Research Topics

Ancient starch remains and prehistoric human subsistence

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Ancient starch remains and prehistoric human subsistence

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Editorial: Ancient starch remains and prehistoric human subsistence

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Editorial on the Research Topic
Ancient starch remains and prehistoric human subsistence

1 Introduction

In recent years, micro-botanical fossil residue research in archaeology and paleoanthropology has grown in popularity (Guan et al., 2014). Invoking evidence from plant microfossils, such as phytoliths and starch granules, in association with various archaeological or paleontological remains (e.g., Barber, 2020; Liu et al., 2019b; Nava et al., 2021; Prebble et al., 2019; Scott et al., 2021; *etc.*), researchers have successfully reconstructed both local and regional plant resource utilization patterns and identified a variety of economic plants that have been processed in a number of Pleistocene and Holocene contexts.

One of the most prevalent plant compounds in the world is starch. The starch granule is therefore considered as essential plant microfossil that has been closely related to humans from prehistoric times (Teaford and Ungar, 2000; Summerhayes et al., 2010; Ungar, 2017). As a form of energy storage, starch is deposited in granules in nearly all green plants and numerous plant tissues and organs, such as leaves, roots, shoots, fruits, grains, and stems (Preiss, 2004). It is composed of discrete granules whose size, shape, morphology, chemical content, and supramolecular structure are dependent on the botanical source (Bertolini, 2010). The generation of a starch granule begins at a place known as the hilum, where successive layers of lamellae are deposited. In several species, the hilum is surrounded by fissures of varying forms (Gott et al., 2006). These visible characteristics, which differ between plant taxa, serve as the foundation for studying ancient starch granules.

Over the past 2 decades, many case studies have examined ancient starch remnants to better understand human behavior and the evolution of the human diet. Researchers extract starch granules from teeth and artifacts found at archaeological sites to investigate ancient carbohydrate diets (e.g., Hardy et al., 2016; Lu et al., 2005; Perry et al., 2007; Piperno et al., 2004; Scott et al., 2021; etc.). However, the process of starch granule analysis can be complicated due to issues like decomposition, preservation, and damage (e.g., García-

Granero, 2020; Haslam, 2004; Henry et al., 2016; Hutschenreuther et al., 2017; Li et al., 2020; Ma et al., 2019; etc.). Identifying the botanical origin of unidentified starch granules is a crucial step, and researchers have built a database of contemporary starch to aid in these identifications (Yang and Perry, 2013; Liu et al., 2014; Arráiz et al., 2016; Mercader et al., 2018a; Liu et al., 2019a; Wan et al., 2020). Starch analysis in archaeology has been challenging due to difficulties in botanical identification. Geometric morphometric analyses of starch granules may yield false positives when the reference Research Topic are small, decreasing the likelihood of identifying starch granules to the species level. (Arráiz et al., 2016; Mercader et al., 2018b). Contamination is also a concern, and it is critical to document contamination in ancient starch laboratories to develop more reliable methods for future research (Crowther et al., 2014).

The Research Topic *Ancient Starch Remains and Prehistoric Human Subsistence* includes 18 original research pieces on advanced techniques, regional studies, and alcoholic beverage consumption, as well as laboratory control and modern starch Research Topic. These works provide updated information and unique viewpoints, enhancing the field's knowledge and serving as a valuable resource for future studies.

2 Prehistoric starch remains, early agriculture, and foodway

After a long era of hunting and gathering, humans developed agriculture, which was a crucial turning point in the history of civilization. The study of the earliest agricultural practices and food production has been extensively researched by scholars as early as one hundred years ago (Candolle, 2011). Starch analysis, although only developed during the recent 20 years, is a key tool for understanding regional patterns of plant domestication and crop origins and spread worldwide. Case studies related to early agricultural practice have been presented in this issue.

Zhang et al.' s study of the Dingsishan Site in the Lingnan region of South China reveals that, during the earliest phase of the Holocene, ancient populations practiced a foraging-based subsistence, with wild plant foods comprising the majority. Huan et al. works also show the selection of plant food resources in South China during 7.3–6.8 ka B.P. In the study by Deng et al., the emergence of agriculture on a south China coast site was dated to 4,800–4,800 cal. BP, with the cultivation of rice and foxtail millet, sheds light on the study of agriculture's Southward dispersal and supports the universality of mixed farming in Southern coastal China.

In China, the lack of archaeobotanical investigations in the Yiluo River Basin hinders our knowledge of the interaction between agriculture and society in China. The study by Yang et al. indicates that millet was the most important crop in the late Yangshao Culture, followed by rice, which became more significant in the following Longshan Culture. During the Qijia period (4,400–3,100 B.P.), the introduction of dry agriculture and globalization of food affected the prehistoric human diet in Northwest China. The study by Ma et al. contributes to our understanding of the subsistence of the Qijia Culture and prehistoric food globalization, which is essential for appreciating East-West Asian connections during the Neolithic and Bronze periods.

Understanding when and how agriculture altered landscapes are essential for the study of human survival strategies and biodiversity. Zhang et al. discovered evidence that farmed rice existed on the Chengdu Plain 7,500 years ago, and that more advanced rice farming became the main survival strategy around 4,200 years ago, having a substantial impact on the local flora. Even in the more densely populated Yellow River watershed, deforestation and the consumption of cereal crops may have happened after 6,000 cal a BP, as suggested by He et al. and Li et al. in this issue.

The study by Wang et al. in the Cai Beo site in Ha Long Bay, northeastern Vietnam's coastal region, uncovered evidence that hunter-gatherers utilized a range of plants, including taros, yams, and acorns, as early as 7,000–6,000 years before present. The study proves the significance of roots and tubers in the ancient subsistence economy of Southeast Asia and confirms one of the earliest known findings of rice in Mainland Southeast Asia between 4,500 and 4,000 years before present.

García-Granero et al. investigation suggests that animal lipids, particularly non-ruminant fats, were predominantly processed in ceramic containers. The investigations also uncovered indications of plant processing, including grains, pulses, and underground storage organs. Despite challenges in conserving and interpreting archaeological material, the study offers a thorough account of past foodways in the region.

The study by Salazar Garca et al. on the diet of Iberian Cardial Neolithic people at the Cova Bonica site reveals a terrestrial C3-based diet with indications of cereal consumption and other plant items. The study sheds light on the Neolithization process in Iberia and transcends the limitations of conventional archaeobotanical and archaeozoological techniques.

3 Early brewing and alcoholic beverages

Recent studies have uncovered evidence of alcohol production and consumption during northern China's Neolithic period, particularly in the Yangshao Culture (ca. 7,000–5,000 cal. BP). Pottery samples from the Yangshao Culture site of Qingtai were analyzed by Liao et al. revealing a mixed filtered alcoholic beverage fermented with fruit and/or honey, and a shift from communal to individual drinking habits in the late Yangshao Culture. In China's Bronze Age, bronze ritual vessels like *gui*, *he* pitchers, and *jue* cups were symbols of high social status and were likely used in ritual feasts. He Y. et al., 2022 study found evidence of fermented beverages made with *qu* starter, emphasizing social status and coinciding with increased social differentiation during early state formation.

4 Functions of neolithic artifacts

Before 4,000 years ago, grooved clay vessels spread to central China from the Yangtze River, where they originated 6,000 years ago. The study by Wang et al. discovered evidence that these vessels were used to grind geophytes and dehull grain seeds, although the

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function of these vessels remains unknown. Similarly, Liu et al. on *li* tripods from Northeast China revealed that they were used for cooking and that the starch granules discovered on them belonged to millets, Triticeae, and tubers. The phytoliths found from the tripods suggested that these plants were utilized in the Chifeng region during the Bronze Age. These studies provide vital information about ancient Chinese people's diets and plant resource management.

5 Research method exploitation

In this Research Topic, Li et al. examined data to determine the possible significance of starch-rich woody plants as food sources in ancient South subtropical China. This work offers information on the role of non-tuberous woody plants as sources of carbohydrates for prehistoric societies in this region. Louderback et al. established diagnostic characteristics for recognizing the starch granules of major North American plant families and developed a dichotomous identification key. This has worldwide significance, as these families have had nutritional significance since prehistory. To investigate starch retention in dental calculus, the experimental model of Bartholdy and Henry introduced biofilms to known amounts of dietary starches. The study indicated that dental calculus provides a limited and skewed view of the original dietary consumption of starches, but the model is effective for verifying methodologies and biases in dental calculus studies pertaining to nutrition.

6 Further perspectives

In recent years, the study of ancient starch has made great progress due to the introduction of new technology and analytical techniques. As the field continues to evolve, several opportunities exist for further research and development. It has been determined that simple picture comparison cannot meet the requirements for accuracy and precision. Therefore, the geometric morphology approach has become a well-recognized, subjectivity-free method of discrimination (Coster and Field, 2015; Chen, 2017; Wan et al., 2020). Using machine learning algorithms such as *Artificial Neural Networks*, *Random Forests*, *Support Vector Machines*, and other prevalent techniques, the enormous geometric morphological data matrix can be evaluated and modeled with greater ease, resulting in more accurate identification of ancient starch granules. Zhang et al.'s work in this Research Topic applies the approach to archaeological case studies and demonstrates its validity for identifying ancient starch. Several experimental articles have been published, however they will not be covered here (e.g., Zhang et al., 2021b, etc.).

Future research on ancient starch analysis should broaden its focus to encompass various time periods and regions, with greater intercultural communication to investigate potential trading and exchange across early civilizations. Discoveries in this field could potentially uncover global patterns and trends in human subsistence and social development. Furthermore, ancient starch analysis has the potential to contribute to contemporary food security and sustainability issues, using old methodologies and expertise. In conclusion, expanding and developing ancient starch analysis may offer valuable insights into the lifestyles, cultural activities, and meals of past civilizations.

Author contributions

The three authors jointly organized and edited this Research Topic, making the publication of this Research Topic possible. The main author of the editorial is the first author YG, and the other two authors have put forward constructive comments and suggestions on it.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Arráiz, H., Barbarin, N., Pasturel, M., Beaufort, L., Domínguez-Rodrigo, M., and Barboni, D. (2016). Starch granules identification and automatic classification based on an extended set of morphometric and optical measurements. *J. Archaeol. Sci. Rep.* 7, 169–179. doi:10.1016/j.jasrep.2016.03.039

Barber, I. G. (2020). Further wet-taro evidence from Polynesia's southernmost Neolithic production margins. *Proc. Natl. Acad. Sci.* 117 (3), 1257–1258. doi:10.1073/pnas.1918374117

Bertolini, A. C. (2010). Trends in starch applications. In *Starches: Characterization, properties, and applications*. A. C. Bertolini, 1–20. Boca Raton, London, New York: CRA Press.

Candolle, A. D. (2011). *The origin of cultivated plants*. Cambridge: Cambridge University Press.

Chen, X. (2017). *Application of geometric morphometry to aquatic organisms*. Beijing: Science Press.

Coster, A. C. F., and Field, J. H. (2015). What starch grain is that? – a geometric morphometric approach to determining plant species origin. *J. Archaeol. Sci.* 58, 9–25. doi:10.1016/j.jas.2015.03.014

Crowther, A., Haslam, M., Oakden, N., Walde, D., and Mercader, J. (2014). Documenting contamination in ancient starch laboratories. *J. Archaeol. Sci.* 49, 90–104. doi:10.1016/j.jas.2014.04.023

García-Granero, J. J. (2020). Starch taphonomy, equifinality and the importance of context: Some notes on the identification of food processing through starch grain analysis. *J. Archaeol. Sci.* 124, 105267. doi:10.1016/j.jas.2020.105267

García-Granero, J. J., Suryanarayan, A., Cubas, M., Craig, O. E., Cárdenas, M., Ajithprasad, P., et al. (2022). Integrating lipid and starch grain analyses from pottery vessels to explore prehistoric foodways in northern Gujarat, India. *Front. Ecol. Evol.* 10. doi:10.3389/fevo.2022.840199

Gott, B., Barton, H., Samuel, D., and Torrence, R. (2006). "Biology of starch," in *Ancient starch research*. Editors R. Torrence and H. Barton (California: Left Coast Press Inc).

Guan, Y., Pearsall, D. M., Gao, X., Chen, F., Pei, S., and Zhou, Z. (2014). Plant use activities during the upper paleolithic in east eurasia: Evidence from the shuidonggou site, northwest China. *Quat. Int.* 347, 74–83. doi:10.1016/j.quaint.2014.04.007

Hardy, K., Radini, A., Buckley, S., Sarig, R., Copeland, L., Gopher, A., et al. (2016). Dental calculus reveals potential respiratory irritants and ingestion of essential plant-based nutrients at Lower Palaeolithic Qesem Cave Israel. *Quat. Int.* 398, 129–135. doi:10.1016/j.quaint.2015.04.033

Haslam, M. (2004). The decomposition of starch grains in soils: Implications for archaeological residue analyses. *J. Archaeol. Sci.* 31 (12), 1715–1734. doi:10.1016/j.jas.2004.05.006

He, Y., Zhao, H., Liu, L., and Xu, H. (2022b). Brewing and serving alcoholic beverages to erlitou elites of prehistoric China: Residue analysis of ceramic vessels. *Front. Ecol. Evol.* 10. doi:10.3389/fevo.2022.845065

Henry, A. G., Debono Spiteri, C., Büdel, T., Hutschenreuther, A., Schmidt, S., and Watzke, J. (2016). Methods to isolate and quantify damaged and gelatinized starch grains. *J. Archaeol. Sci. Rep.* 10, 142–146. doi:10.1016/j.jasrep.2016.09.003

Hutschenreuther, A., Watzke, J., Schmidt, S., Büdel, T., and Henry, A. G. (2017). Archaeological implications of the digestion of starches by soil bacteria: Interaction among starches leads to differential preservation. *J. Archaeol. Sci. Rep.* 15, 95–108. doi:10.1016/j.jasrep.2017.07.006

Li, W., Pagán-Jiménez, J. R., Tsoraki, C., Yao, L., and Van Gijn, A. (2020). Influence of grinding on the preservation of starch grains from rice. *Archaeometry* 62 (1), 157–171. doi:10.1111/arcm.12510

Liu, L., Duncan, N. A., Chen, X., and Cui, J. (2019a). Exploitation of job's tears in Paleolithic and Neolithic China: Methodological problems and solutions. *Quat. Int.* 529, 25–37. doi:10.1016/j.quaint.2018.11.019

Liu, L., Ma, S., and Cui, J. (2014). Identification of starch granules using a two-step identification method. *J. Archaeol. Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

Liu, L., Wang, J., Levin, M. J., Sinnott-Armstrong, N., Zhao, H., Zhao, Y., et al. (2019b). The origins of specialized pottery and diverse alcohol fermentation techniques in Early Neolithic China. *Proc. Natl. Acad. Sci.* 116 (26), 12767–12774. doi:10.1073/pnas.1902668116

Lu, H., Yang, X., Ye, M., Liu, K.-B., Xia, Z., Ren, X., et al. (2005). Millet noodles in late neolithic China. *Nature* 437 (7061), 967–968. doi:10.1038/437967a

Ma, Z., Perry, L., Li, Q., and Yang, X. (2019). Morphological changes in starch grains after dehusking and grinding with stone tools. *Sci. Rep.* 9 (1), 2355. doi:10.1038/s41598-019-38758-6

Mercader, J., Abtosway, M., Bird, R., Bundala, M., Clarke, S., Favreau, J., et al. (2018a). Morphometrics of starch granules from sub-saharan plants and the taxonomic identification of ancient starch. *Front. Earth Sci.* 6 (146). doi:10.3389/feart.2018.00146

Mercader, J., Akeju, T., Brown, M., Bundala, M., Collins, M. J., Copeland, L., et al. (2018b). Exaggerated expectations in ancient starch research and the need for new taphonomic and authenticity criteria. *FACETS* 3 (1), 777–798. doi:10.1139/facets-2017-0126

Nava, A., Fiorin, E., Zupancich, A., Carra, M., Ottoni, C., Di Carlo, G., et al. (2021). Multipronged dental analyses reveal dietary differences in last foragers and first farmers at Grotta Continenza, central Italy (15,500–7000 BP). *Sci. Rep.* 11, 4261. doi:10.1038/s41598-021-82401-2

Perry, L., Dickau, R., Zarrillo, S., Holst, I., Pearsall, D. M., Piperno, D. R., et al. (2007). Starch fossils and the domestication and dispersal of chili peppers (Capsicum spp. L.) in the Americas. *Science* 315 (5814), 986–988. doi:10.1126/science.1136914

Piperno, D. R., Weiss, E., Holst, I., and Nadel, D. (2004). Processing of wild cereal grains in the Upper Palaeolithic revealed by starch grain analysis. *Nature* 430, 670–673. doi:10.1038/nature02734

Prebble, M., Anderson, A. J., Augustinus, P., Emmitt, J., Fallon, S. J., Furey, L. L., et al. (2019). Early tropical crop production in marginal subtropical and temperate Polynesia. *Proc. Natl. Acad. Sci.* 116 (18), 8824–8833. doi:10.1073/pnas.1821732116

Preiss, J. (2004). "Plant starch synthesis," in *Starch in food: Structure, function and applications*. Editor A.-C. Eliasson (Boca, Raton, Boston, New York, washington DC: CRC Press).

Scott, A., Power, R. C., Altmann-Wendling, V., Artzy, M., Martin, M. A. S., Eisenmann, S., et al. (2021). Exotic foods reveal contact between South Asia and the Near East during the second millennium BCE. *Proc. Natl. Acad. Sci.* 118 (2), e2014956117. doi:10.1073/pnas.2014956117

Summerhayes, G. R., Leavesley, M., Fairbairn, A., Mandui, H., Field, J., Ford, A., et al. (2010). Human adaptation and plant use in highland new Guinea 49,000 to 44,000 Years ago. *Science* 330, 78–81. doi:10.1126/science.1193130

Teaford, M. F., and Ungar, P. S. (2000). Diet and the evolution of the earliest human ancestors. *Proc. Natl. Acad. Sci.* 97 (25), 13506–13511. doi:10.1073/pnas.260368897

Ungar, P. S. (2017). *Evolution's bite: A story of teeth, diet, and human origins*. New Jersey: Princeton University Press.

Wan, Z. W., Lin, S. P., Ju, M., Ling, C. H., Jia, Y. L., Jiang, M. X., et al. (2020). Morphotypological analysis of starch granules through discriminant method and application in plant Archaeological samples. *Appl. Ecol. Environ. Res.* 18, 4595–4608. doi:10.15666/aeer/1803_45954608

Yang, X., and Perry, L. (2013). Identification of ancient starch grains from the tribe Triticeae in the North China Plain. *J. Archaeol. Sci.* 40 (8), 3170–3177. doi:10.1016/j.jas.2013.04.004

Zhang, X., Zhu, X., Hu, Y., Zhou, Z., Olsen, J. W., and Guan, Y. (2021b). Ancient starch remains reveal the vegetal diet of the neolithic late dawenkou culture in jiangsu, east China. *Front. Ecol. Evol.* 9 (554). doi:10.3389/fevo.2021.722103

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ORIGINAL RESEARCH
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Neolithic Rice Cultivation and Consequent Landscape Changes at the Baodun Site, Southwestern China

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Identifying when agricultural expansion has occurred and how it altered the landscape is critical for understanding human social survival strategies as well as current ecological diversity. In the present study, phytolith records of three profiles from the Baodun site area were dated to the period from 7,500 to 2,500 aBP by optically stimulated luminescence and ^{14}C dating, providing the first evidence that the Asian cultivated rice (*Oryza sativa*) progenitor was distributed in the Chengdu Plain as early as 7,500 aBP. The percentage of rice bulliform with ≥9 scales and the concentration of rice phytoliths sharply increased by approximately 4,200 aBP, suggesting that rice cultivation occupied a dominant position in survival strategy no later than approximately 4,200 aBP, which might be driven by climate deterioration in eastern China. The results further showed that the proportion of Bambusoideae phytoliths increased synchronously with the increase in the proportion of rice phytoliths, suggesting that the vegetation structure near the site was changed intentionally as a consequence of increasing rice agricultural activity since 4,200 aBP. The present study contributes to a deeper understanding of the distribution of wild rice and rice farming throughout the Baodun culture in the Chengdu Plain, and it also provides a glimpse of how humans intentionally changed the vegetation landscape on a local scale.

Keywords: phytolith, agriculture, Oryza sativa, domestication, bambusoideae, vegetation, luminescence dating, chengdu plain

INTRODUCTION

Agricultural expansion is one of the major drivers of human culture development (Gowdy and Krall, 2014). Reconstructing the time of the spread of domesticated crops can help us understand the climatic and other environmental factors that governed the range of expansion of these crop species (Gutaker et al., 2020), as well as the relationship between farming activities and the consequent landscape changes (Ellis, 2011; Woodbridge et al., 2014; Zheng et al., 2021).

Rice (*Oryza sativa* L.) is a major staple crop for half of the global population (Wang et al., 2018). It is a highly diversified crop cultivated in a wide ecological range, spanning tropical and temperate zones of Asia, from permanent to seasonal wetlands (Fuller et al., 2010; Fuller et al., 2011). However, the distribution of wild rice (*Oryza rufipogon* and/or *O. nivara*) in China remains unclear. The reports of possible wild rice distribution can only be traced back to the Tang and Song Dynasties (You, 1987), while evidence of an earlier distribution can only be obtained from agroclimatic studies (Fuller et al., 2010).

While the origins of rice have been the focus of intensive study, less attention has been paid to its spread after domestication, especially from its cradle Middle-Lower Yangtze to southwest China, which contains a variety of ecosystems and diverse landscapes of the Yunnan-Guizhou Plateau and the low lying Chengdu Plain (d'Alpoim Guedes et al., 2013). There is a further lack of description of the process of landscape modification by rice farming among earlier small-scale societies, as evidence of these processes is sparse (Pavlik et al., 2021).

The Chengdu Plain has a suitable climate for rice cultivation which attracted earlier rice farming populations that widely occupied the area (Sun, 2009; Huang et al., 2017). It is believed that the introduction of rice farming into the Chengdu Plain occurred relatively late, in the time of the Baodun culture (4,600–3,700 cal aBP) from the Middle-Lower Yangtze (Jiang et al., 2002; Jiang et al., 2011; Qin, 2012; d'Alpoim Guedes and Wan, 2015), which was supported by increasing archaeological (Chen et al., 2015) and DNA evidence (Castillo et al., 2016). The development of rice farming in the Chengdu Plain has influenced the regional landscape since the late Neolithic and played a significant role in the formation of prehistoric cultures in Southwestern China, such as Baodun and Sanxingdui cultures (Chen et al., 2015). Although recent studies have outlined the time that rice arrived the plain (d'Alpoim Guedes et al., 2013), however, because of the lack of comparable chronological records from natural deposits and culture layers, the development of rice agriculture and its impact on vegetation are still unclear. In particular, whether wild rice spread into the Chengdu Plain during the Holocene has not been clarified to date.

Phytoliths can be used not only to identify individual species but also to reconstruct the process of crop domestication in order to reveal the intensity of agricultural activities (Piperno, 2006). Specifically, the proportion of bulliform phytoliths with ≥9 fish scale patterns largely differ between wild rice habitats and cultivated rice paddies (Huan et al., 2018; Huan et al., 2020). Bulliform phytoliths can be used to assess the degree and rate of rice domestication to some extent (Huan et al., 2015; Huan et al., 2018). Reconstruction of the quantitative relationship between crop domestication and natural vegetation changes in the same profiles of the same site can provide new evidence for human impact on the ecological environment.

Here, we reported the results of optically stimulated luminescence (OSL) and radiocarbon dating of phytoliths derived from archaeological deposits of the Baodun site approximately 4,500–2,500 cal aBP and a successive natural deposit profile that extends to 7500 aBP in the Chengdu Plain. Based on phytolith assemblage identification, this study revealed the earliest record of wild rice in the Chengdu Plain to date and established a complete rice domestication sequence combined with natural plant changes, which provides new evidence for understanding how humans intentionally influenced their environment.

MATERIALS AND METHODS

Regional Setting

The Chengdu Plain, which covers an area of approximately 8,400 km^2, is the largest fluvial plain in Southwestern China (Liang et al., 2014; Huang et al., 2019a) (**Figure 1A**). It is a composite alluvial plain formed by the Minjiang River, Tuojiang River, and its tributaries in the Northwest Sichuan Plateau. The plain extends from northeast to southwest, with an altitude of approximately 400–750 m, and belongs to the subtropical monsoon climate (Liu et al., 2004). The average annual temperature is 15–16 °C, and the average annual precipitation is 1,000–1,300 mm (Luo et al., 2008). At present, this area is an important rice farming area in Southwestern China.

The chronological order of Neolithic cultures in the Chengdu Plain is: 1) the Baodun culture (4,600–3,700 aBP), which is equivalent to the Longshan period of the Central Plains (Huang et al., 2019b); 2) the Sanxingdui culture (3,700–3,000 aBP), an important archaeological culture after the Baodun culture and is equivalent to the fourth phase of the Erlitou culture in the Central Plains and to the second phase of Yinxu, represented by the Sanxingdui site in Guanghan (Liu et al., 2004) (**Figure 1**); and 3) the Shierqiao culture (3,200–2,600 aBP), from the early Western Zhou Dynasty to the early Spring and Autumn period, represented by the Shierqiao and Jinsha sites (Li et al., 1987; Zhu et al., 2004).

The Baodun culture (4,600–3,700 aBP), which is named by the site of Baodun, is currently the earliest archaeological culture found in the Chengdu Plain in Southwestern China. The Baodun site (E103°45′, N30°26′, 472–474 m) is located in Baodun village, Xinping Town, which is ~5 km northwest of Xinjin County, Chengdu City, Sichuan Province (**Figure 1**). The site is currently the biggest prehistoric site that characterises Baodun culture.

Sample Collection

In the present study, we analysed 105 phytolith samples from three profiles. Among the profiles, two were from excavations T1217 and T2250, and the third was from natural deposits beneath the oldest culture layers of T1215. All three profiles had comparable stratigraphic sequences.

Deposits from the first profile (T1217, 130 cm in thickness) could be divided into 12 layers from bottom to top according to the structure of the stratigraphy, soil colour, and archaeological remains: 1) layers 12–11 (190–170 cm), grey-green clay with brown soil blocks and Fe-Mn nodules, transition layers from natural deposits to the early Baodun culture; 2) layers 10–9 (170–123 cm), brown clay with Fe-Mn nodules, middle to late Baodun culture; 3) layers 8–5 (123–70 cm), grey-brown clay, Han Dynasty; 4) layers 4–3 (70–41 cm), brown clay, Tang-Song Dynasty; 5) layer 2 (41–33 cm), brown clay, Ming-Qing Dynasty; 6) layer 1 (33–0 cm), modern cultivated layer. Four samples were taken at 5 cm intervals in the 12th layer, 30 samples were taken at 2 cm intervals in the 11th–8th layer, 16

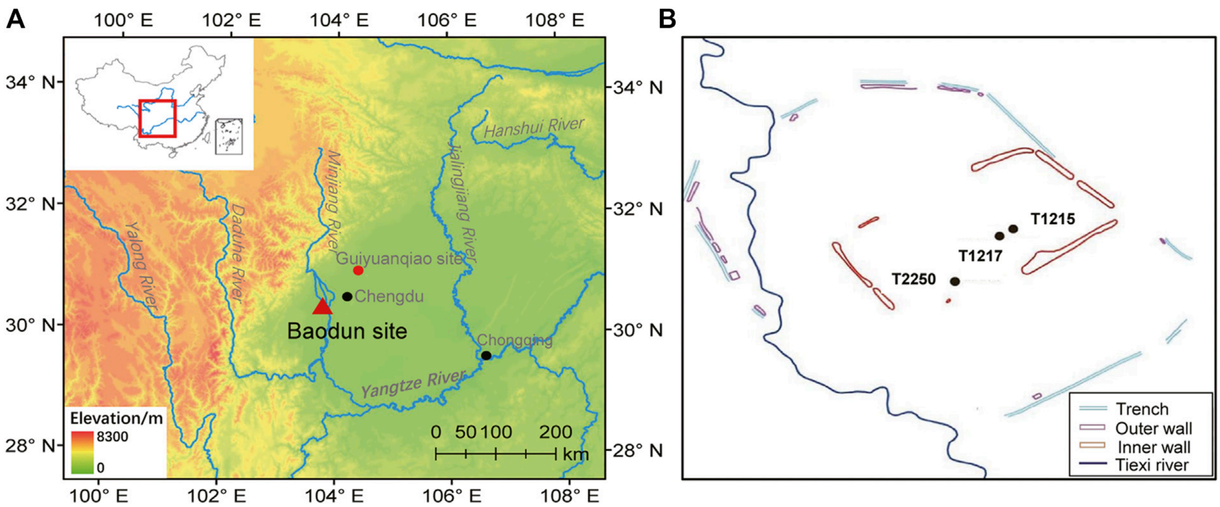

FIGURE 1 | (A) Location of the study site, and **(B)** sampling positions of the three profiles in the Baodun site. The black circles in (A) indicate modern cities, and the red circle indicates the archaeological site in this study.

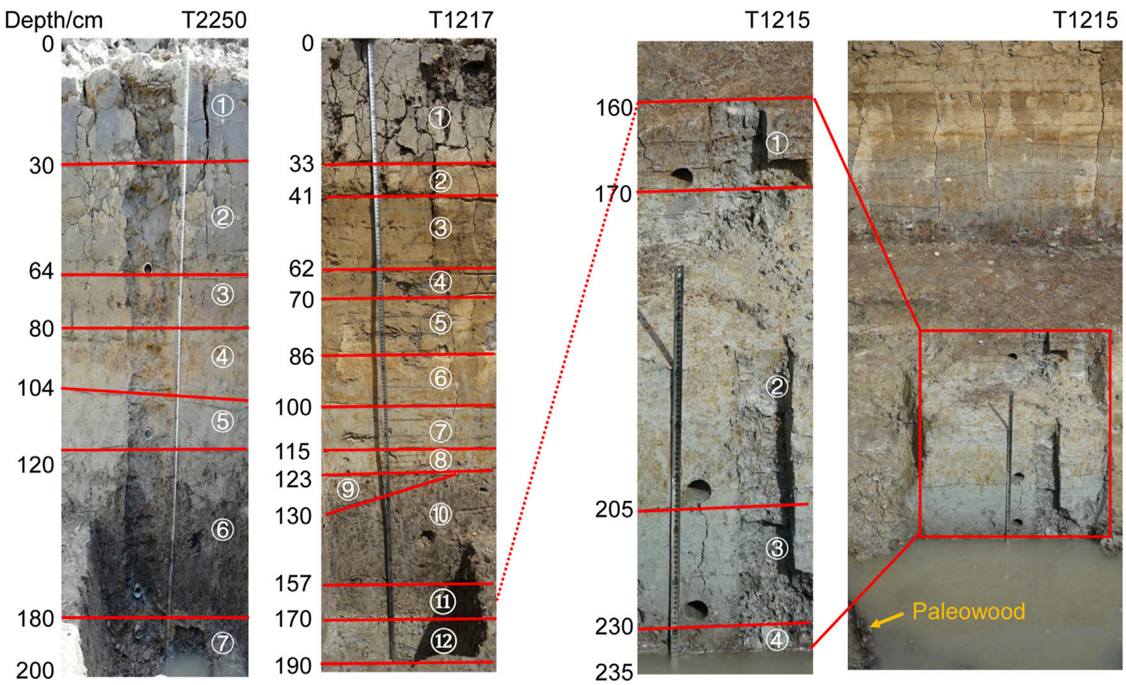

FIGURE 2 | Stratigraphic sequences of the three sampling profiles (T2250, T1217, and T1215).

samples were taken at 5 cm intervals in the 7th–2nd layer, and three samples were taken at 10 cm intervals in the 1st layer. Two charcoals for dating were collected at 140–142 cm and 136–138 cm (**Figure 2**).

T1215 (75 cm in thickness) is a natural deposit profile just beneath the oldest culture layer of the Baodun site. It could be divided into four layers from bottom to top: 1) layers 4 (235–230 cm), black-dark grey clay; 2) layers 3 (230–205 cm), grey clay; 3) layers 2 (205–170 cm), grey clay grey clay with few Fe-Mn nodules; 4) layers 1 (170–160 cm), grey clay with Fe-Mn nodules, which are transition layers from natural deposit to early Baodun culture. Fifteen samples were taken at 5 cm intervals in the 4th–1st layer, and three OSL samples were collected for dating at 228, 204, and 168 cm (**Figure 2**).

Profile T2250 (220 cm in thickness) could be divided into seven layers from bottom to top: 1) layers 7 (200–180 cm),

TABLE 1 | 14C dating results from the Baodun site (Lv et al., 2021).

Sample ID	Depth (cm)	δ^{13}C‰	14C age (aBP)[a]	±	Calibrated 14C dates (2σ) (cal. aBP)
2018BD-T1217-2	136	−25.62	3,690	30	4,146–3,924
2018BD-T1217-1	140	−26.82	3,680	20	4,089–3,926

[a]*The quoted uncalibrated dates are given in radiocarbon years before 1950 (years BP), using the ^{14}C half-life of 5,568 years. The error is quoted as a single value (standard deviation) and reflects both statistical and experimental errors.*

grey-green clay, natural deposit; 2) layers 6 (180–120 cm), grey-green clay with brown soil blocks and Fe-Mn nodules, Baodun culture; 3) layers 5–3 (120–64 cm), grey-green clay to brown clay with few Fe-Mn nodules, Han Dynasty; 4) layers 2 (64–30 cm), grey-green clay, Tang-Song Dynasty; 5) layer 1 (30–0 cm), modern cultivated layer. Five samples were taken at 5 cm intervals in the 7th layer, 30 samples were taken at 2 cm intervals in the 6th–5th layer, 18 samples were taken at 5 cm intervals in the 4th–2nd layer, and three samples were taken at 10 cm intervals in the 1st layer. Four OSL samples were collected for dating at 200, 173, 116, and 62 cm. We analysed a total of 37 samples from layers 7 to 5 in T2250 (**Figure 2**).

Phytolith Extraction and Identification

Phytoliths were extracted from soil samples according to established methods (Zhao and Piperno, 2000; Piperno, 2006) with minor modifications. Initially, approximately 2 g of each sample was weighed and treated with 30% H_2O_2 and 15% HCl to remove organic matter and carbonates. The samples were then subjected to heavy liquid flotation using $ZnBr_2$ (density = 2.35 g/cm^3) to separate the phytoliths, which were subsequently mounted on a slide using Canada Balsam. After air drying, the phytoliths on the slide were counted and identified using a Leica microscope at ×400 magnification. More than 400 phytolith particles were identified in each sample and recorded according to published references and criteria (Wang and Lu, 1993; Lu et al., 2002; Lu et al., 2006). Phytolith abundance was expressed as percentages of all phytoliths counted. In particular, for samples with rice phytoliths, the slides were scanned until 50 rice bulliforms with clear and countable scales were observed (except in 16 out of 105 samples that contained minor phytoliths) in order to calculate the proportion of rice bulliform phytoliths with ≥9 scales (Huan et al., 2015; Huan et al., 2020). The abundance of bulliform with ≥9 scales was expressed as percentages of only bulliform phytoliths counted.

Methods of Chronology Assessment

A total of nine dating samples were collected from the three profiles, of which two were used for ^{14}C dating by the Center for Applied Isotope Studies, University of Georgia, and the rest were tested by OSL from the Luminescence Research Laboratory, Linyi University, China. The dates that were obtained by the ^{14}C method were calibrated by OxCal 4.4 using the IntCal 20 atmospheric curve (Reimer et al., 2020) and are presented in **Table 1** (Lv et al., 2021).

For OSL dating, quartz was purified by the following process: after the removal of carbonates and organic materials, the samples were subjected to wet sieving (63–90 μm), density liquid (SPT, 2.62–2.70 g/cm^3), HF etching (40 min), HCl rinse (40 min), and dry sieving (63 μm) (An et al., 2020). Small aliquots (2 mm diameter) were used to effectively demonstrate the scatter in equivalent dose (D_e). The D_es were measured with a Risø TL/OSL DA-20 reader, and the Single Aliquot Regenerative dose (SAR) (Murray and Wintle, 2000; 2003) protocol and the standardised growth curve (SGC) method (Roberts and Duller, 2004) were applied. The detailed protocol and parameters followed were referred from An et al. (2020). For each sample, five aliquots were measured with the SAR protocol, and the five growth curves of each aliquot of a sample were averaged to build the SGC for each sample. Then normalised natural signals of additional 14–16 aliquots were matched to their SGCs to calculate SGC D_es. Finally, the D_e of each sample was calculated based on the SAR D_es and SGC D_es (n = 19–21) using the central age model (Galbraith et al., 1999). The decay curves (dominated by the fast component), growth curves, and SGCs of samples are displayed in **Figures 3A,B**. D_e distribution plots (Abanico plots) (Dietze and Kreutzer, 2020) of all the samples (**Figures 3C–I**) suggested that the OSL signals were well bleached before the burial. The content of elements was measured using ICP-MS (U and Th) and ICP-OES (K). Considering the high groundwater level, water content was estimated based on the actual measured and saturated values (**Table 1**). The dose rates and ages are calculated by the Dose Rate and Age Calculator (Durcan et al., 2015) and are shown in **Table 2**.

RESULTS

All nine dating samples from the sites successfully yielded credible dates (**Tables 1**, **2**). Generally, all these direct dates fit well with the cultural affiliations of their contexts. Dates of T1215 were found to range from 7,500 to 5,000 BP, which is completely beyond the cultural period of the site. The oldest age of the lowest layer of T1215 (7500 BP) is consistent with that of the palaeowood remains dated at 7,400 cal BP (Huang et al., 2019b). Two dating results of T1217 (4,000 cal aBP) are corresponding with the late Baodun period. Dates of T2250 fell into the range of 4,200–2,500 aBP, covering the periods of middle to late phases of the Baodun culture.

All 105 samples from the Baodun sites yielded abundant phytoliths (**Figure 4**). In total, 26 phytolith morphotypes were identified, of which four could be confirmed from crops, including double-peaked, bulliform, and parallel-bilobate phytolith types of rice, and epidermal long cell phytoliths from the upper lemma and palea of millets (broomcorn and foxtail millet) (**Figure 4**). Other typical morphotypes that could be identified to the subfamily level and lower levels were the long saddle phytoliths of Bambusoideae, rondel and trapeziform sinuate phytoliths of Pooideae, bulliform phytoliths of Arundiaceae, and bilobate phytoliths of Panicoideae, as well as woody phytoliths and Cyperaceae phytoliths.

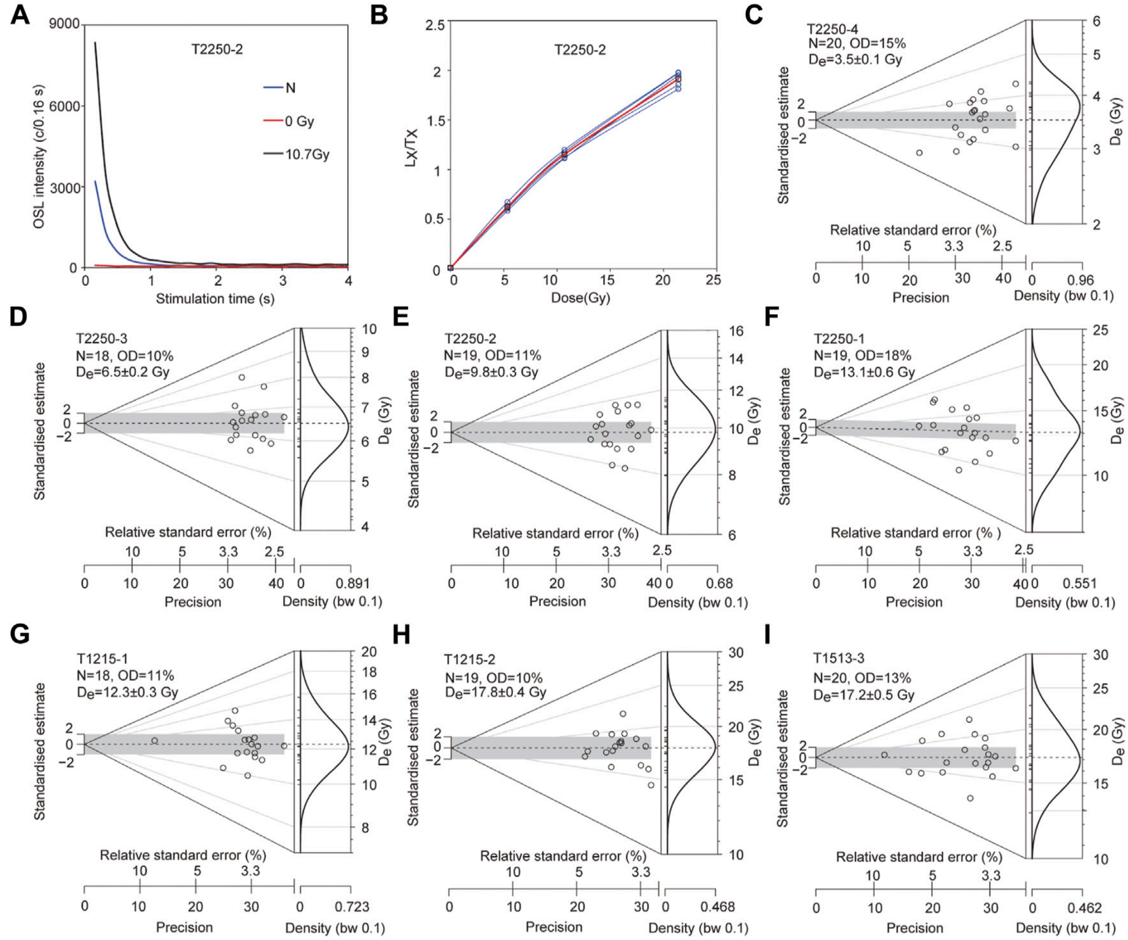

FIGURE 3 | (A) Decay curves, **(B)** growth curves (red for SGC, and **(C–I)** D_e distributions. SGC: Standardised growth curve; De: The scatter in equivalent dose.

TABLE 2 | Optically stimulated luminescence (OSL) dating results of the Baodun Site.

Sample ID	Depth (cm)	U (ppm)	Th (ppm)	K	Water content (%, measured, saturated)	Dose rate (Gy/ka)	D_e (Gy)	Age (a)
T2250-4	62	3.02 ± 0.12	14.20 ± 0.57	1.32 ± 0.03	20 ± 5 (15, 28)	2.7 ± 0.1	3.5 ± 0.1	1,300 ± 100
T2250-3	116	2.94 ± 0.12	14.00 ± 0.56	1.33 ± 0.05	20 ± 5 (20, 27)	2.7 ± 0.1	6.5 ± 0.2	2,500 ± 100
T2250-2	173	3.64 ± 0.15	12.50 ± 0.50	1.19 ± 0.05	30 ± 5 (22, 36)	2.4 ± 0.1	9.8 ± 0.3	4,200 ± 200
T2250-1	200	5.27 ± 0.21	15.60 ± 0.62	1.67 ± 0.07	30 ± 5 (25, 38)	3.2 ± 0.1	13.1 ± 0.6	4,100 ± 200
T1215-1	168	3.60 ± 0.14	14.60 ± 0.58	1.21 ± 0.05	30 ± 5 (18, 33)	2.5 ± 0.1	12.3 ± 0.3	5,000 ± 200
T1215-2	204	3.08 ± 0.12	15.00 ± 0.60	1.44 ± 0.06	35 ± 5 (36, 51)	2.5 ± 0.1	17.8 ± 0.4	7,200 ± 300
T1215-3	228	3.21 ± 0.13	14.10 ± 0.56	1.36 ± 0.05	40 ± 5 (33, 45)	2.3 ± 0.1	17.2 ± 0.5	7,500 ± 300

The most significant feature of crop phytolith assemblages from the three profiles was the presence of abundant rice phytoliths (rice bulliform and double-peaked types), ranging from approximately 0.2–5.5%, in almost every sample 7,500 aBP (**Figure 5**). To reconstruct the farming development process, all 105 samples were further analysed to count the number of scales on the edge of the rice bulliform phytoliths. For each sample, almost 50 bulliform phytoliths with clear and countable scales were carefully observed. The average proportion of bulliform phytoliths with ≥9 scales remained at a low level of 20.2 ± 5.1% between 7,500 and 5,000 aBP in T1215. However, their proportion increased over 25% during 5,000–4,000 aBP in T1217, including the early-middle Baodun period (**Figure 5**), and sharply increased to 48.6 ± 8.5% after 4200 aBP, as evident in T2250 (**Figure 6**).

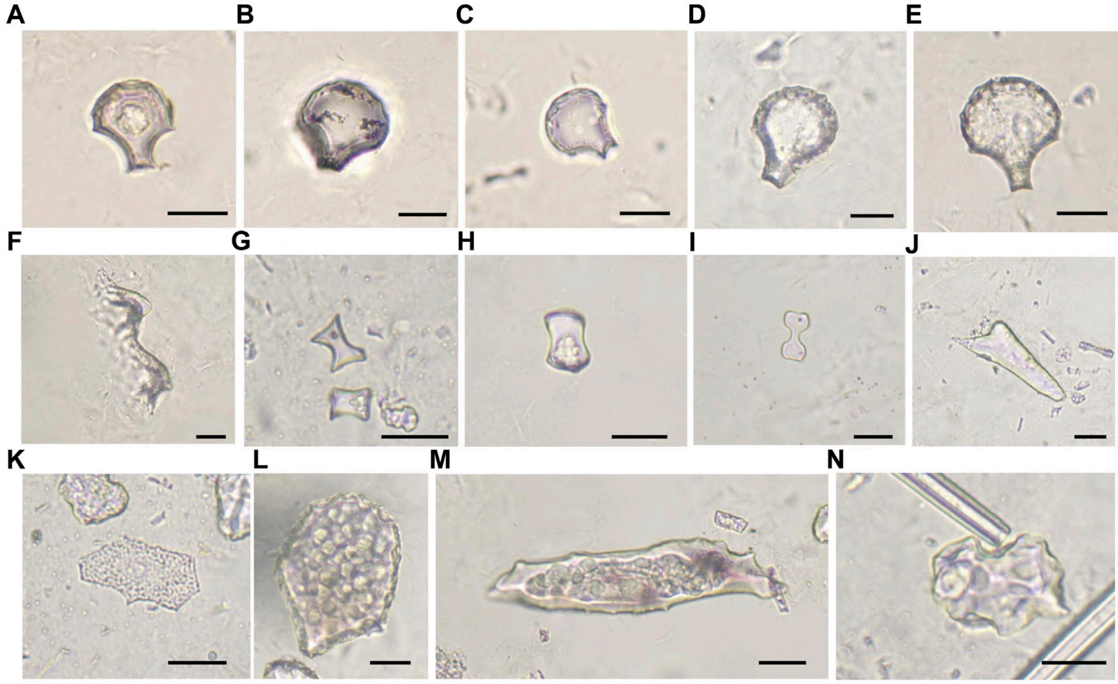

FIGURE 4 | Main phytolith morphotypes at the Baodun site. **(A–E)** rice bulliform phytoliths from rice leaves with increased scale numbers, **(F)** double-peaked cells from rice husk, **(G–H)** long saddle phytoliths, (I) bilobate phytoliths, **(J)** acicular hair cell phytoliths, **(K)** achene phytoliths belonging to Cyperaceae, **(L)** scutiform-bulliform phytoliths belonging to reeds, and **(M–N)** woody phytoliths.

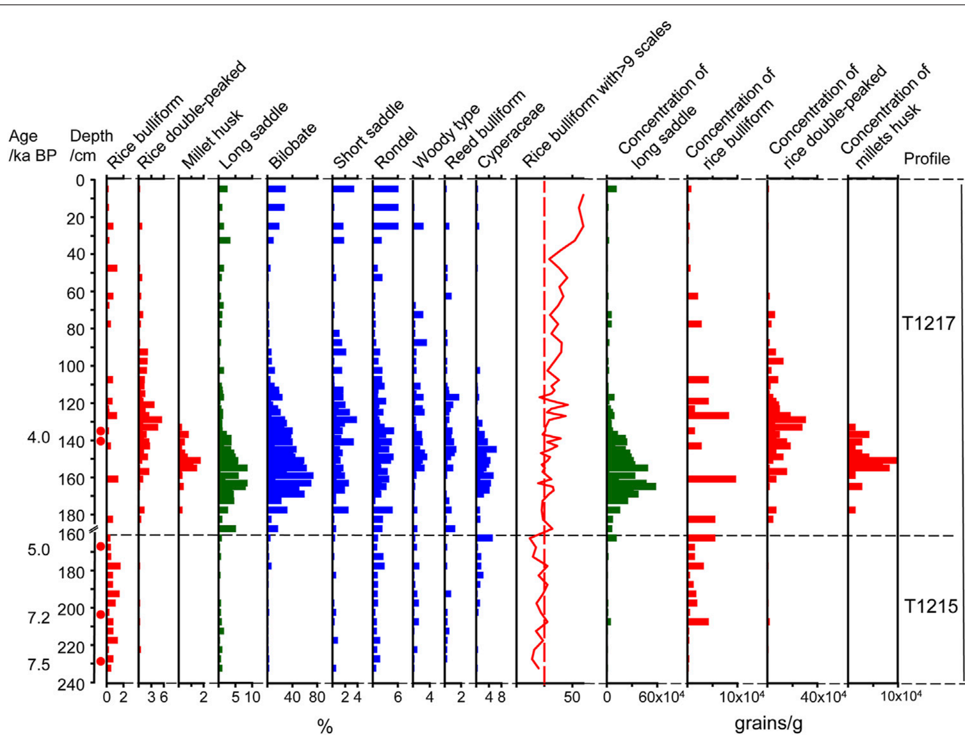

FIGURE 5 | Phytolith assemblages of T1215 and T1217 profiles.

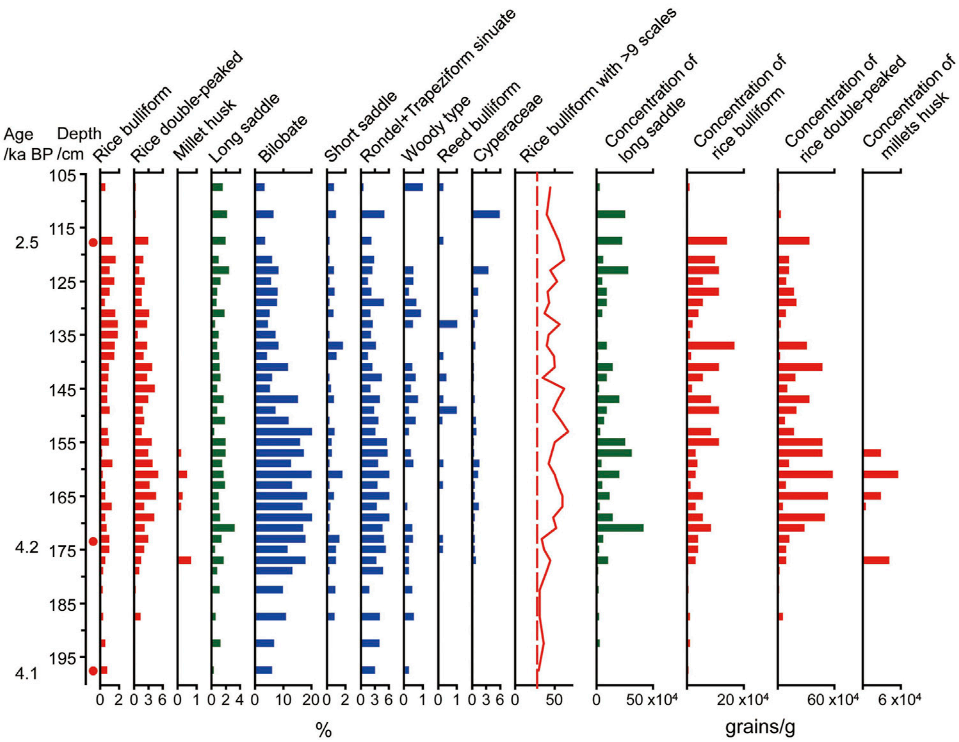

FIGURE 6 | Phytolith assemblages of T2250 profile.

Similarly, the percentage and concentration of rice phytoliths and millet husks increased synchronously in both T1217 and T2250 (**Figures 5**, **6**). Especially, the concentrations of rice bulliform, double-peaked and millet husk in T1217 simultaneously reached the peak of 9.6×10^4, 30×10^4, and 9.6×10^4 grains/g at approximately 4,000 aBP, respectively. The same trend was also evident in T2250, as the concentrations of rice double-peaked and millet husk reached peaks of 57.9×10^4 and 5.5×10^4 grains/g at approximately 4,200 aBP.

It is also worth noting that the assemblage of phytoliths varied greatly from natural to cultural layers (i.e., in T1215 and T1217). It is characterised by a synchronous increase in the abundance of rice phytoliths and long saddle phytoliths belonging to Bambusoideae, bilobate and cross phytoliths belonging to Panicoideae, and rondel and trapeziform sinuate phytoliths belonging to Poaceae, Cyperaceae, and woody phytoliths. In particular, the concentration of rice phytoliths and long saddles also changed synchronously in the profiles of T1217 and T2250, which reached the highest value soon after 4,200 cal aBP, after which it decreased again, revealing a strong relationship between agricultural activities and natural changes in vegetation near the site. The concentration of long saddles sharply increased from 5.8 to 57.9 grains/g and peaked synchronously with the concentration of rice phytoliths at approximately 4,000 aBP in T1217. Similarly, in T2250, the concentration of long saddles obviously increased after 4,200 aBP, from 2.3 to 41.3 grains/g, along with the increase in rice phytolith concentration.

DISCUSSION

Studies on Neolithic agriculture in the Chengdu Plain have focused primarily on documenting the cultivation and spread of crops and were limited to the Baodun period (d'Alpoim Guedes et al., 2013; d'Alpoim Guedes and Wan, 2015). Since there are a limited amount of palaeovegetation records before and after the Baodun period, the introduction and development of rice farming in the Plain, as well as the vegetation evolution history influenced by rice farming are still a subject of discussion (Jiang, 2009; Jiang et al., 2009; Qin, 2012). The phytolith records analysed in the present study dated from 7,500 to at least 2,500 aBP provide chronological records of rice farming development and landscape alteration near the study site.

Wild Rice Distribution

The proportion of bulliform phytoliths with ≥9 fish scale patterns was largely different in wild rice soils (17.5 ± 8.3%) and domesticated rice soils (57.6 ± 8.7%), which can be used as an indicator to assess the degree and rate of rice domestication to some extent (Huan et al., 2015). According to our records, the proportion of rice bulliform phytoliths with ≥9 fish scales was maintained at approximately 15–25% throughout the T1215 profile, which was the first substantial evidence of the presence of wild rice in the Chengdu Plain as early as 7,500 BP. Our results, combined with the presence of Cyperaceae and reed phytoliths from the T1215 profile and other palaeoenvironmental evidence (Huang et al., 2019b), indicated that the environment near the Baodun site was permanent to seasonal wetlands in association with lake and marsh biomes, which contained a spectrum of ecological niches suitable for the rice wild progenitor complex (*Oryza rufipogon* and *O. nivara*).

The Development of Rice Farming

The observed sharp increase in the percentage of rice phytoliths with ≥9 fish scales beyond the distribution range of wild rice data (more than 50%), with simultaneous increases in the percentage of both rice husk and leaves after 4,200 cal BP in T 2250 and T1217 (**Figures 5**, **6**), suggested an increase in the area of domesticated rice habitat and a decrease in the area of wild rice habitat. This, combined with the synchronous increase in the percentage and concentration of rice phytoliths, indicated that rice farming with a minor millet cultivation survival strategy occupied a dominant position no later than 4,200 aBP at the Baodun site.

We suggested that this might be a consequence of social development, and it may more likely be attributed to the rapid expansion of rice cultivation as suggested in a previous study (Gutaker et al., 2020). Gutaker et al. (2020) speculated that the climatic change (aridification) might have resulted in the southward dispersal of rice (agricultural communities). This study used absolute dating and confirmed the abrupt increases in rice abundance and domesticated traits since approximately 4,200 aBP in sedimentary archives in southwestern China, offering archaeological, biological, and chronological evidence for this suspension.

Since 4,200 aBP, Neolithic cultures and rice agriculture (Wang, 2004; An et al., 2005; Guedes et al., 2015; Wang et al., 2016) declined in East Asia/eastern China, and debates remained on what drove the decline, e.g., climate deterioration (An et al., 2005; Marcott et al., 2013), flood and wars (Wang 2004; Shen et al., 2015), and from where the people migrated (Gutaker et al., 2020). The rapid development of rice cultivation in Chengdu Plain in 4,200 aBP may shed light on this question. The aridification since approximately 4,200 aBP could not sustain the former agricultural systems and cause the southward shift of the agricultural belts, which further drove the southward migration of people, resulting in the southward dispersal of rice agricultural communities (Silva et al., 2015; Castillo et al., 2018; Gutaker et al., 2020). At this time, the climate in the Chengdu Plain was relatively mild (Luo et al., 2007; Luo et al., 2008) with ample water sources, presenting an ideal environment for wetland rice production (d'Alpoim Guedes et al., 2013). However, owing to the lack of regional high-resolution evidence of environmental change and population migration, further research is needed.

Although rice has appeared as early as approximately 4,600 BP at the site of study (d'Alpoim Guedes et al., 2013), the present study indicated low proportions of rice bulliform phytoliths with ≥9 scales between 5,000 and 4,200 aBP. We propose that the presence of wild rice had caused inevitable hybridisation with the domesticated rice (Wang et al., 2017) and the wild rice might have degenerated the traits of domesticated rice. Moreover, the area of rice cultivation during the primary stage of the Baodun culture was small, which may also be a reason for the reduced proportion of rice bulliform phytoliths with ≥9 scales. A previous archaeobotanical study also indicated that domesticated rice accounted for only 50% of the total rice remains during the Baodun period (d'Alpoim Guedes et al., 2013). However, rice remains were not distinguished by culture phases in that study. Therefore, the proportion of domesticated rice cultivated during the early stage of the Baodun culture was not exactly determined. Another evidence of a single broken rice spikelet was found in the Guiyuanqiao site in the Chengdu Plain (d'Alpoim Guedes and Wan, 2015). However, it was also difficult to determine whether it was wild or domesticated.

Landscape Changes as a Consequence of Neolithic Rice Cultivation

Our data further indicated that human-induced vegetation changes in our study region can be traced back to as early as 4,200 aBP. The vegetation transformations that occurred at this time involved a simplification of the Poaceae vegetation structure, particularly the increase in Bambusoideae (long saddle phytoliths), Panicoideae (bilobate and cross phytoliths), and Pooideae (rondel, trapeziform sinuate phytoliths), based on a comparison with phytolith records from T1215, T1217 and T2250 (**Figures 5**, **6**). It is suggested that because of the development of rice farming, ancient people altered the surrounding vegetation, for example the irrigation expanded the habitats suitable for hydrophilous plants, such as reeds and some Cyperaceae species. Specifically, agriculture might benefit the expansion of bamboo. On the one hand, the expansion of rice paddy caused deforestation, which provided space for the expansion of bamboo. On the other hand, the utilisation of bamboo as agricultural tools and building materials promoted the development of rice production. Our records, combined with previous charcoal analyses, indicated that bamboo was widely utilised at the Baodun site (Yan et al., 2016).

The combined increase in the amount of evidence of rice and Bambusoideae further suggests that the farmhouse forest (Linpan in Chinese) landscape with the bamboo forest as one of the main vegetation types (Dajiang et al., 2011) may have appeared no later than 4,200 aBP. It also contributes to understanding the evolution of the Linpan community, which is a traditional rural settlement unit that has close relations among rice farming, daily life, and landscape and has outstanding agricultural heritage conservation values in the Chengdu Plain (Fang and Li, 2011; Yuan et al., 2020).

It should be pointed out that information relating to past plant diversity revealed by phytolith data is limited and incomplete, mainly because different plant taxa have different phytolith productivities (Piperno, 2006). In particular, woody taxa yield less phytoliths that can be identified to the genus level (Wang and Lu, 1993). Although the inherent biases of phytolith analyses cannot be eliminated, phytolith records still represent one of the best data sources to describe the surrounding landscape, especially the herbaceous vegetation landscape, in a wide ecological range, from dry to humid regions (Dickau et al., 2013; Zhang et al., 2013; Liu et al., 2021). The phytolith records described in the present study provide a glimpse of the development of rice farming throughout the Baodun culture in the Chengdu Plain and a description of Poaceae floral compositional changes since 7,500 aBP.

CONCLUSION

In the present study, phytolith remains collected from natural deposits and culture layers dated from 7,500 to 2,500 aBP at the Baodun site, Southwestern China, were analysed. The evidence indicated that wild rice was already distributed in the area as early as 7,500 aBP. The comparison of phytolith records from natural and archaeological layers indicated that the survival strategy of rice cultivation for the Baodun people occupied a dominant position no later than approximately 4,200 aBP. We suggest that this may be due to the expansion of rice cultivation to southwestern China caused by late Holocene climate deterioration and the subsequent decline of Neolithic culture in eastern China. The intensified farming activities further induced Poaceae vegetation alternation, particularly the expansion of the bamboo forest in areas near the Baodun site. These findings have implications in understanding the distribution of wild rice during the Holocene, as well as in understanding the development of rice farming and human-induced vegetation and landscape changes in past small-scale societies of Southwestern China. Thus, this study contributed to the understanding of the formation and continuance of the ancient Sichuan civilisation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

JZ conceived and designed the study. JZ and LY compiled archaeological dates. JZ, YL, and LY analysed the data. JZ wrote the manuscript. LY, MT, MH, KS, XH, KH, XY, CW, MY, NW, and HL revised the manuscript.

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REFERENCES

An, C.-B., Tang, L., Barton, L., and Chen, F.-H. (2005). Climate Change and Cultural Response Around 4000 Cal Yr B.P. In the Western Part of Chinese Loess Plateau. *Quat. Res.* 63 (3), 347–352. doi:10.1016/j.yqres.2005.02.004

An, P., Yu, L., Wang, Y., Miao, X., Wang, C., Lai, Z., et al. (2020). Holocene Incisions and Flood Activities of the Keriya River, NW Margin of the Tibetan Plateau. *J. Asian Earth Sci.* 191, 104224. doi:10.1016/j.jseaes.2019.104224

Castillo, C. C., Fuller, D. Q., Piper, P. J., Bellwood, P., and Oxenham, M. (2018). Hunter-gatherer Specialization in the Late Neolithic of Southern Vietnam - the Case of Rach Nui. *Quat. Int.* 489, 63–79. doi:10.1016/j.quaint.2016.11.034

Castillo, C. C., Tanaka, K., Sato, Y.-I., Ishikawa, R., Bellina, B., Higham, C., et al. (2016). Archaeogenetic Study of Prehistoric rice Remains from Thailand and India: Evidence of Early Japonica in South and Southeast Asia. *Archaeol Anthropol. Sci.* 8 (3), 523–543. doi:10.1007/s12520-015-0236-5

Chen, T., Jiang, Z., He, K., Yang, Y., Guedes, J. d. A., Jiang, H., et al. (2015). Phytolith Analysis from the Baodun Archaeological Site, Xinjin, Sichuan. *Acta Anthropol. Sin.* 34 (2), 225–233. 10.16359/j.cnki.cn11-1963/q.2015.0023 .

Dajiang, S., Qibing, C., Tingxing, H., Guangli, L., Dayuan, S., and Qigao, L. (2011). Community Types and the Biodiversity of Farmhouse Forest in Western Sichuan Province. *J. Sichuan Agr. Univ.* 29 (1), 22–28. 10.3969/j.issn.1000-2650.2011.01.005 .

d'Alpoim Guedes, J., Jiang, M., He, K., Wu, X., and Jiang, Z. (2013). Site of Baodun Yields Earliest Evidence for the Spread of rice and Foxtail Millet Agriculture to South-West China. *Antiquity* 87 (337), 758–771. doi:10.1017/s0003598x00049449

d'Alpoim Guedes, J., Jin, G., and Bocinsky, R. K. (2015). The Impact of Climate on the Spread of Rice to North-Eastern China: A New Look at the Data from Shandong Province. *PLOS ONE* 10 (6), e0130430. doi:10.1371/journal.pone.0130430

d'Alpoim Guedes, J., and Wan, J. (2015). Flotation Analysis of Guiyuanqiao Site in Shifang City, Sichuan Province. Sichuan Cult. *Relics* (5), 81–87+94.

Dickau, R., Whitney, B. S., Iriarte, J., Mayle, F. E., Soto, J. D., Metcalfe, P., et al. (2013). Differentiation of Neotropical Ecosystems by Modern Soil Phytolith Assemblages and its Implications for Palaeoenvironmental and Archaeological Reconstructions. *Rev. Palaeobotany Palynology* 193, 15–37. doi:10.1016/j.revpalbo.2013.01.004

Dietze, M., and Kreutzer, S. (2020). "plot_AbanicoPlot(): Function to Create an Abanico Plot. Function Version 0.1.11," in *Luminescence: Comprehensive Luminescence Dating Data Analysis. R Package Version 0.9.10.* Editors S. Kreutzer, C. Burow, M. Dietze, M. C. Fuchs, C. Schmidt, M. Fischer, et al.

Durcan, J. A., King, G. E., and Duller, G. A. T. (2015). DRAC: Dose Rate and Age Calculator for Trapped Charge Dating. *Quat. Geochronol.* 28, 54–61. doi:10.1016/j.quageo.2015.03.012

Ellis, E. C. (20111938). Anthropogenic Transformation of the Terrestrial Biosphere. *Phil. Trans. R. Soc. A.* 369, 1010–1035. doi:10.1098/rsta.2010.0331

Fang, Z., and Li, X. (2011). On Causes for Formation of Linpan Culture in West Sichuan Plain. (5), 45–49. doi:10.3969/j.issn.1004-342X.2011.05.013

Fuller, D. Q., van Etten, J., Manning, K., Castillo, C., Kingwell-Banham, E., Weisskopf, A., et al. (2011). The Contribution of rice Agriculture and Livestock Pastoralism to Prehistoric Methane Levels. *The Holocene* 21 (5), 743–759. doi:10.1177/0959683611398052

Fuller, D., Sato, Y.-I., Castillo, C., Qin, L., Weisskopf, A., Kingwell-Banham, E., et al. (2010). Consilience of Genetics and Archaeobotany in the Entangled History of rice. *Archaeol. Anthrop. Sci.* 2 (SI), 115–131. doi:10.1007/s12520-010-0035-y

Galbraith, R. F., Roberts, R. G., Laslett, G. M., Yoshida, H., and Olley, J. M. (1999). Optical Dating of Single and Multiple Grains of Quartz from Jinmium Rock

Shelter, Northern Australia: Part I, Experimental Design and Statistical Models. *Archaeometry* 41, 339–364. doi:10.1111/j.1475-4754.1999.tb00987.x

Gowdy, J., and Krall, L. (2014). Agriculture as a Major Evolutionary Transition to Human Ultrasociality. *J. Bioecon* 16 (2), 179–202. doi:10.1007/s10818-013-9156-6

Gutaker, R. M., Groen, S. C., Bellis, E. S., Choi, J. Y., Pires, I. S., Bocinsky, R. K., et al. (2020). Genomic History and Ecology of the Geographic Spread of rice. *Nat. Plants* 6 (5), 492–502. doi:10.1038/s41477-020-0659-6

Huan, X., Lu, H., Wang, C., Tang, X., Zuo, X., Ge, Y., et al. (2015). Bulliform Phytolith Research in Wild and Domesticated Rice Paddy Soil in South China. *PLOS ONE* 10 (10), e0141255. doi:10.1371/journal.pone.0141255

Huan, X., Lu, H., Wang, C., and Zhang, J. (2020). Progress of rice Bulliform Phytolith Research on Wild–Domesticated Characteristics. *Acta Palaeontol. Sin*. 59 (4), 467–478. 10.1371/journal.pone.0141255 .

Huan, X., Lu, H., Zhang, J., and Wang, C. (2018). Phytolith Assemblage Analysis for the Identification of rice Paddy. *Sci. Rep.* 8, 10932. doi:10.1038/s41598-018-29172-5

Huang, M., Ma, C., and Zhu, C. (2017). Progress of the Mid-late Holocene Environmental Archaeology in Chengdu plain. *J. Palaeogeog.* 19 (6), 1087–1098. doi:10.7605/gdlxb.2017.06.084

Huang, M., Zhu, C., Ma, C., He, K., and Xu, J. (2019a). Paleoenvironmental Context of the Evolution of the Baodun Culture at Chengdu Plain, Sichuan Province, China. *The Holocene* 29 (11), 1731–1742. doi:10.1177/0959683619862031

Jiang, M., Dai, Y., Zhang, Q., and He, K. (2009). Brief Report on Analysis of Flotation Results of Archaeological Excavation in 2009 Year of Baodun Site in Xinjin. *Archaeol. Discov. Chengdu* (0), 72–86+548–549.

Jiang, M., d'Alpoim Guedes, J., and He, K. (2011). "Flotation Analysis of the Archaeological Excavation of Baodun Site in Xinjin in 2009," in *Archaeological Discoveries of Chengdu in 2009Institute of Cultural Relics and Archaeology of Chengdu* (Beijing: Science Press).

Jiang, Z. (2009). *Agricultural Transformation and Settlement Changes in Chengdu Plain during the Pre-qin Period*. Forum Chinese Cult, China. S2. 187–189.

Jiang, Z., Wang, Y., and Zhang, Q. (2002). A Preliminary Research on the Pre-qin Culture in the Chengdu Plain. *J. Archaeol.* (1), 1–22.

Li, Z., Weng, S., Zhang, X., Jiang, z., Liu, Z., and Zhou, K. (1987). A Brief Report on the First Phase of Excavation of the 12th Qiao Shang Dynasty Architectural Site in Chengdu. Cult. *Relics* (12), 1–23+37+99–101.

Liang, B., Zhu, B., and Wang, Q. (2014). *Quaternary Geology and Environment in the Chengdu Plain*. Beijing: Science Press.

Liu, Y., Chen, J., Chen, Y., Yang, X., Liu, S., Chen, P., et al. (2004). Trial Excavation of Sanxing Village Ruins in Qingbai District, Chengdu. *Archaeol. Discov.*, 255–282.

Liu, Y., Liu, H., Jie, D., Gao, G., Meng, M., and Zhang, G. (2021). Phytolith Morphotypes of Woody Plants and Their Preservation in Soil in the Warm Temperate Humid Zones of China. *Quat. Int.* 599-600, 158–169. doi:10.1016/j.quaint.2021.03.017

Lu, H.-Y., Wu, N.-Q., Yang, X.-D., Jiang, H., Liu, K.-b., and Liu, T.-S. (2006). Phytoliths as Quantitative Indicators for the Reconstruction of Past Environmental Conditions in China I: Phytolith-Based Transfer Functions. *Quat. Sci. Rev.* 25, 945–959. doi:10.1016/j.quascirev.2005.07.014

Lu, H., Jia, J., Wang, W., Wang, Y., and Liu, K.-B. (2002). On the Meaning of Phytolith and its Classification in Gramineae. *Acta Micropalaeontol. Sin.* (4), 389–396. doi:10.3969/j.issn.1000-0674.2002.04.006

Luo, L., Zhu, L., Xiang, F., Yang, W., Kan, A., Luo, H., et al. (2008). Spore-pollen Assemblage and Environmental Changes of the Chengdu Plain during the Late Holocene. *Acta Palaeontol. Sin.* 47 (2), 195–202. doi:10.3969/j.issn.0001-6616.2008.02.006

Luo, L., Zhu, L., Yang, W., Zhang, Q., Wang, C., Kan, A., et al. (2007). Magnetic Susceptibility Characteristics and Paleoclimate Explanation of Chengdu Plain since 4000a. *J. Chengdu Univ. Technol. (Sci. Technol. Edit.)* 34 (3), 327–330. doi:10.3969/j.issn.1671-9727.2007.03.017

Lv, Y., Zhang, J., Tang, M., Yang, Z., Huang, M., Jiang, M., et al. (2021). Phytolith Analysis Reveals the Agricultural Development and Environmental Background of the Pre-qin Period in Chengdu Plain-A Case Study of Baodun and Sanxingcun Sites. *Quat. Sci.* 41 (5), 1475–1488. 10.11928/j.issn. 1001-7410.2021.05.21 .

Marcott, S. A., Shakun, J. D., Clark, P. U., and Mix, A. C. (2013). A Reconstruction of Regional and Global Temperature for the Past 11,300 Years. *Science* 339 (6124), 1198–1201. doi:10.1126/science.1228026

Murray, A. S., and Wintle, A. G. (2003). The Single Aliquot Regenerative Dose Protocol: Potential for Improvements in Reliability. *Radiat. Meas.* 37 (4-5), 377–381. doi:10.1016/s1350-4487(03)00053-2

Murray, A. S., and Wintle, A. G. (2000). Luminescence Dating of Quartz Using an Improved Single-Aliquot Regenerative-Dose Protocol. *Radiat. Measurements* 32 (1), 57–73. doi:10.1016/s1350-4487(99)00253-x

Pavlik, B. M., Louderback, L. A., Vernon, K. B., Yaworsky, P. M., Wilson, C., Clifford, A., et al. (2021). Plant Species Richness at Archaeological Sites Suggests Ecological Legacy of Indigenous Subsistence on the Colorado Plateau. *Proc. Natl. Acad. Sci. USA* 118 (21), e2025047118. doi:10.1073/pnas.2025047118

Piperno, D. R. (2006). *Phytolith: A Comprehensive Guide for Archaeologists and Paleoecologists*. New York: AltaMira Press.

Qin, L. (2012). "Archaeobotany Research and Expectationon on Chinese Agriculture Origin," in *Archaeological ResearchSchool of Archeaology and Museology Peking University & the Center of the Study of Chinese Archaeology in Peking University* (Beijing: Cultural Relics Press), 260–315.

Reimer, P. J., Austin, W. E. N., Bard, E., Bayliss, A., Blackwell, P. G., Bronk Ramsey, C., et al. (2020). The IntCal20 Northern Hemisphere Radiocarbon Age Calibration Curve (0-55 Cal kBP). *Radiocarbon* 62 (4), 725–757. doi:10.1017/rdc.2020.41

Roberts, H. M., and Duller, G. A. T. (2004). Standardised Growth Curves for Optical Dating of Sediment Using Multiple-Grain Aliquots. *Radiat. Measurements* 38 (2), 241–252. doi:10.1016/j.radmeas.2003.10.001

Shen, H., Yu, L., Zhang, H., Zhao, M., and Lai, Z. (2015). OSL and Radiocarbon Dating of Flood Deposits and its Paleoclimatic and Archaeological Implications in the Yihe River Basin, East China. *Quat. Geochronol.* 30, 398–404. doi:10.1016/j.quageo.2015.03.005

Silva, F., Stevens, C. J., Weisskopf, A., Castillo, C., Qin, L., Bevan, A., et al. (2015). Modelling the Geographical Origin of Rice Cultivation in Asia Using the Rice Archaeological Database. *PLOS ONE* 10 (9), e0137024. doi:10.1371/journal.pone.0137024

Sun, H. (2009). Evolution of Prehistoric Grain Species in Sichuan basin: Information Mainly Derived from Archaeological Cultural Interactions. *Forum Chin. Cult* (2), 147–154.

Wang, H., Vieira, F. G., Crawford, J. E., Chu, C., and Nielsen, R. (2017). Asian Wild rice Is a Hybrid Swarm with Extensive Gene Flow and Feralization from Domesticated rice. *Genome Res.* 27 (6), 1029–1038. doi:10.1101/gr.204800.116

Wang, J., Sun, L., Chen, L., Xu, L., Wang, Y., and Wang, X. (2016). The Abrupt Climate Change Near 4,400 Yr BP on the Cultural Transition in Yuchisi, China and its Global Linkage. *Sci. Rep.* 6, 27723. doi:10.1038/srep27723

Wang, W., Mauleon, R., Hu, Z., Chebotarov, D., Tai, S., Wu, Z., et al. (2018). Genomic Variation in 3,010 Diverse Accessions of Asian Cultivated rice. *Nature* 557 (7703), 43–49. doi:10.1038/s41586-018-0063-9

Wang, W. (2004). The Dusscussion on the Causes of Large-Scale Cultural Changes in China Around 2000 BC. *Archaeology* (1), 67–77.

Wang, Y., and Lu, H. (1993). *Phytolith Study and its Application*. Beijing: China Ocean Publishing.

Woodbridge, J., Fyfe, R. M., Roberts, N., Downey, S., Edinborough, K., and Shennan, S. (2014). The Impact of the Neolithic Agricultural Transition in Britain: a Comparison of Pollen-Based Land-Cover and Archaeological 14C Date-Inferred Population Change. *J. Archaeological Sci.* 51, 216–224. doi:10.1016/j.jas.2012.10.025

Yan, X., Wang, S., Jiang, M., and He, K. (2016). Preliminary Study of Charcoal Remains Unearthed from Baodun Site in 2013-2014. *South. Ethno. Archaeo.* 2, 311–328.

You, X. (1987). The Wild rice in Chinese Ancient Records. *Anc. Mod. Agric.* (1), 1–6.

Yuan, L., Qingwen, M., Liyun, Y., Fanxu, M., and Wanquan, Y. (2020). Historical Changes of Plant Community Structure and Biodiversity of Linpan: A Case Study in Pi County of Chengdu. *Chin. Agr. Sci. Bul.* 36 (7), 44–49. 10.11924/j.issn.1000-6850.casb18110111 .

Zhang, J., Lu, H., Wu, N., Qin, X., and Wang, L. (2013). Palaeoenvironment and Agriculture of Ancient Loulan and Milan on the Silk Road. *The Holocene* 23 (2), 208–217. doi:10.1177/0959683612455541

Zhao, Z., and Piperno, D. R. (2000). Late Pleistocene/Holocene Environments in the Middle Yangtze River Valley, China and rice (Oryza Sativa L.) Domestication: The Phytolith Evidence. *Geoarchaeology* 15 (2), 203–222. doi:10.1002/(sici)1520-6548(200002)15:2<203:aid-gea5>3.0.co;2-j

Zheng, Z., Ma, T., Roberts, P., Li, Z., Yue, Y., Peng, H., et al. (2021). Anthropogenic Impacts on Late Holocene Land-Cover Change and Floristic Biodiversity Loss in Tropical southeastern Asia. *Proc. Natl. Acad. Sci. USA* 118 (40), e2022210118. doi:10.1073/pnas.2022210118

Zhu, Z., Wang, F., and Zhang, Q. (2004). Excavation Bulletin of "Meiyuan" Site in Jinsha Site I, Chengdu. Cult. *Relics* (4), 4–65. doi:10.13619/j.cnki.cn11-1532/k.2004.04.001

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Dynamic Interaction Between Deforestation and Rice Cultivation During the Holocene in the Lower Yangtze River, China

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Human activity has transformed the Earth's landscapes pervasively for thousands of years, and the most important anthropogenic alteration was the clearing of forests and the establishment of agriculture. As a center of rice domestication and early population growth, the lower Yangtze River has been extensively transformed in the Holocene. However, the timing, extent, and process of deforestation and its relationship with the intensification of rice cultivation remain controversial. Here, four representative archaeological sites ranging from 8,100 to 4,300 cal a BP, that is, Jingtoushan, Hemudu, Yushan, and Xiawangdu sites, were selected for detailed palynological analysis, and evidence of anthropogenic deforestation and subsistence strategy were also synthesized to investigate dynamic human–forest interaction. Although natural vegetation had already been altered at the Jingtoushan site around 8,000 cal a BP, it was more likely to be the management of acorns by limiting burning to open habitats and increasing yield. As the subsistence shifted from acorn exploitation toward rice cultivation after 6,000 cal a BP, real deforestation for agriculture may occur at the Yushan and Xiawangdu sites due to conflict on labor input and land use. However, these deforestations were just confined to the archaeological sites at local scale, and no consistent vegetation change occurred at regional scales induced by human activities until the last 3,000 years.

Keywords: deforestation, rice cultivation, Jingtoushan, palynological analysis, lower Yangtze River

INTRODUCTION

Human activity has altered the Earth's system pervasively by transforming landscapes, changing vegetational biodiversity, and altering atmospheric composition (Olofsson and Hickler, 2008; Ruddiman et al., 2015; Mottl et al., 2021). Global land-use modeling and ArchaeoGLOBE synthesis revealed that agriculture and pastoralism had become extensive since 6,000 cal a BP, and largely transformed the planet by 3,000 cal a BP (Kaplan et al., 2011; Stephens et al., 2019). In addition, early anthropogenic hypotheses attributed the anomalous increase in atmospheric CO_2 after 7,000 cal a BP and CH_4 after 5,000 cal a BP to prehistoric deforestation and wet-rice farming, respectively (Ruddiman, 2003; Ruddiman et al., 2016). As the most important anthropogenic

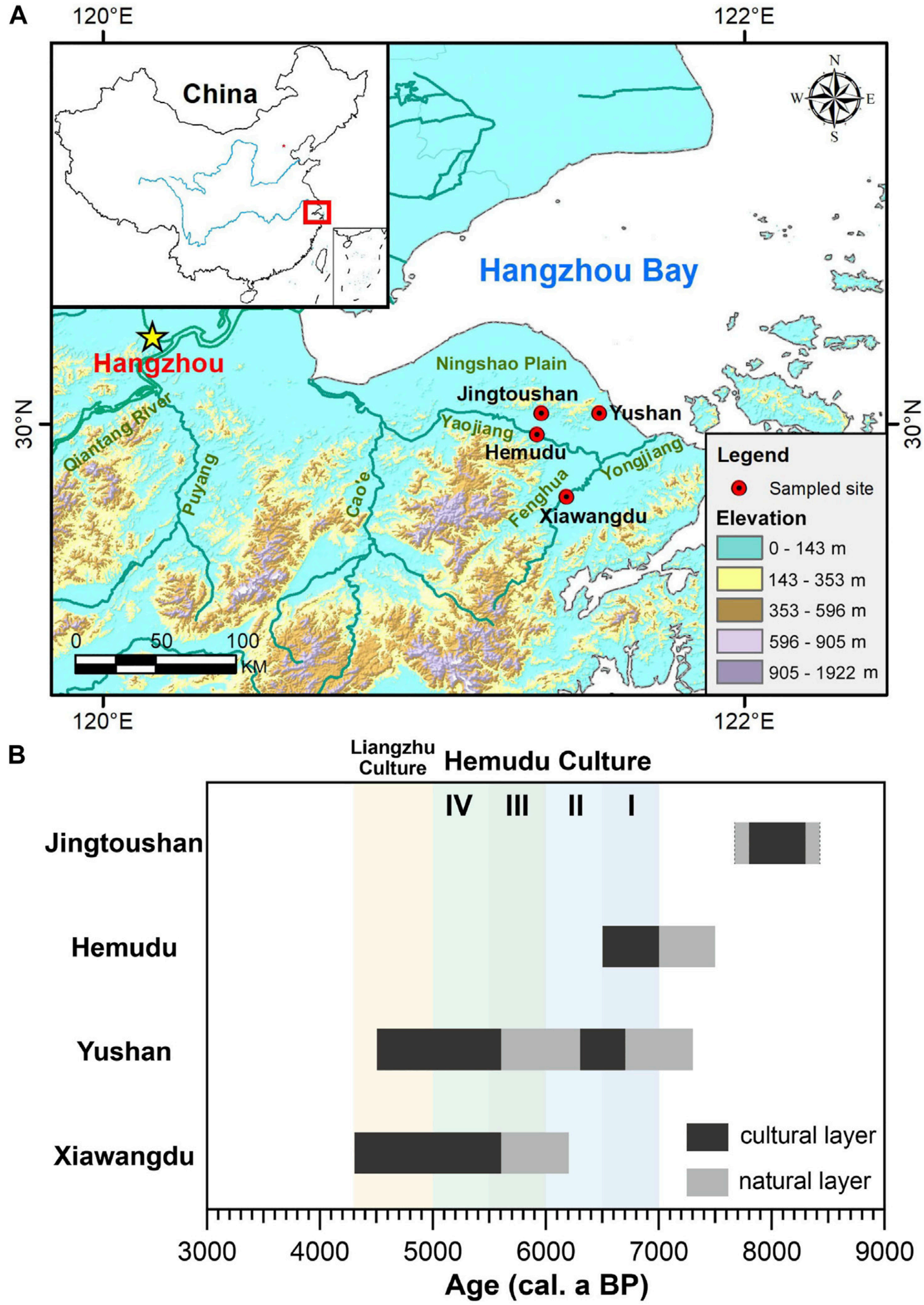

FIGURE 1 | Spatiotemporal distribution of archaeological sites sampled in this study. **(A)** Location of archaeological sites sampled. **(B)** Age range and culture sequence of archaeological sites.

alteration of the natural environment, deforestation and agriculture have been intensively debated by scientists across disciplines (Kaplan et al., 2009; Li et al., 2009).

Although China was widely recognized as a center of rice domestication and early population growth (Lu et al., 2002; Hosner et al., 2016; Zuo et al., 2017), the timing and extent of anthropogenic deforestation were still in dispute. Palynological records indicate human activities have altered the natural vegetated landscapes by deforestation in southern China since 6,000 cal a BP and resulted in a 37% decrease in forest cover in the Yangtze River (Ren, 2006; Cheng et al., 2018). In contrast, synthesis of pollen records in eastern China suggests the effects of human disturbance on vegetation may only occur at some local sites early in the Holocene and have only become an increasingly important factor during the last 2000 years (Liu and Qiu, 1994; Zhao et al., 2009). However, most of these studies have

TABLE 1 | A summary of AMS^{14}C ages from the archaeological sites sampled.

Site	Depth (cm)	Lab code	Material	^{14}C age (a BP, ±1σ)	Calibrated age (a BP, 2σ)	Reference
Jingtoushan	812	Beta412378	Plant[a]	7,150 ± 30	8,019–7,877	This study
Jingtoushan	846	Beta412377	Charcoal	7,180 ± 30	8,025–7,939	This study
Jingtoushan	866	Beta412376	Charcoal	7,210 ± 30	8,165–7,942	This study
Jingtoushan	886	Beta412375	Plant[a]	7,150 ± 30	8,019–7,877	This study
Jingtoushan	906	Beta412374	Charcoal	7,180 ± 30	8,025–7,939	This study
Jingtoushan	857	PLD26540	Fruit	7,130 ± 25	8,011–7,874	This study
Jingtoushan	870	PLD26541	Seed	7,130 ± 25	8,011–7,874	This study
Jingtoushan	887	PLD26542	Wood	7,215 ± 25	8,165–7,958	This study
Jingtoushan	820–870	BA140472	Acorn	6,820 ± 30	7,695–7,584	This study
Jingtoushan	870–920	BA140473	Charcoal	6,995 ± 30	7,932–7,735	This study
Jingtoushan	870–920	BA140474	Acorn	7,060 ± 30	7,961–7,799	This study
Hemudu	172	UGa32508	Charcoal	5,850 ± 35	6,747–6,557	He et al. (2020b)
Hemudu	227	Beta456259	Wood	6,070 ± 30	7,150–6,798	He et al. (2020b)
Hemudu	260	UGa32509	Charcoal	6,190 ± 30	7,235–6,988	He et al. (2020b)
Hemudu	275	Beta456260	Wood	6,010 ± 30	6,943–6,749	He et al. (2020b)
Hemudu	335	UGa32510	Charcoal	6,200 ± 30	7,241–6,995	He et al. (2020b)
Hemudu	484	UGa32511	Charcoal	6,520 ± 30	7,506–7,330	He et al. (2020b)
Yushan	65–70	BA151803	Plant[a]	4,040 ± 30	4,612–4,418	He et al. (2018)
Yushan	100–105	BA151804	Seed	4,365 ± 25	5,030–4,856	He et al. (2018)
Yushan	125–130	BA151805	Seed	4,300 ± 25	4,959–4,830	He et al. (2018)
Yushan	150–155	BA151806	Seed	4,525 ± 25	5,310–5,051	He et al. (2018)
Yushan	180–185	BA151807	Seed	4,785 ± 25	5,585–5,475	He et al. (2018)
Yushan	215–220	BA151808	Seed	5,495 ± 25	6,391–6,211	He et al. (2018)
Yushan	230–235	BA151809	Seed	5,665 ± 25	6,500–6,352	He et al. (2018)
Yushan	245–250	BA151810	Seed	5,860 ± 25	6,745–6,568	He et al. (2018)
Yushan	260–265	BA151811	Seed	6,225 ± 25	7,250–7,010	He et al. (2018)
Xiawangdu	40–35	UGa32514	Charcoal	4,420 ± 30	5,271–4,870	He et al. (2020a)
Xiawangdu	60–55	UGa38688	Rice seed	4,460 ± 20	5,281–4,975	He et al. (2020a)
Xiawangdu	80–75	UGa32515	Rice seed	4,570 ± 30	5,442–5,053	He et al. (2020a)
Xiawangdu	115–110	UGa38687	Rice seed	4,650 ± 20	5,463–5,315	He et al. (2020a)
Xiawangdu	140–135	UGa32516	Rice seed	5,100 ± 30	5,920–5,747	He et al. (2020a)
Xiawangdu	150–145	UGa38686	Rice seed	4,760 ± 20	5,583–5,467	He et al. (2020a)
Xiawangdu	160–155	UGa32517	Rice seed	4,850 ± 30	5,654–5,479	He et al. (2020a)
Xiawangdu	175–165	UGa38685	Rice seed	5,070 ± 20	5,902–5,746	He et al. (2020a)

[a]*Plant indicates fragments of unidentifiable plant that has not been fully charred.*

been conducted on natural sediments and have exhibited indirect signals of human activities, lacking archaeological evidence.

In the lower Yangtze River, pollen and charcoal data from the Kuahuqiao site demonstrated that alder-dominated wetland scrub or oak-pine-dominated forests had been cleared using fire by early rice-cultivators after 7,700 cal a BP (Zong et al., 2007; Innes et al., 2009; Shu et al., 2012). Nevertheless, charcoal analysis from cores near the Kuahuqiao site suggested that human-induced fires were restricted to a small geographic area with no constant large-scale slash-and-burn farming activities (Hu et al., 2020). Pollen data from paddy fields at the Tianluoshan site also suggested no evidence of slash-and-burn agriculture from 7,000 to 4,200 cal a BP (Li et al., 2012). Black carbon analysis of paddy soils from the Chuodun site further demonstrated that the fire was applied to burn crop residue rather than natural vegetation (Hu et al., 2013). Thus, whether the deforestation and slash-and-burn agriculture were adopted in the lower Yangtze River remains controversial.

To investigate the timing, extent, and process of deforestation in the lower Yangtze River and its relationship with the intensification of rice cultivation, four representative archaeological sites (i.e., Jingtoushan, Hemudu, Yushan, and Xiawangdu) (**Figure 1A**) were selected for detailed palynological analysis in this study. Furthermore, evidence of deforestation induced by human activities and the transition of subsistence strategy in this region were also synthesized to offer insight into the dynamic interaction between deforestation and rice cultivation in the Holocene.

