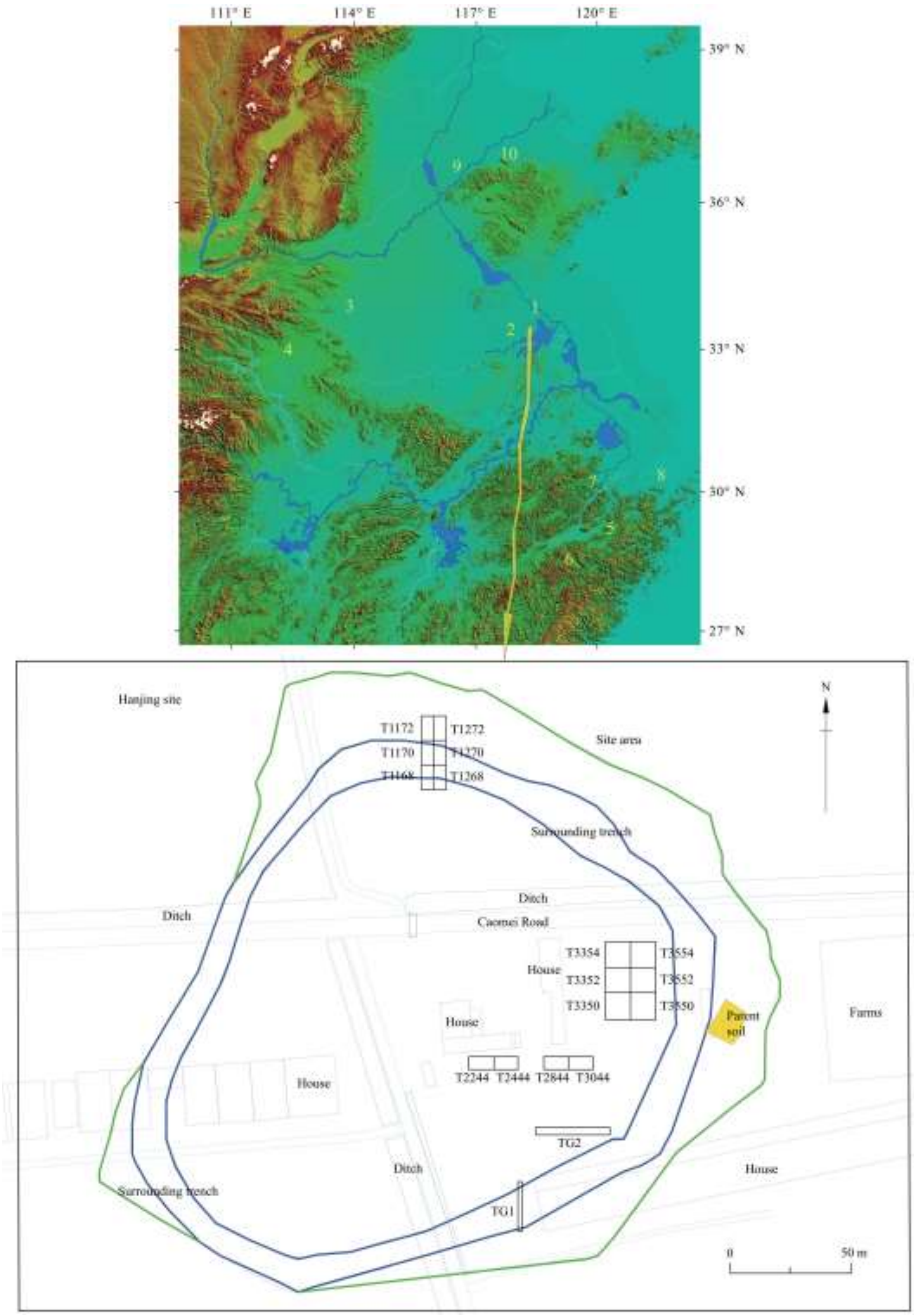

Figure 1 Location of the archaeological sites in the studied and neighbouring regions (upper), and plan of the Hanjing site and excavation area (lower). 1. Hanjing site, 2. Shunshanji site, 3. Jiahu site, 4. Baligang site, 5. Shangshan site, 6. Hehuashan site, 7. Kuahuqiao site, 8. Tianluoshan site, 9. Yuezhuang site, 10. Xihe site.

