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Starch grains from human teeth reveal the plant consumption of proto-Shang people (c. 2000-1600 BC) from Nancheng site, Hebei, China

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Abstract

The founding processes of the first state of ancient China with a known written record, -the Shang dynasty (3600-3046 cal BP), have been poorly understood. Recent discoveries of a host of archaeological sites dating to the proto-Shang culture (4000-3600 cal BP), have helped elucidate the transition to the Shang culture. Nevertheless, there are few investigations about the mode of subsistence and economy of the proto-Shang culture, and how this might have shaped the transition to statehood. In this present study, we analyzed the starch grains preserved in dental calculus and teeth surfaces from 16 samples from the site of Nancheng in order to gain a better understanding of the subsistence strategy and plant consumption of proto-Shang people. We also performed experiments to test how different cooking methods may lead to size changes in the starches of four Poaceae plants, in order to identify the processing methods used by the proto-Shang people. The results indicate that *Triticum aestivum*, *Coix lacryma-jobi*, *Setaria italica*, and some yet-unidentified roots and tubers were consumed by these individuals. These data indicate a broader spectrum of plant consumption than that seen by previous archaeobotanical and stable isotope analyses. Such a broad spectrum of plant consumption provided a substantial economic base for proto-Shang people and might be one of the factors supporting the subsequent development of the Shang state culture.

Keywords: Starch grains; Dental calculus; Cooking; Proto-Shang culture

Introduction

The adoption of agriculture occurred in various times and places around the world, and several authors have linked this development with the formation of large complex political units/organizations (Bellwood, 2005; Fuller and Stevens, 2009). However, the consumption of plants, especially starch-rich plants that eventually became the targets of domestication (Piperno et al., 2004; Yang et al., 2012; Hardy, 2018; Fellows Yates et al., 2021), has been a key part of the human niche since well before the emergence of agriculture. The earliest stages of domestication likely took the form of cultivation, or para-cultivation, of plants that were already well known to foragers from that region. Furthermore, trade or social networks were important in passing along information and new ideas, such as agriculture, among groups who were first experimenting with this new technology. However, our understanding of the use of plants and development of agriculture for these important periods of transition remains limited by the few studies that provide direct evidence of plant food consumption.

This is particularly the case with the proto-Shang culture of China. The later, Shang culture, is the first dynasty known to have a written record and significant development of a political state in present-day China. The roots of this great dynasty are in the proto-Shang culture, but little is known about the subsistence and economy of this earlier group.

The proto-Shang culture, also known as the Xiaqiyan culture, refers to a cluster of sites with similar cultural contexts located in the present-day northern Henan and southern Hebei provinces during the time period between the late Longshan period (4200-4000 cal BP) and the Shang dynasty (3600-3046 cal BP; Li, 1989; Fig. 1). To date, different scholars have proposed four variants of the proto-Shang culture, which present a general pattern that the northern ones are earlier than the southern ones (Shen, 1991; Wei, 1999; Li, 2000; Hu and Wang, 2012).

Despite its importance as the foundation for the subsequent Shang culture, we still know little about the subsistence economy of the proto-Shang people. Previous studies have focused on written records, and the analysis of a handful of artifacts, such as pottery and stone tools. Based mainly on these sources of indirect evidence, some scholars believed that the subsistence strategy of the proto-Shang population went through a gradual change from nomadism to agriculture during the process of their migration southwards in North China Plain (Zhu, 2007; Wang, 2010). Domestic animal bones were commonly excavated from proto-Shang culture sites and provided invaluable information about local livestock husbandry (Hou et al., 2009; Hou and Xu, 2015). In contrast, there have been few investigations about plant use of proto-Shang people, except for one study of the archaeobotanical remains at Zhangdeng. Plant remains including cereal seeds from foxtail millet (*Setaria italica*), common millet (*Panicum miliaceum*), soybean (*Glycine max*), wheat (*Triticum aestivum*) and a few weed taxa were recovered through flotation (Liu, 2012). Other evidence for plant agriculture comes from contemporaneous cultures that appear to have close connection to the proto-Shang, namely the Erlitou culture and Yueshi culture (Li, 1991; Fang, 2010). In the Erlitou site, which is the capital site of Erlitou culture, charred seeds from five crop species, including foxtail millet, broomcorn millet, rice, wheat and soybean were discovered. Zhao and Liu (2019) noted that the ancient agriculture in the Erlitou site was mainly based on the millet crops. For Yueshi culture, which was distributed in today's Shandong province, recent archaeobotanical studies demonstrated that although settlements in different regions had different crop composition, millets always occupied the most important position (Guo and Jin, 2019). Other evidence for plant use among the proto-Shang culture itself comes from recent stable isotope studies of human skeletal remains from several proto-Shang archaeological sites, which have indicated these individuals were heavily reliant on C₄-based food sources, most likely millets (Hou et al., 2013; Ma et al., 2016). The results from the stable isotope analyses and from the single archaeobotanical study provide a rough outline of the likely food sources, but there is urgent need to get direct dietary evidence from several proto-Shang sites. Comparisons of multiple sources of information have the potential to deepen our understanding of dietary spectrum of proto-Shang people. Unfortunately, flotation has not been systematically used during the excavation of archaeological sites dating to the proto-Shang period. Therefore, it is necessary to turn to other archaeobotanical research approaches, especially plant microremain analysis.