MATERIALS AND METHODS

Study Site and Sample Collection

The four archaeological sites are located on the Ningshao Plain on the southern shore of Hangzhou Bay in eastern China (**Figure 1A**). The sequence of Neolithic cultures mainly consisted of the Hemudu Culture (7,000–5,000 cal a BP), which is generally subdivided into four periods at approximately 500-year intervals (Wang, 2000), and the Liangzhu Culture (5,000–4,300 cal a BP) (**Figure 1B**). The mean annual precipitation and temperature in this region are c.1000–1,400 mm and c.15–16°C, respectively. Regional

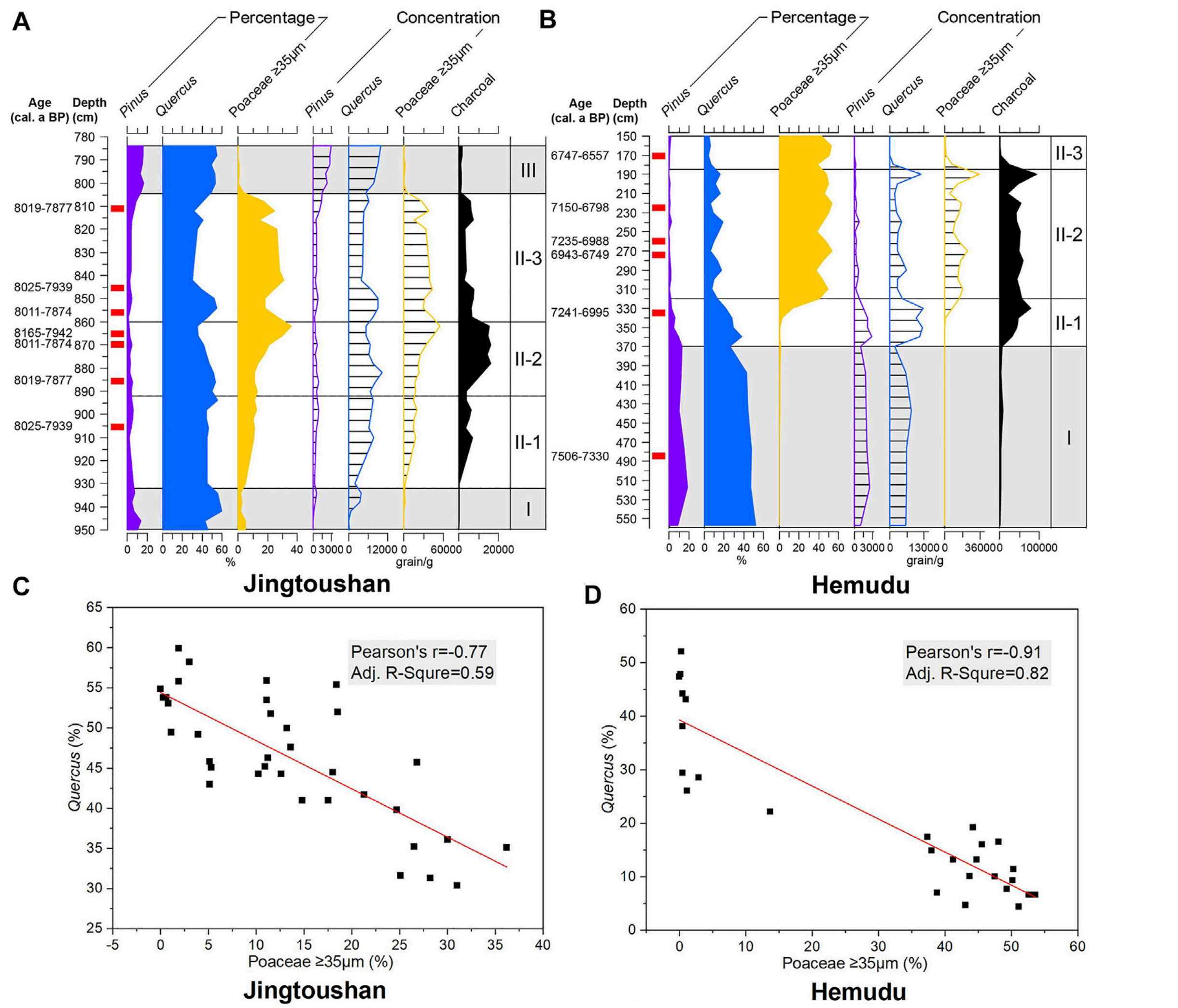

FIGURE 2 | Pollen diagram and relationship between selected taxa at the Jingtoushan and Hemudu sites. **(A,B)** Percentage and concentration pollen diagram with selected taxa. Gray horizontal bars denote the natural layers without human activities. Locations of the dating samples are shown on the right side of the depth scale by red rectangles. **(C,D)** Relationship between percentage of *Quercus* and Poaceae ≥35 μm pollen.

vegetation is under the influence of the East Asian Monsoon and is characterized by subtropical mixed forests of evergreen trees, for example, *Lithocarpus*, *Cyclobalanopsis*, and *Quercus*, and deciduous trees, for example, *Liquidambar*, *Castanea*, and *Celtis*.

Jingtoushan site (30°2′N, 121°22′E) is situated in the Yaojiang Valley, about 2.3 km west of the Tianluoshan site and 7.6 km north of the Hemudu site, with elevation of 2.5 m above local mean sea level (Zhejiang Provicial Institute of Cultural Relics and Archaeology et al., 2021). The Jingtoushan site was first discovered and drilled in 2014, and the cultural layers were about 7–8 m below the ground, which was the deepest archaeological site in the coastal region of China. Three sections of No. 4 core were selected for pollen analysis, that is, 4 h (1,020–920 cm), 4 g (920–820 cm), and 4f (820–720 cm). The cultural layer (930–805 cm) was subdivided into three layers: the silt layer (930–890 cm), the charcoal layer (890–860 cm), and the shell layer (860–805 cm), which was formed upon weathered crust and overlaid by thick marine sediments (**Supplementary Figure S1**). A total of 35 samples were collected at 4 cm intervals, including 25 samples from the cultural layer and five samples from the natural sediment layers below and above, respectively.

The Hemudu site (29°58′N, 121°21′E) is situated beside the Yaojiang River and has been excavated twice in 1973 and 1977, covering approximately 50000 m^2 (Zhejiang Provincial Institute of Relics and Archaeology, 2003). A 715-cm long core HMD1602 was retrieved from the site reservation of pile dwellings southeast of the Hemudu museum in 2016. The upper 140 cm was composed of continental and artificial layers that were not analyzed. The sediment of 370–140 cm was the Hemudu cultural layer and was sampled at 10 cm intervals. The sediment of 558–370 cm containing numerous foraminifera and marine diatoms was interpreted as a neritic layer and sampled at approximately 20 cm intervals (**Supplementary Figure S2**) (He et al., 2020b). A total of 27 samples were

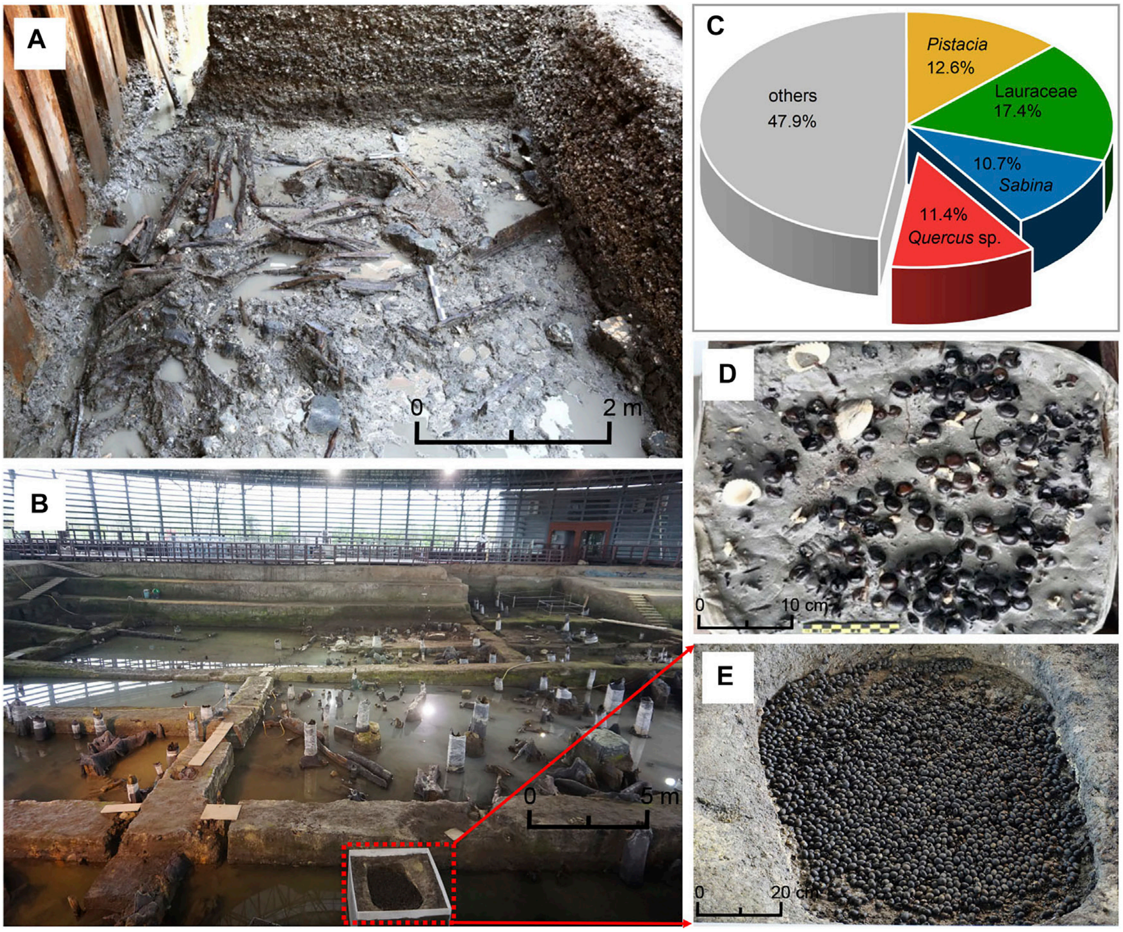

FIGURE 3 | Wooden remains and pits of acorns excavated at the Jingtoushan and Tianluoshan sites. **(A)** Wooden remains excavated below layer 17 in T508 trenches of the Jingtoushan site, including wooden handle and paddle. **(B)** Pile dwellings excavated below layer 6 at the Tianluoshan site. **(C)** Identification of tree species of archaeological woods from the Tianluoshan site (Suzuki et al., 2011). **(D,E)** Pits of acorns from the Jingtoushan (H13) and Tianluoshan sites.

collected for pollen analysis, including 17 samples from the cultural layer and 10 samples from the natural layers below.

The Yushan site (30°02′N, 121°33′E) is situated on the eastern entrance of the Yaojiang Valley, 7.3 km west to the present coastline (Ningbo Municipal Institution of Cultural Relics and Archaeology et al., 2016). The Yushan site was excavated in 2013, and a 275-cm long profile was collected from the south section of trench T0213 (**Supplementary Figure S3**). The upper 60 cm of the profile encompassed historic and modern sediment and was not sampled. The lower 215 cm was subdivided into three natural layers of marine transgression at 275–250 cm, 215–180 cm, and 120–60 cm, and two cultural layers of Hemudu Period II (250–215 cm) and Hemudu Period III-Liangzhu Culture (180–120 cm) (He et al., 2018). A total of 43 samples were collected at 5 cm intervals for pollen analysis.

The Xiawangdu site (29°46′N, 121°26′E) is situated beside the Fenghua River, which merges with Yaojiang River to form Yongjiang River at Ningbo City (Ningbo Municipal Institution of Cultural Relics and Archaeology et al., 2019). This site was excavated in 2017, and a 195-cm long profile sampled was in the west section of trench T0602 (**Supplementary Figure S4**). The upper 10 cm of the profile encompassed historic and modern sediment and was not sampled. The lower 185 cm encompassed three main units, including natural layers of marine transgression at 195–160 cm, cultural layers of Hemudu Period III—IV (160–50 cm), and Liangzhu Culture (50–10 cm) (He et al., 2020a). A total of 40 samples were collected at approximately 5 cm intervals for pollen analysis.

In total, 145 samples were collected from cores/profiles of the four archaeological sites, which were located in the core region of each archaeological site with the most complete cultural sequence and sediments, ranging from the coast to foothills in space and spanning the middle Holocene (8.2–4.2 cal ka BP) in time. Previously, pollen records of profiles from the Yushan and Xiawangdu sites have been reported (He et al., 2018; He et al., 2020a). For this study, palynological analysis of the cores at the Jingtoushan (**Supplementary Figure S5**) and Hemudu (**Supplementary Figure S6**) sites was conducted to get a full view of human–environment interaction during the whole

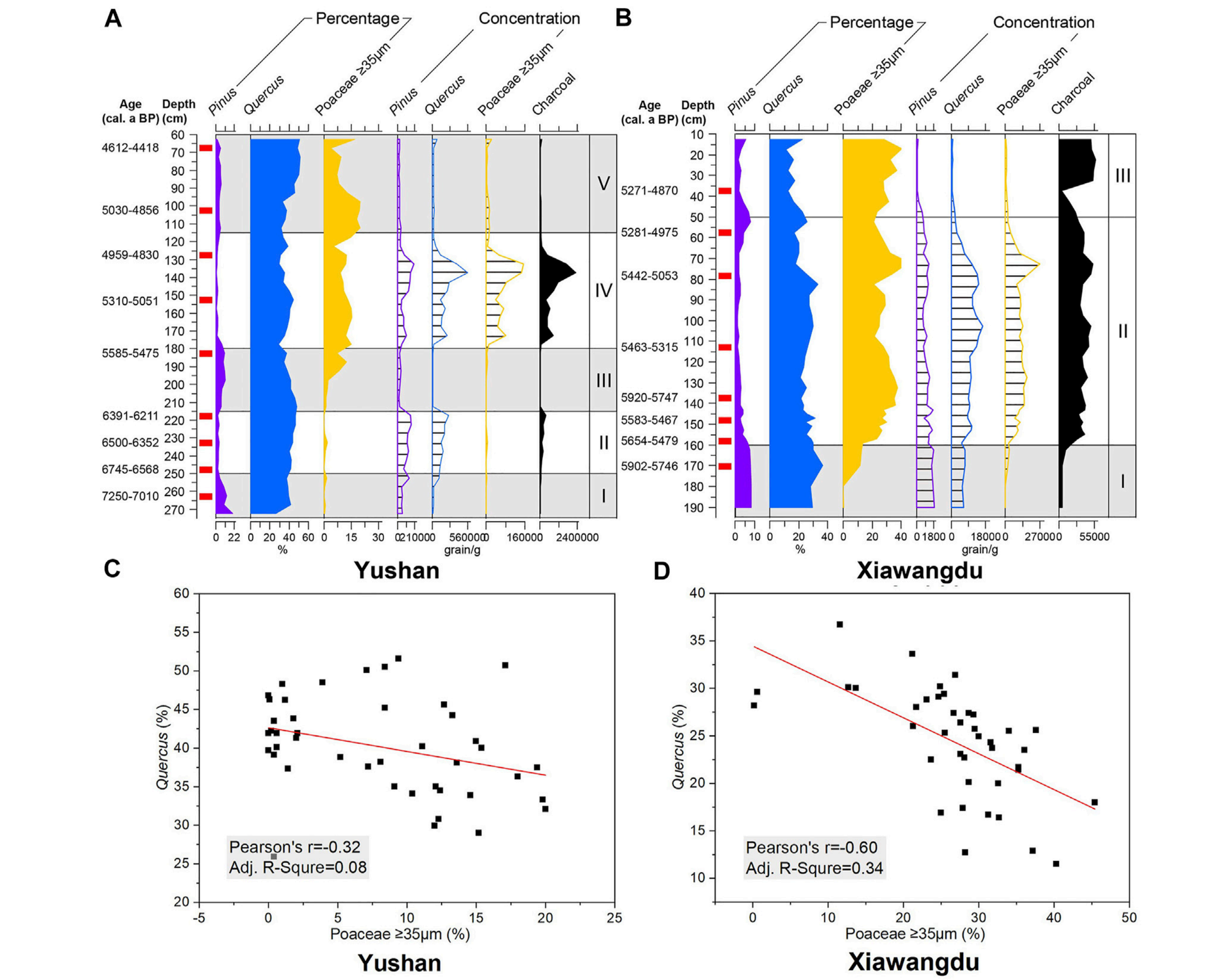

FIGURE 4 | Pollen diagram and relationship between selected taxa at the Yushan and Xiawangdu sites. **(A,B)** Percentage and concentration pollen diagram with selected taxa. Gray horizontal bars denote the natural layers without human activities. Locations of the dating samples are shown on the right side of the depth scale by red rectangles. **(C,D)** Relationship between percentage of Quercus and Poaceae ≥35 μm pollen.

middle Holocene. Each core/profile encompassed both natural (light gray) and cultural layers (dark gray) (**Figure 1B**) for further comparison of environmental change and human activities.

Radiocarbon Dating

Thirty-four samples in total were collected for dating, which have been conducted in four radiocarbon laboratories: Beta Analytic (Beta), Peking University (BA), Paleo Labo Co. (PLD), and the University of Georgia (UGa). With regard to the cores at the Jingtoushan and Hemudu sites, plants and charcoal were collected systematically during sampling; as to the profiles of the Yushan and Xiawangdu sites, samples were collected at the boundaries of each layer and screened to retrieve short-lived seeds and charcoal for dating. The radiocarbon ages were calibrated uniformly using the IntCal20 dataset with the OxCal v4.4 program (Bronk Ramsey, 2009; Reimer et al., 2020). Dates of the Jingtoushan site are first published in this study, and details of all the dates are displayed in **Table 1** and **Figures 2**, **4**.

Pollen Analysis

Two grams of each sample were weighed for pollen analysis. Pollen samples were treated according to the standard procedure developed by Moore et al. (1991), and generally, over 400 grains were counted for each sample using a Leica DM 750 microscope at ×400 magnification. Microscopic charcoal was counted on the pollen slides while identifying pollen. Identification of pollen and spores was made with reference to modern and Quaternary atlases (Institute of Botany and South China Institute of Botany, 1982; Wang et al., 1995; Tang et al., 2016). *Quercus* (oak) pollen included two categories based on the surface, tricolporoidate and size, that is, *Quercus*-deciduous and *Quercus*-evergreen (including *Cyclobalanopsis*) (Tang et al., 2018). Poaceae pollen was divided into three size categories (<35, 35–40, and

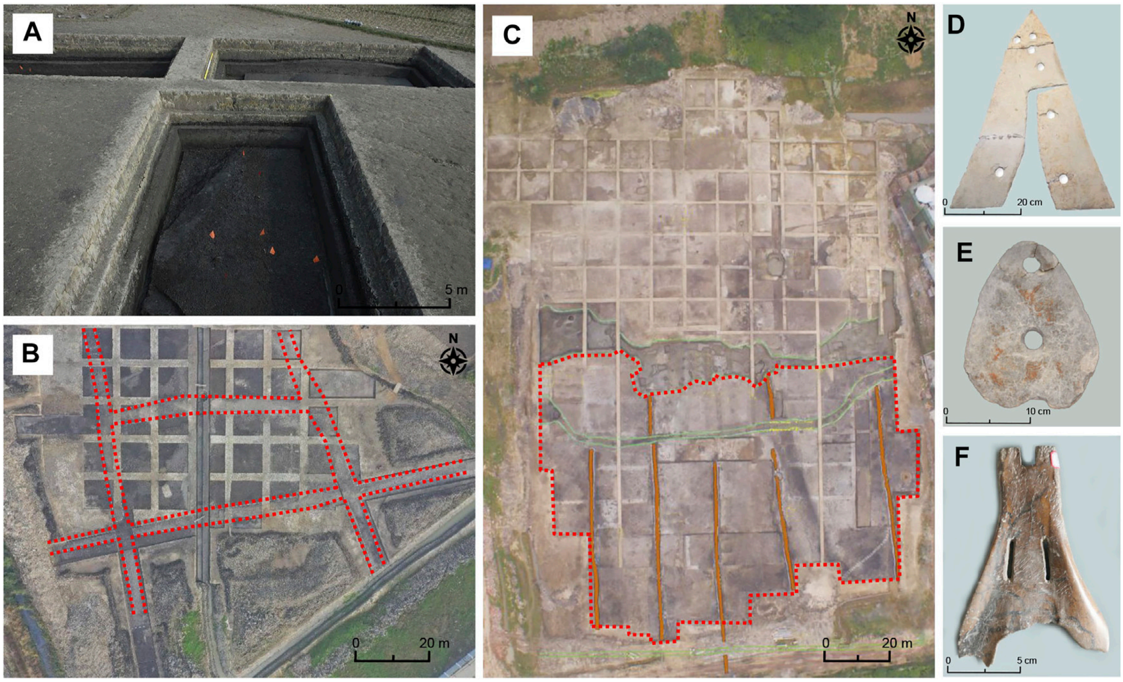

FIGURE 5 | Paddy system and farm tools excavated in the lower Yangtze River. **(A)** Paddy system in T803 and T703 trenches from the Tianluoshan site, dated 5,290 to 5,040 cal a BP (Zheng et al., 2009). **(B)** Paddy system excavated at the Shi'ao site, covering about 4,650 m^2, dated 4,900 to 4,500 cal a BP. **(C)** Paddy system excavated at the Maoshan site, covering about 55000 m^2, dated 4,700 to 4,200 cal a BP (Zhuang et al., 2014). **(D)** Split type stone plow excavated at the Maoshan site. **(E,F)** Integrative type stone plow and bone spade excavated at the Xiawangdu site.

>40 μm), and the large size category (≥35 μm) had been identified as domesticated rice pollen in the sediment of eastern China (Yang et al., 2012). Zones in the pollen diagram were divided according to the sediment and pollen assemblages using C2 software (Juggins, 2007). In addition, a correlation analysis between the percentage of *Quercus* and Poaceae ≥35 μm pollen was conducted by Origin 2021 software.

RESULTS AND DISCUSSION

Deforestation and Rice Cultivation Inside the Archaeological Sites

In this study, the terrestrial pollen assemblages of the four archaeological sites were all predominated by arboreal *Quercus* (average 20.4–46.3%) and herbaceous Poaceae (average 16.9–51.8%), and exhibited diverse patterns with time. In the natural layers of the Jingtoushan (Zones I and III) and Hemudu (Zone I) sites, the pollen diagram was dominated by the primeval vegetation of *Quercus* and *Pinus*. In contrast, the percentage of *Quercus* declined conspicuously and was progressively replaced by that of rice-type Poaceae ≥35 μm in the cultural layers of the Jingtoushan (Zone II, 8,100–7,800 cal a BP) and Hemudu (Zone II, 7,100–6,500 cal a BP) sites (**Figures 2A,B**). However, the percentages were problematic among different sedimentary facies. The decrease of *Quercus* in the cultural layer may not result from deforestation but be caused by the relative rise of Poaceae ≥35 μm instead (Li et al., 2012), which could be corroborated by the strong negative correlation between that of *Quercus* and Poaceae (**Figures 2C,D**).

In addition, the decline in the percentage of *Quercus* and the increase of charcoal in the cultural layer of the Jingtoushan (Zone II-2) and Hemudu (Zone II-1) sites may imply the management of thinning the stands of acorn using fire to harvest efficiently (Pan et al., 2017). Although large quantities of wooden remains, such as pile dwellings, had been excavated at the Jingtoushan and Tianluoshan sites (**Figures 3A, B**), only a few were identified as *Quercus* (11.4%) (**Figure 3C**) (Suzuki et al., 2011), which implied that *Quercus* may have been consciously protected for the collection and storage of acorns (**Figures 3D, E**) rather than deforestation.

In the profiles of the Yushan and Xiawangdu sites, the concentration of *Pinus*, *Quercus*, and Poaceae almost changed synchronously, implying that the relative abundance may not be affected by different sedimentary facies (**Figures 4A, B**). Moreover, the negative correlation between the percentage of *Quercus* and Poaceae was weak (**Figures 4C, D**), indicating these two pollen types change independently and the percentage could reflect the real evolution of local vegetation. In the later stages of the cultural layers of the Yushan (Zone IV, 5,600–5,000 cal a BP) and Xiawangdu (Zone II and III, 5,600–4,300 cal a BP) sites, the decline of the percentage of *Quercus* and *Pinus* coincided with the increase of that of Poaceae ≥35 μm and charcoal, suggesting deforestation of *Quercus* forest induced by the intensification of rice cultivation. Archaeobotanical research suggested that extensive paddy systems, such as Tianluoshan, Shi'ao (ca 4,650 m^2), and Maoshan sites (ca 55000 m^2) (**Figures 5A–C**) (Zheng et al., 2009; Zhuang et al., 2014), and new farm tools, such as stone plow and bone spade (**Figures 5D–F**) (Fuller et al., 2008), had been applied

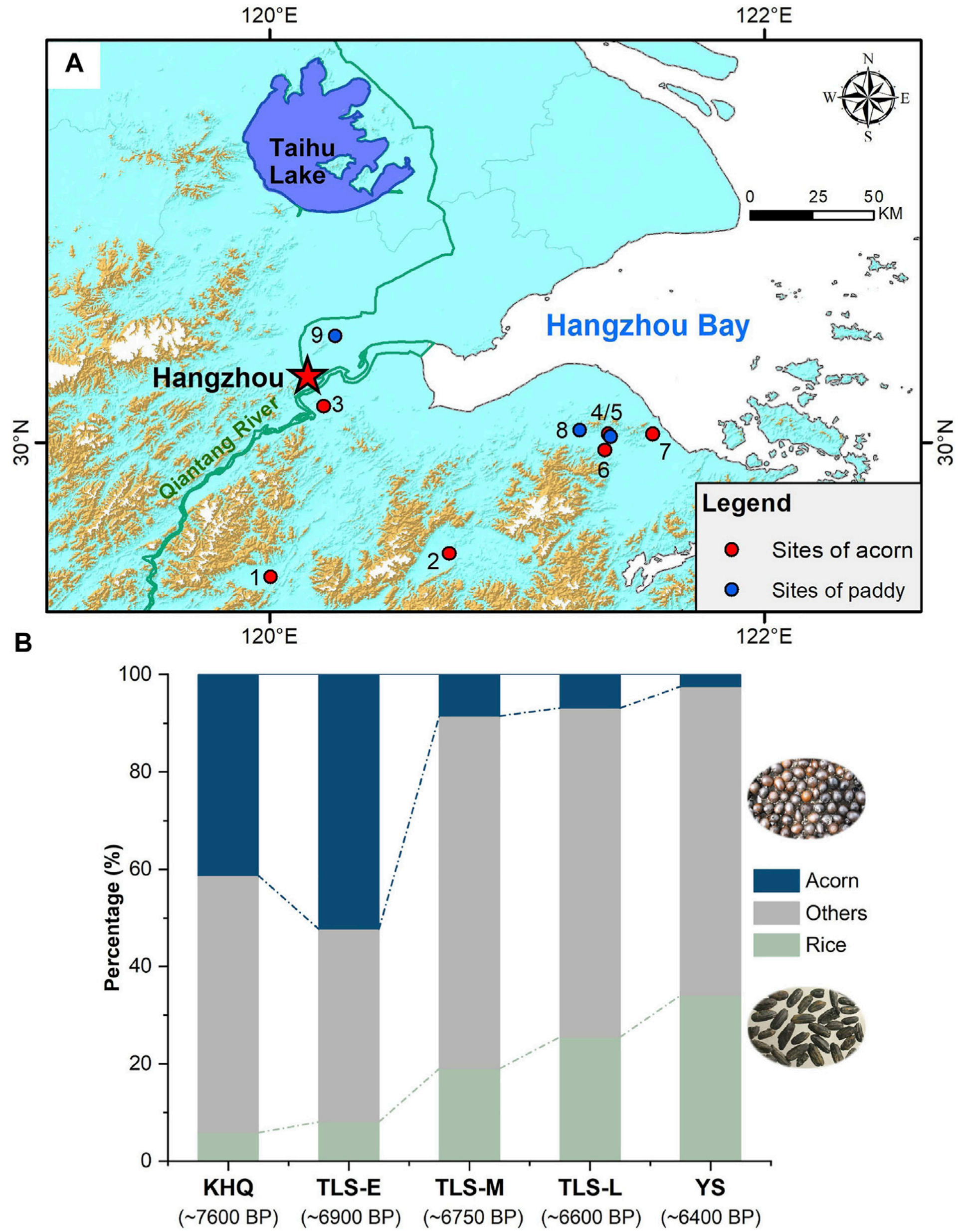

FIGURE 6 | Shift in subsistence from acorn exploitation to rice cultivation. **(A)** Location of sites with acorns (1–7) and paddy (8–9) excavated in the lower Yangtze River. 1. Shangshan, 2. Xiaohuangshan (Liu et al., 2010b), 3. Kuahuqiao, 4. Jingtoushan, 5. Tianluoshan, 6. Hemudu, 7. Yushan, 8. Shi'ao, 9. Maoshan (Zhuang et al., 2014). **(B)** Proportion of plant remains from three sites, Kuahuqiao (KHQ) (Pan, 2011), Tianluoshan (TLS) (Fuller et al., 2009), and Yushan (YS) (Zheng et al., 2019), indicating percentages of rice, acorns, and others.

since late Hemudu-Songze and Liangzhu culture, which further support the hypothesis of slash-and-burn practices due to the possible shortage of cultivated land.

Evolution of Subsistence Strategy: Acorn Exploitation vs. Rice Cultivation

Acorns are the nuts of *Quercus* sp., which are the constructive species of the subtropical mixed forests of evergreen and deciduous trees in southern China, and constitute the main arboreal component in the palynological records since the late Quaternary (Tang et al., 2018). Ethnographic and archaeological evidence suggest that acorn is a significant resource commonly consumed by Native Americans (Anderson, 2005) and recovered from prehistoric sites in Levant Upper-Palaeolithic (Barlow and Heck, 2002), Japanese Jomon (Takahashi and Hosoya, 2002), and Chinese Early-Middle Neolithic (Yang et al., 2009; Liu et al., 2010a). The earliest evidence for the exploitation of acorn in the lower Yangtze River was starch grains extracted from grinding stones of the Shangshan culture (10,000–8,500 cal a BP) (Liu

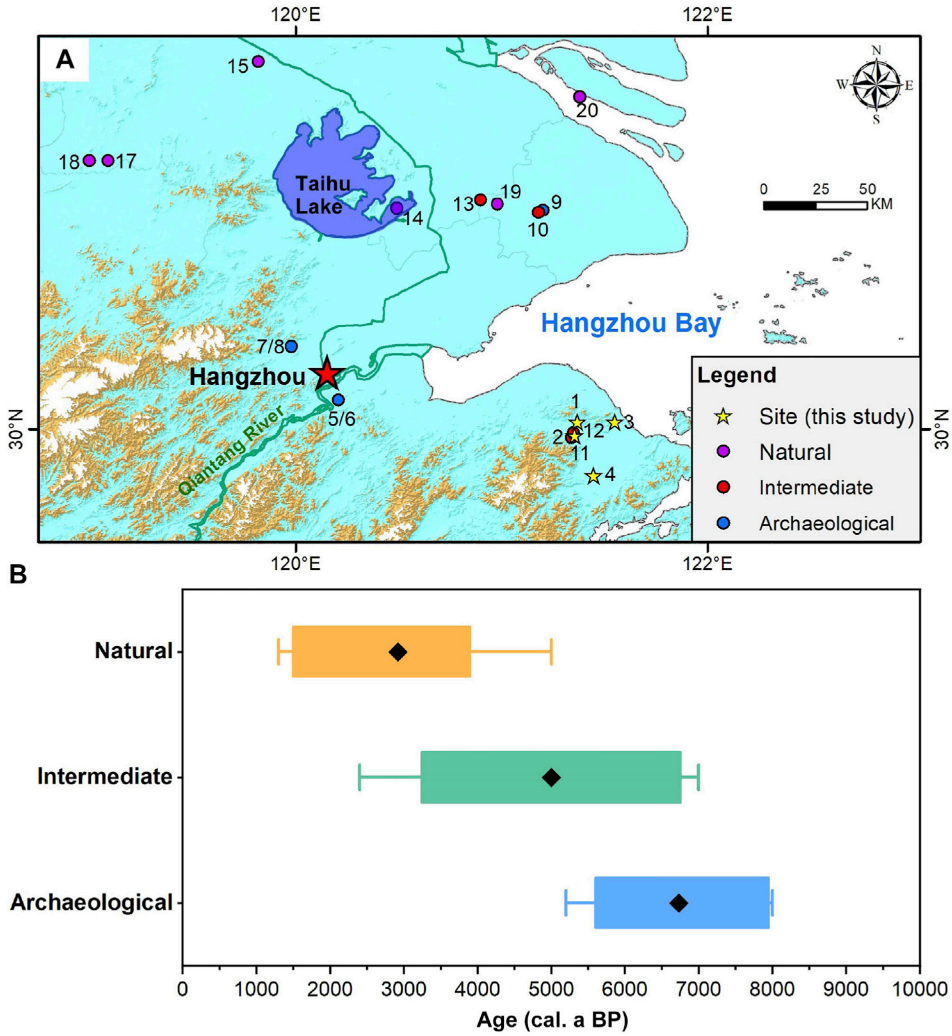

FIGURE 7 | Records of deforestation induced by human activities in the lower Yangtze River. **(A)** Location of sites with records of deforestation. **(B)** Boxplot of deforestation timing recorded in natural, intermediate, and archaeological sites. Detailed information of the sites numbered is shown in **Table 2**.

et al., 2010b; Wang and Jiang, 2021). In addition, large quantities of acorns have been excavated from the storage pits at the Kuahuqiao, Hemudu, and Tianluoshan sites (**Figure 6A**) (Zhejiang Provincial Institute of Relics and Archaeology, 2003; Fuller et al., 2011; Jiang, 2014).

Acorn is a starch-rich resource that was likely to be exploited as a staple food, predating the establishment of rice agriculture. In the early stages of rice cultivation, acorns accounted for approximately 41.2 and 52.2% of the whole plant remains at the Kuahuqiao and Tianluoshan sites (**Figure 6B**), respectively (Fuller et al., 2009; Pan, 2011). In addition, more than twenty storage pits of acorns had been recovered at the Tianluoshan site, and pieces of acorn shells had also been sieved out (Sun, 2013). Thus, in the subsistence strategy, acorn can be used as a staple food for both reserve resources and daily consumption. As a result, the *Quercus* may have been managed consciously by limiting burning to open habitats and increasing per-tree yield (Pan et al., 2017), and the decline of the concentration of *Quercus* in the cultural layer of Jingtoushan (Zone II-2, ca 8,000 cal a BP) and Hemudu (Zone II-1, ca 7,000 cal a BP) sites may have just reflected this human activity. Considering the low-level production of rice cultivation prior to 6,000 cal a BP, ancient humans may not sacrifice the resource of acorns to give way to undependable rice cultivation.

However, several factors may restrict the acorn exploitation. First, acorns are a seasonal resource, concentrated from August to October, and their annual yields vary dramatically (Pan et al., 2017). Second, acorns need special processing methods to remove tannins and an appropriate temperature and humidity during storage (Takahashi and Hosoya, 2002). Finally, a cooling trend through the middle to late Holocene may cause regional declines in oaks (Fuller and Qin, 2010). Therefore, the proportion of acorns in plant remains declined significantly to approximately 6.8 and 2.4% during the later stages of the Tianluoshan and Yushan sites, while that of rice increased progressively to 25.5 and 34.0% (**Figure 6B**), respectively (Fuller et al., 2009; Zheng et al.,

TABLE 2 | Detailed information on the start time of deforestation induced by human activities in the lower Yangtze River (see **Figure 7** for locations).

No.	Site	Start time (cal. a BP)	Category	Location Lat. (N); Long. (E)	Reference
1	Jingtoushan	8,000	Archaeological	30.03°, 121.36°	This study
2	Hemudu	7,000	Archaeological	29.96°, 121.35°	This study
3	Yushan	5,600	Archaeological	30.03°, 121.55°	This study
4	Xiawangdu	5,600	Archaeological	29.77°, 121.44°	This study
5	Kuahuqiao	7,950	Archaeological	30.14°, 120.21°	Shu et al. (2010)
6	Kuahuqiao	7,750	Archaeological	30.14°, 120.21°	Zong et al. (2007)
7	Liangzhu	5,200	Archaeological	30.4°, 119.98°	Li et al. (2010)
8	Liangzhu	4,800	Archaeological	30.4°, 119.98°	Liu et al. (2015)
9	Guangfulin	4,635	Archaeological	31.06°, 121.20°	Tang et al. (2019)
10	Guangfulin	7,000	Intermediate	31.05°, 121.18°	Itzstein-Davey et al. (2007b)
11	Hemudu	6,500	Intermediate	29.96°, 121.34°	Liu et al. (2016)
12	Luojiang	4,100	Intermediate	29.98°, 121.35°	Atahan et al. (2008)
13	Qingpu	2,400	Intermediate	31.11°, 120.9°	Itzstein-Davey et al. (2007a)
14	E2A	5,000	Natural	31.07°, 120.49°	Xu et al. (1996)
15	ZK01	3,900	Natural	31.78°, 119.82°	Shu et al. (2007)
16	ACN	3,750	Natural	31.53°, 117.37°	Chen et al. (2009)
17	Gaochun	3,000	Natural	31.30°, 119.09°	Yao et al. (2017)
18	Caoduntou	2000	Natural	31.30°, 119.00°	Okuda et al. (2003)
19	Dianshan	1,500	Natural	31.09°, 120.98°	Innes et al. (2019)
20	CM97	1,300	Natural	31.61°, 121.38°	Yi et al. (2003)

2019). As the subsistence economy shifts toward an increasing focus on rice, acorns may degenerate from a staple to a famine food reserve (Hosoya, 2011). The labor input and land use of rice cultivation conflicted with that of acorn exploitation, resulting in the local deforestation that is recorded at the Yushan and Xiawangdu sites.

Timing and Extent of Deforestation Induced by Human Activities

Deforestation for farming was the main type of impact on land use made by prehistoric humans on vegetation that has been detected in Europe and China since the middle Holocene (Ren, 2000; Fyfe et al., 2015; Roberts et al., 2018). The entire ecological process of human-induced deforestation could be modeled into five stages, that is, primeval vegetation, deforestation, cultivation, abandonment, and restoration, which can be reflected in the palynological records (Li et al., 2008). The pollen assemblage of anthropogenic deforestation was generally characterized by a decrease of zonal broadleaf wood (e.g., *Quercus*) and a rise of secondary pioneer trees (e.g., *Pinus*), ferns, and herbs (e.g., Poaceae), accompanied by an increase in charcoal (Zheng et al., 2004; Li et al., 2008; Xu et al., 2010). The lower Yangtze River was densely distributed with over 4,000 archaeological sites during the Neolithic and Bronze Age (Hosner et al., 2016) and was substantially transformed by humans. Based on the relative distance to the archaeological sites, palynological research works on the timing of deforestation induced by human activities can be divided into three categories: natural (>3 km), intermediate (50 m-3 km), and archaeological sites (**Figure 7A**), respectively.

The earliest signals of anthropogenic deforestation in the lower Yangtze River could be traced back to ca 8,000 and 7,700 cal a BP from the Jingtoushan and Kuahuqiao sites (**Table 2**) (Zong et al., 2007; Shu et al., 2012); however, the average and median dates of deforestation among archaeological sites were ca 7,000 cal a BP. As to the intermediate site that is situated 50–3,000 m outside the adjacent archaeological site, the timing of deforestation ranged from ca 7,000 to 3,000 cal a BP, with an average and median date of ca 5,000 cal a BP. Finally, the timing of deforestation from the natural sediments ranged from ca 4,000 to 1,500 cal a BP, which was generally later than ca 3,000 cal a BP. Significantly, the timing of deforestation decreased progressively from ca 7,000 cal a BP of the archaeological to ca 5,000 cal a BP of the intermediate site and ca 3,000 cal a BP of the natural sites at the end (**Figure 7B**).

Therefore, the synthesized pattern of pollen records in the lower Yangtze River suggested that possible anthropogenic deforestation in the middle Holocene was confined to local scales (Zhao et al., 2009), and no consistent vegetation change occurred at regional scales induced by human activities as reported previously (Ren, 2006). Instead, the main driver of regional vegetation change at the mid-late Holocene transition in eastern China may be attributed to climatic deterioration (Innes et al., 2014). In general, the effects of human disturbance on vegetation at local scales intensified gradually from archaeological to intermediate sites since the middle Holocene, and became an increasingly important factor in the vegetation of natural sites at regional scales until the last 3,000 years (Liu and Qiu, 1994; Zheng et al., 2021), which coincided with a noticeable increase in the number of archaeological sites after 3,500 cal a BP in southern China (Hosner et al., 2016).

CONCLUSION

In this study, synthesized palynological analysis was applied to the Jingtoushan, Hemudu, Yushan, and Xiawangdu sites, and shed light on the timing, extent, and process of deforestation accompanied by the intensification of rice cultivation in the lower Yangtze River. Although natural vegetation had already been altered at the Jingtoushan site around 8,000 cal a BP, it was more likely to be management of acorns by limiting burning to open habitats and increasing per-tree yield. As the subsistence economy shifted toward rice cultivation after 6,000 cal a BP, real deforestation for agriculture may happen due to conflict of acorn exploitation on labor input and land use, which was just confined to local scale inside the archaeological site. Possible synchronous deforestation that occurred in archaeological, intermediate, and natural sites at regional scales may be postponed until the last 3,000 years.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HL and KH designed the research plan. GS, YZ, HZ, SL, and YL provided the archaeological samples. YW and JZ assisted in the collection and extraction of samples. KH analyzed the data and created the figures. KH and HL wrote and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.849501/full#supplementary-material

REFERENCES

Anderson, M. (2005). *Tending the Wild: Native American Knowledge and Management of California's Natural Resources*. Berkeley: University of California Press.

Atahan, P., Itzstein-Davey, F., Taylor, D., Dodson, J., Qin, J., Zheng, H., et al. (2008). Holocene-aged Sedimentary Records of Environmental Changes and Early Agriculture in the Lower Yangtze, China. *Quat. Sci. Rev.* 27, 556–570. doi:10.1016/j.quascirev.2007.11.003

Barlow, K. R., and Heck, M. (2002). "More on Acorn Eating during the Natufian: Expected Patterning in Diet and the Archaeological Record of Subsistence," in *Hunter–gatherer Archaeobotany: Perspective from the Northern Temperate Zone*. Editors S. L. R. Mason and J. G. Hather (London: University College London Press), 128–145.

Bronk Ramsey, C. (2009). Bayesian Analysis of Radiocarbon Dates. *Radiocarbon* 51, 337–360. doi:10.1017/S0033822200033865

Chen, W., Wang, W., and Dai, X. (2009). Holocene Vegetation History with Implications of Human Impact in the Lake Chaohu Area, Anhui Province, East China. *Veget Hist. Archaeobot* 18, 137–146. doi:10.1007/s00334-008-0173-7

Cheng, Z., Weng, C., Steinke, S., and Mohtadi, M. (2018). Anthropogenic Modification of Vegetated Landscapes in Southern China from 6,000 Years Ago. *Nat. Geosci* 11, 939–943. doi:10.1038/s41561-018-0250-1

Fuller, D. Q., and Qin, L. (2010). Declining oaks, Increasing Artistry, and Cultivating rice: the Environmental and Social Context of the Emergence of Farming in the Lower Yangtze Region. *Environ. Archaeology* 15, 139–159. doi:10.1179/146141010x12640787648531

Fuller, D. Q., Qin, L., and Harvey, E. (2008). "Evidence for a Late Onset of Agriculture in the Lower Yangtze Region and Challenges for an Archaeobotany of rice," in *Past Human Migrations in East Asia: Matching Archaeology, Linguistics and Genetics*. Editors A. Sanchez-Mazas, R. Blench, M. D. Blench, I. Peiros, and M. Lin (London: Routledge), 40–83.

Fuller, D. Q., Qin, L., Zhao, Z., Zheng, Y., Hosoya, L., Chen, X., et al. (2011). "Archaeobotanical Analysis at Tianluoshan: Evidence for Wild-Food Gathering, rice Cultivation and the Process of the Evolution of Morphologically Domesticated rice," in *Integrated Studies on the Natural Remains from Tianluoshan*. Editors Center for the Study of Chinese Archaeology in Peking University and Zhejiang Province Institute of Archaeology and Cultural Heritage (Beijing: Cultural Relics Press), 47–96. (in Chinese).

Fuller, D. Q., Qin, L., Zheng, Y., Zhao, Z., Chen, X., Hosoya, L., et al. (2009). The Domestication Process and Domestication Rate in rice: Spikelet Bases from the Lower Yangtze. *Science* 323, 1607–1610. doi:10.1126/science.1166605

Fyfe, R. M., Woodbridge, J., and Roberts, N. (2015). From forest to farmland: Pollen-inferred Land Cover Change across Europe Using the Pseudobiomization Approach. *Glob. Change Biol.* 21, 1197–1212. doi:10.1111/gcb.12776

He, K., Lu, H., Zheng, H., Yang, Q., Sun, G., Zheng, Y., et al. (2020b). Role of Dynamic Environmental Change in Sustaining the Protracted Process of rice Domestication in the Lower Yangtze River. *Quat. Sci. Rev.* 242, 106456. doi:10.1016/j.quascirev.2020.106456

He, K., Lu, H., Zheng, Y., Zhang, J., Xu, D., Huan, X., et al. (2018). Middle-Holocene Sea-Level Fluctuations Interrupted the Developing Hemudu Culture in the Lower Yangtze River, China. *Quat. Sci. Rev.* 188, 90–103. doi:10.1016/j.quascirev.2018.03.034

He, K., Lu, H., Li, Y., Ding, F., Zhang, J., and Wang, C. (2020a). Cultural Response to Middle Holocene Sea-level Fluctuations in Eastern China: a Multi-proxy Approach. *Boreas* 49, 71–88. doi:10.1111/bor.12421

Hosner, D., Wagner, M., Tarasov, P. E., Chen, X., and Leipe, C. (2016). Spatiotemporal Distribution Patterns of Archaeological Sites in China during the Neolithic and Bronze Age: An Overview. *The Holocene* 26, 1576–1593. doi:10.1177/0959683616641743

Hosoya, L. A. (2011). Staple or Famine Food?: Ethnographic and Archaeological Approaches to Nut Processing in East Asian Prehistory. *Archaeol Anthropol. Sci.* 3, 7–17. doi:10.1007/s12520-011-0059-y

Hu, L., Chao, Z., Gu, M., Li, F., Chen, L., Liu, B., et al. (2013). Evidence for a Neolithic Age Fire-Irrigation Paddy Cultivation System in the Lower Yangtze River Delta, China. *J. Archaeological Sci.* 40, 72–78. doi:10.1016/j.jas.2012.04.021

Hu, Y., Zhou, B., Lu, Y., Zhang, J., Min, S., Dai, M., et al. (2020). Abundance and Morphology of Charcoal in Sediments Provide No Evidence of Massive Slash-

And-Burn Agriculture during the Neolithic Kuahuqiao Culture, China. *PLoS One* 15, e0237592. doi:10.1371/journal.pone.0237592

Innes, J. B., Zong, Y., Chen, Z., Chen, C., Wang, Z., and Wang, H. (2009). Environmental History, Palaeoecology and Human Activity at the Early Neolithic Forager/cultivator Site at Kuahuqiao, Hangzhou, Eastern China. *Quat. Sci. Rev.* 28, 2277–2294. doi:10.1016/j.quascirev.2009.04.010

Innes, J. B., Zong, Y., Wang, Z., and Chen, Z. (2014). Climatic and Palaeoecological Changes during the Mid- to Late Holocene Transition in Eastern China: High-Resolution Pollen and Non-pollen Palynomorph Analysis at Pingwang, Yangtze Coastal Lowlands. *Quat. Sci. Rev.* 99, 164–175. doi:10.1016/j.quascirev.2014.06.013

Innes, J. B., Zong, Y., Xiong, H., Wang, Z., and Chen, Z. (2019). Pollen and Non-pollen Palynomorph Analyses of Upper Holocene Sediments from Dianshan, Yangtze Coastal Lowlands, China: Hydrology, Vegetation History and Human Activity. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 523, 30–47. doi:10.1016/j.palaeo.2019.03.009

Institute of Botany and South China Institute of Botany (1982). *Angiosperm Pollen Flora of Tropical and Subtropical China*. Beijing: Science Press. (in Chinese).

Itzstein-Davey, F., Atahan, P., Dodson, J., Taylor, D., and Zheng, H. (2007b). A Sediment-Based Record of Lateglacial and Holocene Environmental Changes from Guangfulin, Yangtze delta, Eastern China. *The Holocene* 17, 1221–1231. doi:10.1177/0959683607085128

Itzstein-Davey, F., Atahan, P., Dodson, J., Taylor, D., and Zheng, H. (2007a). Environmental and Cultural Changes during the Terminal Neolithic: Qingpu, Yangtze delta, Eastern China. *The Holocene* 17, 875–887. doi:10.1177/0959683607080517

Jiang, L. P. (2014). *Research on Kuahuqiao Culture*. Beijing: Science Press.

Juggins, S. (2007). *C2: Software for Ecological and Palaeoecological Data Analysis and Visualisation (User Guide Version 1.5)*. Newcastle upon Tyne: Newcastle University, 1–77.

Kaplan, J. O., Krumhardt, K. M., Ellis, E. C., Ruddiman, W. F., Lemmen, C., and Goldewijk, K. K. (2011). Holocene Carbon Emissions as a Result of Anthropogenic Land Cover Change. *The Holocene* 21, 775–791. doi:10.1177/0959683610386983

Kaplan, J. O., Krumhardt, K. M., and Zimmermann, N. (2009). The Prehistoric and Preindustrial Deforestation of Europe. *Quat. Sci. Rev.* 28, 3016–3034. doi:10.1016/j.quascirev.2009.09.028

Li, C., Zheng, Y., Yu, S., Li, Y., and Shen, H. (2012). Understanding the Ecological Background of rice Agriculture on the Ningshao Plain during the Neolithic Age: Pollen Evidence from a Buried Paddy Field at the Tianluoshan Cultural Site. *Quat. Sci. Rev.* 35, 131–138. doi:10.1016/j.quascirev.2012.01.007

Li, X., Dodson, J., Zhou, J., and Zhou, X. (2009). Increases of Population and Expansion of rice Agriculture in Asia, and Anthropogenic Methane Emissions since 5000BP. *Quat. Int.* 202, 41–50. doi:10.1016/j.quaint.2008.02.009

Li, Y., Wu, J., Hou, S., Shi, C., Mo, D., Liu, B., et al. (2010). Palaeoecological Records of Environmental Change and Cultural Development from the Liangzhu and Qujialing Archaeological Sites in the Middle and Lower Reaches of the Yangtze River. *Quat. Int.* 227, 29–37. doi:10.1016/j.quaint.2010.05.015

Li, Y., Zhou, L., and Cui, H. (2008). Pollen Indicators of Human Activity. *Sci. Bull.* 53, 1281–1293. doi:10.1007/s11434-008-0181-0

Liu, K.-B., and Qiu, H. (1994). Late-Holocene Pollen Records of Vegetational Changes in China:Climate or Human Disturbance ? *Terr. Atmos. Ocean. Sci.* 5, 393–410. doi:10.3319/tao.1994.5.3.393(PAGES)

Liu, L., Field, J., Fullagar, R., Zhao, C., Chen, X., and Yu, J. (2010a). A Functional Analysis of Grinding Stones from an Early Holocene Site at Donghulin, North China. *J. Archaeological Sci.* 37, 2630–2639. doi:10.1016/j.jas.2010.05.023

Liu, L., Field, J., Weisskopf, A., Webb, J., Jiang, L., Wang, H., et al. (2010b). The Exploitation of Acorn and rice in Early Holocene Lower Yangzi River, China. *Acta Anthropologica Sinica* 29, 317–333.

Liu, Y., Sun, Q., Fan, D., Lai, X., Xu, L., Finlayson, B., et al. (2016). Pollen Evidence to Interpret the History of rice Farming at the Hemudu Site on the Ningshao Coast, Eastern China. *Quat. Int.* 426, 195–203. doi:10.1016/j.quaint.2016.05.016

Liu, Y., Sun, Q., Thomas, I., Zhang, L., Finlayson, B., Zhang, W., et al. (2015). Middle Holocene Coastal Environment and the Rise of the Liangzhu City Complex on the Yangtze delta, China. *Quat. Res.* 84, 326–334. doi:10.1016/j.yqres.2015.10.001

Lu, H., Liu, Z., Wu, N., Berné, S., Saito, Y., Liu, B., et al. (2002). Rice Domestication and Climatic Change: Phytolith Evidence from East China. *Boreas* 31, 378–385. doi:10.1111/j.1502-3885.2002.tb01081.x

Moore, P. D., Webb, J. A., and Collison, M. E. (1991). *Pollen Analysis*. Oxford, U.K: Blackwell scientific publications.

Mottl, O., Flantua, S. G. A., Bhatta, K. P., Felde, V. A., Giesecke, T., Goring, S., et al. (2021). Global Acceleration in Rates of Vegetation Change over the Past 18,000 Years. *Science* 372, 860–864. doi:10.1126/science.abg1685

Ningbo Municipal Institution of Cultural Relics and Archaeology, Department of Archaeology and Museology at Renmin University of China, Fenghua Administrationof Cultural Relics Preservation (2019). Brief Report on the Excavatation of Fangqiao Locality of Xiawangdu Site in Ningbo City, Zhejiang, 2017. *Archaeology*, 3–26. (in Chinese).

Ningbo Municipal Institution of Cultural Relics and Archaeology, Zhenhai Administration of Cultural Relics Preservation, and Research Center for Cultural Heritage Preservation, J.U. (2016). StageⅠ Excavation Report of the Yushan Site in Zhenhai, Ningbo, Zhejiang. *Southeast Cult.*, 23–37. (in Chinese).

Okuda, M., Sato, Y., Tang, L. H., Takahashi, M., Toyama, S., Yano, A., et al. (2003). Late Holocene Vegetation and Environment at Cauduntou, West of Yangtze Delta, SW Jiangsu Province, East China. *Quat. Int.* 105, 39–47. doi:10.1016/s1040-6182(02)00149-0

Olofsson, J., and Hickler, T. (2008). Effects of Human Land-Use on the Global Carbon Cycle during the Last 6,000 Years. *Veget Hist. Archaeobot* 17, 605–615. doi:10.1007/s00334-007-0126-6

Pan, Y. (2011). *Resource Production in the Yangzi delta and Qiantang River Valley from 10000 to 6000 BP: A Palaeoethnobotanical and Human Ecological Investigation. Doctor*. Shanghai: Fudan University.

Pan, Y., Zheng, Y., and Chen, C. (2017). "Human Ecology of the Early Neolithic Kuahuqiao Culture in East Asia," in *Handbook of East and Southeast Asian Archaeology*. Editors J. Habu, P. V. Lape, J. W. Olsen, and A. M. Eastep (New York: Springer), 347–377. doi:10.1007/978-1-4939-6521-2_23

Reimer, P. J., Austin, W. E. N., Bard, E., Bayliss, A., Blackwell, P. G., Bronk Ramsey, C., et al. (2020). The IntCal20 Northern Hemisphere Radiocarbon Age Calibration Curve (0-55 Cal kBP). *Radiocarbon* 62, 725–757. doi:10.1017/RDC.2020.41

Ren, G. (2006). Changes in forest Cover in China during the Holocene. *Veget Hist. Archaeobot* 16, 119–126. doi:10.1007/s00334-006-0075-5

Ren, G. (2000). Decline of the Mid- to Late Holocene Forests in China: Climatic Change or Human Impact? *J. Quat. Sci.* 15, 273–281. doi:10.1002/(sici)1099-1417(200003)15:3<273::aid-jqs504>3.0.co;2-2

Roberts, N., Fyfe, R. M., Woodbridge, J., Gaillard, M.-J., Davis, B. A. S., Kaplan, J. O., et al. (2018). Europe's Lost Forests: a Pollen-Based Synthesis for the Last 11,000 Years. *Sci. Rep.* 8, 716. doi:10.1038/s41598-017-18646-7

Ruddiman, W. F., Ellis, E. C., Kaplan, J. O., and Fuller, D. Q. (2015). Defining the Epoch We Live in. *Science* 348, 38–39. doi:10.1126/science.aaa7297

Ruddiman, W. F., Fuller, D. Q., Kutzbach, J. E., Tzedakis, P. C., Kaplan, J. O., Ellis, E. C., et al. (2016). Late Holocene Climate: Natural or Anthropogenic? *Rev. Geophys.* 54, 93–118. doi:10.1002/2015rg000503

Ruddiman, W. F. (2003). The Anthropogenic Greenhouse Era Began Thousands of Years Ago. *Climatic Change* 61, 261–293. doi:10.1023/b:clim.0000004577.17928.fa

Shu, J., Wang, W., and Jiang, L. (2012). Did Alder (Alnus) Fires Trigger rice Cultivation in the Lower Reaches of the Yangtze River, East China? *Palaeoworld* 21, 69–73. doi:10.1016/j.palwor.2012.02.003

Shu, J., Wang, W., Jiang, L., and Takahara, H. (2010). Early Neolithic Vegetation History, Fire Regime and Human Activity at Kuahuqiao, Lower Yangtze River, East China: New and Improved Insight. *Quat. Int.* 227, 10–21. doi:10.1016/j.quaint.2010.04.010

Shu, J., Wang, W., and Chen, W. (2007). Holocene Vegetation and Environment Changes in the New Taihu Plain, Jiangsu Province, east China. *Acta Micropalaeontologica Sinica* 24, 210–221. (in Chinese with English abstract).

Stephens, L., Fuller, D., Boivin, N., Rick, T., Gauthier, N., Kay, A., et al. (2019). Archaeological Assessment Reveals Earth's Early Transformation through Land Use. *Science* 365, 897–902. doi:10.1126/science.aax1192

Sun, G. P. (2013). "Recent Research on the Hemudu Culture and the Tianluoshan Site," in *A Companion to Chinese Archaeology*. Editor A. P. Underhill (West Sussex, UK: Wiley-Blackwell), 555–573.

Suzuki, S., Zheng, Y., Noshiro, S., Oyama, M., Nakamura, S., and Murakami, Y. (2011). "Identification of Tree Species of Archaeological Woods from Tianluoshan Site in Zhejiang Province," in *Integrated Studies on the Natural Remains from Tianluoshan*. Editors Center for the Study of Chinese Archaeology in Peking University and Zhejiang Province Institute of Archaeology and Cultural Heritage (Beijing: Cultural Relics Press), 108–117. (in Chinese).

Takahashi, R., and Hosoya, L. (2002). "Nut Exploitation in Jomon Society," in *Hunter–gatherer Archaeobotany: Perspective from the Northern Temperate Zone*. Editors S. L. R. Mason and J. G. Hather (London: University College London Press), 146–155.

Tang, L., Shu, J., Chen, J., and Wang, Z. (2019). Mid- to Late Holocene Vegetation Change Recorded at a Neolithic Site in the Yangtze Coastal plain, East China. *Quat. Int.* 519, 122–130. doi:10.1016/j.quaint.2018.12.031

Tang, L., Li, C., Zhang, X., Shao, J., Zhou, Z., and Ling, C. (2018). Pollen Morphology of Fagaceae in Quaternary Deposits of China and its Relationship with Climatic and Geographic Characters. *Acta Palaeontologica Pol.* 57, 387–410. (in Chinese with English abstract).

Tang, L., Mao, L., Shu, J., Li, C., Shen, C., and Zhou, Z. (2016). *Handbook of Quaternary Pollen and Spores in China*. Beijing: Science Press. (in Chinese).

Wang, F., Chien, N., Zhang, Y., and Yang, H. (1995). *Pollen Flora of China*. 2nd ed. Beijing: Science Press. (in Chinese).

Wang, H. M. (2000). The Hemudu Sites and the Hemudu Culture. *Southeast Cult.*, 15–22. (in Chinese with English abstract).

Wang, J., and Jiang, L. (2021). Intensive Acorn Processing in the Early Holocene of Southern China. *The Holocene*, 095968362110417. doi:10.1177/09596836211041732

Xu, D., Lu, H., Wu, N., and Liu, Z. (2010). 30 000-Year Vegetation and Climate Change Around the East China Sea Shelf Inferred from a High-Resolution Pollen Record. *Quat. Int.* 227, 53–60. doi:10.1016/j.quaint.2010.04.015

Xu, X., Chang, W., and Liu, J. (1996). Changes in Vagetation and Climate in the Taihu Lake basin during the Last 11000 Years. *Acta Palaeontologica Sinica* 35, 175–186. (in Chinese with English abstract).

Yang, S., Zheng, Z., Huang, K., Zong, Y., Wang, J., Xu, Q., et al. (2012). Modern Pollen Assemblages from Cultivated rice fields and rice Pollen Morphology: Application to a Study of Ancient Land Use and Agriculture in the Pearl River Delta, China. *The Holocene* 22, 1393–1404. doi:10.1177/0959683612449761

Yang, X., Yu, J., Lü, H., Cui, T., Guo, J., and Ge, Q. (2009). Starch Grain Analysis Reveals Function of Grinding Stone Tools at Shangzhai Site, Beijing. *Sci. China Ser. D-earth Sci.* 52, 1164–1171. doi:10.1007/s11430-009-0089-9

Yao, F., Ma, C., Zhu, C., Li, J., Chen, G., Tang, L., et al. (2017). Holocene Climate Change in the Western Part of Taihu Lake Region, East China. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 485, 963–973. doi:10.1016/j.palaeo.2017.08.022

Yi, S., Saito, Y., Zhao, Q., and Wang, P. (2003). Vegetation and Climate Changes in the Changjiang (Yangtze River) Delta, China, during the Past 13,000 Years Inferred from Pollen Records. *Quat. Sci. Rev.* 22, 1501–1519. doi:10.1016/s0277-3791(03)00080-5

Zheng, Y., Sun, G., Qini, L., Li, C., Wu, X., and Chen, X. (2009). Rice Fields and Modes of Rice Cultivation Between 5000 and 2500 BC in East China. *J. Archaeological Sci.* 36, 2609–2616. doi:10.1016/j.jas.2009.09.026

Zhao, Y., Yu, Z., Chen, F., Zhang, J., and Yang, B. (2009). Vegetation Response to Holocene Climate Change in Monsoon-Influenced Region of China. *Earth-Science Rev.* 97, 242–256. doi:10.1016/j.earscirev.2009.10.007

Zhejiang Provicial Institute of Cultural Relics and Archaeology, Ningbo Institute of Cultural Heritage Management, and Yuyao Hemudu Site Museum (2021). Brief Report on the Excavatation of the Neolithic Site at Jingtoushan in Yuyao City, Zhejiang. *Archaeology*, 3–26. (in Chinese).

Zhejiang Provincial Institute of Relics and Archaeology (2003). *Hemudu Site: The Archaeological Excavation of the Neolithic Sites*. Beijing: Cultural Relics Press. (in Chinese).

Zheng, X., Lei, S., Wang, J., and Zhao, Z. (2019). Analysis of Flotation Results from the Yushan Site in Ningbo City. *Agric. Archaeology*, 21–27. (In Chinese with English abstract).

Zheng, Z., Deng, Y., Zhang, H., Yu, R., and Chen, Z. (2004). Holocene Environmental Changes in the Tropical and Subtropical Areas of the South China and the Relation to Human Activities. *Quat. Res.* 24, 387–393. (in Chinese with English abstract).

Zheng, Z., Ma, T., Roberts, P., Li, Z., Yue, Y., Peng, H., et al. (2021). Anthropogenic Impacts on Late Holocene Land-Cover Change and Floristic Biodiversity Loss in Tropical southeastern Asia. *Proc. Natl. Acad. Sci. USA* 118, e2022210118. doi:10.1073/pnas.2022210118

Zhuang, Y., Ding, P., and French, C. (2014). Water Management and Agricultural Intensification of rice Farming at the Late-Neolithic Site of Maoshan, Lower Yangtze River, China. *The Holocene* 24, 531–545. doi:10.1177/0959683614522310

Zong, Y., Chen, Z., Innes, J. B., Chen, C., Wang, Z., and Wang, H. (2007). Fire and Flood Management of Coastal Swamp Enabled First rice Paddy Cultivation in east China. *Nature* 449, 459–462. doi:10.1038/nature06135

Zuo, X., Lu, H., Jiang, L., Zhang, J., Yang, X., Huan, X., et al. (2017). Dating rice Remains through Phytolith Carbon-14 Study Reveals Domestication at the Beginning of the Holocene. *Proc. Natl. Acad. Sci. USA* 114, 6486–6491. doi:10.1073/pnas.1704304114

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First Farmers in the South China Coast: New Evidence From the Gancaoling Site of Guangdong Province

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The transformation from hunter-gathering to farming in the south China coast has always been a conspicuous topic, as its great significance for the understanding of crop dispersal and human migration into southern China and Southeast Asia. It has been primarily assumed that rice was the only crop cultivated by early farmers in this region since 5,000 cal. BP., but the reliability of this speculation remains ambiguous, owing to the lack of systematic evidence. Based on analysis of macroscopic plant remains and phytoliths, as well as AMS radiocarbon dating at the Gancaoling site in Guangdong province, this study demonstrates the emergence of agriculture in the south China coast could be dated back to as early as 4,800–4,600 cal. BP., with the cultivation of rice and foxtail millet. This subsistence strategy change was an integral part of a more comprehensive social transformation, which started a new era of local history. Moreover, this discovery also provides further evidence supporting the universality of mixed farming in southern China and shed new light on the study of agriculture southward dispersal. The crop package of rice and millets possibly spread into the south China coast from Jiangxi *via* the mountain areas and then into Mainland Southeast Asia by a maritime route along the coastal regions.

Keywords: rice, foxtail millet, agriculture dispersal, southern China, mainland southeast Asia

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INTRODUCTION

The transformation from Paleolithic to Neolithic has long been regarded as the most fundamental revolution in the history of human beings (Childe, 1936; Bar-Yosef, 1998; Olsson and Paik, 2016). With the progress of archaeological research in the past decades, it is becoming more evident that this process was not as simple as imagined before. The related innovations, such as agriculture, pottery, ground stone, and sedentism, did not appear simultaneously, and the order of their appearance varied greatly among different regions (e.g., Kuijt and Goring-Morris, 2002; Fuller et al., 2015; Jordan et al., 2016). Ancient people in many areas with the capability of making pottery and ground stone tools still relied on hunter-gathering for their daily food supplies for thousands of years, while others had gone farther and farther along the pathway of food production. Nevertheless, most of these hunter-gathering communities have ultimately been transformed into or replaced by farming societies, leading to the formation of a farming-dominated world (Bellwood, 2005; Ellis et al., 2013; Stephens et al., 2019). Therefore, how these secondary transformations happened is no less important than the origin of the Neolithic lifeway for our understanding of the grand history of human beings.

The south China coast, along with its adjacent Nanling mountain areas, is one of the typical regions for the investigation of this issue. Current evidence of pottery in this region could be dated back to 17,000 cal. BP. from the Qingtang site (Guangdong Provincial Institute of Archaeology and Museology et al., 2019), while other discoveries before 10,000 cal. BP. have also been reported from Zengpiyan, Niulandong and many other cave sites (Zhang, 2002). Ground stones were also widely found here around 15,000–10,000 cal. BP. (Xiang, 2014). By contrast, no domesticated plants were utilized at the same time, and local people had led a hunter-gathering lifestyle for quite a long time (Zhang and Hung, 2012; Yang et al., 2017; Deng et al., 2019). Regarding the later transformation from hunter-gathering to farming, it has been speculated to be after 5,000 cal. BP. (Zhang and Hung, 2010), while recent evidence from Shixia, Laoyuan, and Chaling of this region reveals a later date of 4,500 cal. BP (Yang et al., 2018; Xia et al., 2019). In this case, more systematic work is still needed to clarify the precise time of agriculture emergence and details of farming practice in this region.

On the other hand, this transformation process in the south China coast is significant for investigating agricultural dispersal into Mainland Southeast Asia. Previous studies have proposed that agriculturalization of Mainland Southeast Asia was based on the introduction of domesticated crops along the terrestrial route from Yunnan province of China, the source region of which could be traced back to Sichuan and then Gansu province in northwest China (Higham, 1996; Higham, 2002; Guedes, 2011; Guedes and Butler, 2014; Deng et al., 2018). However, current earliest evidence of domesticated crops in Mainland Southeast Asia is from the south part of Thailand around 4,500–4,200 cal. BP. (Weber et al., 2010), and it seems possible that rice and millets arrived at the same time in this region. This discovery is almost as old as the earliest evidence from Yunnan (Dal Martello et al., 2018) and thus makes the proposed terrestrial route less convincing. In this case, another maritime route has been raised to reconcile contradictions embedded in current evidence (Higham, 2019; Gao et al., 2020). Nevertheless, because of the absence of mixed farming in the south China coast, the start point of this route is placed further to the southeast coast in Fujian province, the feasibility of which is also not that strong as the distance is too long. Hence, the precise time of agriculture appearance and whether the crop pattern of earliest farming in the south China coast is pure rice or both rice and millet is of great significance to clarify the dispersal of agriculture into Mainland Southeast Asia.

The study presented here is aimed to investigate the general condition of earliest farming in the south China coast, especially the precise time of agriculture emergence, crop pattern and their possible influences on other regions. Systematic samples have been collected from the Gancaoling site of Guangdong province and analyzed by integrating phytoliths, macroscopic plant remains, and direct radiocarbon dating. The new results indicate farming had dispersed into the south China coast around 4,800–4,600 cal. BP. Unlike the previous proposed pure rice agriculture, both rice and foxtail millet were cultivated and consumed by the first farmers in this region. Moreover, this study also emphasizes the significance of the south China coast for the southward dispersal of agriculture into Mainland Southeast Asia.

MATERIALS AND METHODS

Site Description

Gancaoling (23°18′15″N, 113°32′53″E) is situated in the northwest edge of the Pearl River delta, with the Nanling Mountains stretching in the north (**Figure 1**). It was first discovered in 2017 during the prophase archaeological survey for the construction of a new expressway across this region. The whole site covers the top of a small hill, and the total area is nearly 5,000 m^2 (**Figure 2**). After that, a systematic excavation was conducted from 2017 to 2018, covering an area of 3,200 m^2. The excavation reveals that cultural deposits of Gancaoling are mainly late Neolithic remains, including 81 pits, 160 graves and 40 post holes, which are possibly ruins of ancient houses or other constructions. From these contexts, large numbers of ceramics, stone tools and a few jade artefacts have been unearthed. Besides, ten graves of the late Warring States period have also been found.

Sample Collection and Processing

For the analysis of plant remains, 213.5 L of bulk samples were collected from 12 contexts during the excavation, 11 of which were pits with refilled daily refuse and one was earth fill of a tomb. Before being processed, a small sub-sample of roughly 50 ml soils was taken out from each sample and saved for phytolith analysis (except for contexts H55 and M50). The rest sediments were floated at the site using flotation buckets (Pearsall, 2000), and the afloat macroscopic plant remains were collected by mesh bags with 300 × 300 μm^2 apertures. All floats were then dried in shade and sent to the Archaeobotany Lab of Peking University for further analysis. Seeds, fruits and other parts of plant remains were sorted and identified under a stereomicroscope at 10–20× magnification, according to modern collections and published criteria (Wang, 1990; Guo, 2009; Nesbitt, 2016; Cappers and Bekker, 2022).

Phytolith samples were processed referring to established procedures with slight modifications (Pearsall, 2000; Lu et al., 2002). For each sample, 2 g small sample was weighed and treated with 30% H_2O_2 to remove organic matter. After three distilled water rinses, 15% HCl was used to remove carbonates. With another three distilled water rinses, phytoliths were separated from the sediments using heavy liquid (ZnBr2, 2.35 g/cm3) floatation. The suspension with separated phytoliths was removed into a new tube and washed three times with distilled water. It was then mounted on a slide with Canada Balsam. After air drying, phytoliths of each slide were identified and counted under an optical microscope at 400× magnification according to published references and criteria (Wang and Lu, 1992; Lu et al., 2002; Ball et al., 2016). For each sample, at least 500 phytoliths were recorded.

To detect the precise date of each context and chronology of the Gancaoling site, at least two dating samples were selected from each context and sent to the Laboratory of Radiocarbon

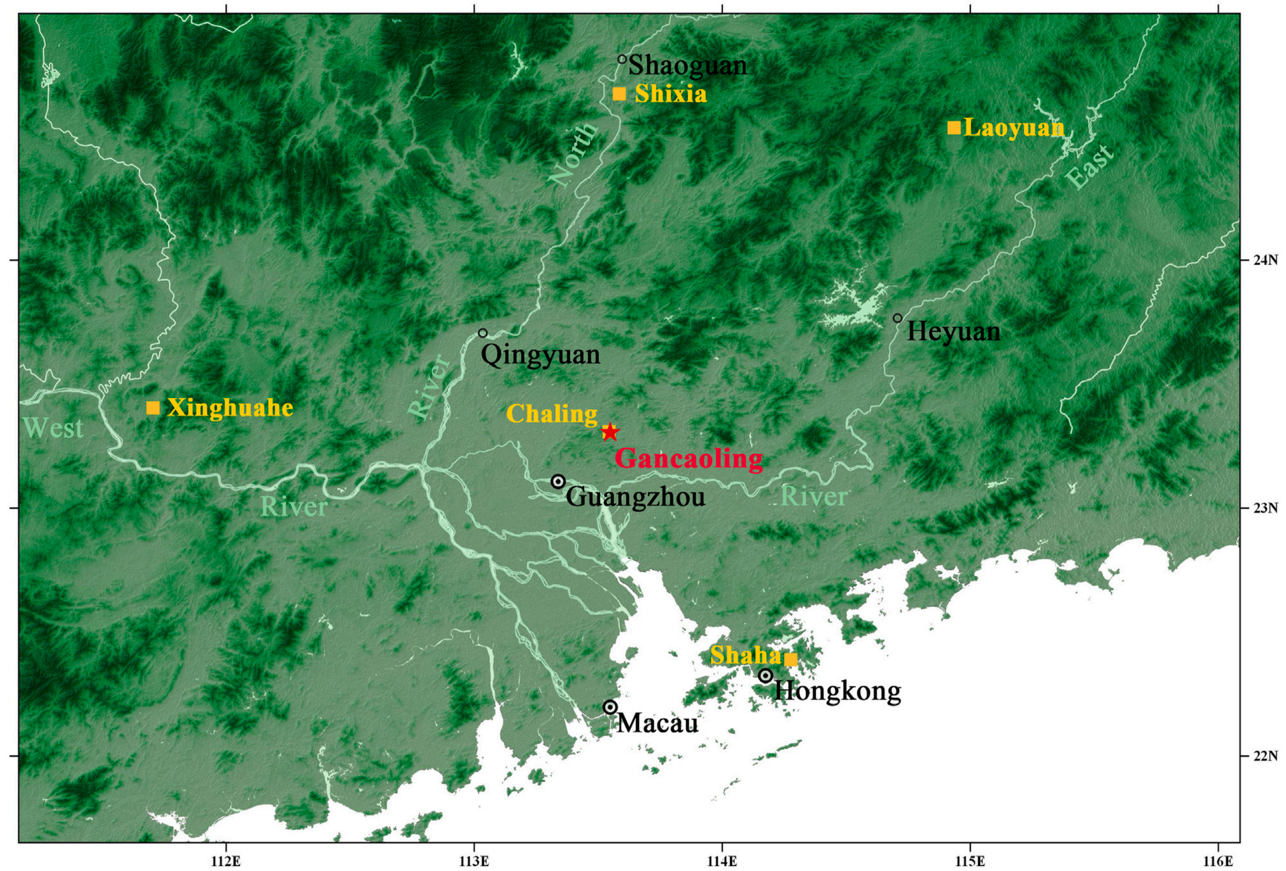

FIGURE 1 | Location of the Gancaoling site and other related sites.

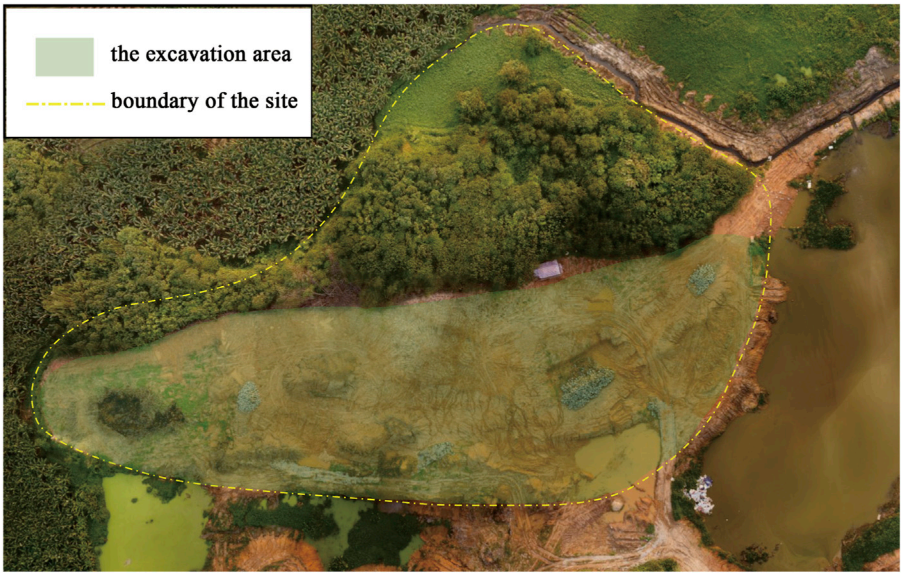

FIGURE 2 | Overview of the Gancaoling site and the excavation area.

TABLE 1 | AMS radiocarbon dating results from the Gancaoling site (All dates are calibrated by OxCal v4.4.4, using the IntCal 20 Atmospheric curve).

Laboratory code	Dated material	Context no	Conventional radiocarbon age	Calibrated date (cal. BP., 2σ range)
BA180578	rice grain	H16	3,855 ± 45	4,413–4,149
BA180579	nut husk fragment	H16	3,875 ± 45	4,417–4,153
BA180581	rice grain	H19	3,970 ± 25	4,523–4,301
BA180580	nut husk fragment	H19	4,030 ± 25	4,571–4,420
BA180582	nut husk fragment	H23	3,930 ± 50	4,521–4,185
BA180583	nut husk fragment	H23	4,060 ± 40	4,800–4,420
BA180584	rice grain	H37	3,895 ± 25	4,413–4,245
BA180585	rice grain	H37	3,835 ± 25	4,401–4,149
BA180586	nut husk fragment	H44	3,975 ± 25	4,524–4,360
BA180587	rice grain	H44	3,940 ± 30	4,515–4,254
BA180588	rice grain	H54	3,965 ± 25	4,521–4,300
BA180589	rice grain	H54	3,900 ± 30	4,417–4,243
BA180590	nut husk fragment	H54	3,885 ± 30	4,415–4,185
Beta - 530,494	foxtail millet grains	H54	3,890 ± 30	4,417–4,188
BA180591	rice grain	H69	4,060 ± 25	4,788–4,425
BA180592	rice grain	H69	3,775 ± 30	4,243–3,998
BA180593	rice grain	M50	3,830 ± 25	4,399–4,099
BA180594	rice grain	M50	3,805 ± 30	4,345–4,089

Dating in Peking University and Beta Analytic Testing Laboratory for direct accelerator mass spectrometry (AMS) radiocarbon dating (**Table 1**). In total, 18 samples were tested, and most of the specimens were single rice grain or nut husk fragment. Only in the case of one sample, four foxtail millet grains from context H54 were combined to ensure enough carbon after pre-treatment.

RESULTS

AMS Radiocarbon Dating Results

All samples processed in this study yielded radiocarbon dates successfully. These dates were calibrated by OxCal 4.4 (Bronk Ramsey, 2009), using the IntCal20 atmospheric curve (Reimer et al., 2020), and presented in **Table 1**. Comparison of results from each context suggests that most of them are highly consistent with each other. According to the dating results, the sampled contexts of Gancaoling generally could be divided into two groups. The dates of the first group are concentrated in the range of 4,600–4,400 cal. BP. Whereas only contexts H19 and H44 could be incorporated into this group. The remaining contexts are all possibly formed a little bit later, around 4,400–4,200 cal. BP., while a few contexts like M50 and H60 could be as late as 4,000 cal. BP. with relatively low probability. Overall, it could be concluded that late Neolithic human activities at this site lasted for hundreds of years, in the range of 4,600 to 4,100 cal. BP., with a very low probability reaching 4,800 cal. BP.

Phytoliths

The preservation condition of Phytoliths in all samples of Gancaoling was relatively poor, many of which were eroded and not easy to be precisely identified. The only crop found in phytolith records of Gancaoling was rice, including double-peaked type from rice grain husk, bulliform flabellate from rice leaf, and paralleled bilobate from rice leaf or stem (**Figures 3A–E**). However, quantities of these identifiable rice morphotypes were deficient, and they were sparsely found in 8 samples, comprising 0.2–0.6% of all phytolith remains in each sample (**Figure 4**, **Supplementary Table S1**).

Besides of these rice remains, 16 morphotypes have been recorded. The most abundant type was spheroid echinate phytolith yielded from palms. It has been found in all contexts, and the highest proportion is 68.8%. Besides, the proportion of bilobate type was also quite high, most of which was more than 10%, and the highest one was 56.1%. Other morphotypes like polylobate and cross from Panicoideae, rondel from pooideae, long saddle from bamboo, middle saddle from Arundinoideae, short saddle from Chloridoideae, and Cyperaceae type, have also been recorded in most samples with quite low proportion. Besides, other common types, such as blocky, tracheary annulate, elongate dentate, elongate entire and acute bulbosus, have been widely found in all samples, but none of them could be identified explicitly to genera level.

Macroscopic Plant Remains

Macroscopic plant remains were generally not rich in the 12 sampled contexts of Gancaoling (**Supplementary Table S2**). In total, 3,373 seeds, fruits and other parts of plants have been recovered, and the average density was only 15.7 specimens per litre. Moreover, there was a significant disparity in density among different contexts, with the highest one reaching 52.3 per litre, and the lowest one was only 3.3 per litre. Overall, 15 taxa of plants have been recognized into genera, species, or family level, while a portion of specimens have not been identified. All these remains could be generally grouped into four categories: crops, fruits, grasses, and weeds (**Figure 5**).

Rice (*Oryza sativa*) was the most common crop in Gancaoling, and has been found in all contexts sampled in this study. These remains comprised rice grains (**Figure 3F**) and fragments, rice spikelet bases (**Figures 3G,H**) and isolated rice grain embryos. Specifically, rice grain fragments were classified into large

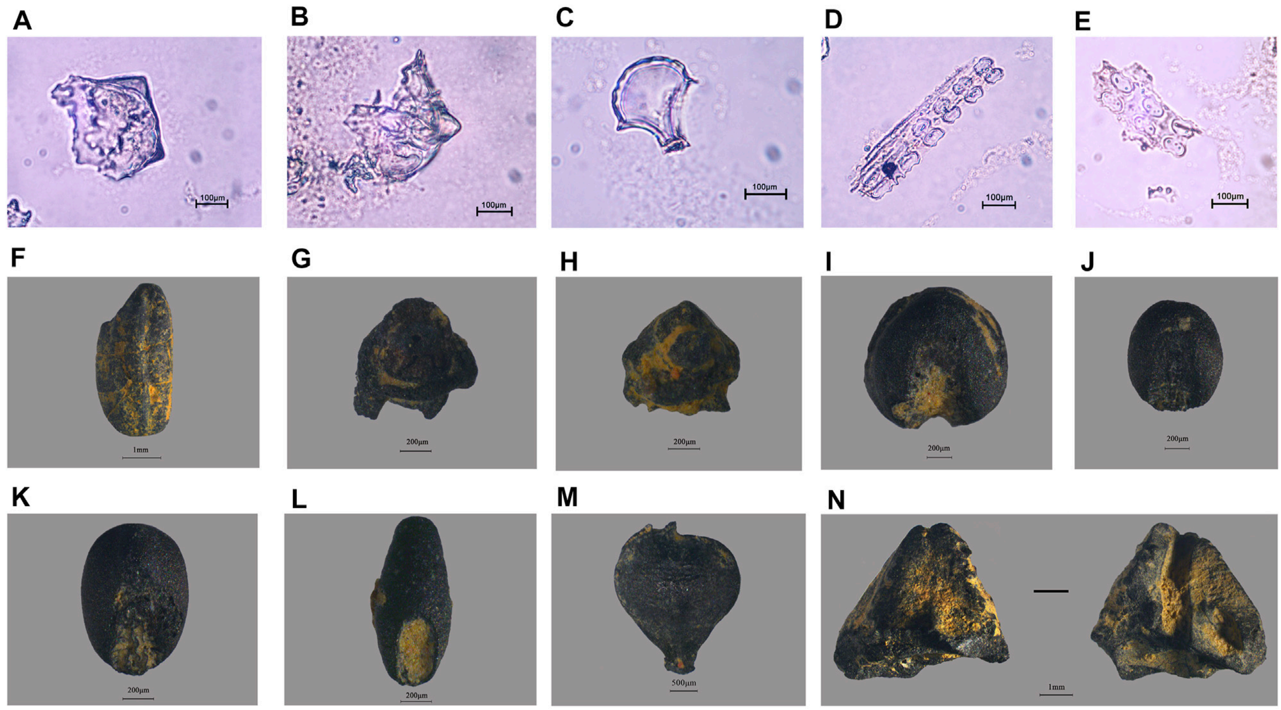

FIGURE 3 | Phytoliths and macroscopic plants remains from Gancaoling [**(A,B)**. double-peaked type; **(C)**. rice bulliform flabellate; **(D,E)**. paralleled bilobate; **(F)**. rice grain; **(G)**. rice spikelet base, non-shattering type; **(H)**. rice spikelet base, immature type; **(I)**. foxtail millet mature grain; **(J)**. foxtail millet immature grain; **(K)**. *Setaria* sp.; **(L)**. *Digitaria* sp; **(M)**. *Scirpus* sp; **(N)**. *Canarium* sp., endocarp fragment].

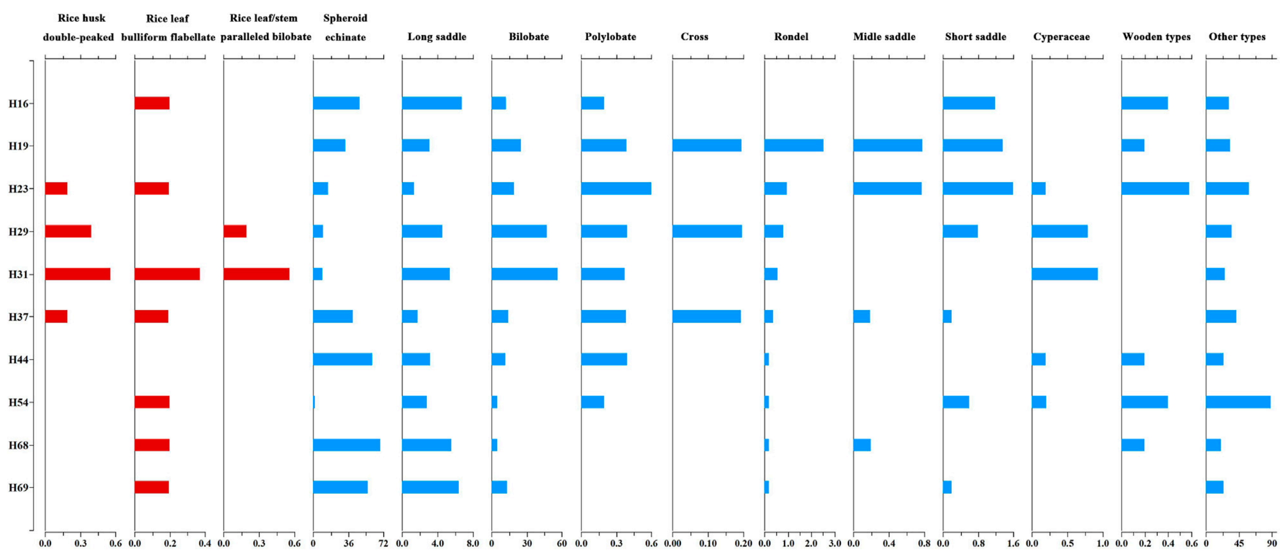

FIGURE 4 | Phytolith assemblages of all contexts from the Gancaoling site.

fragments (larger than half grains), small fragments (smaller than half grains but larger than 1 mm) and tiny fragments (smaller than 1 mm), according to their preservation conditions. According to established criteria, the rice spikelet bases of Gancaoling could also be divided into non-shattering and immature types (Fuller et al., 2009), and 99.29% of them are non-shattering, suggesting they were domesticated. These rice remains in total took up 83.55% of macroscopic plant remains from Gancaoling, with 50 grains, 498 grain fragments, one isolated rice embryo and 2,255 spikelet bases. Foxtail millet (*Setaria italica*) was another crop found in

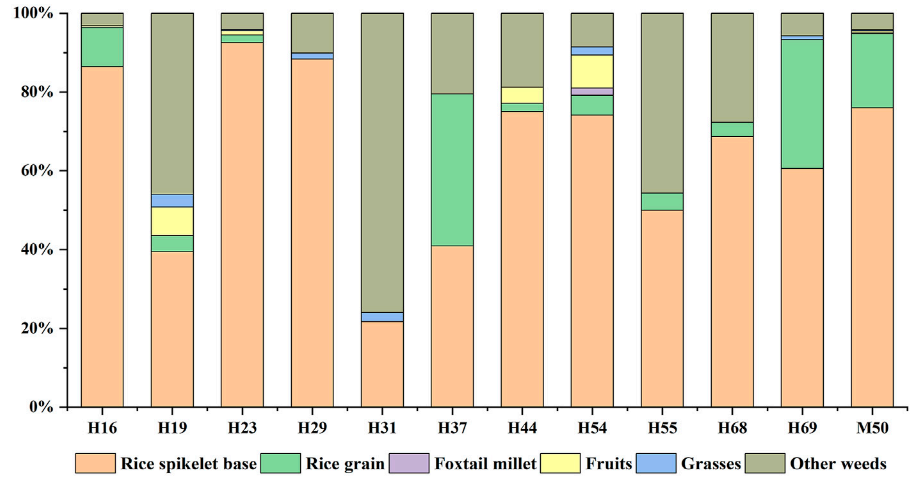

FIGURE 5 | Assemblages of macroscopic plant remains from all contexts of Gancaoling.

Gancaoling (**Figure 3**). It was obviously not widely utilized in the late Neolithic period and has only been found in context H54 and M50, including seven mature grains (**Figure 3l**) and eight immature ones (**Figure 3J**).

Fruits were not common at Gancaling, and *Canarium* sp. (**Figure 3N**) and *Sambucus* sp. were the only two identifiable fruits. 77 nutshell fragments of *Canarium* sp. have been found in 5 contexts (**Supplementary Table S2**), while only one seed of *Sambucus* sp. appeared in context H19. Besides, ten kinds of grasses and other weeds have been recorded, including *Setaria* sp. (**Figure 3K**), *Digitaria* sp. (**Figure 3L**), *Eleusine indica*, *Scirpus* sp. (**Figure 3M**), *Polygonum* sp., and Brassicaceae. However, their quantities were generally quite limited.

DISCUSSION

Subsistence Transformation and Social Shift in the Late Neolithic of South China Coast

Located far from the two agriculture origin centres of China, the Neolithic subsistence strategy in the south China coast has been a conspicuous topic and stimulated different hypotheses. Because of its special environment settings and natural resources, this region has been proposed as the third agriculture origin centre in China, mainly based on roots and tubers, such as taro (Zhao, 2011). While more scholars tend to believe ancient people here were engaged primarily in hunting, fishing and gathering (Chang, 1969; Higham, 2006; Zhang and Hung, 2012). A recent case study at the Xincun site reveals that wild plants like sago-palm, bananas, freshwater roots and tubers were processed by local people as recently as 5,000 cal. BP or even later (Yang et al., 2013). Another research also suggests that tree fruit, especially Canarium, was widely utilized in the Lingnan region (Nanling Mountains and areas to the south, especially the Pearl River valley), Southeast Asia and the Pacific Islands (Deng et al., 2019). No matter these plant resources were totally wild or partly cultivated, it is generally agreed that cereal agriculture was introduced into this region during the late Neolithic period and triggered a groundbreaking social change in local history.

Direct evidence of prehistoric agriculture has long been inadequate in the Lingnan region. The first discovery of such evidence was from the Shixia site in the mountain area, where rice grains and impressions were recovered in two phases, and the earliest one was believed to be no later than 5,000 cal. BP (Yang, 1978; Zhang et al., 2007; Yang et al., 2017). Other occasional discoveries have also been reported from Xinghuahe, Guye in Guangdong and Shaha in Hongkong (Lu et al., 2005; Xiang and Yao, 2006; Relics from the South, 2007). Overall, it is generally recognized that rice farming in this region began around 5,000 cal. BP. and facilitated the later development of local societies (Zhang and Hung, 2010), while a few scholars argue for an earlier emergence of rice agriculture during the Xiantouling culture period (ca. 7,000–6,000 cal. BP.) (Bellwood, 2005). However, all these remains have not been directly dated, and a later re-examination of rice remains from the Guye site reveals none of them was older than 300 years, which were obviously later intrusions (Yang et al., 2017). Therefore, without scientifically rigorous collection of plant remains and direct dating, the emergence of farming and the associated crop pattern in the south China coast is still vague and imprecise.

This condition has been slightly improved with new efforts in the past few years. 20 macroscopic plant remains have been recovered from 6 contexts of the Chaling site, in which 6 were rice grains and one was rice spikelet base. Two rice grains were directly dated to 4,526–4,417 cal. BP. and 4,429–4,248 cal. BP. respectively. Phytolith analysis also disclosed different morphotypes of rice phytoliths from the same site (Xia et al., 2019). Another study also provided phytolith evidence of rice consumption at Laoyuan and Chaling and one direct date of rice grain, which is 4,419–4,246 cal. BP. (Yang et al., 2018). Even so, these pieces of evidence are still too limited to answer this question.

The present study at Gancaoling, for the first time, provided solid evidence and renovated our knowledge on the earliest farming practice in the south China coast. A significant difference from previous understanding is that the earliest agriculture here was not pure rice farming but mixed rice and foxtail millet. The assemblages of macroscopic plant remains clearly demonstrated foxtail millet was cultivated along with rice by first farmers in this region, although rice was no doubt the major crop at that time (**Figure 5**). Furthermore, the presence of large quantities of rice spikelet bases suggested the existence of rice processing activities at the site. Further evidence of local cultivation and processing has also been provided by phytolith morphotypes from different parts of rice, like leaves, stems and grain husks (**Figure 4**). Combined with the systematic AMS radiocarbon dates, it could be confirmed that around 4,800–4,600 cal. BP. rice and foxtail millet had already been cultivated in the south China coast.

Another remarkable discovery from Gancaoling is the appearance of Canarium endocarp fragments. Previous research has revealed that Canarium was a vital food resource in Southern China and the contemporary Asia-Pacific region before the introduction of cereal crops. People used to crack the fruit stones and consume the kernels in these hunter-gathering sites (Deng et al., 2019). The new discovery from Gancaoling reminds us that the agriculturalization of Southern China was possibly not an abrupt substitution of exotic technologies for old traditions. On the contrary, traditional foodways quite possibly continued for some time after the introduction of agriculture. Thus, complicated interactions and competitions could be expected behind this process, especially when considering that fishing and hunting had long been the primary way of obtaining animal resources even until the Bronze age (Yu, 2018).