Overlain by contexts nos. T3352③ and T3354③, the three rice-field-like structures and 18 small-to medium associated ditches were situated to the east of the occupation area. Their dimensions are summarized in Table S1. These features were filled by two layers of archaeological deposits. The lower layer was only found in features nos. T3352k1 and T3352k3, while the upper layer can be seen in most features and generally contains abundant pottery sherds, stone tools and bone artefacts. This stratigraphic context and associated finds such as pottery sherds indicate that these rice-field-like features were used during the first phase of the Shunshanji culture. Through three-dimensional modeling using the Agisoft Photoscan software, we were able to simulate directions of water flows within and around these features. Our results show that water was flowing from southeast of the excavated trench no. T3352 to low-relief areas in the southwest and northwest corners of the trench near the rice-field-like features nos. T3352k2 and T3352k3, and from northeast of the excavated trench no. T3354 to the southern depression area near the rice-field-like feature no. T3352k3 (Figures 2 and 3). Meanwhile, an ancient river channel (no. G16) attributed to the same cultural phase (Figure 2) was excavated near the east of the rice-field-like features, which might have both drainage and irrigation functions.

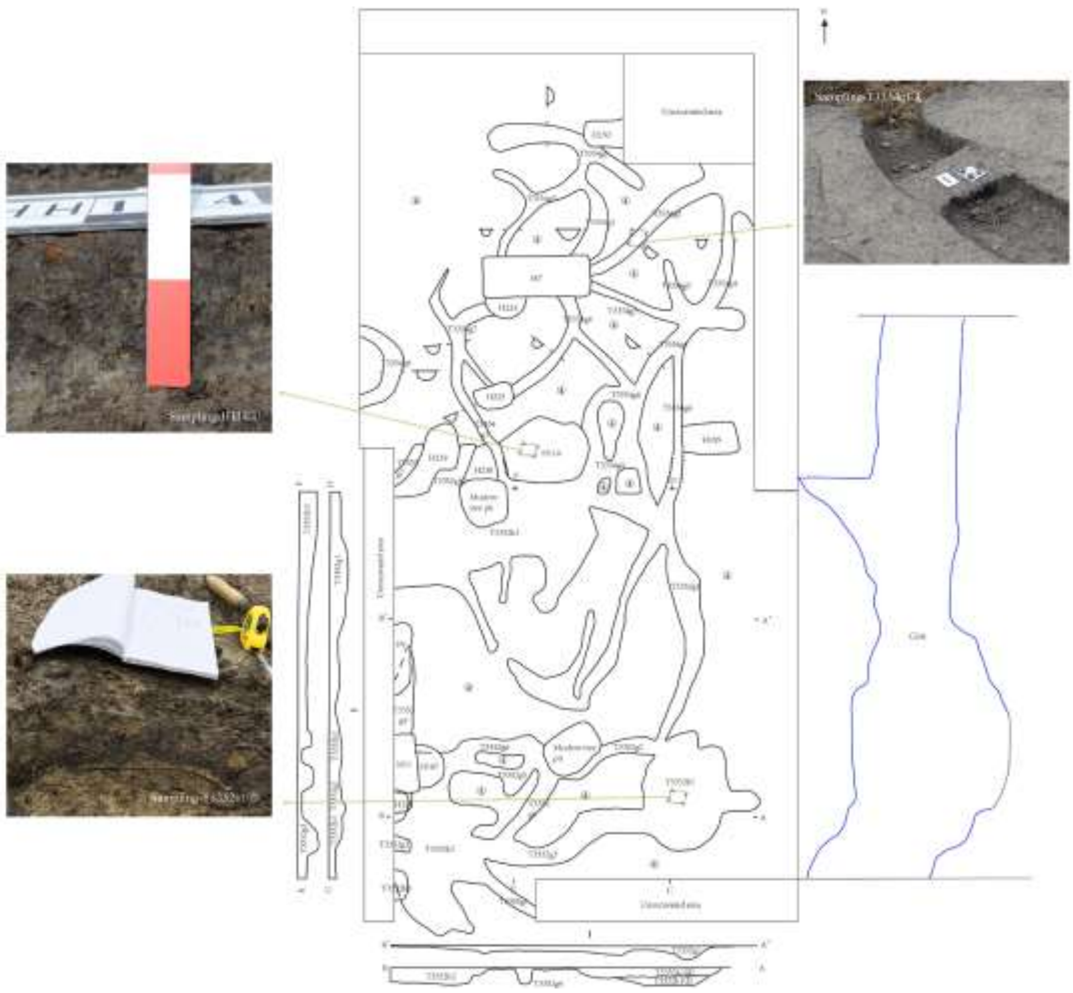

Figure 2 Plans of the excavated archaeological features and sampling locations and sampling photos.