While macrobotanical remains are scarce, many proto-Shang tombs preserved skeletal material. We were able to analyze the plant microremains, specifically the starch grains, that were preserved in the dental calculus of some of these individuals. Starch grain analysis is one of the most important means of archaeobotanical study and has been used to identify artifact function, to trace plant domestication, and to reconstruct human diet (Perry, 2004; Tao et al., 2011; Vinton et al., 2009; Yang et al., 2012; Liu

et al., 2013). Dental calculus is increasingly being used as a target sample type for the analysis of starch grains, as this bio-mineral entraps food particles as it forms, and therefore it represents a direct record of plant consumption (Henry and Piperno, 2008; Piperno and Dillehay, 2008; Wesolowski et al., 2010; Henry et al., 2011; Mickleburgh and Pagán-Jiménez, 2012; Tao et al., 2015; Zhang et al., 2017). In the present study, we intend to get a more comprehensive view of the plant consumption of proto-Shang people using starch evidence from dental calculus. As part of this analysis, we also explored the extent to which different cooking methods may lead to size changes in Poaceae food plants, in order to understand if and how the foods that the proto-Shang people consumed were cooked.

Site description

Nancheng is located on a terrace on the south bank of Gujian River in Ci County, Hebei Province (Fig. 1). This is a mountainous region which forms the transition between the east foot of the Taihang Mountain and North China Plains. The date of the site was mainly based on analysis of the recovered cultural relics and its preserved cultural layers spans from the late Yangshao period (5500-5000 cal BP) to the Qing dynasty (1636-1912 AD). It was excavated between 2007 and 2008 by the Hebei Provincial Institute of Cultural Relics and Archaeology as part of a salvage operation prior to the South-North Water Diversion Project. In total, an area of 6580 m² was excavated, uncovering 205 refuse pits, 5 houses, 5 wells, 5 kilns and 116 tombs. Amongst them, 82 tombs dating to the proto-Shang period are of particular importance. The whole cemetery is nearly 30 m from east to west, and 60 m from north to south. All of the pro-Shang tombs are earthen pits that are shaped like rounded rectangles.

Some of the tombs contained internal structures. One tomb had a wooden inner and outer coffin, two tombs had only an inner coffin, and 22 tombs had a “second-tier platform” but no inner or outer coffins. The remaining 57 tombs contained no internal structures. Numerous funerary objects were unearthed, including pottery, jade artifacts, bone tools, mussel products, and shell ornaments. The differences in tomb structure complexity as well as in the delicacy and quantity of grave goods indicated that social stratification was present in the local society of the proto-Shang culture at this site (Shi et al., 2012).

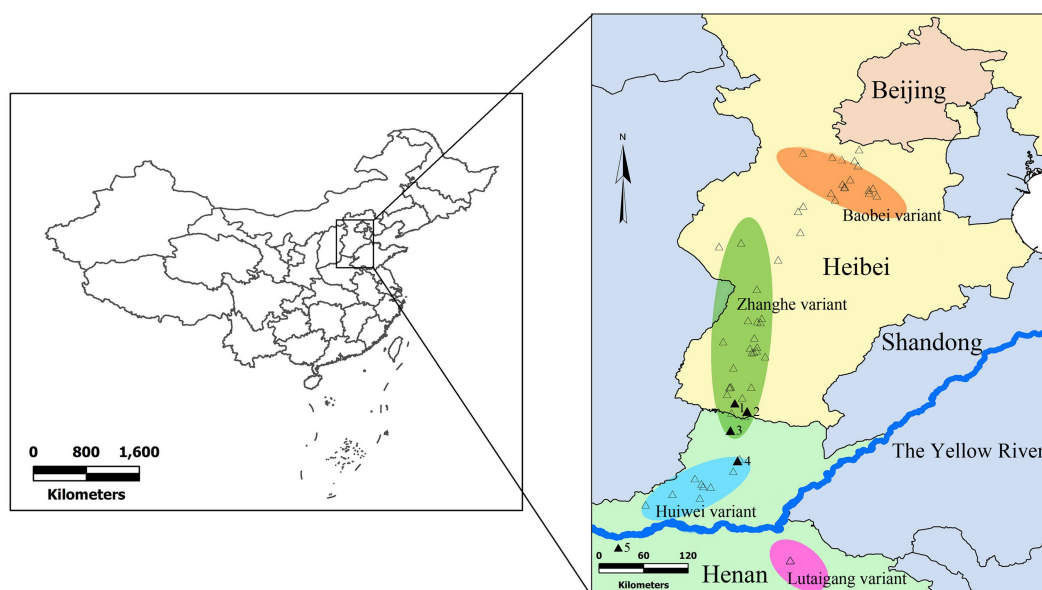


Fig. 1. Map showing the location of archaeological sites of the proto-Shang culture and related sites of other archaeological culture, among which the solid black triangles with numbers indicating the sites

discussed in the text (1. Nancheng; 2. Xiaqiyuan; 3. Zhangdeng; 4. Liuzhuang; 5. Erlitou; adapted from Hou et al. 2013; modified).