Along with the adoption of agriculture, systematic changes have also happened in other aspects of local society (Li and Zhang, 2021). As pointed out by many scholars, the introduction of farming practice in the south China coast was possibly accompanied by human migrations from the Yangtze valley. A noticeable change is that previous flexed-position burial traditions, which was prevalent in hunter-gathering communities of Southern China and other adjacent regions, had been replaced by extended burials (Hung, 2019). Analysis of skeletal remains, especially craniometrics, also suggests distinct physical characters from previous hunter-gathering populations, which should be caused by contributions of large scale immigrants (Matsumura et al., 2019). These speculations could further be supported by recent progress of ancient DNA studies in southern China, which also argued for a mixture of native hunter-gatherers and migrants from the Yangtze valley (Yang et al., 2020; Wang et al., 2021). The mixture model instead of the totally replacement model of population also agrees well with the continuity of traditional foodways like Canarium utilization.

With the development of farming, the size of local population also increased drastically after 6,000 cal. BP., as indicated by the number of archaeological sites. For instance, 265 sites of this period have been discovered in the regional survey of the Liuxi River valley, a tributary of the Pearl River, which is several times of the total number of archaeological sites dated to 7,000–5,000 cal. BP. in the whole Pearl River delta (Han and Xu, 2017). These sites were usually around 5,000 m^2 or smaller, while some regional centres like Shixia and Yanshanzhai emerged in this period, covering 30,000–50,000 m^2 (Guangdong Provincial Institute of Archaeology and Museology et al., 2014). Social differentiations were not only embodied in the divergence among different settlements but also within these communities. Large tombs with rich funerary objects have been found in these regional centres and normal settlements like Gancaoling (Guangdong Provincial Institute of Archaeology and Museology et al., 2014; Li and Zhang, 2021). Meanwhile, jade artefacts such as Cong and Yue of the Liangzhu style had been incorporated into local belief and ritual systems and used as an expression of social status by elites (Tang et al., 2019; Li and Zhang, 2021).

Overall, the adoption of farming is part of a more comprehensive social change in the south China coast around 5,000 cal. BP., which opened a new era for the development of local societies. It was also in this period, the technology of pottery making developed rapidly and led to the invention of stamped hard pottery (Li, 2013). Thereafter, this region, together with Fujian and Jiangxi, became the innovation centre of ceramic technologies and played a new role in the interregional interactions of ancient China.

The Role of South China Coast in the Southward Dispersal of Agriculture

With advances in archaeobotanical research in the past decades, it is becoming more and more apparent that interactions between the two agriculture origin centres in China happened around 7,500 cal. BP., much earlier than expected before (Zhang et al., 2012; Bestel et al., 2018; Wang et al., 2018; Luo et al., 2019). At least around 6,000 cal. BP., foxtail millet had spread into the Yangtze River valley, as indicated by discoveries from the Chengtoushan site (Nasu et al., 2007; Nasu et al., 2012). Plant remains from other parts of Southern China like Jiangxi, Fujian and Taiwan also demonstrated millets were cultivated in small scale along with rice in the late Neolithic period, and the universality of millet cultivation in Southern China has been grossly underestimated before (Tsang et al., 2017; Deng et al., 2018; Ge et al., 2019; Deng et al., 2020; Dai et al., 2021). Therefore, it has been further speculated that millets were accepted by all agricultural regions of southern China in the late Neolithic period, except for the plain areas of the lower Yangtze valley (Deng et al., 2018). Whereas, direct evidence is still absent in many related regions, one of which is the south China coast. Thus, the discovery from Gancaoling again adds new evidence to this hypothesis and shed new light on the reconstruction of the southward dispersal of agriculture.

According to comparisons of pottery styles and other material remains from the south China coast and surrounding regions, external communications of this region show clear periodic features. Around 7,000–6,000 cal. BP., the most representative archaeological culture in this region is the Xiantouling culture, sites with associated remains of which are concentrated in the Pearl River delta (Yang et al., 2015). Material remains from this culture group are famous for their finely made potteries, most of which are with painted or punctated decorations (Shenzhen Municipal

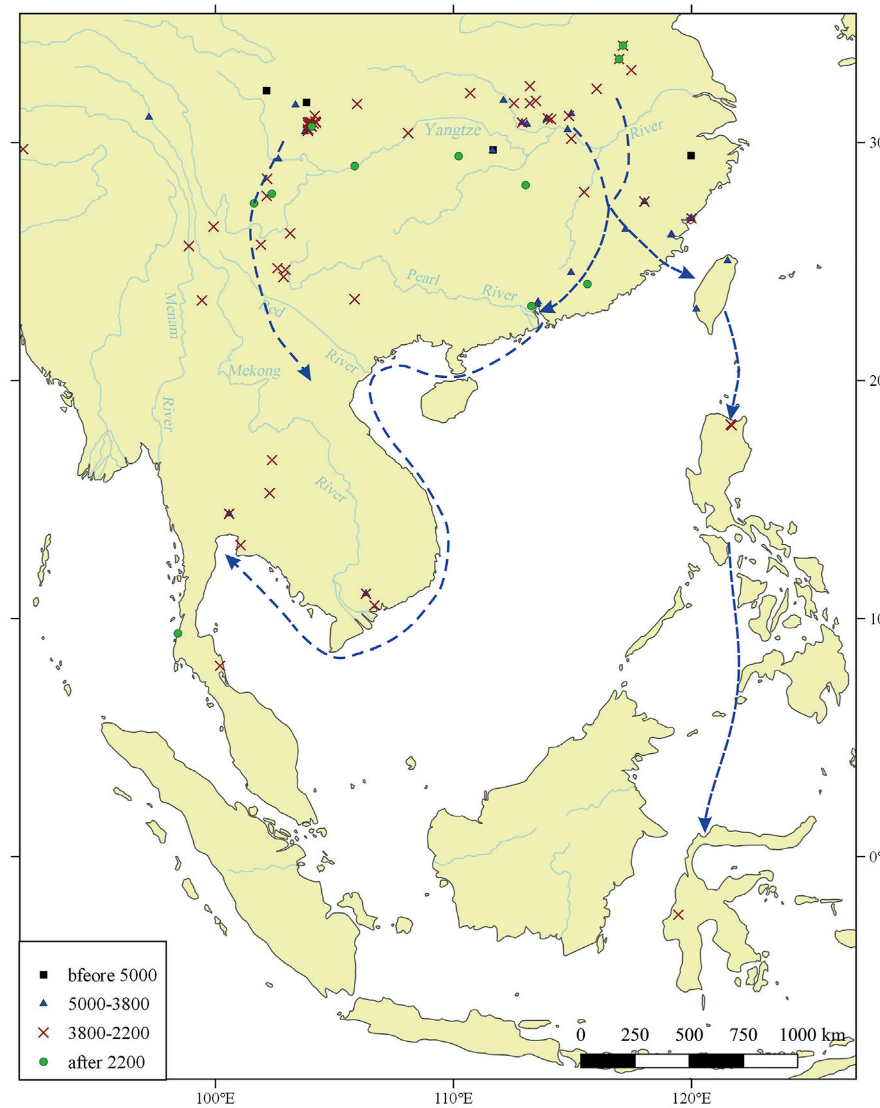

FIGURE 6 | The main southward dispersal routes of agriculture from Yangtze valley into southern China and Southeast Asia.

Institute of Archaeology and Museology, 2013). These remains resembled in features of potteries prevalent in contemporary archaeological cultures of Hunan province, like Gaomiao, Tangjiagang and Daxi (He, 1994). As a result, it is generally agreed that the main channel of external communication between the south China coast and the northerly regions was through the northwest route with Hunan. Another noticeable point is that although rice agriculture had well developed in Hunan province no later than 8,500 cal. BP., farming had not been introduced southward in this period (Zhang and Pei, 1997). Similar conditions could also be observed in the textile materials. Unlike the spinning technologies in Hunan province and other parts of Yangtze valley as indicated by the common use of spindle whorls, ancient residents in the south China coast still used barkcloth instead (Tang, 2003).

After 6,000 cal. BP., the influence from Hunan declined dramatically, while interregional connection through the southeast channel got its start. Comparison of pottery assemblages and morphological features reveals a strong impact of the Fanchengdui culture in Jiangxi province on the Shixia culture and contemporary communities in the coastal region (Li et al., 1989; He, 2010; Li, 2019). Under this background, the introduction of farming was accomplished as an integral part of interregional communications. Therefore, it could be determined that the southward dispersal of rice and foxtail millet was through the mountain areas between Jiangxi and Guandong, just as proposed before (Deng et al., 2018; Deng et al., 2020). However, different from the previous interactions during the Xiantouling period, this transformation seemed more radical and affected all sides of local society. No wonder it is speculated that there were large amounts of immigrants as stated above (Matsumura et al., 2019).

The south China coast is not only a receiver of farming technologies, but also an exporter in a vaster regional configuration. As an integral part of the coastal regions facing Southeast Asia and the Pacific islands, this region has been proposed as one of the possible homeland areas of Austronesian people and agriculture in Island Southeast Asia (Bellwood, 2005; Tsang, 2005). Unfortunately, little evidence has been obtained to support this speculation, while an alternative route from the southeast China coast *via* Taiwan into northern Luzon is preferred (Bellwood and Dizon, 2008; Hung, 2008). Nevertheless, the possible contribution of the south China coast to the agriculturalization of Mainland Southeast Asia need more attentions. In a regular reconstruction of farming dispersal into Mainland Southeast Asia, a terrestrial route from Yunnan and Guangxi has been mentioned frequently (Higham, 1996; Higham, 2002; Deng et al., 2018). However, the existence of farming practice prior to 4,000 cal. BP. in the south part of Yunnan and Guangxi remains questionable, and so is this proposed route. By contrast, in the coastal region of Vietnam and Thailand, some discoveries of rice and/or millets dated back to 4,500–4,000 cal. BP. have been reported, such as An Son, Loc Giang and Non Pa Wai, indicating a possible maritime route for the southward dispersal of agriculture into this region (Weber et al., 2010; Barron et al., 2017). Given the fact that agriculture emergence in Guangxi happened in the Bronze Age and the co-occurrence of rice and foxtail millet in the Pearl River delta (Deng et al., 2019), the nearest start point of this route should be in the south part of Guangdong province, and thus supporting the significance of this region in the southward dispersal of agriculture (**Figure 6**).

CONCLUSION

With systematic archaeobotanical evidence and direct AMS radiocarbon dating, this study demonstrated that agriculture in the south China coast emerged around 4,800–4,600 cal. BP. Different from the previous assumption of pure rice farming, first farmers in this region cultivated rice together with a small portion of foxtail millet as suggested by plant assemblages from Gancaoling. In addition, with the introduction of agriculture, the former hunter-gathering tradition, such as the utilization of Canarium nut, had been maintained for a while, and this transformation possibly happened in a relatively gradual and moderate way, especially when considering the long-lasting utilization of wild mammals, fish and shellfish. Even though, it still needs to be noticed that thorough social change had happened around 5,000 cal. BP. in this region, as reflected in many aspects of local society, like settlement pattern and differentiation, burial practice, ritual and belief system, and quite possibly large amounts of immigrants input.

The new results also provide new evidence supporting the universality of mixed farming in the late Neolithic period of southern China. An interregional comparison reveals this region was not only a receiver of farming technologies but also possibly a junction point on the southward dispersal of agriculture. A package of rice and millets was introduced into this region from Jiangxi Province *via* the mountain areas and further dispersed into Mainland Southeast Asia along a maritime route. Whereas, to confirm this hypothesis, more targeted work is still needed in the coastal regions of Mainland Southeast, especially Vietnam.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZD and QZ designed the study. QZ and BH conducted archaeological excavation and sample collection. ZD and MZ completed sample processing and identification. ZD analyzed the data and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.858492/full#supplementary-material

REFERENCES

Ball, T., Chandler-Ezell, K., Dickau, R., Duncan, N., Hart, T. C., Iriarte, J., et al. (2016). Phytoliths as a Tool for Investigations of Agricultural Origins and Dispersals Around the World. *J. Archaeol. Sci.* 68, 32–45. doi:10.1016/j.jas.2015.08.010

Bar-Yosef, O. (1998). On the Nature of Transitions: the Middle to Upper Palaeolithic and the Neolithic Revolution. *Camb. Archaeol. J.* 8 (2), 141–163. doi:10.1017/s0959774300001815

Barron, A., Turner, M., Beeching, L., Bellwood, P., Piper, P., Grono, E., et al. (2017). MicroCT Reveals Domesticated rice (Oryza Sativa) within Pottery Sherds from Early Neolithic Sites (4150–3265 Cal BP.) in Southeast Asia. *Sci. Rep.* 7:7410. doi:10.1038/s41598-017-04338-9

Bellwood, P. (2005). *First Farmers: The Origins of Agricultural Societies*. Oxford: Blackwell.

Bellwood, P., and Dizon, E. Z. (2008). "Austronesian Cultural Origins: Out of Taiwan, via the Batanes Islands, and Onwards to Western Polynesia, in Past Human Migrations in East Asia," in *Past human migrations in East Asia: matching archaeology, linguistics and genetics*. Editor A. Sanchez-Mazas, R. Blench, M. D. Ross, I. Peiros, and M. Lin (New York: Routledge), 1-17.

Bestel, S., Bao, Y., Zhong, H., Chen, X., and Liu, L. (2018). Wild Plant Use and Multi-Cropping at the Early Neolithic Zhuzhai Site in the Middle Yellow River Region, China. *The Holocene* 28 (2), 195–207. doi:10.1177/0959683617721328

Bronk Ramsey, C. (2009). Bayesian Analysis of Radiocarbon Dates. *Radiocarbon* 51 (1), 337–360. doi:10.1017/s0033822200033865

Cappers, R. T., and Bekker, R. M. (2022). *A Manual for the Identification of Plant Seeds and Fruits*. Groningen: Barkhuis.

Chang, K. C. (1969). *Fengpitou, Tapenkeng and the Prehistory of Taiwan*. New Haven: Yale University.

Childe, V. G. (1936). *Man Makes Himself*. London: Watts & Co.

Dai, J., Cai, X., Jin, J., Ge, W., Huang, Y., Wu, W., et al. (2021). Earliest Arrival of Millet in the South China Coast Dating Back to 5,500 Years Ago. *J. Archaeological Sci.* 129, 105356. doi:10.1016/j.jas.2021.105356

Dal Martello, R., Min, R., Stevens, C., Higham, C., Higham, T., Qin, L., et al. (2018). Early Agriculture at the Crossroads of China and Southeast Asia: Archaeobotanical Evidence and Radiocarbon Dates from Baiyangcun, Yunnan. *J. Archaeological Sci. Rep.* 20, 711–721. doi:10.1016/j.jasrep.2018.06.005

Deng, Z., Hung, H.-c., Fan, X., Huang, Y., and Lu, H. (2018). The Ancient Dispersal of Millets in Southern China: New Archaeological Evidence. *The Holocene* 28 (1), 34–43. doi:10.1177/0959683617714603

Deng, Z., Hung, H.-c., Li, Z., Carson, M. T., Huang, Q., Huang, Y., et al. (2019). Food and Ritual Resources in hunter-gatherer Societies: Canarium Nuts in Southern China and beyond. *Antiquity* 93 (372), 1460–1478. doi:10.15184/aqy.2019.173

Deng, Z., Yan, Z., and Yu, Z. (2020). Bridging the gap on the Southward Dispersal Route of Agriculture in China: New Evidences from the Guodishan Site, Jiangxi Province. *Archaeol. Anthropol. Sci.* 12:151. doi:10.1007/s12520-020-01117-y

Ellis, E. C., Kaplan, J. O., Fuller, D. Q., Vavrus, S., Klein Goldewijk, K., and Verburg, P. H. (2013). Used Planet: A Global History. *Proc. Natl. Acad. Sci.* 110 (20), 7978–7985. doi:10.1073/pnas.1217241110

Fuller, D. Q., Kingwell-Banham, E., Lucas, L., Murphy, C., and Stevens, C. J. (2015). Comparing Pathways to Agriculture. *Archaeol. Int.* 18, 61. doi:10.5334/ai.1808

Fuller, D. Q., Qin, L., Zheng, Y., Zhao, Z., Chen, X., Hosoya, L. A., et al. (2009). The Domestication Process and Domestication Rate in Rice: Spikelet Bases from the Lower Yangtze. *Science* 323 (5921), 1607–1610. doi:10.1126/science.1166605

Gao, Y., Dong, G., Yang, X., and Chen, F. (2020). A Review on the Spread of Prehistoric Agriculture from Southern China to Mainland Southeast Asia. *Sci. China Earth Sci.* 63 (5), 615–625. doi:10.1007/s11430-019-9552-5

Ge, W., Yang, S., Chen, Y., Dong, S., Jiao, T., Wang, M., et al. (2019). Investigating the Late Neolithic Millet Agriculture in Southeast China: New Multidisciplinary Evidences. *Quat. Int.* 529, 18–24. doi:10.1016/j.quaint.2019.01.007

Guangdong Provincial Institute of Archaeology and Museology, School of archaeology and museology of Peking University and Museum of Yingde City (2019). *The Qingtang Site in Yingde City*, 7, 3-15. Archaeo. (Kaogu), (in Chinese).

Guangdong Provincial Institute of Archaeology and Museology, Guangdong Museum, and Qujiang Museum (2014). *The Shixia Site: Report of the Archaeological Excavation in 1973-1978*. Beijing: Cultural Relics Press.

Guedes, J. D. A. (2011). Millets, Rice, Social Complexity, and the Spread of Agriculture to the Chengdu Plain and Southwest China. *Rice* 4 (3), 104–113. doi:10.1007/s12284-011-9071-1

Guedes, J. D. A., and Butler, E. E. (2014). Modeling Constraints on the Spread of Agriculture to Southwest China with thermal Niche Models. *Quat. Int.* 349 (2014), 29–41. doi:10.1016/j.quaint.2014.08.003

Guo, Q. (2009). *The Illustrated Seeds of Chinese Medicinal Plants*. Beijing: Agricultural Publishing House.

Han, W., and Xu, Y. (2017). *Report of the Archaeologcial Surevey in the Liuxi River Vally of Conghua, Guangzhou City*. Guangzhou: Guangzhou Press.

He, G. (2010). Transformation of the Spatial Pattern of Neolithic Cultures in the Lingnan Region. Southeast Cul. *(Dong Nan Wen Hua)* 6, 75–81.

He, J. (1994). *Prehistoric Painted Pottery of the Pearl River Mouth and Daxi Culture," in Ancient Cultures of south China and Neighbouring Regions*. Editor C. Tang (Hong Kong: Chinese University of Hong Kong), 321–330.

Higham, C. F. W. (2006). Crossing National Boundaries: Southern China and Southeast Asia in Prehistory," in Uncovering Southeast Asia's Past: Selected Papers from the 10th International Conference of the European Association of Southeast Asian Archaeologists, Editor E.A.Bacus, I.Glover & V.C.Pigott. (*Singpore: NUS Press.*), 13–21.

Higham, C. F. W. (2002). *Early Cultures of Mainland Southeast Asia*. Bangkok: River Books.

Higham, C. F. W. (1996). *The Bronze Age of Southeast Asia*. Cambridge: Cambridge University Press.

Higham, C. F. W. (2019). "*A Maritime Route Brought First Farmers to Mainland Southeast Asia," in Prehistoric Maritime Cultures and Seafaring in East Asia*. Editor C. Wu & B.V. Rolett. (Singapore: Springer), 41–52. doi:10.1007/978-981-32-9256-7_2

Hung, H. C. (2019). Prosperity and Complexity without Farming: the South China Coast, C. 5000-3000 BC. *Antiquity* 93 (368), 325–341. doi:10.15184/aqy.2018.188

Hung, H. C. (2008). *Migration an Cultural Interaction in Southern Coastal China, Taiwan and the Northern Philippines, 3000 BC to AD 100: The Early History of the Austronesian-Speaking Populations* [dissertation]. Canberra: Australian National University.

Jordan, P., Gibbs, K., Hommel, P., Piezonka, H., Silva, F., and Steele, J. (2016). Modelling the Diffusion of Pottery Technologies across Afro-Eurasia: Emerging Insights and Future Research. *Antiquity* 90 (351), 590–603. doi:10.15184/aqy.2016.68

Kuijt, I., and Goring-Morris, N. (2002). Foraging, Farming, and Social Complexity in the Pre-pottery Neolithic of the Southern Levant: a Review and Synthesis. *J. World Prehist* 16 (4), 361–440. doi:10.1023/a:1022973114090

Li, Y. (2013). A Preliminary Research of the Hutoupu Culture. *Coll. Stu. Archaeo (Kao Gu Xue Yan Jiu)* 10, 603–610. (in Chinese).

Li, Y. (2019). Preliminary Research on the Southward Boundary of Haochuan Culture and Interactions Among Jiangxi, Guandong and Fujian in Late Neolithic Period. J. Hunan Archaeo. *(Hunan Kaogu Jikan)* 13, 248–264. (in Chinese).

Li, Y., and Zhang, Q. (2021). The Civilization Process of Lingnan from the Perspective of Archaeology for a Century. *J. Archaeo. Museo (Wenbo Xuekan)* 2, 4–16. (in Chinese).

Lu, H., Liu, Z., Wu, N., Berné, S., Saito, Y., Liu, B., et al. (2002). Rice Domestication and Climatic Change: Phytolith Evidence from East China. *Boreas* 31 (4), 378–385. doi:10.1080/030094802320942581

Lu, T. L. D., Zhao, Z., and Zheng, Z. (2005). "The Prehistoric and Historic Environments: Vegetations and Subsistence Strategies at Sha Ha, Sai Kung," in The Ancient Culture of Hong Kong-archaeological Discoveries in Sha Ha, Sai Kung. Editor Antiquities and Monuments Office of the Leisure and Cultural Services Department (Hongkong: Antiquities and Monuments Office of the Leisure and Cultural Services Department), 57-64.

Luo, W., Gu, C., Yang, Y., Zhang, D., Liang, Z., Li, J., et al. (2019). Phytoliths Reveal the Earliest Interplay of rice and Broomcorn Millet at the Site of Shuangdun (ca. 7.3-6.8 Ka BP) in the Middle Huai River valley, China. *J. Archaeological Sci.* 102, 26–34. doi:10.1016/j.jas.2018.12.004

Matsumura, H., Hung, H. C., Higham, C., Zhang, C., Yamagata, M., Nguyen, L. C., et al. (2019). Craniometrics Reveal "Two Layers" of Prehistoric Human Dispersal in Eastern Eurasia. *Sci. Rep.* 9: 1451. doi:10.1038/s41598-018-35426-z

Nasu, H., Gu, H., Momohara, A., and Yasuda, Y. (2012). Land-use Change for rice and Foxtail Millet Cultivation in the Chengtoushan Site, central China, Reconstructed from weed Seed Assemblages. *Archaeol. Anthropol. Sci.* 4 (1), 1–14. doi:10.1007/s12520-011-0077-9

Nasu, H., Momohara, A., Yasuda, Y., and He, J. (2007). The Occurrence and Identification of Setaria Italica (L.) P. Beauv. (Foxtail Millet) Grains from the Chengtoushan Site (ca. 5800 Cal B.P.) in central China, with Reference to the Domestication centre in Asia. *Veget Hist. Archaeobot* 16 (6), 481–494. doi:10.1007/s00334-006-0068-4

Nesbitt, M. (2016). *Identification Guide for Near Eastern Grass Seeds*. New York: Routledge.
Olsson, O., and Paik, C. (2016). Long-run Cultural Divergence: Evidence from the Neolithic Revolution. *J. Dev. Econ.* 122, 197–213. doi:10.1016/j.jdeveco.2016.05.003
Pearsall, D. (2000). *Paleoethnobotany: A Handbook of Procedures*. San Diego: Academic Press.
Reimer, P. J., Austin, W. E. N., Bard, E., Bayliss, A., Blackwell, P. G., Bronk Ramsey, C., et al. (2020). The IntCal20 Northern Hemisphere Radiocarbon Age Calibration Curve (0-55 Cal kBP). *Radiocarbon* 62 (4), 725–757. doi:10.1017/rdc.2020.41
Relics from the South (2007). New Archaeoloical Discoveries from the South in 2006. Cul. Relics Southern Chin. *(Nan Fang Wen Wu)* 4, 19-87. (in Chinese).
Shenzhen Municipal Institute of Archaeology and Museology (2013). *Excavation Report of the Xiantouling Site in Shenzhen*. Beijing: Cultural Relics Press.
Stephens, L., Fuller, D., Boivin, N., Rick, T., Gauthier, N., Kay, A., et al. (2019). Archaeological Assessment Reveals Earth's Early Transformation through Land Use. *Science* 365 (6456), 897–902. doi:10.1126/science.aax1192
Tang, C. (2003). Archaeological Study on Tapa Beaters Unearthed from East Asia. Editor C.H. Tsang. in *Prehistory and Classical Civilization*. (Taipei: Academia Sinica), 77–123.
Tang, C., Zhang, Q., and Deng, X. (2019). Preliminary Study on the Southward Boundary of Jade Artefacts of Liangzhu Style. Cul. Relics Southern Chin. *(Nan Fang Wen Wu)* 2, 26–37. (in Chinese).
Tsang, C.-H., Li, K.-T., Hsu, T.-F., Tsai, Y.-C., Fang, P.-H., and Hsing, Y.-I. C. (2017). Broomcorn and Foxtail Millet Were Cultivated in Taiwan about 5000 Years Ago. *Bot. Stud.* 58 (1), 3. doi:10.1186/s40529-016-0158-2
Tsang, C.-H. (2005). "Recent discoveries at the Tapenkeng culture sites in Taiwan: implications for the problem of Austronesian origins", in The peopling of East Asia: putting together archaeology, linguistics and genetics. Editor R. Blench, L. Sagart, and A. Sanchez-Mazas, (New York: Routledge), 63-74.
Wang, C. C., Yeh, H. Y., Popov, A. N., Zhang, H. Q., Matsumura, H., Sirak, K., et al. (2021). Genomic Insights into the Formation of Human Populations in East Asia. *Nature* 591, 413–419. doi:10.1038/s41586-021-03336-2
Wang, C., Lu, H., Gu, W., Zuo, X., Zhang, J., Liu, Y., et al. (2018). Temporal Changes of Mixed Millet and rice Agriculture in Neolithic-Bronze Age Central Plain, China: Archaeobotanical Evidence from the Zhuzhai Site. *The Holocene* 28 (5), 738–754. doi:10.1177/0959683617744269
Wang, Y., and Lu, H. (1992). *The Study of Phytolith and its Application*. Beijing: China Ocean Press.
Wang, Z. (1990). *Farmland Weeds in China: A Collection of Coloured Illustrative Plates*. Beijing: Agricultural Publishing House.
Weber, S., Lehman, H., Barela, T., Hawks, S., and Harriman, D. (2010). Rice or Millets: Early Farming Strategies in Prehistoric central Thailand. *Archaeol. Anthropol. Sci.* 2 (2), 79–88. doi:10.1007/s12520-010-0030-3
Xia, X., Zhang, P., and Wu, Y. (2019). The Analysis of rice Remains from the Chaling Site in the Pearl River Delta, Guangdong Province. *Quat. Sci.* 39 (1), 24–36. (in Chinese).
Xiang, A., and Yao, J. (2006). Cultivated rice in Xinghuahe. Agri. Archaeo. *(Nongye Kaogu)* 1, 33–45. (in Chinese).
Xiang, J. (2014). Differences between the North and the South of the Origin of Grinding Stone in China. Cul. Relics Southern Chin. *(Nan Fang Wen Wu)* 2, 101–109. (in Chinese).
Yang, M. A., Fan, X., Sun, B., Chen, C., Lang, J., Ko, Y.-C., et al. (2020). Ancient DNA Indicates Human Population Shifts and Admixture in Northern and Southern China. *Science* 369 (6501), 282–288. doi:10.1126/science.aba0909
Yang, S. (1978). Cultivated rice Remains from Shixia. Cult. *Relics (Wenwu)* 7, 23–28. (in Chinese).
Yang, S., Qiu, L., Feng, M., and Xiang, A. (2015). *Pre-Qin Archaeology of Guangdong*. Guangzhou: Guangdong People's Publishing House.
Yang, X., Barton, H. J., Wan, Z., Li, Q., Ma, Z., Li, M., et al. (2013). Sago-type Palms Were an Important Plant Food Prior to rice in Southern Subtropical China. *PloS one* 8 (5), e63148. doi:10.1371/journal.pone.0063148
Yang, X., Chen, Q., Ma, Y., Li, Z., Hung, H.-C., Zhang, Q., et al. (2018). New Radiocarbon and Archaeobotanical Evidence Reveal the Timing and Route of Southward Dispersal of rice Farming in south China. *Sci. Bull.* 63 (22), 1495–1501. doi:10.1016/j.scib.2018.10.011
Yang, X., Wang, W., Zhuang, Y., Li, Z., Ma, Z., Ma, Y., et al. (2017). New Radiocarbon Evidence on Early rice Consumption and Farming in South China. *Holocene*. 27 (7), 1045-1051. doi.10.1177/0959683616678465
Yu, C. (2018). Preliminary Research of Subsistence Patterns from Neolithic to Bronze Age in Lingnan and Surrounding Area, China. Cul. Relics Southern Chin. *(Nan Fang Wen Wu)* 2, 180–187. (in Chinese).
Zhang, C., and Hung, H.C. (2012). Later hunter-gatherers in Southern China, 18 000-3000 BC. *Antiquity* 86 (331), 11–29. doi:10.1017/S0003598X00062438
Zhang, C., and Hung, H.C. (2010). The Emergence of Agriculture in Southern China. *Antiquity* 84 (323), 11–25. doi:10.1017/S0003598X00099737
Zhang, C. (2002). The Discovery of Early Pottery in China. Doc. *Praehist* 29, 29–35. doi:10.4312/dp.29.3
Zhang, J., Lu, H., Gu, W., Wu, N., Zhou, K., Hu, Y., et al. (2012). Early Mixed Farming of Millet and rice 7800 Years Ago in the Middle Yellow River Region, China. *PLoS One* 7 (12), e52146. doi:10.1371/journal.pone.0052146
Zhang, W., and Pei, A. (1997). Research on Rice Remains from the Bashidang Site of Li County. Cult. *Relics (Wenwu)* 1, 36–41. (in Chinese).
Zhang, W., Xiang, A., Qiu, L., and Xiao, D. (2007). Research on the Husks of Ancient rice in the Shixia Ruins. J. *South. China Agric.* 28, 20–23. (in Chinese).
Zhao, Z. (2011). New Archaeobotanic Data for the Study of the Origins of Agriculture in China. *Curr. Anthropol.* 52 (S4), S295–S306. doi:10.1086/659308

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Investigating the Plant Microremains on Ceramic *Li* Tripods to Ascertain Their Function and the Plant Resource Exploitation Strategies During the Lower Xiajiadian Culture Period in Chifeng, Northeast China

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In undertaking a functional study of ceramic *Li* tripods, a common archaeological artifact in Northeast China during the Bronze Age, this study provides a crucial insight into both the function of these ceramics, plant resource strategies and human diets during this period. The precise function of the *Li* tripod has to date been controversial due to a lack of direct contextual evidence. Hence, this paper presents analyses of 10 *Li* tripods from six sites in the Chifeng area of Northeast China, all of which can be dated from their excavation contexts to the Lower Xiajiadian cultural period (3,900–3,400 cal a B.P.). The interior and exterior surfaces of the tripods were analyzed using starch grain analysis, phytolith analysis and microfossil charcoal analysis. A total of 133 starch grains were recovered, of which 111 (83.46%) were found on 80% of the interior surfaces examined. Further analysis found that these starch grains had damage features specifically gelatinised characteristics, which were consistent with the starch grains that had been boiled in our cooking simulations. In addition, there were obvious soot traces on the surfaces of the tripods, 2,835 microfossil charcoal grains not completely burned were identified, and 70% of the exterior surface samples had a higher frequency of contact with fire. These findings serve to confirm that *Li* tripods were used in the cooking of food. Of the 133 starch grains found, only 62 could be identified to species level. These were foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum milliaceum*) and plants from Triticeae taxa, as well as roots and tubers. The 3,424 phytoliths recovered from the 10 tripods were found to belong to the Panicoideae and Pooideae genera, of which the husks from foxtail and broomcorn millets from the Panicoideae genus accounted for 6.19% of the total. These results indicate that millets, the Triticeae, roots, and tubers, in addition to plants from Panicoideae and Pooideae genera, were utilized in the Chifeng area of Northeast China during the Bronze Age. This study deepens our understanding of the local subsistence patterns and the social context of early Bronze age civilization in the region.

Keywords: chifeng area, lower xiajiadian culture, early bronze-age civilization, *Li* tripod function, plant resource use, starch grain, phytolith, microfossil charcoal

INTRODUCTION

The West Liao River Basin in Northeast China occupies an important role as the birthplace of the Chinese civilization, and it has been the home of several archaeological cultures since the Neolithic Age, including the Xiaohexi, Xinglongwa, Zhaobaogou, Hongshan, and Lower Xiajiadian cultures (Su and Yin, 1981; Xu et al., 2013; Jia et al., 2017a). Among them, the Lower Xiajiadian culture is represented by the Xiajiadian sites in Chifeng city, which ushered in the Bronze Age and spanned the Erlitou culture to the early Shang Dynasty (ca. 3,900–3,400 cal. B.P.) (Zhang et al., 1987; Li, 1990; Wang et al., 1993). The Lower Xiajiadian culture was widely distributed in Chifeng, and 1,321 Lower Xiajiadian culture sites have been discovered in the Aohan Banner alone (Han, 2010). The archaeological sites of this culture surpass those of any other period in terms of their quantity and density, and the stratum associated with the culture is thick and contains widespread ancient urban settlements (Li and Gao, 1984).

Compared with the cylindrical pottery found in Chifeng during the thousands of years between the Xiaohexi culture and the Xiaoheyan culture in the Neolithic Age, the Lower Xiajiadian culture is mainly characterized by ceramic pottery vessels with three feet that are referred to as *Li* tripods. It has long been acknowledged that *Li* tripods were used for cooking (Liu, 1989; The Palace Museum, 2014). In recent years, ancient books and records, archaeological typology, and residue analyses have provided important clues about the functions of *Li* tripods. For instance, according to a typological study of *Li* tripods in the Yellow River Basin of the Longshan Period, it is believed that the tripod-shaped containers developed in response to practical cooking requirements (Gao, 1996). Moreover, an analysis of the starch grains from the *Li* tripods of the Bronze Age unearthed at the Dongzhao site in Zhengzhou, Henan province indicates that they were used for cooking rice (*Oryza sativa*) and other crops (Sun, 2018). Although large numbers of *Li* tripods from the Lower Xiajiadian cultural period have been unearthed in Chifeng, studies on their functions are still limited. Most studies focus on determining their cultural period and origins (Lin, 1995; Zhao, 2005), e.g., the development of *Li* tripods in Chifeng is believed to have integrated the Hongshan culture and the Erlitou culture (Liaoning Provincial Museum et al., 1983; Li and Gao, 1984).

In terms of the functions of *Li* tripods, the carbonized hulled broomcorn millet (*Panicum milliaceum*) found in a *Li* tripod unearthed at the Sifendi Dongshanzui sites in Chifeng suggests that they were likely used for cooking. However, this does not exclude the possibility that they were used as storage vessels. Therefore, more direct evidence is required to clarify the functions of these tripods. In this respect, plant microremains (including starch grains and phytoliths) can be analyzed to study the functions of pottery (Yang et al., 2014) and accurately identify crop types (Yang et al., 2006), as they are preserved for extensive periods (Torrence and Barton, 2006) and in large quantities (Wang and Lu, 1993). For instance, identifying the phytolith of foxtail millet (*Setaria italica*) and broomcorn millet has provided detailed information about the crop species used (Lu et al., 2009a). In addition, it is possible that starch grains and phytoliths produced by different plant organs remain within artifacts that were used to process husked seeds, roots, and tubers, even when residual phytoliths were lacking (Wu et al., 2011). In recent years, microscopic analyses conducted to identify residues on the surface of pottery and determine the morphology and species of plants used have provided direct evidence that can be used to understand the functions of such artifacts, the ingredients used, and how plant resources were utilized (Yang et al., 2014; Wang et al., 2017). Moreover, scholars have also studied the history of the human use of fire and have reconstructed ancient vegetation using the microfossil charcoal method (Patterson et al., 1987; Innes and Simmons, 2000; Li et al., 2010). Furthermore, the surface of most cooking utensils has traces of soot left from burning. As a charcoal residue produced by incomplete combustion of plant tissue, soot provides a reference or indication of the cooking function of *Li* tripods.

To broaden our knowledge of the subsistence strategies and social landscape of people in the early Bronze Age, starch grain, phytolith and microfossil charcoal residues were extracted from the surfaces of *Li* tripods of the Lower Xiajiadian cultural period unearthed in Chifeng, Northeast China. These residues were then analyzed with the intention of better understanding the functions of *Li* tripods and how the ancient people of Lower Xiajiadian culture used edible plant resources.

MATERIALS AND METHODS

Sample Collection

To analyze plant microremains, samples were collected from the feet of 10 *Li* tripods (**Figure 1A**) that had been previously unearthed from six sites in Chifeng, Northeast China: Mount Aobao Beiliang, Hadagou Nanliang, Fangshen Xiliang, Tuanjie Yingzi Mount Tuzi, Dujiadi, and Gaojia Wazi. The 10 samples were similar in shape, and residues and soot on the surface were visible to the naked eye (**Figure 1B**). All samples were from the Lower Xiajiadian cultural period, and they have been preserved at the Wengniute Museum.

The extraction of residues on the pottery surfaces was undertaken according to the protocols of Yang et al. (2014) and Liu et al. (2014). Twenty samples were analyzed. As the selected samples had been preserved at the Wengniute Museum, the surface was cleaned prior to sampling, that is, both the interior and exterior were rinsed with ultrapure water. To dislodge adhering sediment and starch, the interior surface and exterior surface of each tripod foot were separately shaken in an ultrasonic bath for 10 min, and liquid samples were obtained. To prevent cross-contaminating the samples during sampling process and the experiment, all knives, pipettes, centrifuge tubes, and powder-free gloves used were disposable and none contained starch.

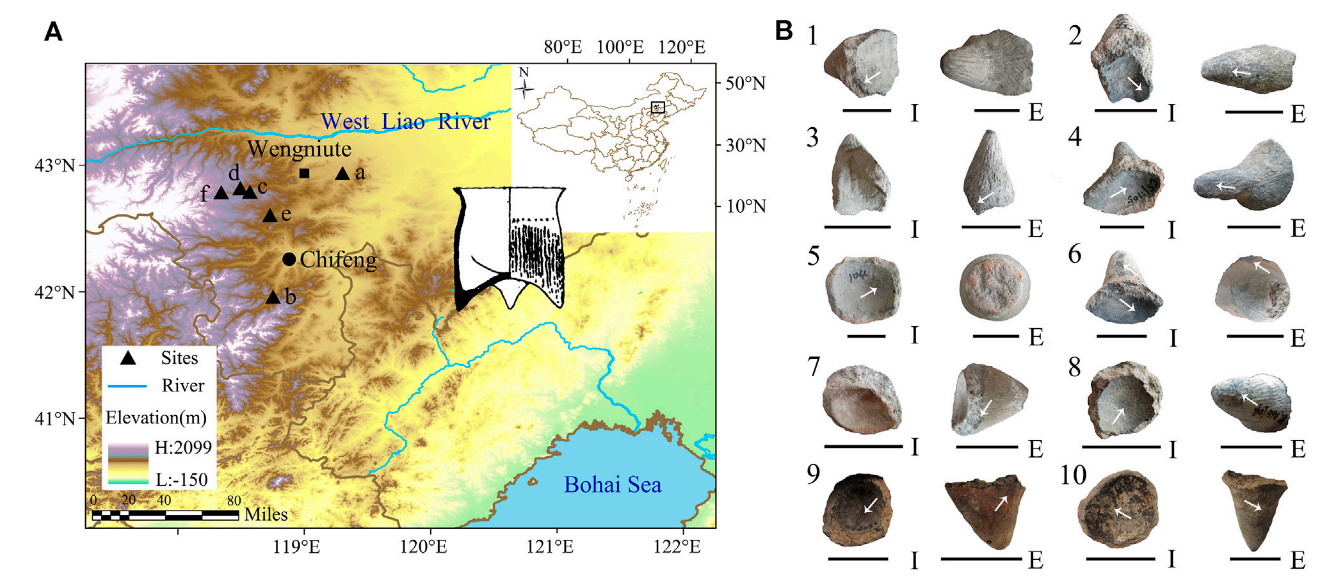

FIGURE 1 | Site locations from which artifacts were obtained and the samples subsequently analyzed. **(A)** Location of study region and archaeological sites: (a), Mount Aobao Beiliang site; (b), Hadagou Nanliang site; (c), Fangshen Xiliang site; (d), Tuanjie Yingzi Mount Tuzi site; (e), Dujiadi site; (f), Gaojia Wazi site. The sketch is of a basin-shaped *Li* Tripod, which is typical of an *Li* Tripod from the Lower Xiajiadian cultural period (Lin, 1995). **(B)** Tripod foot samples from the Lower Xiajiadian cultural period unearthed from Chifeng, Northeast China at the following sites: (1, 2), Mount Aobao Beiliang site; (3, 4), Hadagou Nanliang site; (5, 6), Fangshen Xiliang site; (7, 8), Tuanjie Yingzi Mount Tuzi site; (9) Dujiadi site, Specimen no. 150426-0230; (10) Gaojia Wazi site, Specimen no. 15046-0231. (I indicates the interior surface of *Li* tripods, E indicates the exterior surface of *Li* tripods, and the arrow indicates the sampling location of the residues. Scale bar: 5 cm).

In addition, although previous researchers have taken many images of starch grains from plants commonly used by ancient people, only a few studies have focused on starch grains damaged during processing. Therefore, we collected modern specimens of common Chinese crops (foxtail millet, broomcorn millet, and common wheat), Leguminosae, Liliaceae, and Fagaceae, and conducted processing simulation experiments. These samples were obtained *via* field acquisition and market purchase.

Starch and Phytolith Extraction

The plant microremains in the residues on the surfaces of tripod foot samples were extracted following steps previously reported (Lu et al., 2009b; Yang et al., 2012a; Yang et al., 2012b). Briefly, 6% H_2O_2 was added to the liquid samples of surface residue to break down some of the larger charred particles *via* oxidation and to release any starch grains potentially trapped within the particles or adhering to them. HCl (10%) was then added to remove calcium impurities. Then, the heavy liquid part of 1.8 g/cm^3 CsCl was added, mixed carefully, and centrifuged for 5 min at 2,500 rpm in order to extract the starch grains. The supernatant containing starch granules was then decanted into a fresh tube. Phytoliths were extracted from sediments remaining following starch grain extraction. For the second float, 2.35 g/cm^3 $ZnBr_2$ heavy liquid was added, mixed thoroughly, and centrifuged, and the supernatant containing the phytoliths was decanted into a fresh tube. Starch granule slides were prepared by mounting the recovered residue onto a slide in a solution of 25% glycerin and 75% ultrapure water. The slide was then sealed with neutral gum. Phytolith slides were prepared by mounting the recovered residue in neutral resin and then fixing it with a cover glass.

Analysis of Microfossil Charcoal

We detected microfossil charcoal together with the phytoliths and starch grains in the residue on the surface of the *Li* tripod feet. Next, we counted the total number of microfossil charcoal grains contained in the slides of the starch grains and phytoliths. Subsequently, following the conventions of microfossil charcoal analysis (Wang Z. S. et al., 2020), we randomly extracted 50 grains for each sample. Then we measured the length and width of the microfossil charcoal particles to calculate the length to width ratio (hereinafter referred to as the L/M ratio) for the identification of plant species. Ideally, 50 random grains were measured for the interior and exterior surfaces of each tripod foot sample, starting from the first grain identified. However, if less than 50 grains were identified, all of the grains were measured. Ultimately, we obtained a total of 772 sets of measurement data to determine the L/M ratio range and average L/M ratio for statistical analysis.

Grinding and Cooking Simulation Experiments

The seeds of modern foxtail millet, broomcorn millet, common wheat (*Triticum aestivum*), and lily (*Lilium Brownii*) bulbs were selected as samples. They were cleaned and dried after we confirmed that no traces of other species were present. To observe the influence of grinding on starch grains, 0.5 g of

TABLE 1 | Data of starch grains obtained from the surface residues of *Li* tripods of the Lower Xiajiadian cultural period unearthed from the Chifeng area, Northeast China (*n*).

Sample no		Location	Type I		Type II		Type III	Type IV	Total
			Intact	Damaged	Intact	Damaged			
Mount Aobao Beiliang Site	1	I	1	3	0	4	1	0	9
		E	0	0	0	0	0	0	0
	2	I	2	0	0	0	0	30	32
		E	0	0	0	0	0	0	0
Hadagou Nanliang site	3	I	2	3	0	0	0	20	25
		E	1	0	0	0	0	0	1
	4	I	1	0	0	1	0	6	8
		E	0	0	0	0	0	0	0
Fangshen Xiliang site	5	I	2	0	0	0	0	8	10
		E	0	0	0	0	0	0	0
	6	I	1	6	0	1	0	5	13
		E	0	0	0	0	0	1	1
Tuanjie Yingzi Mount Tuzi Site	7	I	0	0	0	1	0	0	1
		E	0	0	0	0	0	0	0
	8	I	1	2	0	4	0	0	7
		E	0	0	0	1	0	0	1
Dujiadi Site	9	I	2	0	0	1	0	0	3
		E	0	0	0	0	0	0	0
Gaojia Wazi Site	10	I	5	11	2	2	1	1	22
		E	0	0	0	0	0	0	0
Total		—	18	25	2	15	2	71	133
		—	43		17		—	—	—

(I indicates the interior surface of Li *tripods, E indicates the exterior surface of* Li *tripods).*

each sample was placed into a mortar and ground for 2–5 min, thus obtaining four samples for the grinding simulation experiment. The ground samples were then added to a centrifuge tube with 5 ml of ultrapure water and placed in a low-temperature environment for 1 h. To observe the effect of cooking on the starch grains, 0.5 g of each sample was added to a centrifuge tube with 5 ml of ultrapure water for 5–30 min of cooking in a water bath. The timer was started when the water began to boil, and with intervals of 5 min, 24 samples (six groups) were obtained for the cooking simulation experiment. The appropriate amount of starch suspension generated during the grinding and cooking simulation experiment was extracted with a disposable pipette, and a few drops of it were then added to a glass slide before 25% glycerin was added, and the slide was then sealed with neutral gum.

The prepared slides of starch grains and phytoliths were dried at ≤40°C for 48 h. The samples were then observed, identified, photographed, and counted using a Leica DM 750 microscope at ×400 magnification. The analyses were completed at the Environmental Archaeology Laboratory of Northwest University. The identification and classification of starch grains were mainly conducted by referring to the accumulated modern starch grain database of the laboratory and relevant study results (Yang et al., 2010; Wan et al., 2012; Yang andPerry, 2013; Ma et al., 2014). To identify and describe phytoliths and microfossil charcoal, they were compared with published pictures and materials (Wang and Lu, 1993; Lu and Liu, 2003; Piperno, 2006; Zhang and Lu, 2006; Lu et al., 2009a). All starch grains, phytoliths and microfossil charcoal on glass slides were evaluated and C2 v1.7.3 (Juggins, 2003) software was used to process data and obtain the percentage contents of phytoliths.

RESULTS

Starch Grains From Residues on the Surfaces of *Li* Tripod Foot Samples

Starch grains were identified from the interior surfaces of all 10 tripod foot samples. Starch grains were identified in only three samples from the exterior surfaces of the tripod feet. A total of 133 starch grains were extracted from the residues found on the interior and exterior surfaces of the tripod feet. Of these, 71 starch grains were seriously damaged and identification was not possible, these were classified as Type IV. The remaining identifiable 62 starch grains were classified into Types I–III based on their shapes and grain sizes. Of the 62 identifiable starch grains, 40 showed slight damage (**Table 1**). There were 111 damaged starch grains in total, accounting for 83.46% of all extracted starch grains. The definitions of grain types and associated findings are as follows.

Type I starch grains (n = 43) were polyhedron-shaped with centric hila and linear, transverse, star, or Y-shaped fissures

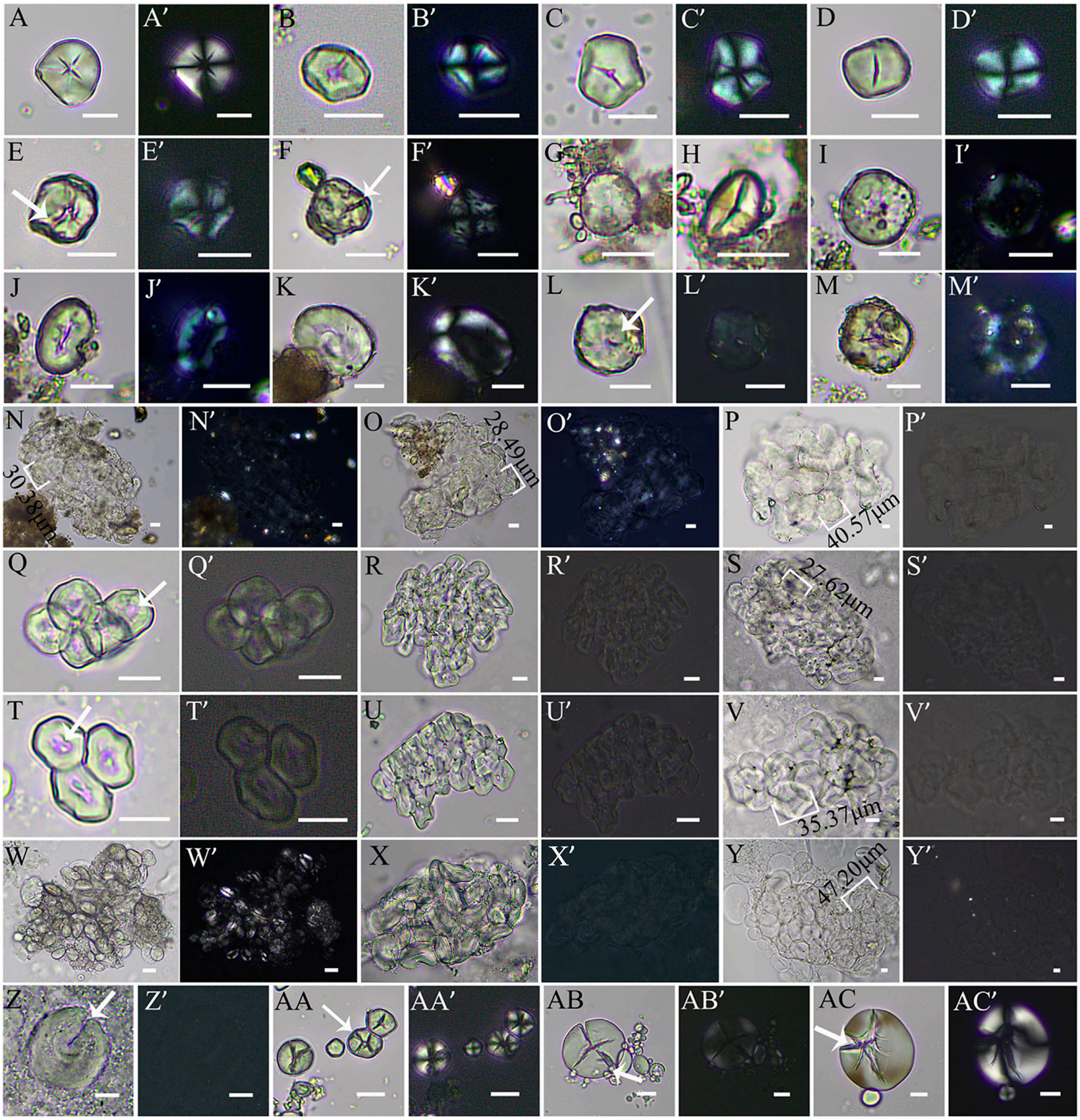

FIGURE 2 | Starch grains extracted from the surface residues of *Li* tripods of the Lower Xiajiadian cultural period unearthed from Chifeng, Northeast China, and starch grain specimens from simulation experiment. **(A–D′)** Type I intact starch grains and **(E–F′)** Type I damaged starch grains. **(G)** Polar view of type II starch grains, **(H)** lateral view of type II starch grains, and **(I–J′)** Type II damaged starch grains. **(K,K′)** Type III starch grains. **(L–O′)** Type IV starch grains. **(P,P′)** Thermally damaged lily starch grains after 5 min of heating. **(Q–S′)** Modern foxtail millet starch damaged by heating: **(Q,Q′)** heated for 5 min, **(R,R′)** heated for 15 min, and **(S, S′)** heated for 30 min **(T–V′)** Modern broomcorn millet starch damaged by heating: **(T,T′)** heated for 5 min, **(U,U′)** heated for 15 min, and **(V,V′)** heated for 30 min **(W–Y′)** Common wheat starch damaged by heating: **(W,W′)** heated for 5 min, **(X,X′)** heated for 15 min, and **(Y–Z′)** heated for 30 min **(AB,AB')** Common wheat starch damaged by grinding; **(AA,AA′)** modern foxtail millet starch damaged by grinding; and **(AC,AC′)** lily starch damaged by grinding. **(A,A′–F, F'; I,I′–AC,AC′)** Each group of photos was taken separately under bright field (left) and cross-polarized light (right). (Arrows indicate the damage characteristics of starch grains, Scale bar: 10 μm)

through the hila of most grains. Extinction crosses were mostly "+"-shaped with straight arms (**Figures 2A–D′**). The maximum lengths ranged from 9.8 to 20.1 μm, with a mean length of 14.8 ± 2.7 μm. The extinction crosses of 25 starch grains were unclear in polarized light (**Figures 2E–F′**) and there were cracks on the surfaces of four grains (**Figures 2E,F**). According to the identification criteria established by Yang et al. (2010), which are based on data analysis of starch grain shapes of Chinese northern modern foxtail and broomcorn millets, and other close wild species, Type I grains were considered to belong to millet, because the surfaces were smooth and the grain sizes were larger than average. The results thus suggest that the starch grains were most likely from foxtail and broomcorn millet crops.

Type II starch grains (n = 17) were characterized by biconvex shapes and a centric hilum. The starch grains were olivary and possessed a longitudinal dent when rotated (**Figures 2G,H**). The extinction crosses of two starch grains were X-shaped in polarized light, and those of the remaining 15 starch grains partially disappeared in polarized light (**Figures 2I–J′**). The grain lengths ranged from 12.9—35.9 μm with a mean of 23.1 ± 4.6 μm. According to the identification criteria established by Piperno et al. (2004) and Yang and Perry (2013) based on the study of such starch grains, it was determined that Type II starch grains were from the tribe Triticeae.

Type III starch grains (n = 2) were oval with an extremely eccentric hilum. There were no cracks, and the arms of extinction crosses were slightly bent (**Figures 2K,K′**).The grain lengths were 19.53 and 30.31 μm, respectively. In modern specimen banks, the grain sizes of roots and tubers are relatively large, and the hilum is skewed to one end (Wan et al., 2011). Therefore, it was determined that Type III starch grains originated from roots and tubers.

Finally, the extinction characteristics of Type IV starch grains (n = 71) were not visible. The surfaces of most were unclear, in an aggregated state, overlaid, and lacked an independent outline (**Figures 2N–O′**). The central parts of some of the starch grains were sunken or hollow, but the basic outline was identifiable (**Figures 2L–M′**). They were classified as Type IV because the species could not be identified.

Starch Grains Obtained From Grinding and Cooking Simulation Experiments

The results of the grinding simulation experiments of modern foxtail and broomcorn millet, common wheat, and lily starch grains are consistent with that of our previous study (Ma et al., 2019). The observational and statistical results identify the following. 1) Grinding caused physical and mechanical damage to some starch grains, resulting in morphological alterations. The main manifestations were that the relatively smooth surface of the starch grains became coarse, the edges became cracked and broken, and/or the central areas on the extinction crosses became dark (**Figures 2AA–AC′**). 2) The grinding strength was positively correlated with the content of the damaged starch grains. 3) After grinding, the extinction crosses of the starch grains were distorted, but the position of the hilum remained unchanged.

The results of starch grains from the cooking simulation experiments are consistent with that of previous studies (Ge et al., 2010; Hong et al., 2013; Lu et al., 2014; Chen et al., 2021). The crystal structure of the starch grains was thermally damaged and their central parts became sunken. The starch molecules gradually became transparent as they expanded and became deformed. As the heating duration increased, the number of small visible grains declined and the number of large grains increased. The average size of the foxtail millet starch grains increased to 15.18, 20.57, and 27.85 μm after being heated for 5, 15, and 30 min, respectively (**Figures 2Q–S′**). This indicates that the grain size of foxtail millet doubled throughout the cooking procedure and became similar to that of broomcorn millet (**Figures 2T–V′**). The starch grain size of common wheat increased to 21.14 and 41.20 μm after being heated for 5 and 30 min, respectively (**Figures 2W–Y′**). The starch grain size of lily increased to 40.88 μm after being heated for 5 min, which is close to its size after being heated for 30 min (48.87 μm; **Figures 2P,P′**).

There was a positive correlation between heating duration, moisture content, and gelatinization of the starch grains. Observations of the seed samples showed that the starch grains became transparent and the extinction crosses were unclear after heating for 5 min (**Figures 2Q,Q′,T,T′,W,W′**). After heating for 15 min, the starch grains were in an aggregated state with increased transparency, and although the extinction crosses had completely disappeared, their basic outlines were maintained (**Figures 2R,R′,U,U′,X,X′**). After heating for 30 min, the independent outline was lost, the starch grains were almost completely transparent, and the extinction characteristics disappeared (**Figures 2S,S′,V,V′,Y,Y′**). Lily starch granules were seriously damaged and their identification characteristics were lost after being heated for 5 min (**Figures 2P,P′**). Finally, grinding damage and cracks were observed in some of the starch grains (**Figures 2Z,Z′**).

Results of Phytolith Analysis

A total of 3,424 phytoliths were extracted from the study samples, of which 107 were damaged and could not be identified. **Figure 3** shows the main phytolith shapes, which include 17 types. There were 692 pointed phytoliths, and they had the highest total phytolith content of 20.21% (**Figure 3O**), followed by smooth elongates (15.13%; **Figure 3M**), sinuate elongates (12.44%; **Figure 3N**), bilobates (11.33%; **Figure 3I**), rondel-shaped phytoliths (10.86%; **Figure 3G**), square phytoliths (8.82%; **Figure 3P**), epidermal cells (3.45%; **Figures 3S,T**), trapezoid phytoliths (1.31%; **Figure 3H**), fan-shaped phytoliths (0.50%; **Figure 3Q**), cross-shaped phytoliths (0.18%; **Figure 3L**), cylindrical polylobate phytoliths (0.09%; **Figure 3K**), silicified stomata (0.09%; **Figure 3R**), and dumbbell-shaped phytoliths arranged vertically (0.06%; **Figure 3J**). In addition, η-type phytoliths from the husks of broomcorn millet (5.46%; **Figures 3B**), *O*-type phytoliths from the husks of foxtail millet (0.73%; **Figure 3A**), and phytoliths from the husks of the family Poaceae (6.22%; **Figures 3C–F**) were extracted from the study samples.

(I indicates the interior surface of *Li* tripods, E indicates the exterior surface of *Li* tripods).

Results of Microfossil Charcoal Analysis

Microfossil charcoal grains were identified on both the interior and exterior surfaces of the 10 *Li* tripod foot samples, and a total of 2,835 grains were detected (**Table 2**).

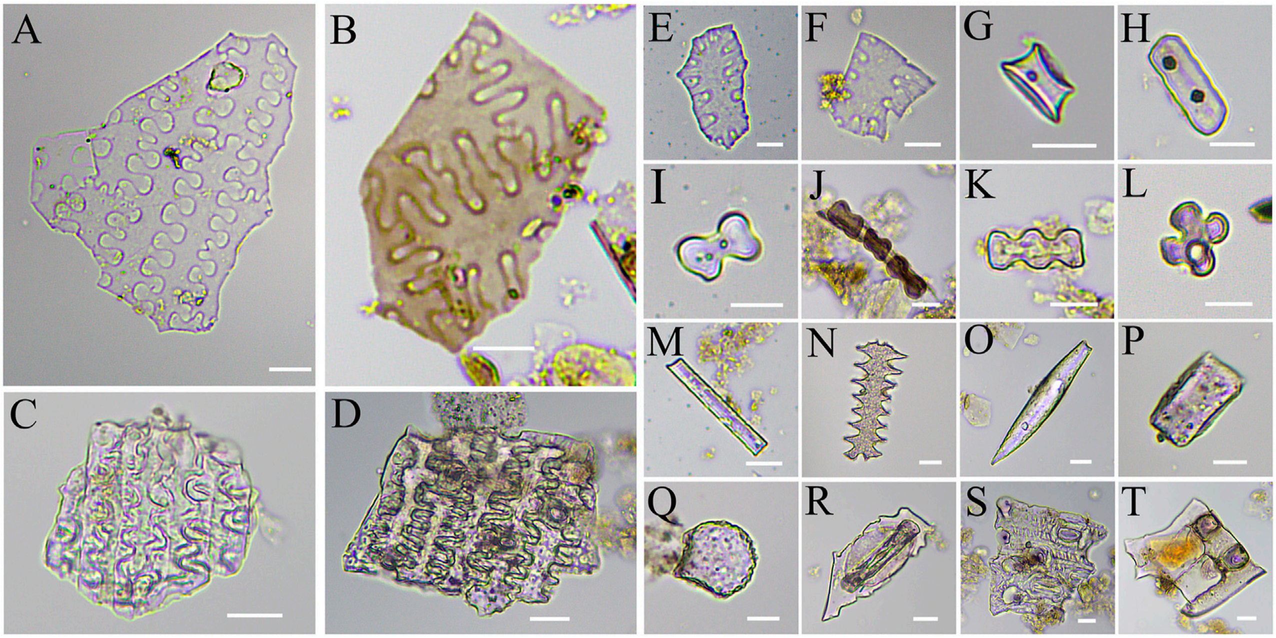

FIGURE 3 | Phytoliths extracted from the surface residues of *Li* tripods from the Lower Xiajiadian cultural period unearthed in Chifeng, Northeast China. **(A)** *Ω* type phytoliths extracted from the husks of foxtail millet, **(B)** *η* type phytoliths extracted from husks of broomcorn mille. **(C–F)** Grain shells of the family Poaceae: **(C)** Phytoliths that are most likely from the husks of Pooideae; **(D–F)** Phytoliths that are most likely from the husks of Panicoideae. **(G)** Rondel, **(H)** Trapezoid, **(I)** Bilobate, **(J)** Dumbbell-shaped phytolith arranged vertically, **(K)** Cylindrical polylobate, **(L)** Cross, **(M)** Smooth-elongate, **(N)** Sinuate-elongate, **(O)** Pointed, **(P)** Square, **(Q)** Fan, **(R)** Silicified stomata, and **(S,T)** Epidermal cells. (Scale bar: 10 μm).

TABLE 2 | Quantitative statistics and measurement data of microfossil charcoal.

Sample no		Location	Number of microfossil charcoal grains	Number of measurements	Range of L/W ratio	Average L/W ratio
Mount Aobao Beiliang Site	1	I	5	5	1.10–2.00	1.55
		E	52	50	1.07–5.79	1.78
	2	I	58	50	1.00–5.26	1.87
		E	251	50	1.00–3.48	1.73
Hadagou Nanliang site	3	I	52	50	1.01–3.97	1.98
		E	217	50	1.00–4.75	1.95
	4	I	58	50	1.04–5.33	1.95
		E	78	50	1.01–3.81	1.77
Fangshen Xiliang site	5	I	9	9	1.34–3.14	2.03
		E	110	50	1.01–4.54	1.84
	6	I	928	50	1.09–3.87	1.86
		E	30	30	1.00–5.17	1.95
Tuanjie Yingzi Mount Tuzi Site	7	I	13	13	1.15–3.02	1.91
		E	114	50	1.07–4.66	2.00
	8	I	12	12	1.00–5.57	2.35
		E	212	50	1.05–4.43	1.88
Dujiadi Site	9	I	232	50	1.01–3.69	1.91
		E	3	3	1.16–1.84	1.50
Gaojia Wazi Site	10	I	216	50	1.02–3.98	1.99
		E	185	50	1.00–4.96	1.90
Total		—	2835	772	1.00–5.79	1.89

(I indicates the interior surface of Li *tripods, E indicates the exterior surface of* Li *tripods, L/M ratio indicates the ratio of length to width of microfossil charcoal).*

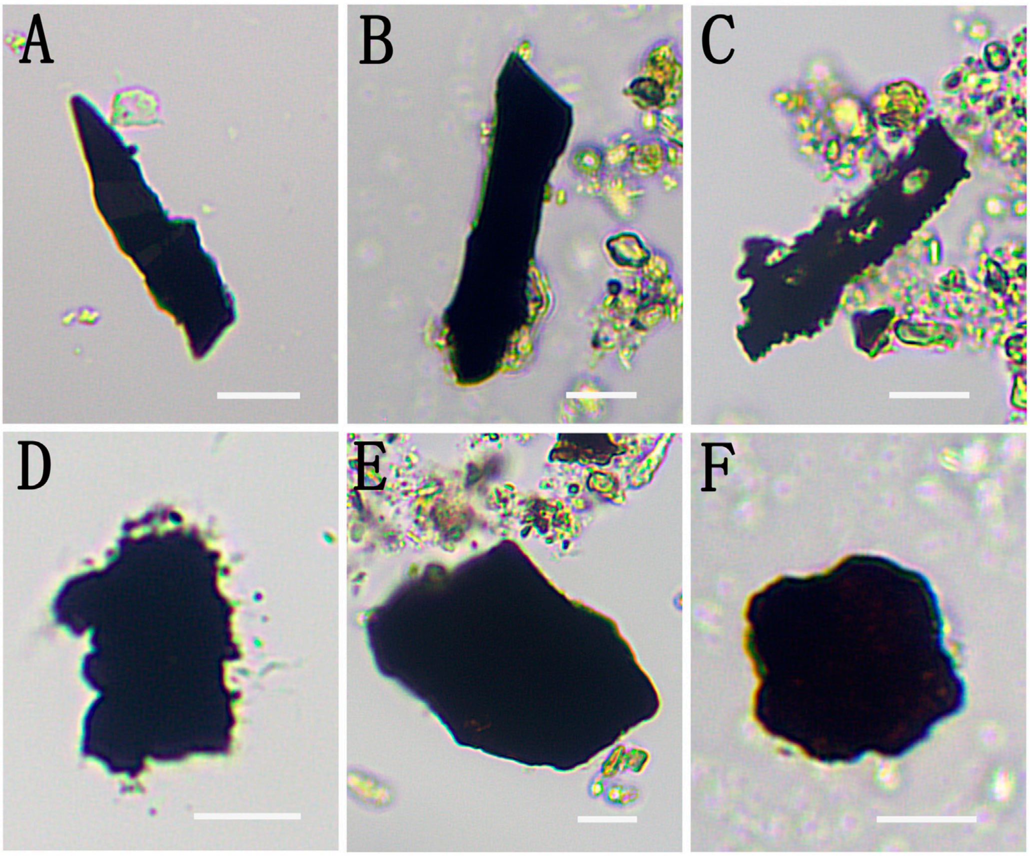

FIGURE 4 | Morphology of microfossil charcoal. **(A–C)** Possible microfossil charcoal from grasses, **(D–F)** Possible charcoal from woody plants. (Scale bar: 10 μm).

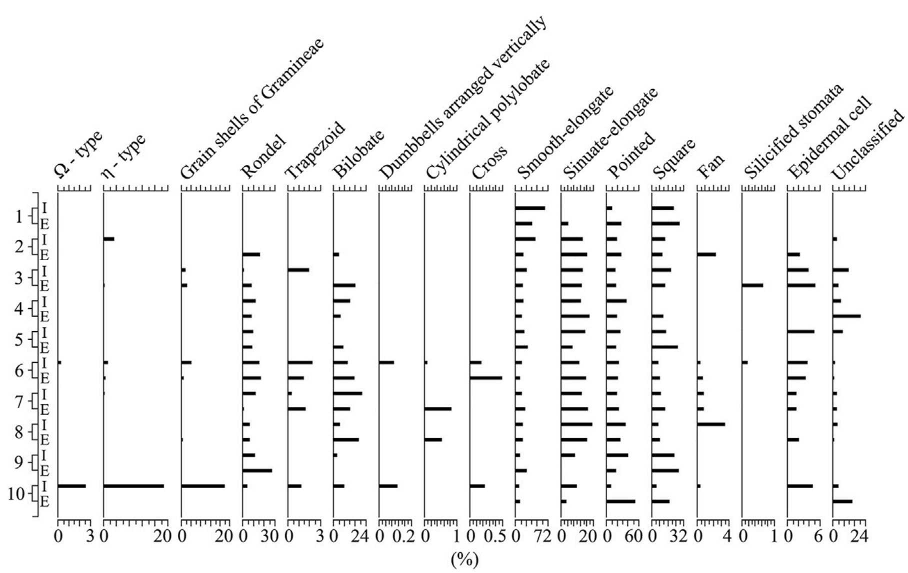

FIGURE 5 | Changes in percentages of phytolith types extracted from the surface residues of *Li* tripods from the Lower Xiajiadian cultural period unearthed in Chifeng, Northeast China.

Microfossil charcoal analysis revealed three main microfossil charcoal shapes: the strip shape, square shape, and approximately circular shape (**Figure 4**. The longitudinal edge and transverse fracture of the strip-shaped charcoal fossils were relatively straight, with many pore structures (**Figures 4A–C**). The square and approximately circular shaped charcoal fossils were relatively dense, with few pores, and the edges were irregular (**Figures 4D–F**). The length of the microfossil charcoal ranged from 5.49 to 148.59 μm, with an average length of 22.26 μm. The L/M ratio ranged from 1 to 5.79 μm, with an average of 1.89 μm (**Table 2**).

According to criteria for the identification of plant species, based on morphological characteristics and the L/M ratio proposed by Zhang and Lu (2006), strip-shaped microfossil charcoal may originate from grasses, while square-shaped or approximately circular specimens may derive from woody plants. After oscillation and centrifugation in the laboratory, the average L/M ratios of microfossil charcoal for grasses and woody plants were 3.90 ± 0.1 μm and 1.8 ± 0.1 μm, respectively. For the microfossil charcoal residue on the surface of the tripod feet, the average L/M ratio was similar to that of microfossil charcoal from woody plants. In the 772 sets of measured data, only 16 microfossil charcoal grains had a length-width ratio greater than 3.90 μm, which accounted for 2.07% of the total measured grains.

DISCUSSION

The tripod foot samples had been preserved in a museum, and the chance of contamination from deposits in the surrounding environment would have been equal for all the tripod surfaces. We therefore extracted samples from both the interior and exterior surfaces of each tripod foot and compared the quantities and assemblages of starch grains and phytoliths separately to determine the possibility of contamination from surrounding deposits. **Table 1** and **Figure 5** show the absolute quantity and appearance frequency of starch grains (n = 130; 100%) and phytoliths (n = 2,388; 100%) that were extracted from the interior surfaces of *Li* tripod foot samples. It is evident that the amounts are considerably greater than those collected from the exterior surfaces (only three starch grains and 1,036 phytoliths were collected). The discrepancies between the numbers thus suggests that most of the starch grains and phytoliths collected from the tripod feet were directly related to the use and behavior of people from the ancient culture.

Functions of *Li* Tripods

A total of 111 damaged starch grains were identified in the residues on the surfaces of the 10 tripod foot samples (**Table 1**). They accounted for 83.46% of the total grains, and they appeared on 80% of the interior surfaces. This result suggests that the starch grains were damaged during processing. To explore processing methods and obtain specimen images for comparison with ancient, damaged starch grains, we referred to previous research (Crowther, 2012; Ge et al., 2020; Chen et al., 2021). Ancient food processing methods are classified into two major types: dry cooking (e.g., roasting and baking) and wet cooking (e.g., boiling). Comparative studies indicate that when the dry cooking method was adopted, the extinction cross characteristic of starch granules may have been lost, but that the limited amount of water absorption involved may have restricted their morphological deformation (Bradbury and Holloway, 1988; Babot, 2003; Chandler-Ezell et al., 2006). In extreme circumstances, since no water is involved, starch granules can be heated to 200°C without any measurable change occurring (Wang et al., 1991; Jang and Pyun, 1996). The form of the starch grains found in the residues left on the interior surfaces of the tripod feet examined in our study is closer to that of the contemporary starch grain samples used in our cooking simulation experiment. These results indicate that the starch grains in the residues on the surfaces of the tripod feet were mainly subjected to thermal (boiling) damage (n = 111) and mechanical (grinding) damage (n = 4).

The degree and rate of starch gelatinization mainly depend on the moisture and temperature (Crowther, 2012). On one hand, when the starch moisture content falls below 60%, heating under normal pressure may fail to cause total gelatinization (Wang et al., 1991). Additionally, the water content of different plant storage organs varies considerably. For example, the water content in fresh roots and tubers (approximately 70–80%) is far higher than that of cereals (approximately 20–30%) (Hoover, 2001; Crowther, 2012). If cooked under the same conditions, the gelatinization speed of roots and tubers is considerably faster than that of cereals, so it is less likely that starch granules in roots and tubers survive and recover (Crowther, 2012). On the other hand, the temperature range from the start of gelatinization to its conclusion is usually 5–20°C. Thus, the extinction cross characteristic of starch granules that are not completely gelatinized within this interval may be retained (Lu et al., 2014). These findings indicate that even when the cooking mode is the same, the survival, recovery and recognition probability of starch granules from different plants or the same plant vary.

Starch grains with mild gelatinization (**Figures 2E–F′,I–J′**) were from foxtail millet, broomcorn millet, and the tribe Triticeae, but it was not possible to identify grains that underwent extreme gelatinization (**Figures 2L–O′**). The shapes and sizes of grains were compared with those from the surface of the tripod, and it was found that those from the tripod feet were closer to the those of the foxtail and broomcorn millets from the cooking simulation specimens (**Figures 2Q,Q′,S,S′,T,T′,V,V′**). In addition, a handful of starch grains were subjected to mechanical damage (**Figure 2Z**) during the cooking simulation experiments, even though grinding was not used, and the mechanical force applied was mainly that of stirring. It was thus inferred that the four damaged starch grains (**Figures 2E,F**) with cracks that were obtained from the residues on the surface of the tripod feet were likely subjected to stirring and squeezing during cooking, or they were from grains that broke during hulling. The identification of gelatinized starch grains in the residues on the surfaces of tripod feet further proves that at least one of the *Li* tripod functions was cooking plant-based food. It also demonstrates that the starch grains extracted from the experiments were mainly associated with processed cooked food rather than surrounding contamination.

According to previous research (Zhang, 2015) and relevant practical knowledge, if a *Li* tripod was used as a cooking vessel, there should be traces of incomplete burning of firewood on its surface as a result of cooking, such as common soot or charcoal residue. An analysis of the microfossil charcoal found on both the interior and exterior surfaces of 10 *Li* tripod foot samples identified different numbers of each. In seven of the samples, the grains detected on the exterior surfaces were far higher than on the interior surfaces (**Table 2**), indicating that the exterior surfaces had a higher frequency of contact with fire. As for the other three samples, the reasons why the microfossil charcoal grains on the interior surfaces were more numerous than on the exterior surfaces is not clear. However, microfossil charcoal remains were found on the interior surfaces of all the samples, and the corresponding species were likely to include woody plants and grasses. Possible reasons for this phenomenon are as follows. 1. Mostly, a *Li* tripod without cover was placed on the fire directly for boiling, and the charcoal grains produced by fuel during cooking were very likely to fall into the *Li* tripod by air. 2, Due to the limited tools available to ancient people for cleaning the tripods, charcoal grains on the outer surface of some *Li* tripods might have become attached to cleaning tools and thus transferred to the inner surface. The *Li* tripod foot was likely not easy to clean for space narrowness, and thus, the charcoal grains likely precipitated and became stuck. The analysis results of microfossil charcoal are consistent with the finding that many starch grains were damaged by boiling, as mentioned above. The residue of incompletely burnt charcoal on the surfaces of *Li* tripods provides further evidence of their cooking function. However, the identification of microfossil charcoal plant species in this study was limited to exploratory experiments, and thus more fundamental experimental studies are required.

In summary, we believe that the *Li* tripods from the Lower Xiajiadian cultural period unearthed in Chifeng were likely used for cooking foxtail and broomcorn millets as well as plants from the tribe Triticeae. Thus, the food processing methods used by this ancient culture at such sites are revealed. The plant microremains obtained support the use of *Li* tripods as an important food processing tool during the Lower Xiajiadian cultural period, and it appears likely that they were typically used as cooking utensils.

Plant Resources Revealed by Ancient Plant Microremains

The results suggest that *Li* tripods of the Lower Xiajiadian cultural period unearthed in Chifeng were used as cooking utensils. The plant microremains on their surfaces signify the food that people used in their recipes. Starch grains of foxtail millet, broomcorn millet, and the tribe Triticeae; roots, and tubers, and phytoliths from the grain husks of foxtail millet and broomcorn millet; and one piece of phytolith that was probably from the husks of Pooideae (**Figure 3C**) were identified in the surface residues of 10 tripod foot samples. The tribe Triticeae belongs to the Pooideae of the Poaceae subfamily. Furthermore, the phytoliths also included bilobate and cross-shaped phytoliths from the leaves of Panicoideae, trapezoid and rondel-shaped phytoliths from Pooideae leaves, and elongate phytoliths and fan-shaped phytoliths from the family Poaceae (Ge, 2016). Therefore, the analytical results of the starch grains and phytoliths are relatively consistent.

The phytoliths from the husks of foxtail and broomcorn millets and those from the husks of Pooideae were mainly extracted from the interior surfaces of the *Li* tripods; hence, these crop husks (6.07% of the total) and the phytoliths of the stems and leaves of the Poaceae are quite likely to represent the limited grain processing techniques of the period. The stems, leaves, and husks of foxtail and broomcorn millets and other grains required separation prior to grinding and cooking (Wang Q. et al., 2020). A previous study simulated the processing methods in prehistoric society using modern grain processing tools. The results indicate that using mortars, pestles, slabs, and mullers as dehusking and grinding tools could not fully hull the grains: the highest dehusking rates achieved in the experiments were 99 and 97.9%. Meanwhile, the husked grains were not 100% intact (Wang et al., 2013). In addition, the apparent soot on the outer surface of the tripod feet analyzed likely contained phytoliths that remained following the process of burning plants. The ancients usually used seeds as food and straw as firewood (Wang and Chai, 2010), hence the influence of the residual phytoliths of foxtail and common millet after burning during the heating process cannot be completely ruled out.

The edible plant resources of the ancient people in the Lower Xiajiadian cultural period included foxtail and broomcorn millets, plants from the tribe Triticeae, roots, and tubers. With respect to their absolute quantities and appearance frequencies, the starch grains and phytoliths from the husks of crops extracted from the residues on the interior surfaces of the tripod feet were mainly millets (starch grains: $n = 42$; 90%, and phytoliths: $n = 208$; 40%). There were comparatively fewer starch grains from the tribe Triticeae ($n = 16$; 70%) and from roots and tubers ($n = 2$; 20%). These results are consistent with those of previous research that suggest that the dry farming structure during the Lower Xiajiadian cultural period was dominated by foxtail and broomcorn millets (Sun et al., 2014; Jia et al., 2016a; Jia et al., 2016b; Ma et al., 2016; Jia et al., 2017b; Jia et al., 2021).

Starch granules from the tribe Triticeae appeared relatively frequently in the residues from the surfaces of the tripod feet. Triticeae has approximately 20 genera and 330 species globally, of which 13 genera and 175 species are found in China, and they are mainly distributed in northern China throughout diverse ecological environments (Liu Y. P. et al., 2013). In addition, Triticeae has been found in many Neolithic Age sites in Northeast China (Ma, 2014; Wu et al., 2018), which indicates that it was universally bred, cultured, and utilized by the ancient people of this region. Furthermore, recent research shows that Triticeae is closely related to human life and plays an important role in wheat crops and fodder (Culman et al., 2013; Pugliese et al., 2019).

A handful of starch grains from roots and tubers were also identified, which indicates that roots and tubers were also utilized in Chifeng during the Lower Xiajiadian cultural period. Roots and tubers are rich in starch and are abundant in the wild. Furthermore, compared with gramineous plants, they have more edible parts. As such, they have been an important edible plant resource since the late Paleolithic period (Liu L. et al., 2013; Yang et al., 2014). It is evident that the selection of multiple plant resources provided choice for these people, which further reduced risks associated with food scarcity from single crop failures.

CONCLUSION

We analyzed starch grains, phytoliths, and microfossil charcoal from residues found on the surfaces of 10 *Li* Tripods from the Lower Xiajiadian cultural period, which were previously unearthed from six sites in Chifeng, Northeast China. In combination with the identification of gelatinized starch grains, the soot traces on *Li* tripod surfaces, and the evidence indicating that 70% of the exterior surface samples had a higher frequency of contact with fire, our results provide further evidence that the *Li* tripods were used for cooking foxtail and broomcorn millets and plants from the tribe Triticeae. In addition, the starch grain and phytolith analyses results suggest that the edible plant resources of the ancient people of Chifeng during the Lower Xiajiadian cultural period included foxtail and broomcorn millets, plants from the tribe Triticeae, roots and tubers. Of these, foxtail and broomcorn millets were dominant. This study provides physical evidence of the functions of *Li* Tripods from the Lower Xiajiadian cultural period, as well as direct evidence of the plant resource exploitation strategies and agricultural patterns of the ancient people of this era, which in turn further promotes knowledge about their subsistence patterns and the social context of early Bronze-Age civilization.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

ZM and SL designed the study and wrote the manuscript. SL collected the samples and conducted the experiments. SL and ZM analyzed the data. XJ, YS, and ZM contributed to the discussion and approved the final manuscript.

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REFERENCES

Babot, M. d. P. (2003). "Starch Grain Damage as an Indicator of Food Processing," in *Phytolith and Starch Research in the Australian-Pacific-Asian Regions: The State of the Art*. Editors D. M. Hart and L. A. Wallis (Canberra: Pandanus Press), 69–81.

Bradbury, J. H., and Holloway, W. D. (1988). *Chemistry of Tropical Root Crops: Significance for Nutrition and Agriculture in the Pacific*. Canberra: Australasian Centre for International Agricultural Research, 39–133.

Chandler-Ezell, K., Pearsall, D. M., and Zeidler, J. A. (2006). Root and Tuber Phytoliths and Starch Grains Document Manioc(Manihot Esculenta), Arrowroot(Maranta Arundinacea), andLlerén (Calatheasp.)at the Real Alto Site, Ecuador. *Econ. Bot.* 60 (2), 103–120. doi:10.1663/0013-0001(2006)60[103:ratpas]2.0.co;2

Chen, T., Hou, L., Jiang, H., Wu, Y., and Henry, A. G. (2021). Starch Grains from Human Teeth Reveal the Plant Consumption of Proto-Shang People (C. 2000-1600 BC) from Nancheng Site, Hebei, China. *Archaeol Anthropol. Sci.* 13, 153. doi:10.1007/s12520-021-01416-y

Crowther, A. (2012). The Differential Survival of Native Starch during Cooking and Implications for Archaeological Analyses: a Review. *Archaeol Anthropol. Sci.* 4 (3), 221–235. doi:10.1007/s12520-012-0097-0

Culman, S. W., Snapp, S. S., Ollenburger, M., Basso, B., and DeHaan, L. R. (2013). Soil and Water Quality Rapidly Responds to the Perennial Grain Kernza Wheatgrass. *Agron.j.* 105 (3), 735–744. doi:10.2134/agronj2012.0273

Gao, T. L. (1996). A Study of the Pottery *Li* Tripod of the Longshan Period in the Yellow River valley. *Acta Archaeol. Sin* 4, 399–442. in Chinese.

Ge, W., Liu, L., Chen, X. C., and Jin, Z. Y. (2010). An Experimental Study on Starch Grain Damage during Food Processing and the Application in Archaeology. *Archaelogy* 7, 77–86. in Chinese.

Ge, W., Liu, L., Zhao, C. C., and Jin, Z. Y. (2020). Analysis of Starch Grain Residues in Utensils Unearthed from Xishan Site. *Archaeol Cult. Relics* 4, 111–119. in Chinese.

Ge, Y. (2016). Phytoliths in Common Modern Plants of China: Morphology and Implication. [Ph.D. Dissertation]. [Beijing]: The university of Chinese Academy of Sciences (In Chinese).

Han, M. L. (2010). A Study on Settlements and Environment of Prehistoric Times in the West Liaohe River valley. *Acta Archaeol. Sin* 1, 1–20. in Chinese.

Hong, J., Zheng, X. L., and Liu, C. (2013). Study on Particle and Gel Properties of Total, A-type, B-type Starch with Different thermal Damage Degrees. *Cereals & Oils* 26 (8), 8–12. in Chinese.

Hoover, R. (2001). Composition, Molecular Structure, and Physicochemical Properties of Tuber and Root Starches: a Review. *Carbohydr. Polym.* 45 (3), 253–267. doi:10.1016/S0144-8617(00)00260-5

Innes, J. B., and Simmons, I. G. (2000). Mid-Holocene Charcoal Stratigraphy, Fire History and Palaoecology at North Gill, North York Moors, UK. *Palaeogeo Palaeocl* 164 (1-4), 151–165. doi:10.1016/S0031-0182(00)00184-X

Jang, J. K., and Pyun, Y. R. (1996). Effect of Moisture Content on the Melting of Wheat Starch. *Starch/Stärke* 48 (2), 48–51. doi:10.1002/star.19960480204

Jia, X., Lee, H. F., Zhang, W., Wang, L., Sun, Y., Zhao, Z., et al. (2016b). Human-environment Interactions within the West Liao River Basin in Northeastern China during the Holocene Optimum. *Quat. Int.* 426, 10–17. doi:10.1016/j.quaint.2016.01.011

Jia, X., Sun, Y. G., Yang, J. G., and Zhao, Z. J. (2017b). Floating Selection Carbonized Plants Result and Analysis of the Upper Xiajiadian Cultural Period of West Liao River Region, Northeast China. *Agric. Archaeol* 6, 33–39. in Chinese.

Jia, X., Sun, Y., Wang, L., Sun, W., Zhao, Z., Lee, H. F., et al. (2016a). The Transition of Human Subsistence Strategies in Relation to Climate Change during the Bronze Age in the West Liao River Basin, Northeast China. *The Holocene* 26 (5), 781–789. doi:10.1177/0959683615618262

Jia, X., Wang, S., Sun, Y., Li, Y., Jiao, Y., Zhao, Z., et al. (2021). Charcoal Evidence for Environmental Change Ca. 3.5 Ka and its Influence on Ancient People in the West Liao River Basin of Northeastern China. *Quat. Res.* 102, 1–11. doi:10.1017/qua.2020.120

Jia, X., Yi, S., Sun, Y., Wu, S., Lee, H. F., Wang, L., et al. (2017a). Spatial and Temporal Variations in Prehistoric Human Settlement and Their Influencing Factors on the South Bank of the Xar Moron River, Northeastern China. *Front. Earth Sci.* 11 (1), 137–147. doi:10.1007/s11707-016-0572-5

Juggins, S. (2003). C2 User Guide: Software for Ecological and Palaeoecological Data Analysis and Visualisation. *Newcastle upon Tyne, UK.* Newcastle: University of Newcastle, 7–72.

Li, B. Q. (1990). *Peking University*. Beijing: Cultural Relics Press, 56–79. in Chinese."A Study of the Lower Xiajiadian Culture," in *Collected Works for the Thirtieth Anniversaryof the Archaeology Speciality of Peking University (1952-1982)*, Compiled by the Department of Archaeology

Li, G. D., and Gao, M. X. (1984). A Study on Several Issues Concerning the Lower Xiajiadian Culture. *J. Liaoning Univ. (Philosophy Soc. Sci. Edition)* 5, 50–56. in Chinese.

Li, Y., Wu, J., Hou, S., Shi, C., Mo, D., Liu, B., et al. (2010). Palaeoecological Records of Environmental Change and Cultural Development from the Liangzhu and Qujialing Archaeological Sites in the Middle and Lower Reaches of the Yangtze River. *Quat. Int.* 227 (1), 29–37. doi:10.1016/j.quaint.2010.05.015

Liaoning Provincial MuseumJuu Uda League Cultural Relic Work StationChifeng Cultural Center (1983). A Preliminary Report on Trial Excavation at the Sifendi Dongshan Site, Chifeng County, Inner Mongolia. *Archaelogy* 5, 420–429. in Chinese.

Lin, X. J. (1995). A Study on *Li* Tripod Families in Northeast Region. *J. Liaohai Cult. Relics.* 1, 72–81. in Chinese.

Liu, L., Bestel, S., Shi, J., Song, Y., and Chen, X. (2013b). Paleolithic Human Exploitation of Plant Foods during the Last Glacial Maximum in North China. *Proc. Natl. Acad. Sci.* 110 (14), 5380–5385. doi:10.1073/pnas.1217864110

Liu, L., Ma, S., and Cui, J. (2014). Identification of Starch Granules Using a Two-step Identification Method. *J. Archaeological Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

Liu, X. L. (1989). Source of Food of Chinese Prehistoric People and the Processing. *Agric. Hist. Chin.* 4, 29–37. in Chinese.

Liu, Y. P., Su, X., Chen, K. L., La, B., and Ke, J. (2013a). Current Taxonomic Status and Major Existing Problems in Triticeae (Poaceae). *J. Biol.* 30 (2), 77–83. in Chinese. doi:10.3969/j.issn.2095-1736.2013.02.077

Lü, H., Li, Y., Zhang, J., Yang, X., Ye, M., Li, Q., et al. (2014). Component and Simulation of the 4,000-Year-Old Noodles Excavated from the Archaeological Site of Lajia in Qinghai, China. *Chin. Sci. Bull.* 59 (35), 5136–5152. doi:10.1007/s11434-014-0663-1

Lu, H. Y., and Liu, K-B. (2003). Morphological Variations of Lobate Phytoliths from Grasses in China and the South-Eastern United States. *Divers. Distrib* 9 (1), 73–87. doi:10.1046/j.1472-4642.2003.00166.x

Lu, H., Zhang, J., Liu, K.-b., Wu, N., Li, Y., Zhou, K., et al. (2009b). Earliest Domestication of Common Millet (Panicum Miliaceum) in East Asia Extended to 10,000 Years Ago. *Proc. Natl. Acad. Sci.* 106 (18), 7367–7372. doi:10.1073pnas.090015810610.1073/pnas.0900158106

Lu, H., Zhang, J., Wu, N., Liu, K.-b., Xu, D., and Li, Q. (2009a). Phytoliths Analysis for the Discrimination of Foxtail Millet (*Setaria Italica*) and Common Millet (*Panicum Miliaceum*). *PLoS ONE* 4 (2), e4448. doi:10.1371/journal.pone.0004448

Ma, Z. K. (2014). *The Process of Millet Domestication and Dispersal*. [Ph.D. dissertation]. Beijing: University of Chinese Academy of Sciences (in Chinese.

Ma, Z., Li, Q., Huan, X., Yang, X., Zheng, J., and Ye, M. (2014). Plant Microremains Provide Direct Evidence for the Functions of Stone Knives from the Lajia Site, Northwestern China. *Chin. Sci. Bull.* 59 (11), 1151–1158. doi:10.1007/s11434-014-0174-0

Ma, Z., Perry, L., Li, Q., and Yang, X. (2019). Morphological Changes in Starch Grains after Dehusking and Grinding with Stone Tools. *Sci. Rep.* 9 (1), 2355. doi:10.1038/s41598-019-38758-6

Ma, Z., Yang, X., Zhang, C., Sun, Y., and Jia, X. (2016). Early Millet Use in West Liaohe Area during Early-Middle Holocene. *Sci. China Earth Sci.* 59 (8), 1554–1561. doi:10.1007/s11430-015-5257-5

Patterson, W. A., Edwards, K. J., and Maguire, D. J. (1987). Microscopic Charcoal as a Fossil Indicator of Fire. *Quat. Sci. Rev.* 6 (1), 3–23. doi:10.1016/0277-3791(87)90012-6

Piperno, D. R. (2006). *Lanham MD*. Altamira Press.Phytolith: a Comprehensive Guide for Archaeologist and Paleoecologists

Piperno, D. R., Weiss, E., Holst, I., and Nadel, D. (2004). Processing of Wild Cereal Grains in the Upper Palaeolithic Revealed by Starch Grain Analysis. *Nature* 430 (7000), 670–673. doi:10.1038/nature02734

Pugliese, J. Y., Culman, S. W., and Sprunger, C. D. (2019). Harvesting Forage of the Perennial Grain Crop Kernza (*Thinopyrum Intermedium*) Increases Root Biomass and Soil Nitrogen Cycling. *Plant Soil* 437, 241–254. doi:10.1007/s11104-019-03974-6

Su, B. Q., and Yin, Z. W. (1981). The Issues Concerning the Types of Archaeological Areas in Archaeological Culture. *Cult. Relics* 5, 10–17. in Chinese.