Figure 3 Simulation of the directions of water flow in excavated trench no. T3352.

3. Materials and methods

3.1 Materials

A total of 140 soil samples attributed to the Hanjing Phase I and II were collected from the eastern excavated part of the Hanjing site. Bulk samples for phytolith analysis were collected at 5 cm intervals from a range of archaeological contexts. This was to allow a more robust assessment of the taphonomical issues and the representativeness of phytolith samples (see Figure 2 and Table S2 for detail). In particular, considering that the rice-field-like structures cover a substantial area but the deposits of them are generally thin, multiple sampling points in the rice-field-like structures were selected for sampling. Whilst only one sample was collected per sampling unit from other archaeological features, within each sampling point, multiple samples were collected at different depths. Flotation samples were obtained from the deposits of all archaeological features during excavation to increase the representativeness of the macro-botanical samples. Organic residues were also collected from selected pottery sherds discovered from ash pits and other archaeological contexts.

3.2 Methods

Flotation (Zhao, 2004) and manual wet-washing in buckets were combined to recover plant remains. Macro-plant remains were collected, identified and measured under a Nikon SMZ1000 stereo microscope. Phytoliths were extracted following the protocol established by Piperno (1988, 2006). The assessment of morphological features of the *Oryza*-type bulliform and double-peaked *Oryza*-type glume cells were performed using traditional methods proposed and improved by Zhao et al. (1998) and Huan et al. (2015). Potsherd samples were examined for plant remains and plant impressions under a stereo microscope. Lipid residues were extracted from both the food crusts and ceramic matrices using the protocol outlined by Evershed et al. (1990) and Craig et al. (2013), and then analyzed by GC-MS and GC-C-IRMS at the Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences. For more details of the methods, see *Supplementary material*.

4. Results

The results of the taxonomic identification, statistics and measurements of rice grains and phytoliths are given in the *Supplementary material*.

4.1 Dating

As shown in Figure 4 and Table S3, these AMS ^{14}C dates of charred rice grains and some charcoal from the archaeological contexts were calibrated (Reimer et al., 2020; Bronk Ramsey, 2021) and fall between 8400-8000 cal. BP, corresponding to that are designated to the Hanjing Phase I and II based on pottery typology. In addition, as an experimental reference, there are two samples of charcoal attributed to the Hanjing Phase Ⅲ whose AMS ^{14}C dates fall between 7800-7700 cal. BP.