Materials and methods

Sixteen teeth belonging to 13 individuals were retrieved from the proto-Shang tombs at Nancheng (Table 1). We chose teeth that had the largest bands of calculus, without considering their sex or grave type. The teeth were cleaned of loose debris with disposable tooth brushes and this sediment was retained as a control sample. A dental pick was used to detached visible calculus from all the teeth. Calculus fragments from each individual sample were collected in a piece of aluminum foil and then immediately transferred into 1.5ml centrifuge tubes. 500µl of 2% HCl were added to the tubes, and left for about one hour, or as long as needed until there were no more bubbles. Subsequently, the samples were centrifuged for 10min at 3, 000 rpm and then the supernatant was pipetted off. 500µl of water was added to rinse, and the centrifugation and removal of the supernatant was repeated, three times in total. We note that the preferred method for calculus sampling now uses EDTA rather than HCl and this may have reduced the number of starches we recovered (Tromp et al., 2017). Long exposure to high concentration HCl can also damage starches. However, our previous work (Henry, 2010) has shown that low concentration HCl, at room temperature, for a short duration (less than 24 hours) does not cause significant damage to starches. While there is the possibility that HCl caused some of the damaged starches we observed (see results below), we believe our methods minimized this potential harm. Furthermore, we observed damaged starches on samples that had not undergone HCl treatment (the rinse samples, see below).

Table 1 Context of the analyzed teeth at Nancheng.

Tomb	Burial form	Age	Sex	Tooth
M9	Simple inhumation	Adolescent, <20	Indeterminate	Molar
M21	Second-tier platform burials	Adult, 20 to <40	Male	Molar
M21	Second-tier platform burials	Adult, 20 to <40	Male	Molar
M28	Simple inhumation	Adult, 20 to <40	Male	Molar
M30	Second-tier platform burials	Unable to refine	Indeterminate	Molar
M44	Second-tier platform burials	Adult, 20 to <40	Male	Molar
M52	Simple inhumation	Adult, 20 to <40	Male	Molar
M55	Simple inhumation	Adult, 20 to <40	Female	Molar
M58	Simple inhumation	Adult, >40	Male	Molar
M59	Second-tier platform burials	Adult, >40	Female	Molar
M59	Second-tier platform burials	Adult, >40	Female	Molar
M75	Second-tier platform burials	Adult, 20 to <40	Male	Molar
M80	Simple inhumation	Adult, 20 to <40	probably female	Molar

M83	Simple inhumation	Adult, 20 to <40	Female	Molar
M88	Second-tier platform burials	Unable to refine	Indeterminate	Molar
M88	Second-tier platform burials	Unable to refine	Indeterminate	Molar

The sediment sample was mixed with 5% sodium hexametaphosphate for 24 hours. After the sample was rinsed two times with distilled water, a heavy liquid solution of CsCl (density 1.8) was added to the sample to float starch grains. After centrifuging at 3,000 rpm for 10 minutes, the supernatant was carefully withdrawn and transferred into a new tube. The transferred sample was rinsed three times with distilled water, and the remaining residue was mounted on a slide for further examining.

After removing the calculus, all of the teeth were placed inside separate 50 ml beakers with distilled water to barely cover them, and shaken in an ultrasonic water bath for 10 minutes. The residues in the beakers were transferred to labeled 15 ml centrifuge tubes, centrifuged for 10min at 3,000 rpm. Then pipetted off the supernatant and mounted the remaining residue.

From all sample types, the retrieved residue was mounted in 10% glycerin and 90% water on a slide and examined under polarized and unpolarized light on a Zeiss Axioscope microscope at 400×magnification. Each starch grain was photographed, described, and documented, and the entire slide was examined. To identify the ancient starch grains, we made one-to-one comparisons with modern reference collections of economic and other plants, especially cereals, that are native to the study region and time period. Other available comparative data from published literature were also consulted (Ge et al., 2010; Wan et al., 2011a; Yang et al., 2009; Yang et al., 2010a; Yang et al., 2012; Yang and Perry, 2013; Zhang et al., 2017).