Sun, Y. G., Zhao, Z. J., Cao, J. E., Sun, J. S., and Dang, Y. (2014). 2009 Floating Selection and Analysis Report on Erdao Jingzi Ruins of Inner Mongolia. *Agric. Archaeol.* 6, 1–9. in Chinese.

Sun, Y. N. (2018). *Plant Food Sources and Pottery Function at the Site of Qingtai and Dongzhao from Yangshao Period to the Bronze Age Based on Starch Grain Analysis*. Master's thesis (Hefei: University of Science and Technology of China (in Chinese).

The Palace Museum. (2014).*A Study On the Families of Chinese Li Tripods*. Beijing: The Forbidden City Publishing House (In Chinese).

Torrence, R., and Barton, H. J. (2006). *Ancient Sarch Research*. Walnut Creek: Left Coast Press, 1–256.

Wan, Z. W., Yang, X. Y., Ge, Q. S., and Jiang, M. X. (2011). Morphological Characteristics of Starch Grains of Root and Tuber Plants in south China. *Quat. Sci.* 31 (4), 36–745. doi:10.3969/j.issn.1001.74lO.2011.04.18

Wan, Z. W., Yang, X. Y., Li, M. Q., Ma, Z. K., and Ge, Q. S. (2012). China Modern Starch Grain Database. *Quat. Sci.* 32 (2), 371–372. doi:10.3969/j.issn.1001-7410.2012.02.24

Wang, C. X., Cheng, J. T., Cao, J. E., Ta, L., Xiong, Z. L., and Guan, Y. (2017). Diet and Related Issues Revealed by Starch Grain Analysis on Pottery Unearthed from the Weijiawopu Site, Inner Mongolia. Acta Anthropol. *Sin* 36 (3), 406–414. in Chinese. doi:10.16359/j.cnki.cn11-1963/q.2017.0055

Wang, L. X., Qi, X. G., and Xiao, B. G. (1993). A Tentative Discussion of the Origin of Lower Xiajiadian Culture. *North. Cult. Relics.* 2, 5–16. in Chinese.

Wang, Q., Kamijyou, O., and Luan, F. S. (2013). The Study of Stone Saddle Querns and Rollers at Beiqian Site, Jimo, Shandong Province. *Dongfang Archaeology* 1, 154–179. in Chinese.

Wang, Q., Ma, Z. K., Chen, Q. H., Ma, Y. C., Yang, X. Y., Ruan, Q. R., et al. (2020b). Microbotanical Remains Provide Direct Evidence for the Functional Study of Stone Tools from Jartai Pass Site in Nilka County, Xinjiang. *Quat. Sci.* 40 (2), 450–461. doi:10.11928/j.issn.1001-7410.2020.02.15

Wang, S. S., Chiang, W. C., Zhao, B., Zheng, X. G., and Kim, I. H. (1991). Experimental Analysis and Computer Simulation of Starch-Water Interactions during Phase Transition. *J. Food Sci.* 56 (1), 121–124. doi:10.1111/j.1365-2621.1991.tb07990.x

Wang, X. G., and Chai, G. S. (2010). On the Types and Utilization of Biomass Energy in Ancient China. *Stud. Hist. Nat. Sci.* 29 (4), 421–436. in Chinese.

Wang, Y. J., and Lu, H. Y. (1993). *The Study of Phytolith and its Application*. Beijing: China Ocean Press (in Chinese.

Wang, Z. S., Zhao, Y. T., Miao, Y. F., Zou, Y. G., and Tang, G. Q. (2020a). Statistical Problem of Microcharcoal in Loess Sediments Based on the Pollen Methodology. *Arid Land Geo.* 43 (3), 661–670. in Chinese. doi:10.12118/j.issn.1000-6060.2020.03.12

Wu, W. W., Qing, G. L., and Jin, G. Y. (2018). Preliminary Analysis Results of Starch Granule Collected from Tabuobo Site in Inner Mongolia. *Huaxia Archaeology* 5, 52–58. 96 (in Chinese).

Wu, W. W., Yang, X. Y., and Jin, G. Y. (2011). The Application of Starch Analysis in Archaeology: Case Studies on Yuezhuang and Other Sites. *Dongfang Archaeology* 1, 330–348. in Chinese.

Xu, D., Lu, H., Wu, N., Liu, Z., Li, T., Shen, C., et al. (2013). Asynchronous marine-terrestrial Signals of the Last Deglacial Warming in East Asia Associated with Low- and High-Latitude Climate Changes. *Proc. Natl. Acad. Sci.* 110 (24), 9657–9662. doi:10.1073/pnas.1300025110

Yang, X., Ma, Z., Wang, T., Perry, L., Li, Q., Huan, X., et al. (2014). Starch Grain Evidence Reveals Early Pottery Function Cooking Plant Foods in North China. *Chin. Sci. Bull.* 59 (32), 4352–4358. doi:10.1007/s11434-014-0500-6

Yang, X., and Perry, L. (2013). Identification of Ancient Starch Grains from the Tribe Triticeae in the North China Plain. *J. Archaeological Sci.* 40 (8), 3170–3177. doi:10.1016/j.jas.2013.04.004

Yang, X., Wan, Z., Perry, L., Lu, H., Wang, Q., Zhao, C., et al. (2012a). Early Millet Use in Northern China. *Proc. Natl. Acad. Sci.* 109 (10), 3726–3730. doi:10.1073/pnas.1115430109

Yang, X. Y., Kong, Z. C., Liu, C. J., and Ge, Q. S. (2010). Morphological Characteristics of Starch Grains of Millets and Their Wild Relatives in North China. *Quat. Sci.* 30 (2), 364–371. in Chinese. doi:10.3969/j.issn.1001-7410.2010.02.13

Yang, X. Y., Lu, H. Y., and Xia, Z. K. (2006). The Application of Plant Starch Grains Analysis in Archaeology. *Archaeol Cult. Relics* 3, 87–91. in Chinese.

Yang, X., Zhang, J., Perry, L., Ma, Z., Wan, Z., Li, M., et al. (2012b). From the Modern to the Archaeological: Starch Grains from Millets and Their Wild Relatives in China. *J. Archaeological Sci.* 39 (2), 247–254. doi:10.1016/j.jas.2011.09.001

Zhang, H. M. (2015). The Relationship between the Primitive Pottery Product Design and the Food Culture of Li Tribe in Hainan Island. *Art Des.* 12, 112–114. (in Chinese). doi:10.17265/1539-8072/2015.12.012

Zhang, J. P., and Lu, H. Y. (2006). Preliminary Study of Charcoal Morphology and its Environmental Significance. *Quat. Sci.* 26 (5), 857–863. (in Chinese).

Zhang, Z. P., Kong, Z. S., Zhang, W. J., and Chen, Y. (1987). in *Analects Archaeological Culture (1)*. Editor B. Q. Su (Beijing: Cultural Relics Press), 58–78. in Chinese.*"Study of the Lower Xiajiadian Culture"*

Zhao, B. F. (2005). *Study on Archeology Culture of Xia Dynasty to Warring States in Northeast Region of China*. [Ph.D. dissertation]. Changchun: Jilin University (in Chinese.

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Integrating Lipid and Starch Grain Analyses From Pottery Vessels to Explore Prehistoric Foodways in Northern Gujarat, India

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This study attempts a holistic approach to past foodways in prehistoric northern Gujarat, India, by considering evidence of food production, distribution, preparation and consumption. We present here the results of a pilot residue study, integrating lipid and starch grain analyses, conducted on 28 ceramic vessels from three Chalcolithic/Harappan settlements (c. 3300–2000 cal. BC) in northern Gujarat, which are discussed in the light of previous evidence of plant and animal acquisition and preparation strategies in this region. We aim to explore how the prehistoric inhabitants of northern Gujarat transformed ingredients into meals, focusing on how different foodstuffs were processed. When assessed on their own, the lipid and compound-specific isotopic data suggest that animal fats were primarily processed in ceramic vessels, specifically non-ruminant fats. However, lipid residue analysis favors the detection of fat-rich animal products and is often unable to disentangle signatures resulting from the mixing of plant and animal products. The incorporation of starch grain analyses provides evidence for the processing of a range of plants in the vessels, such as cereals, pulses and underground storage organs. Together, the results provide a holistic perspective on foodways and a way forward in overcoming preservational and interpretational limitations.

Keywords: food, culinary practices, archeology, lipids, starch grains, South Asia

INTRODUCTION

Archeological research has long focused on the cultural, social and economic practices concerning food production and consumption (often referred to as "foodways"; Staller and Carrasco, 2009; Peres, 2017), especially since the systematic recovery of macrobotanical and microfaunal remains through flotation techniques became widespread in the 1970s. Through the analysis of plant and animal remains, often combined with the analysis of food-related artifacts such as ceramics and grinding stones, archeologists have explored issues such as the social division of labor during food production (e.g., Bolger, 2010), distribution (e.g., Welch and Scarry, 1995) and preparation (e.g., VanDerwarker and Detwiler, 2002), intra- and inter-cultural culinary preferences (e.g., Kirch and O'Day, 2003) and the role of food in social aggregation and cooperation (e.g., Bray, 2003), among other topics.

Despite the diversity of methodological and theoretical approaches used to explore prehistoric foodways and the abundant literature discussing this topic, few studies have considered both plant and animal resources holistically (e.g., Spielmann, 2002; Bogaard et al., 2009; Kansa et al., 2009; Twiss et al., 2009; Ivanova et al., 2018; Gaastra et al., 2019; McClatchie et al., 2019; Dunne et al., 2021). However, the acquisition, preparation and consumption of both plants and animals are firmly tied together in integrated agro-pastoral systems and, therefore, both resources need to be considered together in order to explore prehistoric foodways and pursue a better understanding of how food systems operated in the past.

In the last few decades, past food preparation and consumption activities have been explored through the analysis of residues from archeological artifacts and human dental calculus. Chemical (lipids and proteins; e.g., Craig et al., 2015; Hendy et al., 2018) and microbotanical (starch grains and phytoliths; e.g., Lu et al., 2005) analyses have greatly contributed to our understanding of the use of plant and animal ingredients in prehistoric cuisines, often highlighting the presence of foodstuffs not detected through conventional archeobotanical and zooarcheological methods (e.g., Högberg et al., 2009; Salque et al., 2012; Saul et al., 2013). Phytoliths are often found on non-edible plant parts but can also represent the use of taxa often underrepresented in conventional archeobotanical analyses, such as spices (Saul et al., 2013); whereas starch grains are mainly produced in the edible plants parts (seeds, fruits, underground storage organs, etc.) and are usually regarded as a direct evidence of the consumption of starchy plants, including, among others, cereals, pulses and tubers (Torrence and Barton, 2006). In parallel, the analysis of lipids from pottery vessels has thrown light on our understanding of the exploitation of animal fats and the origin of dairying practices (e.g., Craig et al., 2005; Evershed et al., 2008), as well as on the use of leafy vegetables, plant oils (Charters et al., 1993, Charters et al., 1997; Copley et al., 2001, 2005; Dunne et al., 2016) and apicultural products (Roffet-Salque et al., 2015).

Individually, phytoliths, starch grains and lipids provide valuable information on the consumption of plant and animal resources. However, their independent analysis might underestimate the importance of certain resources. For example, fat-rich animal products are more easily identified in lipid residue analysis, while the contribution of lipid content of plant-based products, generally at least tenfold lower, is likely masked by animal fats (Charters et al., 1995; Hammann and Cramp, 2018; Miller et al., 2020). Plant waxes, sterols and seed oils have been identified in pottery vessels, but generally in archeological settings with good organic preservation (e.g., Copley et al., 2001, 2005; Dunne et al., 2016). Additionally, there are only a few established biomarkers for specific plant products (brassica leaf waxes, maize and some millet species; Charters et al., 1997; Reber and Evershed, 2004; Heron et al., 2016). These preservational and interpretational biases can potentially skew our interpretation of past resource- and vessel-use in respect to plants, rendering the use of plant products in pottery vessels "invisible".

At present, microbotanical remains and lipid residues are seldom analyzed as part of the same study, and when this happens they often come from different vessels, thus impeding an effective integration of the results (see for example, microbotanical and lipid residue analyses conducted at the Neolithic site of Stavroupoli, in northern Greece; García-Granero et al., 2018; Whelton et al., 2018). The integrated analysis of lipid residue and microbotanical proxies has the potential to widen the spectrum of identifiable food resources and examine differential pathways of the processing and consumption of food (Kooiman et al., 2021). Moreover, an integrated analysis can help overcome the interpretative limitations of individual proxies.

We present here the results of a pilot study aiming at assessing the potential of an integrated lipid residue and microbotanical analysis to explore prehistoric foodways in Chalcolithic/Harappan northern Gujarat, western India (**Figure 1**). The materials analyzed in this study comprise 28 ceramic vessels potentially used for food preparation and consumption from three settlements in northern Gujarat: Chalcolithic deposits from Datrana (c. 3300–3000 cal. BC) and Loteshwar (c. 2700–2300 cal. BC), and late Urban Harappan deposits from Shikarpur (c. 2200–2000 cal. BC)—see **Table 1** for a summary of the archeological contexts and **Supplementary Material** for a more detailed description of the full stratigraphy of the sites. The results are discussed in the light of previous evidence of plant and animal acquisition and preparation strategies in this region to explore how the Chalcolithic/Harappan inhabitants of northern Gujarat transformed ingredients into meals, thinking through the potential culinary pathways of different foodstuffs.

AREA OF STUDY

For the purposes of this study, we define northern Gujarat broadly, encompassing the northern part of mainland Gujarat (often referred to as North Gujarat), the island of Kachchh and the northern part of the peninsula of Saurashtra, a region characterized by a semiarid climate (400–600 mm of average annual precipitation), with most of the rainfall occurring during the Indian summer monsoon (June-September). Several ceramic traditions co-exist in northern Gujarat during the Chalcolithic/Harappan period, including the Anarta (c. 3700–2250 cal. BC), the Pre-Prabhas (c. 3300–2900 cal. BC) and the Classical and Sorath Harappan (c. 2500–1900 cal. BC). Anarta pottery has been recovered in different proportions from over 60 prehistoric sites, but it is most common in seasonal camps occupied by semi-nomadic agro-pastoralists, such as Loteshwar (Ajithprasad and Sonawane, 2011; Rajesh et al., 2013a), whereas Pre-Prabhas pottery has only been recovered at Datrana, a lithic blade workshop (Ajithprasad, 2011; Gadekar et al., 2013;

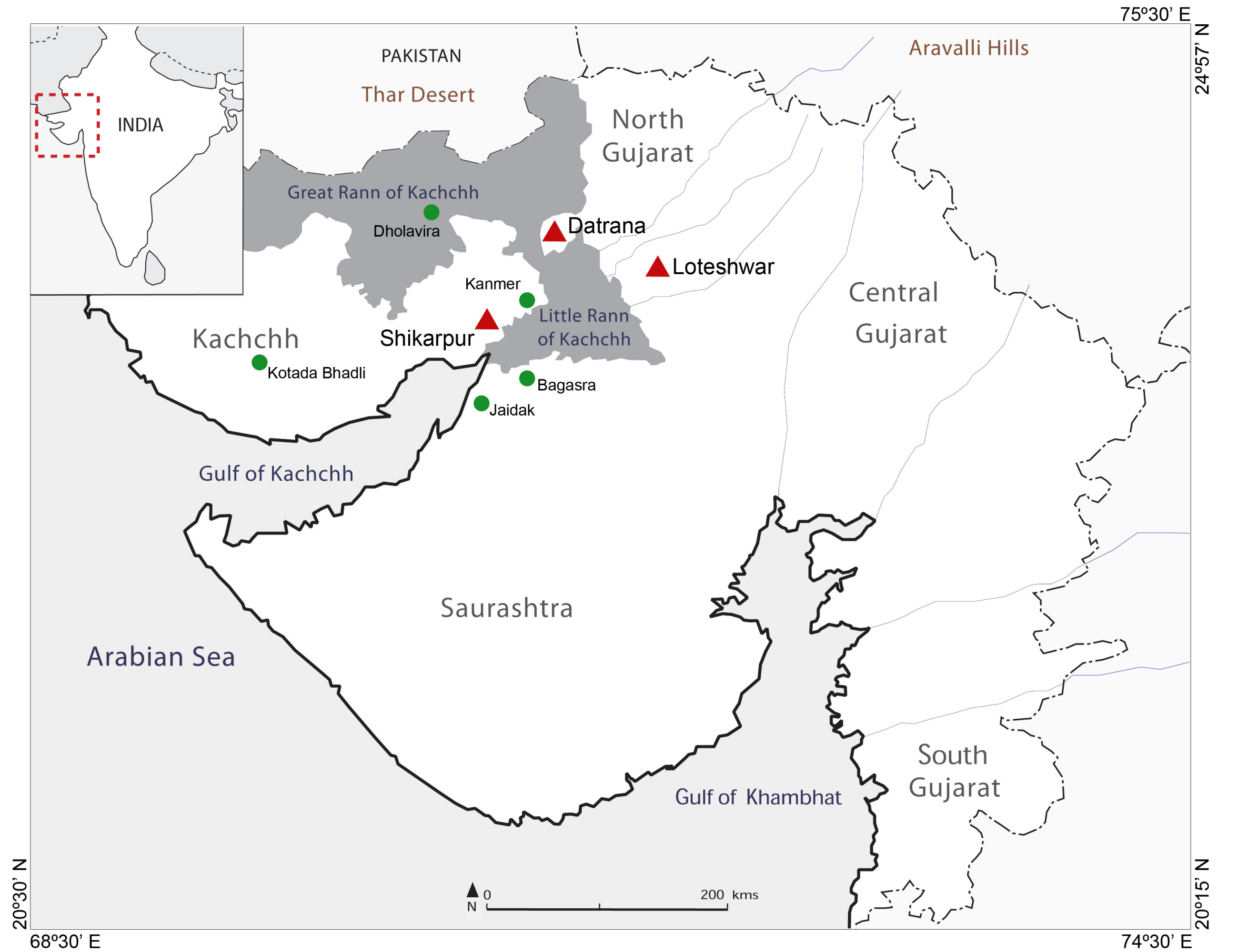

FIGURE 1 | Map of Gujarat showing the case studies (red triangles) and other archeological sites mentioned in the text (green circles). Background map prepared by Francesc C. Conesa.

TABLE 1 | Summary of the available archeological information for the deposits where the samples analyzed in this study were recovered.

Site	Chronology of the analyzed samples	Site description	Ceramics	Lithics	Other material culture	References
Datrana	c. 3300–3000 BC	Dune occupied by agro-pastoral groups focused on the production of lithic blades	Mostly pre-prabhas, very little Early Harappan Sindh and Anarta	Tools and debitage, mostly made of chalcedony, some Rohri chert blades, grinding tools	Carnelian beads	Ajithprasad, 2002, 2011; Gadekar et al., 2013; Rajesh et al., 2013b; García-Granero et al., 2017a
Loteshwar	c. 2700–2300 BC	Dune seasonally occupied by semi-nomadic agro-pastoral groups	Mostly Anarta, very little Harappan	Tools and debitage, mostly made of chalcedony and chert, grinding tools	Terracotta, shell and copper objects, steatite and semi-precious stone beads	Rajesh et al., 2013a; Gadekar et al., 2014a; García-Granero et al., 2016
Shikarpur	c. 2200–2000 BC	Fortified rural settlement	Mostly Classical and Sorath Harappan, some Anarta	Chert blades and cores (including Rohri chert), grinding tools	Terracotta, shell and copper objects, steatite and semi-precious stone beads	Bhan and Ajithprasad, 2009; Gadekar et al., 2014b; Chase et al., 2020

Rajesh et al., 2013b), and a few other sites (Rajesh et al., 2018). Classical Harappan pottery of the Indus Civilization is found mostly in walled settlements, with the characteristic Indus city plan and associated material culture, ranging from villages such as Shikarpur (Bhan and Ajithprasad, 2009) to major urban centers such as Dholavira (Bisht, 2015). Finally, Sorath Harappan pottery is the dominant regional ceramic tradition associated with the Harappan sites (mostly) of Saurashtra (see e.g., Farooqui et al., 2013, p. 2632 and references therein).

Recent archeobotanical and zooarcheological research in northern Gujarat has provided extensive evidence for the production and distribution of plant and animal resources during the Chalcolithic and Harappan period. The analysis of macroscopic plant remains (charred and mineralized fruits and seeds) from Anarta and Harappan sites shows that prehistoric populations relied on the cultivation of monsoon-adapted crops native to South Asia, particularly small millets such as little millet (*Panicum sumatrense*), browntop millet (*Brachiaria ramosa*), bristly foxtail (*Setaria verticillata*), green foxtail (*S. viridis*), Kodo millet (*Paspalum scrobiculatum*) and barnyard millet (*Echinochloa* spp.), as well as tropical pulses such as horsegram (*Macrotyloma uniflorum*), mung bean (*Vigna radiata*) and black gram (*V. mungo*) (Fuller, 2011; Pokharia et al., 2011, 2017; García-Granero et al., 2015, 2016). Sesame (*Sesamum indicum*) was also recovered from Loteshwar (García-Granero et al., 2016) and a few Harappan sites in the region (Pokharia et al., 2011, 2017). Small amounts of barley (*Hordeum vulgare*) and, to a lesser degree, free-threshing wheat (*Triticum aestivum*/*turgidum*) are also recovered from all studied sites; these were probably not cultivated locally but traded in from other Indus regions more suitable for the cultivation of winter crops (García-Granero, 2015). Wild plants are also normally recovered from Chalcolithic/Harappan sites in northern Gujarat but there exists no clear evidence of their use for human consumption, with the possible exception of Egyptian crowfoot grass (*Dactyloctenium aegyptium*) at Loteshwar (García-Granero et al., 2016).

There is abundant evidence for animal husbandry in Chalcolithic and, especially, Harappan northern Gujarat, where hunting appears to have played a minor role, particularly during the Harappan period. Cattle (*Bos* sp.) herding seems to have been the main animal-related activity during the Anarta period (Patel, 2009), later complemented with sheep (*Ovis aries*) and goats (*Capra hircus*) in Harappan settlements—and, possibly, water buffaloes (*Bubalus bubalis*) and pigs (*Sus* sp.), the domestic status of which has not been clarified to date (Thomas et al., 1996, 1997; Chase, 2010, 2014; Joglekar and Goyal, 2011; Goyal, 2013; Joglekar et al., 2013). The domestic animal slaughter patterns at Harappan Bagasra and Shikarpur suggest that cattle and buffalo were generally kept for secondary products and/or animal traction prior to consumption, whereas sheep and goats were raised primarily for meat (Chase, 2010, 2014). Fishing, particularly of marine habitats, also seems to have been an important activity in Harappan Gujarat (Abhayan, 2016). Judging by the abundance of otoliths and other fish bones at archeological sites in northern Gujarat, fish must also have been an important resource for prehistoric populations, although its role in their subsistence is currently unknown due to the scant attention fish remains have received in South Asian zooarcheology (Abhayan et al., 2016).

Husbandry practices were further explored at Harappan Bagasra, Shikarpur, Jaidak and Kotada Bhadli through stable isotope analyses ($^{87}Sr/^{86}Sr$, $\delta^{13}C$, and $\delta^{18}O$) of animal tooth enamel. At Bagasra, strontium ratios suggest that most sheep and goats were raised locally, whereas around half of the analyzed cattle were raised further afield, in several locations throughout central Saurashtra (Chase et al., 2014, 2018). Carbon and oxygen values further suggest that cattle were predominantly fed agricultural fodder (millet cultivation by-products), as shown by the presence of C_4 plants in their diet, whereas sheep and goats consumed a mixed diet made of both agricultural fodder and wild plants (mixed C_3–C_4 diet) (Chase et al., 2014). Results from nearby Shikarpur and Jaidak (Chase et al., 2020) seem to confirm the patterns observed at Bagasra. $\Delta^{13}C$-values from Kotada Bhadli, however, showed that both cattle/buffaloes and sheep/goats consumed a mixed C_3–C_4 diet (Chakraborty et al., 2018), thus suggesting that livestock management practices were not uniform throughout Harappan northern Gujarat.

Despite the relatively rich literature discussing food production and distribution in Chalcolithic northern Gujarat, less attention has been devoted to food preparation activities, and the remains of food consumption have been largely unexplored. Evidence for the pre-consumption preparation of plant resources comes from the analysis of microbotanical remains (starch grains and phytoliths) from grinding stones from Loteshwar, Datrana and Shikarpur, which show that these were mostly used to grind small millets and pulses, with a minor presence of other resources such as wheat/barley and underground storage organs (García-Granero et al., 2015, 2016, 2017a,b). Evidence for the preparation of animal resources comes from the analysis of cut-marks in cattle/buffalo and sheep/goat from Bagasra, which showed different butchery practices between those residing within and without the walled settlement (Chase, 2012). Using a different approach, Goyal (2017) suggested that preparation via roasting was more common in wild animals, particularly deer, than in domestic animals at Kanmer. Presence of burnt marks and the scarcity of cut marks in the fish assemblages from Bagasra, Kanmer and Shikarpur suggest that at least some fish resources were also prepared via roasting (Abhayan, 2016: 298).

MATERIALS AND METHODS

Sample Details

A total of 28 pottery vessels were analyzed in this study: 11 from Datrana (excavated in 2010), six from Loteshwar (excavated in 2009) and 11 from Shikarpur (excavated in 2012). All samples from Datrana come from bases of medium or large pots/dishes characterized as Pre-Prabhas. Samples from Loteshwar come from body sherds of medium pots, five characterized as Anarta and one characterized as probably Sorath Harappan (but definitely not Anarta). Finally, samples from Shikarpur come from bases of a variety of Classical Harappan vessels, including four pots/vases, four goblets, two pots/jars and one flat platter (a detailed description of the vessels can be found in **Supplementary Table 1**).

After retrieval from the archeological matrix potsherds were wrapped in aluminum foil and stored at the Department of

Archeology and Ancient History, M.S. University of Baroda, Vadodara, India, where they were sampled for lipid residue and microbotanical analyses in November 2013. Sampling took place in a controlled environment—a closed room with no airstream. A small portion of each sherd (c. 1 cm^2) was detached from the main sherd, wrapped in aluminum foil and sent to the BioArch Laboratory, University of York, United Kingdom, for lipid residue analyses.

Microbotanical residue recovery consisted of a two-step process in which the outer layer of sediment was first dry brushed from the inner surface of the vessel (dry sample), and then the inner layer of sediment was brushed with deionized water (wet sample). By removing the outer layer of sediment the likelihood of contamination from the burial environment decreases considerably (Hart, 2011), so our analysis focused on the wet samples. Microbotanical samples were transferred to the BioGeoPal Laboratory, IMF-CSIC, Barcelona, Spain, where wet samples were immediately dried at 40°C and stored. Gloves were not used during the sampling of potsherds or the extraction and analysis of starch grains to prevent starch contamination (Crowther et al., 2014).

Lipid Residue Analysis: Extraction and Analytical Protocol

Lipid extracts were obtained and prepared from the pottery sherds (n = 28) at the BioArch Laboratory using previously reported protocols of extraction and methylation (Craig et al., 2013). Each sherd was first cleaned with a high-speed drill to eliminate any surface contamination. Ceramic was then drilled from the interior surface (1 g). The ceramic powder was weighed and sealed in glass vials prior to all analyses. Methanol (4 ml) was added to the powdered pottery and the mixture was sonicated for 15 min. Then, sulfuric acid (800 μl) was added and heated at 70°C for 4 h. Lipids were extracted from centrifuged pottery powder with n-hexane (3 × 2 ml) and dried under a gentle stream of N_2. Internal standard (10 μl of hexatriacontane $C_{36:0}$) was added in all the samples prior analysis to quantify the relative abundance of lipids. All samples were analyzed by Gas Chromatography-Flame Ionisation Detection (GC-FID) (n = 28), Gas Chromatography-Mass Spectrometry (GC-MS) (n = 28) and Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) (n = 27).

GC-FID was carried out using an Agilent 7890S gas chromatograph (Agilent Technologies, Cheadle, Cheshire, United Kingdom). The sample (1 μl) was injected into the GC at 300°C with a splitless injector, using helium as carrier gas (2 ml min^{-1}). The GC column was a polymide coated fused-silica DB-1 (15 m × 320 μm × 0.1 μm; J&W Scientific, Folsom, CA, United States). The GC oven was set at 100°C for 2 min, then increased by 20°C min^{-1} until 325°C, where it was held for 3 min.

GC-MS was carried out using an Agilent 7890 B Series Gas Chromatograph attached to an Agilent 5977 B Mass Spectrometer with a quadrupole mass analyzer (Agilent technologies, Cheadle, Cheshire, United Kingdom). All samples were initially screened using a split/splitless injector in splitless mode, which was maintained at 300°C. The GC carrier gas was helium, configured at a constant flow rate of 1 ml min^{-1}. The column (HP-5MS) was coated with 5% phenyl-methylpolysiloxane (30 m × 0.25 mm × 0.25 μm; Agilent technologies, Cheadle, Cheshire, United Kingdom). The oven temperature was set at 50°C for 2 min, then raised by 10°C min^{-1} until 325°C was reached, where it was held for 15 min until the end of the run. The ionization energy of the mass spectrometer was 70 eV and spectra were obtained in scanning mode between m/z 50 and 800.

In order to assess the presence of miliacin, each Total Ion Chromatogram (TIC) was scanned for m/z 189, m/z 204, m/z 231, m/z 425, m/z 440, corresponding to miliacin fragmentation. Additionally, ω-(o-alkylphenyl) alkanoic acids (APAAs) were screened by searching the TIC for the molecular ions (M +) for APAAs of C_{16} –C_{22} at m/z 262, 290, 318, and 346 and the fragment ion of the base peak m/z 105.

The stable carbon isotope values of palmitic and stearic FAMES were measured by GC-c-IRMS, using an Agilent 7890B series GC (Agilent Technologies, Santa Clara, CA, United States), linked by an Isoprime GC5 interface (Isoprime Cheadle, United Kingdom) to an Isoprime 100 (Isoprime, Cheadle, United Kingdom) and to an Agilent 5975C inert mass spectrometer detector (MSD). Samples were re-dissolved in hexane and 1 μl was injected into DB-5MS ultra-inert fused-silica column (60 m × 0.250 mm × 0.25 μm, J&W Scientific, Folsom, CA, United States). The temperature program was 50°C for 0.5 min, 25°C min^{-1} to 175°C, 8°C min^{-1} to 325°C, isothermal hold for 20 min. The carrier gas used was ultra-high purity grade helium (3 ml min^{-1}). The gas flow eluting from the column was split into one stream that was directed to the MSD for compound identification, and another stream that was directed through the CuO furnace tube at 850°C to convert all the carbon species to CO_2. Ion intensities (44, 45, and 46 m/z) of eluted products were recorded and the corresponding $^{13}C/^{12}C$ ratios were computed. Data was analyzed using IonVantage and IonOS software (Isoprime, Cheadle, United Kingdom) and the samples were compared with a standard reference gas (CO_2) of known isotopic composition. The results are expressed in per mill (‰) relative to an international standard (VPDB). Within each batch, a mixture of n-alkanoic acid ester standards of known isotopic composition (Indiana standard F8-3) was used to check instrument accuracy (± 0.3‰) and precision on repeated measurements (± 0.5‰). Each sample was measured in duplicate. The resulting data were corrected to account for methylation of the carboxyl group through comparisons with a $C_{16:0}$ and $C_{18:0}$ fatty acid standard of known isotopic composition that were processed with each batch under identical conditions. As only a couple modern reference dairy fats from South Asia are available (Craig et al., 2005), data obtained from other published modern ruminant, dairy, non-ruminant fats, marine fats and plant oils (Copley et al., 2003; Craig et al., 2005; Spangenberg et al., 2006; Gregg et al., 2009; Outram et al., 2009; Steele et al., 2010; Dunne et al., 2012) were compared with the obtained results.

Microbotanical Remains

Processing and analysis of the wet samples from potsherds took place at the BioGeoPal Laboratory, IMF-CSIC, Barcelona, Spain. Samples were chemically processed for the extraction of starch grains and phytoliths following the protocols described in García-Granero et al. (2017b), which involve the use of 5% sodium hexametaphosphate [$(NaPO_3)_6$] to deflocculate clays, sodium polytungstate [$Na_6(H_2W_{12}O_{40})$] at a specific gravity of 1.8 g/cm^3 to isolate starch grains, 5% hydrochloric acid (HCl) to dissolve calcium carbonate, 33% oxygen peroxide (H_2O_2) to oxidize organic matter and sodium polytungstate at a specific gravity of 2.35 g/cm^3 to isolate phytoliths. Samples were observed under plain and cross-polarized transmitted light in a Leica DM2500 optical microscope with a Leica DFC490 camera for microphotography. 10% of the starch residue from each sample was mounted in 50% glycerine and fully scanned at × 200 magnifications, whereas c. 1 mg of the phytolith residue from each sample was mounted in Entellan® and scanned at × 630 magnifications until 250 identifiable single-cell phytoliths were observed. Samples where less than 100 identified phytoliths had been encountered after scanning 10% of the slide were considered sterile and the analysis did not proceed any further. Microbotanical remains encountered in archeological samples were compared with the modern plant reference collection housed at the BioGeoPal Laboratory (**Supplementary Figure 1**).

RESULTS

Lipid Preservation and Molecular Evidence (Gas Chromatography-Flame Ionisation Detection and Gas Chromatography-Mass Spectrometry)

Lipid yields ranged from 4.6 to 339.1 μg/g ($\bar{x}$ = 74.5 μg/g) (**Table 2**). All but one sample (DTR116) had lipid yields above 5 μg/g (n = 27, 96%). The lipid yields are relatively higher than those reported from other lipid residue studies in South Asia, such as at Kotada Badli, a Sorath Harappan settlement in Gujarat (Chakraborty et al., 2020, using a slightly different extraction protocol; Correa-Ascencio and Evershed, 2014), and Harappan sites in northwest India (Suryanarayan et al., 2021, using the same extraction protocol as in this study). Comparisons of lipid yields suggest there are no differences in lipid yields across sites {Kruskal-Wallis test of effect of site [$\chi^2(2) = 2.6$, $p = 0.27$]}.

All lipid profiles were dominated by mid-chain ($C_{14:0}$, $C_{16:0}$ and $C_{18:0}$) fatty acids and small peaks of long-chain fatty acids ($C_{20:0}$–$C_{24:0}$). All the profiles also contained odd-chain fatty acids such as $C_{15:0}$ and $C_{17:0}$, as well as branched-chain fatty acids such as C_{15} and C_{17}. Such profiles are characteristic of degraded animal fats (Dudd and Evershed, 1998; Dudd et al., 1999; Halmemies-Beauchet-Filleau et al., 2013, 2014; **Supplementary Figure 2**). The palmitic/stearic (P/S) ratio of the extracts ranged between 0.7 and 1.2, which is also indicative of animal products.

No peaks of n-alkanes, which can be indicative of plant waxes (Kolattukudy, 1970), were detected in the extracts. Similarly, miliacin, the chemical "biomarker" for *Panicum* spp. and some other small millets (Lu et al., 2009; Bossard et al., 2013), could not be identified in the extracts. Other chemical biomarkers, such as compounds formed during exposure to high temperatures or those indicating heated fish products—mid-chain ketones and long-chain (> C_{18}) ω-(o-alkylphenyl) alkanoic acids and isoprenoid fatty acids, respectively—could also not be identified in the lipid extracts using the methods described.

Compound-Specific Isotopic Evidence (Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry)

The fatty acid $\delta^{13}C$-values from the analyzed extracts ranged between –30.5 and –24.5‰ ($C_{16:0}$) and between –29.9 and –23.5‰ ($C_{18:0}$), reflecting a relatively narrow spread of values. The $\Delta^{13}C$-values ranged from 3.2 to –2.7‰, which are consistent with modern reference fats from non-ruminant and ruminant adipose fats (**Figure 2**, **Table 2**, and **Supplementary Figure 3**). Most of the extracts (n = 21, 78%) had $\Delta^{13}C$-values which fall within the range of reference non-ruminant fats, while the remaining (n = 6, 22%) fall within the range for ruminant adipose fats, suggesting they were predominantly used to process the carcass fats of cattle/buffalo, sheep/goat or wild ruminant animals such as deer or nilgai (*Boselaphus tragocamelus*). None of the extracts had values consistent with reference dairy-based products. Some extracts also have fatty acid $\delta^{13}C$-values that are consistent with those reported for plant oils, especially C_3 oilseed plants such as sesame (Steele et al., 2010). However, plants have a much higher palmitic/stearic ratio than animal fats, and they produce significant deviations in $\Delta^{13}C$-values depending on the absolute $\delta^{13}C$-values of the end-members (Steele et al., 2010; Hendy et al., 2018). Clear evidence for plant products was not detected in the lipid extracts in this study.

The $\delta^{13}C_{16:0}$ values of the extracts indicate the input of both C_3 and C_4 plants, likely routed through animal diet (Halmemies-Beauchet-Filleau et al., 2013, 2014). A moderate negative correlation coefficient between the $\delta^{13}C_{16:0}$ and $\Delta^{13}C$ values was observed (–0.35, p = 0.012), suggesting that samples with more negative $\Delta^{13}C$-values produced fatty acids enriched in ^{13}C, likely from tissues of ruminant animals that were consuming C_4 plants. However, there was no effect of site or vessel form on $\delta^{13}C_{16:0}$ values [$\chi^2(2) = 1.3$, $p = 0.53$ and $\chi^2(6) = 4.6$, $p = 0.59$, respectively] or on $\delta^{13}C_{18:0}$ values [$\chi^2(2) = 0.4$, $p = 0.82$ and $\chi^2(6) = 2.3$, $p = 0.89$, respectively]. This indicates that no significant differences in the usage of vessels across sites and vessel forms can be detected (see **Supplementary Figure 4**).

Microbotanical Evidence

Starch grains were generally scarce in most samples (0–8 grains per sample) but very abundant in sample DTR116 (n = 256) (**Table 2**). The presence and relative abundance of starch types varies greatly among sites (**Figure 3** and **Table 3**). Thus, whereas at Datrana most starch grains (99% of the assemblage) belong to the Hordeeae tribe (Poaceae, grasses), these are a minor component of the assemblage at Shikarpur (7%) and completely absent at Loteshwar. Plants from the Hordeeae tribe are not

TABLE 2 | Results of the chemical and starch grain analyses from pottery vessels from Datrana, Loteshwar and Shikarpur.

	Molecular evidence		GC-c-IRMS			Starch grains						
	Lipid conc. (μ g/g)	P/S ratio	$\delta^{13}C$ $C_{16:0}$	$\delta^{13}C$ $C_{18:0}$	$\Delta^{13}C$ ($C_{18:0}$-$C_{16:0}$)	Small millets	Job's tears	Wheat/barley	Pulses	Ginger	USO (other)	UNID starch
Datrana												
DTR101	130.4	0.8	–28.3	–25.8	2.5	0	0	0	0	0	0	0
DTR104	107.5	0.9	–29.6	–29.0	0.6	0	0	0	0	0	0	0
DTR105	43.0	1.1	–25.4	–28.1	– 2.7	0	0	2	0	0	0	1
DTR106	67.0	0.7	–25.0	–23.5	1.5	0	0	0	0	0	0	0
DTR107	41.7	0.9	–29.7	–28.5	1.2	0	0	0	0	0	0	0
DTR109	17.4	0.8	–30.2	–29.8	0.4	0	0	0	0	0	0	0
DTR113	30.0	1.1	–30.0	–29.6	0.3	0	0	1	0	0	0	0
DTR115	9.2	0.9	–29.8	–29.6	0.2	0	0	0	0	0	0	0
DTR116	4.6	–	–	–	–	0	0	256	0	0	0	0
DTR117	94.8	0.9	–30.0	–29.8	0.2	0	0	0	0	0	0	0
DTR120	27.9	1.2	–24.5	–26.0	– 1.5	1	0	0	0	1	0	1
Total Datrana	– –	–	–	–	–	1	0	259	0	1	0	2
Ubiquity Datrana	– –	–	–	–	–	9%	0%	27%	0%	9%	0%	18%
Loteshwar												
011109/4	34.6	0.9	–28.2	–27.5	0.7	0	1	0	0	0	0	0
021109/2	52.4	1.1	–28.5	–29.0	– 0.5	0	0	0	4	0	0	2
021109/8	12.8	1.0	–30.3	–29.6	0.7	0	0	0	0	0	0	0
031109/11	130.6	0.9	–30.1	–29.5	0.6	0	1	0	0	0	0	0
071109/19	60.3	0.9	–30.2	–29.7	0.5	0	0	0	0	0	0	0
071109/20	42.8	1.0	–28.4	–29.1	– 0.7	0	0	0	4	0	0	0
Total Loteshwar	– –	–	–	–	–	0	2	0	8	0	0	2
Ubiquity Loteshwar	– –	–	–	–	–	0%	33%	0%	33%	0%	0%	17%
Shikarpur												
240112/23	99.2	0.9	–29.9	–29.5	0.4	0	0	1	0	0	0	0
240112/29	97.7	0.9	–26.9	–28.5	– 1.6	0	0	0	2	0	0	0
250112/8	12.8	1.0	–30.5	–29.8	0.7	0	0	0	0	0	1	0
270112/8	86.1	0.9	–30.2	–29.6	0.6	0	0	0	0	0	0	0
010212/16	339.1	1.0	–26.3	–28.0	– 1.8	0	0	0	0	0	0	0
010212/19	79.8	0.9	–26.6	–27.8	– 1.2	0	0	0	1	0	0	0
020212/9	52.2	0.9	–29.9	–29.5	0.3	0	0	0	0	0	0	0
050212/12	44.9	0.9	–29.4	–29.3	0.1	0	0	0	1	0	0	0
050212/8	25.4	1.0	–27.2	–29.9	– 2.7	0	0	0	7	1	0	0
070212/12	139.1	0.9	–30.3	–29.6	0.7	0	0	0	0	0	0	1
090212/7	135.1	0.7	–26.6	–23.5	3.2	0	0	0	0	0	0	0
Total Shikarpur	– –	–	–	–	–	0	0	1	11	1	1	1
Ubiquity Shikarpur	– –	–	–	–	–	0%	0%	9%	36%	9%	9%	9%

The description of the features used to taxonomically identify starch grains can be found in ***Table 3****.*

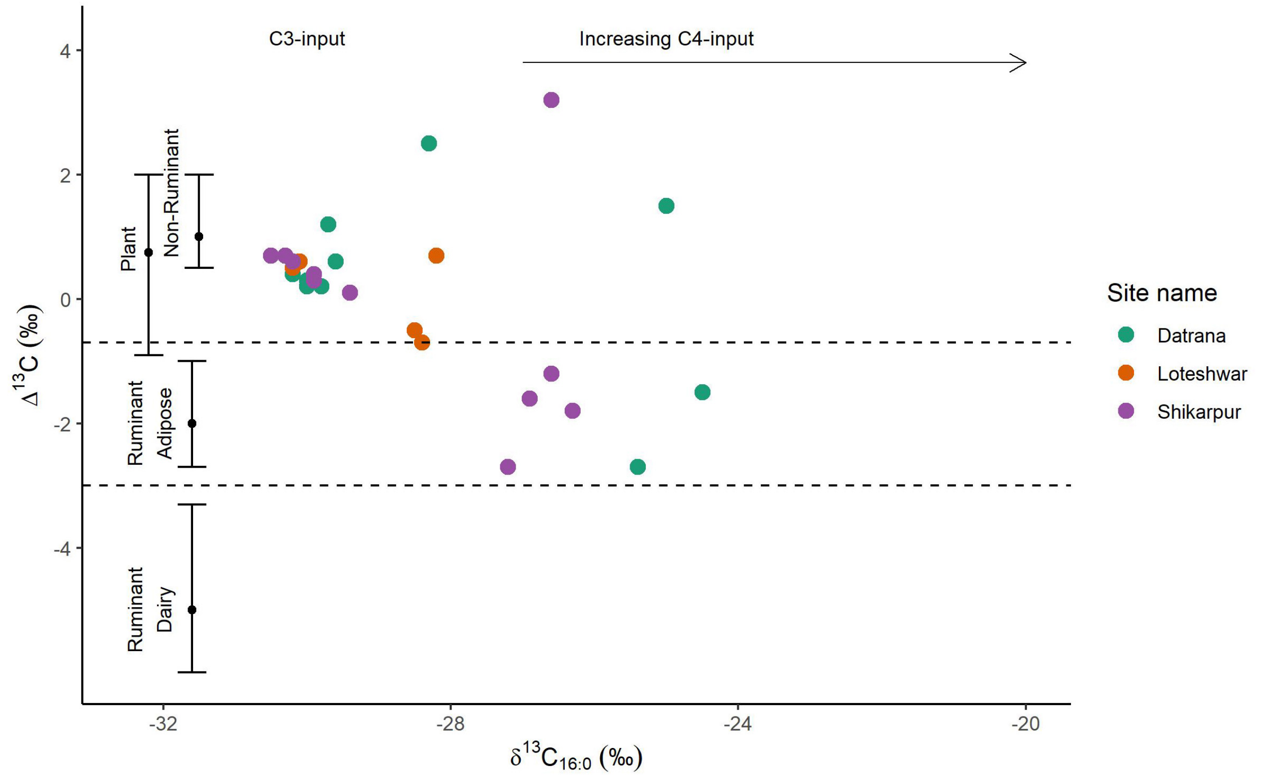

FIGURE 2 | Compound-specific isotopic values by site. The difference in $\delta^{13}C$-values for $C_{16:0}$ and $C_{18:0}$ fatty acids ($\Delta^{13}C$) are plotted against $\delta^{13}C$-values for $C_{16:0}$ fatty acids against a global reference range for modern porcine, ruminant carcass, dairy fats and plant oils (Copley et al., 2003; Spangenberg et al., 2006; Gregg et al., 2009; Outram et al., 2009; Steele et al., 2010; Dunne et al., 2012; Hendy et al., 2018).

native to Gujarat, suggesting these starch grains most likely represent wheat (*Triticum* sp.) and/or barley *(Hordeum vulgare)* traded in from other areas of the Indus Civilization, such as the Indus plain in present-day Sindh (Pakistan). Conversely, starch grains from the Faboideae subfamily (Fabaceae, pulses) predominate at both Shikarpur (73%) and Loteshwar (67%) but are completely absent from Datrana. The starch assemblage further includes one small millet (Paniceae tribe, Poaceae) grain from Datrana, two Job's tears (*Coix lacryma-jobi*, Andropogoneae tribe, Poaceae) grains from Loteshwar, two ginger (*Zingiber* sp., Zingiberaceae) grains (one from Datrana and one from Shikarpur), one grain from an unidentified underground storage organ from Shikarpur and a few grains that could not be identified due to severe damage ($n = 1$), the lack of comparable modern reference material ($n = 1$) or the lack of diagnostic features in the starch grains ($n = 3$). Phytoliths were virtually absent from all but three vessels (**Supplementary Table 3**) and are therefore not considered in the discussion of the results.

DISCUSSION

Direct archeological evidence for food preparation and consumption is often limited to exceptional archeological finds (e.g., Lu et al., 2005). In most cases, the reconstruction of past foodways is hampered by the poor survival of organic remains, taphonomic pathways and depositional and post-depositional processes. In this study, the low preservation of starch grains and survival of only free fatty acids within the lipid extracts limits the extent to which past foodways can be reconstructed. The interpretation of the results from northern Gujarat is further hindered by few previous biomolecular studies and lack of modern reference fats from the region; in fact, this is one of the few lipid residue studies on South Asian archeological material (see also Chakraborty et al., 2020; Suryanarayan et al., 2021) and the only study in South Asian archeology integrating chemical and microbotanical analysis from the same vessels. Nevertheless, a holistic approach to past foodways has the potential to overcome biases by integrating evidence, and opens up new questions to examine the production, distribution, preparation and consumption of both animal and plant resources.

Plant and Animal Ingredients in Vessels?

The obtained lipid residue results suggest the presence of degraded animal fats in the vessels. When compared with available modern reference fats from other parts of the world, the fatty acid isotopic values suggest the dominance of the processing of non-ruminant fats in the vessels, possibly porcine carcass fats or fats of other monogastric animals, such as birds. However, the presence of non-ruminant fats in vessels does not correlate with the faunal record from

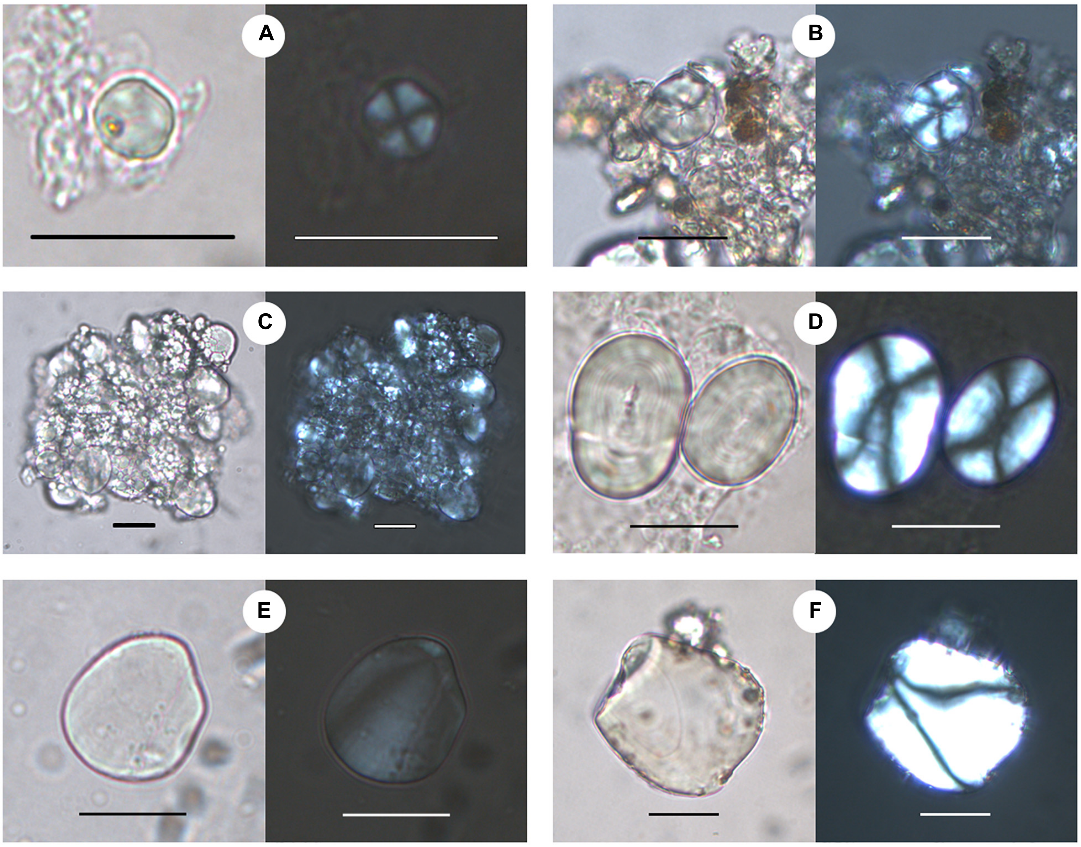

FIGURE 3 | Starch grains observed in samples from pottery vessels from Datrana, Loteshwar and Shikarpur: **(A)** Paniceae (small millet) grain form sample DTR120; **(B)** Andropogoneae (Job's tears) grain from sample 031109/11; **(C)** cluster of Hordeeae (wheat/barley) grains from sample DTR116; **(D)** Faboideae (pulses) grains from sample 071109/20; **(E)** *Zingiber* sp. (ginger) grain from sample 050212/8; and **(F)** undetermined underground storage organ from sample 250112/8. Scale bar: 20 μm (for comparative reference material, see **Supplementary Figure 1**).

TABLE 3 | Shape and size of the starch grain morphotypes found in pottery vessels from Datrana, Loteshwar and Shikapur.

Taxonomy	N_o	Shape	Size (μ m)		
			Mean (*SD*)	Range	N_m
Paniceae (small millets)	1	Polyhedral	7,851	–	1
Andropogoneae (Job's tears)	2	Polyhedral	18,734 (1,699)	17,533–19,936	2
Hordeeae Type A (wheat/barley)	24	Rounded/ovate, discoidal	23,552 (3,193)	17,942–26,666	7
Hordeeae Type B (wheat/barley)	239*	Rounded, spherical	6,302 (1,743)	3,779–7,785	4
Faboideae (pulses)	19	Ovate, ovoid	22,797 (8,049)	10,907–36,994	17
Zingiber sp. (ginger)	2	Ovate, discoidal	23,653 (6,339)	19,171–28,136	2
Unidentified underground storage organ	1	Globose	Broken, not measured	–	–
Unknown	1	Ovate, ovoid	14,305	–	1
Damaged unidentified	1	–	Broken, not measured	–	–

This morphotype occurs in numerous plants and therefore was only assigned to wheat/barley when found in association with Hordeeae Type A starch grains (e.g.,* ***Figure 3C**). N_o = number of observed starch grains. N_m = number of measured starch grains.*

Chalcolithic/Harappan northern Gujarat, as there is limited evidence for the exploitation of pigs (domestic or wild) or other omnivorous taxa. Pig remains are relatively scarce (0–15% NISP) in Chalcolithic/Harappan settlements in Gujarat compared to the remains of ruminants, particularly sheep/goat (15–40% NISP) and cattle/buffalo (50–70% NISP), which dominate the faunal assemblages (Thomas et al., 1996, 1997; Patel, 2009; Chase, 2010, 2014; Joglekar and Goyal, 2011; Goyal, 2013; Joglekar et al., 2013). Similar results were reported from Harappan sites in northwest India, where porcine remains are similarly scarce in the zooarcheological assemblage (Suryanarayan et al., 2021). While it is possible that non-ruminant animal resources were selectively processed in pots, it is also worth exploring alternative explanations for their predominance in the pottery vessels from northern Gujarat.

Mixing models to investigate how mixtures of plant and animal products processed in ancient vessels can affect compound-specific isotope results have demonstrated that it can be challenging to interpret fatty acids isotopic results when plant and animal products are mixed in vessels, particularly from environments where both C_3 and C_4 plants are available (Hendy et al., 2018; Bondetti et al., 2020; Suryanarayan, 2020; Suryanarayan et al., 2021). Hendy et al. (2018) showed that $\Delta^{13}C$-values similar to non-ruminant fats can be created when vessels are used to process mixtures of ruminant adipose products and C_3 plants. Considering the available zooarcheological evidence and the predominance of C_3 plants in the starch assemblage from pottery vessels from Datrana, Loteshwar and Shikarpur, it is possible that the $\Delta^{13}C$-values of the extracts resulted from mixtures of plant and animal products rather than non-ruminant animal fats. Although the low lipid contribution of plants may not be enough to influence the $\delta^{13}C$-value of the animal-derived lipids (Miller et al., 2020), a likely possibility could be the use of C_3 plant oils, such as sesame, which has been found at Loteshwar (García-Granero et al., 2016), which have higher lipid content. These hypotheses, however, need to be tested through additional mixing models and experimental research.

A small number of vessels from Datrana and Shikarpur have extracts with isotopic values that fall within the range of ruminant carcass fats and have $\delta^{13}C_{16:0}$ values between −27 and −24‰. These values suggest that fats processed in these vessels were from animals with a mixed C_3–C_4 plant diet (e.g., Roffet-Salque et al., 2016). The zooarcheological record in prehistoric northern Gujarat includes both domestic (cattle, sheep and goats) and wild ruminants (e.g., nilgai). The stable carbon isotope signature of animals grazing on wild vegetation would reflect the consumption of mostly C_3 plants, which form the majority of the local flora (Chase et al., 2014: 4). On the other hand, the stable carbon isotope signature of animals grazing on agricultural fodder would reflect the consumption of both C_4 (e.g., small millets) and C_3 plants (e.g., tropical pulses), as shown by stable carbon isotope analyses of tooth enamel of domestic ruminants at Bagasra (Chase et al., 2014, 2018), Shikarpur (Chase et al., 2020), Jaidak (Chase et al., 2020) and Kotada Bhadli (Chakraborty et al., 2018). Therefore, the presence of C_4 plants in the diet of the ruminants processed at Datrana and Shikarpur seems to indicate that they were partly fed agricultural by-products, and thus the molecular evidence likely represents the processing of domestic animals in vessels. Moreover, the study of burnt animal bones from Kanmer suggested that, unlike domestic taxa, wild mammals such as deer were regularly prepared via roasting (Goyal, 2017), further suggesting that the ruminant fats from Datrana and Shikarpur derive from domestic animals.

Prehistoric Foodways in Northern Gujarat

Archeobotanical and zooarcheological research has shown that the inhabitants of Chalcolithic/Harappan northern Gujarat consumed a wide array of plant and animal resources. Lipid residue and starch grain analyses, however, only identified a few of these resources in the pottery vessels from Datrana, Loteshwar and Shikarpur. Although we acknowledge that the small set of samples analyzed may not be generalizable, the results open up questions about the culinary pathways of certain foodstuffs.

Among the food ingredients potentially consumed by prehistoric populations in northern Gujarat, two were not detected in the extracts: fish and dairy products. Molecular markers for fish products were not detected in the analyzed extracts, and the compound-specific isotopic results do not suggest aquatic input. In contrast, aquatic products were tentatively identified from a single vessel at Kotada Bhadli (Chakraborty et al., 2020). Although "absence of evidence is not evidence of absence"—and the techniques required to detect fish products in lipid extracts may need to be more sensitive in nature—fish might have had a specific culinary pathway at the sites examined in this study. Fish (both marine and riverine) seem to have been an important component of the Harappan diet, but how this resource was part of the Harappan cuisine has rarely been explored. Ichthyoarcheological studies on the materials from several Indus Civilization sites highlighted the paucity of cut marks on the recovered bones ($<$ 2% of the assemblages in all studied sites; Abhayan, 2016; Belcher, 1998). At Bagasra, Kanmer and Shikarpur, most cut marks were noticed on the vertebral elements, which probably indicates standardization in chopping (Abhayan, 2016; Abhayan et al., 2018) and suggests that fish might have been cooked mostly whole and not dismembered. Visible signs of heat alteration on some of the fish bones (Abhayan, 2016, p. 298) is also commensurate with roasting or frying rather than cooking in vessels, potentially explaining why fish products were not detected in the vessels analyzed in this study.

None of the fatty acid $\delta^{13}C$-values from the lipid extracts were consistent with modern references of dairy products, including an ethnographic dairy vessel from Gujarat (Craig et al., 2005). Our results are similar to those obtained from northwest India, where only a small percentage of vessels had evidence of dairy products (Suryanarayan et al., 2021), but contrast with lipid residue analysis of vessels from Kotada Bhadli, where 6 out 21 vessels (mostly bowls) had evidence of dairy use (Chakraborty et al., 2020). Although the origins of dairying in prehistoric South Asia are still unknown, it is assumed that secondary products such as dairy were an important part of the economy, especially during the Harappan period (Gouin, 1990; Bourgeois and Gouin, 1995; Miller, 2004; Wright, 2010; Chase et al., 2014). Once again, "absence of evidence is not evidence of absence"; however, a number of possibilities must be explored. It is possible that the Chalcolithic semi-nomadic agro-pastoralists at Loteshwar and inhabitants of Datrana were not engaging in dairying; however, this is harder to explain for Shikarpur, where zooarcheological analysis suggests that cattle and buffalo were probably kept for secondary products and/or animal traction (Chase, 2010, 2014). It may also be possible that at Datrana, Loteshwar and Shikarpur milk and dairy products were processed in containers, such as bowls, that were not part of this study, or in

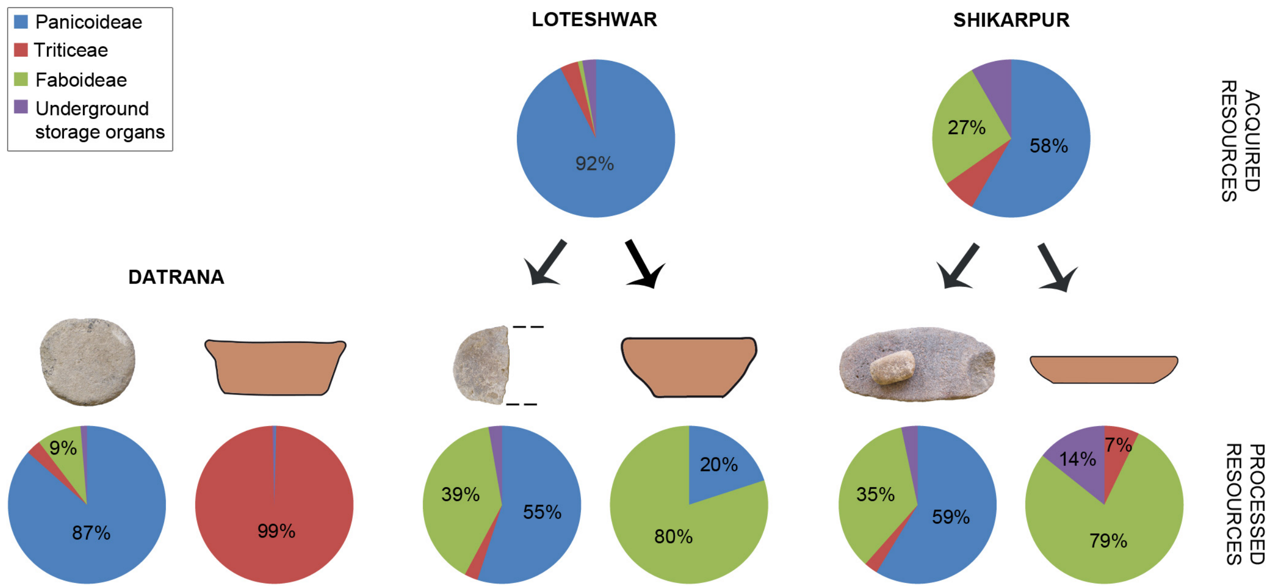

FIGURE 4 | Plant foodways at Datrana, Loteshwar and Shikarpur. Comparison of the main groups of starch-rich plants identified in archeological sediments (acquired resources), grinding stones (ground resources) and pottery vessels (cooked resources). Very few macrobotanical remains were found at Datrana (García-Granero et al., 2017a) and therefore have not been included in this figure. Raw data can be found in **Supplementary Table 2**. Panicoideae: several small millet species and Job's tears; Hordeeae: wheat and barley; Faboideae: several pulses, including horsegram and *Vigna* sp.; Underground storage organs: ginger and other unidentified resources.

TABLE 4 | Plant foodways at Datrana, Loteshwar and Shikarpur.

Acquired resources	Ground resources	Cooked resources
Datrana		
Cultivated: – *Gathered*: underground storage organs (USOs) *Traded*: **small millets**, Job's tears, **barley** (and wheat?), pulses and ginger	**Small millets**, **Job's tears**, wheat/barley, pulses and USOs (incl. ginger)	Small millets, **wheat/barley** and USOs (incl. ginger)
Loteshwar		
Cultivated: **browntop millet**, ***Setaria* spp.**, other small millets, Job's tears, horsegram and sesame *Gathered*: Egyptian crowfoot grass and USOs *Traded*: wheat (and barley?)	**Small millets**, **Job's tears**, wheat/barley, Egyptian crowfoot grass, **pulses** and USOs	Job's tears and **pulses**
Shikarpur		
Cultivated: **browntop millet**, ***Setaria* spp.**, Job's tears and ***Vigna* sp.** *Gathered*: USOs *Traded*: barley (and wheat?), rice and ginger	**Small millets**, **Job's tears**, wheat/barley, **pulses** and USOs	Wheat/barley, **pulses** and USOs (incl. ginger)

Comparison of macroscopic remains of cultivated, gathered and traded edible plants most commonly recovered from archeological sediments (acquired resources), starch grains recovered from grinding stones (ground resources) and starch grains recovered from pottery vessels (cooked resources). Most common plant resources in each category are highlighted in bold.

vessels that have not survived in the archeological record (e.g., wooden vessels). In present-day traditional northern Gujarat milk is collected in small or medium pots with a slightly wide, open mouth and consumed in bowls or cups (P. Ajithprasad personal observation), and milk collection vessels are seldom used for any other purpose. Thus, it is possible that their representation in archeological contexts might also be limited. Finally, it is also possible that the mixing of resources in vessels has made it harder to detect the direct processing of dairy products in vessels.

By comparing the starch evidence from the ceramic vessels with the evidence for the use of starch-rich plant parts from previously published macro and microbotanical analyses at Datrana, Loteshwar and Shikarpur we can attempt to disentangle the different culinary pathways potentially followed by each plant resource (**Figure 4** and **Table 4**). The starch evidence from potsherds was generally scarce and therefore any interpretation using this proxy must be considered with caution. Only 10% of the starch residue was analyzed from each sample to allow comparability with previous starch analyses on grinding stones, which may have contributed to the low number of starch grains recovered in this study—which suggests that grinding stones might provide a more suitable environment for the preservation of starch grains, although such a discussion is beyond the scope of the present study. In any case, the marked taxonomic differences observed between the assemblages from grinding stones and pottery vessels in all studied sites suggests culinary choices and the different pathways in which plant ingredients were processed and prepared. At Datrana, macrobotanical remains and phytoliths were extremely scarce. However, the starch evidence from grinding stones suggested the inhabitants of this lithic blade workshop consumed mainly small millets and Job's tears, complemented by small amounts of pulses, wheat/barley and ginger (García-Granero et al., 2017a). In striking contrast, small millet and Job's tears starch grains were virtually absent from pottery vessels, which mostly included wheat/barley and a single ginger starch grain. At Loteshwar and Shikarpur, both the macrobotanical assemblage and the microbotanical evidence from grinding stones suggested that locally cultivated small millets and, to a lesser degree, Job's tears and pulses formed the basis of the diet of the inhabitants of these settlements, complemented by wheat and barley traded in from other regions within the greater Indus Valley more prone to the cultivation of winter cereals (García-Granero et al., 2015, 2016). The starch evidence from pottery vessels, on the other hand, was mostly composed of pulses, with a minor presence of Job's tears at Loteshwar and underground storage organs (including ginger) and wheat/barley at Shikarpur.

The evidence from the analyzed pottery vessels thus suggests that plant resources were not only acquired in different ways (cultivated, gathered or traded in) but also followed different culinary pathways. Small millets were probably ground to prepare flour-based products. Job's tears seems to have been consumed in a similar way, possibly mixed with small millet flour. Miliacin was not detected in the lipid extracts (although perhaps more sensitive techniques are required), which might suggest millets were not cooked in vessels. Starch grains of pulses, on the other hand, are detected in both grinding stones and from pottery vessels, which might indicate they were incorporated in a wider range of meals using different processing techniques. Grinding stones may have been used both for grinding pulses into flour or used for preparing other dishes—and for splitting the seeds to form the basis of stews or soups. Starches of wheat and barley are not common in the studied sites but appear in abundance in vessel DTR116 from Datrana. Other types of starch grains were not found in this vessel, and it had a very low lipid concentration (below 5 μg/g). The detection of cereals via lipid residue analysis remains challenging, since biomarkers such as plant sterols and alkylresorcinols are highly susceptible to degradation, and their uptake into the ceramic matrix is very limited (Hammann and Cramp, 2018). All the available evidence, thus, seems to suggest that vessel DTR116 was used exclusively to prepare wheat/barley-based foods. Finally, ginger may have been ground on grinding stones and also incorporated during cooking. Based on a qualitative assessment of our reference collection, taxa within the ginger family produce notably less starch grains than cereals and pulses. Therefore, the presence of ginger at Datrana and Shikarpur, though minor, attests to its culinary use at these sites. Ginger starch grains were also documented in human dental calculus from Harappan Farmana, in Haryana (northern India) (Weber and Kashyap, 2010; Kashyap and Weber, 2013), highlighting that their use as food condiments may have been widespread across the greater Indus Valley during the Chalcolithic/Harappan period.

CONCLUSION

The combination of methods used in this study provides a unique means to explore the culinary use of both plant and animal ingredients that may not be detectable via conventional techniques in archeobotany and zooarcheology. They also provide a way to overcome interpretational limitations posed by individual methods, especially for the detection of plant remains within vessels. This preliminary study suggests the dominance of animal fats in vessels, although the interpretation of the compound-specific results is challenging, and pottery vessels may have been used to process C_3 plants/oils and fats from ruminants, which is supported by microbotanical analyses. The presence of $\Delta^{13}C$-values similar to non-ruminant fats or other omnivorous taxa might have resulted from the combination of these ingredients, either as part of a single dish combining plant and animal foodstuffs or as a result of multiple cooking events. No differences in culinary practices can be detected across the studied sites, which had markedly different ceramic traditions and relied on different subsistence strategies. In particular, the predominance of non-ruminant fats and/or admixtures of plants and ruminant fats in pottery vessels and the use of small millets for producing flour have been observed in all studied sites. Overall, the interpretations offered are, of course, tentative given the sample size and issues of differential preservation of the molecular and microbotanical evidence but nonetheless illustrative of how finer grained interpretations may be gleaned from multi-proxy approaches of this nature. Future research in this and neighboring areas will assess whether the observed culinary continuity (spanning over a thousand years) is representative of deeper cultural practices common to prehistoric populations in northern Gujarat and other Indus regions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

PA and MM acquired funding, provided samples, and designed the study. JG-G, AS, MCu, OC, and MCá analyzed the data. JG-G, AS, MCu, OC, and MM interpreted the data. JG-G and AS drafted the main manuscript and prepared the figures. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.840199/full#supplementary-material

REFERENCES

Abhayan, G. S. (2016). *Preparation and Application of Fish Bone Identification Keys for Harappan Sites in Gujarat with Special Reference to Bagasra, Kanmer and Shikarpur*. Ph.D. thesis. Gurgaon: Deemed University.

Abhayan, G. S., Joglekar, P. P., Ajithprasad, P., Krishnan, K., Bhanand, K. K., and Rajesh, S. V. (2018). "Exploitation during the Harappan Period at Bagasra in Gujarat, India in An Ichthyoarchaeological Approach," in *Walking with the Unicorn: Social Organization and Material Culture in Ancient South Asia. Jonathan Mark Kenoyer Felicitation*, eds D. Frenez, G. M. Jamison, and R. W. Law (Oxford: Archaeopress Archaeology), 1–18. doi: 10.2307/j.ctv19vbgkc.6

Abhayan, G. S., Joglekar, P. P., Rajesh, S. V., Gopan, A. S., and Chase, B. (2016). Fish Otoliths from Navinal, Kachchh, Gujarat: identification of Taxa and Its Implications. *Herit. J. Multidiscip. Stud. Archaeol.* 4, 218–227.

Ajithprasad, P. (2002). "The pre-Harappan cultures of Gujarat," in *Indian archaeology in retrospect, vol II. Protohistory: archaeology of the Harappan Civilization*, eds S. Settar and R. Korisettar (New Delhi: Manohar), 129–158.

Ajithprasad, P. (2011). "Chalcolithic Cultural Patterns and the Early Harappan Interaction in North Gujarat," in *Cultural Relations between the Indus and the Iranian Plateau during the Third Millennium BCE*, eds T. Osada and M. Witzel (Cambridge: Harvard University), 11–40.

Ajithprasad, P., and Sonawane, V. H. (2011). "The Harappa culture in North Gujarat: A regional paradigm," in *Linguistics, Archaeology and Human Past*, eds T. Osada and H. Endo (Kyoto: Research Institute for Humanity and Nature), 223–269.

Belcher, W. B. (1998). *Fish exploitation of the Baluchistan and Indus Valley traditions: an ethnoarchaeological approach to the study of fish remains*. Ph.D. thesis. Madison: University of Wisconsin-Madison.

Bhan, K., and Ajithprasad, P. (2009). *Excavations at Shikarpur, Gujarat 2008-2009*. Available online at: https://www.harappa.com/sites/default/files/pdf/Shikarpur-2008-2009.pdf (accessed February 28, 2022).

Bisht, R. S. (2015). *Excavations at Dholavira (1989-90 to 2004-05)*. Available online at: https://www.scribd.com/document/262316120/Excavations-at-Dholavifra-1989-2005-RS-Bisht-2015 (accessed February 28, 2022).

Bogaard, A., Charles, M., Twiss, K. C., Fairbairn, A., and Yalman, N. (2009). Private pantries and celebrated surplus: storing and sharing food at Neolithic Çatalhöyük. Central Anatolia. *Antiquity* 83, 649–668. doi: 10.1017/S0003598X00098896

Bolger, D. (2010). The dynamics of gender in early agricultural societies of the Near East. *Signs J. Women Cult. Soc.* 35, 503–531. doi: 10.1086/605512

Bondetti, M., Lucquin, A., Savel'ev, N. A., Weber, A. W., and Craig, O. E. (2020). Resource processing, early pottery and the emergence of Kitoi culture in CisBaikal: insights from lipid residue analysis of an Early Neolithic ceramic assemblage from the Gorelyi Les habitation site, Eastern Siberia. *Archaeol. Res. Asia* 24:100225. doi: 10.1016/j.ara.2020.100225

Bossard, N., Milbeau, C. L., Sauze, J., and Terwilliger, V. (2013). Distribution of miliacin (olean-18-en-3β-ol methyl ether) and related compounds in broomcorn millet (*Panicum miliaceum*) and other reputed sources: implications for the use of sedimentary miliacin as a tracer of millet. *Org. Geochem.* 63, 48–55. doi: 10.1016/j.orggeochem.2013.07.012

Bourgeois, G., and Gouin, P. R. (1995). ésultats d'une analyse de traces organiques fossiles dans une «faisselle» harappéenne. *Paléorient* 21, 125–128. doi: 10.3406/paleo.1995.4613

Bray, T. L. (ed.) (2003). *The Archaeology and Politics of Food and Feasting in Early States and Empires*. London: Kluwer Academic/Plenum Publishers. doi: 10.1007/b100538

Chakraborty, K. S., Chakraborty, S., Le Roux, P., Miller, H. M. L., Shirvalkar, P., and Rawat, Y. (2018). Enamel isotopic data from the domesticated animals at Kotada Bhadli, Gujarat, reveals specialized animal husbandry during the Indus Civilization. *J. Archaeol. Sci. Rep.* 21, 183–199. doi: 10.1016/j.jasrep.2018.06.031

Chakraborty, K. S., Slater, G. F., Miller, H. M. L., Shirvalkar, P., and Rawat, Y. (2020). Compound specific isotope analysis of lipid residues provides the earliest direct evidence of dairy product processing in South Asia. *Sci. Rep.* 10:16095. doi: 10.1038/s41598-020-72963-y

Charters, S., Evershed, R. P., Quye, A., Blinkhorn, P. W., and Reeves, V., (1997). Simulation Experiments for Determining the Use of Ancient Pottery Vessels: the Behaviour of Epicuticular Leaf Wax During Boiling of a Leafy Vegetable. *J. Archaeol. Sci.* 24, 1–7. doi: 10.1006/jasc.1995.0091

Charters, S., Evershed, R. P., Blinkhorn, P. W., and Denham, V. (1995). Evidence for the Mixing of Fats and Waxes in Archaeological Ceramics. *Archaeometry* 37, 113–127.

Charters, S., Evershed, R. P., Goad, L. J., Leyden, A., Blinkhorn, P. W., and Denham, V. (1993). Quantification and distribution of lipid in archaeological ceramics: implications for sampling potsherds for organic residue analysis and the classification of vessel use. *Archaeometry* 35, 211–223.

Chase, B. (2010). Social change at the Harappan settlement of Gola Dhoro: a reading from animal bones. *Antiquity* 84, 528–543. doi: 10.1017/s0003598x00066758

Chase, B. (2012). "Crafting Harappan Cuisine on the Saurashtran Frontier of the Indus Civilization," in *The Menial Art of Cooking: Archaeological Studies of Cooking and Food Preparation*, eds S. Graff and E. Rodriguez-Alegria (Boulder: The University Press of Colorado), 145-172.

Chase, B. (2014). On the pastoral economies of Harappan Gujarat: faunal analyses at Shikarpur in context. *Herit. J. Multidiscip. Stud. Archaeol.* 2, 1–22.

Chase, B., Meiggs, D., and Ajithprasad, P. (2020). Pastoralism, climate change, and the transformation of the Indus Civilization in Gujarat: faunal analyses and biogenic isotopes. *J. Anthropol. Archaeol.* 59:101173. doi: 10.1016/j.jaa.2020.101173

Chase, B., Meiggs, D., Ajithprasad, P., and Slater, P. A. (2014). Pastoral land-use of the Indus Civilization in Gujarat: faunal analyses and biogenic isotopes at Bagasra. *J. Archaeol. Sci.* 50, 1–15. doi: 10.1016/j.jas.2014.06.013

Chase, B., Meiggs, D., Ajithprasad, P., and Slater, P. A. (2018). What is left behind: advancing interpretation of pastoral land-use in Harappan Gujarat using herbivore dung to examine biosphere strontium isotope ($^{87}Sr/^{86}Sr$) variation. *J. Archaeol. Sci.* 92, 1–12. doi: 10.1016/j.jas.2018.01.007

Copley, M. S., Berstan, R., Dudd, S. N., Docherty, G., Mukherjee, A. J., Straker, V., et al. (2003). Direct chemical evidence for widespread dairying in prehistoric Britain. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1524–1529. doi: 10.1073/pnas.0335955100

Copley, M. S., Bland, H. A., Rose, P., Horton, M., and Evershed, R. P. (2005). Gas chromatographic, mass spectrometric and stable carbon isotopic investigations of organic residues of plant oils and animal fats employed as illuminants in archaeological lamps from Egypt. *Analyst* 130, 860–871. doi: 10.1039/b500403a

Copley, M. S., Rose, P. J., Clapham, A., Edwards, D. N., Horton, M. C., and Evershed, R. P. (2001). Detection of Palm Fruit Lipids in Archaeological Pottery from Qasr Ibrim, Egyptian Nubia. *Proc. R. Soc. Lond. B Biol. Sci.* 268, 593–597. doi: 10.1098/rspb.2000.1394

Correa-Ascencio, M., and Evershed, R. P. (2014). High throughput screening of organic residues in archaeological potsherds using direct acidifed methanol extraction. *Anal. Methods* 6, 1330–1340. doi: 10.1039/c3ay41678j

Craig, O. E., Chapman, J., Heron, C., Willis, L., Bartosiewicz, L., Taylor, G., et al. (2005). Did the first farmers of central and eastern Europe produce dairy foods?. *Antiquity* 79, 882–894. doi: 10.1017/S0003598X00115017

Craig, O. E., Saul, H., Lucquin, A., Nishida, Y., Taché, K., Clarke, L., et al. (2013). Earliest evidence for the use of pottery. *Nature* 496, 351–354. doi: 10.1038/nature12109

Craig, O. E., Shillito, L. M., Albarella, U., Viner-Daniels, S., Chan, B., Cleal, R., et al. (2015). Feeding Stonehenge: cuisine and consumption at the Late Neolithic site of Durrington Walls. *Antiquity* 89, 1096–1109. doi: 10.15184/aqy.2015.110

Crowther, A., Haslam, M., Oakden, N., Walde, D., and Mercader, J. (2014). Documenting contamination in ancient starch laboratories. *J. Archaeol. Sci.* 49, 90–104. doi: 10.1016/j.jas.2014.04.023

Dudd, S. N., and Evershed, R. P. (1998). Direct demonstration of milk as an element of archaeological economies. *Science* 282, 1478–1481. doi: 10.1126/science.282.5393.1478

Dudd, S. N., Evershed, R. P., and Gibson, A. M. (1999). Evidence for varying patterns of exploitation of animal products in different prehistoric pottery traditions based on lipids preserved in surface and absorbed residues. *J. Archaeol. Sci.* 26, 1473–1482. doi: 10.1006/jasc.1998.0434

Dunne, J., Evershed, R. P., Salque, M., Cramp, L., Bruni, S., Ryan, K., et al. (2012). First dairying in green Saharan Africa in the fifth millennium BC. *Nat. Lett.* 486, 390–394. doi: 10.1038/nature11186

Dunne, J., Jórdeczka, M., Chłodnicki, M., Hardy, K., Kubiak-Martens, L., Hoyo, M.-M.-D., et al. (2021). Holocene resource exploitation along the Nile: diet and subsistence strategies of Mesolithic and Neolithic societies at Khor Shambat 1, Sudan. *Antiquity* 95, 1426–1445. doi: 10.15184/aqy.2021.141

Dunne, J., Mercuri, A., Evershed, R., Bruni, S., and di Lernia, S. (2016). Earliest direct evidence of plant processing in prehistoric Saharan pottery. *Nat. Plants* 3:16194. doi: 10.1038/nplants.2016.194

Evershed, R. P., Payne, S., Sherratt, A., Coolidge, J., and Urem-Kotsu, D. (2008). Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* 455, 528–531. doi: 10.1038/nature07180

Farooqui, A., Gaur, A. S., and Prasad, V. (2013). Climate, vegetation and ecology during Harappan period: excavations at Kanjetar and Kaj, mid-Saurashtra coast, Gujarat. *J. Archaeol. Sci.* 40, 2631–2647. doi: 10.1016/j.jas.2013.02.005

Fuller, D. Q. (2011). Finding plant domestication in the Indian subcontinent. *Curr. Anthropol.* 52, S347–S362. doi: 10.1086/658900

Gaastra, J. S., de Vareilles, A., and Vander Linden, M. (2019). Bones and seeds: an integrated approach to understanding the spread of farming across the Western Balkans. *Environ. Archaeol.* 27, 44–60. doi: 10.1080/14614103.2019.1578016

Gadekar, C., Ajithprasad, P., and Madella, M. (2013). Crested ridge technique and lithic assemblage from Datrana, Gujarat. *Herit. J. Multidiscip. Stud. Archeol.* 1, 16–28.

Gadekar, C., Ajithprasad, P., Madella, M., Balbo, A., Rajesh, S. V., Rondelli, B., et al. (2014a). Continuation of a tradition over five thousand years: lithic assemblage from Loteshwar, North Gujarat, western India. *Herit. J. Multidiscip. Stud. Archaeol.* 2, 283–304.

Gadekar, C., Rajesh, S. V., and Ajithprasad, P. (2014b) Shikarpur lithic assemblage: new questions regarding Rohri chert blade production. *J. Lithic Stud.* 1, 137–149.

García-Granero, J. J. (2015). *From gathering to farming in semi-arid northern Gujarat (India): a multi-proxy approach.* Ph.D. thesis. Barcelona: University of Barcelona. Available online at: https://www.tdx.cat/handle/10803/351960#page=1

García-Granero, J. J., Gadekar, C., Esteban, I., Lancelotti, C., and Madella, M. (2017a). What is on the craftsmen's menu? Plant consumption at Datrana, a 5000-year-old lithic blade workshop in North Gujarat, India. *Archaeol. Anthropol. Sci.* 9, 251–263. doi: 10.1007/s12520-015-0281-0

García-Granero, J. J., Lancelotti, C., and Madella, M. (2017b). A methodological approach to the study of microbotanical remains from grinding stones: a case study in northern Gujarat (India). *Veget. Hist. Archaeobot*. 26, 43–57. doi: 10.1007/s00334-016-0557-z

García-Granero, J. J., Lancelotti, C., and Madella, M. (2015). A tale of multi-proxies: integrating macro-and microbotanical remains to understand subsistence strategies. *Veget. Hist. Archaeobot*. 24, 121–133. doi: 10.1007/s00334-014-0486-7

García-Granero, J. J., Lancelotti, C., Madella, M., and Ajithprasad, P. (2016). Millets and herders: the origins of plant cultivation in semiarid north Gujarat (India). *Curr. Anthropol*. 57, 149–163. doi: 10.1086/685775

García-Granero, J. J., Urem-Kotsou, D., Bogaard, A., and Kotsos, S. (2018). Cooking plant foods in the northern Aegean: microbotanical evidence from Neolithic Stavroupoli (Thessaloniki, Greece). *Quat. Int.* 496, 140–151. doi: 10.1016/j.quaint.2017.04.007

Gouin, P. (1990). Rapes, jarres et faisselles: la production et l'exportation des produits laitiers dans l'Indus du 3e millénaire. *Paléorient* 16, 37–54. doi: 10.3406/paleo.1990.4531

Goyal, P. (2013). Multiple Role of Cattle in the Harappan Economy at Kanmer Gujarat. *Herit. J. Multidiscip. Stud. Archeol.* 1, 63–77.

Goyal, P. (2017). The preparation and discard of animal products at Kanmer, Gujarat. *Shodha Patrika* 68, 52–61.

Gregg, M. W., Banning, E. B., Gibbs, K., and Slater, G. F. (2009). Subsistence practices and pottery use in Neolithic Jordan: molecular and isotopic evidence. *J. Archaeol. Sci.* 36, 937–946. doi: 10.1016/j.jas.2008.09.009

Halmemies-Beauchet-Filleau, A., Vanhatalo, A., Toivonen, V., Heikkilä, T., Lee, M. R., and Shingfield, K. J. (2013). Effect of replacing grass silage with red clover silage on ruminal lipid metabolism in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. *J. Dairy Sci.* 96, 5882–5900. doi: 10.3168/jds.2013-6872

Halmemies-Beauchet-Filleau, A., Vanhatalo, A., Toivonen, V., Heikkilä, T., Lee, M. R., and Shingfield, K. J. (2014). Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. *J. Dairy Sci.* 97, 3761–3776. doi: 10.3168/jds.2013-7358

Hammann, S., and Cramp, L. J. E. (2018). Towards the detection of dietary cereal processing through absorbed lipid biomarkers in archaeological pottery. *J. Archaeol. Sci.* 93, 74–81. doi: 10.1016/j.jas.2018.02.017

Hart, T. C. (2011). Evaluating the usefulness of phytoliths and starch grains found on survey artifacts. *J. Archaeol. Sci.* 38, 3244–3253. doi: 10.1016/j.jas.2011.06.034

Hendy, J., Colonese, A. C., Franz, I., Fernandes, R., Fischer, R., Orton, D., et al. (2018). Ancient proteins from ceramic vessels at Çatalhöyük West reveal the hidden cuisine of early farmers. *Nat. Commun.* 9:4064. doi: 10.1038/s41467-018-06335-6

Heron, C., Shoda, S., Breu Barcons, A., Czebreszuk, J., and Eley, Y. (2016). First molecular and isotopic evidence of millet processing in prehistoric pottery vessels. *Sci. Rep.* 6:38767. doi: 10.1038/srep38767

Högberg, A., Puseman, K., and Yost, C. (2009). Integration of use-wear with protein residue analysis – a study of tool use and function in the south Scandinavian Early Neolithic. *J. Archaeol. Sci.* 36, 1725–1737. doi: 10.1016/j.jas.2009.03.030

Ivanova, M., De Cupere, B., Ethier, J., and Marinova, E. (2018). Pioneer farming in southeast Europe during the early sixth millennium BC: climate-related adaptations in the exploitation of plants and animals. *PLoS One* 13:e0197225. doi: 10.1371/journal.pone.0197225

Joglekar, P., and Goyal, P. (2011). Faunal remains from Shikarpur, a Harappan site in Gujarat, India. *Iran. J. Archaeol. Stud.* 1, 15–25.

Joglekar, P. P., Abhayan, G. S., Mungur-Medhi, J., and Nath, J. A. (2013). Glimpse of Animal Utilization Pattern at Khirsara, Kachchh, Gujarat, India. *Herit. J. Multidiscip. Stud. Archeol.* 1, 1–15.

Kansa, S. W., Kennedy, A., Campbell, S., and Carter, E. (2009). Resource exploitation at late Neolithic Domuztepe: faunal and botanical evidence. *Curr. Anthropol.* 50, 897–914. doi: 10.1086/605910

Kashyap, A., and Weber, S. (2013). "Starch Grain Analysis and Experiments Provide Insights into Harappan Cooking Practices," in *Connections and Complexity: New Approaches to the Archaeology of South Asia*, eds S. A. Abraham, P. Gullapalli, T. P. Raczek, and U. Z. Rizvi (California: Left Coast Press), 177–194.

Kirch, P., and O'Day, S. J. (2003). New archaeological insights into food and status: a case study from pre-contact Hawaii. *World Archaeol.* 34, 484–497. doi: 10.1080/0043824021000026468

Kolattukudy, P. (1970). Plant Waxes. *Lipids* 5, 9–275. doi: 10.1007/BF02532477

Kooiman, S. M., Albert, R. K., and Malainey, M. E. (2021). Multiproxy Analysis of Adhered and Absorbed Food Residues Associated with Pottery. *J. Archaeol. Method Theory* doi: 10.1007/s10816-021-09537-3 [Epub ahead of print].

Lu, H., Yang, X., Ye, M., Liu, K. B., Xia, Z., Ren, X., et al. (2005). Culinary archaeology: millet noodles in late Neolithic China. *Nature* 437:967. doi: 10.1038/437967a

Lu, H., Zhang, J., Liu, K. B., Wu, N., Li, Y., Zhou, K., et al. (2009). Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. *Proc. Natl. Acad. Sci. U. S. A.* 106, 7367–7372. doi: 10.1073/pnas.0900158106

McClatchie, M., Schulting, R., McLaughlin, R., Colledge, S., Boggard, A., Barratt, P., et al. (2019). Food Production, Processing and Foodways in Neolithic Ireland. *Environ. Archaeol.* 27, 80–92. doi: 10.1080/14614103.2019.1615215

Miller, L. (2004). *Urban economies in urban states: the secondary products revolution in the Indus Civilisation*. Ph.D. thesis. New York: New York University.

Miller, M., Whelton, H. L., Swift, J. A., Maline, S., Hammann, S., Cramp, L. J. E., et al. (2020). Interpreting ancient food practices: stable isotope and molecular analyses of visible and absorbed residues from a year-long cooking experiment. *Sci. Rep.* 10:13704. doi: 10.1038/s41598-020-70109-8

Outram, A. K., Stear, N. A., Bendrey, R., Olsen, S., Kasparov, A., Zaibert, V., et al. (2009). The earliest horse harnessing and milking. *Science* 323, 1332–1335. doi: 10.1126/science.1168594

Patel, A. K. (2009). Occupational histories, settlements, and subsistence in western India: what bones and genes can tell us about the origins and spread of pastoralism. *Anthropozoologica* 44, 173–188. doi: 10.5252/az2009n1a8

Peres, T. M. (2017). Foodways archaeology: a decade of research from the southeastern United States. *J. Archaeol. Res.* 25, 421–460. doi: 10.1007/s10814-017-9104-4

Pokharia, A. K., Agnihotri, R., Sharma, S., Bajpai, S., Nath, J., Kumaran, R. N., et al. (2017). Altered cropping pattern and cultural continuation with declined prosperity following abrupt and extreme arid event at˜ 4,200 yrs BP: evidence from an Indus archaeological site Khirsara, Gujarat, western India. *PLoS One* 12:e0185684. doi: 10.1371/journal.pone.0185684

Pokharia, A. K., Kharakwal, J. S., Rawat, R. S., Osada, T., and Nautiyal, C. M. (2011). Archaeobotany and archaeology at Kanmer, a Harappan site in Kachchh, Gujarat: evidence for adaptation in response to climatic variability. *Curr. Sci.* 100, 1833–1846.

Rajesh, S. V., Gadekar, C., Abhayan, G. S., Ajithprasad, P., and Sharma, B. P. (2018). Janan: a Pre-Urban Harappan site on Khadir Island, Kachchh District, Gujarat. *Puratattva* 48, 173–183.

Rajesh, S. V., Krishnan, K., Ajithprasad, P., and Sonawane, V. H. (2013a). Evaluating the Anarta tradition in the light of material culture from Loteshwar and other sites in Gujarat. *Man Environ.* 38, 10–45.

Rajesh, S. V., Krishnan, K., Ajithprasad, P., and Madella, M. (2013b). Pre-Prabhas assemblage from Gujarat, western India. *Herit. J. Multidiscip. Stud. Archaeol.* 1, 181–209.