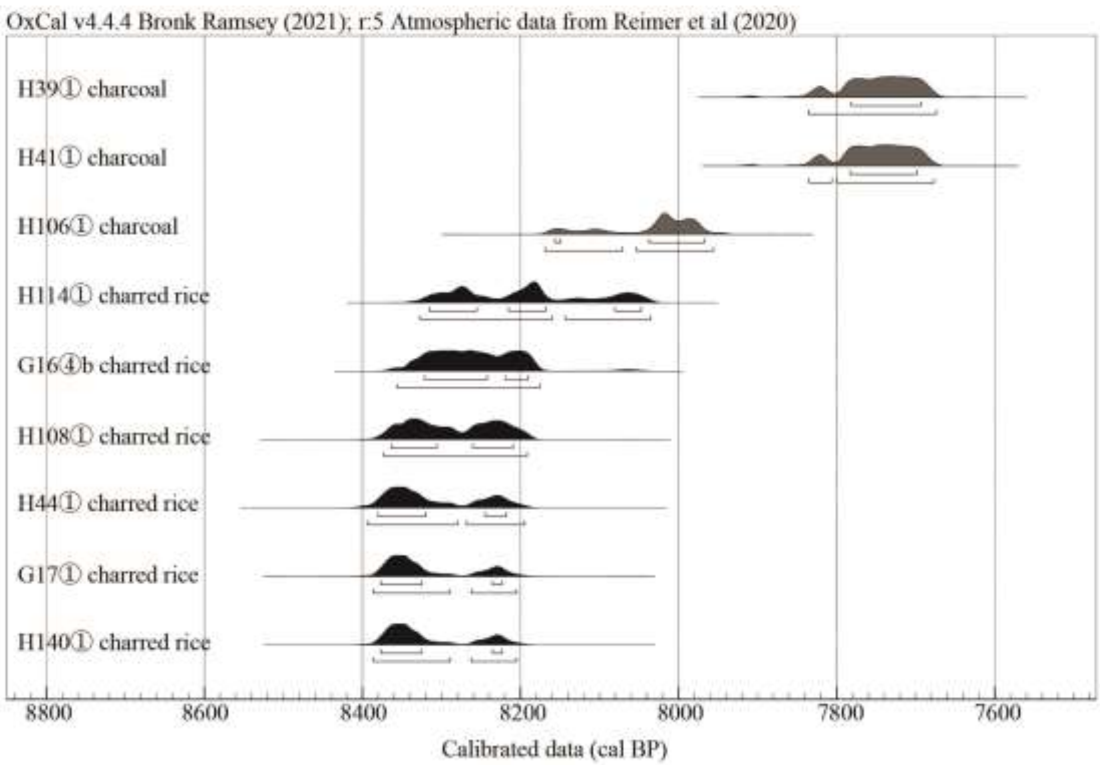

Figure 4 Plot of calendar age of the Hanjing site calibrated from AMS ^{14}C date.

4.2 Carbonized rice remains

56 charred rice grains and three pieces of rice spikelet bases were found (Figure S2) in the floatation samples collected from the phases I and II archaeological contexts. Both the percentage and frequency of rice remains increased from the Phase Ⅰ to Phase Ⅱ (Figure S3). Two pieces of rice spikelet bases display an uneven profile, dimpled appearance, and less symmetrical scar on the bases (Figure S2). Better preserved rice grains (with more than half of the grain) were selected for grain size measurement. The results show that they are overall of relatively small grain sizes (Table S4). The morphological features of the rice spikelet bases discovered at the Hanjing site resembles the domesticated ones published by Fuller and colleagues (Fuller et al., 2009). Meanwhile, the size range of some small rice grains at Hanjing are close to those at the Jiahu site, which might be considered as an indicator that they were wild rice (Fuller et al., 2007). This indicates that domesticated rice and wild rice likely co-existed at the Hanjing site.

4.3 Microstructure of and impressions on potsherd

Plant remains associated with pottery unearthed from a range of archaeological contexts (T3350④, T3350⑤, H122①, H128① and H135①) at the Hanjing site showed that some potsherds were tempered with rice husks and straws. Charred remains of rice plants or impressions can be found on the inner and/or outer surfaces as well as in the cross-section of the potsherds. Careful microstructure observation of a piece of potsherd tempered with plant material found at context no. H135① of the Hanjing Phase Ⅰ was carried out and the phytolith remains, adhering to some areas of the pot's red inner and grey outer walls, shows a loose texture and porous structure. Impressions of rice husk were mostly found in the outer walls, while some translucent residues were present in the inner walls and cross sections (Figure 5). The phytoliths belong to the double-peaked *Oryza*-type. According to a study on the impact of heat on the morphology of rice phytoliths (Wu et al., 2012), it is likely that the residue of double-peaked *Oryza*-type phytoliths were impartially fired at temperature < 1000 ℃, thus not experiencing significant morphological alteration.