The size of starch grains is often used for identifying plant species, but previous studies have shown that cooking and other processing methods can alter the size and morphology of starch grains (Babot, 2003; Samuel, 2006; Henry et al., 2009; Messner and Schindler, 2010). We tested the extent to which different cooking methods may lead to size changes in four Poaceae plants, i.e., *Setaria italica*, *Panicum miliaceum*, *Sorghum bicolor* and *Coix lacryma-jobi*. These plant species may have been used for thousands of years in China and their starch grains were often encountered in archaeological research. The starch grains from these species overlap in shape and size, making it somewhat challenging to distinguish them based on morphological characteristics, especially when they have been processed or subjected to diagenetic processes for those ancient processed starch grains. We processed starches from these plants using a variety of different methods (Table 2), and two hundred grains of each sample were measured to obtain data on the length of the starch grains.

Table 2 Different cooking methods applied to the caryopses of four cereals.

Method	Duration	Which samples	Description
Boiled whole	1, 5, 10, 30 min	All four plants	Three milliliters of sample added to 15 ml boiling water in 25 ml beaker, boiled for certain time (boiling

Ground then boiled	1, 5, 10, 30 min	All four plants	water added during the period to prevent it from drying) Three milliliters of sample ground to a medium powder with mortar and pestle, then added to 15 ml boiling water in 25 ml beaker, boiled for certain time (boiling water added during the period to prevent it from drying)
Ground then baked	5-10 min	All four plants	Five milliliters of sample ground to a medium powder with mortar and pestle, mixed with water to form a paste, then baked in a muffle furnace at 200°C for certain time
Ground then soaked	3 days	All four plants	Three milliliters of sample ground in mortar and pestle to a medium powder, then added to 15 ml water in 25 ml beaker, lightly covered and left at room temperature

Results

Microfossil analysis

A total of 40 starch grains were retrieved from 14 out of 16 calculus samples. Another 45 starch grains were extracted from 13 teeth using the ultrasonic method. 79 of these 85 starch grains could be categorized into four broad types (Table 3), described below with details of the characteristic features. These types could be identified at either the genus or the species level. The remaining starches could not be identified in any way due to post- or pre-depositional damage that had altered the granule morphology, among which there are a few cooked starches.

Type 1: 43 starch grains with size range 11.29-25.5µm were simple, sub-rounded and polygonal overall in shape, without lamellae. The hilum was centric and faint, often traversed by deep, pronounced, Y-shaped or stellate fissures (Fig. 2a-d). Our modern reference material and previous studies all demonstrate that starch grains with polygonal shape are generally, but not always from the

grass family Poaceae (Ge et al., 2010; Yang et al., 2012; Dong et al., 2014; Tao et al., 2015);. Nevertheless, inspecting all potential candidates, the sizes of rice, common millet (*Panicum miliaceum*) and foxtail millet (*Setaria italica*) are much smaller, while the diameter of type 1 starch grains falls within the range of Job’s tears (*Coix lacryma-jobi*) and sorghum (*Sorghum bicolor*). Published papers and microscopic observation results show a proportion of starch grains from sorghum are decorated with lamellae (Li et al., 2010; Yang and Jiang, 2010b). Additionally, the origin of sorghum in China is still controversial and no scientifically-identified archaeobotanical remain has been reported (Liu et al., 2012). We therefore argue that sorghum is highly unlikely, but cannot firmly conclude that Type 1 starches are only from Job’s tears. Our processing experiments (discussed below) indicate that the size of millet increases during processing, so Type 1 starches may also represent a mix of Job’s tears, millet, and/or other Poaceae.

Type 2: This group contained 29 lenticular or discoid starch grains. They were 13.27-34.35µm in size, with centric hilum and no fissure. The extinction cross was symmetrical, with arms that widened towards the ends. Some had demonstrable lamellae (Fig. 2e-h). When rotated into side view, their lenticular shape was apparent. These starch grains clearly resemble many taxa in the Triticeae tribe. A small amount of them have crater-like depressions on surface, which is also a prominent characteristic of starch grain from the Triticeae tribe (Piperno et al., 2004; Yang and Perry, 2013). Considering that wheat was introduced into China during this time period, and that wheat macroremains were recovered at the proto-Shang site of Zhangdeng (Liu, 2012), we believe that this type of starch grains represents wheat (*Triticum aestivum*).

Type 3: Six starch grains were classified to this type, with a characteristic large size range (14.29-46.77µm). They were oval in shape, with an eccentric hilum and visible lamellae in most cases. Their extinction crosses were eccentric and bent (Fig. 2i-l). The characteristics of this type are consistent with those of a large number of tuber and root plants (Wan et al., 2011a; Li et al., 2013). Nevertheless, given that retrieved number of type 3 is too rare and our modern reference from underground storage organs is quite limited, further precise identification is not currently possible.

Type 4: One starch grain belonging to this type was recovered. It is 19.63µm in size, triangular ovate in shape, with a centric hilum and radiating fissures. Lamellae are invisible (Fig. 2m-n). These morphological features are consistent with starches from *Castanea* spp. and *Quercus* spp. (Yang et al., 2009). Due to the limited number of starch grain, type 4 is tentatively classified as tree nut, possibly from Fagaceae.