Reber, E. A., and Evershed, R. P. (2004). How did Mississippians prepare maize? The application of compound-specific carbon isotope analysis to absorbed pottery residues from several Mississippi Valley sites. *Archaeometry* 46, 19–33. doi: 10.1111/j.1475-4754.2004.00141.x

Roffet-Salque, M., Lee, M. R. F., Timpson, A., and Evershed, R. P. (2016). Impact of modern cattle feeding practices on milk fatty acid stable carbon isotope compositions emphasise the need for caution in selecting reference animal tissues and products for archaeological investigations. *Archaeol. Anthropol. Sci.* 9, 1343–1348. doi: 10.1007/s12520-016-0357-5

Roffet-Salque, M., Regert, M., Evershed, R. P., Outram, A. K., Cramp, L. J., Decavallas, O., et al. (2015). Widespread exploitation of the honeybee by early Neolithic farmers. *Nature* 527, 226–230. doi: 10.1038/nature15757

Salque, M., Radi, G., Tagliacozzo, A., Uria, B. P., Wolfram, S., Hohle, I., et al. (2012). New insights into the Early Neolithic economy and management of animals in Southern and Central Europe revealed using lipid residue analyses of pottery vessels. *Anthropozoologica* 47, 45–63. doi: 10.5252/az2012n2a4

Saul, H., Madella, M., Fischer, A., Glykou, A., Hartz, S., and Craig, O. E. (2013). Phytoliths in pottery reveal the use of spice in European prehistoric cuisine. *PLoS One* 8:e70583. doi: 10.1371/journal.pone.0070583

Spangenberg, J. E., Jacomet, S., and Schibler, J. (2006). Chemical analyses of organic residues in archaeological pottery from Arbon Bleiche 3, Switzerland – evidence for dairying in the late Neolithic. *J. Archaeol. Sci.* 33, 1–13. doi: 10.1016/j.jas.2005.05.013

Spielmann, K. A. (2002). Feasting, craft specialisation, and the ritual mode of production in small-scale societies. *Am. Anthropol.* 104, 195–207. doi: 10.1525/aa.2002.104.1.195

Staller, J., and Carrasco, M. (eds) (2009). *Pre-Columbian Foodways: Interdisciplinary Approaches to Food, Culture, and Markets in Ancient Mesoamerica*. Berlin: Springer Science & Business Media. doi: 10.1007/978-1-4419-0471-3

Steele, V., Stern, B., and Stott, A. W. (2010). Olive oil or lard? Distinguishing plant oils from animal fats in the archeological record of the eastern Mediterranean using gas chromatography/combustion/isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* 24, 3478–3484. doi: 10.1002/rcm.4790

Suryanarayan, A. (2020). *What's cooking in the Indus Civilisation? Investigating Indus food through ceramic lipid residue analysis*. Ph.D. thesis. Cambridge: University of Cambridge. doi: 10.17863/CAM.50249

Suryanarayan, A., Cubas, M., Craig, O. E., Heron, C. P., and Shinde, V. S. (2021). Organic residues in pottery from the Indus Civilisation in northwest India. *J. Archaeol. Sci.* 125:105291. doi: 10.1016/j.jas.2020.105291

Thomas, P. K., Joglekar, P. P., Deshpande, A., Pawankar, S. J., and Matsushima, Y. (1997). Subsistence based on animals in the Harappan culture of Gujarat, India. *Anthropozoologica* 2, 767–776.

Thomas, P. K., Matsushima, Y., and Deshpande, A. (1996). "Faunal remains," in *Kuntasi: A Harappan Emporium on West Coast*, eds M. K. Dhavalikar,

M. H. Raval, and Y. M. Chitalwala (Maharashtra: Deccan College), 297-330.

Torrence, R., and Barton, H. (eds) (2006). *Ancient Starch Research*. Walnut Creek: Left Coast Press.

Twiss, K. C., Bogaard, A., Charles, M., and Henecke, J. (2009). Plants and animals together: interpreting organic remains from building 52 at Çatalhöyük. *Curr. Anthropol.* 50, 885–895. doi: 10.1086/644767

VanDerwarker, A. M., and Detwiler, K. R. (2002). Gendered practice in Cherokee foodways: a spatial analysis of plant remains from the Coweeta Creek Site. *Southeast. Archaeol.* 21, 21–28.

Weber, S., and Kashyap, A. (2010). Harappan plant use revealed by starch grains from Farmana, India. *Antiquity* 84:326.

Welch, P. D., and Scarry, C. M. (1995). Status-related variation in foodways in the Moundville chiefdom. *Am. Antiq.* 60, 397–419. doi: 10.2307/282257

Whelton, H. L., Roffet-Salque, M., Kotsakis, K., Urem-Kotsou, D., and Evershed, R. P. (2018). Strong bias towards carcass product processing at Neolithic settlements in Northern Greece revealed through absorbed lipid residues of archaeological pottery. *Quat. Int.* 496, 127–139. doi: 10.1016/j.quaint.2017.12.018

Wright, R. (2010). *The Ancient Indus: Urbanism, Economy and Society*. Cambridge: Cambridge University Press.

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Brewing and Serving Alcoholic Beverages to Erlitou Elites of Prehistoric China: Residue Analysis of Ceramic Vessels

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The Bronze Age in China is characterized by the appearance of bronze ritual vessels, such as *gui* and *he* pitchers and *jue* cups, which were symbols of high social status and likely used in ritual feasting events. Their forms imitate similar ceramic vessels made of white clay. This transformation of such ceramic vessels into their bronze counterparts took place at the Erlitou site in the Yiluo basin, North China (ca. 1,800–1,500 BC). Such white pottery types are commonly regarded as alcohol-related vessels, but there is a lack of scientific analysis of organic remains on vessels' interior surfaces to understand their functions. In this study, we analyzed microfossil remains on 16 ceramic vessels unearthed from Erlitou and discovered direct evidence of the production and consumption of fermented beverages that were prepared using *qu* starter as a saccharification agent. *Dakouzun* wide-orifice vats may have been used for fermentation, likely in semi-solid-state fermentation conditions; narrow-orifice jars *zun* for storage; *gui* and *he* pitchers for heating and/or pouring the beverages; and *jue* cups for drinking. *Monascus* mold and herbs were probably used to make *qu* starter. Fermentation ingredients were primarily rice and wheat, sometimes mixed with broomcorn millet, Job's tears, roots of snake gourd, among other plants. Rice and wheat were minor crops in the region, probably cultivated for special uses and received by the Erlitou elites as tributary items for making alcoholic beverages. This research demonstrates that Erlitou feasting activities involved serving luxury drinks with prestige utensils in socially exclusive spaces, which emphasized social status, wealth, and power. The development of such drinking materiality and social values coincided with increased social differentiation at the time of early state formation.

Keywords: starch granules, phytoliths, *Monascus* mold, yeast, *qu* starter, rice, wheat, white pottery

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INTRODUCTION

China's long history of making and drinking alcohol can be traced back to the early Neolithic time (ca. 9,000–7,000 cal. BP). Residue analyses of ceramic globular jars from the Jiahu site in Henan province and the Qiaotou site in Zhejiang province indicate that such vessels once contained mixed fermented beverages of rice, honey and fruit at Jiahu (McGovern et al., 2004) and rice-based fermented beverages mixed with Job's tears and tubers at Qiaotou (Wang et al., 2021). During the middle Neolithic period (ca. 7,000–4,900 cal. BP), fermentation and drinking vessels became more diverse regionally. In the middle Yellow River region, a rather limited number of forms of *jiandiping* amphorae were used for fermentation and communal drinking in the Yangshao culture (Liu, 2021). In the lower Yellow River region, on the other hand, a variety of vessels with exquisite craftmanship were used for brewing, heating, pouring, and drinking alcoholic beverages, coinciding with the development of social differentiation in the Dawenkou culture (ca. 6,100–2,600 cal. BP) (Liu, 2017; Liu et al., 2021b). As societies became more stratified during the late Neolithic period of the Longshan culture (ca. 5,000–3,900 cal. BP), the alcohol-related vessels also became more elaborate in form, specialized in function, and often appeared in concentration at large sites or elite burials. These parallel developments suggest that consumption of alcoholic beverages was an important part of feasting ritual activities at political and economic centers (McGovern et al., 2005; Underhill, 2018; He et al., 2021).

Feasts were a significant part of various rituals in prehistory, and the artifacts associated with such activities are important material remains for studying ritual behaviors and social relations in the past (Dietler and Hayden, 2001; Hayden, 2014). The ceramic assemblages for alcohol consumption, which include serving vessels (e.g., *gui* and *he* pitchers for heating and pouring liquid) and drinking vessels (e.g., *jue* and *bei* cups), became widespread in most of the Yellow River valley from the late Neolithic to the early Bronze Age. Chinese archeologists have studied these vessels for decades, exploring their origins and development mainly by studying typological changes (Gao and Shao, 1981; Du, 1990, 1992). These studies demonstrate that these vessels were the prototypes of the first bronze ritual vessels, such as *gui, he,* and *jue*, found at the Erlitou site in Henan province, the development of which marked the beginning of the Bronze Age in north China. Thus, ceramic drinking vessels laid the foundation for the emergence and institutionalization of bronze ritual vessel types, and this transformation first took place at the Erlitou site.

Despite of the importance of these vessels, there has been little scientific analysis of their functions. It is still unclear how these hypothetically alcohol-related vessels were used and what brewing methods were practiced in the Erlitou culture. To address these issues, this paper aims to provide direct evidence of the alcohol-making technology and drinking practices at the Erlitou site. By analyzing the residues on ceramic vessels and their archeological contexts, the results will shed new light on the production and consumption of alcoholic beverages at Erlitou, and on the social function of ritual feasting in the emergence of state-level civilization.

MATERIALS AND METHODS

Archeological Background

The Erlitou site in Henan province (c. 1,900/1,800–1,500 BC) was the first Bronze Age urban center and is also regarded by many as a capital of Xia, the first dynasty in China. For over 60 years, excavations have revealed a well-designed political and economic center (300 ha in area) with a clear social hierarchy in mortuary practices and residential arrangements. Elaborate ceramic drinking vessels have also been found in elite burials and in the palatial area (Liu and Chen, 2012; **Figure 1**). These vessels include *gui* and *he* pitchers for serving and *jue* cups for drinking, and are mostly made of non-local white clays (Li et al., 2010). The vessels are white, light yellow, and light pink in color (**Figure 2**), and the rarity of the raw materials and unusual aesthetic designs may have been symbols of the high social status of the Erlitou elites (Liu, 2003). Therefore, our sampling strategy was to primarily focus on white ceramic serving and drinking vessels unearthed from the palatial area and from an elite cemetery. Since it is also important to understand what brewing methods involved in alcohol production, we focused on two vessel types, wide-orifice vats (*dakouzun*; vat hereafter) and narrow-orifice jars (*zun*; jar hereafter), that are often assumed to be used in the fermentation process (Bao and Zhou, 2007, p. 13–23).

The ceramic samples analyzed were mostly found in close proximity to the palace foundations in the eastern section of the palatial zone. Some samples come from a tomb (2015YLVM7) ranked in the first tier of the Erlitou elite burial assemblage. This tomb is about 3 m^2 in area, furnished with cinnabar on the bottom, and associated with many grave goods, including jades, turquoise beads, laquear wares, and ceramic vessels. We studied residue remains adhering to the interior surfaces of 16 pottery sherds, including one *dakouzun* vat, five *zun* jars, five *he* pitchers, three *gui* pitchers, and two *jue* cups, which are considered alcohol-related pottery vessels. We collected three control samples to compare with the residue samples. Two were sediments from the external surfaces of two *he* pitchers, which likely reflect the soil matrix around the artifacts in the archeological deposits. The third control sample was from the interior surface of a *kecaopen* grooved basin, unlikely related to an alcohol fermentation process. This vessel type has been found in many Neolithic and early Bronze Age sites, and may have been used for grinding/processing foodstuffs based on residue analysis (Sun et al., 2019; Reinhart, 2020). All these sherds come from the deposits from the Erlitou Phases II, IV and Upper Erligang phases, dating to around 1,800/1,700–1,400 BC (**Figure 2** and **Table 1**).

Methods

Four sherds (from *he* pitchers and a *jue* cup) from the elite tomb were sampled *in situ* during the excavation, while the rest of the sherds were cleaned by the excavators before our sampling. The process of collecting and analyzing the residue samples is as follows. Each pottery sherd was first cleaned with a new toothbrush to remove loose soil on the interior surface of the vessel. Residues adhering to the vessel interior surface were then extracted using a clean ultrasonic toothbrush and distilled water for 3 min. The residue liquid from each sample was kept in a test tube. We also used clean blades to scrape off visible residue sediments on the vessels' interior surfaces and stored the sediments in small plastic bags.

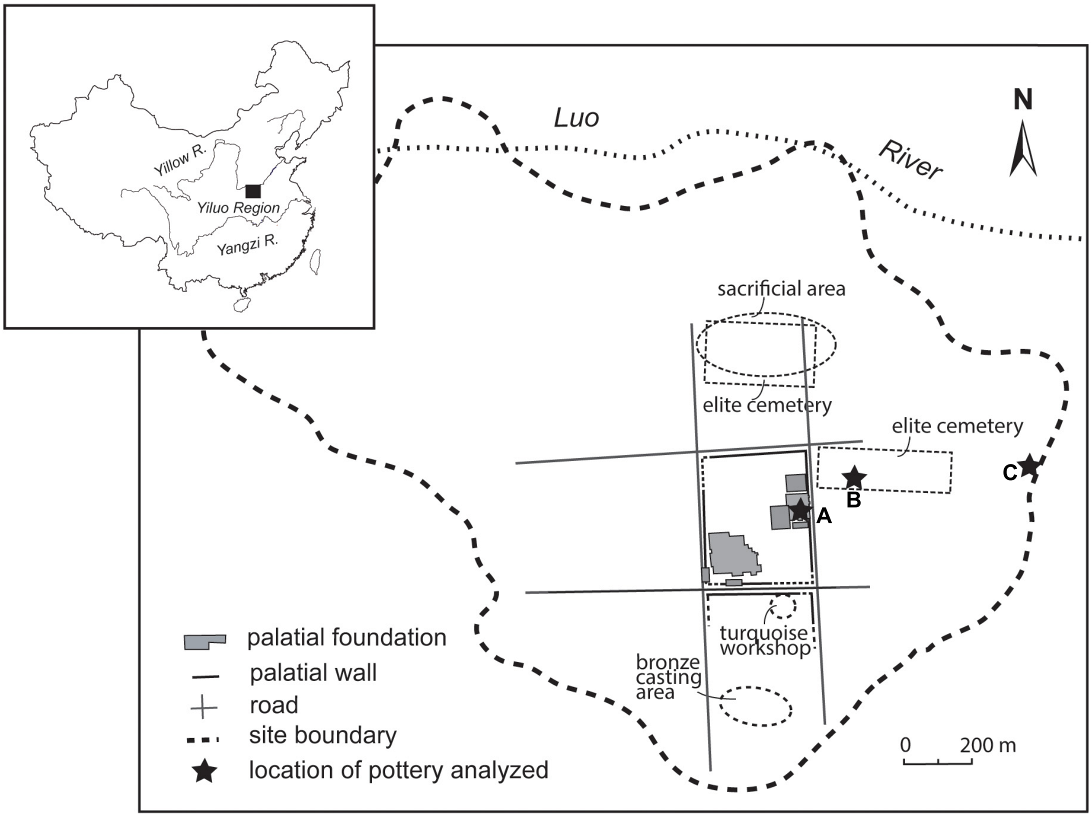

FIGURE 1 | Locations of pottery samples excavated from the Erlitou site in the Yiluo region. **(A)** General area where pottery sherds were unearthed from the eastern part of the palatial zone; **(B)** location of the elite tomb 2015YLVM7; **(C)** location of Jar4.

Residue samples, including the control samples, were processed using protocols established by the Stanford Archeology Center that involve two procedures: EDTA (ethylenediaminetetraacetic acid; 0.1%) dispersion and heavy liquid (sodium polytungstate, density 2.35) separation (Liu et al., 2019). Congo red (0.1%, 1 mg / ml) was used to dye a small part of the residue of some samples to identify the presence of gelatinized starch granules (Lamb and Loy, 2005). Extractions obtained from residue samples were mounted in 50% (vol/vol) glycerol and 50% (vol/vol) distilled water on glass slides and scanned under a Zeiss Axio Scope A1 microscope fitted with polarizing filters and differential interference contrast (DIC) optics, at 200 × and 400 × to identify starch, phytoliths, and fungi. Photographs were taken using a Zeiss Axiocam HRc3 digital camera and Zeiss Axiovision software version 4.9.1.

Identification of microfossil remains was based on modern reference collections, which contain more than 1,300 plant specimens, in the Stanford Archeology Center, Stanford University. We have conducted experimental brewing of many types of plants to generate a database for identifying fermentation-related starch morphologies, which are partially published (Wang et al., 2017). We also consulted other studies on morphological alterations of cooked starch (Henry et al., 2009). Phytolith identification was based on our reference database and published information (Piperno, 2006; Lu et al., 2009). Fungi were identified according to our microbial database and published sources (Young, 1931; Bao and Zhou, 2007; St-Germain and Summerbell, 2011).

RESULTS

Our analysis revealed abundant microfossil remains, including starch granules, phytoliths and fermentation-related fungi (molds and yeast cells).

Starch Granules

A total of 743 starch granules were found in the 16 residue samples, of which 534 (71.9%) were classified into five types that correspond to certain taxa in modern reference data (**Figure 3**). We recorded starch counts and ubiquity measures [percentage presence, calculated as (the total number of samples that one plant taxon is identified)/(the total number of residue samples)] (Pearsall, 1989, p. 212–217) to indicate relative abundances of plant taxa. Six granules (0.8%) exhibit characteristics of underground storage organs (USOs) but could not be further identified taxonomically. Two hundred and three (27.3%) starch granules were either badly damaged or lacked diagnostic characteristics and thus were classified as unidentifiable (UNID). A great majority of starches were damaged (n = 682; 91.8% of the

FIGURE 2 | Selected pottery sherds analyzed **(A–J)** and corresponding complete vessels **(J–O)**. **(A)** *Dakouzun* Urn, body; **(B)** *zun* Jar2, base; **(C)** *he* Pitcher1, spout; **(D)** *he* Pitcher4, spout; **(E)** *he* Pitcher5, foot; **(F)** *gui* Pitcher1, foot; **(G)** *gui* Pitcher3, body; **(H)** *jue* Cup1, body; **(I)** *jue* Cup2, spout; **(J)** *kecaopen* grooved basin, body; **(K)** *dakouzun* urn (2001VH272:19); **(L)** *zun* jar (2001VH12:7); **(M)** *he* pitcher (2002VM4:6); **(N)** *gui* pitcher (82YLIX M10:6); **(O)** *jue* cup (2001VM1:10); **(P)** 2002VH87:18 (circles indicate the vessel parts corresponding to the sherds analyzed).

total; 93.8% ubiquity); causes of their morphological alternation included enzyme digestion, fermentation, and cooking (steaming or boiling) (**Table 2**). The damage patterns observed included the disappearance of extinction crosses (**Figures 4E–H**); pitting, deep channels, and broken edges (**Figures 4B,D,I,J**); central depressions (**Figures 4F,G,J**); and gelatinization (**Figure 4K**). These damage patterns are consistent with those appearing on fermented cereal starch granules in our reference data (**Figures 3B,D,F–H,J,K**) and with published information (Henry et al., 2009; Wang et al., 2017).

When we used Congo red to stain the residue samples, the gelatinized starch granules were easily identified, showing red color in bright-field microscopy and red or orange-yellow gloss under polarized light (**Figure 4L**). Smoke marks were observed on the external surface of the baggy foot of the *he* pitcher (**Figure 2E**). These marks, along with the gelatinized starch granules in the pitcher samples, indicate that the liquid foods inside the vessels were heated at a high temperature.

Type I starch, identified as Panicoideae, including foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*), and probably small granules from Job's tears (*Coix lacryma-jobi* L.) (n = 69; 9.3% of the total), was found in four samples (25.0% ubiquity). Starch granules from these three species share some common characteristics, making it difficult to separate them

TABLE 1 | Ceramic samples analyzed.

Vessel #	Vessel type	Sample #	Element	Clay color	Artifact #	Chronology	Location
Vat	*Dakouzun*	POT27	Body	Gray	2003T35G14⑦	ELT-IV	Outside eastern wall of palatial area
Jar1	*Zun*	POT26	Body	White	2015T2G1①	ELT-II	Eastern part of palatial area
Jar2	*Zun*	POT28	Base	Gray	2002T12G10①	ELT-II	Eastern part of palatial area
Jar3	*Zun*	POT29	Body	Gray	2017T3525PH192③	ELT-II	Eastern part of palatial area
Jar4	*Zun*	POT40	Body	Gray	2000IIIT3H24⑧	ELT-II	Eastern periphery of the site
Jar5	*Zun*	POT25	Base	White	2015T1H6	ELT-IV	Eastern part of palatial area
He Pitcher1	*He*	POT8	Spout	White	2017T3023H205②	ELT-II	Eastern part of palatial area
He Pitcher2	*He*	POT10	Foot	White	2017T3023H205②	ELT-II	Eastern part of palatial area
He Pitcher3	*He*	POT33	Body	White	2015YLVM7	ELT-IV	Elite burial area to the east of palatial area
He Pitcher4	*He*	POT35	Spout	White	2015YLVM7	ELT-IV	Elite burial area to the east of palatial area
He Pitcher5	*He*	POT36	Foot	Gray	2015YLVM7	ELT-IV	Elite burial area to the east of palatial area
Gui Pitcher1	*Gui*	POT12	Foot	White	2015T1G2	ELT-II	Eastern part of palatial area
Gui Pitcher2	*Gui*	POT6	Body	White	2017T3325H170 top road	ELT-II	Eastern part of palatial area
Gui Pitcher3	*Gui*	POT7	Base	White-brown	2017T3131H159	Upper ELG	Eastern part of palatial area
Jue Cup1	*Jue*	POT1	Body	White	2017T3325H170 topsoil	ELT-II	Eastern part of palatial area
Jue Cup2	*Jue*	POT34	Spout	White	2015YLVM7	ELT-IV	Elite burial area to the east of palatial area
Grooved basin	*Kecaopen*	POT32	Body	Gray	2000IIIT2H13④	ELT-IV	Eastern periphery of the site

precisely (Liu et al., 2014). The size range of Type I granules was 7.23–19.84 μm, and the shapes were polygonal or near circular. The hila were centric, and fissures were often observed. The damaged granules showed diagnostic features consistent with fermentation in our modern reference data (**Figures 4A,B** compared with **Figures 3A,B**).

Type II starch, identified as Job's tears (*C. lacryma-jobi* L.) (n = 9; 1.2% of the total), was found in only one sample (Jar2; 6.2% ubiquity). These granules were polygonal in shape, and their size range was 14.22–23.31 μm. Their morphology differed from that of millets, but had certain characteristics consistent with Job's tears, such as larger sizes, eccentric hila, and the extinction crosses with zig-zag arms (Liu et al., 2014; **Figures 4C,D** compared with **Figures 3C,D**).

Type III starch, identified as Triticeae (n = 129; 17.4% of the total), appeared in eight samples (50.0% ubiquity). The granules' size range was 4.56–55.46 μm, shape was lenticular, and hila were centric. Type III starch granules were consistent with wheat or barley (**Figures 4E,F** compared with **Figures 3E,F**). However, due to damages caused by fermentation and gelatinization, some granules were larger than granules of native Triticeae starches from our modern reference data (size ranges: 6.31–35.49 μm for barley; 5.84–36.41 μm for wheat).

Type IV starch, identified as rice (*Oryza* sp.) and appearing in a compound form consisting of multiple small granules (2.28–9.67 μm), was found in 14 samples (87.5% ubiquity). We counted visible individual granules in order to calculate the percentage of Type IV starch present (n = 321 granules, 55 compounds; 43.2% of the total). Most individual granules were blurry without clear morphology. When the starch granules' morphology was discernible, they were polygonal in shape. The birefringence of the granules was weak, without extinction crosses. These morphological features were consistent with fermented rice starch from our modern reference data (**Figures 4G,H** compared with **Figures 3G,H**). Rice starch granules are very small, and most of them lose their original integrity after fermentation, making it difficult to identify them. Even though some rice compounds are preserved, few granules exhibit a complete shape. Since our rice starch counts were based on the number of measurable granules, the recorded number of rice starch granules is likely to be far lower than the actual number. Despite these problems in preservation, rice starch had the most numerous counts and the highest ubiquity in the assemblage.

Type V starch, identified as the root of snake gourd *Trichosanthes kirilowii* of the Cucurbitaceae family (n = 6; 0.8% of the total), was found in two samples (ubiquity 12.5%). The size range was 9.95–22.33 μm. The granules' forms included round, bell-shaped, and semi-circular. Their hila were centric or eccentric, and extinction crosses often had curved arms (**Figures 4I,J** comparable with **Figure 3I**). This plant is distributed widely in China (Wu et al., 2011b), and its root was traditionally used as famine food (Zhu, 1406) and as an ingredient for making medicinal alcohol (Li, 1981).

Only one starch granule was found in the two control samples from exteriors of *he* pitchers, confirming that the starches in the residues found on the interior walls of the pottery were not contaminated by the surrounding soil. In the control sample from the grooved basin, 21 starch granules were found, with a great majority (n = 20) showing characteristics of gelatinization caused by cooking (steaming or boiling) and unidentifiable taxonomically (**Table 2**). This basin may have been used for processing various foodstuffs, including cooked ones, thus serving as a non-alcohol related food vessel.

Phytoliths

A total of 754 phytoliths were recorded (**Table 3**). The morphotypes found were mainly associated with Poaceae. The most abundant morphotype, elongated psilate/sinuate in silica skeletons (**Figure 5M**) and in single cells from leaves and stems (n = 296; 39.7% of the total), was found in 15 samples (93.8%

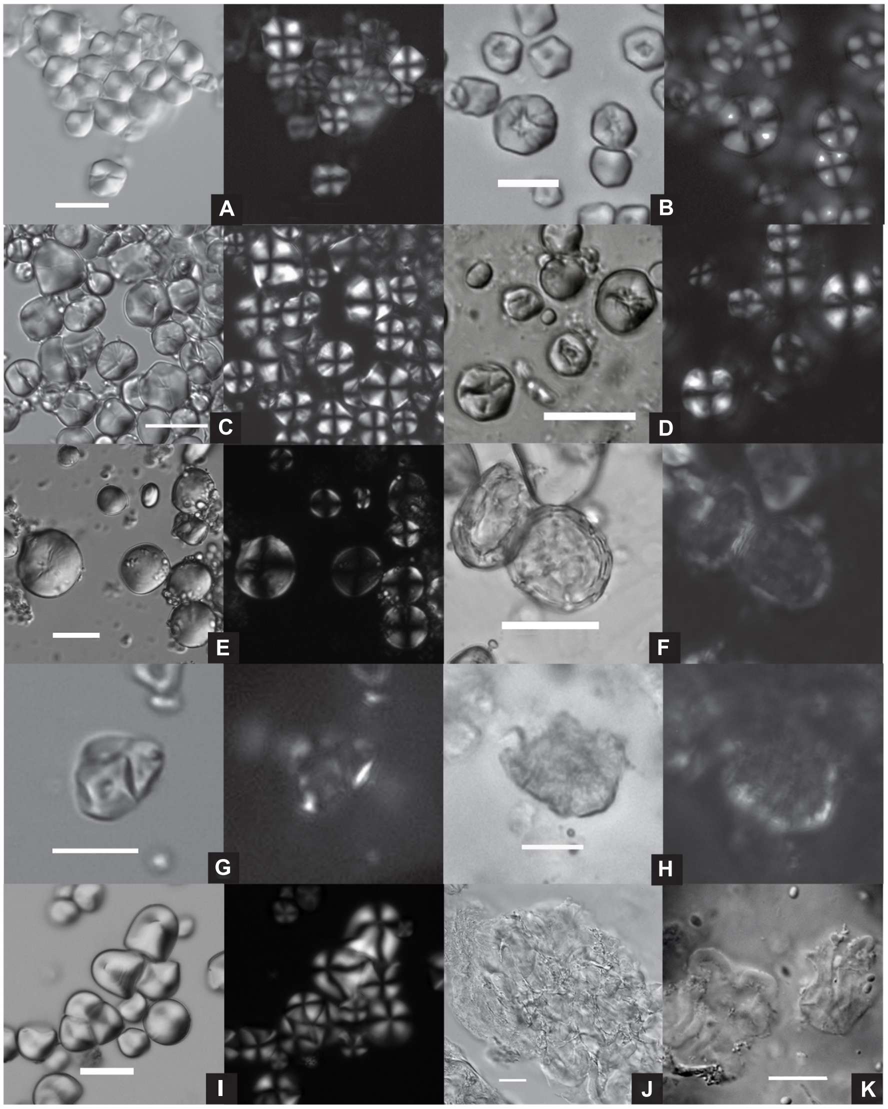

FIGURE 3 | Modern starch reference examples related to this paper (each starch image is shown in DIC and polarized views). **(A)** Broomcorn millet *Panicum miliaceum*; **(B)** mashed broomcorn millet with deep fissures and concave center in bright field; **(C)** job's tears *Coix lacryma-jobi*; **(D)** mashed Job's tears with deep fissures and concave center in bright field; **(E)** barley *Hordeum vulgare*; **(F)** barley fermented for 40 days with concave center, deep fissures, and pits in bright field, and disappeared birefringence in polarized light; **(G)** rice *Oryza sativa*, fermented for 18 days, concave center in bright field and blurry birefringence in polarized light; **(H)** rice, fermented for 1 year, blurry granule edge in bright field, birefringence only shown at the edge in polarized light; **(I)** snake gourd root *Trichosanthes kirilowii*; **(J)** gelatinized barley, boiled for 3 min; **(K)** gelatinized starch granules from the modern-day millet beer, *hunjiu*, produced in Yulin, Shaanxi. (Scales, **A,B,G,H**: 10 μm; rest: 20 μm).

ubiquity). The second most numerous morphotype, double-peaked phytoliths from rice husks (n = 125; 16.7% of the total, **Figure 5A**), was found in seven samples (43.8% ubiquity). We also identified η-type phytoliths from broomcorn millet husk (n = 2; **Figure 5N**); a Paniceae husk type (n = 1); bilobates (n = 88; **Figure 5E**), polylobates (n = 2), and crosses (n = 30) likely from Panicoideae; and, in four samples, elongated dendriform phytoliths (n = 7; **Figure 5I**) that may have come from Triticeae (possibly wheat or barley) husks. Other morphotypes found include rondel (n = 16; **Figure 5F**), common bulliform (n = 30; **Figure 5G**), rice type bulliform (n = 4; **Figure 5H**), scooped parallel bilobate (n = 15; **Figure 5D**), and hair cell (n = 22; **Figures 5J,K**).

It has been reported that the glumes and leaves of Job's tears produce great varieties of bilobate, polylobate, cross, and rondel phytoliths, with particularly abundant Variant-1 crosses (quadrilobate forms) in medium (11.5–15.97 μm), large (15.98–20.60 μm), and extra-large (≥20.61 μm) widths. In contrast, broomcorn millet and foxtail millet produce smaller Variant-1 crosses (<13 μm) (Duncan et al., 2019). In our samples, several crosses were classified as large-size and medium-size Variant-1 type; one was found in Jar2 (**Figure 5B**), in which

TABLE 2 | Erlitou starch record.

	Type I	Type II	Type III	Type IV		Type V				Damaged starch			
	Panicoideae	**Job's tears**	**Triticeae**	**Rice grain**	**Rice compound**	**Snake gourd**	**USOs**	**UNID**	**Total**	**Enzyme**	**Ferment**	**Cooked**	**Total**
Vat				9	3			25	34	1	18	15	34
Jar1			28	15	3			7	50	3	17	6	26
Jar2	43	9	28	75	15	5	2	25	187	76	79	6	161
Jar3				23	4		1	11	35		27	8	35
Jar4				18	4				18		18		18
Jar5				19	4			2	21		20	1	21
He Pitcher1			2	3	1	1	1	11	18	4	7	3	14
He Pitcher2				22	3			7	29	1	22	6	29
He Pitcher3	4		19	6	1			39	68	40	26	2	68
He Pitcher4			1	19	4			7	27	2	24	1	27
He Pitcher5	21		46	4	1			43	114	69	43	2	114
Gui Pitcher1				44	6			8	52		49	2	51
Gui Pitcher2	1							1	2				
Gui Pitcher3			2				1	2	5	1	1		2
Jue Cup1				48	5			4	52	1	48	3	52
Jue Cup2			3	16	1		1	11	31	9	21		30
Total n	69	9	129	321	55	6	6	203	743	207	420	55	682
Total %	9.3	1.2	17.4	43.2		0.8	0.8	27.3	100.0	27.9	56.5	7.4	91.8
Ubiquity n	4	1	8	14	14	2	5	15	16	11	15	12	15
Ubiquity %	25.0	6.2	50.0	87.5	87.5	12.5	31.3	93.8	100	68.8	93.8	75.0	93.8
MIN (m)	7.23	14.22	4.56	2.28		9.95	8.65	4.02					
MAX (m)	19.84	23.31	55.46	9.67		22.33	33.32	45.42					
Average (m)	12.54	19.53	21.43	5.14		16.13	15.77	17.91					
Control samples													
Kecaopen basin				8	1		1	12	21			20	20
He pitcher4-Ext									0				
He pitcher5-Ext								1	1				

we also identified Job's tears starch granules. These correlated findings strongly support the existence of Job's tears in this sample (**Tables 2**, **3**). In addition, husk phytoliths from broomcorn millet, rice and Triticeae were identified in several samples, as were their corresponding starches, providing mutual confirmation of the existence of these cereals. Hair cells were primarily found in Jar2, as were snake gourd root starch granules. Since hair cells are often from eudicots, including Cucurbitaceae, those identified in our sample may be correlated with the snake gourd root starch granules identified (**Tables 2**, **3**). However, the origins of hair cells remain uncertain as they have not yet been studied. One source may be snake gourd root, but more research is needed.

Opaque perforated platelet phytoliths (n = 42; **Figure 5L**) were discovered in eight samples (50.0% ubiquity), the majority of which were from Jar1 and Jar3. This type of phytolith exhibits regular or irregular perforations, and commonly exists in the inflorescences of daisies (Asteraceae) (Piperno, 2006:196). Asteraceae is a large family, with 18 genera and 1,145 species native to China (Wu et al., 2011a); therefore, we were unable to further identify the taxonomical origins of these phytoliths.

Abundant micro-charcoal fragments and blackened phytoliths were found in the residues from the body and foot of two *he* pitchers (3, 5) (**Figure 5M**), indicating that the vessels were in contact with fire. These findings are consistent with the presence of smoke marks on the outer wall of the foot of *he* Pitcher3 and with the large number of gelatinized starch granules in its residue sample, supporting the previous speculation that *he* pitchers were used to heat alcoholic beverages.

In the two control samples from the *he* pitchers, only small numbers of phytoliths from grass stems and leaves were found (n = 8, 28), and no phytoliths of millet husk and rice husk were present. The contrast between the phytolith findings in these two control samples and in the residue samples rules out the possibility of soil contamination. In the sample from the grooved basin, 60 phytoliths were recorded, including husks from broomcorn millet, rice, and Triticeae, along with other grass phytoliths (**Table 3**). These may be related to the various foodstuffs processed in this basin.

Fungi

Many fungal particles (n = 203), including molds (n = 177) and yeast cells (n = 26), were found in 15 samples (**Table 4**). The most numerous elements found were cleistothecia, which are consistent with genus *Monascus* molds (n = 102; 50.2% of the total; 86.7% ubiquity). Hyphae and mycelia (n = 52; 25.6% of the

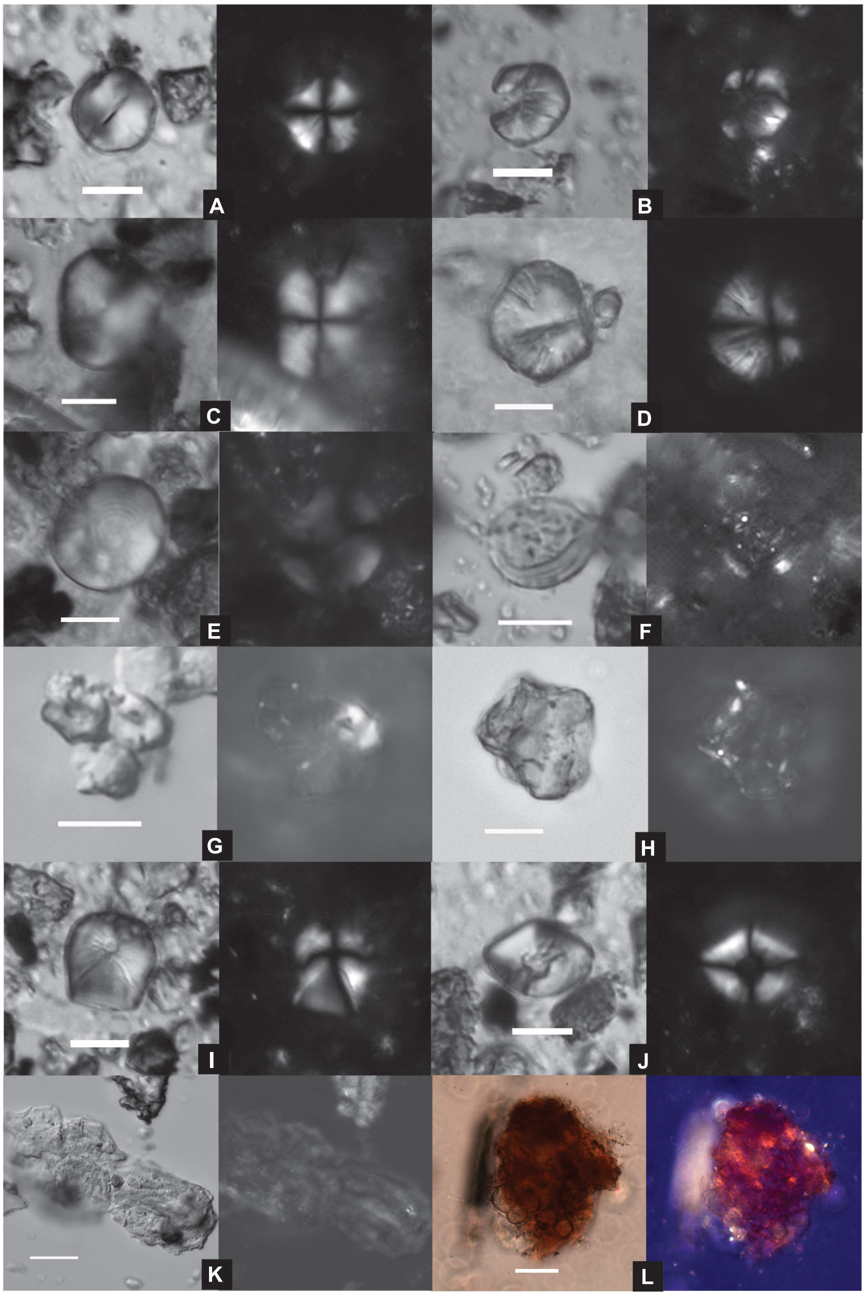

FIGURE 4 | Ancient starch granules from Erlitou pottery vessels (each starch image is shown in DIC and polarized views). **(A)** Paniceae; **(B)** damaged Paniceae with edge partially missing and deep fissures; **(C)** job's tears; **(D)** damaged Job's tears with pitting and deep fissures in bright field; **(E)** damaged Triticeae, blurry birefringence in polarized light; **(F)** damaged Triticeae with pitting, deep fissures, and concave center in bright field, birefringence only shown at the edge in polarized light; **(G)** damaged rice cluster, concave center in bright field and disappeared birefringence in polarized light; **(H)** damaged rice cluster, partially gelatinized, some showing complete granule shapes; **(I)** damaged snake gourd root with pitting; **(J)** damaged snake gourd root with disappeared center and deep fissures; **(K)** unidentified gelatinized starch; **(L)** starch cluster, dyed with Congo red (Scales, **A–J**: 10 μm; **K,L**: 20 μm).

total) were found in 10 vessels (66.7% ubiquity); they were septate or aseptate, white or brown, and many were also consistent with *Monascus* molds (**Figure 6**).

Monascus is characterized by a cleistothecium, which consists of curved ascogenous hyphae enclosing asci that contain ascospores. The cleistothecium is spherical or oval (25–75 μm) in shape, and normally orange or red in color. Hyphae are branched and septate and ascospores are spherical (ca. 5 μm in diameter) or ovoid (6 × 5 μm). The mycelium is white during the early stages of development, but rapidly changes to orange and later to red or crimson (Young, 1931; Manan et al., 2017). The genus *Monascus* is divided into more than a dozen species, which are mainly found

TABLE 3 | Erlitou phytolith record.

Phytolith morphotype	Taxonomic attribution (likely)	Residue samples																				Control samples		
		Vat	Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	*He* Pit1	*He* Pit 2	*He* Pit 3	*He* Pit 4	*He* Pit 5	*Gui* Pit 1	*Gui* Pit 2	*Gui* Pit 3	*Jue* Cup1	*Jue* Cup2	Total n	Total %	Ubiq. n	Ubiq. %	*Kecao-pen* basin	*He* Pit4–Ext	*He* Pit5–Ext
Silica skeletons																								
η-type	*P. miliacaeum* husk		1														1	2	0.3	2	12.5	2		
Undetermined Paniceae	Paniceae husk								1									1	0.1	1	6.3			
Elongate dendriform	Pooideae husk		3			1	1	1										6	0.8	4	25.0	2		
Elongate echinate	Grass husk						2	3										5	0.7	2	12.5	2		
Elongate crenate	Grass husk	1					1	2	4									8	1.1	4	25.0			
Elongate columellate	Grass husk										1					1	2	4	0.5	3	18.8	1		
Scooped bilobate parallel	Oryzoideae		1				1	3					1				3	9	1.2	5	31.3	6		5
Elongate psilate/sinuate	Grass leaf/ culm	4	44	1	2	11	13	2	59	19	29	29	2			1	25	241	32.3	14	87.5	5		
Opaque perforated platelets	Asteraceae	1	18	4	12				1		2	2					2	42	5.6	8	50.0			
Stoma sheet						1			1		1						3	6	0.8	4	25.0			
Jigsaw									5				1					6	0.8	2	12.5			
Undetermined multi-cell			1			3		6	1	1	3	3	2				6	26	3.5	9	56.3	2		
Single-cell phytolith																								
Double-peak	*Oryza* husk		2			9		1		6	49	3					55	125	16.7	7	43.8	3		
Oryza type bulliform	*Oryza* leaf					2						1			1			4	0.5	3	18.8			
Scooped bilobate	Oryzoideae			1				6					5				3	15	2.0	4	25.0	7		
Bilobate	Poaceae	7	17			25	1	11	15	6	1	2	2			1		88	11.8	11	68.8			6
Polylobate	Poaceae											1						1	0.1	1	6.3			
Cross/quadra-lobate	Poaceae		7	2		7	1	2	4	1		1	3	2				30	4.0	10	62.5			
Rondel	Poaceae		1			5		6				2	2					16	2.1	5	31.3	3		
Common bulliform	Poaceae					8				1	4	4	2				11	30	4.0	6	37.5	6	5	2
Elongate dendriform	Pooideae husk						1											1	0.1	1	6.3	1		

(Continued)

TABLE 3 | (Continued)

Phytolith morphotype	Taxonomic attribution (likely)	Residue samples																				Control samples		
		Vat	Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	*He* Pit1	*He* Pit 2	*He* Pit 3	*He* Pit 4	*He* Pit 5	*Gui* Pit 1	*Gui* Pit 2	*Gui* Pit 3	*Jue* Cup1	*Jue* Cup2	Total n	Total %	Ubiq. n	Ubiq. %	*Kecao-pen* basin	*He* Pit4–Ext	*He* Pit5–Ext
Elongate psilate/sinuate	Grass leaf/culm		3	2		1	3	5	4	7	12	6	3	2		1	6	55	7.4	13	81.3	19	3	15
Trichome			3														1	4	0.5	2	12.5			
Hair cell	Eudicots		2	12		1		1		1	1	1	1				2	22	2.9	9	56.3	1		
Total		16	103	22	16	74	24	51	95	42	103	55	24	4	1	4	120	754	100.0	16	100.0	60	8	28

in oriental food. This fungus produces a bright red pigment, and has been used as a fermentation starter, a food pigment, and in traditional Chinese medicine. *Monascus purpureus* is known to have been used to make the red rice *qu* starter for brewing traditional rice-based red beer (Huang, 2000, p. 192–203; Bao and Zhou, 2007, p. 166–169). The morphology of the cleistothecia found in the Erlitou vessels match very well that of *Monascus* sp. in our reference sample (**Figures 6A–D,I,M** compared with **Figures 6E–H,J,N**) and in published information (Bao and Zhou, 2007, p. 166–169).

Yeast cells ($n = 26$) were found in six vessels. They were round or oval and measured 4.66–9.83 μm in size with an average of 6.76 μm. Some cells in the vat were clustered, and some appeared to be in the process of budding, characterized by one or more small protuberances on the parent cell, or by a smaller cell attached to a bigger parent cell. Their morphology is similar to that of *Saccharomyces cerevisiae*, which is the most commonly used yeast for alcohol fermentation (Boulton and Quain, 2001). However, we were unable to identify their taxonomy based on morphology alone in this study (**Figure 6K** compared with **Figure 6L**).

The presence of *Monascus* molds and yeast cells provide strong evidence that these vessels were used for alcohol fermentation and drinking. *Monascus* appears to have been the mold type primarily used as the fermentation agent. Jar5 had no fungal remains, suggesting that its function was different from that of other vessels.

Only two fungal elements were recovered from the three control samples. This observation is in clear contrast to the residues in the alcohol-related vessels, indicating that the abundant fungal remains in the residues were associated with alcohol production and consumption as their original function.

DISCUSSION

Based on the above analyses of various microbotanical and microbial remains in the residues, we observed the following phenomena.

(1) Fermentation method: The two most common methods of brewing cereal-based alcoholic beverages in China include the use of malts and the use of *qu* starter as saccharification agents (Ling, 1958). According to previous studies, if malts were used there may be husks left in the fermentation liquid, so there may be many husk phytoliths in the residues of fermentation vessels. If *qu* starter was used, there may be fermentation-related molds in the residues (Wang et al., 2016; Liu et al., 2019; Liu, 2021). *Monascus* has traditionally been used for brewing rice-based fermented beverages in China (Bao and Zhou, 2007, p.166–169). We found mold components in 15 out of 16 vessels, among which cleistothecia and hyphae of *Monascus* were the most common elements. The co-existence of rice starches and phytoliths with *Monascus* components in our samples suggests that the brewing method at Erlitou likely used *qu* starter with *Monascus* molds and rice as the fermentation agent.

The fermented beverages made by *Monascus* molds and rice are red, and are called *hongqujiu*, red mold beer. In modern times, red beer is mainly produced in Zhejiang and Fujian provinces in

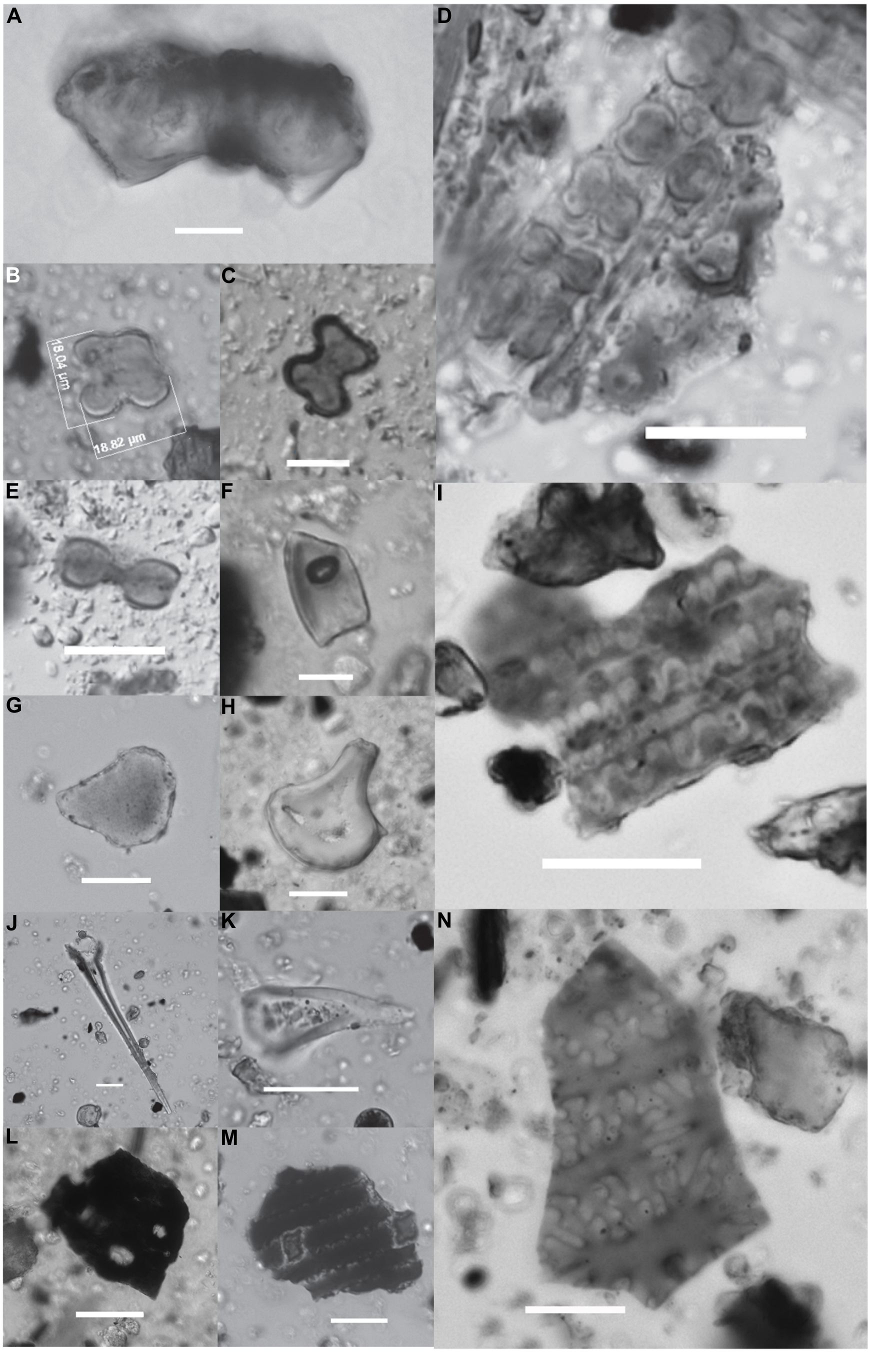

FIGURE 5 | Phytolith remains from Erlitou pottery vessels. **(A)** Rice double-peak husk; **(B)** Job's tears cross; **(C)** cross; **(D)** Oryzoideae scooped parallel bilobate; **(E)** bilobate; **(F)** rondel; **(G)** common bulliform; **(H)** rice bulliform; **(I)** dendriform husk, possibly Triticeae; **(J,K)** hair cell; **(L)** opaque perforated platelets; **(M)** burnt Elongate sinuate skeleton; **(N)** broomcorn millet η-type husk. (Scales, **C,F**: 10 μm; L: 50 μm; rest: 20 μm).

southern China. It is generally believed that this brewing method originated in the lower Yangtze River region and later dispersed to other regions (Bao and Zhou, 2007, p. 104–110). According to the published archeological data currently available, the history of using *Monascus* molds as starter for brewing beer in the Yellow River Basin can be traced back at least to the middle phase of the Yangshao culture, such as the Xipo site in Lingbao and the Dingcun site in Mianchi, all in Henan Province, dating to ca. 6,000–5,100 years ago (Feng et al., 2021; Liu et al., 2021a). Thus, the alcohol-making method at Erlitou apparently inherited the fermentation tradition that had already existed for thousands of years in the Central Plains.

TABLE 4 | Erlitou fungi record.

	Vat	Jar 1	Jar 2	Jar 3	Jar 4	*He* Pit1	*He* Pit2	*He* Pit3	*He* Pit4	*He* Pit5	*Gui* Pit1	*Gui* Pit2	*Gui* Pit3	*Jue* Cup1	*Jue* Cup2	Total n	Total %	Ubiq. n	Ubiq. %	*Kecao-pen* basin	*He* Pit4-Ext	*He* Pit5-Ext
Hypha			18	1	2			1		4	4	1	1	5	4	41	20.2	10	66.7	1		
Mycelium	4			2										5		11	5.4	3	20.0			
Cleistothecium	15	15	25	2	19	1	1		1		5	3	1	13	1	102	50.2	13	86.7			
Sporangia (UNID)			4								1	1			1	7	3.4	4	26.7			
Sporangia with hypha (UNID)			7		3		2		1					3		16	7.9	5	33.3		1	
Mold total	19	15	54	5	24	1	3	1	2	4	10	5	2	26	6	177	87.2	15	100.0		1	
Yeast	12		1		4			1	2	6						26	12.8	6	40.0			
Fungi total	31	15	55	5	28	1	3	2	4	10	10	5	2	26	6	203	100.0	15	100.0	1	1	0

It is also interesting to note that some wide-orifice vats and narrow-orifice jars both were likely related to the brewing process, given that residues from such vessels tested in this study contained abundant *Monascus* mold components and yeast cells. These vessels are most likely to have facilitated the semi-solid-state fermentation (semi-SSF) process, during which vats were used for primary fermentation, and jars for secondary fermentation and storage. This brewing technique is still practiced in southern China today (Bao and Zhou, 2007, p. 155–260).

The semi-SSF method is different from the earlier liquid-state fermentation technique, which used globular jars and *jiandiping* amphorae as fermenters during the early and middle Neolithic periods (Liu et al., 2019; Liu, 2021). The Erlitou brewing method appears to be consistent with the brewing tradition that uses *Dakougang* wide-orifice vats as the fermenter for making *hongqujiu* red beer, which may have originated in the Lower Yangzi River valley and spread to northern China. This hypothesis is based on the evidence that *Monascus* molds and rice starches have been found in the residues from wide-orifice vats at Xipo in Henan (Feng et al., 2021) and a Dawenkou culture site at Yuchisi in Anhui (Liu et al., under review[1]).

(2) Rice for beer brewing: Rice starch and rice husk phytoliths were found in 14 vessels, accounting for 87.5% in ubiquity, indicating that rice was the basic raw material for alcohol making. The microfossil analysis of the floors from a large public building and associated ceramic vessels of the Yangshao culture at the Huizui site, about 15 km east of Erlitou, showed that rice was one of the ingredients for alcohol making (Liu et al., 2018). Therefore, using rice to make alcohol may have been a local tradition.

In the Yiluo region, where Erlitou is located, rice has never been a major crop. Prior studies have identified limited remains of carbonized rice grains in only a few locations at late Yangshao, late Longshan, and Erlitou culture sites. In the flotation samples from 15 sites contemporary with Erlitou in the Yiluo region, only 19 rice grains have been recovered from five sites (Lee et al., 2019). In contrast, flotation samples from the Erlitou site have revealed abundant rice, totaling 5,687 grains, and accounting for 23.9% of the total unearthed plant seeds and 30.8% of the total unearthed crop seeds, second only to millet (11,059 grains) (Institute of Archaeology CASS, 2014). Such a clear discrepancy between Erlitou and other sites in the quantity of rice remains raises a question about the sources of rice. It is unlikely that the large amount of rice unearthed in the central area of Erlitou was produced by Erlitou urban residents themselves; thus, rice may have been a tributary item obtained from small and medium-sized settlements that mainly engaged in agricultural production in the Yiluo region and beyond.

(3) Wheat for beer brewing: Triticeae starches and/or dendriform phytoliths (from husk of Pooideae, possibly Triticeae) were found in ten vessels, accounting for 62.5% in ubiquity. They may come from wild Triticeae grasses or domesticated wheat/barley. Wild Triticeae seeds or domesticated

[1]Liu, L., Wang, J., Chen, X., and Liang, Z. (under review). The quest for red rice beer: transregional interactions and development of competitive feasting in Neolithic China. *Archaeol. Anthropol. Sci.*

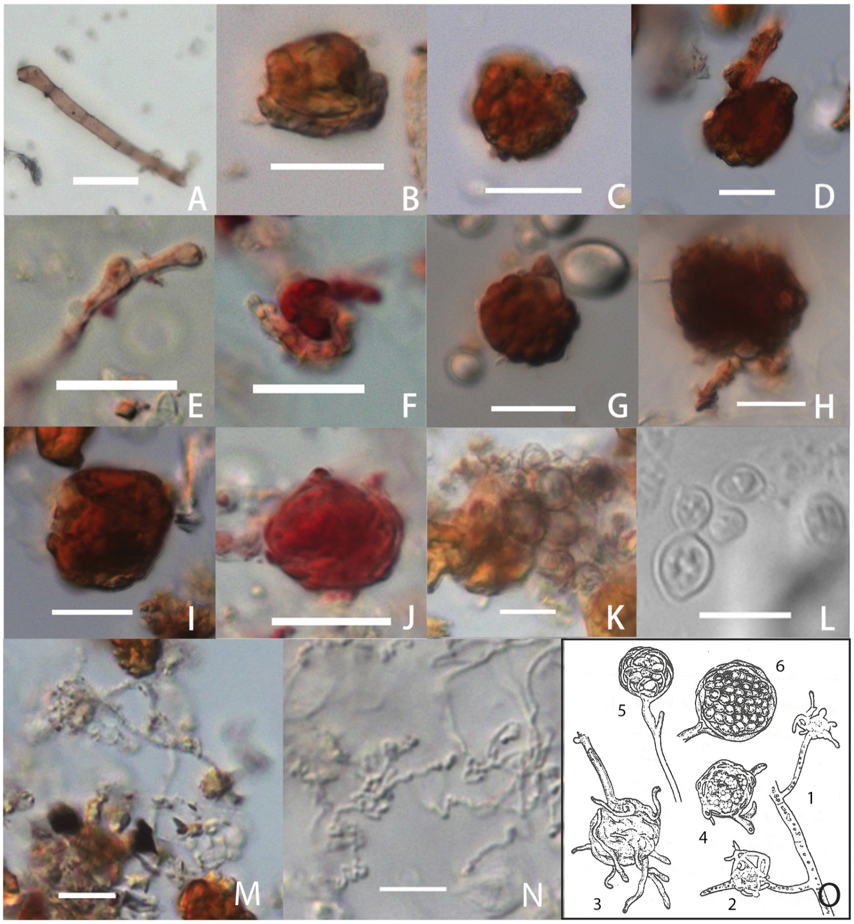

FIGURE 6 | Fungi from Erlitou pottery vessels **(A–D,I,K,M)** compared to modern reference **(E–H,J,L,N,O)**. *Erlitou fungi:* **(A)** Septate hyphae, with protruding part on the left, an indication of reproduction; **(B)** *Monascus* cleistothecium; **(C)** *Monascus* cleistothecium with ascospores; **(D)** *Monascus* cleistothecium connected to hyphae; **(I)** *Monascus* cleistothecium with clear peridial wall cell, asci, and ascospores; **(K)** clustered yeast cells; **(M)** mycelia. *Modern fungi:* **(E)** Hyphae, with protruding part on the right, an indication of reproduction; **(F)** hyphae surrounding the *Monascus* ascogonium, in the formation process of cleistothecium; **(G)** *Monascus* cleistothecium with ascospores; **(H)** *Monascus* cleistothecium connected to hyphae; **(J)** *Monascus* cleistothecium with clear peridial wall, asci, and ascospores; **(L)** *S. cerevisiae* yeast cells; **(N)** *Monascus* mycelia; **(O)** *Monascus* in various development states (1–3: Ascogenous cleistothecia; 4.5: Immature cleistothecia; 6: Mature cleistothecium; after Bao and Zhou, 2007; **Figures 4–6**) (Scales, **K–N**: 10 μm; rest: 20 μm).

barley were not found in flotation samples from sites dating to Erlitou period in the Yiluo basin (Institute of Archaeology CASS, 2014; Lee et al., 2019), so it is reasonable to rule out these two taxa as possible origins of these microfossil remains. Wheat was introduced to the Central Plains from West Asia through Central Asia in the late Neolithic times, around the third millennium BC (Liu et al., 2017). In the Yiluo region, carbonized wheat seeds dating to the Erlitou period have been uncovered from flotation samples, but in very small quantity. Only three wheat grains were found in Phase IV deposits at the Erlitou site, while four wheat grains were unearthed from two other Erlitou culture sites, suggesting that wheat may have been cultivated locally in limited quantities (Institute of Archaeology CASS, 2014; Lee et al., 2019). Therefore, it is likely that the Triticeae starches and dendriform phytoliths in the vessels were from wheat, which may have been produced locally in the Yiluo region and/or was obtained from places farther away. We speculate that in this early stage of the introduction of wheat to the Yiluo region, its function may have been special, perhaps primarily related to alcohol making.

(4) Millet and Job's tears for beer brewing: Residues containing millet starches and husk phytoliths appeared in six vessels, with two husk phytoliths identifiable as broomcorn millet, together accounting for only 37.5% in ubiquity. In contrast, the abundance of millets found in flotation samples from the Erlitou site show that millets were the main crops, of which foxtail millet accounts for 60.0% of unearthed crop seeds, and broomcorn millet accounts for 8.3%. According to previous studies, the raw materials for alcohol-brewing in the Yellow River region during the Neolithic period were primarily broomcorn millet, rice and Triticeae (Liu et al., 2019; He et al., 2021; Liu, 2021). Therefore, we speculate that broomcorn millet may have also been one of the main ingredients in alcohol-making at Erlitou, although it was used less frequently than were rice and wheat.

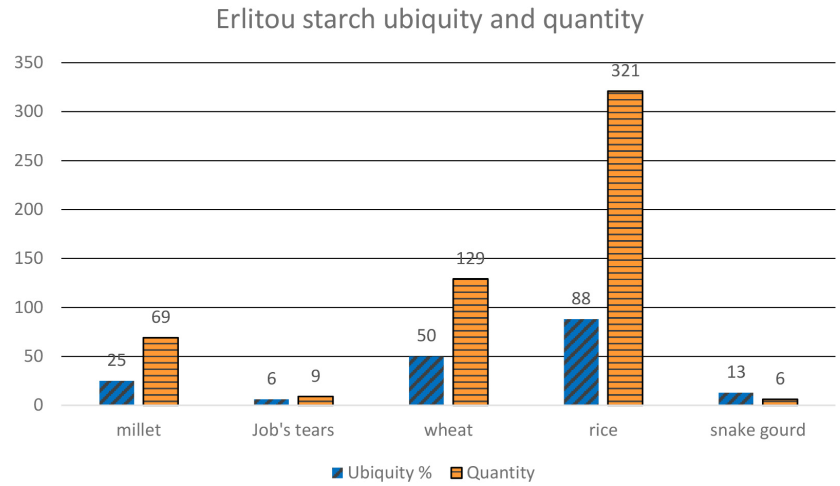

FIGURE 7 | Comparison of starch ubiquity (%) and quantity among alcohol-brewing ingredients at Erlitou.

(5) Job's tears and snake gourd roots for beer brewing: The use of Job's tears, snake gourd root, and other tubers for alcohol production can be traced back to the early and middle Neolithic periods (Liu et al., 2019; Liu, 2021). We found very low quantities of these in this study: Job's tears starches and phytoliths (large Variant 1 type) were found in only one vessel, and snake gourd root starches in two vessels. These observations suggest that these traditional alcohol-making ingredients were still in use, but infrequently, at Erlitou.

(6) The relationship between starch and phytolith remains: In several cases, starch granules and phytoliths from rice, wheat, millet, and Job's tears coexisted in the same vessels, increasing the reliability of taxonomical identification. However, there were far greater numbers of starch granules identifiable as rice, wheat, millet, and Job's tears (n = 321, 129, 25, 9, respectively) than there were husk phytoliths from the corresponding plants (n = 125, 7, 2, 1, respectively). This observation suggests that rice, wheat, millet, and Job's tears were probably hulled before entering these vessels, and that the existence of small amounts of husk phytolith is likely the result of insufficient hulling, rather than a result of using germinated cereals as saccharifying agents. This, along with the abundant *Monascus* mold elements found in the residues, indicate that *qu* starter was used for alcohol fermentation.

(7) Use of herbal *qu* starter: Most vessels revealed many phytoliths from stems and leaves (e.g., elongated psilate/sinuate morphotype), which cannot be used for taxonomical identification. Some of them may come from herbs related to making *qu*, commonly referred to as *caoqu*, or herbal *qu* starter. Molds and yeasts naturally attach to the stems and leaves of some plants, and the traditions of making *qu* by adding stems, leaves, and seeds of various wild plants can be found today in the Yangzi River valley and among the indigenous peoples in Taiwan (Ling, 1958; Yu, 2003). At present, we cannot further identify these phytoliths in our samples.

(8) Alcohol-brewing ingredients and luxury foods: When further comparing the plant types in different vessels, we can see that Jar2 has the most diverse ingredients, including rice, millet, Job's tears, Triticeae and snake gourd root, while other vessels revealed mainly rice, Triticeae and millet. The starch types with highest counts and ubiquity were from rice (321, 87.5%) and from Triticeae (129, 50.0%), suggesting that rice and Triticeae (likely wheat, as discussed above) were the most common basic raw materials, while other plants were used much less frequently (**Figure 7**). In the Yiluo region, which is part of the Loess Plateau suitable for dryland farming, millets were the major cultivars in subsistence economies, while rice and wheat were minor crops (Lee et al., 2019). It is possible that rice and wheat were produced mainly for alcohol production, given that rice has been essential for making *qu* starter with *Monascus* mold and also used as the main ingredient for brewing red rice beer (Bao and Zhou, 2007), and wheat has also been a traditionally preferred cereal for making *qu* starter (Jin et al., 2017). The importance of rice and wheat in alcohol production suggests that they were especially needed for feasting activities of the Erlitou elites. Such characteristics, i.e., being in high demand by elites but not widely available to commoners, are typical traits of luxury foods (Veen, 2003).

(9) Drinking practice and social relations in transition: Compared to the alcohol production and consumption in the previous Neolithic period, several changes in raw materials of fermentation, in drinking vessels, and in consumption practices appeared during the Erlitou period. First, using multiple grains and tubers/roots to make alcoholic beverages was a long-lasting tradition during the Neolithic times in the Yellow River region. Based on the analysis of the *jiandiping* amphorae of the Yangshao culture, brewing ingredients included mainly broomcorn millet, together with rice, Job's tears, Triticeae, snake gourd root, yam, and lily, among others (Liu, 2021). This Neolithic tradition, using mostly staple foods for alcohol making, was gradually replaced by methods that used only grains as brewing ingredients in the historical period (Bao and Zhou, 2007). This transition may have taken place in the Erlitou culture, when rice and

wheat became primary ingredients, while millet and roots/tubers declined in use. If rice and wheat, being luxury foods, were obtained by Erlitou elites from tribute goods, the ability to make alcoholic beverages with such food items would have demonstrated their prestige, wealth, and power. Second, during the Neolithic Yangshao culture, *jiandiping* amphorae used for both production and consumption of alcoholic beverages were ordinary vessels, frequently found in all archeological contexts. Communal drinking through reed straws may have been a common practice in feasting activities which took place in public buildings or central plazas in settlements. Such a feasting tradition apparently emphasized group solidarity and social inclusion (Liu, 2021). In contrast, the alcohol-related pottery assemblage at Erlitou is composed of specialized vessel forms used for brewing, storing, serving (heating and pouring), and drinking, suggesting more elaborated procedures involved in feasting activities. Serving and drinking vessels made of white clay were likely also luxury items, which have been found mainly in the walled palatial areas and elite burials. These changes indicate that the feasting activities may have been socially exclusive in nature. Considered together, serving luxury drinks with prestige utensils in socially exclusive spaces may have become the focus of ritual feasting held by the Erlitou elites, which also emphasized social status, wealth, and power. These changes in drinking materiality and social values occurred in the late Neolithic Longshan culture (Underhill, 2018), and became more formalized and perhaps institutionalized at Erlitou, coinciding with greater social differentiation and heightened elite power at the time of early state formation.

CONCLUSION

Based on multiple lines of evidence from microfossil analyses in this preliminary study of Erlitou pottery vessels, we can tentatively reconstruct the processes of alcohol production and consumption at Erlitou, the first Bronze Age urban center in north China. Various ceramic vessels were involved in these processes. *Dakouzun* wide-orifice vats were used for fermentation, likely in semi-solid-state fermentation conditions; *zun* narrow-orifice jars for storage of the beverages; *gui* and *he* pitchers for heating and pouring; and *jue* cups for drinking. The brewing method likely first prepared *qu* starter with *Monascus* mold, also added herbs to aid fermentation. The brewing ingredients may have included primarily rice and wheat, sometimes mixed with broomcorn millet, Job's tears and roots of snake gourd. Rice and wheat may have been partially received as tributes from the surrounding settlements in the Yiluo region and beyond.

The use of precious white clay to produce alcohol-related serving and drinking vessels also indicates that alcohol consumption was a high-ranking elite activity. Such feasting events involving fermented beverages, held in palaces and associated with elite burial rituals, coincided with the growth of elite power and social stratification at Erlitou. Alcohol apparently played a significant role in the emergence of the first Bronze Age state in China, a research topic which has not yet been explored adequately. This study opens a new window for further investigation of alcohol-related material remains and social activities, as well as their relation to state formation in prehistoric China.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LL conceptualized and designed the study. LL and YH analyzed samples and wrote the manuscript. HZ and HX excavated the site and provided the materials and data. All authors contributed to the article and approved the submitted version.

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REFERENCES

Bao, Q., and Zhou, J. (2007). *Niangzao (Fermentation)*. Zhengzhou: Daxiang Press.

Boulton, C., and Quain, D. (2001). *Brewing Yeast and Fermentation*. Oxford: Blackwell Science Ltd.

Dietler, M., and Hayden, B. (eds) (2001). *Feasts: Archaeological and Ethnographic Perspectives on Food, Politics, and Power*. Washington: Smithsonian Institution Press.

Du, J. (1990). Tao jue: Zhongguo gudai jiuqi yanjiu zhiyi (Pottery jue pitcher: study of ancient drinking vessels in China). *Kaogu* 6, 519–530.

Du, J. (1992). Fengdinghe yanjiu (On the *fengdinghe* vessels). *Kaogu Xuebao* 1, 1–33. doi: 10.1163/24684791-12340011

Duncan, N. A., Starbuck, J., and Liu, L. (2019). A Method to identify cross-shaped phytoliths of job's tears, Coix lacryma-jobi L., in Northern China. *J. Archaeol. Sci. Rep.* 24, 16–23. doi: 10.1016/j.jasrep.2018.12.010

Feng, S., Liu, L., Wang, J., Levin, M. J., Li, X., and Ma, X. (2021). Red beer consumption and elite utensils: the emergence of competitive feasting in the Yangshao culture, North China. *J. Anthropol. Archaeol.* 64:101365. doi: 10.1016/j.jaa.2021.101365

Gao, G., and Shao, W. (1981). Shiqian tao gui chulun (On the pottery *gui* vessels in prehistory). *Kaogu Xuebao* 4, 427–457.

Hayden, B. (2014). *The Power of Feasts: From Prehistory to the Present*. Cambridge: Cambridge University Press.

He, Y., Liu, L., Sun, Z., Shao, J., and Di, N. (2021). "Proposing a toast" from the first urban center in the north Loess Plateau, China: alcoholic beverages at Shimao. *J. Anthropol. Archaeol.* 64:101352. doi: 10.1016/j.jaa.2021.101352

Henry, A. G., Hudson, H. F., and Piperno, D. R. (2009). Changes in starch grain morphologies from cooking. *J. Archaeol. Sci.* 36, 915–922. doi: 10.1016/j.jas.2008.11.008

Huang, H. T. (2000). *Science and Civilisation in China: Vol 6, Biology and Biological Technology, Part V: Fermentations and Food Science*. Cambridge: Cambridge University Press.

Institute of Archaeology CASS (2014). *Erlitou 1999-2006*. Beijing: Wenwu Press.

Jin, G., Zhu, Y., and Xu, Y. (2017). Mystery behind Chinese liquor fermentation. *Trends Food Sci. Technol.* 63, 18–28. doi: 10.3389/fmicb.2019.00696

Lamb, J., and Loy, T. (2005). Seeing red: the use of Congo Red dye to identify cooked and damaged starch grains in archaeological residues. *J. Archaeol. Sci.* 32, 1433–1440. doi: 10.1016/j.jas.2005.03.020

Lee, G.-A., Crawford, G. W., Walsh, R. C., Kim, H., Kneisly, A., Lee, H., et al. (2019). "Archaeobotanical analysis on sites surveyed in the Yiluo region (1998-2011)," in *Luoyang Pendi Zhong Dong Bu Diqu Xian-Qin Shiqi Yizhi Diaocha Baogao (Survey Report of Pre-Qin Sites in East-central Luoyang Basin)*, eds Institute of Archaeology CASS and Sino-Australian Yiluo Region Archaeology Team (Beijing: Kexue Press), 204–228.

Li, B.-P., Liu, L., Chen, X.-C., Zhao, J.-X., Drennan, J., Greig, A., et al. (2010). Chemical comparison of rare Chinese white pottery from four sites of the Erlitou state: results and archaeometrical implications. *Archaeometry* 52, 760–776. doi: 10.1111/j.1475-4754.2009.00510.x

Li, S. (1981). *Bencao Gangmu (Materia Medica, Arranged According to Drug Descriptions and Technical Aspects)*. Beijing: Public Health Press.

Ling, C. (1958). Zhongguo jiu zhi qiyuan (The origins of alcohol in China). *Bull. Inst. Hist. Philol.* 29, 883–901.

Liu, L. (2003). "The products of minds as well as of hands": production of prestige goods in the Neolithic and early state periods of China. *Asian Perspect.* 42, 1–40. doi: 10.1353/asi.2003.0025

Liu, L. (2017). Zaoqi taoqi, zhuzhou, niangjiu yu shehui fuzahua de fazhan (Early pottery, porridge, and development of social complexity). *Zhongyuan Wenwu* 2, 24–34.

Liu, L. (2021). Communal drinking rituals and social formations in the Yellow River valley of Neolithic China. *J. Anthropol. Archaeol.* 63:101310. doi: 10.1016/j.jaa.2021.101310

Liu, L., and Chen, X. (2012). *The Archaeology of China: From the Late Palaeolithic to the Early Bronze Age*. Cambridge: Cambridge University Press.

Liu, L., Ma, S., and Cui, J. (2014). Identification of starch granules using a two-step identification method. *J. Archaeol. Sci.* 52, 421–427. doi: 10.1016/j.jas.2014.09.008

Liu, L., Wang, J., Chen, X., and Liang, Z. (2021b). Shandong Dawenkou wenhua jiuqi chutan (Investigation of alcohol-related vessels of the Dawenkou culture in Shandong). *Huaxia Kaogu* 1, 49–61.

Liu, L., Li, Y., and Hou, J. (2021a). Mianchi Dingcun yizhi Yangshao wenhua de qujiu he guyajiu (Alcoholic beverages made of qu and mults discovered at the Yangshao culture site of Dingcun in Mianchi). *Zhongyuan Wenwu* 5, 75–85.

Liu, L., Wang, J., Chen, X., Li, Y., and Zhao, H. (2018). Yangshao wenhua dafangzi yu yanyin chuantong: Henan Yanshi Huizui yizhi F1 dimian he taoqi canliuwu fenxi (Large houses and feasting tradition of the Yangshao Culture: Starch and phytolith analyses of the residues from pottery vessels and floors of House No.1 at Huizui in Yanshi, Henan). *Zhongyuan Wenwu* 1, 32–43.

Liu, L., Wang, J., Levin, M. J., Sinnott-Armstrong, N., Zhao, H., Zhao, Y., et al. (2019). The origins of specialized pottery and diverse alcohol fermentation techniques in Early Neolithic China. *Proc. Natl. Acad. Sci. U. S. A.* 116, 12767–12774. doi: 10.1073/pnas.1902668116

Liu, X., Lister, D. L., Zhao, Z., and Petrie, C. A. (2017). Journey to the east: diverse routes and variable flowering times for wheat and barley en route to prehistoric China. *PLoS One* 12:e0209518. doi: 10.1371/journal.pone.0209518

Lu, H., Zhang, J., Wu, N., Liu, K.-B., Xu, D., and Li, Q. (2009). Phytolith analysis for the discrimination of Foxtail millet (*Setaria italica*) and Common millet (*Panicum miliaceum*). *PLoS One* 4:e4448. doi: 10.1371/journal.pone.0004448

Manan, M. A., Mohamad, R., and Ariff, A. (2017). The morphology and structure of red pigment producing fungus: *Monascus purpureus*. *J. Microbiol. Exp.* 5:00138.

McGovern, P. E., Underhill, A., Fang, H., Luan, F., Hall, G., Yu, H., et al. (2005). Chemical identification and cultural implications of a mixed fermented beverage from late prehistoric China. *Asian Perspect.* 44, 249–275. doi: 10.1353/asi.2005.0026

McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., et al. (2004). Fermented beverages of pre- and proto-historic China. *Proc. Natl. Acad. Sci. U. S. A.* 101, 17593–17598. doi: 10.1073/pnas.0407921102

Pearsall, D. M. (1989). *Paleoethnobotany: A Handbook of Procedures*. San Diego: Academic Press.

Piperno, D. R. (2006). *Phytoliths: A Comprehensive Guide for Archaeologists and Paeoecologists*. Lanham: Altamira Press.

Reinhart, K. (2020). Analysis of grooved ceramic vessels from the early Chinese Bronze Age site of Yanshi Shangcheng: preliminary evidence of food preparation methods through starch analysis. *J. Archaeol. Sci. Rep.* 29:101852. doi: 10.1016/j.jasrep.2019.05.017

St-Germain, G., and Summerbell, R. (2011). *Identifying Fungi: A Clinical Laboratory Handbook*. Belmont: Star Publishing Company.

Sun, Q., Shuo, Z., Wu, Y., and Yang, Y. (2019). Anhui Hanshan Lingjiatan yizhi chutu kecaopen de dianfenli fenxi (Anaysis of starch granules on grooved basins from the Lingjiatan site in Hanshan, Anhui). *Acta Anthropol. Sin.* 38, 132–147. doi: 10.16359/j.cnki.cn11-1963/q.2018.0036

Underhill, A. P. (2018). Urbanization and new social contexts for consumption of food and drink in northern China. *Archaeol. Res. Asia* 14, 7–19. doi: 10.1016/j.ara.2017.07.004

Veen, M. V. D. (2003). When is food a luxury? *World Archaeol.* 34, 405–427. doi: 10.1080/0043824021000026422

Wang, J., Jiang, L., and Sun, H. (2021). Early evidence for beer drinking in a 9000-year-old platform mound in southern China. *PLoS One* 16:e0255833. doi: 10.1371/journal.pone.0255833

Wang, J., Liu, L., Ball, T., Yu, L., Li, Y., and Xing, F. (2016). Revealing a 5,000-y-old beer recipe in China. *Proc. Natl. Acad. Sci. U. S. A.* 113, 6444–6448. doi: 10.1073/pnas.1601465113

Wang, J., Liu, L., Georgescu, A., Le, V. V., Ota, M. H., Tang, S., et al. (2017). Identifying ancient beer brewing through starch analysis: a methodology. *J. Archaeol. Sci. Rep.* 15, 150–160. doi: 10.1016/j.jasrep.2017.07.016

Wu, Z. Y., Raven, P. H., and Hong, D. Y. (eds) (2011b). *Flora of China Vol. 19 (Cucurbitaceae through Valerianaceae, with Annonaceae and Berberidaceae)*. Beijing: Science Press.

Wu, Z. Y., Raven, P. H., and Hong, D. Y. (eds) (2011a). *Flora of China Volume 20-21 (Asteraceae)*. Beijing: Science Press.

Young, E. M. (1931). The morphology and cytology of Monascus ruber. *Am. J. Bot.* 18, 499–517. doi: 10.1002/j.1537-2197.1931.tb09606.x

Yu, W. (2003). Niangzao Jiangnan mijiu de caoqu (The herb-starter for making rice wine in southern China). *Dongfang Meishi Xueshuban* 4, 75–80.

Zhu, S. (1406). *Jiuhuang Bencao (Herbal for Relief of Famines)*, 1982 Edn. Taipei: Shangwu Press.

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Plant Consumption by Early-Middle Neolithic Peoples in Guangxi, South China: Archaeobotanical Evidence From the Dingsishan Site

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The Dingsishan Site, located in Nanning City, Guangxi Zhuang Autonomous Region, is one of the most important Neolithic archaeological sites in the Lingnan region of China's southeastern seaboard. Plant microfossil remains recovered from excavated artifacts and human teeth suggest that the site's ancient inhabitants practiced a subsistence system based on foraging. Wild plant food resources dominated their vegetal diet. Starch granules extracted from residue samples represent various taxa, including plant roots and tubers, aquatic plant fruits, beans, and wild cereals, defining the primary vegetal diet of Dingsishan's Neolithic occupants. In addition, residue samples from shell artifacts yielded starch granules and phytolith remains, providing significant clues as to the function of these tools. We also identified millet starch granules from Dingsishan Cultural Phases 3-4 (ca. 7,000–6000 BP), albeit in extremely low quantities. Holocene paleoecological conditions in the Lingnan area provided diverse and plentiful wild food resources, allowing the relatively late emergence of agriculture. Our study suggests that Middle Neolithic human groups in the Yong River drainage consumed various plants, and their subsistence pattern was relatively stable through the four Cultural Phases identified at Dingsishan. Our conclusions enhance understanding the diversity of plant food resources exploited by foraging societies and interpretations of differences in patterns of agricultural origins in different ecological regions of China.

Keywords: ancient starch, prehistoric subsistence, foraging, residue analysis, phytolith analysis, Dingsishan site

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1 INTRODUCTION

The Lingnan area refers to the Nanling (or Wuling) Mountains and geographical areas to the south, mainly encompassing the Chinese province-level subdivisions of Guangdong, Guangxi, Fujian, and all administrative districts south of the Nanling Mountains. The Lingnan area today is a mixed subtropical and tropical monsoon zone characterized by a warm and humid climate with great biodiversity that is exceptionally plentiful. Abundant natural resources and a salubrious climate provided a favorable environment for the development of prehistoric human cultures. Ancient humans have occupied the Lingnan area since the Paleolithic period (>12,000 BP), forming prehistoric cultures with distinctive regional characteristics. Extensive archaeological fieldwork has been carried out in the Lingnan area since the 1930s, accumulating a substantial database (Gong and Gong, 2013). Early-Middle Neolithic cultural characteristics and sequence of development in the Lingnan area have long been important topics for scholars from many related disciplines.

The cultural behavior of prehistoric people is closely related to their ecological context. Neolithic cultural groups in the Lingnan region and neighboring Southeast Asia expressed complex and diverse subsistence patterns, which were quite different from those of contemporaneous cultures in the Chinese Central Plains and the middle-lower reaches of the Yangtze River (Han, 2012). A subtropical climate provided abundant and diverse biological resources for ancient people, facilitating the acquisition of animal and plant resources essential for human survival. However, this paleoecological situation also dampened the development of agriculture in the Lingnan area, where foraging subsistence systems based on hunting-fishing-gathering persisted well into the Holocene.

The economic strategies of prehistoric foragers in southern China and greater Southeast Asia, as well as the transition to agriculture in these regions, have been the subject of intensive investigation in recent years (Higham, 2013; Yang et al., 2013a; Cheng et al., 2018; Denham et al., 2018; Oxenham et al., 2018; Yang et al., 2018; Deng et al., 2019). Carbonized plant remains are essential evidence to facilitate discussion of the human use of ancient plant resources. However, moist and acidic subtropical soils in the Lingnan region often prevent plant remains from being well preserved. Evidence directly documenting the foodways of ancient people is very rare, thus hindering our understanding of the subsistence patterns of this area's prehistoric inhabitants (Chen, 2016; Yu, 2018). These problems have thus far prevented the systematic and complete understanding of Early-Middle Neolithic Cultures in this area. As a result, plant microfossils become particularly important. Phytolith and starch granules in plants, especially, can provide solid evidence and support for the resolution of the questions outlined above.

Archaeological fieldwork conducted at the Dingsishan Site uncovered numerous artifacts and human remains, which has facilitated the application of multidisciplinary research approaches and provided a body of materials to enhance our understanding of the essential characteristics of Early-Middle Neolithic culture and ancient human economic activities in the Lingnan area (Fu et al., 1998; Fu, 2002). In our study, plant residue analysis was applied to materials unearthed from the Dingsishan Site. Starch remains from archaeological contexts at Dingsishan are fully described in this paper.

2 ARCHAEOLOGICAL CONTEXT

The Dingsishan Site (22°43′48″ N, 108°28′6″ E, ca. 70 m above mean sea level) is a well-preserved shell midden located on the first terrace of the right bank of the Bachi River, a tributary of the Yong River, approximately 3 km south of the Yongning District center, in the Guangxi Zhuang Autonomous Region, South China (**Figure 1**). The site was found in 1994 and excavated in 1997; subsequently, the Institute of Archaeology of the Chinese Academy of Social Sciences conducted excavations there between 1998–2000. The site extends over an area of roughly 5,000 square meters at present. Its stratigraphic profile is divisible into seven layers which yielded a total of 331 human burials and a large number of artifacts, including pottery, lithics, animal bones, and shell artifacts (Fu et al., 1998; Fu, 2002). Based on artifact typology, stratigraphic correlations, and chronological work conducted at adjacent archaeological sites, the Dingsishan cultural sequence can be roughly divided into four chrono-phases: Phase 1, ca. 10,000 BP; Phases 2-3, 8,000–7000 BP; and Phase 4, ca. 6000 BP (Fu et al., 1998; Chen, 2021). Artifacts unearthed from Phase 1 deposits include perforated stone tools and tektite flakes, as well as a small number of pottery sherds mixed with fine sand. Phases 2-3 are referred to as Dingsishan Culture, part of a complex of similar material culture widely distributed in southern Guangxi, especially in Yong River Valley, during the Early-Middle Neolithic. Human burials and substantial quantities of pottery, lithics, shell and bone artifacts were unearthed from these Dingsishan Culture layers. Various modes of human interment are known, especially the unique burial custom of dismemberment. Very few funerary objects were discovered in these graves. In Phase 4, the quantity of lithic artifacts decreases significantly over those of Phases 2-3 and shell implements disappear completely. Ceramic technology improved dramatically with the adoption of the potter's wheel and higher firing temperatures (Fu et al., 1998; Fu, 2002).

3 METHODS AND MATERIALS

3.1 Plant Residue Analysis

Plant microfossil residue analysis has been comprehensively applied in archaeology during recent years (McGovern et al., 2017; Prebble et al., 2019; Wang et al., 2019; Barber, 2020; Zhang et al., 2021; Guan et al., 2022). In our study, residue samples were collected from the surfaces of artifacts and human teeth unearthed from the Dingsishan Site. Human teeth were selected from 78 individuals unearthed from 76 graves belonging to Phases 2-4 (Fu et al., 1998) (**Supplementary Table S1**). The artifacts sampled derive from all the four phases. A total of 91 lithic, 20 shell, and 11 bone artifacts were selected for sampling. We collected both dental and artifact residue samples according to protocols established by Pearsall et al. (2004) and Guan et al. (2014). The residue samples comprise three sediments: Sediment I (Sed I), deposits attached to the surface of human teeth and artifacts; Sediment II (Sed II), liquid samples obtained by washing the specimens' surfaces with distilled water; Sediment III (Sed III), liquid samples derived by ultrasonic cleansing of teeth and artifacts. Soil was rarely found adhering to dental specimens so only Sed III samples were collected in those cases.

Residue samples were gathered from different sediment-types for several reasons. Sed I samples reflect a micro-residue originating in soil, while Sed II samples, which are obtained by wet brushing, are thought to contain matter from both soil and the deep surfaces of the sampled specimens. The main aim of the wet brushing is to isolate Sed I and Sed III, to mitigate cross-contamination from Sed I to Sed III. In this case, Sed I samples are equally as important as Sed III samples since both can provide valid indications, while Sed II samples are difficult to analyze because they are, by definition, mixtures of multiple points of origination. Therefore, Sed II samples are not discussed further here. The taxa and amounts of starch granules in each sediment sample are used as indicators to evaluate which taxon/taxa were derived from soil contamination and which were formed as the result of ancient human behaviors.

Finally, 79 dental and 285 artifact residue samples were collected and processed in the Key Laboratory of Vertebrate Evolution and Human Origins of the Chinese Academy of Sciences in Beijing. The experimental process followed that of Guan et al. (2010), integrating several laboratory operations (Chandler-Ezell and Pearsall, 2003; Pearsall et al., 2004). Processing included the following steps: concentration, deflocculation, and heavy liquid flotation. Starch granule and phytolith extraction slides were observed with a Nikon Ni-E biological polarizing microscope. 100% glycerol was used as a mounting medium for starch and phytolith extractions. NIS-Elements D3.2 software was applied for the photography. Both phytolith and starch slides were viewed at 200–3×00 magnification and photographed at ×400 magnification.

3.2 Geometric Morphometric Analysis of Starch Granules

Geometric morphometry analysis has been widely used recently in many disciplines such as entomology, aquatic biology, medical science, paleoanthropolgy, and archeology (e.g.,.Slice, 2007; Mitteroecker and Gunz, 2009; Addis et al., 2010; Webster and Sheets, 2010; Adams and Otárola-Castillo, 2013; Park et al., 2013; McNulty and Vinyard, 2015; Savriama, 2018) with accompanying advances in the anslysis of objects' shapes. This method allows us to visualize differences among complex shapes with nearly the same facility. Moreover, it avoids the shortcomings of varying data sources, non-repeatability, and the size and shape data can be calculated altogether (Chen, 2017). The technological detail see Bookstein (1997) and Zelditch et al. (2004), thus will not be elaborated in this paper. In our study, 35 landmarks were assigned on each single starch granule, presenting the contour, location of hilum, and the extinction cross curvature of individual starch granule (**Figure 2**) (Zhang et al., 2021). Thin Plate Spline (TPS) files (landmark configurations) were imported into *MorphoJ* software (Klingenberg, 2011), for General Procrustes Analysis (GPA) and Canonical Variate Analysis (CVA). CVA assumes that the covariance structure within all groups is the same, therefore, a pooled within-group covariance matrix is used throughout for CVA and for computing distances between pairs of groups. The Mahalanobis distance matrix and Procrustes distance matrix may help explain group similarities and differences. In addition to CVA, our study also applied a supervised machine learning method to modern starch geometric morphometric data for model training (for technical

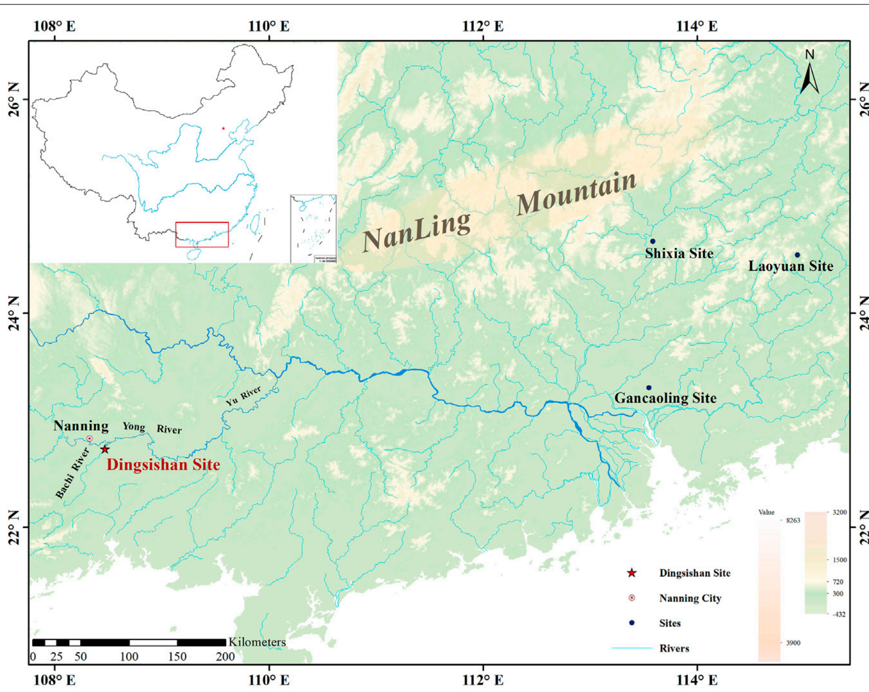

FIGURE 1 | Geographic location of the Dingsishan site and other Neolithic Site mentioned in this paper.