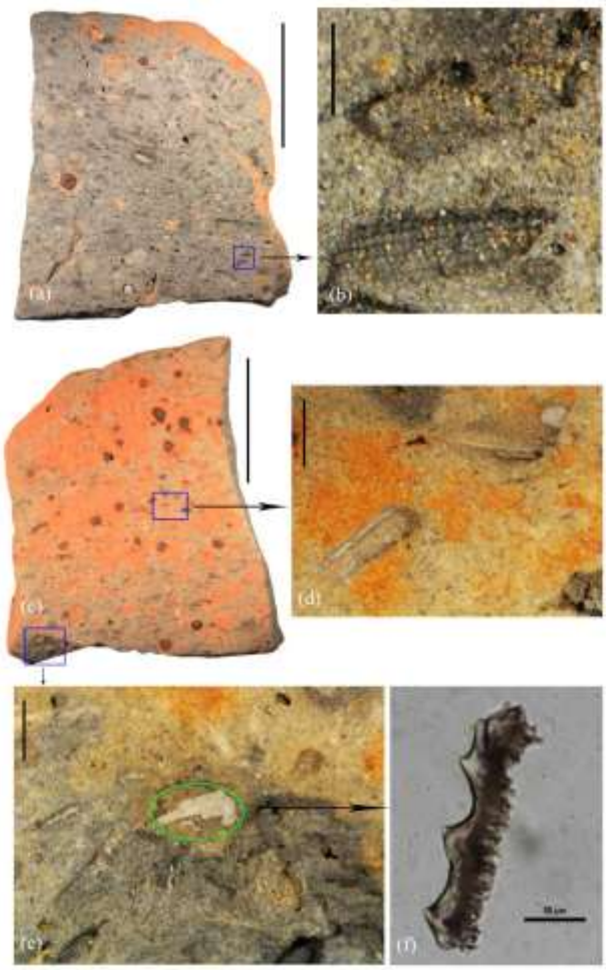

Figure 5 Impressions and phytolith remains in potsherd from cultural deposit no. H135①. a, outer wall of the pot. b, impressions of rice husks in outer wall. b, inner wall of the pot. d, impressions of rice husks in inner wall. e, impressions of rice husks and phtolith remains in cross section. f. double-peaked *Oryza*-type phytolith. Scale bars: a, c, 2 cm. b, d, e, 1mm. f, 50 μm.

4.4 Morphological features of rice phytoliths

The measurement of *Oryza*-type bulliform from the Hanjing site suggested a minute decrease in the size of the phytoliths (Table S5, Figure S4) from the phase I to phase II. Statistical models based on the dimension of rice phytoliths help predict the domestic status of rice (Zhao et al., 1998; Huan et al., 2015) (*See Supplementary material for detail*). Applying these methods, we identify that the percentages of domestic *Oryza*-type bulliform cells with nine or more fish-scale decorations decreased from the Hanjing Phase Ⅰ to Ⅱ, while the predicted double-peaked *Oryza*-type glume cells suggested an increasing percentage of 4.9% domestic types from phase I to phase II (Table 1).

Table 1 Predicted domestic and wild types of rice phytoliths from the Hanjing site.

Cultural stage	Predictions by double-peaked *Oryza*-type glume cells				Predictions by *Oryza*-type bulliform cells		
	Domestic	Uncertain	Wild	N	Domestic	Wild	N
Hanjing Phase Ⅰ	31.0%	46.8%	22.2%	171	44.4%	55.6%	196
Hanjing Phase Ⅱ	35.9%	45.3%	18.8%	329	39.6%	60.4%	308

4.5 Organic residues analysis

Combining the preliminary lipid molecular markers and compound-specific isotopic results, we inferred that the pottery at the Hanjing site was mainly used to process C3 plants (possibly rice), aquatic foods and terrestrial non-ruminant animals (unpublished data).

5. Discussion

5.1 Subsistence economy at Hanjing

Amongst the Early Neolithic sites with evidence of rice consumption and cultivation in China, it is not uncommon to find rice husks used as tempering material for pottery production as has been found at the Shangshan site (Liu et al., 2007; Zheng et al., 2016). Whilst this is often considered a sign of intensified rice consumption which created abundant byproducts of rice food processing for pottery production, to what extent this could be seen as definite evidence of intensified rice production remains to be demonstrated. On the other hand, detailed examination of rice husk impressions in pottery could also reveal other characteristics of early rice economies. Our analysis of pottery sherds and phytoliths discovered at Hanjing confirms that rice husks were broken before being used as tempering material for pottery production. This appears to be a deliberate practice. We suggest that more quantitative studies should be conducted to understand the relationship between early rice cultivation and pottery production at the Hanjing and elsewhere.