In contrast to the starches recovered from the calculus and rinse samples, only two starch grains were discovered from the adhering sediment samples. One starch grain had morphology matching that of type 1. The other one was round grain, 11µm in diameter, with no hilum and lamellae. This starch grain was damaged, showing widened extinction cross and central depression. Its features were not consistent with the four morphotypes.

Table 3 Number of different types of extracted starch grain using two sampling methods.

Sampling Method	Type1	Type2	Type3	Type4	Unidentified
Direct sampling	18	16	3	0	3
Ultrasonic water bath	25	13	3	1	3

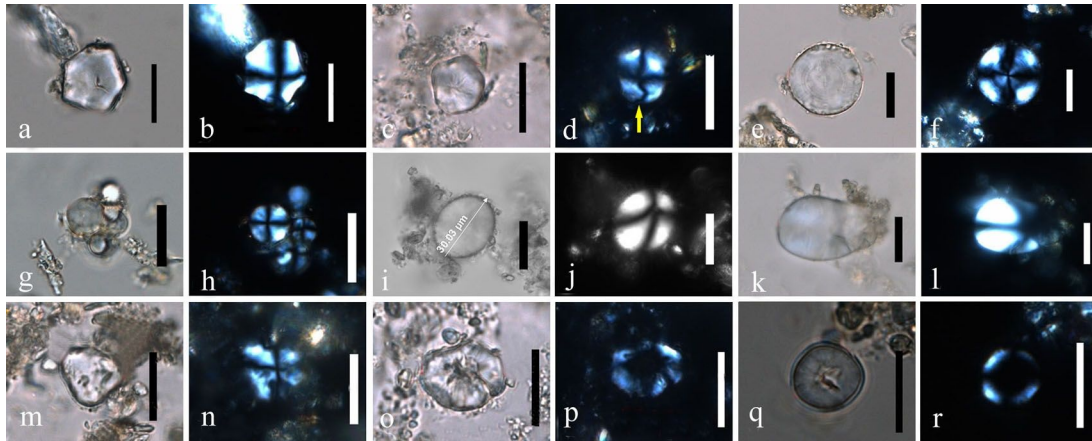


Fig.2. Starch grains extracted from human teeth at Nancheng site (each grain shown in unpolarized and polarized views). (a-b) Type 1, likely foxtail millet (*Setaria italica*); (c-d) Type 1, likely Job's tears (*Coix lacryma-jobi*), the arrow points to the Z-shaped arm, which is a typical morphological characteristics of starch from Job's tears; (e-h) Type 2, likely wheat (*Triticum aestivum*); (i-l) Type 3, possible tuber or root; (m-n) Type 4, possible tree nut; (o-r) Damaged starch grains (Scale bar= 20µm).

Cooking experiments

Using length as a means to distinguish among starch types is problematic, given the large standard deviation within species. However, our measurements on processed starches indicate that grinding and cooking cause an increase in starch size. As table 4 shows, starch grains from common millet, foxtail millet, sorghum and Job's tears increase by 6-31%, 11-53%, 5-25%, 6-15% respectively, depending on the processing method (Fig. 3). Importantly, starch grains from foxtail millet increase significantly and show obvious overlap with Job's tears starches after processing. Given the morphological similarities between these two taxa, and with the knowledge that foxtail millet was recovered during flotation at Zhangdeng, as well as being common in North China during this period (Lee et al., 2007; Liu, 2012), we expect that several of the type 1 starch grains are processed millet. Recent stable carbon and nitrogen isotopic analyses applied on human bones from Nancheng also strongly indicated that the indigenous people relied primarily on C4 food, most likely from millet agriculture (Ma et al., 2016).

Table 4 Size change of starch grain due to different cooking methods.

Species	Method	Duration	Mean length (µm)	Increase in mean length (%)	Range length (µm)	of Count
Common millet	Raw		7.92±1.25		5.31-11.59	205
Common millet	Ground then soaked	3 days	8.43±1.32	6	5.84-13.22	200
Common millet	Boiled whole	1 min	8.97±1.47	13	6.16-14.55	197
Common millet	Boiled whole	5 min	8.93±1.54	13	5.44-14.27	204
Common millet	Boiled whole	10 min	10.02±1.89	27	6.67-17.63	195
Common millet	Ground then	5-10 min	10.36±2.10	31	6.48-16.43	204