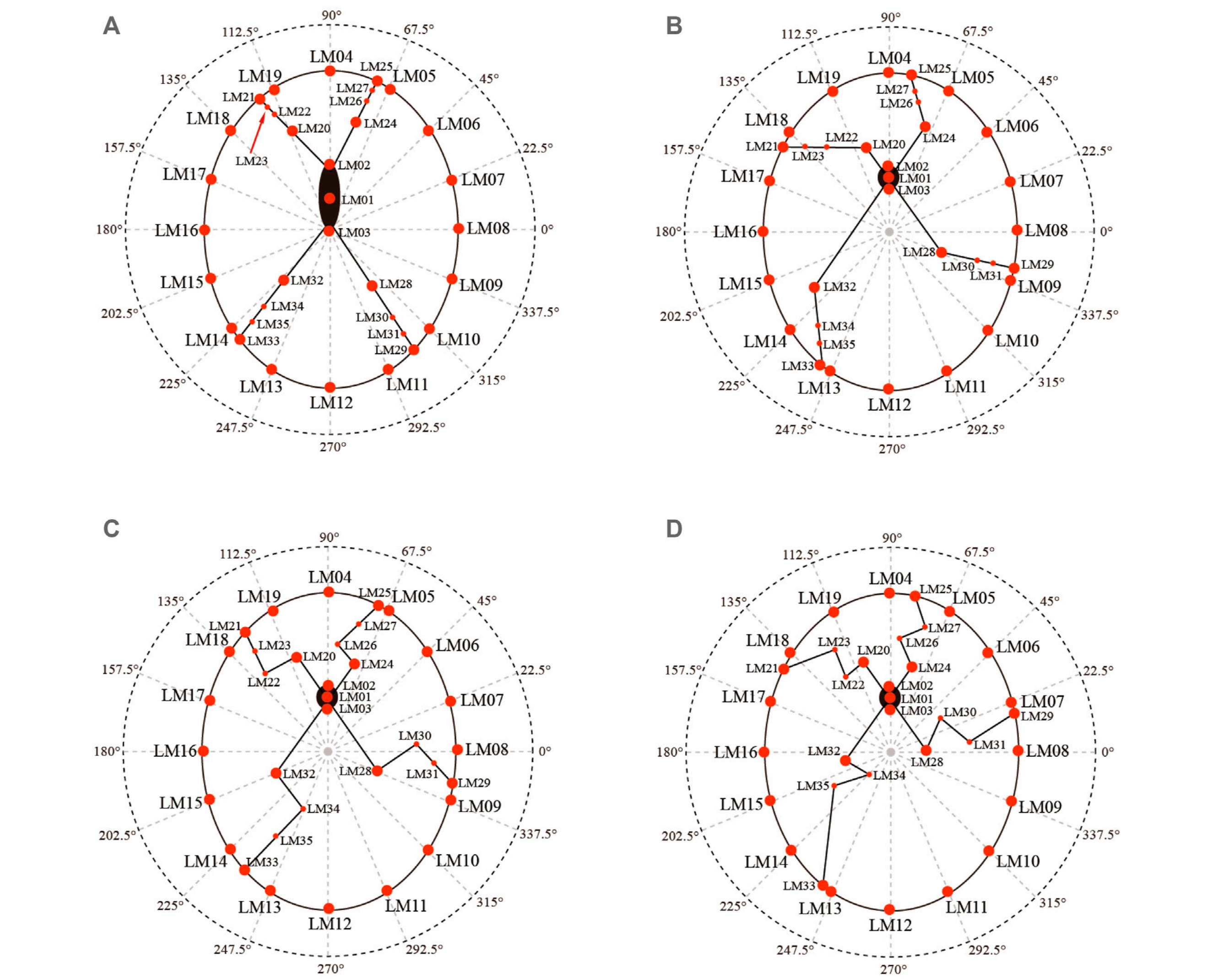

FIGURE 2 | Definition of the 35 landmarks on starch granule. **(A)** Extinction cross with no bend; **(B)** each arm with one bend; **(C)** each arm with two bends; and **(D)**, each arm with three bends (based on Zhang et al., 2021).

details, see Zhang et al. (2021). In order to maximize the reliability of the machine learning model results, we adopted 95% accuracy as the standard of model usability, therefore, we regarded labels with an accuracy of 95% or more in the predicted results as valid, while labels with an accuracy of less than 95% were doubtful and are not presented here.

No matter what starch ideintification method is used, the contal groups, datasets derived from modern starch granules, are extremely essential (**Figure 3**). We have thus far compiled a modern reference database of more than 98 starch-producing taxa including both domesticated and wild taxa. For the geometric morphometric data extraction, some plants, such as certain species of *Colocasia*, produce extremely small (<5 μm) starch granules, and are thus inappropriate for the acquisition of landmark configurations. As a result, 57 taxa were included in our quantitative examination. Phytolith classification was based completely on published resources (Yang et al., 2009; Liu et al., 2011; Wan et al., 2011a; Wan et al., 2011b; Yang et al., 2013b; Yang and Perry, 2013; Liu et al., 2014; Wan et al., 2016; Liu et al., 2019; Ma et al., 2019; Li et al., 2020).

4 RESULTS

Starch granules, phytoliths and small quantities of plant tissue fragments were revealed from the extracts (**Table 1**). Pollen, bordered pits, epidermal fibers and cells of unknown biological origin, and other organic fragments were also recovered from all three sediment types in extremely low frequencies and lacking distinguishing biological attributes, thus they are not included in this paper.

4.1 Starch Granule Analysis

A total of 887 starch granules was recovered from all three sediments types of residue samples (**Figure 4**), among which

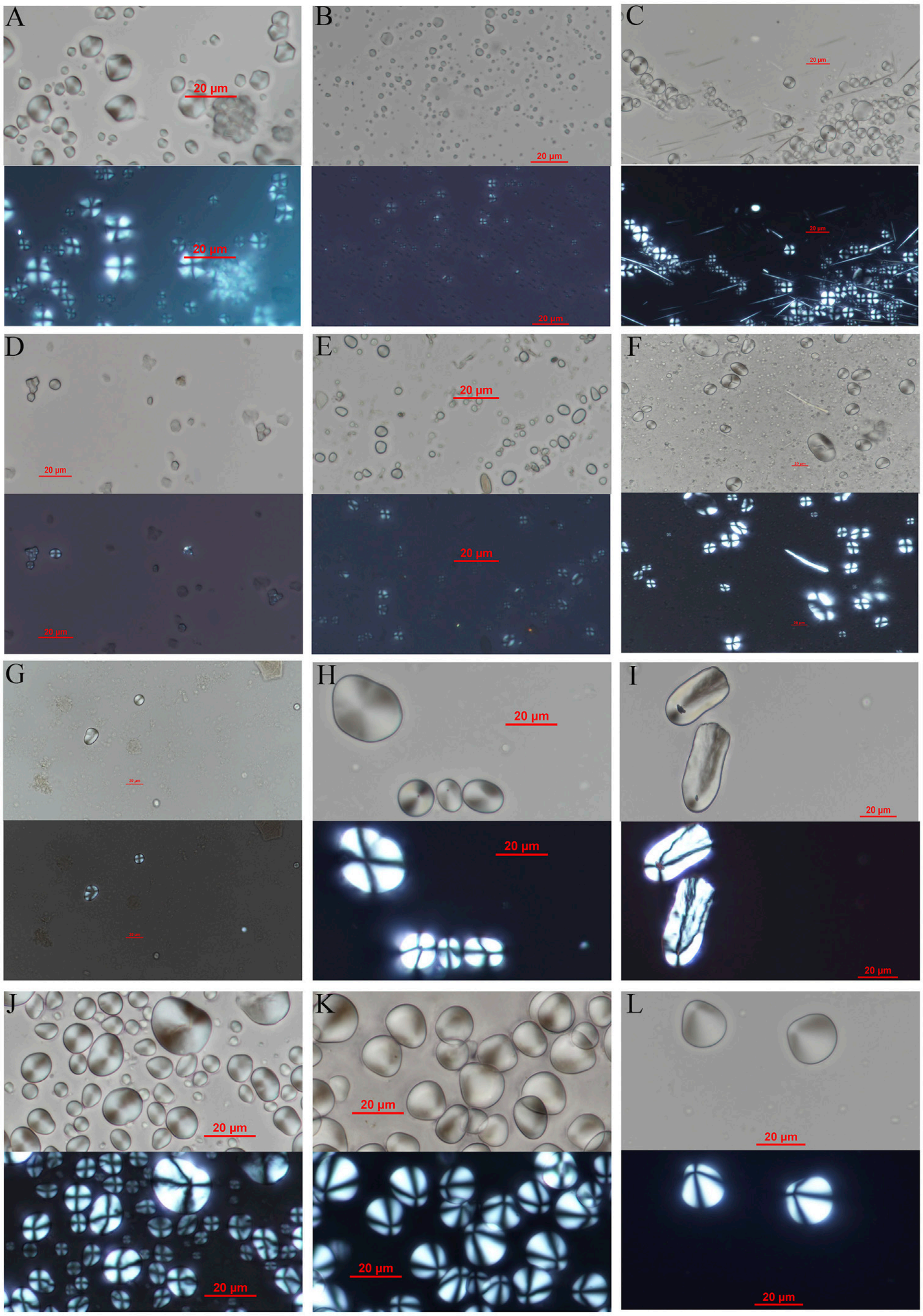

FIGURE 3 | Modern plant starch references included in this paper. **(A)** *Pueraria lobata* **(B)** *Amorphophallus virosus* **(C)** *Pinellia ternata* **(D)** *Zizania aquatica* (seed) **(E)** *Acorus tatarinowii* **(F)** *Canavalia gladiata* **(G)** *Vigna umbellata* **(H)** *Trapa bispinosa* **(I)** *Nelumbo nucifera* (root) **(J)** *Sagittaria trifolia* **(K)** *Bolbostemma paniculatum* **(L)**: *Polygonatum sibiricum*.

32 were extracted from Sed I, 147 from Sed II, and 708 from Sed III (**Table 1**). Among these starch remains, 221 are seriously damaged or lack of identifiable features. Therefore, 666 granules (75.08% of the total) are examined in detail. We compared granules from Sed I with III by image comparison and geometric morphometric analysis to exclude contamination in Sed III, resulting in starch granules originating from human behavior during the occupation of the site.

4.1.1 Morphological Classification

All recovered starch granules can be divided into the following categories by simple geometric and other visible characteristics:

TABLE 1 | Frequency of plant microfossil remains at the Dingsishan Site.

Specimen Type	Sediment I		Sediment II		Sediment III		Total	
	Starch	Phytolith	Starch	Phytolith	Starch	Phytolith	Starch	Phytolith
Human teeth	\	\	\	\	178	235	178	235
Shell artifacts	\	\	2	0	315	461	317	461
Bone artifacts	0	0	3	9	18	20	21	29
Stone artifacts	32	3	142	48	197	100	371	151
Total	32	3	147	57	708	816	887	876

Type 1, Semi-spheroidal, ellipsoidal, and elongated-ellipsoidal granules. The main two-dimensional shapes are oblong, ovate and irregular fan-shaped. The diameter range of these granules is 5.84–36.27 μm. This granule type always exhibits an eccentric hilum, remarkable lamellae and bent extinction crosses, therefore this type is easily distinguished from others. Granules of this type may be produced by roots and tubers of both terrestrial and aquatic plants.

Type 2, Polyhedral starch granules. The two-dimensional shapes are polygonal or circular with invisible lamellae, pronounced fissures and centric to slightly eccentric hilums, ranging 4.91–22.86 μm in size. Starch granules of this type were probably produced by *Panicum* sp., *Setaria* sp. (including wild species and domesticated species such as millet) and other Poaceae seeds. In addition, **Figures 4K–M** and o could be identified as the tribe Triticeae based on morphology and size (Yang and Perry, 2013). And the bell-shaped starch grains such as **Figure 4I** are mainly associated with roots and tubers in southern China, but some acorn, such as *Cyclobalanopsis*, contain similar starch grains, it is difficult to distinguish these granules according to traditional typology method. Therefore, more precise quantitative analytical methods are used in our project and the additional results are displayed at 4.1.2 (Wang, 2017).

Type 3, Polyhedral or spheroidal body with an extremely small diameter range. Most of the two-dimensional shapes are rounded polygons, and the rest are almost circular. These granules have smooth surfaces and invisible lamellae. No clear extinction cross or hila position can be observed due to the small size of the granule bodies. The diameter range of these granules is 4.25–9.18 μm. These starch granules resemble the morphology of modern specimens of the Araceae, according to our reference database (**Figure 3B**) and published literature (Wan et al., 2011a).

Type 4, Kidney bean shaped granules, which include starch from legumes (Family Fabaceae), ranging 8.56–17.28 μm in size. according to our modern reference database (**Figure 3F**) and published literature (Wang et al., 2013). These granules exhibit visible fissures and lamellae, with mostly invisible hilums. Furthermore, the center of the extinction crosses appears as a dark linear area and the extinction crosses themselves are "χ" shaped.

Type 5, Drop-shaped granules. This type features close and eccentric hila, visible lamellae and almost no fissures. The two-dimensional shapes are mostly tri-rounded corners ovoids or drop-shaped ovoids. The diameter range of these granules is 6.75–26.52 μm. According to recent studies (Yang et al., 2009), starch granules probably derive from *Quercus* acorns and, especially, nuts of the Chinese chestnut, *Castanea mollissima*.

This classification does not effectively assess the differences between Sed I and III, thus more precise quantitative analytical methods are needed.

4.1.2 Geometric Morphometric Analysis and Evaluation of Contamination

Landmark configurations of all recovered starch granules were applied to CVA and machine learning algorithm analysis. The distribution of canonical variates (CVs) showed that the geometric morphometric characteristics of starch granules in Sed I and Sed III defined two peak values (**Figure 5**), and the Mahalanobis distance also showed apparent differences between the groups (p value < 0.0001), suggesting different dominant sources of the two samples. In this case, most of the starch granules from Sed III are considered the result of human use of artifacts and chewing food. For a better understanding, we displayed the canonical variates calculated from Sed III starch granules and modern starch granules on a two-dimensional scatter plot, thereafter, compared the overlap scatters to infer which plant taxa the Sed III starch granules belong to. Scatter plot results of CVA with confidence ellipses (probability = 0.95) (**Figure 6**) reflect a clustered relationship and distribution region that may be formed by geometric data of different types of starch granules, and provide important reference data for further distinguishing starch granule groups with obvious or unique morphological characteristics. However, the results presented here cannot cover all the canonical variates simultaneously and, as a result, we cannot quantitatively count and analyze species information on starch granules according to CVA data, and the mathematical distance between groups does not support detailed classification of starch granules in a more quantitative way. In order to present more accurate and specific recognition results and exlude soil contamination in Sed III samples, a SVM model was applied from which a list was acquired (**Table 2**), suggesting taxa possibly derived from soil sediments.

These results establish that *Castanea mollissima, Bolbostemma paniculatum, Maranta arundinacea, Panicum miliaceum, Polygonatum sibiricum, Pueraria lobata, Sagittaria trifolia, Saururus chinensis, Setaria italica, Trapa bispinosa,* and *Zizania aquatica* may be considered positive taxa (model accuracy ≥95%) (**Table 3**), among which wild species dominate the assemblage. These taxa are regarded as indicators of human activity during the period of the site's

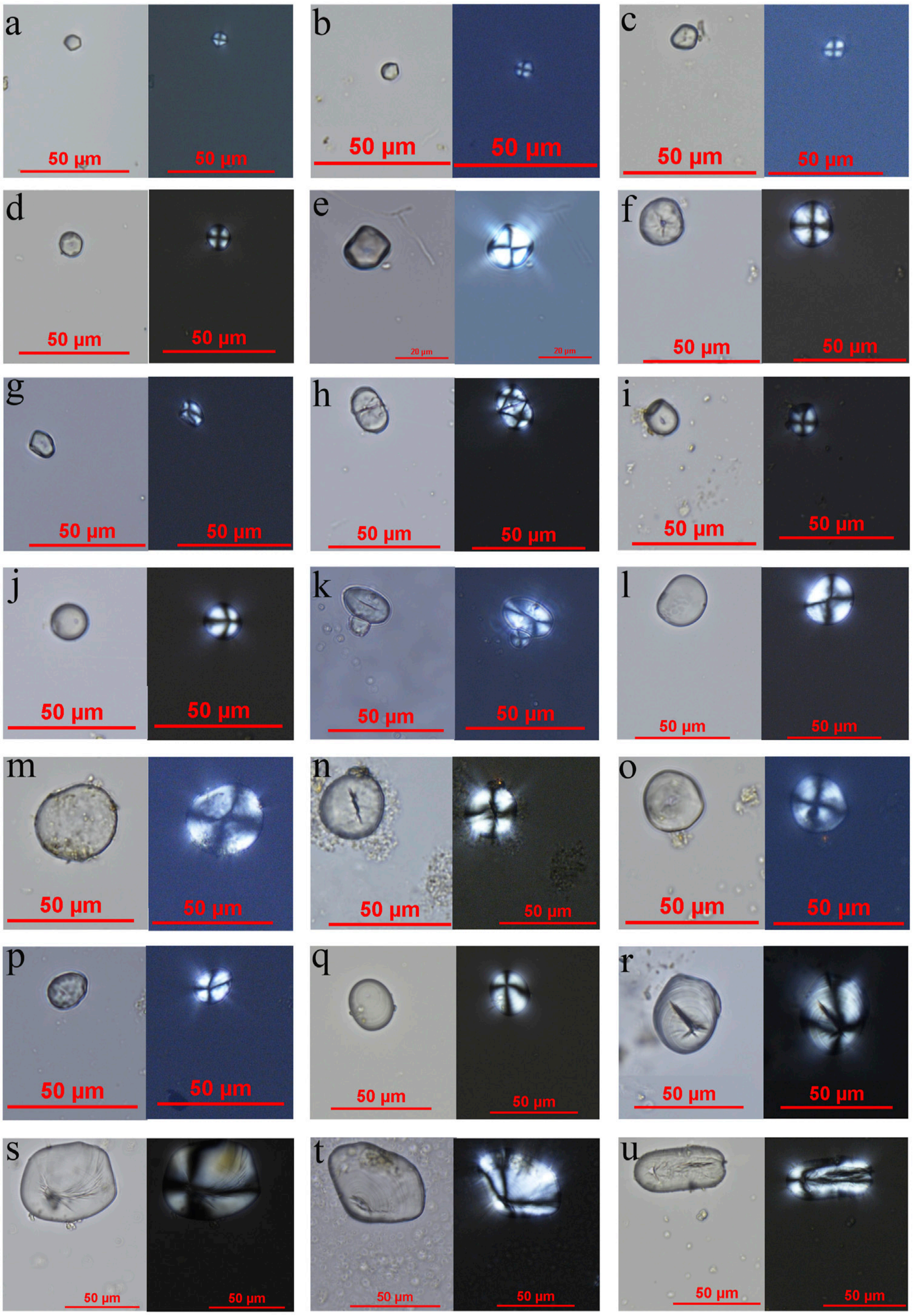

FIGURE 4 | Starch granules from Dingsishan Sed III plant residue samples. **(A)** Polyhedron starch granule **(B)** Polyhedron starch granule **(C)** Polyhedron starch granule **(D)** Polyhedral spheroid starch granule **(E)** Polyhedron starch granule **(F)** Polyhedral spheroid starch granule **(G)** Polyhedron starch granule **(H)** Semi-ellipsoid starch granule **(I)** Bell-shaped starch granule **(J)** Ellipsoid starch granule **(K)** Ellipsoid starch granule **(L)**: Ellipsoid starch granule **(M)**: Ellipsoid starch granule **(N)**: Drop-shaped starch granule **(O)**: Ellipsoid starch granule **(P)**: Drop-shaped starch granule **(Q)**: Ellipsoid starch granule **(R)**: Ellipsoid starch granule **(S)**: Ellipsoid starch granule **(T)**: Fan-shaped starch granule **(U)**: Elongated-ellipsoid starch granule.

occupation. The taxa possibly derived from soil will not be discussed below.

One issue should be considered that, the size of starch granules is an important index when identifying foxtail millet and broomcorn millet (Yang et al., 2012). In view of its low quantity, and weakly positive in geometric morphometric analysis, we suggest to consider the five granules of foxtail and broomcorn millets as the subfamily Panicoideae.

4.1.3 Starch Taxa Associated With Different Cultural Stages at Dingsishan

As previously stated, the Dingsishan archaeological deposit can be separated into four cultural stages, among which Phases 2-3 are defined as Dingsishan Culture. In consequence, positively identified Sed III starch granule taxa are categorized into six groups according to cultural stage and specimens sampled: 1) artifacts from Cultural Phase 1 (A01); 2) artifacts from Cultural Phase 2 (A02); 3) artifacts from Cultural Phase 3 (A03); 4) artifacts from Cultural Phase 4 (A04); 5) human teeth from Cultural Phase 2-3 (T02-03); and 6) human teeth from Cultural Phase 4 (T04). SVM predictions for each group (see **Table 3**) suggest that the Dingsishan starch granules (n = 465) likely derive from terrestrial plant roots and tubers (58.49%), aquatic plants (32.26%), legumes (6.67%), nuts (1.29%) and cereal grains (Panicoideae seeds) (1.08%). The Panicoideae seeds were present only in Phase 3 and 4 at low percentages. Cultural Phase 1 shows a pattern distinguishable from Phase 2 and 3. Wild taxa represented are mainly subtropical species, among which *Manihot esculenta* (cassava or manioc) and *Maranta arundinacea* (arrowroot) are not native to China. They appear on the list of identified species because, in the process of determining unknown starch granules, the SVM model searched for the most similar subset in the control group and output the label of that most similar group for the unknown starch granules. This does *not* mean that these two plants are actually *Manihot esculenta* and *Maranta arundinacea* but, rather, that their starch granule morphologies are highly similar to those taxa. Due to the great diversity of plants in Guangxi, our modern starch database does not include all the wild root and rhizome resources that may have existed during the Neolithic period, thus this problem occurs. One way to solve this problem is to expand the scale of the control group and provide more accurate predictive models in future work.

4.2 Phytolith Remains and Plant Tissue Fragments

At Dingsishan, 235 phytoliths from tooth samples and 641 phytoliths from three stratigraphic levels of artifacts were recovered (**Figure 7**). In summary, 816 specimens were extracted from Sed III samples. These phytoliths were classified into eight types based upon criteria provided by Lu et al. (2006) including Elongate, Fan-shaped, Saddle, Square, Bilobate short cells, Cylindrical polylobate, Globular echinate and other irregular-shaped. Fan-shaped and Elongate forms appeared most frequently, suggesting these phytoliths might belong to the Bambusoideae (bamboos), Palmae (palms), or Chloridoideae (tropical and sub-tropical grasses) taxonomic groups. We also identified several unknown taxa with conspicuous morphological characteristics. Phytolith analysis failed to yield evidence of cultivation, indicating that foraging may have been the most sustainable subsistence pattern for Dingsishan's Neolithic inhabitants.

5 DISCUSSION

In this study, starch granules and other plant microfossils extracted from human dental remains and artifacts provide considerable evidence for exploring human diet and subsistence patterns at Dingsishan. We suggest that the Neolithic inhabitants of the site utilized underground storage organs as their primary source of vegetal food (**Table 4**). Roots and tubers, rich sources of energy, are usually easy to gather and process, and are thus one of the most common plant food resources exploited in both prehistoric and modern times. Fruit of the aquatic plant, *Trapa bispinosa* (water caltrop) and tubers of *Sagittaria trifolia* (Chinese arrowhead) are rich in starch, which can provide the necessary energy for human survival. Nuts and grains also occupy a place in human plant recipes, but in relatively low percentages. In addition, in terms of the identifiable starch number among all the results, except for *Amorphophallus virosus*, *Maranta arundinacea*, and *Sagittaria trifolia*, the identifiable quantity of other plant species is tiny under the strictly statistical standards, which limits our discussion about how Dingsishan people consume and utilize these plants.

The utilization of wild plant resources was an essential part of ancient human subsistance behavior for millennia at Dingsishan, throughout all four Cultural Phases. However, during Cultural Phases 3 and 4, Panicoideae seeds evidenced by the starch remains are present, while this discovery shows low quantity

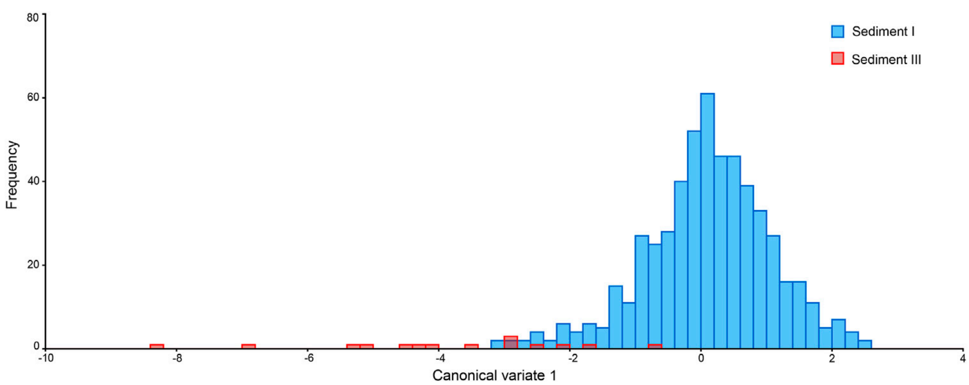

FIGURE 5 | Canonical variable frequency bar chart of Sed I and Sed III samples from the Dingsishan Site.

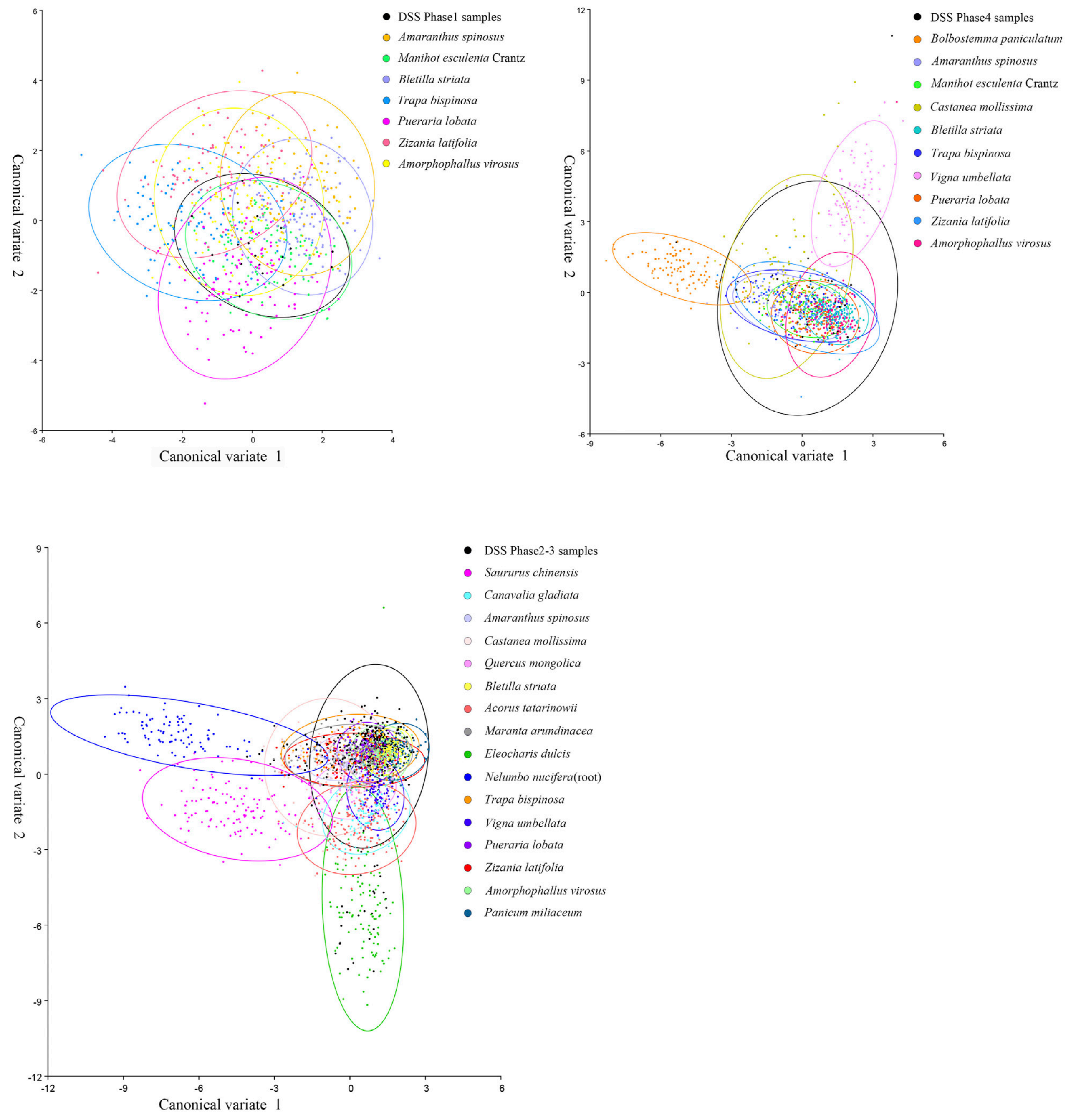

FIGURE 6 | Confidence ellipses (probability = 0.95) based on Canonical Variate scatter plot of CV1 and CV2.

(n = 5) and weakly positive characteristic in geometric morphometric analysis, it surely suggesting a subtle change during Cultural Phase 3. Therefore, current evidence is insufficient to determine whether cultivation behavior leading to the subsequent millet agriculture appeared at that time. Nevertheless, these data may indicate that Dingsishan's Neolithic occupants were in a transitional period of plant resource utilization during Cultural Phases 3-4 when they attempted to intensify the utilization of seed plants, paying greater attention to the acquisition and utilization of cereal grain resources than previously.

It is noteworthy that 315 starch granules in Sed III were extracted from shell knives (or spatulas), which accounted for 59.4% of the total starch granule yield from artifacts (n = 530) in Sed III. Taxonomically identifiable starch granules recovered from shell implements came mainly from roots and tubers, perhaps indicating that implements such as shell knives were used mainly for gathering and processing roots and tubers.

TABLE 2 | Extrapolated identification of archaeologically-derived starch granules from the Dingsishan Neolithic site.

Starch taxon	Count (sed I)	Count (sed III)	SVM model accuracy (%)	Sed III sample Confidence
Amorphophallus virosus	0	131	93.18	Positive
Acorus tatarinowii	0	10	91.43	Positive
Bolbostemma paniculatum	0	1	100	Weakly Positive
Castanea mollissima	0	6	97.22	Positive
Canavalia gladiata	0	21	91.18	Positive
Maranta arundinacea	0	125	97.5	Positive
Manihot esculenta	0	14	92.5	Positive
Nelumbo nucifera (root)	0	19	93.94	Positive
Panicum miliaceum	0	2	100	Weakly Positive
Pinellia ternata	13	1	100	Negative
Polygonatum sibiricum	0	1	100	Weakly Positive
Pueraria lobata	0	5	100	Weakly Positive
Sagittaria trifolia	0	89	100	Positive
Saururus chinensis	0	7	95	Positive
Setaria italica (72-h soak)	0	3	96.88	Weakly Positive
Trapa bispinosa	0	2	100	Weakly Positive
Vigna umbellata	0	5	90.91	Weakly Positive
Vicia faba	2	0	100	Negative
Zizania aquatica (seed)	0	23	100	Positive

Scholars have argued that South China may have been a relatively stable tropical agricultural zone with roots and tubers constituting the principal cultivated crops (Li, 1990; Zhao, 2006). However, carbonized or otherwise preserved roots and tubers are rarely found in archaeological contexts due to the high moisture and acidity of tropical soils. It is difficult to demonstrate the existence of root- and tuber-based agriculture based on the quantity of plant remains available for study. Morphological standards for distinguishing domesticated root and tuber remains are still unclear, which limits our ability to provide extensive discussion of this issue. Our results suggest that roots and tubers existed for a long period (Phases1-4) at Dingsishan, and in larger proportions.

A foraging strategy dominated subsistence systems in the Lingnan region for a long period during the Early-Middle Neolithic. Previous studies have proposed that rice agriculture and planting techniques were introduced into this area from the Middle and Lower Yangzi Valley about 5,000–4000 BP (Zhang and Hung, 2009; Chi and Hung, 2012). Currently, the earliest direct evidence of rice agriculture in the Lingnan region comes from the Shixia and Laoyuan sites in Guangdong Province where carbonized rice grains have been dated directly to 4,347–4,090 and 4,419–4,246 Cal BP, respectively (Yang et al., 2017; Yang et al., 2018). Moreover, Deng et al. (2022) published their new discovery of Gancaoling Site, for where the ancient human cultivated rice together with a small portion of foxtail millet around 4,800–4,600 cal. BP. So far, no clear evidence of early agriculture at contemporaneous sites farther south in the Yong River Valley in Guangxi has been detected; the earliest evidence of rice agriculture in Guangxi is a diagnostic *Oryza* phytolith found at Dingsishan in Cultural Phase 4 deposits (Zhao et al., 2005). However, our study detected no clear evidence of rice farming, such as rice phytoliths and starch, which may be a function of the number and type of samples selected for analysis. Since the distribution of plant residues is random, and the microfossils which attached to artifacts and human teeth are easily destroyed by human activities and the preservation condition, we suppose that the different source of samples selected by us and Zhao et al. (2005) led to the different results. Current archaeological evidence and our research suggest that Phase 4 at Dingsishan was probably a transitional period with respect to human subsistence patterns (Chen, 2016). Therefore, plant remains associated with Dingsishan Phase 4 are considered significant evidence for exploring this transformation. We expect accumulating follow-up research results to clarify this critical transition to developed agriculture.

The Guangxi Zhuang Autonomous Region, where the Dingsishan Site is located, is bisected by the Tropic of *Cancer* and is bordered by tropical seas to the south, the Nanling Mountains to the north, and the Yunnan-Guizhou Plateau to the west. Mountains and hills dominate the terrain. Numerous rivers, abundant water resources, and a complex coastline are all typical. The region has a warm climate with high average annual rainfall and sunshine amounts. The average annual temperature is 17.5–23.5°C. According to paleoclimatic and paleoenvironmental studies conducted in Guangxi (e.g., Li, 1998; Zhang et al., 2000; Zhang et al., 2003), the Lingnan region began to enter a warming period in the early Holocene about 10,000 years ago, during which various plants, especially broad-leaved species, rhizomes and herbs, greatly increased, providing abundant fruits, green leaves and tubers for human consumption. Our results indicate that such wild plant resources were crucial to the prehistoric occupants of Dingsishan. They apparently gathered and consumed wild plant resources such as roots and tubers, beans and aquatic plants, nuts and grains, indicating the extensive use of diverse plant resources. Roots and tubers are usually easy to gather and process; thus, they are considered one of the most common food resources in both prehistoric and modern times. Aquatic plants are widely distributed in the subtropical zone. For Dingsishan's

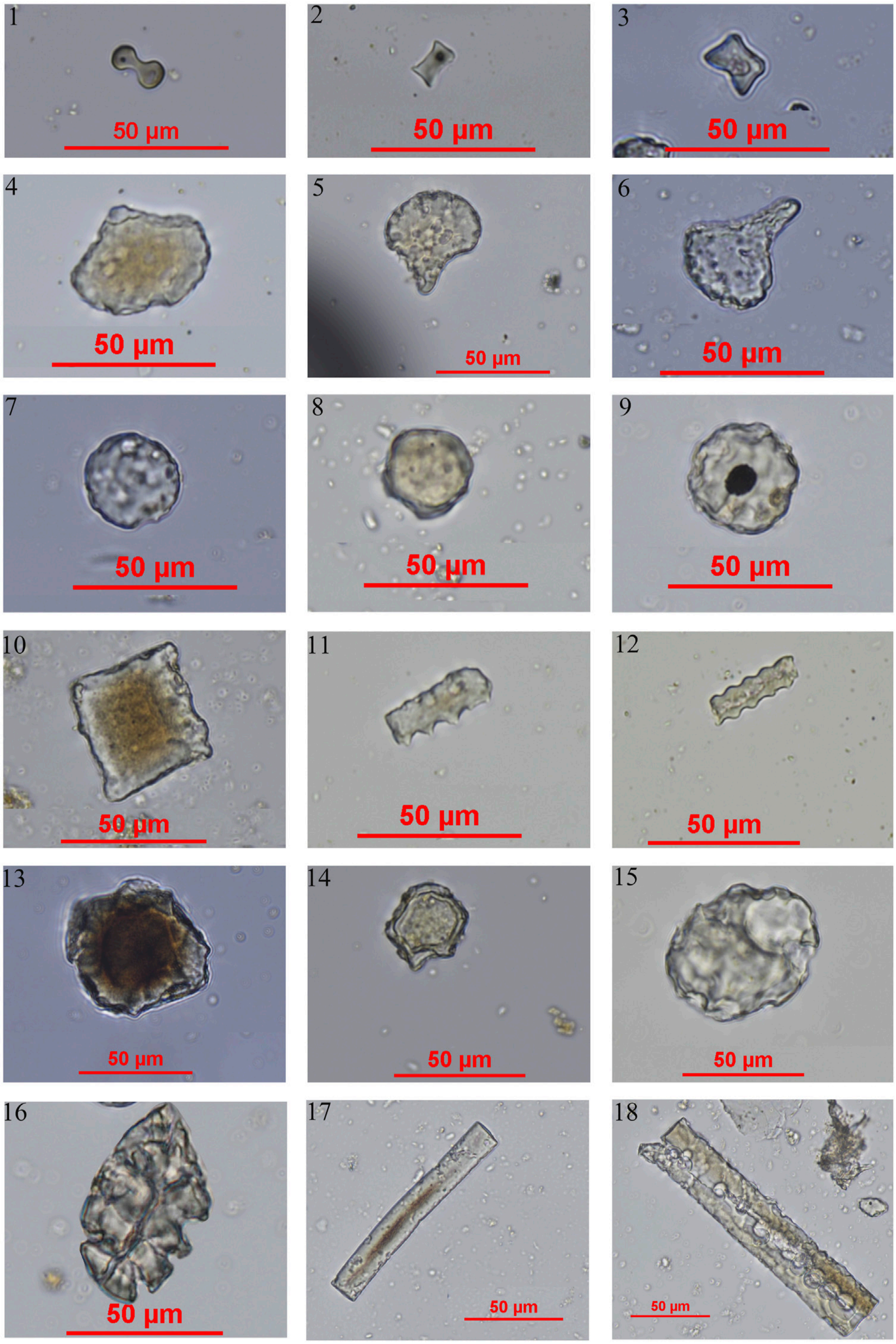

FIGURE 7 | Phytoliths recovered from Dingsishan plant residues. 1. Bilobate 2–3. Short cell (Two-spiked rondels) 4–6. Bulliform cells 7–9. Globular echinate 10. Rectangle 11. Elongate echinate; 12. Elongate sinuate 13–16. Polyhedron from wood 17. Smooth elongate 18. Elongate from wood.

prehistoric inhabitants, living as they did along a river, obtaining such resources was very time- and labor-efficient, with a high resource return rate. Nuts and grains also occupied a place in plant recipes, but the proportions of these two plant resources are relatively low. Our analysis confirms that the Lingnan region's hydrothermal conditions and natural environment fostered a

TABLE 3 | Positively identified plant taxa (SVM model accuracy ≥95%) from the Dingsishan site.

Starch taxon	A01	A02	A03	A04	T02-03	T04	Total	%	Remarks
Castanea mollissima	6						6	2.27	Wild
Pueraria lobata	4					1	5	1.89	Wild
Trapa bispinosa	2						2	0.76	Wild
Maranta arundinacea		125					125	47.35	Wild
Saururus chinensis		1	6				7	2.65	Wild
Zizania aquatica (seed)					23		23	8.71	Wild
Polygonatum sibiricum					1		1	0.38	Wild
Setaria italica						3	3	1.14	Domesticated
Panicum miliaceum			1	1			2	0.76	Domesticated
Sagittaria trifolia					89		89	33.71	Wild
Bolbostemma paniculatum				1			1	0.38	Wild
Total	12	126	7	2	113	4	264	100	

TABLE 4 | Starch taxa associated with different cultural stages at the Dingsishan Site.

Cultural phase	Age (years BP)	Plant Taxa	Edible Portion	Remarks
1	ca. 10,000	*Castanea ollissima*	Nut	Wild
		Pueraria lobata	Root	Wild
		Trapa bispinosa	Fruit	Wild
2	ca. 8,000	*Maranta arundinacea*?	Tuber	Wild
		Saururus chinensis	Root	Wild
		Zizania aquatica	Seed	Wild
		Polygonatum sibiricum	Tuber	Wild
		Sagittaria trifolia	Corm	Wild
3	ca. 7,000	*Saururus chinensis*	Root	Wild
		Zizania aquatica	Seed	Wild
		Polygonatum sibiricum	Tuber	Wild
		Sagittaria trifolia	Corm	Wild
		Panicum miliaceum	Seed	Cultivated?
4	ca. 6,000	*Panicum miliaceum*	Seed	Cultivated?
		Bolbostemma	Tuber	Wild
		paniculatum	Tuber	Wild
		Setaria italica	Seed	Cultivated?

level of biodiversity that gave the region's ancient inhabitants ready access to diverse food sources and delayed the process of agricultural development.

6 CONCLUSION

We identified several types of plant starch from Dingsishan plant residue samples. Geometric morphometric analysis indicates that these starch granules might derive from plant underground storage organs, aquatic plant fruits and tubers, and a small number of nuts and cereals. These plant micro-remains reflect the diversity of the vegetal food resources exploited by the Lingnan region's prehistoric inhabitants between roughly 10,000 and 6,000 years ago. Terrestrial plant roots and tubers, and aquatic plant edible parts occupy the most crucial position in the diet structure of human beings during various cultural periods. In brief, gathering was the principal means by which prehistoric humans obtained plant food resources.

Dingsishan is the best-preserved Early-Middle Neolithic shell midden site yet discovered in Guangxi. It provides important information to enhance our understanding of the cultural characteristics and chronology of prehistoric Guangxi and the greater Lingnan region. Conducting a systematic study of the animal and plant remains in this site can reveal the unique economic patterns of prehistoric people in the Lingnan region and provide better understanding of regional diversity and common developmental trajectories of prehistoric human subsistence patterns.

What pathways did the emergence and development of agriculture in prehistoric South China follow? This question has been common among archaeologists for the past several decades. Today, many scholars are still focused on reconstructing the processes of subsistence change in their entirety. Plant residue analysis provides a unique perspective to enhance discussions of these issues. Archaeobotanical studies tend to concentrate on evidence of specialized agricultural food production, and such research has focused predominantly on data regarding farming economies, while problems such as the use of plant foods in hunter-gatherer contexts and how foraging peoples obtained and cultivated plant foods are discussed less or such discussions are at least limited by a relative paucity of substantiating evidence. Research on plant micro-remains from archaeological contexts needs to continuously accumulate, embracing the latest investigative technology and information efficiently and comprehensively to inexorably establish an effective and comprehensive interpretive database.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XZ: Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - Original Draft; CH, ZZ and QH: Resources, Supervision; JO: Writing - Review andamp; Editing; YG: Conceptualization, Funding Acquisition, Supervision, Writing - Review andamp; Editing.

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REFERENCES

Adams, D. C., and Otárola-Castillo, E. (2013). Geomorph: Anrpackage for the Collection and Analysis of Geometric Morphometric Shape Data. *Methods Ecol. Evol.* 4, 393–399. doi:10.1111/2041-210X.12035

Addis, P., Melis, P., Cannas, R., Secci, M., Tinti, F., Piccinetti, C., et al. (2010). A Morphometric Approach for the Analysis of Body Shape in Bluefin Tuna: Preliminary Results. *Collective Volume Scientific Pap.* 65, 982–987.

Barber, I. G. (2020). Further Wet-Taro Evidence from Polynesia's Southernmost Neolithic Production Margins. *Proc. Natl. Acad. Sci. U.S.A.* 117, 1257–1258. doi:10.1073/pnas.1918374117

Bookstein, F. L. (1997). Landmark Methods for Forms without Landmarks: Morphometrics of Group Differences in Outline Shape. *Med. Image Anal.* 1 (3), 225–243. doi:10.1016/s1361-8415(97)85012-8

Chandler-Ezell, K., and Pearsall, D. M. (2003). Piggyback Microfossil Processing: Joint Starch and Phytolith Sampling from Stone Tools. *The Phytolitharien* 15, 2–8.

Chen, W. (2016). Research on Neolithic Chronologies andSubsistential Strategies of Lingnan Area. Unpublished Ph.D. dissertation. Changchun: Jilin University.

Chen, X. (2017). *Application of Geometric Morphometry to Aquatic Organisms*. Beijing: Science Press.

Chen, W. (2021). Zailun "Huanan zaoqi Xinshiqi ^{14}C niandaishuju yinqi de kunhuo" wenti (Discussion on the issue of the confusion caused by the ^{14}C Dates of Early Neolithic remains from South China again) (in Chinese). *Wenbo* 4, 42–50. doi:10.3969/j.issn.1000-7954.2021.04.005

Cheng, Z., Weng, C., Steinke, S., and Mohtadi, M. (2018). Anthropogenic Modification of Vegetated Landscapes in Southern China from 6,000 Years Ago. *Nat. Geosci* 11, 939–943. doi:10.1038/s41561-018-0250-1

Chi, Z., and Hung, H.-c. (2012). Later hunter-gatherers in Southern China, 18 000-3000 BC. *Antiquity* 86, 11–29. doi:10.1017/S0003598X00062438

Deng, Z., Hung, H.-c., Li, Z., Carson, M. T., Huang, Q., Huang, Y., et al. (2019). Food and Ritual Resources in hunter-gatherer Societies: *Canarium* Nuts in Southern China and beyond. *Antiquity* 93, 1460–1478. doi:10.15184/aqy.2019.173

Deng, Z., Huang, B., Zhang, Q., and Zhang, M. (2022). First Farmers in the South China Coast: New Evidence from the Gancaoling Site of Guangdong Province. *Front. Earth Sci.* 10, 858492. doi:10.3389/feart.2022.858492

Denham, T., Zhang, Y., and Barron, A. (2018). Is There a centre of Early Agriculture and Plant Domestication in Southern China? *Antiquity* 92 (365), 1165–1179. doi:10.15184/aqy.2018.156

Fu, X., Li, X., Li, Z., Zhang, L., and Chen, C. (1998). Guangxi Yongningxian Dingsishan yizhi de fajue (Discovery of Dingsishan Site in Yongning County, Guangxi) (in Chinese). *Archaeology* 11, 11–33. CNKI:SUN:KAGU.0.1998-11-001.

Fu, X. (2002). The Dingsishan Site and the Prehistory of Guangxi, Southern China. *Bull. Indo-Pacific Prehistory Assoc.* 22, 63–72.

Gong, W., and Gong, Q. (2013). Huanan Xinshiqi shidai zaoqi wenhuayicun de faxian ji xiangguan wenti de tantao (Discovery and discussion of Early Neolithic Cultural remains in South China) (in Chinese). *Shiqian Yanjiu*, 125–150.

Guan, Y., Pearsall, D. M., Gao, X., and Zhou, Z. (2010). Shizhipin zhiwu canliuwu fenxi de shiyanshi fangfa yi Shuidonggou shizhipin weili (Plant residue analysis method of stone artifacts in the laboratory: Based on the Shuidonggou lithic materials) (in Chinese). *Acta Anthropologica Sinica* 29, 395–404.

Guan, Y., Pearsall, D. M., Gao, X., Chen, F., Pei, S., and Zhou, Z. (2014). Plant Use Activities during the Upper Paleolithic in East Eurasia: Evidence from the Shuidonggou Site, Northwest China. *Quat. Int.* 347, 74–83. doi:10.1016/j.quaint.2014.04.007

Guan, Y., Wang, C., Zhou, Z., Cheng, J., Cao, J., Ta, L., et al. (2022). Evidence from Plant Starch Residues of the Function of Early Pottery and the Plant Diet of Neolithic Inhabitants of Inner Mongolia, North China. *Quat. Int.* 608-609, 215–225. doi:10.1016/j.quaint.2020.10.010

Han, J. (2012). Zhongguo Xinshiqishidai zaozhongqi wenhua de quxi yanjiu (Research of Cultural Regions from Early-Middle Neolithic in China) (in Chinese). *Kaoguxue Yanjiu*, 24–36.

Higham, C. (2013). Hunter-gatherers in Southeast Asia: from Prehistory to the Present. *Hum. Biol.* 85, 21–43. doi:10.13110/humanbiology.85.1-3.0021

Klingenberg, C. P. (2011). MorphoJ: an Integrated Software Package for Geometric Morphometrics. *Mol. Ecol. Resour.* 11, 353–357. doi:10.1111/j.1755-0998.2010.02924.x

Li, W., Pagán-Jiménez, J. R., Tsoraki, C., Yao, L., and Van Gijn, A. (2020). Influence of Grinding on the Preservation of Starch Grains from rice. *Archaeometry* 62, 157–171. doi:10.1111/arcm.12510

Li, F. (1990). Shilun Huanan diqu yuanshinongye de qiyuan (The origin of primitive agriculture in South China) (in Chinese). *Nongye Kaogu* 2, 84–95.

Li, W. (1998). *Zhongguo Disiji Zhibei Yu Huanjing (Quaternary Vegetation and Environment in China) (In Chinese)*. Beijing: Science Press.

Liu, Z., Wang, L., Zhou, W., Chen, Y., and Wang, Z. (2011). The Surface of the Geometric Characteristics Analysis for rice Endosperm Starch Granules by Using Image J (In Chinese). *J. Chin. Electron Microsc. Soc.* 30, 466–471. doi:10.3969/j.issn.1000-6281.2011.04.035

Liu, L., Duncan, N. A., Chen, X., and Cui, J. (2019). Exploitation of Job's Tears in Paleolithic and Neolithic China: Methodological Problems and Solutions. *Quat. Int.* 529, 25–37. doi:10.1016/j.quaint.2018.11.019

Liu, L., Ma, S., and Cui, J. (2014). Identification Of Starch Granules Using A Two-Step Identification Method. *J. Archaeol. Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

Lu, H.-Y., Wu, N.-Q., Yang, X.-D., Jiang, H., Liu, K.-b., and Liu, T.-S. (2006). Phytoliths as Quantitative Indicators for the Reconstruction of Past Environmental Conditions in China I: Phytolith-Based Transfer Functions. *Quat. Sci. Rev.* 25, 945–959. doi:10.1016/j.quascirev.2005.07.014

Ma, Z., Perry, L., Li, Q., and Yang, X. (2019). Morphological Changes in Starch Grains after Dehusking and Grinding with Stone Tools. *Sci. Rep.* 9, 2355. doi:10.1038/s41598-019-38758-6

McGovern, P., Jalabadze, M., Batiuk, S., Callahan, M. P., Smith, K. E., Hall, G. R., et al. (2017). Early Neolithic Wine of Georgia in the South Caucasus. *Proc. Natl. Acad. Sci. U.S.A.* 114, E10309–E10318. doi:10.1073/pnas.1714728114

McNulty, K. P., and Vinyard, C. J. (2015). Morphometry, Geometry, Function, and the Future. *Anat. Rec.* 298, 328–333. doi:10.1002/ar.23064

Mitteroecker, P., and Gunz, P. (2009). Advances in Geometric Morphometrics. *Evol. Biol.* 36, 235–247. doi:10.1007/s11692-009-9055-x

Oxenham, M. F., Trinh, H. H., Willis, A., Jones, R. K., Domett, K., Castillo, C., et al. (2018). Between Foraging and Farming: Strategic Responses to the Holocene Thermal Maximum in Southeast Asia. *Antiquity* 92, 940–957. doi:10.15184/aqy.2018.69

Park, P., Aguirre, W., Spikes, D., and Miyazaki, J. (2013). Landmark-based Geometric Morphometrics: What Fish Shapes Can Tell Us about Fish Evolution. Tested Studies for Laboratory Teaching. *Proc. Assoc. Biol. Lab. Educ.* 34, 361–371.

Pearsall, D. M., Chandler-Ezell, K., and Zeidler, J. A. (2004). Maize in Ancient Ecuador: Results of Residue Analysis of Stone Tools from the Real Alto Site. *J. Archaeological Sci.* 31, 423–442. doi:10.1016/j.jas.2003.09.010

Prebble, M., Anderson, A. J., Augustinus, P., Emmitt, J., Fallon, S. J., Furey, L. L., et al. (2019). Early Tropical Crop Production in Marginal Subtropical and Temperate Polynesia. *Proc. Natl. Acad. Sci. U.S.A.* 116, 8824–8833. doi:10.1073/pnas.1821732116

Savriama, Y. (2018). A Step-by-step Guide for Geometric Morphometrics of floral Symmetry. *Front. Plant Sci.* 9, 1433. doi:10.3389/fpls.2018.01433

Slice, D. E. (2007). Geometric Morphometrics. *Annu. Rev. Anthropol.* 36, 261–281. doi:10.1146/annurev.anthro.34.081804.120613

Wan, Z., Yang, X., Ge, Q., and Jiang, M. (2011a). A. Morphological Characteristics of Starch Grains of Root and Tuber Plants in South China. *Quat. Sci.* 31 (4), 10. doi:10.3969/j.issn.1001-7410.2011.04.18

Wan, Z., Yang, X., Ma, Z., and Liu, G. (2011b). B. Morphological Change of Starch Grain Based on Simulated experiment and its Significance of Agricultural Archaeology - Taking Wheat as an Example. *Agric. Sci. Tech.* 12, 1621–1624. doi:10.3969/j.issn.1009-4229-B.2011.11.017

Wan, Z., Li, M., and Li, H. (2016). Study on Morphological Characteristics of Starch Granules in the Triticeae (In Chinese with English Abstract). *J. Triticeae Crops* 36, 1020–1027. doi:10.7606/j.issn.1009-1041.2016.08.07

Wang, Q., Jia, X., Li, M., and Yang, X. (2013). Zhongguo changjian doulei dianfenli xingtai fenxi jiqi zai nongyekaogu zhong de yingyong (The morphological analysis of common edible beans starch grains and the application in agricultural Archaeology) (in Chinese). *Wenwu Chunqiu* 2013, 3–11. doi:10.3969/j.issn.1003-6555.2013.03.001

Wang, J., Zhao, X., Wang, H., and Liu, L. (2019). Plant Exploitation of the First Farmers in Northwest China: Microbotanical Evidence from Dadiwan. *Quat. Int.* 529, 3–9. doi:10.1016/j.quaint.2018.10.019

Wang, W. W. (2017). The Utilization of Roots and Tubers in Prehistoric China. Undergraduate Dissertation. Beijing: University of Chinese Academy of Sciences.

Webster, M., and Sheets, H. D. (2010). A Practical Introduction to Landmark-Based Geometric Morphometrics. *Paleontol. Soc. Pap.* 16, 163–188. doi:10.1017/S1089332600001868

Yang, X., and Perry, L. (2013). Identification of Ancient Starch Grains from the Tribe Triticeae in the North China Plain. *J. Archaeological Sci.* 40, 3170–3177. doi:10.1016/j.jas.2013.04.004

Yang, X., Kong, Z., Liu, C., Zhang, Y., and Ge, Q. (2009). Characteristics of Starch Grains from Main Nuts in North China. *Quat. Sci.* 29 (1), 153–158. doi:10.1007/s11461-009-0031-5

Yang, X., Zhang, J., Perry, L., Ma, Z., Wan, Z., Li, M., et al. (2012). From the Modern to the Archaeological: Starch Grains from Millets and Their Wild Relatives in china. *J. Archaeological Sci.* 39 (2), 247–254. doi:10.1016/j.jas.2011.09.001

Yang, X., Barton, H. J., Wan, Z., Li, Q., Ma, Z., Li, M., et al. (2013a). Sago-Type Palms Were an Important Plant Food Prior to Rice in Southern Subtropical China. *PLoS One* 8 (5), e63148. doi:10.1371/journal.pone.0063148

Yang, X., Ma, Z., Li, Q., Perry, L., Huan, X., Wan, Z., et al. (2013b). Experiments with Lithic Tools: Understanding Starch Residues from Crop Harvesting. *Archaeometry* 56, 828–840. doi:10.1111/arcm.12034

Yang, X., Wang, W., Zhuang, Y., Li, Z., Ma, Z., Ma, Y., et al. (2017). New Radiocarbon Evidence on Early rice Consumption and Farming in South China. *The Holocene* 27 (7), 1045–1051. doi:10.1177/0959683616678465

Yang, X., Chen, Q., Ma, Y., Li, Z., and Hung, H. (2018). New Radiocarbon and Archaeobotanical Evidence Reveal the Timing and Route of Southward Dispersal of rice Farming in south China. *Sci. Bull.* 63, 43–49. doi:10.1016/j.scib.2018.10.011

Yu, C. (2018). Xinshiqi shidai zhi Qingtong shidai Lingnan ji zhoubiandiqu de shengye chutan (Preliminary research of subsistence patterns from Neolithic to Bronze Age in Lingnan and surrounding Area China) (in Chinese). *Nanfang Wenwu* 2, 180–187.

Zelditch, M., Swiderski, D., Sheets, H. D., and Fink, W. (2004). *Geometric Morphometrics for Biologists: A Primer*. New York and London: Elsevier Academic Press.

Zhang, C., and Hung, H. (2009). Huanan he Xinan diqu nongye chuxian de shijian ji xiangguanwenti (The time and issues of agricultural origin in South and Southwest China) (in Chinese). *Nanfang Wenwu* 3, 64–71.

Zhang, M., Lin, Y., Qin, J., and Li, B. (2000). The Formation Environment and Paleoclimatic Significance of No.1 Stalagmite in Xianshui Cave, Guanyang County, Guangxi. *Geology-Geochemistry* 1, 34–40.

Zhang, M., Yuan, D., Lin, Y., Qin, J., and Zhang, C. (2003). High Resolution Climatic Records of Stalagmite from Xianshui Cave in Guilin since 6.00 Ka BP. *Acta Geoscientia Sinica* 5, 439–444. doi:10.1016/S0955-2219(02)00073-0

Zhang, X., Zhu, X., Hu, Y., Zhou, Z., Olsen, J., and Guan, Y. (2021). Ancient Starch Remains Reveal the Vegetal Diet of the Neolithic Late Dawenkou Culture in Jiangsu, East China. *Front. Ecol. Evol.* 9, 722103. doi:10.3389/fevo.2021.722103

Zhao, Z., Fu, x., and Lu, L. (2005). Guangxi Yongningxian Dingsishan yizhi chutu zhiguishi de fenxi yu yanjiu(Analysis and research on phytoliths unearthed from Dingsishan Site in Yongning, Guangxi) (in Chinese). *Archaeology* 11, 76104–76184.

Zhao, Z. (2006). "Dui Huanan diqu yuanshinongye de zairenshi (Rethinking of primitive agriculture in South China) (in Chinese)," in Huanan ji Dongnanya diqu shiqian kaogu jinian Zengpiyan yizhi fajue 30 zhounian guoji xueshu yantaohui lunwenji (International seminar symposium to Commemorate the 30th Anniversary of the Excavation of Zengpiyan Site), Guilin city, Guangxi Province, December 10–14, 2003 (Beijing: Institute of Archaeology, Chinese Academy of Social Sciences Cultural relics publishing house), 145–156.

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The Emergence of Rice and Millet Farming in the Zang-Yi Corridor of Southwest China Dates Back to 5000 Years Ago

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The Zang-Yi Corridor is of pivotal significance for the interactions between northwest China, southwest China, and mainland Southeast Asia. It has been hypothesized that the formation of mixed farming in this region and its surrounding areas was based on multiple waves of crop dispersal, with foxtail millet and broomcorn millet arriving first from northwest China around 5,300 cal. BP and rice from middle Yangtze valley after 4,700 cal. BP. Based on the systematic sampling and direct dating conducted at the Guijiabao site, Sichuan Province, this study demonstrates that by no later than 5,000 cal. BP, mixed farming had already emerged in the south part of Zang-Yi corridor, which was much earlier than expected before. With this new evidence, it is argued that the transformation into farming in Southwest China was based on the dispersal of a crop package comprising foxtail millet, broomcorn millet, and rice instead of different waves of introduction. A further comparison of all archaeobotanical data in this region revealed that crop patterns varied significantly between different sites because of their diverse environmental conditions.

Keywords: rice, millet, phytolith, mixed farming, crop dispersal, southwest China, mainland Southeast Asia

INTRODUCTION

The emergence of agriculture is of profound significance in human history, as it brought thorough change to the lifestyle of human beings and set a solid economic foundation for the further progress of human societies (Childe, 1936; Bellwood, 2005; Bellwood, 2013; Fuller and Stevens, 2019). However, the transformation into farming for most parts of the world was counted on the diffusion of domesticated plants and related technologies (Bellwood, 2013; Fuller and Lucas, 2017), as the origin of agriculture only happened in limited centers around the world (Diamond, 2002; Fuller, 2010). Continued interactions and exchanges between these independent agriculture origin centers brought local and exotic crops together, enabling a more diverse dietary and stable crop production to support a larger population (Jones et al., 2016; Liu et al., 2016). Hence, the study of agricultural diffusion is of crucial significance to our understanding of ancient social development and interregional communications.

With two distinct agricultural systems, China is a key center for the investigation of agriculture origins (Fuller et al., 2014; Larson et al., 2014; Lu, 2017). Early farming practice in northern China

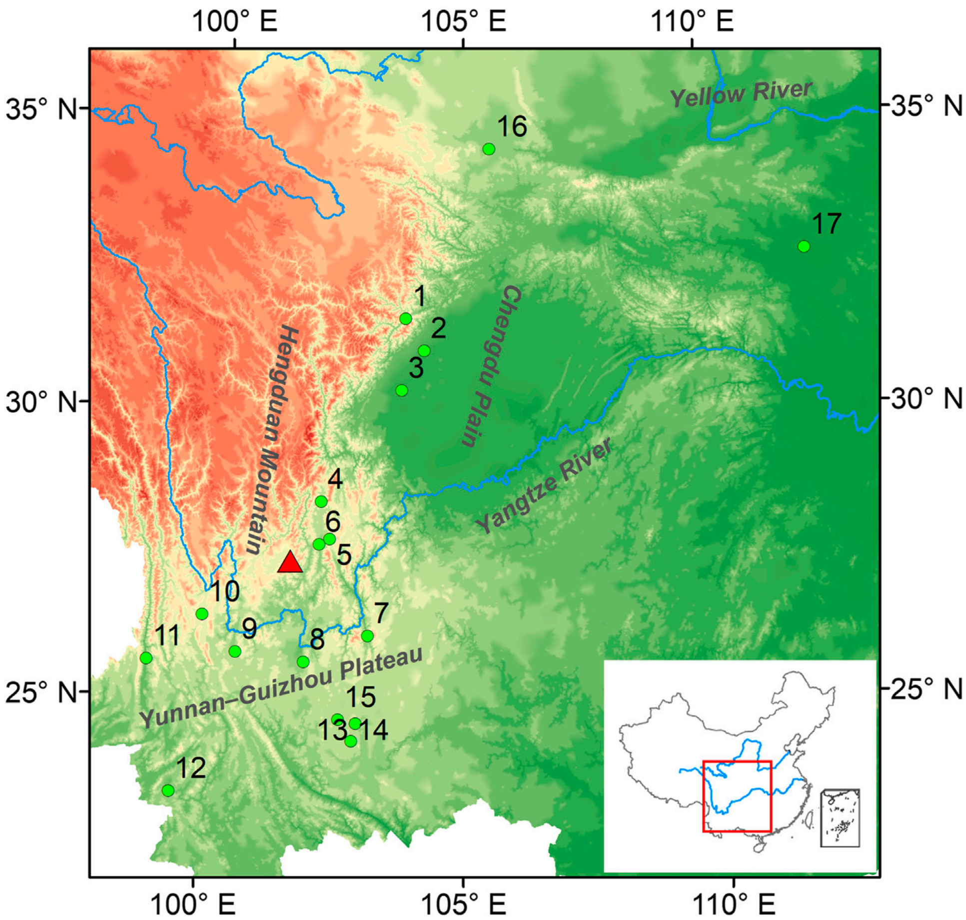

FIGURE 1 | Location of the Guijiabao site (red triangle) and other sites mentioned in the study. 1-Yingpanshan, 2-Guiyuanqiao, 3-Baodun, 4-Gaopo, 5-Henglanshan, 6-Shapingzhan, 7-Yubeidi, 8-Dadunzi, 9-Baiyangcun, 10-Haimenkou, 11-Shilinggang, 12-Shifodong, 13-Xueshan, 14-Guangfentou, 15-Heposuo,16-Xishanping, 17-Baligang.

was based on local domestication of foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum miliaceum*) (Lu et al., 2009a; Yang et al., 2012), while in southern China, especially the middle and lower Yangtze Valley, rice (*Oryza sativa*) had been domesticated no later than 8,500 cal. BP and cultivated as a staple crop since then (Deng et al., 2015; Zuo et al., 2017; Huan et al., 2021). In this case, the agriculturalization of other regions was all realized through population expansion and agriculture dispersal from these two centers. With the progress in archaeological research over the past decades, it has become increasingly evident that the southward dispersal of agriculture in China was mainly along three main routes, among which the Zang-Yi Corridor is of critical significance in subsistence transformation and social development in southwest China, including the Chengdu Plain, the Yunnan-Guizhou Plateau, and the Tibetan Plateau (He et al., 2017; Deng et al., 2018; Gao et al., 2021). Further influence of this route could even have reached mainland Southeast Asia and joined the long-distance communications along the southern foothills of the Himalayan Mountains with south Asia and beyond (Gao et al., 2020). Therefore, many researchers have been dedicated to investigating the dispersal and development of farming in this region.

To date, the earliest evidence of agriculture in the Zang-Yi Corridor is from the Yingpanshan site on the northern edge of this region. Large amounts of foxtail millet and broomcorn millet have been recovered there and traced back to ca. 5,300 cal. BP (Zhao and Chen, 2011). Typological comparisons of pottery from this site revealed the strong influence of contemporary Majiayao culture in the Gansu province. Hence, it is widely believed that these earliest Neolithic populations migrated from northwest China. A detailed chemical composition analysis of painted pottery from Yingpanshan also supports close interactions between local people and the Majiayao communities (Hung, 2011). Whereas, plant remains from many later sites of southwest China all suggest a mixed pattern of crop assemblages, comprising foxtail millet, broomcorn millet, and rice (Guo, 2011; Huang et al., 2011; Yan et al., 2013; Jin et al., 2014; Jiang et al., 2016a; Jiang et al., 2016b; Dal Martello et al., 2018). Among these discoveries, the earliest evidence came from Guiyuanqiao and Baodun, where rice was directly dated to ca. 4,700 cal. BP (Guedes et al., 2013; Guedes and Wan, 2015).

TABLE 1 | AMS Radiocarbon dating results of Neolithic contexts from the Guijiabao site. All dates were calibrated by OxCal v4.4.4, using the IntCal 20 Atmospheric curve (Reimer et al., 2020).

Lab code	Context no	Dated Material	Uncalibrated ^{14}C Date (BP)	Calibrated Dates (2σ)
BA170230	H1	Foxtail millet	4,480 ± 40	5,300–4,975
BA192710	TN22E39②	Broomcorn millet	4,455 ± 45	5,290–4,883
BA170249	H20	Rice	4,365 ± 25	5,030–4,856
BA190411	TN32E37⑦	Broomcorn millet	4,205 ± 25	4,845–4,626
BA190424	F21	Broomcorn millet	4,065 ± 20	4,787–4,442
BA190402	TN30E34③	Broomcorn millet	4,045 ± 25	4,612–4,421
BA190436	H127	Broomcorn millet	3,885 ± 25	4,413–4,239
BA190403	TN30E34④	Foxtail millet	3,770 ± 35	4,245–3,988
BA190437	H135	Broomcorn millet	3,635 ± 30	4,081–3,849
BA190389	H166	Rice	3,505 ± 25	3,847–3,693

In this regard, it is hypothesized that the formation of mixed farming in southwest China is based on two waves of agricultural dispersal: first from northwest China with the introduction of foxtail millet and broomcorn millet around 5,300 cal. BP, and second from the middle Yangtze valley with the introduction of rice after 4,700 cal. BP (Guedes et al., 2013). However, this speculation is still debatable for two reasons. Firstly, whether the earliest crop pattern only consists of foxtail millet and broomcorn millet or the entire package of millets and rice is still ambiguous, as there is no evidence from other contemporary sites of Yingpanshan. Secondly, no evidence reveals interregional communications between the middle Yangtze Valley and Southwest China prior to 4,500 cal. BP (Jiang et al., 2020), making the speculated introduction of rice from the middle Yangtze valley questionable. Therefore, a more targeted study should be conducted at these early sites in the region to test or modify this model.

Here, we report the systematic phytolith analysis at the Guijiabao site in Sichuan Province. The new results, along with radiocarbon dating, reveal mixed farming had already emerged in the southern part of the Zang-Yi Corridor no later than 5,000 cal. BP, which is much earlier than expected before. With this new discovery, it is quite possible that all these crops, including foxtail millet, broomcorn millet, and rice, were introduced into this region as a package, and the source of agriculture introduction into this region should also be re-examined, especially the possibility of middle Yangtze valley.

MATERIALS AND METHODS

Sample Collection

The Guijiabao site (101.6°E, 27.45°N) (**Figure 1**) is situated in the Yanyuan County of Sichuan Province and is surrounded by the middle part of the Hengduan Mountains (Zhong et al., 2016; Hao et al., 2022). As a crossroad in the southern part of the Zang-Yi Corridor, this region has played a crucial role in interregional interactions along this route. Guijiabao was first discovered in 2015, and a systematic field survey and small-scale excavation were conducted to confirm the preservation conditions and major remains in the site. Subsequently, excavations were carried out in three seasons from 2016 to 2018.

According to archaeological investigations, the total area of Guijiabao is around 30,000 m^2, but cultural deposits have been heavily disturbed by modern activities. Even though, a large number of ancient ruins have been unearthed, including house foundations, pits, tombs, and many artifacts such as pottery, stone tools, and spindle whorls (Hao et al., 2022). All of these remains could be grouped into three periods, namely: Neolithic, Bronze Age, and historical period, among which the Neolithic remains could further be divided into two phases. According to the result of systematic radiocarbon dating at the site (**Table 1**), Phase I of the Neolithic period at this site occurred around 5,000–4,500 cal. BP, and Phase II around 4,500–3,700 cal. BP. Moreover, the beginning of human activities in this period probably could reach 5,300 cal. BP as revealed by radiocarbon dates of foxtail millet grains from context H1 and broomcorn millet grains from context TN22E39②. The date of the Bronze Age remains at the site is roughly 3,200–2,700 cal. BP, where a hiatus of 500 years after the Neolithic period could be observed. Historical relics occurred much later, mainly stretching from the late Northern Song dynasty to the Ming dynasty (ca. 1,200–500 cal. BP).

In order to detect Neolithic agriculture practices at the Guijiabao site, soil samples for phytolith analysis have been collected during the excavations. In total, 154 samples from the Neolithic period were obtained, of which 16 were from Phase I and 138 from Phase II (details in **Supplementary Table S1**). These sampled contexts cover different types of archaeological features of the site, including cultural layers (sample codes start with T), pits (sample codes start with H), house foundations (sample codes start with F), and ditches (sample codes start with G).

Phytolith Extraction and Identification

Phytoliths were extracted from soil samples according to established methods (Piperno, 1988; Lu et al., 2002) with minor modifications. Initially, approximately 2 g of each sample was weighed and treated with 30% H_2O_2 and 15% HCl to remove organic matter and carbonate. The samples were then subjected to heavy liquid flotation using $ZnBr_2$ (density, 2.35 g/cm^3) to separate the phytoliths, which were subsequently mounted on a slide using Canada Balsam. After air-drying, the phytoliths on the slide were counted and identified using a Leica microscope at ×400 magnification. More than 400 phytolith particles in each sample were identified and recorded according to previously published

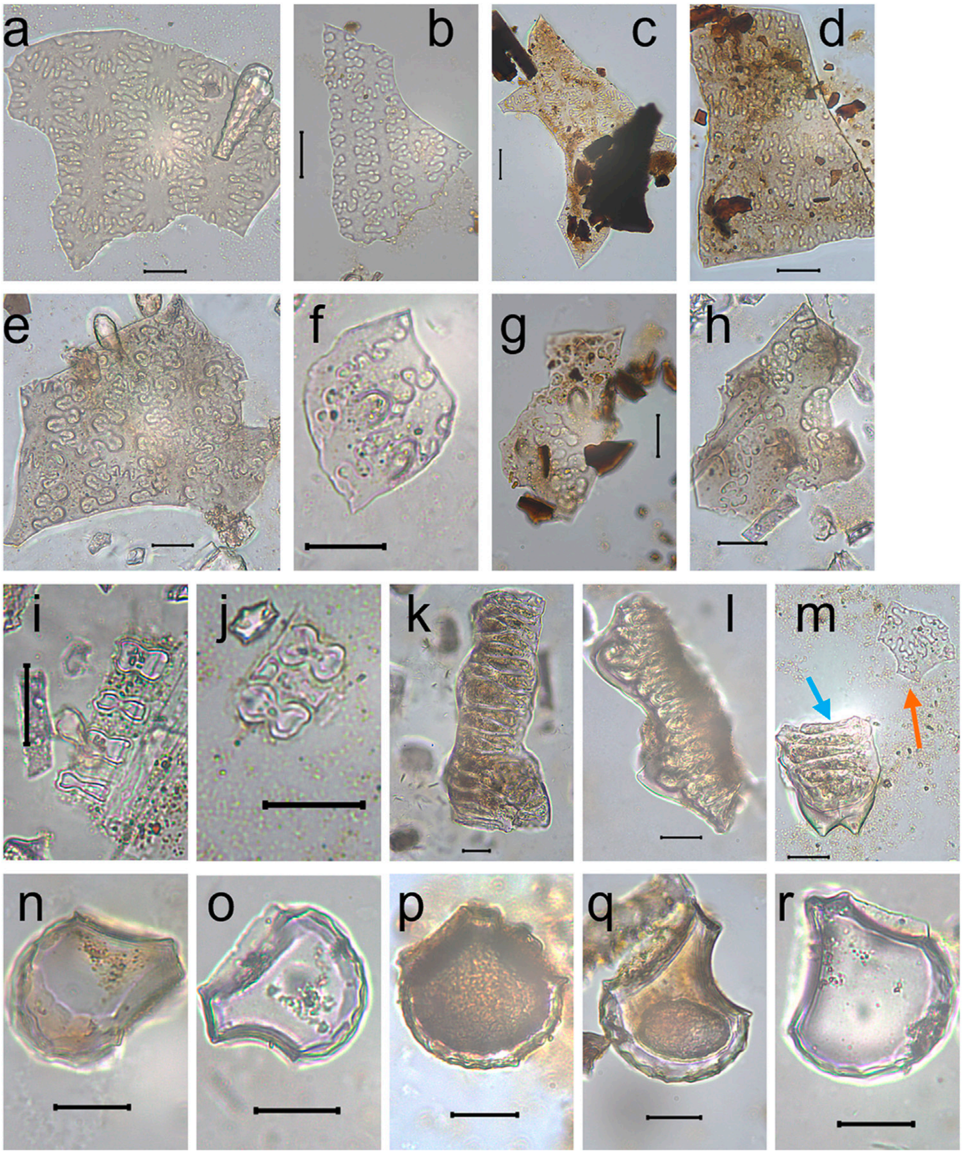

FIGURE 2 | Crop phytoliths obtained from the Guijiabao site. **(A–D)**: broomcorn millet η-type; **(E–H)**: foxtail millet Ω-type; **(I,J)**: rice parallel bilobate; **(K,L)**: rice double-peaked; **(M)**: rice double-peaked (blue arrow) and broomcorn millet η-type (orange arrow); **(N,O)**: rice bulliform with <9 scales; **(P–R)**: rice bulliform with ≥9 scales (scale bar = 20 μm).

references and criteria (Wang and Lu, 1993; Lu et al., 2006; Lu et al., 2007; Lu et al., 2009b; Ge et al., 2018; Ge et al., 2020a; Ge et al., 2020b). In particular, for samples with rice phytoliths, the slides were scanned until 50 rice bulliform phytoliths with clear and countable scales were observed to calculate the proportion of rice bulliform phytoliths with ≥9 scales (Wang and Lu, 2012; Huan et al., 2015; Huan et al., 2020).

RESULTS

All 154 samples yielded abundant phytoliths. In total, 26 phytolith morphotypes were identified, of which five were confirmed from crops, including double-peaked, bulliform, and parallel-bilobate types from rice, η-type epidermal long cell phytoliths from the upper lemma and palea of broomcorn millet, and Ω-type from foxtail millet (**Figure 2**). Other main phytolith types include elongate psilate, square, rondel, hair cell, rectangle, bilobate, elongate echinate, bulliform, and long saddle.

Phytolith assemblages in the Guijiabao site were characterized by high proportions of elongate psilate, elongate echinate, square, rectangle, rondel, hair cell, and bilobate, along with a low proportion of rice and a relatively lower proportion of broomcorn millet morphotypes (**Figure 3**; **Supplementary Table S1**). Foxtail millet phytoliths appeared in Phase II, but their proportions were much lower than those of broomcorn

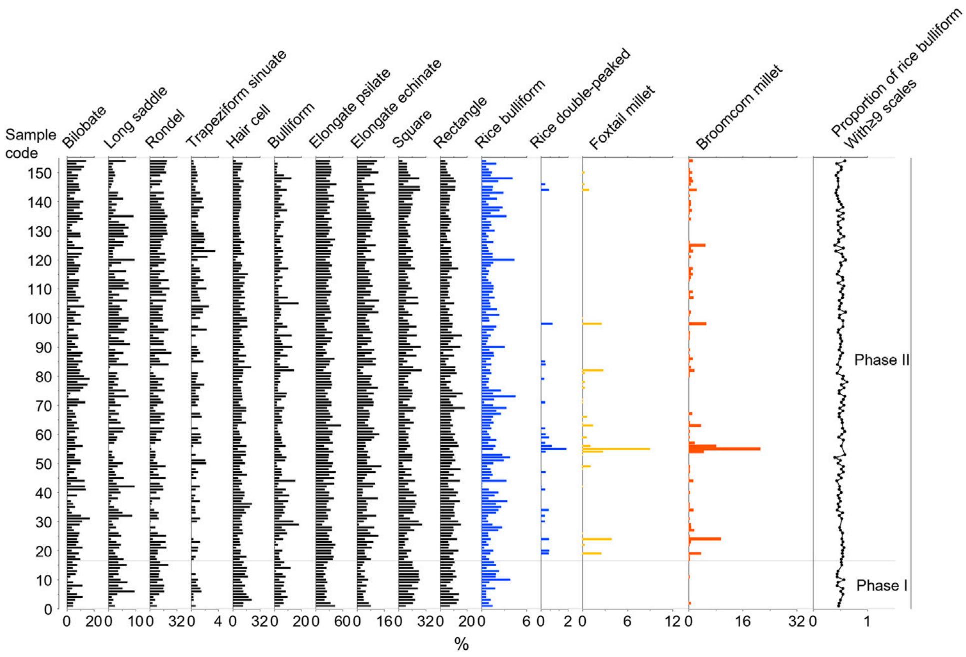

FIGURE 3 | Percentage diagram of major phytolith types at the Guijiabao site. Sample code in **Supplementary Table S1**.

millet and rice (**Figure 3**). It is also worth noting that the phytolith assemblage of Phase II was characterized by the presence of double-peaked types of rice husk, revealing a strong relationship between cultural deposits in these contexts and rice dehusking activities at the site (Harvey and Fuller, 2005). The presence of bulliform and parallel-bilobate types from rice leaves and stems also demonstrates local cultivation of rice.

Based on the preservation conditions, 139 samples were selected for further analysis of scales on the edge of rice bulliform phytoliths, including 14 from Phase I and 125 from Phase II (**Figure 3**; **Supplementary Table S1**). For each sample, at least 50 bulliform phytoliths with clear and countable scales were carefully observed. The average proportion of bulliform phytoliths with ≥9 scales was 49.40 ± 4.44% in Phase I and 50.63 ± 4.63% in Phase II.

DISCUSSION

The Emergence of Mixed Farming in Southwest China

The Zang-Yi Corridor is a significant channel for interregional communications and human movements in southwest China during historical and modern times (Fei, 1980). Archaeological investigations in the past decades further suggest that cultural interactions, population migration, and the flow of raw materials and technologies along this route started much earlier than previously thought (Huo, 2005). The Neolithization process of the entire southwest region was based on the introduction of these innovations and possible human migrations, among which the emergence of agriculture was no doubt in the central place.

The new data presented in this study clearly show that abundant rice bulliform phytoliths were present in all samples from Phase I (5,000–4,500 cal. BP) at the Guijiabao site, together with the typical η-type phytoliths of broomcorn millet. A detailed analysis of scales on the edge of rice bulliform phytoliths suggested that the proportion of rice bulliform phytoliths with ≥9 scales in Phase I was 49.40 ± 4.44% (**Figure 3**), which was close to Phase II level (50.63 ± 4.63%) and modern domesticated rice (Huan et al., 2015; Huan et al., 2020). Considering the current AMS radiocarbon dates of this phase, especially one direct date of rice grain (BA170249, 5,030–4,856 cal. BP, 95.4% probability), these lines of evidence could confirm that rice had already been cultivated together with broomcorn millet in the southern part of Zang-Yi Corridor prior to 5,000 cal. BP.

The continuation of this mixed farming strategy could be demonstrated by crop assemblages from Phase II (4,500–3,700 cal. BP) of Guijiabao, when phytoliths of rice, broomcorn millet, and foxtail millet were found together in nearly all samples (**Figure 3**). A comparison of these phytoliths revealed that their proportions varied greatly among different samples, but generally rice and broomcorn millet were more important than foxtail millet. Nevertheless, given the different preservation conditions of these phytoliths, the specific crop pattern needs to be further confirmed by other evidence, such as macroscopic plant remains and stable isotope analyses of human bones. Another point that needs to be clarified here is the absence of foxtail millet phytoliths in all samples of Phase I in this study, which may have resulted from the relatively poor preservation conditions of foxtail millet phytoliths, as foxtail millet grains have been recovered in this phase and directly dated (**Table 1**). Another possible reason is that the samples from Phase I in this study were limited.

Overall, the present study clearly suggests that rice was cultivated together with foxtail millet and broomcorn millet from 5,000 cal. BP to 3,700 cal. BP at the Guijiabao site. Along with discoveries from other sites, it could be confirmed that they were introduced simultaneously as a package into the Zang-Yi Corridor and then other parts of southwest China at the very beginning of the Neolithic period in the region, which differs from the previously assumed process of multiple waves of crop introduction (d'Alpoim Guedes, 2011). Actually, the pure millet crop pattern of Yingpanshan, which is the main evidence of the previous model, is also questionable. The absence of rice at the Yingpanshan site might have resulted from insufficient sampling, as only 45 L of soil samples from nine contexts were processed (Zhao and Chen, 2011). Another piece of evidence in support of this query is that stable isotope analysis of human bones from two contexts of Yingpanshan indicated that their long-term diets after childhood consisted of both C_3 and C_4 foods instead of pure C_4 millets (Lee et al., 2020). In this case, these lines of evidence all tend to support a dispersal of crop package including foxtail millet, broomcorn millet and rice into this region.

On the other hand, the hypothesis that the middle Yangtze region as the source of rice for Southwest China is also unreliable, as no evidence supports the early communication between these two regions prior to 4,500 cal. BP. Even around 4,000 cal. BP, except for the sparse traits of pottery styles that are speculated to be influenced by the middle Yangtze region, no other evidence has been found (Jiang et al., 2020). In contrast, the southern part of Gansu Province is not a pure millet farming region as assumed before. Previous studies have shown that mixed farming has been carried out in many regions of Northern China since 7,500 cal. BP (Zhang et al., 2012; Wang et al., 2017; Wang et al., 2018), and the westernmost discovery was at the Xishanping site of Gansu Province, where charred seeds and phytoliths of foxtail millet, broomcorn millet, and rice have been dated back to 5,300–4,800 cal. BP (Li et al., 2007a; Li et al., 2007b). Plant remains from a regional survey in the Li County of southern Gansu also found rice grains in three sites (Ji, 2007). Therefore, it is quite possible that mixed farming was practiced at many sites in the southern part of Gansu province, and the crop package of millets and rice in southwest China was introduced from this region at the same time.

From a broader view, another possible region related to the emergence of mixed farming in Southwest China is the upper Han River valley, where mixed farming should also have been practiced prior to the Yangshao period, referring to the discoveries in the middle Han River Valley (Deng et al., 2015). Typological comparisons of pottery from the Luojiaba site in the Jialing River valley revealed influences of the Yangshao culture in the upper Han River valley (Li, 2018), thus this channel may serve as another parallel dispersal route in the eastern part of the Sichuan Basin. To clarify the emergence of mixed farming in Southwest China, more targeted research in these regions is needed in the future.

Crop Patterns and Regional Diversity in the Zang-Yi Corridor of Southwest China

With the flow of populations and emergence of innovative technologies, the adoption, adaptation, and integration of exotic elements has been a unique cultural feature of the Zang-Yi Corridor, which could also be observed in the farming strategies. The mixed cropping system has been widely practiced not only in the Neolithic Age, but also in later periods, owing to its complex ground features and environmental conditions. There are seven mountains and six large rivers in the Hengduan Mountainous region, which can be divided into 13 geomorphological zones, covering 19 climatic zones from the tropical zone to plateau frigid zone (Li, 1989; Zhang, 1989). The flexibility of farming practices is clearly reflected in the regional diversity and chronological changes in the cropping systems of this region.

During the Neolithic Period (ca. 5,300–3,600 cal. BP), although foxtail millet, broomcorn millet, and rice were widely cultivated in most sites, the proportion of each crop varied considerably among the four sites with systematic archaeobotanical work (**Figure 4**). Foxtail millet and broomcorn millet were cultivated at the Yingpanshan site and played almost equivalent roles in its farming system (Zhao and Chen, 2011). However, in Phase I of Baiyangcun (4,600–4,300 cal. BP), rice was undoubtedly dominant in the farming system, and foxtail millet was also important. Nevertheless, broomcorn millet only accounted for 2.21% of all crop remains (Dal Martello et al., 2018). This pattern is similar to that of Henglanshan, but plant remains of this site are too limited, and the data are not sufficiently representative (Jiang et al., 2016a; Jiang et al., 2016b). In Phases II and III of Baiyangcun (4,200–4,050 cal. BP), a noticeable change occurred when foxtail millet became the most important crop, followed by rice. The Dadunzi site in Yunnan Province (4,000–3,600 cal. BP) presented a different pattern dominated by foxtail millet, where broomcorn millet was also important. However, the proportion of rice was relatively low (Jin et al., 2014). In addition, wild soybeans were also found at Yingpanshan and Baiyangcun during this period, which was possibly utilized on a small scale.

Archaeobotanical evidence from the early phase of Bronze Age (ca. 3,600–2,800 cal. BP) was more limited as macroscopic plant remains were only recovered from four sites. Unlike the previous period, rice was the main cereal crop in all these sites, accounting for 62.5 to 97.7% of all crop remains. The proportion of foxtail millet in the early phase of Haimenkou was more than 22.88% (Xue, 2010; Xue et al., 2022), but less than 5% in Shifodong and Shapingzhan (Zhao, 2010; Yan et al., 2016b). Broomcorn millet was very rare during this period, with only one grain from Gaopo and two grains from Haimenkou reported (Xue, 2010; Jiang et al., 2013). Another significant change in farming practices of this period is the adoption of wheat and barley, as indicated by the plant remains from Haimenkou, where 261 wheat and seven barley grains have been found. Although wheat and barley were dispersed into the northwestern part of Xinjiang around 5,000 cal. BP (Zhou et al., 2020), the earliest evidence in the Hexi Corridor was only approximately 4,000 cal. BP (Dodson et al., 2013) and 3,600 cal. BP in Central China (Deng et al., 2020). Therefore, the mountainous area of the Zang-Yi Corridor was one of the earliest regions to adopt wheat and barley in southern China. By contrast, no solid evidence of wheat has been reported in the contemporary Chengdu Plain. Cannabis and buckwheat were also found in Haimenkou, revealing more diversified crop patterns since early Bronze Age.

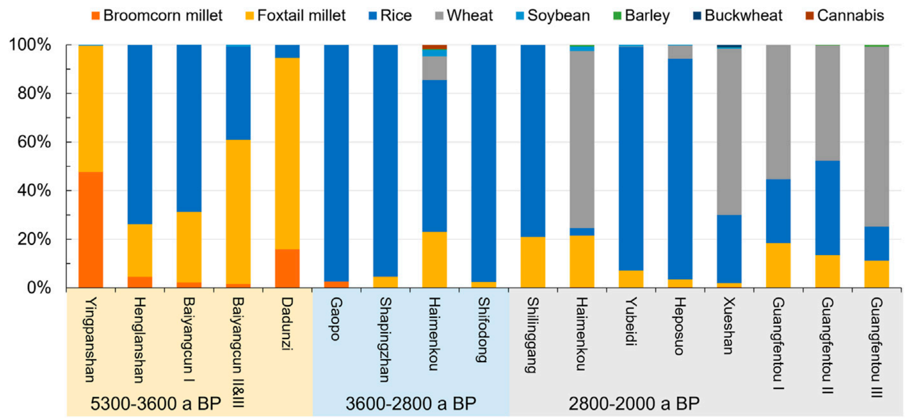

FIGURE 4 | Diachronic charts of crop patterns in the Zang-Yi Corridor of southwest China.

During the late Bronze Age (ca. 2,800–2000 cal. BP), the importance of wheat was greatly improved, which has been found in nearly all sites except for Shilinggang, and is dominant in the crop assemblages of Haimenkou, Guangfentou, and Xueshan (Xue, 2010; Li et al., 2016; Li and Liu, 2016; Wang et al., 2019). Even though, the specific crop patterns at these sites were still different from each other. Foxtail millet was the secondary important crop in the late phase of Haimenkou, with rice and broomcorn millet accounting for a small portion of the entire assemblage (Xue, 2010; Xue et al., 2022). However, in the three phases of Guangfentou and Xueshan, the proportion of rice is much higher than that of foxtail millet (Li and Liu, 2016; Wang et al., 2019). In addition, plant remains from Heposuo and Yubeidi both demonstrated a rice-dominated crop pattern, and the proportions of foxtail millet and rice were slightly different from each other (Yang et al., 2020; Yang et al., 2021). In contrast, broomcorn millet, barley, and soybean were only sparsely found at these sites, which could possibly be used as risk-buffering crops by the locals.

Overall, a comparison of crop patterns from all sites in the different periods of the Zang-Yi Corridor clearly demonstrates that mixed farming and regional diversity are the main characteristics of the agricultural practices in the region. During the Neolithic period, foxtail millet, broomcorn millet, and rice were all staple crops, although their proportions greatly varied in different sites. With the introduction of wheat in approximately 3,600 cal. BP, this exotic crop became the most fundamental crop along with foxtail millet and rice in the Bronze Age, while broomcorn millet was only discovered sparsely and accounted for a low proportion. The conditions were similar for the newly introduced barley. Soybeans, cannabis, and buckwheat have also been utilized on a small scale by local people, forming a more diversified crop pattern.

CONCLUSION

Based on systematic phytolith analysis and direct dating of plant remains from Guijiabao in the south part of the Zang-Yi Corridor, this study shed new light on our understanding of the emergence of farming practice in Southwest China. The new data demonstrated that prior to 5,000 cal. BP, rice, foxtail millet, and broomcorn millet were cultivated and consumed together at the Guijiabao site. With new evidence, the previous hypothesis on the dispersal of agriculture in Southwest China needs to be reconsidered. Rice should have been introduced into this region together with foxtail millet and broomcorn millet as a package around 5,000 cal. BP or even earlier, instead of from the two waves of dispersal from Northwest China and the middle Yangtze Valley, respectively. Plant remains from the late period of Guijiabao and comparison with archaeobotanical remains from other sites further revealed that mixed cropping systems had been widely practiced throughout the Neolithic and Bronze Age in the Zang-Yi corridor. Moreover, great regional diversity could be observed with specific crop patterns, which could have resulted from adaptation to complex ground features and environmental conditions. With the introduction of wheat around 3,600 cal. BP, local crop patterns became more complex, starting a new era in the history of agricultural development in this region.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XHu and ZD designed the study. ZD, ZZ, XY, XHa, and QB conducted archaeological excavation and sample collection. XHu, ZD, and HL completed sample processing and identification. XHu and ZD analyzed the data and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.874649/full#supplementary-material

REFERENCES

Bellwood, P. S. (2013). *First Migrants: Ancient Migration in Global Perspective*. Wiley Blackwell.

Bellwood, P. S. (2005). *The First Farmers: The Origins of Agricultural Societies*. Malden: Blackwell Pub.

Childe, V. G. (1936). *Man Makes Himself*. London: Watts.

Dal Martello, R., Min, R., Stevens, C., Higham, C., Higham, T., Qin, L., et al. (2018). Early Agriculture at the Crossroads of China and Southeast Asia: Archaeobotanical Evidence and Radiocarbon Dates from Baiyangcun, Yunnan. *J. Archaeological Sci. Rep.* 20, 711–721. doi:10.1016/j.jasrep.2018.06.005

d'Alpoim Guedes, J. (2011). Millets, rice, Social Complexity, and the Spread of Agriculture to the Chengdu Plain and Southwest China. *Rice* 4, 104–113. doi:10.1007/s12284-011-9071-1

Deng, Z., Fuller, D. Q., Chu, X., Cao, Y., Jiang, Y., Wang, L., et al. (2020). Assessing the Occurrence and Status of Wheat in Late Neolithic central China: the Importance of Direct AMS Radiocarbon Dates from Xiazhai. *Veget Hist. Archaeobot* 29, 61–73. doi:10.1007/s00334-019-00732-7

Deng, Z., Hung, H.-c., Fan, X., Huang, Y., and Lu, H. (2018). The Ancient Dispersal of Millets in Southern China: New Archaeological Evidence. *The Holocene* 28 (1), 34–43. doi:10.1177/0959683617714603

Deng, Z., Qin, L., Gao, Y., Weisskopf, A. R., Zhang, C., and Fuller, D. Q. (2015). From Early Domesticated Rice of the Middle Yangtze Basin to Millet, Rice and Wheat Agriculture: Archaeobotanical Macro-Remains from Baligang, Nanyang Basin, Central China (6700-500 BC). *Plos One* 10 (10), e0139885. doi:10.1371/journal.pone.0139885

Diamond, J. (2002). Evolution, Consequences and Future of Plant and Animal Domestication. *Nature* 418, 700–707. doi:10.1038/nature01019

Dodson, J. R., Li, X., Zhou, X., Zhao, K., Sun, N., and Atahan, P. (2013). Origin and Spread of Wheat in China. *Quat. Sci. Rev.* 72, 108–111. doi:10.1016/j.quascirev.2013.04.021

Fei, X. (1980). Distinguishment of Ethnic Groups in China. *Social Sci. China* 1, 147–162. (in Chinese).