Preliminary zooarchaeological study of the faunal remains discovered at the Hanjing site suggests that pigs (including both wild and domesticated types) accounted for the highest percentage in the faunal assemblage, followed by deer. Many aquatic species such as fish and *Trionyx* were also present. This is consistent with our preliminary result of organic residues analysis of the Hanjing pottery sherds. Combined this zooarchaeological evidence with the archaeobotanical data we have obtained, it is clear that hunting, fishing, rice cultivation and wild plant food collection (unpublished data) were all important parts of subsistence at Hanjing. This subsistence economy is similar to that of the Shunshanji site, which according to several studies (Luo et al., 2016; Yang et al., 2016), also consisted of hunting-gathering as well as rice cultivaiton. This similarity points to a shared characteristic of the subsistence strategies amongst the Early to Mid-Neolithic sites in the Huai valley, in which hunting and gathering still played a very important role in food production.

5.2 The earliest supportive evidence of rice cultivation and domestication in the Huai River

Studies on the *Oryza*-type bulliform cells from the Shunshanji site have shown a size increase of the bulliform cells from the Shunshanji Phase I to Phase III (ca. 8500-7500 BP) (Luo et al., 2016). This trend is different from what we observed at Hanjing. Moreover, the *Oryza*-type bulliform cells from the Hanjing site have a smaller average size than those at the contemporaneous Shunshanji site, as confirmed by our measurement of their VL and HL ratios (Figure S4). These dimensional variations of bulliform cells between different archaeological contexts and sites in the studied region might be associated with several factors that merit further investigation. For instance, were these variations associated with early stage of rice cultivation and domestication or changes of ecological conditions of rice growth? Or are these related to our sampling strategies?

Our measurements of *Oryza*-type bulliform cells and double-peaked Oryza-type glume cells show that these two types of phytoliths hold different significance to predict rice domestication at Hanjing (Figure S5). The exceptionally high percentage (45%) of the 'uncertain type' based on the prediction model indicates that further improvement should be made to allow more robust prediction of rice domestication using phytolith as a proxy to reconstruct rice farming history. Nevertheless, considering the proportions of ≥9 fish-scale decorations of bulliform cells from Hanjing Phase I to II (39.61-44.39%), which are situated between the range of wild (17.46%±8.29%) and domesticated rice (63.70%±9.22%) as proposed by Huan et al. (2015), it can be surmised that the rice from Hanjing was at a relatively early stage of rice domestication (Figure S5).

Controversy regarding the timing of rice domestication persists, but many studies have shown that by ca. 7000 BP, rice domestication already occurred and rice cultivation had reached a critical stage of development (e.g. Fuller et al., 2007, 2008; Wang et al., 2010; Zhao, 2010). Studies of rice phytoliths from Early and Mid-Neolithic sites (>7000 BP) in the Lower Yangtze River, including Shangshan, Hehuashan, Kuahuqiao and Tianluoshan (Wu et al., 2014; Luo et al., 2016; Qiu et al., 2019) suggest that domestication was at an early stage due to the fluctuating situations of domestic predictions amongst the different rice phytolith assemblages at these sites (Figures 6 and 7). Compared with the Lower Yangtze River, the Shunshanji and Hanjing sites of the Huai River valley showed a relatively higher proportion of domesticated rice bulliform cells in the phytolith assemblages as predicted by the measurement of the *Oryza*-type bulliform cells (Figure 6), although the double-peaked *Oryza*-type glume cells might potentially show an opposite trend (Figure 7). This discrepancy of phytolith identification and prediction of rice domestication might be attributed to several analytical and methodological factors, including analytical approaches and sampling issues, statistical methods applied during measurement and data analysis.