millet	baked					
Foxtail millet	Raw		9.71±1.64		5.84-17.62	204
Foxtail millet	Ground then soaked	3 days	10.80±2.58	11	6.42-22.91	202
Foxtail millet	Boiled whole	1min	11.75±2.79	21	5.34-22.26	201
Foxtail millet	Boiled whole	5 min	14.88±3.21	53	6.78-22.90	197
Foxtail millet	Ground then baked	5-10 min	12.90±3.26	33	7.37-25.54	200
Sorghum	Raw		15.76±3.80		8.14-24.54	204
Sorghum	Ground then soaked	3 days	16.61±4.32	5	7.76-28.27	210
Sorghum	Boiled whole	1 min	16.71±3.64	6	8.53-26.73	202
Sorghum	Boiled whole	5 min	17.78±3.99	13	7.97-28.03	198
Sorghum	Boiled whole	10 min	18.20±3.52	15	7.80-27.77	199
Sorghum	Boiled whole	15 min	18.71±3.96	19	10.20-29.68	199
Sorghum	Boiled whole	30min	19.64±4.70	25	10.02-31.13	204
Sorghum	Ground then baked	5-10 min	18.43±4.08	17	10.21-33.78	203
Job's tears	Raw		13.10±2.41		8.27-22.50	200
Job's tears	Ground then soaked	3 days	13.88±2.70	6	7.62-23.12	203
Job's tears	Boiled whole	1 min	14.35±2.68	10	8.07-22.42	197
Job's tears	Boiled whole	5 min	14.63±2.32	12	9.76-22.14	196
Job's tears	Boiled whole	10 min	14.51±2.78	11	9.19-23.37	202
Job's tears	Boiled whole	15 min	15.02±2.90	15	8.38-25.98	202
Job's tears	Boiled whole	30 min	14.87±2.80	14	9.71-26.48	202
Job's tears	Ground then baked	5-10 min	14.16±3.25	8	7.29-25.61	201

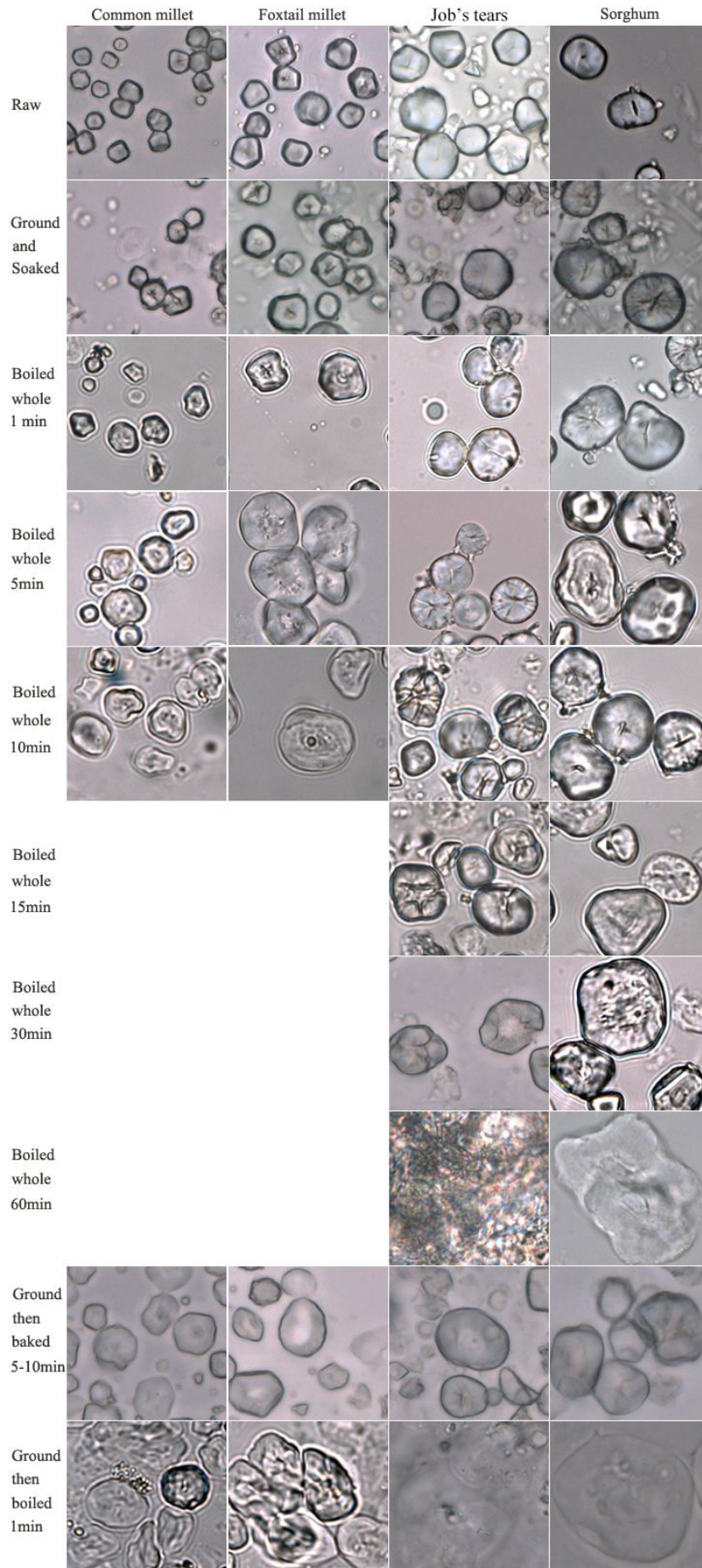


Fig.3. Different characteristic of starch grains from four Poaceae plants, both raw and after processing. Each square is 50 μm wide.