Fuller, D. Q. (2010). An Emerging Paradigm Shift in the Origins of Agriculture. *Gen. Anthropol.* 17 (2), 1–12. doi:10.1111/j.1939-3466.2010.00010.x

Fuller, D. Q., Denham, T., Arroyo-Kalin, M., Lucas, L., Stevens, C. J., Qin, L., et al. (2014). Convergent Evolution and Parallelism in Plant Domestication Revealed by an Expanding Archaeological Record. *Proc. Natl. Acad. Sci. U.S.A.* 111 (17), 6147–6152. doi:10.1073/pnas.1308937110

Fuller, D. Q., and Lucas, L. (2017). "Adapting Crops, Landscapes, and Food Choices: Patterns in the Dispersal of Domesticated Plants Across Eurasia," in *Human Dispersal and Species Movement: From Prehistory to the Present*. Editor N. Boivin, R. Crassard, and M. Petraglia (Cambridge: Cambridge University Press), 304–331.

Fuller, D. Q., and Stevens, C. J. (2019). Between Domestication and Civilization: the Role of Agriculture and Arboriculture in the Emergence of the First Urban Societies. *Veget Hist. Archaeobot* 28 (3), 263–282. doi:10.1007/s00334-019-00727-4

Gao, Y., Dong, G., Yang, X., and Chen, F. (2020). A Review on the Spread of Prehistoric Agriculture from Southern China to mainland Southeast Asia. *Sci. China Earth Sci.* 63 (5), 615–625. doi:10.1007/s11430-019-9552-5

Gao, Y., Yang, J., Ma, Z., Tong, Y., and Yang, X. (2021). New Evidence from the Qugong Site in the central Tibetan Plateau for the Prehistoric Highland Silk Road. *The Holocene* 31 (2), 230–239. doi:10.1177/0959683620941144

Ge, Y., Lu, H., Wang, C., and Gao, X. (2020a). Phytoliths in Selected Broad-Leaved Trees in China. *Sci. Rep.* 10 (1), 15577. doi:10.1038/s41598-020-72547-w

Ge, Y., Lu, H., Zhang, J., Wang, C., and Gao, X. (2020b). Phytoliths in Inflorescence Bracts: Preliminary Results of an Investigation on Common Panicoideae Plants in China. *Front. Plant Sci.* 10, 1736. doi:10.3389/fpls.2019.01736

Ge, Y., Lu, H., Zhang, J., Wang, C., He, K., and Huan, X. (2018). Phytolith Analysis for the Identification of Barnyard Millet (Echinochloa sp.) and its Implications. *Archaeol Anthropol. Sci.* 10 (1), 61–73. doi:10.1007/s12520-016-0341-0

Guedes, J. d. A., Jiang, M., He, K., Wu, X., and Jiang, Z. (2013). Site of Baodun Yields Earliest Evidence for the Spread of rice and Foxtail Millet Agriculture to South-West China. *Antiquity* 87, 758–771. doi:10.1017/s0003598x00049449

Guedes, J. D. A., and Wan, J. (2015). Flotation Results and Analysis on Guiyuanqiao Site in Shifang City, Sichuan. *Sichuan Cult. Relics* 5 (81–87), 94. (in Chinese).

Guo, F. (2011). The Excavation of Longwangmiao Site in Hanyuan County, Sichuan in 2009. *East Asia Archaeology* 3, 406–442. (in Chinese).

Hao, X., Zhou, Z., Liu, L., and Tian, J. (2022). The Neolithic Occupants in the Yanyuan Basin: Excavation of the Burials at the Guijiabao Site, Sichuan Province, Southwest China. *Archaeological Res. Asia* 29, 100341. doi:10.1016/j.ara.2021.100341

Harvey, E. L., and Fuller, D. Q. (2005). Investigating Crop Processing Using Phytolith Analysis: the Example of rice and Millets. *J. Archaeological Sci.* 32 (5), 739–752. doi:10.1016/j.jas.2004.12.010

He, K., Lu, H., Zhang, J., Wang, C., and Huan, X. (2017). Prehistoric Evolution of the Dualistic Structure Mixed rice and Millet Farming in China. *The Holocene* 27 (12), 1885–1898. doi:10.1177/0959683617708455

Huan, X., Lu, H., Jiang, L., Zuo, X., He, K., and Zhang, J. (2021). Spatial and Temporal Pattern of rice Domestication during the Early Holocene in the Lower Yangtze Region, China. *The Holocene* 31 (9), 1366–1375. doi:10.1177/09596836211019090

Huan, X., Lu, H., Wang, C., Tang, X., Zuo, X., Ge, Y., et al. (2015). Bulliform Phytolith Research in Wild and Domesticated Rice Paddy Soil in South China. *Plos One* 10 (10), e0141255. doi:10.1371/journal.pone.0141255

Huan, X., Lu, H., Wang, C., and Zhang, J. (2020). Progress of rice Bulliform Phytolith Research on Wild–Domesticated Characteristics. *Acta Palaeontologica Sinica* 59 (4), 467–478. (in Chinese).

Huang, F., Guo, F., and Jin, P. (2011). The Discovery and its Significance of rice Phytoliths at Maiping Neolithic Site. *Sichuan Cult. Relics*, 79–83. (in Chinese).

Hung, L. (2011). *Pottery Production, Mortuary Practice, and Social Complexity in the Majiayao Culture, NW China (Ca. 5300-4000 BP)*. St. Louis: Washington University.

Huo, T.-I., Huang, Y.-H., and Wu, J.-C. (2005). Percutaneous Ablation Therapy for Hepatocellular Carcinoma: Current Practice and Future Perspectives. *J. Tibetology* 68, 155–159. (in Chinese). doi:10.1016/s1726-4901(09)70239-4

Ji, D. (2007). Environmental Archaeological Perspective: Dispersal of Anatomically Modern Human and Origin of Agriculture in Northern China. PhD thesis. Lanzhou: Lanzhou University.

Jiang, M., Geng, P., Liu, L., and Zuo, Z. (2013). Flotation Results and Analysis on Gaopo Site in Mianning County in 2011. *Archaeological Discoveries in Chengdu*, 317–330. (in Chinese).

Jiang, M., Hu, T., Bu, Q., and Wang, H. (2016a). Flotation Results on Henglanshan Site in Xichang City in 2011 and 2013. *Archaeological Res. Chengdu* (3), 503–515. (in Chinese).

Jiang, M., Yan, X., Liu, X., Huang, Y., and Wang, H. (2016b). Flotation Results on Henglanshan Site in Xichang City in 2014. *Archaeological Discoveries in Chengdu*, 115–139. (in Chinese).

Jiang, Z., Wang, Y., and Zhang, Q. (2020). A Study on Pre-qin Archaeological Cultures in the Chengdu plain. *Acta Archaeologica Sinica*, 1–22. (in Chinese).

Jin, H., Liu, X., Min, R., Li, X., and Wu, X. (2014). Flotation Results and Analysis on Dadunzi Site in Yuanmou County, Yunnan. *Jianghan Archaeology* 3, 109–114. (in Chinese).

Jones, M., Hunt, H., Kneale, C., Lightfoot, E., Lister, D., Liu, X., et al. (2016). Food Globalisation in Prehistory: The Agrarian Foundations of an Interconnected Continent. *Jba* 4, 73–87. doi:10.5871/jba/004.073

Larson, G., Piperno, D. R., Allaby, R. G., Purugganan, M. D., Andersson, L., Arroyo-Kalin, M., et al. (2014). Current Perspectives and the Future of Domestication Studies. *Proc. Natl. Acad. Sci. U.S.A.* 111 (17), 6139–6146. doi:10.1073/pnas.1323964111

Lee, C. Y., Lin, K. C., Chen, J., and Czermak, A. (2020). Dietary History of Two Human Individuals at the Yingpanshan Site, Sichuan Province, Revealed by Carbon and Nitrogen Isotope Analysis of Serial Samples of Dentinal Collagen. *Int. J. Osteoarchaeol* 30 (4), 565–574. doi:10.1002/oa.2871

Li, B. (1989). Geomorphologic Regionalization of the Hengduan Mountains Region. *Mountain Res.* 7 (1), 21–28. (in Chinese).

Li, H., Zuo, X., Kang, L., Ren, L., Liu, F., Liu, H., et al. (2016). Prehistoric Agriculture Development in the Yunnan-Guizhou Plateau, Southwest China: Archaeobotanical Evidence. *Sci. China Earth Sci.* 59 (8), 1562–1573. doi:10.1007/s11430-016-5292-x

Li, S. (2018). An Analysis and Speculation on the Sources of Prehistoric Remains from the Luojiaba Site. *Sichuan Cult. Relics* 3, 33–37. (in Chinese).

Li, X., and Liu, X. (2016). Flotation Results and Analysis on Gunagfentou Site in Jiangchuan, Yunnan. *Agric. Archaeology* 3, 20–27. (in Chinese).

Li, X., Zhou, X., Zhang, H., Zhou, J., Shang, X., and Dodson, J. (2007a). The Record of Cultivated rice from Archaeobiological Evidence in Northwestern China 5000 Years Ago. *Chin. Sci Bull* 52 (10), 1372–1378. doi:10.1007/s11434-007-0186-0

Li, X., Zhou, X., Zhou, J., Dodson, J., Zhang, H., and Shang, X. (2007b). The Earliest Archaeobiological Evidence of the Broadening Agriculture in China Recorded at Xishanping Site in Gansu Province. *Sci. China Ser. D* 50 (11), 1707–1714. doi:10.1007/s11430-007-0066-0

Liu, X., Reid, R. E., Lightfoot, E., Matuzeviciute, G. M., and Jones, M. K. (2016). Radical Change and Dietary Conservatism: Mixing Model Estimates of Human Diets along the Inner Asia and China's Mountain Corridors. *The Holocene* 26 (10), 1556–1565. doi:10.1177/0959683616646842

Lu, H., Liu, Z., Wu, N., Berné, S., Saito, Y., Liu, B., et al. (2002). Rice Domestication and Climatic Change: Phytolith Evidence from East China. *Boreas* 31 (4), 378–385. doi:10.1080/030094802320942581

Lu, H. (2017). New Methods and Progress in Research on the Origins and Evolution of Prehistoric Agriculture in China. *Sci. China Earth Sci.* 60 (12), 2141–2159. doi:10.1007/s11430-017-9145-2

Lu, H., Wu, N., Liu, K., Jiang, H., and Liu, T. S. (2007). Phytoliths as Quantitative Indicators for the Reconstruction of Past Environmental Conditions in China II: Palaeoenvironmental Reconstruction in the Loess Plateau. *Quat. Sci. Rev.* 26 (5), 759–772. doi:10.1016/j.quascirev.2006.10.006

Lu, H., Wu, N., Yang, X., Jiang, H., Liu, K., and Liu, T. (2006). Phytoliths as Quantitative Indicators for the Reconstruction of Past Environmental Conditions in China I: Phytolith-Based Transfer Functions. *Quat. Sci. Rev.* 25 (9), 945–959. doi:10.1016/j.quascirev.2005.07.014

Lu, H., Zhang, J., Liu, K.-b., Wu, N., Li, Y., Zhou, K., et al. (2009a). Earliest Domestication of Common Millet (Panicum Miliaceum) in East Asia Extended to 10,000 Years Ago. *Proc. Natl. Acad. Sci. U.S.A.* 106 (18), 7367–7372. doi:10.1073/pnas.0900158106

Lu, H., Zhang, J., Wu, N., Liu, K.-b., Xu, D., and Li, Q. (2009b). Phytoliths Analysis for the Discrimination of Foxtail Millet (Setaria Italica) and Common Millet (Panicum Miliaceum). *Plos One* 4 (2), e4448. doi:10.1371/journal.pone.0004448

Piperno, D. R. (1988). *Phytolith Analysis: An Archaeological and Geological Perspective*. London: Academic Press.

Wang, C., Lu, H., Gu, W., Wu, N., Zhang, J., Zuo, X., et al. (2017). The Spatial Pattern of Farming and Factors Influencing it during the Peiligang Culture Period in the Middle Yellow River valley, China. *Sci. Bull.* 62 (23), 1565–1568. doi:10.1016/j.scib.2017.10.003

Wang, C., Lu, H., Gu, W., Zuo, X., Zhang, J., Liu, Y., et al. (2018). Temporal Changes of Mixed Millet and rice Agriculture in Neolithic-Bronze Age Central Plain, China: Archaeobotanical Evidence from the Zhuzhai Site. *The Holocene* 28 (5), 738–754. doi:10.1177/0959683617744269

Wang, C., and Lu, H. (2012). Research Progress of Fan-Shaped Phytolith of Rice and Relvent Issues. *Quat. Sci.* 32 (2), 269–281. (in Chinese).

Wang, Q., Jiang, Z., Yang, W., and Chen, X. (2019). Plant Remains from the Xueshan Site in Chengjiang County of Yunnan Province. *Agric. Hist. China* 2, 3–11. (in Chinese).

Wang, Y., and Lu, H. (1993). *The Study of Phytolith and its Application*. Beijing: China Ocean Press, 1–228.

Xue, Y. (2010). A Preliminary Investigation on the Archaeobotanical Material from the Site of Haimenkou in Jianchuan County. Master thesis. Beijing: Peking University, 1–157.

Xue, Y., Dal Martello, R., Qin, L., Stevens, C. J., Min, R., and Fuller, D. Q. (2022). Post-neolithic Broadening of Agriculture in Yunnan, China: Archaeobotanical Evidence from Haimenkou. *Archaeological Res. Asia* 30, 100364. doi:10.1016/j.ara.2022.100364

Yan, X., Guo, F., Wang, Y., and Guo, X. (2013). Flotation Results and Analysis on Zhengjiaba Site in Langzhong City, Sichuan-On the Carbonized Archaeobotanic Remains of Pre-qin Period in Sichuan Sichuan Cultural. *Relics* 4, 74–85. (in Chinese).

Yan, X., Jiang, M., Liu, X., Liu, L., Bu, Q., and Li, T. (2016a). The Report of Plant Remains Unearthed at the Sites of Guijiabao and Daozuomiao in Yanyuan County in 2015. *Archaeological discoveries in Chengdu*, 147–154. (in Chinese).

Yan, X., Jiang, M., Zuo, Z., and Bu, Q. (2016b). Flotation Results and Analysis on Shapingzhan Site in Xichang City in 2014. *Archaeological Discoveries in Chengdu*, 155–162+531532. (in Chinese).

Yang, W., Jiang, Z., and Chen, X. (2020). A Preliminary Study on Plant Remains from the Yubeidi Site. *Agric. Hist. China* 39 (1), 3–11. (in Chinese).

Yang, W., Jiang, Z., Yao, H., and Chen, X. (2021). A Study on Agriculture Structure in the Shizhaishan Period of Dianchi Region, Yunnan. *Agric. Hist. China* 40 (2), 36–47. (in Chinese).

Yang, X., Wan, Z., Perry, L., Lu, H., Wang, Q., Zhao, C., et al. (2012). Early Millet Use in Northern China. *Proc. Natl. Acad. Sci. U.S.A.* 109 (10), 3726–3730. doi:10.1073/pnas.1115430109

Zhang, J., Lu, H., Gu, W., Wu, N., Zhou, K., Hu, Y., et al. (2012). Early Mixed Farming of Millet and Rice 7800 Years Ago in the Middle Yellow River Region, China. *Plos One* 7 (12), e52146. doi:10.1371/journal.pone.0052146

Zhang, Y. (1989). Climatic Division of the Hengduan Mountains Region. *Mountain Res.* 7 (1), 13–20. (in Chinese).

Zhao, Z., and Chen, J. (2011). Flotation Results and Analysis on Yingpanshan Site in Mao County. *Sichuan Cult. Relics South. China* 3, 60–67. (in Chinese).

Zhao, Z. (2010). *Report of Plant Remains at the Shifodong Site, Shifodong in Gengma County*. Beijing: Cultural Relics Press, 368–373. (in Chinese).

Zhong, Y., Lu, D., Yan, B., Zuo, Z., Sun, C., Liu, X., et al. (2016). The Report of Investigation and Excavation of Guijiabao Site in Yanyuan County in 2015. *Archaeological Discoveries in Chengdu*, 18–52. (in Chinese).

Zhou, X., Yu, J., Spengler, R. N., Shen, H., Zhao, K., Ge, J., et al. (2020). 5,200-year-old Cereal Grains from the Eastern Altai Mountains Redate the Trans-eurasian Crop Exchange. *Nat. Plants* 6, 78–87. doi:10.1038/s41477-019-0581-y

Zuo, X., Lu, H., Jiang, L., Zhang, J., Yang, X., Huan, X., et al. (2017). Dating rice Remains through Phytolith Carbon-14 Study Reveals Domestication at the Beginning of the Holocene. *Proc. Natl. Acad. Sci. U.S.A.* 114 (25), 6486–6491. doi:10.1073/pnas.1704304114

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A New Filtered Alcoholic Beverage: Residues Evidence From the Qingtai Site (*ca*. 5,500–4,750 cal. BP) in Henan Province, Central China

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Recent studies have provided evidence of alcohol production and consumption in 16 sites in northern China during the Neolithic period, focusing on the Yangshao Culture (*ca*. 7,000–5,000 cal. BP). Yet, the comparison of similarities and differences in brewing technology and drinking patterns within the Yangshao Culture still needs more supporting information from case studies in different regions. In this paper, 17 pottery samples excavated from the Yangshao Culture site of Qingtai (*ca*. 5,500–4,750 cal. BP) in the central part of Henan Province were analyzed for microfossils (starch grains, phytoliths, fungi) and organic acids, on the basis of the theoretical model constructed from our simulation experiments. The results revealed a mixed filtered alcoholic beverage, likely to be fermented by fruit and/or honey. The ingredients were mainly foxtail millet, rice, Job's tears, Triticeae, snake gourd roots, lotus roots, legumes, nuts, fruits, and/or honey. What's more, we found that the *jiandiping* amphora from Qingtai was not likely used for brewing or drinking. In terms of prehistoric drinking habits, in the large-scale settlement of the late Yangshao Culture in China, it is possible that people drank filtered alcohol alone or that a few people drank filtered alcohol poured from the painted bottle, indicating a switch from communal drinking to individual drinking. This study sheds light on the similarities and differences in brewing techniques, fermentation ingredients, and drinking patterns among different regions of the late Neolithic Yangshao Culture, and deepens our understanding of alcoholic beverages in the early Chinese civilized societies.

Keywords: prehistoric alcoholic beverages, qingtai site, yangshao culture, microfossil and organic acid analyses, brewing technology, drinking pattern

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1 INTRODUCTION

Alcoholic beverages have always been given special status because of their miraculous medical, religious, social, and political value (McGovern et al., 2004). With the prevalence of religious ceremonies and feasting activities, as well as the expansion of the population and the progress of agriculture, the production and consumption of alcoholic beverages became more and more common in the Neolithic period (Hayden, 2003; Liu, 2021a). Since the late Neolithic period,

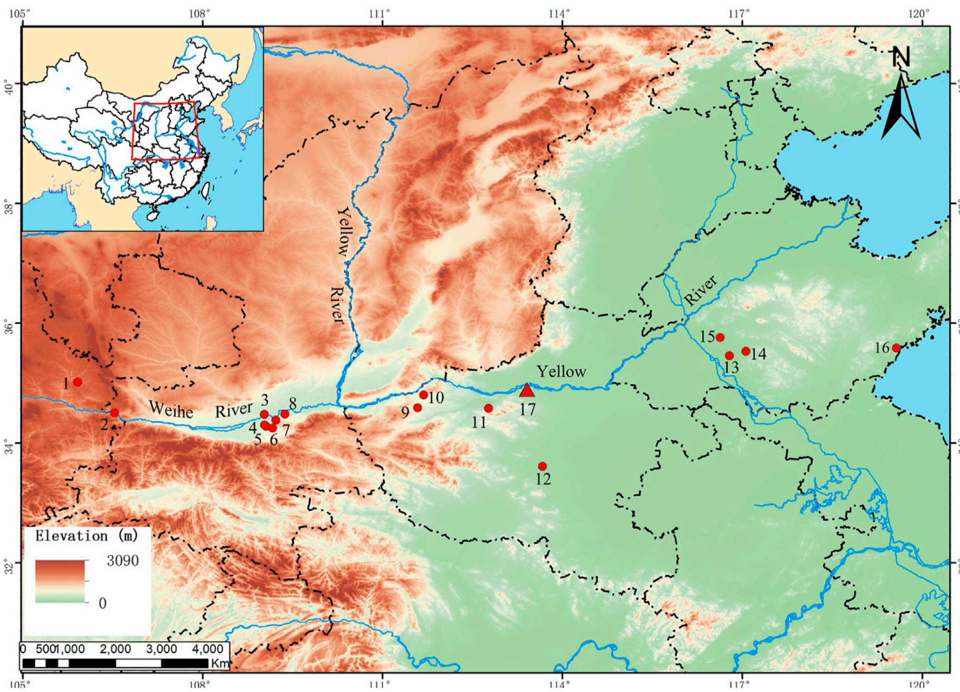

FIGURE 1 | Location of the Qingtai site and other related archaeological sites in north China. 1) Dadiwan; 2) Guantaoyuan; 3) Yangguanzhai; 4) Mijiaya; 5) Banpo; 6) Xinjie; 7) Jiangzhai; 8) Lingkou; 9) Xipo; 10) Dingcun; 11) Huizui; 12) Jiahu; 13) Wangyin; 14) Xixiahou; 15) Dongjiabai; 16) Liangchengzhen; 17) Qingtai.

China gradually entered an early civilized society, and the ritual system was about to emerge (Wang, 2008; Gao, 2019; Wang, 2020). At that time, although the pottery vessels associated with alcohol had not completely lost their practical function, some were in the transformation stage into special ritual vessels (Xu, 2004). In the historical period, alcohol became an important part of the ritual system, laying the foundation of Chinese ritual and music civilization (Huang, 2002; Huang, 2008). Therefore, as a unique carrier of civilization, the evolution of alcoholic beverages, to a certain extent, reflected the origin of Chinese civilization and the process of social complexity.

Recently, considerable archaeological evidence about prehistoric alcohol brewing in China has been reported based on the chemical and microfossil (starches, phytoliths, and fungi) analyses on pottery vessels, almost spanning the entire Neolithic period (**Figure 1**; McGovern et al., 2004; Wang et al., 2016; Liu et al., 2017a; Liu et al., 2018a; Liu et al., 2019a; Liu et al., 2019b; Liu et al., 2020a; Liu et al., 2020b; Liu et al., 2020c; Liu et al., 2020d; Feng et al., 2021; Zhao and Liu, 2021; Liu et al., 2021b). In the early Neolithic of central China, the analyses of residues from Jiahu, Guantaoyuan, and Lingkou sites have shown that globular jars were used to make cereal-based fermented beverages, and two brewing methods, including the use of malted cereal and *qu* starter, had already been developed and used throughout the Neolithic period (McGovern et al., 2004; Liu et al., 2019b; Liu, 2021a). By the middle and late Neolithic, several archaeological studies proved that the brewing and drinking vessels in the Yangshao Culture had evolved from globular jars to flat-based jars (pingdiqi), then to conical-based jars (*jiandiping*) in the Weihe River valley and the western Henan, central China (Wang et al., 2016; Liu et al., 2017a; Liu et al., 2018a; Liu et al., 2019a; Liu et al., 2020a; Liu et al., 2020b; Liu et al., 2020c), while in Beixin culture in eastern China, the globular jars were still be used (Liu et al., 2020d). During the late and end Neolithic period, various pottery, including goblets and cups, were related to alcohol drinking in the Haidai region, eastern China (McGovern et al., 2005; Liu et al., 2021b). These two types of fermentation and drinking patterns in central and eastern China reflected different development trends of alcoholic culture. However, the brewing materials in the above sites were similar, containing a mixture of cereals (foxtail millet, common millet, Job's tears, rice, Triticeae), roots, tubers, nuts, and honey or fruits. Comparing Paleoethnobotanical research of these sites, it has been proved that the fermentation ingredients were the same as daily food (Liu, 2021a).

Previous studies have made significant achievements on regional variation in fermentation techniques, drinking rituals, and socio-political developments in northern China during the Neolithic period, with a focus on the Yangshao Culture. In the middle and late stages of Yangshao Culture, the central Henan region became the most representative and influential political center, and the large-scale regional center settlements such as Qingtai, Shuanghuaishu, and Xishan sites entered the early civilized society (Han, 2019; Wang, 2020; Wei, 2021). However, the alcoholic culture in this core region is still unclear. What's more, most of the published data were on the results of microfossil residues analysis or only conducted chemical analysis; only a few studies combined both two approaches for experiments. To obtain more information on brewing materials and techniques, it is necessary to employ multi-disciplinary research.

FIGURE 2 | Pottery samples from the Qingtai site (scale bar: 5 cm). S1, *Jiandipng*; S2, *jiandipng*; S3, funnel (a hole in the bottom); S4, lip; S5, jar; S6, jar; S7, jar; S8, jar; S9, *jiandipng*; S10, *jiandiping*; S11, jar; S12, cup; S13, *jiandipng*; S14, *jiandipng*; S15, lip; S16, *jiandipng*; S17, painted bottle.

Thus, microfossils and chemical analyses of the pottery unearthed from the Qingtai site in central Henan were conducted in this study. The aim of this paper is to provide more evidence for a better understanding of the production and consumption of alcoholic beverages in the early civilized societies at the core region of the Late Neolithic period, as well as the similarities and differences in brewing technology and drinking patterns among different regions within the Yangshao Culture.

2 MATERIALS AND METHODS

2.1 Materials

The Qingtai site is located on the mound of Qingtai Village, Xingyang City, Henan Province, central China (**Figure 1**). In 1981, Zhengzhou Municipal Museum and Xingyang County Cultural Center excavated the Qingtai site, and ^{14}C dates placed the time period between *ca.* 5,500–4,750 cal. BP, a large-scale settlement site in the political hinterland of the late Yangshao culture (Zhang and Zhao, 1987). In 2015, to explore the origin of Chinese silk, the Zhengzhou Municipal Institute of Cultural Relics and Archaeology conducted a systematic investigation, exploration, and excavation at this site again (Fang, 2018). The excavation uncovered significant relics, such as three-fold ring trenches, adobe houses, tombs, and astronomical sacrifice areas, representing the emergence of civilization in central China. In addition, *jiandiping*, jars, cups, and funnels, suspected to be related to alcoholic beverages, were also discovered. It was worth noting that a painted bottle was found in a tomb with niches, which was also the only complete painted pottery unearthed from Qingtai during this excavation period.

In this study, we selected 17 pottery samples from the Qingtai site for starches, phytoliths, fungi, and organic acids analyses, including seven *jiandiping* amphorae, one funnel, two lids, five flat-based jars, one cup, and one painted bottle (**Figure 2**).

2.2 Methods

Residue sampling of the pottery was completed in the field laboratory at the Qingtai site. First, each surface of the sample was washed separately with distilled water. Then, an ultrasonic tooth cleaner was used to clean the pottery inside; the ultrasonic water samples were then transferred to the 50 ml centrifugal tube as the experimental samples. To test potential contamination, we also collected the control samples by gathering dust on shelves in the field laboratory and soil on each pottery surface. Further extraction was conducted at the Bio-archaeology laboratory, University of Science and Technology of China.

2.1.1 Extraction of Starches, Phytoliths, and Fungi

The liquid samples were treated with 5% $(NaPO_3)_6$, 10% HCl, and 30% H_2O_2 to disperse carbonates and minerals. After that, two kinds of heavy liquid (CsCl at a specific gravity of 1.89 and $ZnBr_2$ at a specific gravity of 2.35) were used for the centrifuge procedure, the former to extract starch grains and fungi, and the latter to extract phytoliths. In addition, to prevent contamination from the experimental environment, instruments, or reagents, a blank control sample was also set up and treated using the same protocol. Extractions obtained from residue samples were examined under the Leica DM4500P polarizing microscope. Starch and phytolith identifications were based on published studies (Madella et al., 2005; Wei et al., 2008; Lu et al., 2009; Yang et al., 2009; Ge et al., 2010; Yang et al., 2010;

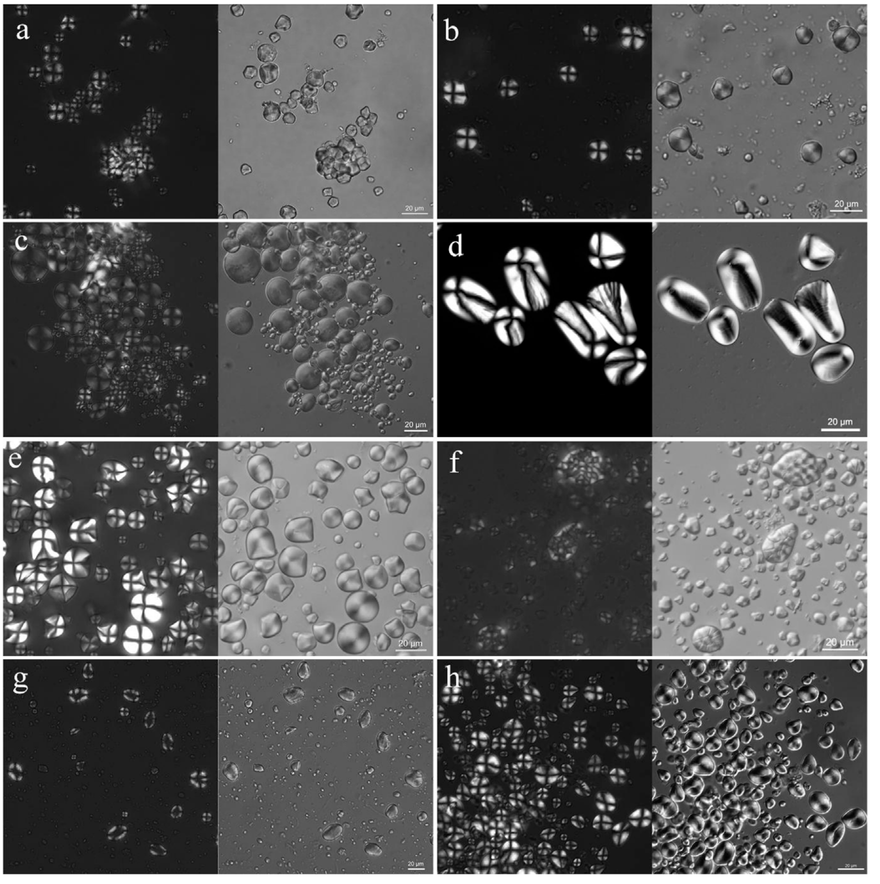

FIGURE 3 | Examples of starch grains morphology from the modern reference (scale bar: 20 μm). **(A)**: *Setaria italica*; **(B)**: *Coix lacryma-jobi;* **(C)**: Wheat; **(D)**: *Nelumbo nucifera*; **(E)**: *Trichosanthes kirilowii;* **(F)**: *Oryza sativa*; **(G)**: Leguminosae; **(H)**: *Quercus* sp.

Wan et al., 2011; Wang et al., 2013; Yang and Perry, 2013; Neumann et al., 2019; Henry, 2020) and our lab modern reference collection of common plant species (**Figure 3**). Fungi identification was based on the published book (St-Germain and Summerbell, 2011).

2.1.2 Extraction of Organic Acid

First, all the samples were centrifuged at 3,000 r/min for 15 min, then transferred to new 15 ml centrifuge tubes. Subsequently, samples were filtered by 0.45 μm microfiltration membrane to 5 ml sample bottles. Next, a vacuum centrifugal concentrator was used to centrifuge at 1,000 r/min and concentrate to 100 μl at 55°C. Finally, the samples were sent to the Center for Science and Chemical Science Experiments of the University of Science and Technology of China for liquid chromatography-mass spectrometry (LC-MS) testing.

2.1.3 Simulation Experiments of Brewing

Previous simulation experiments have proved that starch grains can be damaged by saccharification and fermentation, so the evidence of alcohol residues would be found by observing the morphology of starch grains (Henry et al., 2009; Wang et al., 2017). This discovery has been applied to the analysis of the archaeological context in recent years, and experimental evidence for the brewing of *qu* wine, malted wine, and *koujiao* wine has been reported in 14 prehistoric sites in China (Liu, 2021a). To further understand the fermentation technology, scholars have established an analysis method: based on the residues of microbotanical and microbial remains to determine the brewing function and methods (Liu et al., 2019b; Liu et al., 2020a; Liu et al., 2020b; Liu et al., 2020d; Liu et al., 2021b; Feng et al., 2021; Zhao and Liu, 2021). However, this method has not been demonstrated by simulation experiments.

Therefore, a simulation experiment was designed to verify the different combinations of microfossils in three kinds of alcohol residues. Six common grain alcohol ingredients, including rice, foxtail millet, wheat, yam, lotus root, and mung bean, were selected and brewed into malted alcohol, *qu* alcohol, and *koujiao* alcohol separately under the same experimental conditions in our laboratory. Given the turbid state of the fermented liquor, the fermentation samples were divided into filtered and unfiltered residues to observe starch grains, phytoliths, and fungi under a microscope, respectively.

The results showed that starch grains do unique damage during saccharification and fermentation (**Figure 4**). However, different brewing methods have similar damage to the same kind

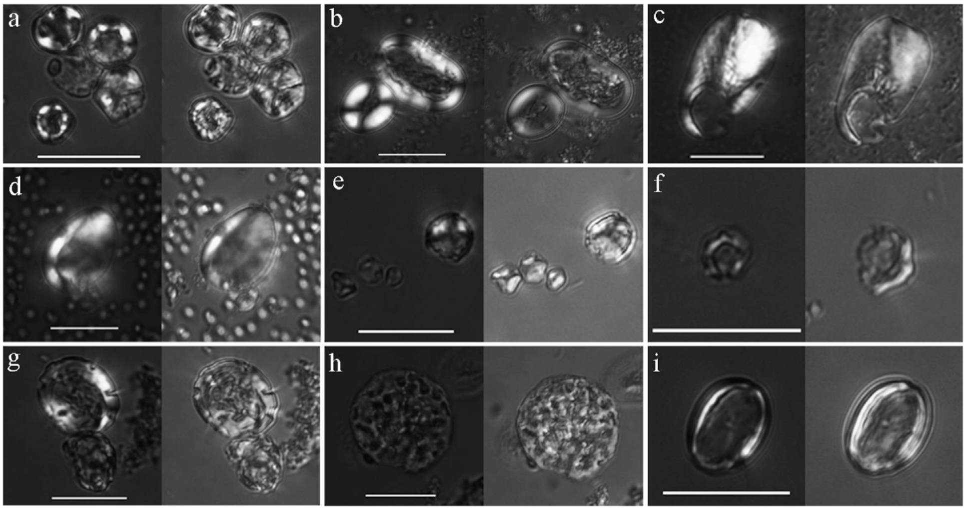

FIGURE 4 | Damaged starch grains in the three alcohol (scale bar: 20 μm). **(A)** foxtail millet in *koujiao* alcohol; **(B)** mung bean in *qu* alcohol; **(C)** lotus root in *qu* alcohol; **(D)** yam in *koujiao* alcohol; **(E,F)** rice in malted alcohol; **(G–I)** wheat in malted alcohol.

of starch grains, making it difficult to distinguish brewing methods by the different morphological changes of starch grains. Large amounts of husk phytoliths were found in all unfiltered malted and *qu* alcohol, small amounts were found in some unfiltered *koujiao* liquors, and none or minute amounts were found in all filtered *qu* alcohol, malted alcohol, and *koujiao* alcohol. The results indicated that the presence of husk phytoliths remains is not only related to the brewing method but also the filtration behavior. Fungi are present in all filtered and unfiltered liquor after fermentation. In sum, the microbotanical and microbial remains can be used as a basis for judging whether pottery was exposed to alcohol; however, it is hard to accurately speculate on the brewing method.

3 RESULTS

Among the 17 experiment samples of pottery, 218 starch grains were extracted from the painted bottle S17, the funnel S3, and the lid S4. No starch grain was found in all the control samples, and only one starch grain was yielded from the unused surface of the jar S8. As the amounts of starch grains from the experiment samples were much higher than the control samples and unused surfaces, we believe that starch grains recovered from the three potteries are associated with human behavior in processing foods. Therefore, this study focused on these three pottery pieces in statistical analysis. Among them, organic acids were also detected in these three pottery pieces, but not fungal remains. In addition, 32 phytoliths were only found on the funnel.

3.1 Starch Remains

Among the 218 starch grains, 19 starch grains could not be identified due to the damage or lack of typical characteristics. The remaining 199 starch grains were classified into nine broad categories based on their morphology and size (**Table 1**; **Figure 5**).

Type A: starch grains (n = 48, 8.72–20.41 μm) are mostly faceted, hilum is centric with a "−" or "+" or "Y" or star-shaped fissures, some parts of starch have depressions (**Figure 5A**). The extinction arms are crossed and vertical under polarizing light, and lamellae are invisible. This type of starch grains is mostly from Panicoideae plants (Ge et al., 2010; Yang et al., 2010), such as foxtail millet (*Setaria italica*), common millet (*Panicum miliaceum*), as well as Job's tears (*Coix lacryma-jobi*). However, the size of a common millet is relatively small, while the size of Job's tears is relatively large, and the end of the extinction arm of Job's tears starch grains is mostly similar to a "Z" shaped curve. The size range and morphological structure of this type of starch granules are consistent with those of foxtail millet, so it is identified as *Setaria italica*.

Type B: starch grains (n = 27, 12.18–26.03 μm) are polygonal and nearly circular. Its characterized by a centric hilum, linear fissures, and invisible lamellae. The extinction arms are crossed and vertical under polarizing light, and the end of the extinction arms presents a "Z" shaped curve (**Figure 5B**). In modern plants, the starch grains of Panicoideae plants generally have a polyhedral structure, and the extinction arms cross vertically. According to our lab modern sample database (**Figure 3B**), the size of foxtail millet and common millet starch grains are relatively small, and the end of the cross-extinction arm of Job's tears has a unique "Z" shaped curve (Ge et al., 2010). Thus, type B starch granules come from *Coix lacryma-jobi*.

Type C: starch grains (n = 92, 5.37–39.65 μm) are nearly round or convex lens-shaped, the hilum is centric and closed, the extinction arm is "Z" shaped; if turning over the starch after tapping the glass slide, a long "—" shaped fissure appeared. This type can observe two kinds of starch grains of different sizes. The larger starch grains (11.23–39.65 μm) have clear lamellae (**Figure 5C**), while the smaller ones (5.37–9.4 μm) are not obvious. Combined with related (Yang and Perry, 2013) research on the morphology of ancient and modern starch grains in Triticeae. We judged that this kind of starch grains come from the Triticeae plants.

TABLE 1 | Starches from the Qingtai site.

Sample no	Artifact no	Name of pottery	Type A	Type B	Type C	Type D	Type E	Type F	Type G	Type H	Type I	Unknown	Total	Brewing damage
S3	H183②	funnel	28	9	27	5	8	1		1	5	16	100	37
S4	H183②	lip			1	3						3	7	6
S17	M145①:2	Painted bottle	20	18	64	2	3	2	2				111	57
		Total	48	27	92	10	11	3	2	1	5	19	218	100

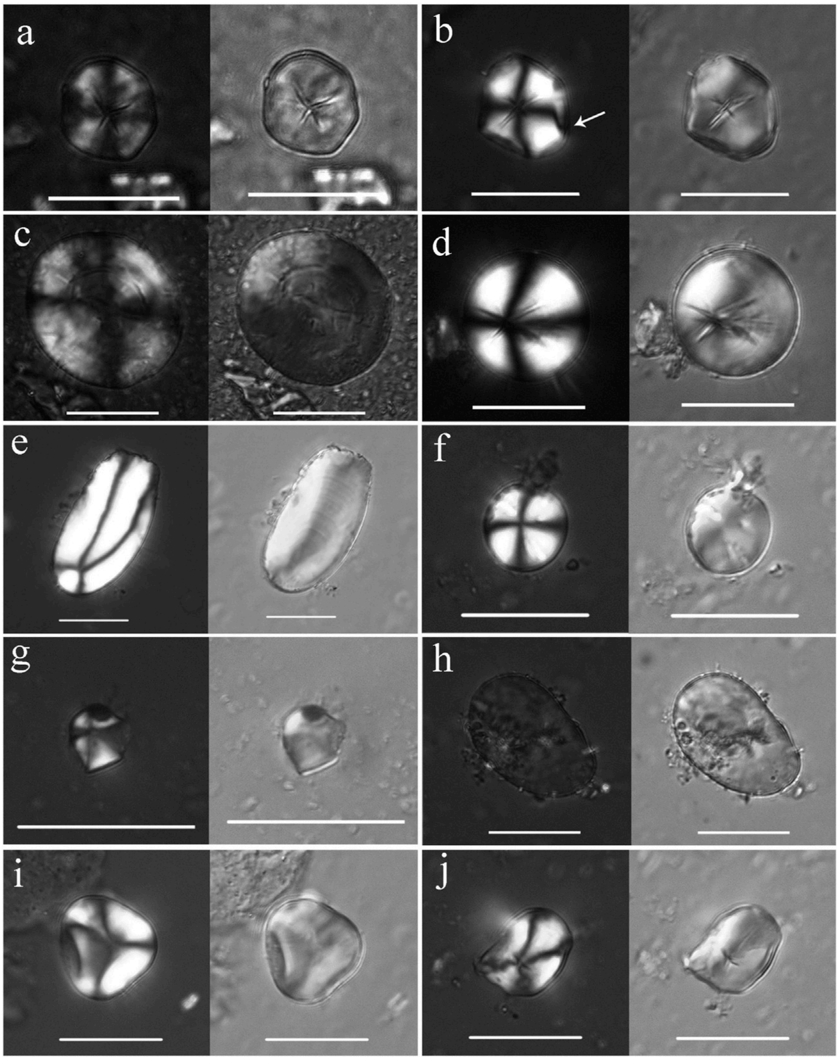

FIGURE 5 | Types of starch grains at the Qingtai site (scale bar: 20 μm). **(A)** *Setaria italica*; **(B)** *Coix lacryma-jobi*; **(C)** Triticeae; **(D,E)** *Nelumbo nucifera*; **(F)** *Trichosanthes kirilowii;* **(G)** *Oryza sativa*; **(H)** Leguminosae; **(I)** *Quercus* sp.; **(J)** tubers.

Type D: starch grains (n = 10, 11.46–47.24 μm) are the circle (**Figure 5D**) and long oval (**Figure 5E**), which have a large size distribution range. Short "-" shaped or "X" shaped fissures can be observed at the hilum, and the extinction arms under polarizing light are cross. The lamellae of this kind of starch grains gradually spread outward around the hilum is obvious, and the hilum of the long oval starch grains is eccentric. Comparing our laboratory's modern database (**Figure 3D**) and related analysis (Wan et al., 2011) of the starch grains in roots and tubers in modern southern China, this type of starch grains should come from lotus root (*Nelumbo nucifera*).

Type E: starch grains (n = 11, 17.35–23.18 μm) are round, semi-circular, and bell-shaped with a smooth surface and no fissure, the hilum is centric or eccentric, and the lamellae are invisible. Extinction arms are crossed vertically or curved under polarizing light (**Figure 5F**). Compared with the modern sample database of our laboratory (**Figure 3E**), the shape and length of this type of starch granules are similar to those of the root of snake gourd (*Trichosanthes kirilowii*), so this type of starch granule is identified as the *Trichosanthes kirilowii*.

Type F: starch grains (*n* = 3, 5.26–8.63 μm) are polyhedral structures, with the centric hilum and invisible lamellae and the cross-extinction arm is "X" shaped (**Figure 5G**). All these grains have small sizes of less than 10 μm, and most of them are closely arranged. Based on the morphological characteristics and the microscopic observation of starch grains of modern rice by Wei et al. (2008), we infer that Type F starch grains originate from rice (*Oryza sativa*).

Type G: starch grains (*n* = 2, 9.62–32.52 μm) are kidney-shaped and oval, and the hilum is invisible. One of them has a fissure through the long axis, and the other has no fissure (**Figure 5H**). According to our laboratory database and related study on legumes starch grains (Wang et al., 2013), this type of starch granules should come from Leguminosae.

Type H: starch grain (*n* = 1, 19.36 μm) is triangular oval, the hilum is closed and centric, the lamellae are invisible, and the cross-extinction arm is "X" shaped (**Figure 5I**). The starch grains of nut plants are mostly triangular oval and drop-shaped, such as *Castanea* sp. of the Fagaceae tribe and *Quercus* sp. However, the hilum of the *Castanea* sp. starch grains is mostly eccentric, and the *Castanea* sp. starch grains with a long axis greater than 10 μm can be observed with lamellae, while the *Quercus* sp. starch grains basically have no lamellae (Yang et al., 2009). Thus, these starch grains can be identified as *Quercus* sp.

Type I: starch grains (*n* = 5, 16.43–27.79 μm) are mainly oval-shaped, lamellae are unclear, cross-extinction arms are "X" shaped, hilum is eccentric, and the part of them have "-" shaped fissures (**Figure 5J**). According to the database of our laboratory, one of the typical characteristics of starch grains in roots and tubers is the eccentric hilum. These five starch grains extracted lacked further species characteristics and cannot be further distinguished, so this type can be classified as a roots or tubers plant.

Compared with the results of our simulation experiments, nearly half of the starches show signs of morphological alterations to some extent and were related to brewing behavior (*n* = 100, 45.9% of the total). These damage characteristics (*n* = 58) included small pits and cracks, missing edges, hazy extinction cross, and weakened birefringence which was the direct result of enzymatic hydrolysis of starch grains (**Figure 6A,B**). Simultaneously, there are also some other damage characteristics (*n* = 42) caused by fermentation and gelatinization, such as enlarged fissures radiating outward from the hilum, further expansion of the central depression, swelling, and melting (**Figure 6C–H**). Some starch grains only have the edge, and the central area almost disappears, forming a ring structure (**Figure 6G**). As the starch grains continue to decompose, the extinction cross arm gradually disappears until the crystal structure is lost (**Figure 6G,H**).

3.2 Phytolith Remains

Of the three pottery samples (S3, S4, S17) yielding brewing damaged starch grains, 32 phytoliths were found only on the funnel S3, and most of which were from Poaceae plants (**Table 2**). Two double-peak phytoliths (**Figure 7**: g) from rice husk and dumbbell with scooped ends paralleled arrangement (**Figure 7A**) phytoliths from rice stem or leaf were present, corresponding to the presence of rice starch grains on the funnel. One Ω-type phytolith (**Figure 7H**) of foxtail millet husks was also found, but no η-type phytolith typical or starch of common millet was recovered. Other Poaceae families include two fan-shaped (**Figure 7E**), three cross (**Figure 7F**), 14 bilobate (**Figure 7D**), and two square saddle (**Figure 7B**) phytoliths, also found under the microscope. Further, hair cells (*n* = 1, **Figure 7C**) were found, mainly in the stem or leaf of eudicots.

3.3 Organic Acid Remains

Through the LC-MS analysis, 11 kinds of organic acids, including lactic acid, acetic acid, amber acid, fumaric acid, propanoic acid, butyric acid, citric acid, 3-hydroxybutyric acid, malic acid, pyruvic acid, and oxalic acid were detected from the funnel, lid, and painted bottle (The metrical data for organic acids remains is listed in **Supplementary Table S1**). Among them, eight kinds of organic acids were found in the funnel except for citric acid, 3-hydroxybutyric acid, and malic acid. Eight kinds of organic acids were found in the lid except for lactic acid, fumaric acid, and malic acid. Nine kinds of organic acids were found in the pottery bottle except for pyruvic acid and oxalic acid.

4 DISCUSSION

As mentioned in the previous article, some scholars believed that the combination of microbotanical and microbial can determine the fermentation methods based on experimental research and experience in the analysis of archaeological materials (Wang et al., 2017; Liu et al., 2019b; Liu et al., 2020a; Liu et al., 2020b; Liu et al., 2020d; Liu et al., 2021b; Feng et al., 2021; Zhao and Liu, 2021): 1) In the case of malted alcohol, the husk of the cereals and the starch grains with fermentation characteristics (saccharification and gelatinization) might be preserved on the brewer. 2) If it is *qu* alcohol, fungi related to *qu* and starch grains with fermentation characteristics might be detected by microscopy. 3) The production of *koujiao* alcohol does not require the malted grains or *qu*, so the residue might mainly contain starch grains with fermentation characteristics. Our simulation results revealed that these data need to be interpreted with caution, as husk phytolith residues are also associated with filtration behavior.

4.1 Fermentation Methods and Ingredients

Among the 17 pottery samples selected from the Qingtai site, three pottery samples (funnel, lid, and painted bottle) with brewing damaged starch demonstrated that they were used as alcoholic vessels. The brewing materials at Qingtai included foxtail millet, rice, Triticeae, Job's tear, legumes, lotus root, and Quercus, which correspond to the daily food sources as

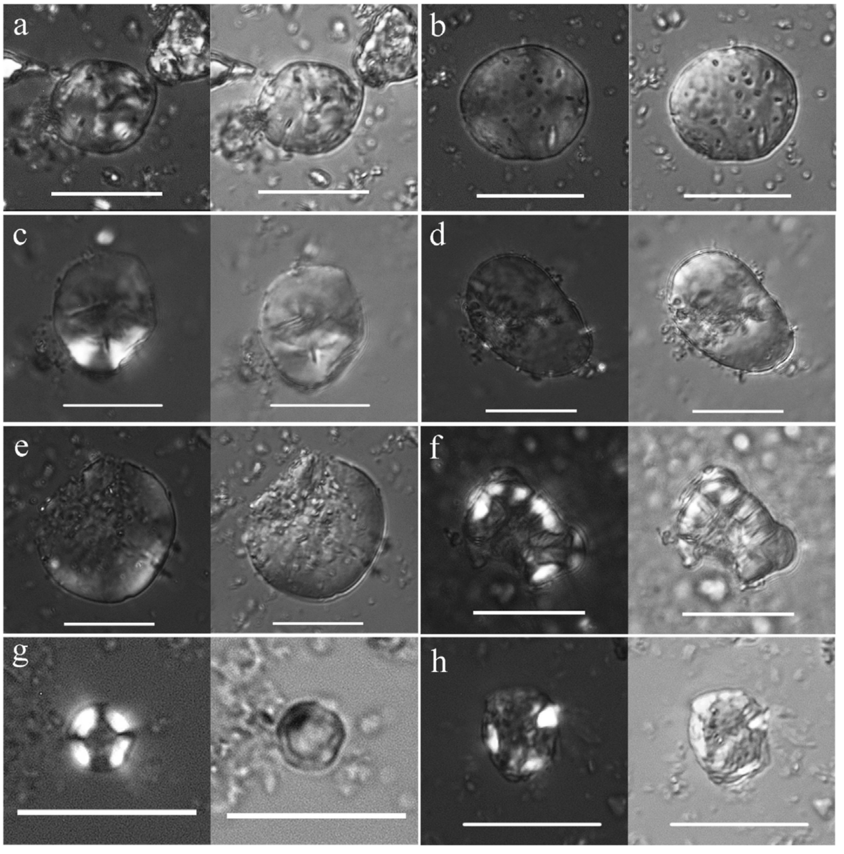

FIGURE 6 | Damaged starch grains from the Qingtai site (scale bar: 20 μm). **(A,B)**: showing small pits and cracks, hazy extinction cross; **(C)**: showing swelling, melting, and hazy extinction cross; **(D)**: showing pits, melting, and hazy extinction cross; **(E)**: showing missing edges; **(F–H)**: showing central depression and swelling.

TABLE 2 | Phytoliths from the Qingtai site funnel.

Phytolith morphotype	Taxonomic attribution	funnel
Scooped ends paralleled arrangement	Oryza stem/leaf	1
Double-peak	Oryza husk	2
Square saddle	Poaceae	2
Silicified cells		6
Hair cells		1
Fan-shaped	Poaceae	2
Cross	Poaceae	3
Bilobate	Poaceae	14
Ω-type	Foxtail millet husk	1
Total		32

indicated by the microbotanical remains from Qingtai (Sun, 2018). This phenomenon also accords with the selection of fermentation ingredients in other Yangshao sites (Liu, 2021a).

The types of starch grains and phytoliths extracted from the funnel, lip, and painted bottle can match each other. The species of starch grains were mainly foxtail millet (22.02% of the total) and Triticeae (42.20% of the total). Correspondingly, Ω-type phytoliths from foxtail millet husks were found, and the total number of Poaceae phytoliths accounted for the highest proportion, up to 62.50%. Besides, small amounts of starch grain (n = 3) and phytoliths (n = 3) of rice were present. Although husk phytoliths were found in the funnel, it is hard to define whether the sprouting or chewing, or *qu* method was used, because simulation experiments showed that the husk phytoliths existed in these three saccharification methods of alcohol. What is surprising is that no fungi remain was seen in any of the three alcoholic vessels. This result is likely attributable to the preservation environment of the microorganisms. Studies have shown that the perishable nature of fungi makes it more difficult to preserve archaeological records than other plant or animal remains (Dugan, 2008; Berihuete-Azorin et al., 2018).

From the result of LC-MS analysis, four common alcoholic organic acids, including lactic acid, acetic acid, amber acid, and fumaric acid (Li, 2017; Coelho et al., 2018) were found in three pottery. Lactic acid and fumaric acid are produced by the fermentation of sugar contained in starchy raw materials such as rice and wheat owing to the action of lactic acid bacteria (Hao et al., 2021). Acetic acid is commonly found in various types of alcohol, and it is converted from different organic matters by *Acetobacter* through fermentation (Barata et al., 2012). Lactic acid is the most common organic acid in grain wine, followed by amber acid (Zhao et al., 2020). These results are consistent with data obtained in starch grains and phytoliths analyses, suggesting

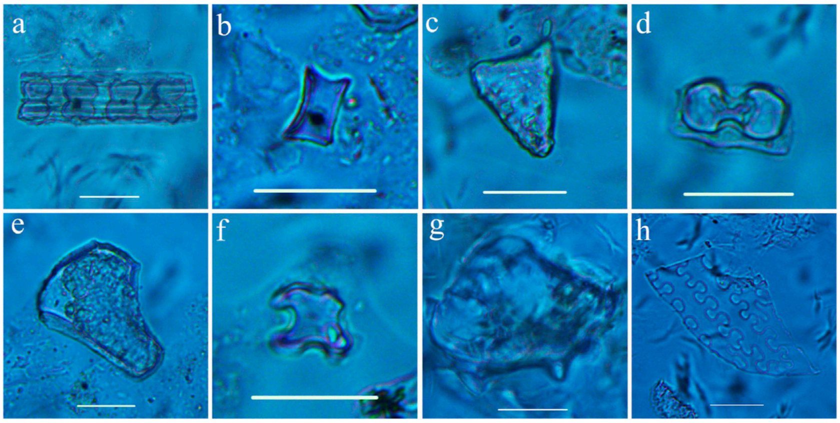

FIGURE 7 | Phytoliths from the Qingtai site (scale bar: 20 μm). **(A)**: dumbbell with scooped ends paralleled arrangement; **(B)**: square saddle; **(C)**: hair cell; **(D)**: bilobate; **(E)**: fan-shape; **(F)**: cross; **(G)**: double-peak; **(H)**: Ω-type.

the three potteries were related to the cereal-based beverages. However lactic acid and amber acid are also present in fruit and honey wines (He, 2017; Zeng et al., 2018). Meanwhile, malic acid, citric acid, oxalic acid, and pyruvic acid are the main organic acids in fruit and honey wine; citric acid is also commonly found in honey wine (He, 2017; Li, 2017; Zeng et al., 2018). The macro-botanical remains of fruit such as jujube, grape, and hawthorn were recovered from the Qingtai site (data from Miaomiao Yang's result, unpublished). Due to the absence of tartaric acid, which is the strongest biomarker of grape wine (Cheng et al., 2013), the addition of grapes might be ruled out. As the surfaces of fruits and honey contain yeast which can convert sugars to ethanol and carbon dioxide (McGovern et al., 2005), it can be seen that the Qingtai people were likely to add fruits and/or honey as fermentation agents.

Based on multiple lines of evidence in the current data, the wine from Qingtai was likely a mixed alcoholic beverage made of various cereals as main ingredients, fruit and/or honey as a starter, as well as supplemented by roots, tubers, beans, and nuts.

4.2 Brewing Vessels

First, phytoliths were detected only on the funnel among the three wine vessels of the Qingtai site. Our simulation experiments show that filtration will affect the amount of phytoliths residues in wine. Given that silk relics have been unearthed from the Qingtai site, it is believable that people at the site were able to separate the dregs from the wine (Zhang and Gao, 1999). Combining the shapes of the three vessels, the combination of residues, and the brewing simulation experiment results, we speculated that the unhulled cereals were indeed used in the production of alcoholic beverages, and then the funnel was used to filter the dregs. Therefore, starch grains, phytoliths of husk, stems, and leaves, as well as organic acids were left on the surface of the funnel. The painted bottle was likely used to store filtered alcohol, so no phytolith was found in it. Because the sample size of the lid residue was too tiny to make a clear judgment, it might be covered on the wine storage container to prevent the evaporation of alcohol. What's more, three pottery strainers were unearthed from the layers of the late Yangshao Culture in Dahecun, Huizui, and Yangshaocun sites in central and western Henan, and alcohol residues were found on one of the strainers (Liu et al., 2019a). It can be said that the filtering behavior had appeared in the political center of late Yangshao Culture, which represents the progress of brewing technology and people's higher pursuit for the taste of alcoholic beverages.

Second, throughout the Yangshao period, the shape of fermentation and storage vessels gradually evolved from globular jars to *pingdiqi*, then to *jiandiping* and vats (Liu, 2021a; Feng et al., 2021). In our experiment, it is a remarkable fact that no starch grain was observed in seven *jiandiping* and five flat-based jars collected at the Qingtai site, suggesting that the functions of these vessels were probably not related to alcoholic beverages. The existing statistics data show that the Yangshao sites where *jiandiping* and *pingdiqi* were used as wine vessels were distributed in the Weihe River valley (**Figure 1**: Lingkou, Jiangzhai, Banpo, Yangguanzhai, Mijiaya, Xinjie) and the western Henan (**Figure 1**: Dingcun), so the findings at the Qingtai site might have reflected the inconsistency among different regions. In addition, no alcohol residue was found in 10 *jiandiping* or flat-based jars collected from the Shuanghuaishu site in central Henan (mid-late Yangshao Culture), but alcohol residues were detected in one *jiandiping* and one vat from Chengyan site in western Henan (early Yangshao Culture) base on residue analysis of other sites in our laboratory (data from Jingwen Liao's master thesis, unpublished). These data further confirmed that the fermentation vessels in Yangshao Culture were probably not a single linear evolutionary model, but a more complex multi-mode between different regions and stages.

4.3 Drinking Patterns

Yangshao Culture is famous for its painted images. This 36 cm-high painted bottle was painted with black patterns of zigzag, octagonal, and circular electric fans on its surface. The leader of the Qingtai excavation team believed the images were symbolic of the Sun and reproductive worship, and partly reflected the religious beliefs (Gu, 2018). Speculated that the geometric patterns on the Yangshao amphorae were likely to be the earliest symbol of alcoholic beverages in prehistoric China, which expressed a functional significance Liu (2021a). Whether as a religious or functional expression, such an exquisite wine vessel was a funerary object and carefully buried in a niche (the only niche in the Qingtai), highlighting the significance of alcoholic beverages in the late Neolithic.

Previous studies have pointed out that the drinking patterns in the Yangshao Culture were likely to communal siphoning drinking with group dancing (Liu, 2017b; Liu et al., 2018b; Liu et al., 2020a; Liu et al., 2020c). In the early Yangshao period, this kind of communal feast was mainly to maintain community solidarity. While in the late period, it was the competitive feasting for gaining and maintaining personal status (Feng et al., 2021). However, the phenomenon of siphoning drinking has not been observed in Qingtai. Funnel for filtering and small painted bottle for storage from Qingtai indicated a new way of drinking pattern, which might have evolved from communal siphoning drinking to drinking alone, or a few people using cups to drink the clear liquid poured from the bottle. Although no evidence of wine cups was found in this study, which may be related to the small sample size, scientific evidence has been provided for the use of wine cups at the Shuanghuaishu, the same period and area site as the Qingtai site (data from Jingwen Liao's master thesis, unpublished). In general, the appearance of these new wine vessel types reflected the changes in drinking methods of prehistoric humans in the early civilized society of the late Yangshao culture. But it is unclear whether this change in drinking patterns implied a new form of social organization in the late Neolithic period.

5 CONCLUSION

This paper provides a scientific case study of the production and consumption of alcoholic beverages in the early civilized Chinese society by analyzing microfossils and organic acids on 17 pieces of late Neolithic pottery from the Qingtai site. Multiple shreds of evidence suggest that the painted bottle, funnel, and lid at the Qingtai site, a large-scale settlement of the late Yangshao culture, were associated with brewing and serving mixed filtered alcohol beverages, which were probably fermented by fruits and/or honey. The ingredients were mainly foxtail millet, rice, Job's tears, Triticeae, snake gourd root, lotus root, legumes, nuts, fruits, and/or honey. What's more, this research has also shown that the *jiandiping* amphora or flat-based jars at the Qingtai site were probably not used for brewing or drinking. Another major finding is that people in Qingtai preferred to drink filtered alcohol alone, or a few people drink the filtered alcohol poured from the painted bottle.

As a pilot study, our simulation experiments propose a more cautious model for the interpretation of alcohol residues, and offer the first comprehensive archaeobotanical and organic chemical analyses of Chinese prehistoric alcoholic vessels, highlighting the regional differences in the Yangshao Culture alcoholic vessels, brewing techniques, and drinking patterns. Unfortunately, although a variety of artifacts potentially associated with the brewing process were sampled, the fermenter and wine cup were not found in Qingtai yet, probably because of the limited sample size. Moreover, the links between the consumption of fermented beverages and social changes at a critical time in the origin of Chinese civilization were not sufficiently discussed in our paper. Thus, a further expansion of the sample size and the integration of cultural context and archaeological data are expected in future work to enhance our understanding on the role of prehistoric alcoholic beverages in the origins of world civilization.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YY, JZ, and JL designed the research. JL, YY, JZ, and YG completed the writing. WG, QW, and LD completed the excavation of the Qingtai Site. JL, WL, LY, YG, and CG completed sampling in the field and experiments in the laboratory.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.884630/full#supplementary-material

REFERENCES

Barata, A., Malfeito-Ferreira, M., and Loureiro, V. (2012). The Microbial Ecology of Wine Grape Berries. *Int. J. Food Microbiol.* 153, 243–259. doi:10.1016/j.ijfoodmicro.2011.11.025

Berihuete-Azorín, M., Girbal, J., Piqué, R., Palomo, A., and Terradas, X. (2018). Punk's Not Dead. Fungi for Tinder at the Neolithic Site of La Draga (NE Iberia). *PLoS One* 13, e0195846. doi:10.1371/journal.pone.0195846

Cheng, B., Zhang, J., Xu, H., Hou, L., Wang, Y., Zhang, Y., et al. (2013). Analysis of Organic Acid Contents in Wine Grape from Different Cultivars. *Food Sci.* 34, 223–228.

Coelho, E. M., da Silva Padilha, C. V., Miskinis, G. A., de Sá, A. G. B., Pereira, G. E., de Azevêdo, L. C., et al. (2018). Simultaneous Analysis of Sugars and Organic Acids in Wine and Grape Juices by HPLC: Method Validation and Characterization of Products from Northeast Brazil. *J. Food Compost. Anal.* 66, 160–167. doi:10.1016/j.jfca.2017.12.017

Dugan, F. M. (2008). *Fungi in the Ancient World: How Mushrooms, Mildews, Molds, and Yeast Shaped the Early Civilizations of Europe, the Mediterranean, and the Near East*. Minnesota: American Phytopathological Society (APS Press.

Fang, Y. (2018). Five Major Archaeological Discoveries in Henan Province in 2017. *Huaxia Archaeology* 3, 122–128.

Feng, S., Liu, L., Wang, J., Levin, M. J., Li, X., and Ma, X. (2021). Red Beer Consumption and Elite Utensils: The Emergence of Competitive Feasting in the Yangshao Culture, North China. *J. Anthropological Archaeology* 64, 101365. doi:10.1016/j.jaa.2021.101365

Gao, J. (2019). On the Pattern and Motive Force of the Early State Formation in China. *J. Hist. Sci.* 6, 21–33.

Ge, W., Liu, L., and Jin, Z. (2010). Morphological Analyses on Starch Granules of Five Grass Species and Their Significance for Archaeology. *Quat. Sci.* 30, 377–384.

Gu, W. (2018). A Painted Gourd Bottle Found at Qingtai Site in Xingyang, Henan Province. *Yellow River Yellow Earth Yellow Race* 10, 61–62.

Han, J. (2019). The Earliest China: On the Formation of Early China as a Mono-Centered Complex. *Cult. Relics Cent. China* 5, 60–65.

Hao, J., Ke, F., Liang, Z., Zhang, L., Dai, Z., Shi, S., et al. (2021). Determination of 18 Kinds of Organic Acids in rice-flavor Liquor by Liquid Phase Method. *Sci. Technology Food Industry* 42, 283–290.

Hayden, B. (2003). Were Luxury Foods the First Domesticates? Ethnoarchaeological Perspectives from Southeast Asia. *World Archaeology* 34, 458–469. doi:10.1080/0043824021000026459a

He, R. (2017). *Studies On Brewing Technology, Flavor Components And Antioxidant Activity Of Mead*. Master's Thesis. Chongqing (China): Southwest University.

Henry, A. G. (2020). *Handbook for the Analysis of Micro-particles in Archaeological Samples*. Switzerland: Springer International Publishing.

Henry, A. G., Hudson, H. F., and Piperno, D. R. (2009). Changes in Starch Grain Morphologies from Cooking. *J. Archaeological Sci.* 36, 915–922. doi:10.1016/j.jas.2008.11.008

Huang, X. (2002). Wine Culture and Social Politics in Ancient China. *J. Chin. Cult.* 2, 127–131.

Huang, Y. (2008). *Wine, Winest And Traditional Culture: The Study Of the Wine Culture Of Ancient China*. PhD Thesis. Xiamen (China): Xiamen University.

Li, J. (2017). *The Application Of The Organic Acids And Proteins Determination On Wine Residues Research*. Master's Thesis. Xi'an (China): Northwest University.

Liu, L. (2021a). Communal Drinking Rituals and Social Formations in the Yellow River valley of Neolithic China. *J. Anthropological Archaeology* 63, 101310. doi:10.1016/j.jaa.2021.101310

Liu, L. (2017b). Early Pottery, Porridge, Wine Making and the Development of Social Complexity. *Cult. Relics Cent. China* 2, 24–34.

Liu, L., Li, Y., and Hou, J. (2020b). Making Beer with Malted Cereals and Qu Starter in the Neolithic Yangshao Culture, China. *J. Archaeological Sci. Rep.* 29, 102134. doi:10.1016/j.jasrep.2019.102134

Liu, L., Wang, J., Chen, X., and Li, Y. (2019a). Analysis of Neolithic Pottery Strainers in the Middle Yellow River valley. *Cult. Relics Cent. China* 6, 55–61.

Liu, L., Wang, J., Chen, X., Li, Y., and Zhao, H. (2018b). Large Houses and Feasting Tradition of the Yangshao Culture: Starch and Phytolith Analyses of the Residues from Pottery Vessels and Floors of House No.1 at Huizui in Yanshi, Henan. *Cult. Relics Cent. China* 1, 32–43.

Liu, L., Wang, J., Chen, X., and Liang, Z. (2021b). Investigation of Alcohol-Related Vessels of the Dawenkou Culture in Shandong. *Huaxia Archaeology* 1, 49–61.

Liu, L., Wang, J., Chen, X., and Liang, Z. (2020d). The Fermentation Function of Globular Jars in the Beixin Culture. *Southeast Cult.* 5, 74–84.

Liu, L., Wang, J., and Di, N. (2020c). From Flatbase Amphorae to Conical-Base Amphorae – Changes in Fermentation Vessels and Brewing Methods in Neolithic Middle Yellow River Region. *Cult. Relics Cent. China* 3, 94–106.

Liu, L., Wang, J., Levin, M. J., Sinnott-Armstrong, N., Zhao, H., Zhao, Y., et al. (2019b). The Origins of Specialized Pottery and Diverse Alcohol Fermentation Techniques in Early Neolithic China. *Proc. Natl. Acad. Sci. U.S.A.* 116, 12767–12774. doi:10.1073/pnas.1902668116

Liu, L., Wang, J., and Liu, H. (2020a). The Brewing Function of the First Amphorae in the Neolithic Yangshao Culture, North China. *Archaeol Anthropol. Sci.* 12. doi:10.1007/s12520-020-01069-3

Liu, L., Wang, J., Zhao, H., Shao, J., Di, N., and Feng, S. (2018a). Residue Analyses on Pottery from the Late Yangshao Culture Site of Xinjie in Lantian, Shaanxi: New Evidence of Beer Brewing. *Agric. Archaeology* 1, 7–15.

Liu, L., Wang, J., Zhao, Y., and Yang, L. (2017a). Beer in the Yangshao Culture: Decoding the Function of Pottery at the Yangguanzhai Site. *Agric. Archaeology* 6, 26–32.

Lu, H., Zhang, J., Wu, N., Liu, K.-b., Xu, D., and Li, Q. (2009). Phytoliths Analysis for the Discrimination of Foxtail Millet (Setaria Italica) and Common Millet (Panicum Miliaceum). *PLoS One* 4, e4448. doi:10.1371/journal.pone.0004448

Madella, M., Alexandre, A., and Ball, T. (2005). International Code for Phytolith Nomenclature 1.0. *Ann. Bot.* 96, 253–260. doi:10.1093/aob/mci172

McGovern, P. E., Underhill, A. P., Fang, H., Luan, F., Hall, G. R., Yu, H., et al. (2005). Chemical Identification and Cultural Implications of a Mixed Fermented Beverage from Late Prehistoric China. *Asian Perspect.* 44 (2), 249–275. doi:10.1353/asi.2005.0026

McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., et al. (2004). Fermented Beverages of Pre- and Proto-Historic China. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17593–17598. doi:10.1073/pnas.0407921102

Neumann, K., Neumann, K., Strömberg, C. A. E., Ball, T., Albert, R. M., Vrydaghs, L., et al. (2019). International Code for Phytolith Nomenclature (ICPN) 2.0. *Ann. Bot.* 124, 189–199. doi:10.1093/aob/mcz064

St-Germain, G. S., and Summerbell, R. (2011). *Identifying Fungi: A Clinical Laboratory Handbood*. Belmont, CA: Star Publishing Company. Guys,Summerbell.

Sun, Y. (2018). *Plant Food Sources and Pottery Function at the Site of Qingtai and Dongzhao from Yangshao Period to the Bronze Age Based on the Starch Grain analysisMaster's Thesis*. Hefei (China): University of Science and Technology of China.

Wan, Z., Yang, X., Ge, Q., and Jiang, M. (2011). Morphological Characteristics of Starch Grains of Root and Tuber Plants in South China. *Quat. Sci.* 31, 736–744.

Wang, J., Liu, L., Ball, T., Yu, L., Li, Y., and Xing, F. (2016). Revealing a 5,000-Y-Old Beer Recipe in china. *Proc. Natl. Acad. Sci. U.S.A.* 113, 6444–6448. doi:10.1073/pnas.1601465113

Wang, J., Liu, L., Georgescu, A., Le, V. V., Ota, M. H., Tang, S., et al. (2017). Identifying Ancient Beer Brewing through Starch Analysis: A Methodology. *J. Archaeological Sci. Rep.* 15, 150–160. doi:10.1016/j.jasrep.2017.07.016

Wang, Q., Jia, X., Li, M., and Yang, X. (2013). Morphological Analysis of Starch Grains of Common Edible Legumes in China and its Application in Agricultural Archaeology. *Wenwu Chunqiu* 3, 3–11.

Wang, W. (2008). An Understanding of the Concepts Related to the Study of the Origin of Chinese Civilization. *J. Hist. Sci.* 1, 10–13.

Wang, W. (2020). Archaeological Evidence of Chinese Civilization of More Than 5,000 Years. *QiuShi* 2, 70–76.

Wei, C., Zhang, J., Zhou, W., Chen, Y., and Liu, Q. (2008). Degradation of Amyloplast Envelope and Discussion on the Concept of Compound Starch Granule in Rice Endosperm. *Chin. J. Rice Sci.* 22, 377–384.

Wei, X. (2021). From Culture to Civilization: On Yangshao Culture and Chinese Archaeology in the Past Century & Civilization Achievements in Yangshao Culture. *Huaxia Archaeology* 4, 3–10.

Xu, H. (2004). The Remains of Ritual System and the Origin of Ritual Music Culture. *Archaeology of Xia, Shang and Zhou dynasties* 1, 21–33.

Yang, J., Ge, J., Liu, L., Ding, Y., and Tan, Y. (2009). Gap Phase Regeneration Recruitment of Mixed conifer-broadleaf Forests in Wolong Nature Reserve. *Front. For. China* 4, 153–158. doi:10.1007/s11461-009-0031-5

Yang, X., Kong, Z., Liu, C., and Ge, Q. (2010). Morphological Characteristics of Starch Grains of Millets and Their Wild Relatives in North China. *Quat. Sci.* 30, 364–371.

Yang, X., and Perry, L. (2013). Identification of Ancient Starch Grains from the Tribe Triticeae in the North China Plain. *J. Archaeological Sci.* 40, 3170–3177. doi:10.1016/j.jas.2013.04.004

Zeng, J., Ma, Y., Qin, D., and Zeng, L. (2018). Research on Effect and Detection Method of Organic Acids in Fruit Wine. *China Brewing* 37, 183–187.

Zhang, S., and Gao, H. (1999). Observation and Study on Silk and Linen Unearthed from Qingtai Site in Xingyang. *Cult. Relics Cent. China* 3, 10–16.

Zhang, S., and Zhao, Q. (1987). Excavation of the Yangshao Culture Site at Qingtai in the First Half of 1981. *Cult. Relics Cent. China* 1, 3–9.

Zhao, P., Cai, J., Gu, S., Qian, B., Wang, L., Lu, F., et al. (2020). Analysis of Characteristic Flavor Substances of Traditional Shaoxing Rice Wines of Different Ages. *Food Sci.* 41, 231–237.

Zhao, Y., and Liu, L. (2021). Investigation of Alcoholic Fermentation Methods in the Yangshao Culture in Eastern Gansu Region. *Cult. Relics Cent. China* 1, 49–63.

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Description of Starch Granules From Edible Acorns (Oak), Palms, and Cycads in Southern China

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A review of ethnological and archaeobotanical evidence shows the potential for a range of starch-rich woody plants, beyond tuberous plants, to have been important foods in prehistoric south subtropical China. In this paper we review the size and shape characteristics of starch granules non-tuberous woody plants (Palms, Cycads and Acorns) that our research has identified as important sources of carbohydrates for prehistoric communities. The study sample consists of 34 modern starch reference samples across eight genera (Palms: *Arenga, Caryota,*; Cycads:*Cycas*; and, Acorns: *Castanopsis*, *Fagus, Lithocarpus, Quercus,* and *Quercus* section *Cyclobalanopsis*). Our descriptive criteria are developed granule descriptors standard in the literature and then assessed for their utility using multiple correspondence analysis. The results demonstrate that both morphometric characteristics and the maximum size of granules are valuable for distinguishing starch granules at various taxonomic levels. Of the five morphometric characteristics recorded in this study sample, granule shape is the most effective variable for granule identification.

Keywords: starch identification, non-parametric test, multiple correspondence analysis, woody plants, southern China

[1]Recent molecular studies of the oak grouping, *Cyclobalanopsis*, has revised this Southeast Asian group to the section level within the genus Quercus (see Denk et al., 2017; Hipp et al., 2020; Zhou et al., 2022). As we understand it, the use of the term *Cyclobalanopsis* refers in part, to the particular phenotypic expression of acorns in having acorns with distinctive cups bearing concrescent rings of scales; they commonly also have densely clustered acorns, though this does not apply to all of the species. Phenotypic differences will have mattered to hunter-gatherers collecting these plantswhile they may have processed and eaten them in similar ways to all other *Quercus*, the visual differences in the acorns, while not significant in terms of their molecular evolution, are likely to have featured in ethno-taxonomies. Differences in growth habitat will also have mattered to hunter-gatherers and be significant in our consideration of their use as a food in prehistory. To align our paper with previous studies and to reduce confusion in comparisons of starch granules, we continue to refer to *Cyclobalanopsis* sp. in this paper, while recognising it is technically inaccurate in current botanical scholarship.

1 INTRODUCTION

Starch grain analysis contributes to the understanding of several archaeological issues including prehistoric diet (e.g. Loy et al., 1992; Barton 2005; Lu et al., 2005; Liu et al., 2010; Yang and Jiang 2010; Yao et al., 2016), tool use (e.g. Piperno et al., 2004; Revedin et al., 2010; Buckley et al., 2014; Ma et al., 2014) and the emergence of agriculture and plant domestication (e.g. Denham et al., 2003; Pearsall et al., 2004; Perry et al., 2007; Yang et al., 2012). To contribute to these fields, it is normally necessary to attempt a taxonomic identification of recovered starch granules by direct comparison with available reference material and sometimes with published granule descriptions from other studies (e.g. Torrence and Barton 2006; Yang and Perry 2013; Yao et al., 2016; Guan et al., 2020; Lucarini and Radini 2020; Tsafou and García-Granero 2021). A morphometric analysis by granule description is the most common approach (e.g. Loy 1994; Piperno and Holst 1998; Torrence and Barton 2006; Liu et al., 2014; Mercader et al., 2018; Brown and Louderback 2020), but some limitations are recognized including the experience of the analyst, the breadth of the reference collection, the state of granule preservation (e.g. Lamb and Loy 2005; Barton 2009; Yang and Perry 2013; Ma et al., 2019), and complications arising from a similarity of granule size and shape between species (e.g. Liu et al., 2014; Guan et al., 2020). The development of expert systems for granule identification, or at least an increased reliance on quantitative approaches that are not so dependent on subjective decisions made by the analyst, have been recommended (Torrence et al., 2004; Wilson et al., 2010; Liu et al., 2014; Coster and Field 2015; Arráiz et al., 2016; Louderback et al., 2016). To date, no expert system has replaced the need for an experienced analyst to identify granules at a taxonomic level. With the growing importance of this technique in archaeology, and the potential it has to resolve important questions about tool use and diet in prehistoric China, here we apply some statistical procedures to assess the reliability of our recording variables so that we may improve the reliability of identification of ancient starch granules from our study region of south subtropical China.

Starch residue analyses of stone tools from a range of archaeological sites in south subtropical China, dating between 10,000 and 2,000 BP, has already provided direct evidence for the critical horticultural hypothesis, which emphasized the importance of tuberous plants, such as taros (e.g. *Colocasia* spp.), yams (e.g. *Dioscorea* spp.), and lotus root (*Nelumbo nucifera* Gaertn), in the indigenous diet and agricultural system (e.g. Li and Lu 1987; Yin 2000; Lu 2003; Zhao 2005; Yang et al., 2013; Li et al., 2016). Our review of the available historical texts and some archaeological finds from southern China, however, reveal that some lesser-known starch-rich woody plants, including acorns, palms and cycads, may have also contributed significant calories to the communities in this region (Cao et al., 2011; Ge 2015; Geng 2019; Liu 1983; Liu et al., 2012; Zhou et al., 2014; Fuller and Qin 2010; Yang et al., 2013).

Edible acorns (nuts of the Fagaceae family) were once an important food staple for hunter-gatherer and farming communities in the northern hemisphere. Archaeological records document the consumption of large quantities of acorns in the woodlands of Europe, North Africa, Southeast Asia and North America (e.g. Fuller and Qin 2010; Higham 2014; Messner 2011; Humphrey et al., 2014; Kawashima 2016; Lentfer et al., 2013; Noshiro 2016; Sasaki and Noshiro 2018; Stevens and Mcelreath 2015; Tushingham and Bettinger 2013). Preserved acorn exocarps (the tough outer shell) have been recovered from several waterlogged sites in southern China, including the Tianluoshan site (7,000–6,000 BP) in the Yangtze River Basin and the Guye site (5,900–5,000 BP) in the Pearl River Delta (Fuller et al., 2011; Institute of Cultural Relics and Archaeology of Zhejiang Institute of Cultural Relics and Archaeology 2004; Yang et al., 2018). At the Tianluoshan site, acorns (*Cyclobalanopsis/Lithocarpus/Quercus*) were stored in special purpose subterranean storage pits (Fuller and Qin 2010). Acorn starches have been identified from the surfaces of stone tools at the Xiaohuangshan site (9,000–7,700 BP), charred residues inside the ceramic pottery at the Kuahuqiao site (8,000–7,000 BP), and dental calculus at the Qihe Cave site (c. 9,500 BP), indicating long-term use of this plant resource in the local and regional diets of southern China (Guan et al., 2018; Liu et al., 2010; Yang and Jiang 2010; Yao et al., 2016). However, acorns, as food staples, were gradually replaced by other crops, including domesticated rice (Fuller and Qin 2010). In the historic period, acorns remained important in seasonal diets, but were relegated to a supplementary food or famine food, as noted, for example, in *Lu's Commentaries* of History (finished c. 239 BC), or the Compendium of Materia Medica (finished in 1578). Today, some ethnic minorities in southwest China, such as the Dong people and Wa people, still preserve the tradition of acorn consumption, albeit on a small scale (**Supplementary Table S1**) (Cao et al., 2011; Liu et al., 2012; Zhou et al., 2014).

Another important, though sidelined starchy plant foods in southern China is the palms which store starch in the trunk pith, recorded as *Guanglang* (桄榔), *Suomu* (莎木, the possible phonetic name of "sago"), and *Mianmu* (面木, meaning the trees with flour) (Ge 2015; Geng 2019; Lin 1997). The archaeological evidence for this foodstuff is confirmed only at the Xincun site in the Pearl River Estuary (5,300–4,400 BP) by recovered starch granules from at least two genera of palms (*Caryota* Lour. and *Coryphya* L.) (Yang et al., 2013). However, Chinese scholars have identified several genera of palms with edible pith, including guanglang (*Arenga* spp.), fishtail palms (*Caryota* spp.), as well as some species of cycads (*Cycas* spp.), from a series of Chinese historic texts (Ge 2015; Geng 2019; Lin 1997). For example, *Hua Yang Guo Zhi* (Eastern Jin Dynasty, 317–420 AD) recorded guanglang flour being consumed in the cities of the Yunnan-Guizhou Plateau where cereal crops were lacking. According to *Shu Du Fu*, this flour was already one of the critical commodities in the markets of the Sichuan basin, a region outside the natural range of these plants, at the time of the Wei-Jin dynasties (c. 220–315 AD), (Flora of China, online at: http://www.iplant.cn/info/%E6%A1%84%E6%A6%94?t=foc). This record shows the importance of this food, and that it was a traded commodity in some regions of southern China in the historic period. The Zhuang people in Longzhou, Guangxi still

TABLE 1 | Modern reference study sample.

Family	Genus	Species	Granules Size			
			Length Range/μm	Mean Length/μm	Median	Granule Count
Fagaceae	*Lithocarpus*	*Lithocarpus cleistocarpus*[a]	5.91–15.61	9.2 ± 1.9	8.7	135
Fagaceae	*Quercus*	*Quercus franchetii*[b]	5.13–24.93	10.0 ± 3.3	9.4	111
Fagaceae	*Quercus*	*Quercus oxyphylla*[b]	5.07–21.58	10.1 ± 3.0	9.5	107
Fagaceae	*Quercus*	*Quercus variabilis*[b]	5.07–21.17	10.2 ± 2.7	9.9	116
Fagaceae	*Lithocarpus*	*Lithocarpus litseifolius*[a]	5.54–17.69	10.5 ± 2.3	9.9	100
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis phanera*[a]	6.84–19.88	11.5 ± 2.5	11.0	110
Fagaceae	*Castanopsis*	*Castanopsis fargesii*[a]	6.6–19.5	12.0 ± 2.2	12.0	101
Fagaceae	*Castanopsis*	*Castanopsis platyacantha*[a]	8.3–19.2	12.5 ± 2.0	12.3	113
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis chapensis*[a]	6.29–20.83	12.9 ± 2.5	12.7	117
Fagaceae	*Quercus*	*Quercus cocciferoides*[b]	5.01–28.46	13.0 ± 3.8	12.7	121
Fagaceae	*Castanopsis*	*Castanopsis sclerophylla*[a]	6.5–22.1	13.2 ± 3.0	13.1	101
Cycadaceae	*Cycas*	*Cycas pectinata*	5.3–30.3	14.1 ± 4.8	13.6	112
Fagaceae	*Castanopsis*	*Castanopsis hystrix*[a]	6.1–28.7	14.3 ± 4.1	14.4	105
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis glauca*[a]	7.63–22.48	14.6 ± 2.6	14.6	122
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis gambleana*[a]	7.69–24.88	14.7 ± 3.6	14.7	110
Palmae	*Caryota*	*Caryota obtusa*	8.0–47.3	18.1 ± 8.2	15.4	105
Cycadaceae	*Cycas*	*Cycas panzhihuaensis*	11.2–37.0	20.6 ± 4.9	20.2	113
Palmae	*Arenga*	*Arenga westerhoutii* (white)[d]	7.4–52.0	26.4 ± 11.3	24.8	100
Palmae	*Arenga*	*Arenga westerhoutii* (red)[d]	11.7–65.8	29.5 ± 11.3	27.3	116
Fagaceae	*Fagus*	*Fagus sylvatica*[c]	<5	-	-	>6
Fagaceae	*Lithocarpus*	*Lithocarpus chrysocomus*[a]	15.89	-	-	1
Fagaceae	*Castanopsis*	*Castanopsis fleuryi*[a]	-	-	-	0
Fagaceae	*Castanopsis*	*Castanopsis kweichowensis*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus crenata*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus engleriana*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus grandifolia*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus longipetiolata*[c]	-	-	-	0
Fagaceae	*Fagus*	*Fagus longipetiolata*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus lucida*[c]	-	-	-	0
Fagaceae	*Fagus*	*Fagus orientalis*[c]	-	-	-	0
Fagaceae	*Lithocarpus*	*Lithocarpus balansae*[a]	-	-	-	0
Fagaceae	*Lithocarpus*	*Lithocarpus corneus*[a]	-	-	-	0
Fagaceae	*Lithocarpus*	*Lithocarpus fohaiensis*[a]	-	-	-	0
Fagaceae	*Quercus*	*Quercus* semecarpifolia[b]	-	-	-	0

[a]*Provided by Herbarium, Kunming Institute of Botany, CAS.*
[b]*Collected in the field with CAS, botanists.*
[c]*provided by Herbarium, Institute of Botany, CAS.*
[d]*Two landraces of guanglang palm flour were purchased from Longzhou, Guangxi. Red flour is extracted from guanglang palm trees over 10 m in height, which have low starch content, while white flour comes from high-yielding trees that grow to around five to 6 m tall.*

value guanglang flour as a traditional food and a medicinal supplement for treating diarrhoea (Ge 2015).

From the above review, we expand our range of important food plants in prehistoric southern China beyond roots and tubers to incorporate starchy palms, cycads and acorns, which may have been underestimated as important foods in previous studies. We therefore seek to expand our range of identifiable starches and look to the statistical analysis of modern reference materials to improve methods of identification as well as establish a reliable identification key for these species.

2 MATERIALS AND METHODS

2.1 Modern Starch Reference Collections

The starch reference collection for this study comes from a total of 31 reference samples of oak trees (Fagaceae, $n = 29$) and two new collections of guanglang landraces (*Arenga westerhoutii*). These specimens are supplemented for analysis by species of palm (*Caryota obtusa*) and cycads (*Cycas pectinata* and *Cycas panzhihuaensis*) already included in the Chinese Modern Starch Reference Database held at the Institute of Geographical Sciences and Natural Resources Research, Beijing (**Table 1**).

The oaks, Fagaceae, were primarily obtained from the Herbarium of the Kunming Institute of Botany while some *Fagus* samples were provided by the Herbarium of the Institute of Botany, Chinese Academy of Science. We also collected some *Quercus* nuts in the field with botanists, such as *Q. semecarpifolia* nuts from the woodland behind the Huanglong Temple in Yunnan Province and *Q. variabilis*/*Q. franchetii* from the Kunming Botanical Garden (**Table 1**). With the help of botanists, we selected as "intact and mature" samples as possible for this study. All of them were identified to species level by botanists from the Kunming Institute of Botany, Chinese Academy of Sciences. We

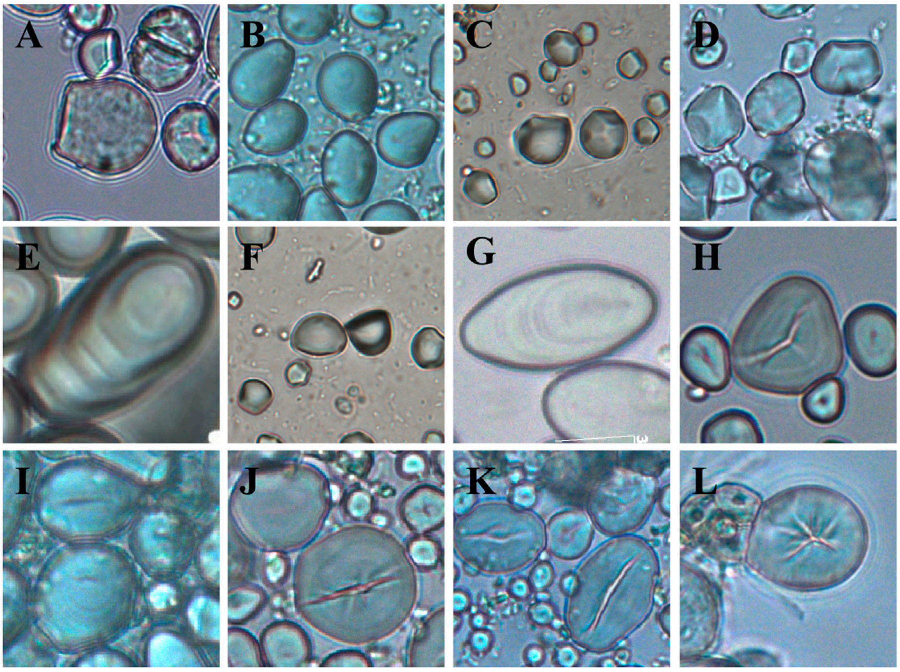

FIGURE 1 | Some of the morphological features considered in this paper: **(A)** bell-shaped granule with rough surface (*Cyclobalanopsis glauca*); **(B)** drop-shaped granule (*Quercus cocciferoides*); **(C)** faceted spherical granule (*Cycas pectinata*); **(D)** irregular polyhedron granule (*Castanopsis hystrix*); **(E)** pestle-shaped granule with visible lamellae (*Caryota obtusa*); **(F)** spherical cap granule (*Cycas pectinata*); **(G)** spindle-shaped granule with visible lamellae (*Arenga westerhoutii* (red)); **(H)**, triangle granule with v-shaped fissure (*Quercus variabilis*); **(I)** linear fissure; **(J)** and **(k)** longitudinal fissure; **(L)**, stellate fissure.

purchased two "landrace" samples of guanglang flour (*Arenga*) from local markets in Longzhou, Guangxi. The red sample is the reddish flour extracted from guanglang palm trees over 10 m in height, which have low starch content. It is said that this guanglang landrace is a rare wild landrace which is difficult to cultivate. The white flour is from high-yielding cultivated trees that grow to around five to 6 m tall. Other starch materials including *Caryota* and *Cycas* were analyzed from slides held in the China Modern Starch Grain Morphological Database.

2.2 Method of Extraction of Starches From Specimens

Following the protocols developed by Yang et al. (2009), we released the starch grains from the acorn kernels by the following method: 1) the nut, in a small plastic sealed bag, was broken with a hammer; 2) small pieces of broken nut were transported to new sterile test tubes with pure water for 24 h soaking; 3) after soaking, the samples were crushed with clean glass stirring rods to fully release the starches. The two purchased samples of guanglang palms were collected as ground flour. We dissolved 1 g flour into 20 ml of distilled water for the sample preparation.

All the starch solutions of acorns and guanglang flour were pipetted onto clean glass slides and mounted in a 50%/50% glycerine/water solution. The prepared slides were observed using a Zeiss AxioMAT at the Starch and Residue laboratory, School of Archaeology and Ancient History, University of Leicester. Images of the starch granules were taken with Zeiss AxioCam and Zeiss AxioVision software. For statistical purposes, a minimum of 100 granules of each sample were recorded. We measured the longest orientable measurement of each granule and recorded the following morphological features: granule shape; hilum position; presence/absence of fissures; the form of the fissure; presence or absence of lamellae; and surface texture (smooth/rough) (**Supplementary Table S2**).