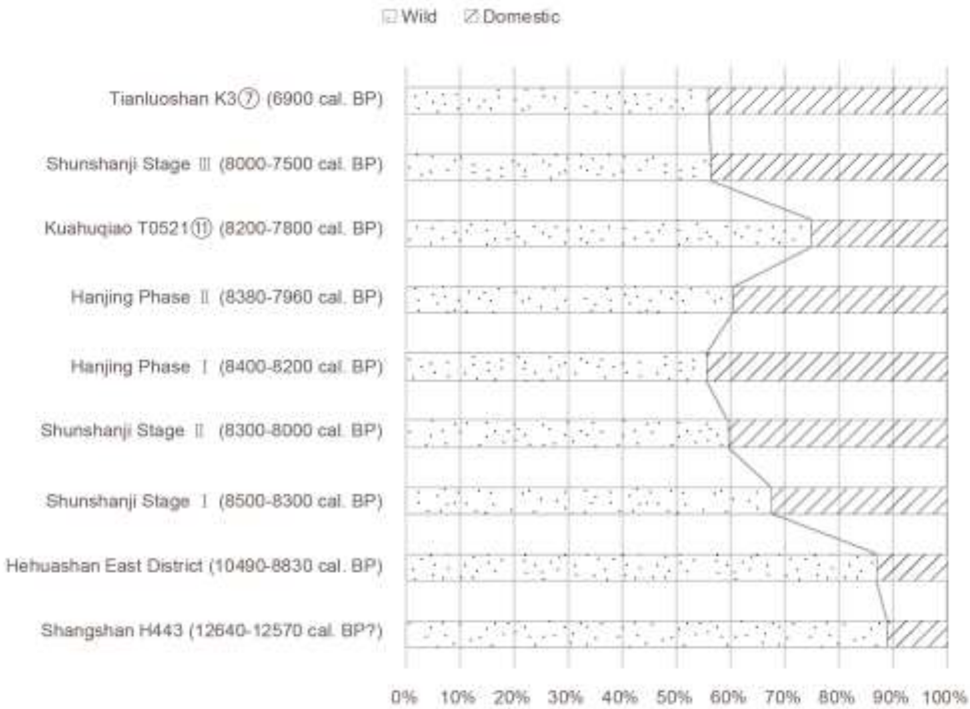

Figure 6 Rice domestication predictions in prehistoric China before ca. 7000 BP by *Oryza*-type bulliform cells. Raw data of the Shangshan, Hehuashan, Shunshanji, Kuahuqiao and Tianluoshan sites in this plot were collected from Luo et al. (2016), Qiu et al. (2019) and Wu et al. (2014).

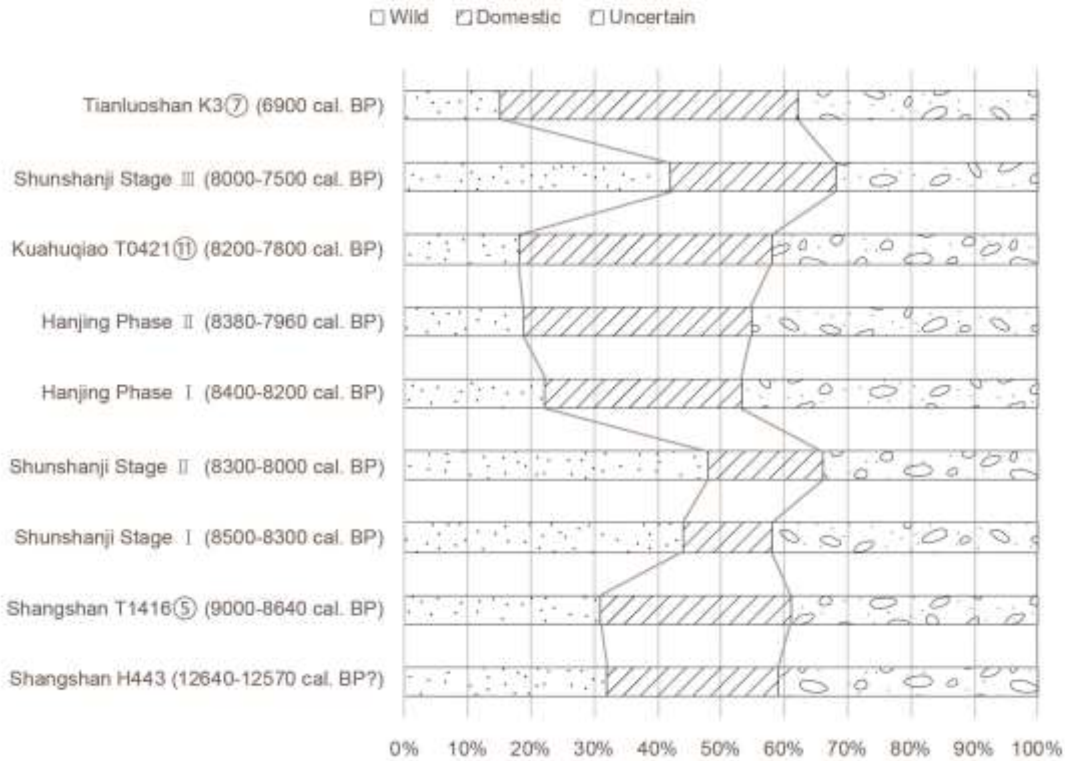

Figure 7 Rice domestication predictions in prehistoric China before ca. 7000 BP by double-

peaked *Oryza*-type glume cells. Raw data of the Shangshan, Shunshanji, Kuahuqiao and Tianluoshan sites in this plot were collected from Luo et al. (2016) and Wu et al. (2014).