Comparisons among samples:

We anticipated that there might be differences in plant remains depending on the sex of the individuals, or on the associated tomb type. Surprisingly, the samples were remarkably heterogeneous. Most of the samples yield starches of type 1 and type 2 (Table 5), which likely record the presence of two common food sources. We ran a MANOVA test (using the `manova` function in `r`, using the “Pillai” test) to see if tomb type, sex, and the interaction between the two, influenced the number of each type of starch. Our results suggested that sex had a significant influence on the starch results (Pillai = 1.45, $p = 0.013$). This significant result was the result of finding type 4 starches only on two individuals for whom we were unable to assign sex (individuals M9 and M88). Because of the unknown sex of these individuals, we can not conclude that there is a true difference between males and females. Overall, therefore, we see no difference in plant use among the tomb types and sexes. .

Table 5 Number of different types of extracted starch grain from the analyzed teeth

Tomb	Type1	Type2	Type3	Type4	Unidentified
M9	5	2	0	1	0
M21	2	0	0	0	0
M28	4	2	1	0	0
M30	4	2	1	0	0
M44	3	5	0	0	0
M52	6	1	0	0	1
M55	0	0	0	0	1
M58	3	1	0	0	1
M59	1	6	1	0	1
M75	4	6	2	0	2
M80	2	0	0	0	0
M83	3	2	0	0	0
M88	6	2	1	1	0

Discussion

To date, there are several methods to extract microfossils from dental calculus. In most cases, the direct sampling method using a dental pick is recommended. However, it is quite usual to encounter archaeological teeth with faint dental calculus that is difficult to sample. Boyadjian and colleagues (2007) found that using an acid to wash the surface of the tooth was effective in extracting starch grains, but that the acid eroded enamel surfaces. To maximize our sample of starch grains, we both directly sampled the calculus, but also placed the teeth in an ultrasonic water bath, as is often used for the starch grain analysis of stone tools. The ultrasonic water bath method yielded 45 starch grains, even more than the direct sampling method, indicating that the water bath is an effective means to recover starch grains for study.

Meanwhile, there were only two starch grains in the sediment control samples which previously covered on the teeth. The number is much less than the amount of starch grains obtained by the ultrasonic water bath method. Therefore, contamination from the adjacent sediment, transport processes, and laboratory environment may be considered negligible. However, we were unable to

examine other contamination control samples from the site, such as animal bone surfaces, animal calculus or other materials. Only by including a larger number of control samples could we more firmly suggest that the water wash samples do not represent contamination.

Recent studies have shown that the starch grains recovered from calculus do not represent the entire diet of an individual, and that analysis should therefore be at a population level (Leonard et al., 2015; Power et al., 2015). Nevertheless, the assemblage of starch grains that we recovered were remarkably consistent, both with each other and with our expectations of what foods would have been eaten based on other lines of evidence.

Most of our samples included starches from wheat. While wheat was originally domesticated in the Near East, it has been cultivated in China for more than 4,000 years (Zhao et al., 2015). Early findings of wheat remains were predominantly from the northwestern part of China and the lower Yellow River region. For example, caryopses of *T. aestivum* were discovered in the Gumugou tombs (about 3,800 BP) and coeval Xiaohe Cemetery (about 3,600 BP) (Yang et al., 2014; Zhang et al., 2017), and an older charcoal date associated with wheat was reported by Li et al. (2007) from Xishanping. In eastern China, wheat remains were also unearthed from several archaeological sites dating to Shangdong Longshan culture (Zhao, 2004; Crawford et al., 2005). To date, the only archaeobotanical wheat remains belonging to proto-Shang culture were discovered from Zhangdeng (Liu, 2012). However, wheat remains were recovered by flotation from archaeological sites dating to the contemporaneous Erlitou culture, which indicate that wheat had been transported into the Central Plains during this time period (Zhao and Fang, 2007; Zhao, 2015). Archaeological evidence has indicated that the proto-Shang culture was strongly influenced by the Erlitou culture when the former moved southwards. In addition, as we mentioned previously, the subsistence strategies of proto-Shang population changed from nomadism to agriculture during their migration. Along with the macrofossil evidence from Zhangdeng, the finding of wheat starches in our study further verified that wheat was consumed and cultivated by proto-Shang people. In the future, more archaeobotanical work should be applied in order to solve the question about the source of wheat agriculture in proto-Shang culture and the extent of the influence of the Erlitou culture, which was the preceding dynasty in the Central Plains.

Starches consistent with Job's tears (our Type 1) were the most common ones in the sample from Nancheng. It is generally considered that Job's tears was domesticated in China and that this plant has been used for thousands of years. To date, the earliest evidence of Job's tears was unearthed from Shangshan (9400-8000 calBP) and Xiaohuangshan (9000-7000 cal BP) (Liu et al., 2010; Yao et al., 2016; Zuo et al., 2017). As archaeobotanical research has recently increased in China, more and more Job's tears remains have been discovered, at Kuahuqiao (8000-7000 cal BP), Hemudu (7000-6500 cal BP), Baodun (4700-4000 cal BP), Sampula Cemetery (2100-1800 cal BP) and several typical Late Neolithic sites (5000-4000 cal BP) located in the middle reaches of the Ganjiang River (Yu and Xu, 2000; Jiang et al., 2008; Yang and Jiang, 2010b; Wang et al., 2012; Guedes et al., 2013). At all of these sites, the seeds were collected for consumption, except at Sampula Cemetery where they were used as beads. Our study further emphasizes the importance of this plant as a food source over a long period of time by the inhabitants of North China.

There is no doubt that the inhabitants of Nancheng processed and cooked their food. Studies have illustrated that gelatinized starch grains tend to be damaged and degraded, and that many partially gelatinized starches tend to increase in size relative to the native state (Babot, 2003; Samuel, 2006; Henry et al., 2009; Messner and Schindler, 2010; Gong et al., 2011; Wang et al., 2011b; Liu et al., 2013). This obviously will cause some confusion and uncertainty on the identification of these starch

grains. In view of that the morphology of Poaceae starch grains are rather difficult to distinguish, we chose four plant species of this kind and applied systematic simulated cooking experiments. Our results indicated that, when exposed to similar cooking methods, the starch grains from foxtail millet and common millet show a greater change in size than those from the other examined plants. Importantly, foxtail millet increased to the point that it cannot be separated from the Job's tears starches on the basis of size. Furthermore, recent stable isotope analysis of human bones from Nancheng indicated that a diet based mainly on C4 food, likely millet (Ma et al., 2016). Considering the macrobotanical results from coeval Zhangdeng site and the general situation of agriculture in North China during this period of time, it is reasonable to conclude that the spectrum of plant consumption of proto-Shang people also included foxtail millet, and that some of our Type 1 starches come from this plant. The results of this experiment with modern cereal plants shed new light on how different processing methods affect starch size, further improving our understanding of ancient starch grain identification. This study is a powerful reminder that researchers must use caution when trying to identify processed starch grains extracted from archaeological remains. The size of these starches may have been significantly altered, and identification should not be based on size alone, particularly for Poaceae cereals.

Plant roots and tubers have played an indispensable role in diet of Chinese people currently and in the past. Nevertheless, it is quite difficult to find macrobotanical remains from tubers in archaeological sites owing to their fragile structure. Fortunately, many roots and tubers are rich in starch and therefore starch grain analysis has the potential to reveal much about this currently under-studied food resource. In the flotation work in Zhangdeng, no root or tuber remains were discovered. Our study provided the first archaeobotanical evidence for the consumption of this kind of plants by proto-Shang people. Additionally, both modern and archaeological sample investigation indicated that root and tuber starches were more susceptible to damage due to cooking owing to their physical and compositional properties compared with cereal starch (Crowther, 2012). This fragility might explain why we found so few starches of this type, but the finding of few starches should not be interpreted to mean that roots and tubers were unimportant foods.

As we noted before, written records and archaeological studies revealed that the subsistence strategy of proto-Shang people had transformed from nomadic to agricultural during the process of their migration southwards. Combined with other archaeobotanical and stable isotope evidence, our study indicated that grass seeds, including millets, Job's tears and wheat played important part in the spectrum of plant consumption of proto-Shang people. It is generally believed that agriculture is one of the most important factors promoting the formation of ancient civilization. Thus, the adopting of agricultural subsistence probably facilitated the development of the Shang tribe and the later establishment of Shang dynasty. Furthermore, Zhao (2011) deemed that multi-crop farming could maximize the agricultural production and mitigate the effects of natural disaster. This special pattern of agricultural development had some corresponding relationship with the formation of Chinese civilization. To clarify this issue clearly, more archaeobotanical, zooarchaeological and stable isotopic analyses should be applied on the investigation of proto-Shang culture.

Conclusion

Microfossil analysis of dental calculus is still in its emerging stage in China, but this and other recent studies have shown the potential of this method, especially for those archaeological sites where flotation was not applied and/or human bones were well preserved. At Nancheng, the finding of starches from wheat, Job's tears, and possibly foxtail millet suggests that these three species played important role in the diet of proto-Shang people. Additionally, starches that likely represent roots and

tubers were also found, and although the number was small, we should not underestimate the potential importance of these foods. These findings show that proto-Shang people had a subsistence economy based on a variety of plant foods and practiced food production. Indeed, such a rich variety of plant food resources no doubt greatly improved the living standards of the proto-Shang people and also provided the impetus for the formation and prosperity of Shang dynasty. Finally, the results of our cooking experiment highlight the need to take into account the enlargement of size when identifying different species of cooked or processed starch grains. This is particularly significant for four Poaceae cereals with polygonal starches, namely foxtail millet, common millet, Job's tears, and sorghum.

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