However, such a caveat in phytolith analysis is mitigated by our discovery of macro plant remains and excavation of archaeological features related to early rice cultivation. Indeed, the direct evidence of rice cultivation and domestication at Hanjing comes from the increasing percentages of charred rice grains from the Hanjing Phase Ⅰ to Ⅱ (ca. 8400-8000 cal. BP) and the presence of domesticated rice spikelet bases. As discussed above, the possible rice fields and related archaeological features suggest deliberate management of local hydrology at Hanjing. Meanwhile these features would have facilitated irrigation and drainage in the field, which were crucial for rice cultivation. The presence of domesticated rice phytoliths in these rice-like features might be considered direct evidence for their close association to early rice cultivation. Combining all this evidence, we believe that the Hanjing site presents one of the earliest evidence of rice cultivation and domestication in the Huai River valley, making this region another important center for rice domestication.

In parallel to the well-established case of early rice cultivation and domestication in the Lower Yangtze River and possibly Middle Yangtze River (Zhao, 2010; Wu et al., 2014; Ma et al., 2016; Qiu et al., 2019), archaeobotanical studies at the Jiahu site (Cheng, 2016) and Baligang site (Deng et al., 2015) convincingly confirm that the Upper Huai region and adjacent areas of the Hanshui River was another center of early rice cultivation and domestication at around 8500 BP. Our archaeobotanical data from Hanjing and Shunshanji adds further supportive evidence to this opinion. More importantly, since the Hanjing and Shunshanji sites were located in the Middle and Lower Huai River valley, it is clear that by 8500-8000 BP, rice farming had begun to spread across the Huai River region. As suggested by Qin (2012), this rapid expansion of rice coincided with the onset of the Holocene warm period which not only provided favorable conditions for rice farming, but also population growth and economic development. A similar case can be seen at the Yuezhuang (Crawford et al., 2013) and Xihe (Jin et al., 2014) (Houli cultural sites, 8000-7500 cal. BP) of the Lower Yellow River in which expansion of early rice farming might have been related to a warmer and wetter period during the Holocene. Our new evidence supports the Huai River valley as another important center for early development of rice economies that was less well understood, compared to the Middle and Lower Yangtze River valleys and that might have experienced a different pathway to the beginning and development of rice farming to the other two centers.

6. Conclusions

Our examination of macro- and micro-plant remains, food residues and archaeological features possibly related to initial water management has convincingly demonstrated that rice cultivation had begun at the early phase of the Hanjing site and was accompanied by significant morphological changes of spikelet bases and rice phytoliths. Our results firmly suggest that the Huai River region was another center for early cultivation and domestication of rice in prehistoric China and that rice utilization began to spread rather early within the region. This dataset also sheds new light on the process of earliest modification of local ecosystems of rice which led to cultivation and eventual domestication of rice in the Huai River valley.

However, the co-existence of domesticated and wild rice spikelet bases and the different trends of dimensional changes of double-peaked *Oryza*-type and *Oryza*-type bulliform rice phytoliths confirm that rice cultivation at the Hanjing site was at an early stage. Furthermore, the determination of the domestication status of *Oryza*-type bulliform cells based on fish-scale decorations disagrees with the results based on the morphometric of double-peaked *Oryza*-type glume cells. Such discrepancy merits further investigation into whether it is related to the nature of rice plant physiology in initial rice cultivation or to some other factors.

Our reconstruction of the palaeo-diet and food processing at Hanjing also accord with many other studies suggesting that rice cultivation, as well as animal husbandry, hunting and wild food gathering were all integral parts of subsistence economies in the Huai River valley and its neighboring regions between 8500-7000 cal. BP. Future research should aim to further unpack the pathways from early cultivation to the fully establishment of agricultural economies in the Huai River region, investigate the unique role of climate and environment as well as social factors in this process, and compare this Huai-river case with other centers of prehistoric rice economies such as the Middle and Lower Yangtze River regions.

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