2.3 Analyzing Method of Selected Variables

We observed all 34 samples including those newly collected for the database (**Table 1**), and expect at least 100 starch grains to be measured in each sample. If not enough granules were observed, we continued to sample until exhaustion of the sample itself. The collected data, including maximum granule length and the morphological variables (granule shapes, hilum position, lamellae visibility, fissure types, and surface texture) were recorded. The maximum granule sizes were measured using Zeiss Axiovision. We then tested the utility of granule size as a discriminating feature for starch identification statistically, despite the fact that much granule size overlap makes it difficult to use granule size as an independent discriminator. An assessment of the maximum granule sizes indicates that the values of most species tested do not have a normal distribution (**Supplementary Table S4**), violating the assumption required for parametric analysis (Wang 2013; Zhang 2014). Accordingly, we

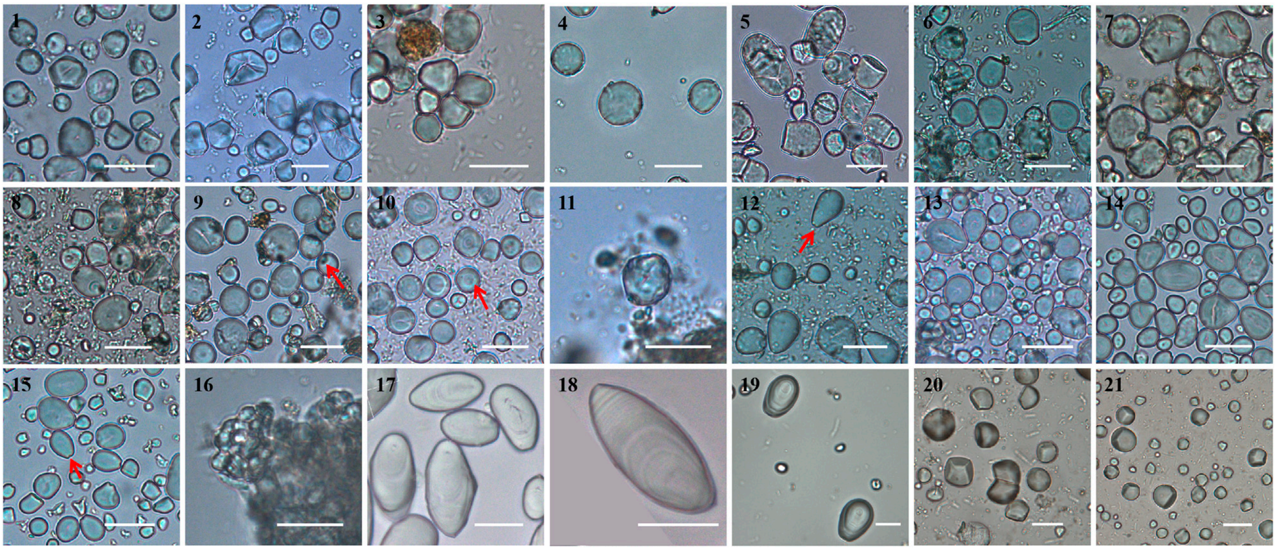

FIGURE 2 | Starch granules from woody starch plants. 1–16, acorn kernels:1, *Castanopsis platyacantha*; 2, *Castanopsis hystrix*; 3, *Castanopsis fargesii*; 4, *Castanopsis sclerophylla*; 5, *Cyclobalanopsis glauca*; 6, *Cyclobalanopsis chapensis*; 7, *Cyclobalanopsis gambleana*; 8, *Cyclobalanopsis phanera*; 9, *Lithocarpus cleistocarpus*; 10, *Lithocarpus litseifolius*; 11, *Lithocarpus chrysocomus*; 12, *Quercus cocciferoides*; 13, *Quercus oxyphylla*; 14, *Quercus variabilis*; 15, *Quercus franchetii*; 16, *Fagus sylvatica*; 17–18, guanglang palm: 17, *Arenga westerhouti* (red); 18, *Arenga westerhouti* (white); 19, fishtail palm: *Caryota obtusa*; 20–21, cycads: 20, *Cycas panzhihuaensis*; 21, *Cycas pectinata*. Scale, 20 μm. The red arrows shows the pits in the granules.

employ a non-parametric test to the dataset, the Kruskal–Wallis test for multiple independent samples. These statistical procedures are performed with SPSS v25.0.

We counted the proportion of each category of the recorded morphological variables to observe and summarize their distribution. In our descriptions of granule morphotypes, we follow the approach of previous studies (e.g. Torrence et al., 2004; Torrence and Barton 2006; Yang and Perry 2013; Liu et al., 2014; Mercader et al., 2018), including overall shape, fissure types, lamellae visibility, hilum position and surface texture (**Figure 1** and **Supplementary Table S2**). We further use a geometric typology that separates overall granule shapes into four categories: irregular-shape type; ovate types; polygonal types; and faceted types. The ovate types include drop-shaped (an ovate with a wide shoulder and one pointed end; **Figure 1B**), pestle-shaped (an ovate with a shoulder at the hilum, tapering toward the distal end; **Figure 1E**), spindle-shaped (an ovate with two symmetrically pointed ends; **Figure 1G**), sphere and oval-shaped. And the faceted types are granules with a spherical or ellipsoid surface but abutting each other in the plant cell causing facets to form (**Figure 1**), including bell-shaped (**Figure 1A**), faceted spheres (**Figure 1C**), and spherical caps (**Figure 1F**). We then calculate the percentage of all the features observed in the starch samples at a species level, in order to identify diagnostic features.

Besides the granule description, we applied statistical procedures to reduce dependence on subjective decisions. As multivariate analysis has been effectively applied in previous starch reference analyses (Devaux et al., 1992; Liu et al., 2014; Torrence et al., 2004), we also select a multivariate analysis approach, multiple correspondence analysis (MCA), to analyse our morphometric data through SPSS v25.0. The MCA results are scatter plots in two dimensions (e.g. **Figure 5**), presenting a data set with categorized variables in a two-dimensional graph (Beh and Lombardo 2014; Devaux et al., 1992; Greenacre 2007; Le Roux and Rouanet 2005; Macheridis 2017). The variables (shown in the columns) are plant taxa, granule shape, fissure pattern, lamellae visibility, hilum position and surface texture, and each object (shown in the rows) represents an individual granule from our dataset. Inertia measures an approximate level of homogeneity within the dataset, the higher the value the greater the correspondence among the data (Greenacre 2007). The centroid of the result plot represents the average distribution in each row, which means that the closer the point comes to the centroid, the less different it is from the rest of the observations (Zhang and Dong 2013). The closer the distribution of the category points in the plot, the more relevant they are in the dataset (Franco 2016). The input dataset for the analysis of all productive species is a 2115*6 matrix. As the main body of our samples is from Fagaceae samples, we therefore analyzed the Fagaceae samples separately. To avoid outliers, we exclude 107 rows where the shape features have high frequency in palm and cycad starches, as distributional patterns of these plots would give emphasis to these uncommon features (Greenacre 2007). Hence, a 1462*6 matrix was analyzed using SPSS 25.0.

3 RESULTS AND DISCUSSION

3.1 Broad Trends in the Observation Data

In our study sample, a total of 19 species, from seven genera, produced slides with over 100 granules (**Table 1**). We note that

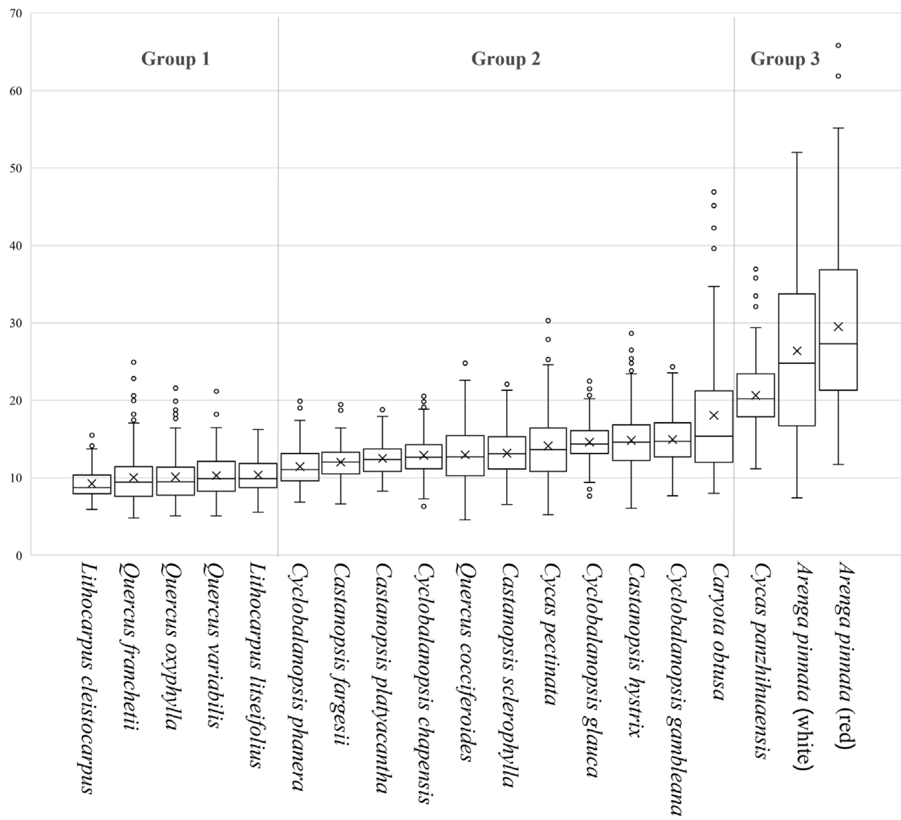

FIGURE 3 | Box plot of the granule sizes of the starches examined.

FIGURE 4 | Percentage distribution of morphological features among plant species (Data shown in **Supplementary Table S2**).

for 15 species of Fagaceae nuts, no, or only few, starch granules were recorded. The *Lithocarpus chrysocomus* sample released only one 15.89 μm granule (**Figure 2**: 11) and *Fagus sylvatica* produced only a few tiny agglomerated granules (**Figure 2**: 16). The lower starch content of some species, such as *Fagus* species, is in accordance with published data (**Supplementary Table S3**).

TABLE 2 | Adjusted significance values of pairwise comparison results of the Kruskal–Wallis test.

Table 2 Adjusted significance values of pairwise comparison results of the Kruskal-Wallis test

	Lithocarpus cleistocarpus	*Quercus franchetii*	*Quercus oxyphylla*	*Quercus variabilis*	*Lithocarpus litseifolius*	*Cyclobalanopsis phanera*	*Castanopsis fargesii*	*Castanopsis platyacantha*	*Cyclobalanopsis chapensis*	*Quercus cocciferoides*	*Castanopsis sclerophylla*	*Cycas pectinata*	*Castanopsis hystrix*	*Cyclobalanopsis glauca*	*Cyclobalanopsis gambleana*	*Caryota obtusa*	*Cycas panzhihuaensis*	*Arenga westerhoutii*(white)	*Arenga westerhoutii*(red)
Lithocarpus cleistocarpus		1.000	1.000	0.339	0.156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus franchetii	1.000		1.000	1.000	1.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus oxyphylla	1.000	1.000		1.000	1.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus variabilis	0.339	1.000	1.000		1.000	0.213	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lithocarpus litseifolius	0.156	1.000	1.000	1.000		0.454	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyclobalanopsis phanera	0.000	0.009	0.021	0.213	0.454		1.000	0.147	0.004	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Castanopsis fargesii	0.000	0.000	0.000	0.000	0.000	1.000		1.000	1.000	1.000	0.644	0.307	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Castanopsis platyacantha	0.000	0.000	0.000	0.000	0.000	0.147	1.000		1.000	1.000	1.000	1.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Cyclobalanopsis chapensis	0.000	0.000	0.000	0.000	0.000	0.004	1.000	1.000		1.000	1.000	1.000	0.038	0.003	0.001	0.000	0.000	0.000	0.000
Quercus cocciferoides	0.000	0.000	0.000	0.000	0.000	0.011	1.000	1.000	1.000		1.000	1.000	0.015	0.001	0.000	0.000	0.000	0.000	0.000
Castanopsis sclerophylla	0.000	0.000	0.000	0.000	0.000	0.000	0.644	1.000	1.000	1.000		1.000	0.413	0.049	0.017	0.001	0.000	0.000	0.000
Cycas pectinata	0.000	0.000	0.000	0.000	0.000	0.000	0.307	1.000	1.000	1.000	1.000		1.000	1.000	1.000	0.127	0.000	0.000	0.000
Castanopsis hystrix	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.038	0.015	0.413	1.000		1.000	1.000	1.000	0.000	0.000	0.000
Cyclobalanopsis glauca	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.049	1.000	1.000		1.000	1.000	0.000	0.000	0.000
Cyclobalanopsis gambleana	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.017	1.000	1.000	1.000		1.000	0.000	0.000	0.000
Caryota obtusa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.127	1.000	1.000	1.000		0.004	0.039	0.000
Cycas panzhihuaensis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004		1.000	1.000
Arenga westerhoutii (white)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039	1.000		1.000
Arenga westerhoutii (red)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	

Asymptotic significance (2-sided tests) is displayed. The significance level is 0.05. Significance values are adjusted by the Bonferroni correction for multiple tests. p < 0.05 suggest that the two species have statistically significant differences in size distribution.

Ida et al. (2017) tested the nutritional content of some edible beechnuts (*Fagus* spp.) which were once consumed in central Honshu, Japan, indicating that these nuts are high in fats, but low in carbohydrate. But there are some inconsistencies (**Supplementary Table S3**). For example, *Lithocarpus corneus*, which has been found to have a starch content of 47.78% of the kernel (Yang 2014), did not release any granules in our study (**Table 1**).

From the productive starch samples, we first review the broad trends in the data, specifically the morphological data and granule size for each category of plant in our sample. In terms of maximum length, *Lithocarpus cleistocarpus* has the smallest starch range in our sample, both in terms of mean size (9.2 μm) and median size (8.7 μm). The starch granules of the *Arenga landraces* are much larger than those of other plants (**Table 1**). However, there is, generally, a lot of overlap in the sizes of our samples, especially of acorns, which may make it difficult to identify them by granule size alone (**Figure 3** and **Table 1**).

The first feature we note is surface texture, which differs between acorn and palm/cycad starch granules (**Figure 4**). It is clear that all palm/cycad starch grains have smooth surfaces, whereas in the acorn samples, there are some granules with a rough surface (**Figure 4**). Among the starches examined, the palm granules are most likely to have visible lamellae. They also have a higher percentage of granules with an eccentric hilum. Conversely, the fissure patterns make it difficult to classify the granules recorded, as the majority do not bear any fissures (**Figure 4**).

We chart the four categories of granule shapes with different colors (**Figure 4**). The grey type includes irregular-shape granules. The faceted types are in blue, while the ovate-types in orange, the polygonal types (e.g. **Figure 1D**) in red. Of the facet types, bell-shaped granules are concentrated in the *Cyclobalanopsis*and *Casntanospsis* sample, especially *Cyclobalanopsis glauca* (**Figure 2**: 5) which accounts for 56% of its total sample (**Figure 4** and **Supplementary Table S2**). The faceted spherical granules are mainly found in the cycad samples, which separately account for 46 and 56% of the study sample (**Figure 4** and **Supplementary Table S2**). Spherical cap granules occur in small proportions in both *Cycas* and *Lithocarpus*. For ovate types, oval and spherical granules are widely distributed in every sample. Drop-shaped granules are primarily observed in *Quercus* while pestle-shaped and spindle-shaped granules are most frequent in palms. Most species produce some form of polygonal granules (**Figure 4**). However, we note an irregular type, with wavy edges or concave/convex surfaces (**Figure 1D**), mostly observed in *Castanopsis*. The two species of this genus, *C. hystrix* (**Figure 2**: 2) and *C. fargesii* (**Figure 2**: 3) also have a high percentage of normal polygonal granules.

3.2 Non-Parametric Test of Maximum Granule Size

Although the overlap in size of our samples cannot be ignored (**Figure 3**), the Kruskal–Wallis test indicates significant differences among the granule size data (H = 1422.199; df = 18; $p < 0.05$). Further pairwise comparison results (**Table 2**) reveal the differences in the size distributions. Combining the median granules size (**Table 1**) and adjusted significance values (**Table 2**), we first note the large-sized granule group containing *Cycas panzhihuaensi* and two landraces of *Arenga* (**Figure 3**, Group 3), for which the median size is larger than 20 microns (**Table 1**). However, there is no significant difference between the three samples. The group of two *Lithocarpus* samples and three *Quercus* samples (**Figure 3**, Group 1) have the same size distributions with a median size smaller than 10 microns (**Table 1**).

The remaining species produce medium-sized granules (**Figure 3**, Group 2), which can be roughly classified into two groups. The first contains five species, *Castanopsis fargesii, Castanopsis platyacantha, Cyclobalanopsis chapensis, Quercus cocciferoides*, and *Castanopsis sclerophylla*, which have similar size distributions, but can be distinguished from other species (**Table 2**). The second contains four species, *Castanopsis hystrix, Cyclobalanopsis glauca, Cyclobalanopsis gambleana*, and *Caryota obtusa* (**Table 2**). The median size of the former group (12.0–13.1 microns) is smaller than the latter (14.4–15.4 microns) (**Table 1**).

The size of *Cyclobalanopsis phanera* has a similar distribution to *Quercus variabilis, Lithocarpus litseifolius, Castanopsis fargesii*, and *Castanopsis platyacantha,* the median size of which is 11.0 microns, while *Cycas pectinata* (median size = 13.6 microns) has no statistically significant difference in size from the most abundant species (n = 9) (**Table 2**).

3.3 Multiple Correspondence Analysis of Morphological Data

3.3.1 Discrimination of all Starches at Species Level

The first-time analysis indicates that the variable fissure pattern has the least discrimination value within the dataset (**Supplementary Table S5**), which corresponds to our overview of the data (**Figure 4**). Hence, we exclude this column from the second analysis. The second-time analysis at species level shows the differences in our reference starches, with the first dimension explaining 52.3% of the inertia and the second dimension explaining 38.8% (**Supplementary Table S5**). The result plot (**Figure 5**) shows that we can distinguish species within the genus Fagaceae.

The Cycadaceae cluster is located in the first quadrant, i.e. the positive direction of both dimensions. This cluster contains two granule shapes, faceted spherical type and spherical cap type, indicating the close correlation between these and the two cycad samples. The other morphological features, according to the discrimination measures (**Supplementary Table S5**) and the point locations (**Figure 5**), which characterise the granules in this cluster are no lamellae, centric hilum, and smooth surfaces. These characteristics are fully consistent with our observations of the overall trends (**Figure 4**).

Along the first dimension, we note another cluster of three Palmae samples, with the points of pestle-shaped, spindle-shaped, and visible lamellae indicating the major morphological features of the Palmae granules (**Figure 5**). As this cluster is also located in the second quadrant, the Palmae granules are distinguishable by other variables, including those that strongly affect the negative direction of the first dimension, eccentric hilum, and the positive direction the second dimension, smooth surfaces. But the results of the two guanglang landraces show a high degree of consistency, so we cannot distinguish them further by their morphological features.

The points representing Fagaceae species are far away from the palm and cycad samples, revealing evident differences in morphological features (**Figure 5**). The most consistent difference is that the granules from edible acorns tend to have rough surfaces, which can be seen in the percentage distribution of the morphological features (**Figure 4**). We also note that the four *Cyclobalanopsis* species and *Castanopsis platyacantha*, in the dashed circle of **Figure 5**, may be distinguishable from other species, as they are relatively far away from the centroid. The granules may have a bell shape or irregular polygonal shape, with rough surfaces. However, the Fagaceae species are relatively close to the centroid of the plot, suggesting that they may represent the average features of our dataset. This may be because the sample of Fagaceae is larger than the other two genera. Hence, we decide to analyse the Fagaceae independently, to find their morphological differences.

3.3.2 Discrimination of Fagaceae Granules at Both Species Level and Genus Level

The analysis of the Fagaceae starches, at both the species level and the genus level, shows that the variables fissure type and lamellae visibility have weak discriminant value (**Supplementary Table S5**), consistent with **Figure 4**, that is, the Fagaceae starches commonly have no fissures or lamellae. Therefore, we focus on the variables of shape, hilum position and granule surface texture within the dataset which could be used to discriminate acorn starches.

The results at the species level show the differences in our reference starches, with the first dimension explaining 51.7% of the inertia and the second dimension explaining 41.0% (**Supplementary Table S5**). Of the two binary variables, based on the discrimination measures (**Supplementary Table S5**), dimension 1 is related to hilum position, i.e. species along the positive direction of dimension 1 tend to have centric hilum, while dimension 2 is surface texture. In this case, we note five rough clusters of Fagaceae starches in the plot (**Figure 6A**).

Cluster 1 contains the points for two *Lithocarpus* species with spherical granules. This indicates a correspondence between spherical granules and these two species. These granules also have a centric hilum and smooth surface. Cluster 2 shows the associations between bell-shaped granules and the species *Castanopsis platyacantha, Cyclobalanopsis glauca* and *Cyclobalanopsis gambleana*. These granules tend to have a centric hilum but rough surface. Along dimension 1, there are two clusters in the negative direction, representing granules with eccentric hilum. Cluster 3 indicates that the granules of *Cyclobalanopsis chapensis* and *Cyclobalanopsis phanera* are similar in morphology, characterized as oval with rough surfaces. Cluster 4 includes *Quercus cocciferoides, Q. variabilis* and *Q. franchetii*. These granules mainly have a drop shape but can be classified further. Compared to the other two species, the starches of *Q. cocciferoides* are more likely to have rough surfaces, consistent with the percentage calculation (**Figure 4**).

Cluster 5 is located in the positive direction of the second dimension, representing an overall trend for granules with smooth surfaces. This cluster contains two species of *Castanopsis*, *C. hystrix* and *C. fargesii*, and has polygonal features, showing their correspondence. According to the point locations, *C. hystrix* tends to produce normal polygonal granules, but *C. fargesii* may produce irregular types. This result is consistent with the trends represented by the granule shape percentages. We note that the *C. hystrix* granules may have a higher occurrence of an eccentric hilum. Based on the percentage calculation (**Supplementary Table S2**), the difference in this variable between these two species is minor.

The result at the genus level (**Figure 6B**) show that discrimination is possible at this taxonomic level, with the first dimension explaining 48.8% of the inertia and the second dimension explaining 38.6% (**Supplementary Table S5**). In the results plot, the feature hilum position, which has a large measure of discrimination in the first dimension, distinguishes *Quercus* from others, specifically, *Quercus* granules have a higher probability of having an eccentric hilum than the other three genera. Similarly, *Cyclobalanopsis* starches are more likely to have rough surfaces.

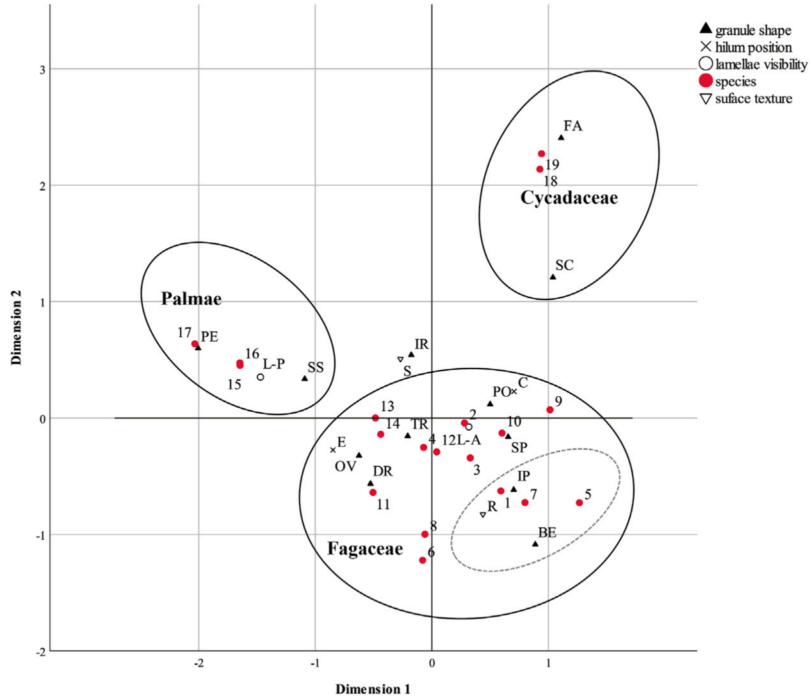

FIGURE 5 | MCA result of morphometric data of starches at the species level. Labels: Species: 1, *Castanopsis platyacantha*; 2, *Castanopsis hystrix*; 3, *Castanopsis fargesii*; 4, *Castanopsis sclerophylla*; 5, *Cyclobalanopsis glauca*; 6, *Cyclobalanopsis chapensis*; 7, *Cyclobalanopsis gambleana*; 8, *Cyclobalanopsis phanera*; 9, *Lithocarpus cleistocarpus*; 10, *Lithocarpus litseifolius*; 11, *Quercus cocciferoides*; 12, *Quercus oxyphylla*; 13, *Quercus variabilis*; 14, *Quercus franchetii*; 15, *Arenga westerhoutii* (white); 16, *Arenga westerhoutii* (red); 18, *Caryota obtusa*; 18, *Cycas pectinata*; 19, *Cycas panzhihuaensis*. Shapes: BE: bell shape, DR: drop shape, FA: faceted sphere, IP: irregular polyhedron, IR: irregular shape, OV: oval shape, PE: pestle shape, PO: polygonal shape, SP: sphere, SC: spherical cap, SS: spindle shape, TR: triangle. Lamellae visibility: L-A: lamellae absence, L-P: lamellae presence. Surface texture: R: rough surface, S: smooth surface. Ellipses are not statistical, but hand-drawn to isolate key woody plant forms (Acorns, Cycads, Palms).

This result also reveals the most identifiable shape features of the four Fagaceae genera. The identification keys include bell-shaped granules for *Cyclobalanopsis* and drop-shaped granules for *Quercus*. The *Castanopsis* and *Lithocarpus* granules seem to be similar in shape, irregular polyhedrons, polygons and spheres. However, according to the percentage distribution of the shape features (**Figure 4**), we suggest that spherical granules are common in *Lithocarpus* starch sample, while polygonal granules, especially irregular types, are *Castanopsis*. This result matches our previous analysis of morphological characteristics at the species level.

4 Identification Scheme of Woody Plant Starches

The identification of productive woody starch granules in this study combines two methods of mutual verification: a traditional morphmetric analysis made by an expert and automated computer-based statistical analyses. The statistical analyses confirms that both the numerical variables of maximum granule size and nominal morphological variables are useful to discriminate starches of various plant taxa, supporting previous attempts to identify plants using a morphometric method (e.g. Torrence et al., 2004; Yang and Perry 2013; Liu et al., 2014; Coster and Field 2015). Non-parametric tests statistically evaluate the distribution differences of granule sizes between the samples examined. Multiple correspondence analysis can be used to visualize the correlation between morphometric data and plant taxa in a two-dimensional plot and calculate the contribution of each categorized variable, which can help an analyst select those that are most critical for discriminating starches.

In our cases, the fissure types of the starches from woody plants make the least contribution in starch identification. However, we can reasonably identify granules from tested edible acorn kernels, cycad pith and palm pith in southern China on the basis of size and other morphological features. The identification scheme is summarized as follows.

(1) The spindle-shaped or pestle-shaped granules, with eccentric hilum, clear visible lamellae, smooth surface and non-fissure are the typical starches from starchy palm pith including *Caryota obtuse* (**Figure 2**:19) and the two landrace samples of *Arenga westerhoutii* (**Figure 2**:17–18). The size data can be used to make an inter-genus distinction, as the maximum granule sizes of the two guanglang landraces (mean size = 26.4/29.5 microns) are statistically larger than those of fishtail palm (mean size = 18.1 microns) (**Table 1**).
(2) The diagnostic morphological features of the two *Cycas* species are fully identical. They have faceted sphere or spherical cap shapes with a smooth surface and centric hilum, but without any fissures or visible lamellae (**Figure 2**: 20–21). However, the two *Cycas* are statistically

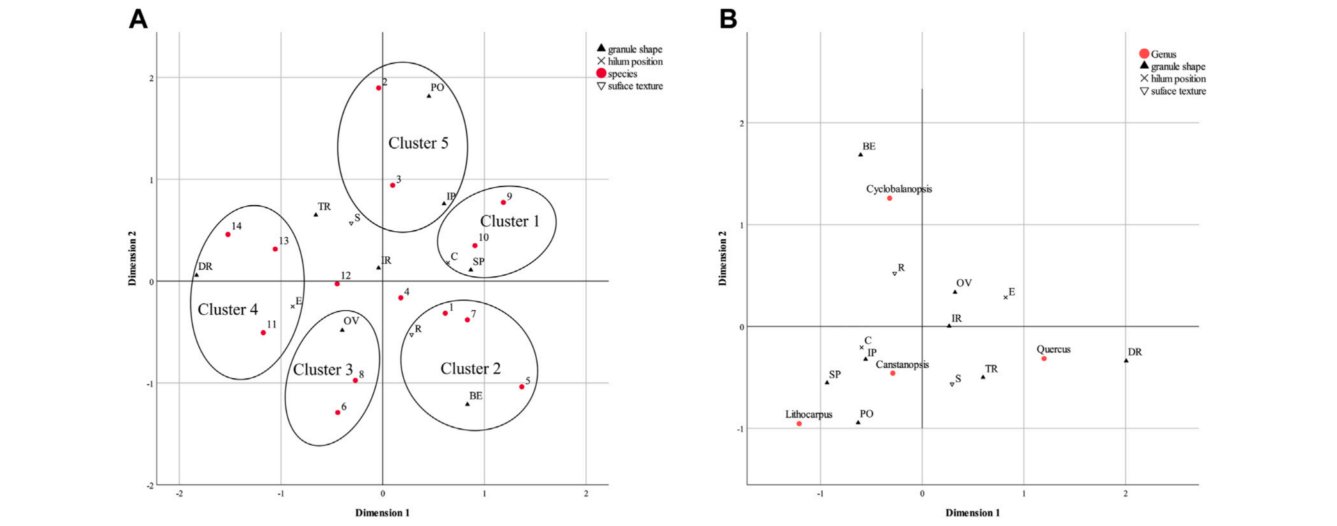

FIGURE 6 | MCA result of morphometric data of Fagaceae starches: **(A)** at the species level; **(B)** at the genus level. Labels: Species: 1, *Castanopsis platyacantha*; 2, *Castanopsis hystrix*; 3, *Castanopsis fargesii*; 4, *Castanopsis sclerophylla*; 5, *Cyclobalanopsis glauca*; 6, *Cyclobalanopsis chapensis*; 7, *Cyclobalanopsis gambleana*; 8, *Cyclobalanopsis phanera*; 9, *Lithocarpus cleistocarpus*; 10, *Lithocarpus litseifolius*; 11, *Quercus cocciferoides*; 12, *Quercus oxyphylla*; 13, *Quercus variabilis*; 14, *Quercus franchetii*. Shapes: BE: bell-shape, DR: drop-shape, FA: faceted sphere, IP: irregular polyhedron, IR: irregular shape, OV: oval shape, PE: pestle shape, PO: polygonal shape, SP: sphere, SC: spherical cap, SS: spindle-shape, TR: triangle. Lamellae visibility: L–A: lamellae absence, L–P: lamellae presence. Surfaces texture: R: rough surface, S: smooth surface.

different in size, with granules from *C. panzhihuaensis* (mean size = 20.6 microns) larger than those from *C. pectinata* (mean size = 14.1 microns) (**Table 1**), which can be used to make an inter-species identification.

(3) Starch granules from acorn kernels are more likely to have rough surfaces and various morphological features by comparison. Based on our analyses, though, we can discriminate further at genus level.

(a) *Quercus* granules (**Figure 2**:12–15) are most distinguishable within the acorn starches, as they have a typical granule form, which has drop shape, eccentric hilum, but no fissures or visible lamellae. In our observations, some of them have a small pit at their pointed ends (**Figure 2**:12), making them more identifiable. Among the species collected, *Q. cocciferoides* are distinguishable, because the maximum granule size of this species (mean size = 13.1 microns) is statistically larger than the other three species (mean size = 10.0/10.1/10.2 microns) which are in the smallest granule sample of our dataset.

(b) A rough bell-shape granule with centric hilum is the most identifiable type of *Cyclobalanopsis* at the genus level (**Figure 2**: 5–8). Of the four species studied, the starch samples of *C. glauca* (**Figure 2**: 5) and *C. gambleana* (**Figure 2**: 7) are dominated by these typical granules. The maximum size data do not show a statistically significant difference between them (**Table 2**). The other two species, *C. chapensis* (**Figure 2**: 6) and *C. phanera* (**Figure 2**: 8), have identifiable granules, but are more likely to produce oval granules, according to both traditional analyses (**Figure 4**) and the MCA results (**Figure 6A**). Their granule sizes are also smaller than the former two species, of which *C. phanera* is the smallest (mean size = 11.5 microns).

(c) The granules of *Lithocarpus* are statistically smaller (with size medians smaller than 10 microns) (**Figure 3**), while the two species *L. litseifolius* and *L. cleistocarpus* cannot be distinguished by size. They are also similar in morphology, having spherical shapes, centric hilum and smooth surfaces, without any fissures or visible lamellae (**Figure 2**: 9–10). Such granule form seems to be widespread in many other plants, for example, lotus seeds (*Nymphaea tetragone*) (Wan et al., 2011). Therefore, we return to the granule images, and note that there is a small pit at the centre of the granule (**Figure 2**: 10) which may help to distinguish this genus.

(d) The *Castanopsis* granules (**Figure 2**: 1–4) are characterized by irregular polygonal shapes, with centric hilum and smooth surfaces, although the percentage distribution of shape features suggests that this is not the major form in some species (**Figure 4**). On the basis of size, *C. hystrix* granules (mean size = 14.3 microns) are larger than those of the other three *Castanopsis* species.

The study shows that it is possible to identify starches among plant taxa using both numeric size data and nominal morphological features. Traditional identification is effective for setting up an identification scheme, and multiple correspondence analysis applied to morphological data is also a useful approach in the early stage of discriminating identifiable features of modern reference collections.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HB and XY. Performed the experiments: ZL and WW. Analyzed the data: ZL, HB and XY. Contributed reagents/materials/analysis tools: XY and HB. Wrote the paper: ZL, HB and XY. Collected the samples in fieldwork: ZL, WW and XY.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.815351/full#supplementary-material

REFERENCES

Arráiz, H., Barbarin, N., Pasturel, M., Beaufort, L., Domínguez-Rodrigo, M., and Barboni, D. (2016). Starch Granules Identification and Automatic Classification Based on an Extended Set of Morphometric and Optical Measurements. *J. Archaeol. Sci. Rep.* 7, 169–179. doi:10.1016/j.jasrep.2016.03.039

Barton, H. (2009). "Starch Granule Taphonomy: the Results of a Two Year Field Experiment," in *Archaeological Science under a Microscope: Studies in Residue and Ancient DNA Analysis in Honour of Thomas H. Loy*. Editors M. Haslam, G. Robertson, A. Crowther, S. Nugent, and L. Kirkwood (Canberra: ANU E Press), 129–140. doi:10.22459/ta30.07.2009.09

Barton, H. (2005). The Case for Rainforest Foragers: the Starch Record at Niah Cave, Sarawak. *Asian Perspect.* 44, 56–72. doi:10.1353/asi.2005.0005

Beh, E. J., and Lombardo, R. (2014). *Correspondence Analysis: Theory, Practice and New Strategies*. Chichester: John Wiley & Sons.

Brown, G. H., and Louderback, L. A. (2020). Identification of Starch Granules from Oak and Grass Species in the Central Coast of California. *J. Archaeol. Sci. Rep.* 33, 102549. doi:10.1016/j.jasrep.2020.102549

Buckley, S., Usai, D., Jakob, T., Radini, A., and Hardy, K. (2014). Dental Calculus Reveals Unique Insights into Food Items, Cooking and Plant Processing in Prehistoric Central Sudan. *PLOS ONE* 9, e100808. doi:10.1371/journal.pone.0100808

Cao, L., Xu, H., and Xi, S. (2011). Ethnobotanical Research on Edible Plants of Gannan the Hakkas in Jiangxi Province. *J. Gannan Normal Univ.* 3, 53–56. doi:10.1016/j.jep.2008.01.016

Coster, A. C. F., and Field, J. H. (2015). What Starch Grain Is that? A Geometric Morphometric Approach to Determining Plant Species Origin. *J. Archaeol. Sci.* 58, 9–25. doi:10.1016/j.jas.2015.03.014

Denham, T. P., Haberle, S. G., Lentfer, C., Fullagar, R., Field, J., Therin, M., et al. (2003). Origins of Agriculture at Kuk Swamp in the Highlands of New Guinea. *Science* 301, 189–193. doi:10.1126/science.1085255

Denk, T., Grimm, G. W., Manos, P. S., Deng, M., and Hipp, A. L. (2017). "An Updated Infrageneric Classification of the Oaks: Review of Previous Taxonomic Schemes and Synthesis of Evolutionary Patterns," in *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus Quercus L*. Editors E. Gil-Pelegrín, J. J. Peguero-Pina, and D. Sancho-Knapik (Cham, Switzerland: Springer International Publishing AG). doi:10.1007/978-3-319-69099-5_2

Devaux, M. F., Qannari, E. M., and Gallant, D. J. (1992). Multiple-correspondence Analysis Optical Microscopy for Determination of Starch Granules. *J. Chemom.* 6, 163–175. doi:10.1002/cem.1180060307

Di Franco, G. (2016). Multiple Correspondence Analysis: One Only or Several Techniques? *Qual. Quant.* 50, 1299–1315. doi:10.1007/s11135-015-0206-0

Fuller, D., Qin, L., Zhao, Z., Zheng, Y., Hosoya, L., Chen, X., et al. (2011). "Archaeobotanic Analysis for the Tianluoshan Site," in *The Center for the Study of Chinese Archaeology in Peking University and Institute of Cultural Relics and Archaeology of ZhejiangA Comprehensive Study on the Natural Remains of Tianluoshan Site* (Beijing: Cultural Relics Press), 47–96.

Fuller, D. Q., and Qin, L. (2010). Declining Oaks, Increasing Artistry, and Cultivating Rice: the Environmental and Social Context of the Emergence of Farming in the Lower Yangtze Region. *Environ. Archaeol.* 15, 139–159. doi:10.1179/146141010x12640787648531

Ge, W. (2015). Study on the Use of Guanglang (Sago-type Palms) Among Ethnic Groups in South china. *Agric. Archaeol.* 6, 253–259.

Geng, Z. (2019). Study on the Policy Adjustment of Ancient Staple Crops and Environmental Changes: Case Study of Guanglang Palms (Sago-type Palms) in the Southern Areas of China. *China Agric. Univ. J. Soc. Sci. Ed.* 36, 109–115. doi:10.13965/j.cnki.gzmzyj10026959.2020.12.014

Greenacre, M. J. (2007). *Correspondence Analysis in Practice*. second edition. Boca Raton: CRC Press.

Guan, Y., Tian, C., Peng, F., Guo, J., Wang, H., Zhou, Z., et al. (2020). Plant Diet during the Pleistocene-Holocene Transition in Northwest China: Evidence from Starch Remains from Pigeon Mountain Site in Ningxia Province. *Quat. Int.* 559, 110–118. doi:10.1016/j.quaint.2020.03.016

Guan, Y., Zhou, Z., Fan, X., and Gao, X. (2018). Neolithic Human Diet Revealed by the Dental Residues in West Fujian. *Acta Anthropol. Sin.* 37, 631–639. doi:10.16359/j.cnki.cn111963/q.2017.0056

Higham, C. (2014). *Early Mainland Southeast Asia: From First Humans to Angkor*. Bangkok: River Books Press.

Hipp, A. L., Manos, P. S., Hahn, M., Avishai, M., Bodénès, C., Cavender-Bares, J., et al. (2020). Genomic Landscape of the Global Oak Phylogeny. *New Phytol.* 226, 1198–1212. doi:10.1111/nph.16162

Humphrey, L. T., De Groote, I., Morales, J., Barton, N., Collcutt, S., Bronk Ramsey, C., et al. (2014). Earliest Evidence for Caries and Exploitation of Starchy Plant Foods in Pleistocene Hunter-Gatherers from Morocco. *Proc. Natl. Acad. Sci. U.S.A.* 111, 954–959. doi:10.1073/pnas.1318176111

Ida, H., Takeuchi, S., and Takasaki, S. (2017). Beechnuts as a Local Specialty in a Snowy Semi-mountainous Area in Central Japan Based on Their Production and Nutritional Traits. *J. Jpn. For. Soc.* 99 (1), 10–17. doi:10.4005/jjfs.99.10

Kawashima, T. (2016). Food Processing and Consumption in the Jōmon. *Quat. Int.* 404, 16–24. doi:10.1016/j.quaint.2015.08.040

Lamb, J., and Loy, T. (2005). Seeing Red: the Use of Congo Red Dye to Identify Cooked and Damaged Starch Grains in Archaeological Residues. *J. Archaeol. Sci.* 32, 1433–1440. doi:10.1016/j.jas.2005.03.020

Le Roux, B., and Rouanet, H. (2005). Multiple Correspondence Analysis (MCA). In*Geometric Data Analysis*. Dordrecht: Springer.

Lentfer, C., Matthews, P., Gosden, C., Lindsay, S., and Specht, J. (2013). Prehistory in a Nutshell: a Lapita-Age Nut-Cracking Stone from the Arawe Islands, Papua New Guinea. *Archaeol. Ocean.* 48, 121. doi:10.1002/arco.5008

Li, G., and Lu, X. (1987). The Original Agriculture Form of Ethnic Minorities in South China *(Beijing*. Beijing: Agriculture Press).

Li, Z., Zhao, J., Li, Q., Ma, Z., Liu, C., Shang, J., et al. (2016). Study on Plant Remains from the Shixiongshan Site of Qin-Han Dynasties in Hilly Area of Guangdong

Province. *Quat. Sci.* 36 (5), 1253–1262. doi:10.11928/j.issn.1001-7410.2016.05.20

Lin, H. (1997). The Study of *Cycas* Plants in Chinese Historic Documents. *Agric. Hist. China* 16, 96–102.

Liu, C., Du, F., Wang, J., Guo, S., Xi, Z., and Leng, T. (2012). An Ethnobotanical Study on the Wild Edible Plant Resources of Wa Nationality. *J. West China For. Sci.* 41, 42–49. doi:10.16473/j.cnki.xblykx1972.2012.05.001

Liu, L., Field, J., Weisskopf, A., Webb, J., Wang, H., and Chen, X. C. (2010). The Exploitation of Acorn and Rice in Early Holocene Lower Yangzi River china. *Acta Anthropol. Sin.* 29, 317–336. doi:10.16359/j.cnki.cn111963/q.2010.03.006

Liu, L., Ma, S., and Cui, J. (2014). Identification of Starch Granules Using a Two-step Identification Method. *J. Archaeol. Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

Liu, Y. (1983). Ancient Woody Foods: Acorns. *Yunnan For.* 03, 31.

Louderback, L. A., Herzog, N. M., and Pavlik, B. M. (2016). A New Approach for Identifying Starch Granules of Wild Food Plants from Arid Western North America. *Starch – Stärke* 68, 1–7. doi:10.1002/star.201600167

Loy, T. H., Spriggs, M., and Wickler, S. (1992). Direct Evidence for Human Use of Plants 28,000 Years Ago: Starch Residues on Stone Artefacts from the Northern Solomon islands. *Antiquity* 66, 898–912. doi:10.1017/s0003598x00044811

Loy, T. (1994). “Methods in the Analysis of Starch Residues on Prehistoric Stone Tools,” in *Tropical Archaeobotany*. Editor J. Hather (London: Routledge), 86–114.

Lu, H., Yang, X., Ye, M., Liu, K.-B., Xia, Z., Ren, X., et al. (2005). Millet Noodles in Late Neolithic China. *Nature* 437, 967–968. doi:10.1038/437967a

Lu, L. (2003). “A Preliminary Analysis for the Residues on the Surface of Unearthed Stone Tools in Zengpiyan Site,” in *Institute of Archaeology, Chinese Academy of Social Sciences, Antiquities Task Force in Guangxi Zhuang Autonomous Region, Museum of Zengpiyan Site in Guilin, and Antiquities Task Force in GuilinGuilin Zengpiyan.* (Beijing: Cultural Relics Press), 646–651.

Lucarini, G., and Radini, A. (2020). First Direct Evidence of Wild Plant Grinding Process from the Holocene Sahara: Use-Wear and Plant Micro-residue Analysis on Ground Stone Tools from the Farafra Oasis, Egypt. *Quat. Int.* 555, 66–84. doi:10.1016/j.quaint.2019.07.028

Ma, Z., Perry, L., Li, Q., and Yang, X. (2019). Morphological Changes in Starch Grains after Dehusking and Grinding with Stone Tools. *Sci. Rep.* 9, 2355–2413. doi:10.1038/s41598-019-38758-6

Ma, Z., Li, Q., Huan, X., Yang, X., Zheng, J., and Ye, M. (2014). Plant Microremains Provide Direct Evidence for the Functions of Stone Knives from the Lajia Site, Northwestern China. *Chin. Sci. Bull.* 59, 1151–1158. doi:10.1007/s11434-014-0174-0

Macheridis, S. (2017). The Use of Multiple Correspondence Analysis (MCA) in Taphonomy: the Case of Middle Helladic Asine, Greece. *Int. J. Osteoarchaeol.* 27, 477–487. doi:10.1002/oa.2571

Mercader, J., Abtosway, M., Bird, R., Bundala, M., Clarke, S., Favreau, J., et al. (2018). Morphometrics of Starch Granules from Sub-saharan Plants and the Taxonomic Identification of Ancient Starch. *Front. Earth Sci.* 6, 146. doi:10.3389/feart.2018.00146

Messner, T. C. (2011). *Acorns and Bitter Roots Starch Grain Research in the Prehistoric Eastern Woodlands*. (Tuscaloosa: University of Alabama Press).

Noshiro, S. (2016). Change in the Prehistoric Use of Arboreal Resources in japan: From Their Sophisticated Management in the Jomon Period to Their Intensive Use in the Yayoi to Kofun Periods. *Quat. Int.* 397, 484–494. doi:10.1016/j.quaint.2015.05.037

Pearsall, D. M., Chandler-Ezell, K., and Zeidler, J. A. (2004). Maize in Ancient Ecuador: Results of Residue Analysis of Stone Tools from the Real Alto Site. *J. Archaeol. Sci.* 31, 423–442. doi:10.1016/j.jas.2003.09.010

Perry, L., Dickau, R., Zarrillo, S., Holst, I., Pearsall, D. M., Piperno, D. R., et al. (2007). Starch Fossils and the Domestication and Dispersal of Chili Peppers (Capsicum Spp. L.) in the Americas. *Science* 315, 986–988. doi:10.1126/science.1136914

Piperno, D. R., and Holst, I. (1998). The Presence of Starch Grains on Prehistoric Stone Tools from the Humid Neotropics: Indications of Early Tuber Use and Agriculture in Panama. *J. Archaeol. Sci.* 25, 765–776. doi:10.1006/jasc.1997.0258

Piperno, D. R., Weiss, E., Holst, I., and Nadel, D. (2004). Processing of Wild Cereal Grains in the Upper Palaeolithic Revealed by Starch Grain Analysis. *Nature* 430, 670–673. doi:10.1038/nature02734

Revedin, A., Aranguren, B., Becattini, R., Longo, L., Marconi, E., Lippi, M. M., et al. (2010). Thirty Thousand-Year-Old Evidence of Plant Food Processing. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18815–18819. doi:10.1073/pnas.1006993107

Sasaki, Y., and Noshiro, S. (2018). Did a Cooling Event in the Middle to Late Jomon Periods Induced Change in the Use of Plant Resources in japan? *Quat. Int.* 471, 369–384. doi:10.1016/j.quaint.2017.10.037

Stevens, N. E., and Mcelreath, R. (2015). When Are Two Tools Better Than One? Mortars, Millingslabs, and the California Acorn Economy. *J. Anthropol. Archaeol.* 37, 100–111. doi:10.1016/j.jaa.2014.12.002

Torrence, R., and Barton, H. (2006). *Ancient Starch Research*. California: Left Coast Press.

Torrence, R., Wright, R., and Conway, R. (2004). Identification of Starch Granules Using Image Analysis and Multivariate Techniques. *J. Archaeol. Sci.* 31, 519–532. doi:10.1016/j.jas.2003.09.014

Tsafou, E., and García-Granero, J. J. (2021). Beyond Staple Crops: Exploring the Use of ‘invisible’ Plant Ingredients in Minoan Cuisine through Starch Grain Analysis on Ceramic Vessels. *Archaeol. Anthropol. Sci.* 13, 1–16. doi:10.1007/s12520-021-01375-4

Tushingham, S., and Bettinger, R. L. (2013). Why Foragers Choose Acorns before Salmon: Storage, Mobility, and Risk in Aboriginal California. *J. Anthropol. Archaeol.* 32, 527–537. doi:10.1016/j.jaa.2013.09.003

Wan, Z., Yang, X., Ge, Q., and Jiang, M. (2011). Morphological Characeteristics of Starch Grains of Root and Tuber Plant in South China. *Quat. Sci.* 31 (4), 736–745. doi:10.3969/j.issn.10017410.2011.04.18

Wang, X. (2013). *Non-parametric Statistics*. Beijing: Tsinghua University Press.

Wilson, J., Hardy, K., Allen, R., Copeland, L., Wrangham, R., and Collins, M. (2010). Automated Classification of Starch Granules Using Supervised Pattern Recognition of Morphological Properties. *J. Archaeol. Sci.* 37, 594–604. doi:10.1016/j.jas.2009.10.024

Yang, J., Ge, J., Liu, L., Ding, Y., and Tan, Y. (2009). Gap Phase Regeneration Recruitment of Mixed Conifer-Broadleaf Forests in Wolong Nature Reserve. *Front. For. China* 4, 153–158. doi:10.1007/s11461-009-0031-5

Yang, S. (2014). The Research and Exploitation of Starch Plants of Fagaceae in China. *Jiangsu Agric. Sci.* 42 (05), 324–327. doi:10.15889/j.issn.10021302.2014.05.004

Yang, X., Barton, H. J., Wan, Z., Li, Q., Ma, Z., Li, M., et al. (2013). Sago-type Palms Were an Important Plant Food Prior to Rice in Southern Subtropical China. *PLoS ONE* 8, e63148. doi:10.1371/journal.pone.0063148

Yang, X., and Jiang, L. (2010). Starch Grain Analysis Reveals Ancient Diet at Kuahuqiao Site, Zhejiang Province. *Chin. Sci. Bull.* 55, 1150–1156. doi:10.1007/s11434-009-0545-0

Yang, X., Li, Z., Wang, W., and Cui, Y. (2018). South Dispersal of Rice Farming: the Beginning Time and Subsistence Background of Rice Farming in Lingnan Region. *J. Archaeol. Museology* 01, 33–47.

Yang, X., and Perry, L. (2013). Identification of Ancient Starch Grains from the Tribe Triticeae in the North china Plain. *J. Archaeol. Sci.* 40, 3170–3177. doi:10.1016/j.jas.2013.04.004

Yang, X., Zhang, J., Perry, L., Ma, Z., Wan, Z., Li, M., et al. (2012). From the Modern to the Archaeological: Starch Grains from Millets and Their Wild Relatives in China. *J. Archaeol. Sci.* 39, 247–254. doi:10.1016/j.jas.2011.09.001

Yao, L., Yang, Y., Sun, Y., Cui, Q., Zhang, J., and Wang, H. (2016). Early Neolithic Human Exploitation and Processing of Plant Foods in the Lower Yangtze River, China. *Quat. Int.* 426, 56–64. doi:10.1016/j.quaint.2016.03.009

Yin, S.Kunming (2000). *Man and Forest: Swidden Agriculture in the Perspective of Ecological Anthropology*. (Kunming: Yunnan Education Publishing House).

Zhang, L. (2014). Principles of the Kruskal-Wallis Test and its Empirical Analysis. *J. Suzhou Univ. Sci. Technol. Nat. Sci.* 1, 14–15. doi:10.3969/j.issn.16720687.2014.01.003

Zhang, W., and Dong, W. (2013). *Advanced Tutorials of Statistical Analyses Using SPSS*. Beijing: Higher Education Press.

Zhao, Z. (2005). Archaeobotanical Studies on the Origin of Agriculture and Civilization. *Soc. Sci. Manag. Comment* 2, 82–91.

Zhejiang Institute of Cultural Relics and Archaeology (2004). *Zhejiang Provincial Institute of Cultural Relics and Archaeology and Xiaoshan Museum*. Beijing: Cultural Relics Press, 241–270.

Zhou, B.-F., Yuan, S., Crowl, A. A., Liang, Y.-Y., Shi, Y., Chen, X.-Y., et al. (2022). Phylogenomic Analyses Highlight Innovation and Introgression in the Continental Radiations of Fagaceae across the Northern Hemisphere. *Nat. Commun.* 13, 1320. doi:10.1038/s41467-022-28917-1

Zhou, C., Lu, T., Wang, J., Chen, X., Luo, S., and Tang, J. (2014). The Traditional Knowledge of Dong Nationality Utilizing Plant Resources in Southeast Jiangxi. *Guihaia* 34, 614–621. doi:10.1016/s0254-6272(14)60062-5

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Before Rice and the First Rice: Archaeobotanical Study in Ha Long Bay, Northern Vietnam

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Mainland Southeast Asia experienced a long, sustained period of foraging economy before rice and millet farming spread into this area prior to 4,000 years BP. Although hundreds of individuals from dense cemeteries are found in several hunter-gatherer sites in Guangxi, Southern China, and Northern Vietnam, dating from the early to middle Holocene (ca. 9,000–4,500 years BP), so far, little has been known about food sources in these pre-farming contexts. In particular, plant food resources have been unclear, although they likely were crucial to supporting rather large populations of hunter-gatherers in this region. To investigate this issue, micro plant remains, including starches and phytoliths, were recovered from stone tools excavated at the Cai Beo site in Ha Long Bay of coastal Northeastern Vietnam, and those findings revealed new understanding of the ancient diet. Examinations of those residues indicated that the hunter-gatherers at Cai Beo as early as 7,000–6,000 years BP exploited a broad spectrum of plants, such as taros, yams, acorns, palms, and more. This study exemplifies how maritime hunter-gatherers interfaced with the local plants and generated population growth from about 7,000 to 4,500 years BP. The results help us to conceptualize the early exploitation, management, and potential cultivation of subtropical and tropical plants over the broad geography of Asia and the Pacific before the arrival of rice and millet farming. In particular, the result validates the significance of roots and tubers in the ancient subsistence economy of Southeast Asia. Moreover, from the archaeological context of 4,500 to 4,000 years BP, the rice discovered in this study represents one of the earliest known in Mainland Southeast Asia.

Keywords: Hunter-gatherers, starch, phytolith, Vietnam, Southeast Asia, rice, Ha Long Bay

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1 INTRODUCTION

Mainland Southeast Asia (MSEA) experienced a long, sustained period of foraging economy before agriculture spread into this area more than 4,000 years ago (Bellwood et al., 2011; Castillo et al., 2018; Higham, 2021). Before rice in coastal Southern China and MSEA, several complex hunter-gatherer groups and affluent village settlements emerged around 7,000 years BP or even earlier. They are remarkable in terms of their population size and social development (Zhang and Hung, 2012; Hung, 2019). This phenomenon probably was related to an advanced subsistence economy, wherein people obtained marine resource supplies and exploited various starch-rich plants, which provided carbohydrates and produced energy to support the stable growth of coastal societies.

Historical records and modern observations have shown clearly about the importance of tropical, starch-rich plants, such as roots and tubers, palms, bananas, and others, among the traditional societies living in the subtropical and tropical areas of Asia and the Pacific islands (Barton, 2012; Matthews et al., 2012). Therefore, these same food sources may have supported the initial cultural development and population growth in these areas. However, until now, the archaeobotanical evidence has been limited across the region, other than in Papua New Guinea, which has been considered as the forerunner in exploiting, managing, and domesticating taros, yams, and bananas (Denham et al., 2003; Fullagar et al., 2006; Loy et al., 2015). Nonetheless, the hypothesis of the original domestication of taro (*Colocasia esculenta*, Araceae) in Papua New Guinea has been challenged by recent DNA analysis (Ahmed et al., 2020) that instead indicated an origin generally within Southeast Asia (Matthews, 1991; Yoshino, 2002; Matthews, 2014; Ahmed et al., 2020).

Through archaeobotanical studies at a few sites in coastal Southern China and Island Southeast Asia (ISEA), the discovery of a broad spectrum of plants, including taros and yams, sago palms, bananas, tree nuts, and others (Barton and White, 1993; Barker et al., 2007; Oliveira, 2008; Yang et al., 2013; Yang et al., 2017) has provided new insights into the exploitation of subtropical and tropical plants from the late Pleistocene to middle Holocene periods.

In the previous studies in MSEA, most efforts concentrated on the emergence of rice and millet agriculture (Weber et al., 2010; Nguyen, 2013; Barron et al., 2017; Castillo et al., 2018). Therefore, the knowledge about ancient plant usage beyond rice was limited to a few woody plants, palms, bamboos, and wild fruits (Pyramarn, 1989; Bowdery, 1999; Nguyen, 2008). In such a research background, systematic studies of the pre-farming period (i.e., prior to 4,500–4,100 years BP) in MSEA can fill the knowledge gap in the Asia-Pacific archaeobotanical record.

Cai Beo, a representative ancient coastal settlement in Northeastern Vietnam dating to ca. 7,000 to 4,000 years BP, is ideal for the archaeobotanical studies that can address such a research question. Furthermore, the integrated findings of ancient starches and phytolith analyses could clarify the subsistence strategies and human-environment relationships of the ancient coastal people in Southeast Asia (SEA).

2 MATERIALS AND METHODS

2.1 Site Description and Sample Collection

The Cai Beo site (N 20°43′8″, E 107°3′2″), nearly 4 m above current sea level, is located on Cat Ba Island, the largest island in Ha Long Bay in the Hai Phong Municipality (**Figure 1**). Cai Beo was reported first by French archaeologist Madeleine Colani who then conducted a small-scale test excavation in 1938 (Colani, 1938). Later, in 1972, 1973, 1981, 1986, and 2006, the site was excavated five times by Vietnamese archaeologists, opening a total excavated area of 449 m^2 (Nguyen, 2005; Nguyen, 2009). The known distribution area of Cai Beo is around 18,000 square meters.

Several hundreds of kg of large fish bones and marine shells were reported from the excavations at Cai Beo, indicating a sea-oriented economy (Nguyen, 2019). Additionally, rich terrestrial animal remains, such as masked civet, brown bear, deer, elk, wild boar, elephant and monkey, were reported from the site (Li, 2019), indicating an ancient forest environment at that time.

The deposit of Cai Beo contains three cultural layers, named as Pre-Halongian, Proto-Halongian, and Ha Long Culture during the 1973 excavation. However, since 1981 excavation, it has been generally suggested that the deposit of the site involves a local "Cai Beo phase" (subdivided into Cai Beo phases 1 and 2) and the later "Ha Long phase" (**Figure 2**). The lower two cultural layers of Cai Beo, phases 1 and 2, span about 7,000 through 5,000 years BP. This estimate is based on a radiocarbon date (ZK-328-0, see below) and comparisons with similar findings at another five Cai Beo cultural sites in Ha Long Bay (Nguyen, 2005; Nguyen, 2009).

The lowest layer (Cai Beo phase 1, see **Figure 2**: Cultural Layer I) represents the earliest occupation of the site. This layer is 2.4–3.2 m deep, consisting of sand deposits mixed with round gravels, debris, marine fish bones, oysters, and other seashells. Large numbers of stone tools were discovered, including many grinding tools (**Figure 3A**, all of which exhibited visible usage wear. Most stone tools are flaked chopping tools produced by the direct strike method. Additional items were disc-shaped tools, pointed tools, ¼ round pebble tools, stone hammers, stone anvils, and others. The so-called "polished stone tools" unearthed from this layer had shown sharpening only on the blade part. The discovered potsherds are small in number and size, characterized as thick and coarse. The middle cultural layer (see **Figure 2**: Cultural Layer II/Cai Beo Phase 2) is 1.2–2.4 m deep, and polished stone axes appeared in this layer.

The radiocarbon date (ZK-328-0) of 6,893–6,391 cal. years BP (5,810 ± 115 uncal. years BP, half-life: 5,730 years) (or 5,645 ± 115 uncal. years BP, half-life: 5,570 years) was obtained from fish bones (Laboratory of the Institute of Archaeology, 1977) excavated from the Cai Beo cultural layer. Other time indicators include the characteristics of human burial practice, craniometry, pottery and lithic remains (Nguyen, 2009). For instance, similar *Haolizhuo* (oyster picks) lithic tools were found at Cai Beo cultural layers widely distributed along the coastal southern China sites in Fujian, Guangdong, and Guangxi from 7,000 to 5,000 years BP (Hung, 2019). A flexed burial unearthed from the Cai Beo cultural phase has been identified as the Australo-Papuan affinity by Nguyen Lan Cuong (Nguyen, 2009). Both the flexed burial and the Australo-Papuan affinity are the key cultural indicators of hunter-gatherers in pre-Neolithic Southern China and Southeast Asia (Higham, 2013; Hung et al., 2017; Matsumura et al., 2019).

During the 1981 excavation, the lowest cultural layer was noticed in a position beneath and therefore pre-dating a marine

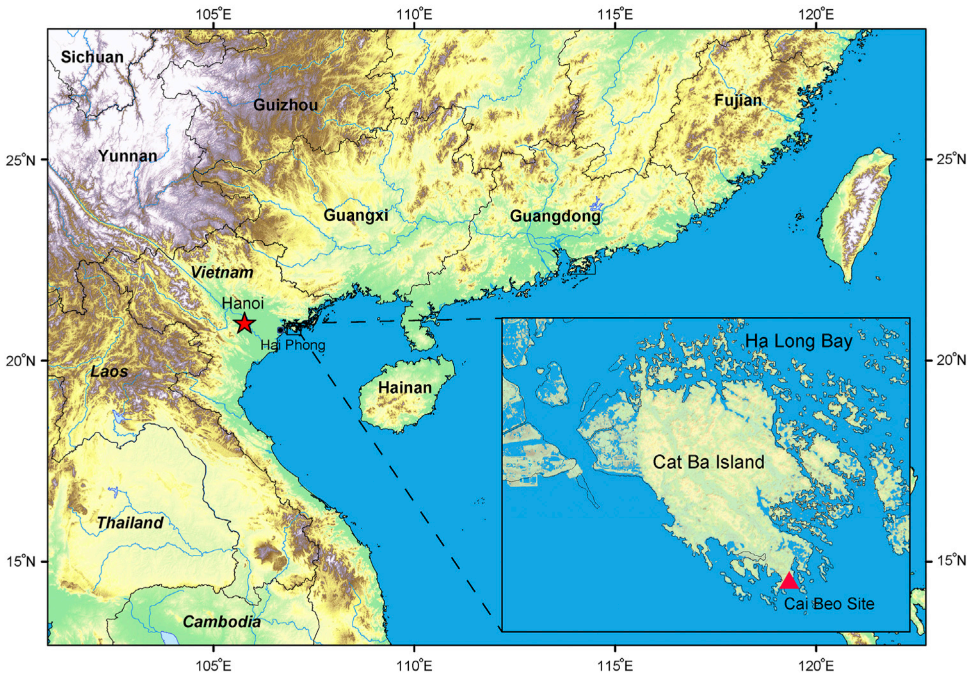

FIGURE 1 | Location of the Cai Beo site. The site is shown by a red triangle.

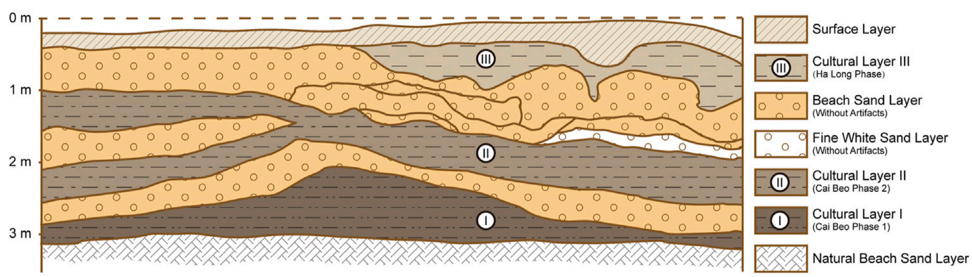

FIGURE 2 | Trench profile of south wall of H3, Cai Beo site (1973 excavation, after Nguyen, 2009: 62).

FIGURE 3 | Typical stone tools collected from the Cai Beo site (Scale bar: 5 cm). **(A)** Grinding stone, **(B)** Muller/Pounder, **(C)** Pitted stone, **(D)** Stone tool with "Ha Long Mark", **(E)** Scraper, **(F)** Pointed tool.

transgression layer (about 20 centimeter thickness, a sterile gravel layer) that appeared in the depth of 2.4 meters under the square surface. Therefore, the earliest occupation at the site must have pre-dated this marine transgression (Nishimura, 2006) that had occurred at 6000 to 5500 years ago in the area of Ha Long Bay (Nguyen and Tran, 2009). This dating constraint further supports the proposed age of the Cai Beo cultural phase 1 as preceding the range of 6000–5500 cal. years BP.

The upper layer of the Cai Beo site represents the Ha Long cultural phase (**Figure 2**: Cultural Layer III), generally considered as starting around 4,500 years BP (Nishimura, 2006; Nguyen, 2009; Peng, 2018). At this site in particular, the associated layer is 0.2–1.2 m deep. New forms of wholly polished stone tools included well polished shouldered axes and quadrangular adzes. Other stone objects were chisels, hammers, sandstones with the so-called "Ha Long mark", and stone rings. Compared with the findings from the preceding Cai Beo cultural layers, the

TABLE 1 | Types and numbers of starches recovered from the study of stone tools from the Cai Beo site.

Tool	Field Number	Tool Type	Starch Type																	
			I			II					III				IV		V	VI	VII	Total
			Ia	Ib	Ic	IIa	IIb	IIc	IId	IIe	IIIa	IIIb	IIIc	IIId	IVa	IVb				
The Ha Long Phase (4,500–4,000 years BP)																				
1	86 CB H1 (II) 24	Grinding stone		12	16	13	2				3	2		1						49
2	86 CB H1 (II) M21	Grinding stone		24	18	32						6					1	1		82
3	86 CB H2 (II) 37 M21	Grinding stone		2	1	3			1											7
4	86 CB H1 (II) 50 M21	Grinding stone		55	17	52	1				2									127
5	86 CB H2 (II) 38 M21	Muller/Pounder		11	3	9						1								24
6	73 CB H3 (4) 82	Muller/Pounder		26	26	5	18		8	3	21	14			2				1	124
7	73 CB H4 (2) 17	Pitted stone		85	51	10	19		2	7	29	13			2	1				219
8	86 CB 10 (II) M21	Pitted stone	10	73	46	73					4									206
9	73 CB H1 (7) 2	Stone tool with "Ha Long Mark"		13	49	10	3		1	2	17			5	1					101
10	86 CB H1 (II) 40	Scraper		12	14	6			1			2								35
The Cai Beo Phase (7,000–5,000 years BP)																				
11	73 CB H1 (12) 81	Grinding stone		3	2	1	22	2	7	12	7	16	1		3	2				78
12	73 CB H2 (11) 104	Grinding stone		14	5	3	15	1	9	7	6	6			1					67
13	73 CB H3 (6) 143	Grinding stone		61	41	3	20		6	10	33	11	5	2	1	1	5			199
14	86 CB H1 (I) 12	Grinding stone	2	14	13	22							1	1				1		54
15	86 CB H1 (I) 16 M21	Grinding stone		112	46	78	2				4	3						5		250
16	86 CB H1 16–2 M21	Grinding stone		33	4	67	3				2	1		2	1			1		114
17	73 CB H2 (11) 115	Muller/Pounder		21	9	5	10		4	4	4	4								61
18	86 CB H2 (I) 5 M21	Muller/Pounder	1	7	15	8						1	2	1						35
19	73 CB H3 (6) 106	Pitted stone		27	15	6	4		1	3	9	5								70
20	73 CB H3 (6) 152	Pointed stone		21	3	10	1		1	1	4		2							43
21	86 CB H2 (I) 6	Pointed stone		65	58	54	1					1								179
22	86 CB H2 (I) 18	Scraper		31	16	17														64
Total	13	722	468		487 121	3	41	49		145 86	11	12		11 4		6	8	1		2,188
	55%		32.04%		11.61%	0.68%		0.27%		0.37%	0.05%		100%							
Total %																				

pottery in the Ha Long cultural layer appeared in large quantities and in more varied forms and decorations, including the use of a wheel-thrown technique in some cases.

More than 38 sites around Ha Long Bay have been grouped into the Ha Long Culture in terms of the artifact association and time period (Nguyen, 2019). Among those Ha Long cultural sites, radiocarbon dates show an age post-dating the older "Cai Beo" context. The available C14 dates from several Ha Long cultural sites concentrated from ca. 4,500 through 3,500 cal. years BP (Chen, 2007; Nguyen, 2009; Nguyen and Tran, 2009; Nguyen and Clarkson, 2013; McColl et al., 2018; Peng, 2018). For instance, one of the C14 dates from Ba Vung site is 4,727–4,517 cal. years BP (4,100 ± 40 uncal. years BP, charcoal sample) (Chen, 2007), and another Ha Long cultural site of Hon Hai Co Tien is dated to 4,381–3,926 cal. years BP (3,755 ± 60 uncal. years BP, human bone sample) (McColl et al., 2018). At the Cai Beo site, the pottery and other artifacts from the Ha Long layer generally accord with the expectations of the early Ha Long Culture association.

During this project, we had submitted 12 samples from Cai Beo, including one human skull, one deer horn, one deer tooth, five mammal bones, and four marine fish bones, to Beta Analytic for AMS dating. Unfortunately, a reliable collagen fraction could not be isolated and purified from any of these samples. As a result, the bone material cannot be dated. However, the basic chronology and two major cultural phases of the site can be reconstructed through multiple lines of evidence as has been outlined here.

2.2 Micro Plant Extraction and Identification

In total, 22 stone tools from Cai Beo were examined in this study for micro-plant remains. The samples include grinding stones, mullers/pounders, pitted stones, stone tools with the so-called "Ha-Long Mark", scrapers, and pointed tools (**Figure 3**; **Table 1**). In addition, four control samples were collected from the non-use surface of stone tools and the storage in Hai Phong and Hanoi to detect modern contamination. All of the stone tools analyzed in this study had been excavated by Kim Dung Nguyen and colleagues from 1972 to 1986 from the Cai Beo site, and they were stored at the Hai Phong Museum and Institute of Archaeology in Hanoi.

The sediments and dust on each tool's surface were first rinsed with distilled water and then cleaned in an ultrasonic bath with distilled water for 5 minutes to recover the residues. Next, the ultrasound mixtures were transferred to test tubes, which were processed to recover the micro plant remains, including starches and phytoliths, in the Department of Archaeology and Natural History laboratory, The Australian National University.

The extraction process of starch and phytolith followed the process of previous studies (Lu et al., 2008; Yang et al., 2013; Pearsall, 2016; Deng et al., 2017). First, the starch samples were isolated with LST (lithium heteropolytungstate) heavy liquid (density 1.9), mounted on a slide in a solution of 10% glycerine and 90% distilled water, then sealed with nail polish. For the preparation of the phytolith samples, the residues were rinsed three times with distilled water, and then the samples were separated with heavy liquid (density 2.35). After the above steps, the samples were rinsed twice with distilled water and then one more time with 30% ethyl alcohol. Finally, the phytolith samples were mounted on the slide with Canada Balsam. The slides of starch and phytolith were observed, measured, and counted under the optical microscope (Machine model: Olympus BX-51), respectively.

The identification of ancient starches is based on the modern reference collections from Vietnam by the authors of this study, the database (http://cmsgd.igsnrr.ac.cn/) built by the Institute of Geographic Sciences and Natural Resources Research (IGSNRR) (Yang et al., 2018), and other related published studies from tropical and subtropical areas of Asia and the Pacific (Fullagar et al., 2006; Lentfer, 2009). Furthermore, the identification of phytolith types was in accordance with the International Code for Phytolith Nomenclature and published documents (Wang and Lu, 1993; Katharina et al., 2019).

3 RESULTS

3.1 Starch Grains

In total, 2,364 starch grains were recovered from the 22 stone tools (**Table 1**). Only very few starches were found in the control samples of non-use contexts, indicating that the starches recovered from the use surfaces of stone tools were indeed related with the ancient use. One hundred seventy-six starch grains could not be categorized due to the lack of diagnosable characteristics. In comparison, the other 2,188 starch grains can be classified into seven types and 17 subtypes based on their morphological features and biological attributes after comparison with modern references (**Figures 4–6**). The details of each type are described below.

3.1.1 Type I Starch

Type I starch grains (N=1,203, 55% of the total) are identified as Aroids. They are classified into three subtypes, mainly according to their sizes.

Type Ia starch grains (N=13) are usually smaller than 5 μm in dimension, and their shapes are difficult to distinguish. As a result, we could not identify all of them under the microscope. However, if the grains appeared in sheets or as clusters (compound grains), then they were easier to find (**Figures 4A–A'**). Type Ia starch grains best matched the features of taros (*Colocasia esculenta*) after comparing them to our reference material (**Figures 5A–A'**). Notably, large quantities of small granules were extracted from 21 stone tools. Although the small granule sizes created difficulty in observing their cross-arms under the polarized light, they nonetheless resembled the starch grains of wild taro (*Colocasia antiquorum*) or *Alocasia* sp. (**Figures 5B,C**).

Type Ib starch grains (N=722) are spherical, sub-rounded, or rounded polygonal with multiple facets (**Figures 4B–B'**). Their sizes range from 5.67 to 12.59 μm, with the mean size being 8.87 ± 1.19 μm. These features are most consistent with the *Colocasia* spp. (**Figures 5D–D'**).

Type Ic starch grains (N=468) exhibit shapes similar to Ib starch grains (**Figures 4C–C'**), except that their sizes are much larger, with the mean size of 12.73 ± 1.92 μm. About 96% of starch grains are larger than 10 μm. Many of the underground storage organs from the Araceae plants produce this kind of starch. However, this type best matches the features of the *Alocasia* spp. (**Figures 5E–E'**).

3.1.2 Type II Starch

Type II starch grains (N=701, 32.04% of the total) exhibit the distinct morphology that characterizes them into the *Dioscorea* genus, including five subtypes.

Type IIa starch grains (N=487) are characterized by their small size, polygonal shape, and centric hilum (**Figures 4D–D'**). Only a few species of *Dioscorea* produce this kind of starch (Fullagar et al., 2006; Hang et al., 2006). Type IIa starch grains are most comparable with tubers of the lesser yam (*Dioscorea esculenta*) (**Figures 5F–G'**).

Type IIb starch grains (N=121) are generally triangular or elliptical in shape with eccentric hilum (**Figures 4E–E'**). The mean size is 20.26 ± 7.1 μm. They best match the characteristics of the purple yam (*Dioscorea alata*) (**Figures 5H–H'**).

Three starch granules are classified as Type IIc, resembling angular triangles with flat bases (**Figures 4F–F'**). This granule type was easy to distinguish from others due to its angular shape. This type could be identified as "air potatoes" (*Dioscorea bulbifera*) (**Figures 5I–I'**).

Type IId starch grains (N=41) are irregular ovate shapes, with highly eccentric hilum and bent cross-arms (**Figures 4G–G'**). This type closely resembles a modern reference belonging to the *Dioscorea* genus, collected from local markets in the Yunnan Province of China (**Figures 5J–J'**).

Type IIe starch grains (N=49) resemble elongated ovals with eccentric hilum (**Figures 4H–H'**). Their grain sizes range from 14.9 to 46.68 μm, with the mean size being 26.15 ± 6.59 μm. This type most likely comes from a kind of yam (*Dioscorea* sp.), but more comparable reference material will need to be collected in order to ascertain a species.

3.1.3 Type III Starch

Type III starch grains (N=254, 11.61% of the total) share the same features with acorns (Fagaceae). Therefore, they are classified into four subtypes.

Type IIIa starch grains (N=145) are irregularly oval or droplet-shaped (**Figures 4I–J'**), which are the typical features of the *Quercus* sp. (**Figures 5K–K'**).

Type IIIb starch grains (N=86) are oval or bell-shaped. The hilum is eccentric, and sometimes the lamellae are visible (**Figures 4K–K'**). Compared with our reference material, this type most closely resembles the species of the *Cyclobalanopsis* genus (**Figures 5L–L'**).

Type IIIc starch grains (N=11) are round with centric hilum. Some granules present linear fissures and visible lamellas (**Figures 4L–L'**), showing the same features as the *Lithocarpus* sp. (**Figures 5M–M'**).

Type IIId starch grains (N=12) are characterized by their polygonal shape, centric hilum, and visible lamellae (**Figures 4M–M'**). This type best matches the feature of the *Castanopsis* sp. (**Figures 5N–N'**).

3.1.4 Type IV Starch

Type IV starch grains (N=15, 0.68% of the total) can be subdivided into two groups. Eleven of the 15 starch grains (Type IVa), ranging 16.2–40.93 μm in size, with an elongated, irregular oval shape and highly eccentric hilum (**Figures 4N–N'**), best match those from sugar palm (*Arenga pinnata*) (**Figures 5O–O'**) from our reference collection. The other four starch grains (Type IVb) are small ovate in shape with faint lamellae, similar to the starch from Kitul Palm (*Caryota urens*) (**Figures 4O–O', Figures 5P–P'**).

3.1.5 Type V Starch

Type V starch grains (N=6, 0.27% of the total) have a large oval shape with well defined lamellae or irregularly compounded with complex extinction crosses (**Figures 4P–P'**). Based on the comparative collections, Type V starch grains share the typical features of the terrestrial fern (*Angiopteris* sp.) (**Figures 5Q–Q'**), from which the starches in rhizomes are known as substitutes for staple foods (Liu et al., 2012).

3.1.6 Type VI Starch

Eight starch grains are classified as Type VI starch. They are oval in shape with highly eccentric hilum, ranging 10.94–26.03 μm in size (**Figures 4Q–Q'**). The morphological characteristics of Type VI starch grains are typical in gingers (Zingiberaceae), and they most closely resemble the rhizomes of the *Kaempferia* sp. (**Figures 5R–R'**) from our reference material.

3.1.7 Type VII Starch

Only one starch grain belonging to Type VII. It is 28.61 μm in length, with an irregular ovoid shape and wrinkled texture (**Figures 4R–R'**) similar to the starch characteristics of bananas (*Musa* sp.) (**Figures 5S–S'**).

3.2 Phytoliths

Given the fact that most roots and tubers do not produce phytoliths, the phytoliths extracted from the surface of the stone tools are scarce. However, 80 phytoliths were recovered (**Figure 7**), the majority of which could be classified taxonomically only to the family level.

Fifteen globular echinate phytoliths are from palms (Aracaceae) (**Figure 8A**. Additionally, phytoliths from rice were discovered, including the scale decorated bulliform produced by rice leaf tissue, double-peaked phytolith produced by rice glumes, and parallel bilobate from stems (**Figures 8B–E**). The percentage of rice bulliform phytoliths with ≥9 fish-scale decorations can be used to differentiate wild rice from domesticated rice (Saxena et al., 2006; Huan et al., 2015). However, the numbers of rice bulliform phytoliths recovered in Cai Beo is small (n=9) and the decorations were obscure to observe, and therefore we could not ascertain whether they were wild or domesticated. One volcaniform phytolith was identified, originally from the leaf of banana (*Musa* sp.) (**Figure 8F**).

4 DISCUSSION

The microfossil studies of plant residues recovered from Cai Beo indicate that the coastal hunter-gatherers utilized a broad spectrum of plants, including taros, yams, acorns, palms, and

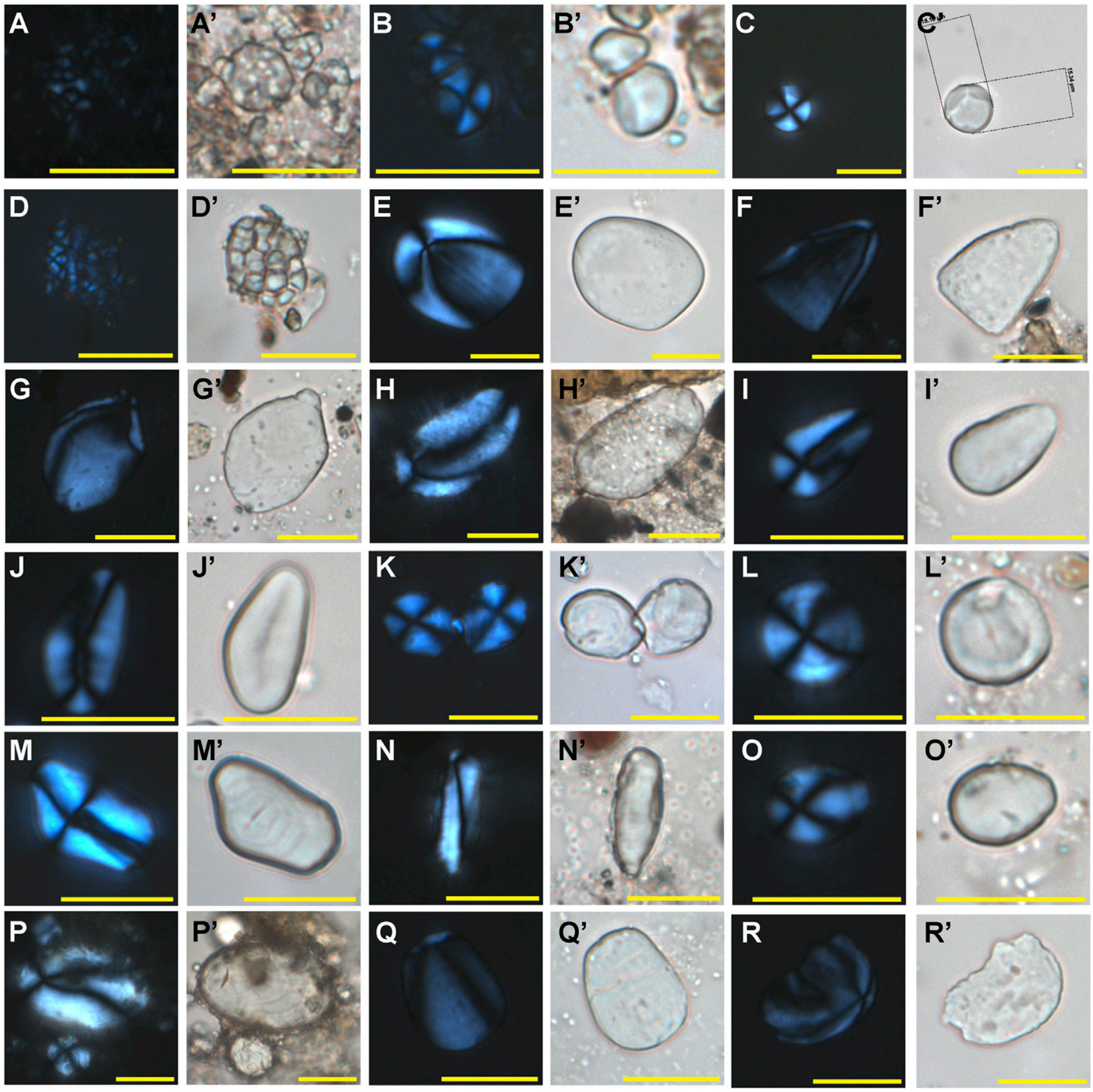

FIGURE 4 | Ancient starches recovered from residues on the stone tools (under polarized and brightfield light). **(A-A′)** Type Ia, *Colocasia esculenta*, **(B-B′)** Type Ib, *Colocasia* spp., **(C-C′)** Type Ic, *Alocasia* spp., (**D-D′)** Type IIa, *Dioscorea esculenta*, **(E-E′)** Type IIb, *Dioscorea alata*, **(F-F′)** Type IIc, *Dioscorea bulbifera*, **(G-G′)** Type IId, *Dioscorea* sp., **(H-H′)** Type IIe, *Dioscorea* sp., **(I-J′)** Type IIIa, *Quercus* sp., **(K-K′)** Type IIIb, *Cyclobalanopsis* sp., **(L-L′)**Type IIIc, *Lithocarpus* sp., **(M-M′)** Type IIId, *Castanopsis* sp., **(N-N′)** Type IVa, *Arenga pinnata*, **(O-O′)** Type IVb, *Caryota urens*, **(P-P′)** Type V, *Angiopteris* sp., **(Q-Q′)** Type VI, *Kaempferia* sp., **(R-R′)** Type VII, *Musa* sp. Scale bar, 20 μm.

others. More than 80% of the total starch grains are identified as yams and taros, reflecting the practical economic significance of roots and tubers in the ancient subsistence system of MSEA. This finding constitutes the first validation that taros and yams were essential plant food sources during the pre-farming ancient contexts in the tropical coastal area of MSEA.

4.1 Edible Aroids (55% of the Total Starches)

Edible aroids (family Araceae) comprise many underground food crops grown in tropical and sub-tropical regions. They are essential food crops in India, SEA, and the Pacific islands (Opara, 2003). At several sites in the ISEA and the Pacific, aroids often are discovered together with yams, in which *Colocasia*, *Alocasia*, and *Cyrtosperma* are reported (Barton and White, 1993; Barton, 2005; Fullagar et al., 2006; Barker et al., 2007; Loy et al., 2015). A study of ancient starch remains from the Niah cave in Borneo have identified grains of aroids (*Alocasia* spp.) from the upper layer of the Hell Trench sequence dating to less than 40,000 years BP (Barker et al., 2002; Barton, 2005).

Taro (*Colocasia esculenta*) is one of the oldest and most important cultigens in the Indo-Pacific region (Blench, 2012; Spriggs and Matthews, 2012). Nowadays, taro has persisted as one

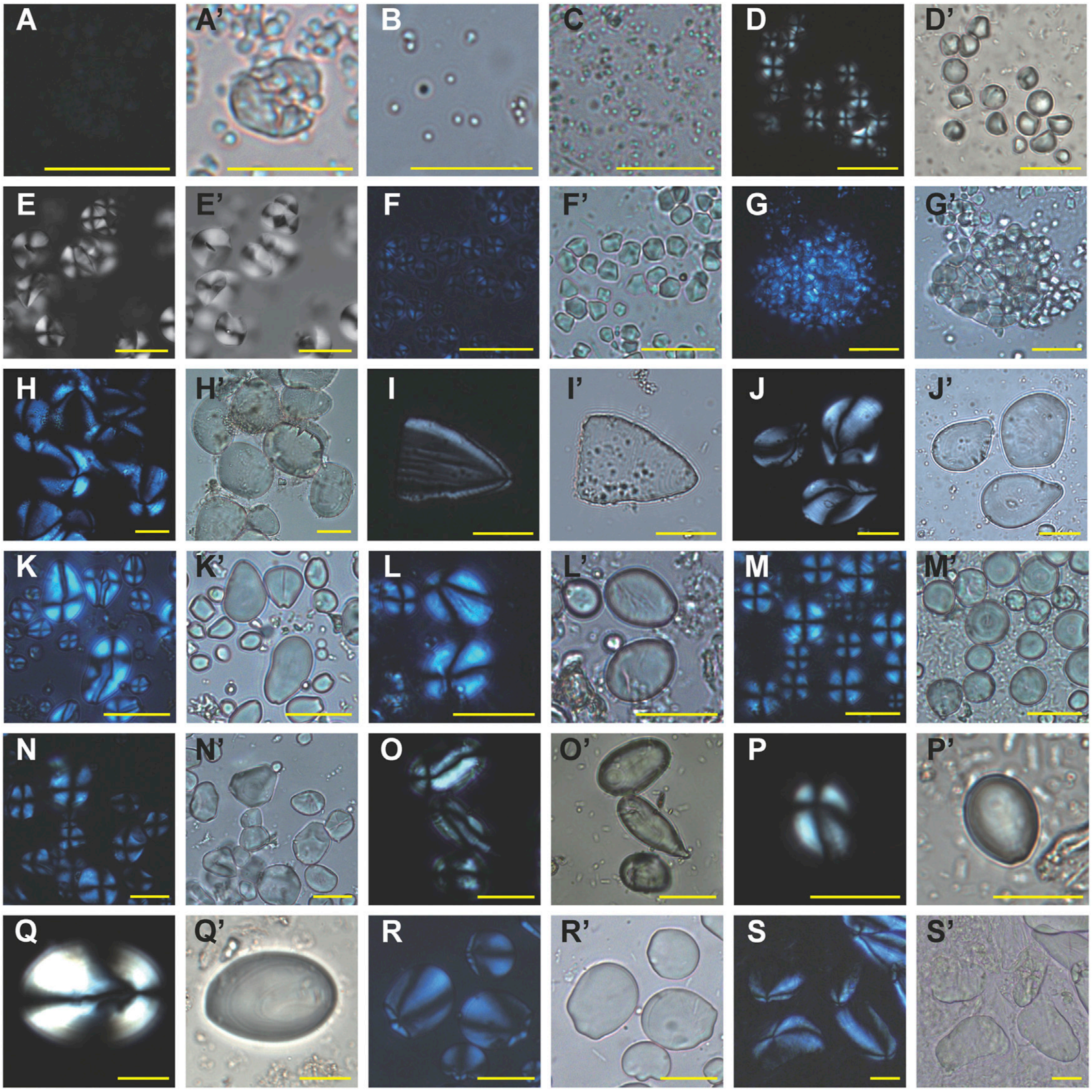

FIGURE 5 | Modern starch references relevant to this study (under polarized and brightfield light). **(A-A′)** *Colocasia esculenta*, **(B)** *Colocasia antiquorum*, **(C)** *Colocasia* sp., **(D-D′)** *Colocasia konishii*, **(E-E′)** *Alocasia macrorrhizos* (picture is sourced from (Fullagar et al., 2006)), **(F-G′)** *Dioscorea esculenta*, **(H-H′)** *Dioscorea alata*, **(I-I′)** *Dioscorea bulbifera*, **(J-J′)** *Dioscorea* sp., **(K-K′)** *Quercus franchetii*, **(L-L′)** *Cyclobalanopsis phanera*, **(M-M′)** *Lithocarpus litseifolius*, **(N-N′)** *Castanopsis hystrix*, **(O-O′)** *Arenga pinnata*, **(P-P′)** *Caryota urens*, **(Q-Q′)** *Angiopteris yunnanensis*, **(R-R′)** *Kaempferia galanga*, **(S-S′)** *Musa* sp. Scale bar, 20 μm.

of the most significant staple diets and cultural elements in many parts of SEA. Still, the role of taro in Southeast Asian prehistory has been underestimated due to the lack of palaeobotanical study. Furthermore, the original domestication center of taro is still disputed (Oliveira, 2012). The greatest diversity of wild *Colocasia* species appears to extend from northeast India to southern China, within the Himalayan region of MSEA (Matthews, 1991). As mentioned, although some suggested independent domestication of taro occurred in Papua New Guinea during the early to middle Holocene (Matthews, 1991; Fullagar et al., 2006; Golson et al., 2017; Golson, 2020), a recent study of chloroplast DNA of taro does not support this hypothesis. Instead, it reveals that the cultivated taro was introduced into Papua New Guinea from SEA after an early or middle Holocene domestication (Ahmed et al., 2020).

Previously, a study in Guangxi of southwest China has recovered taro (*Colocasia esculenta*) starches from the Zengpiyan cave site (ca. 12,500 to 7,600 years BP). Starches were discovered on the excavated stone tools (Lu, 2003). Our new finding from Cai Beo confirms the early and long-term exploitation of taro in MSEA, which in accordance with archaeological, ethnobotanical, taxonomic, and genetic studies, helping to reconceptualize the management and domestication process of taro on a larger scale. A future archaeobotanical survey will concentrate on the remains found in older cave sites in Southern China and MSEA.

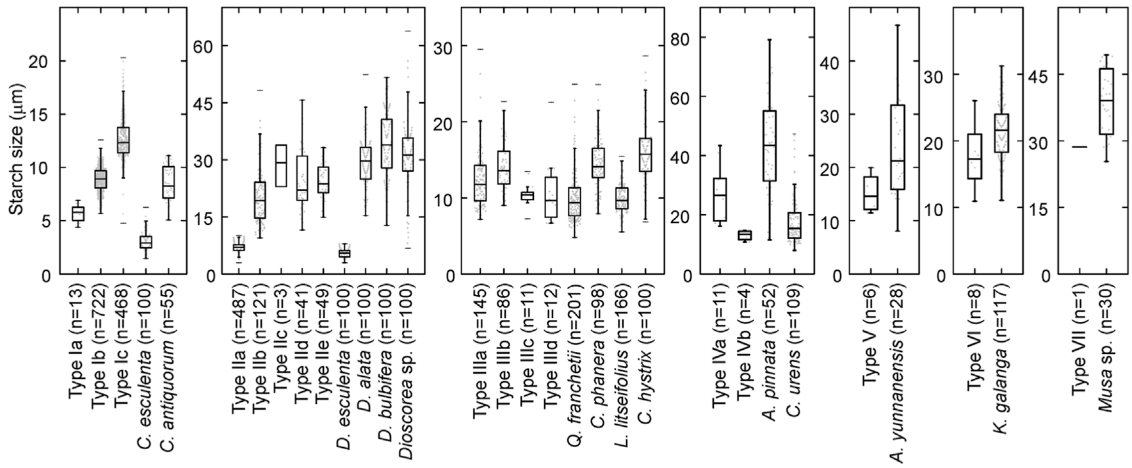

FIGURE 6 | Starch sizes from Cai Beo compared with modern reference samples.

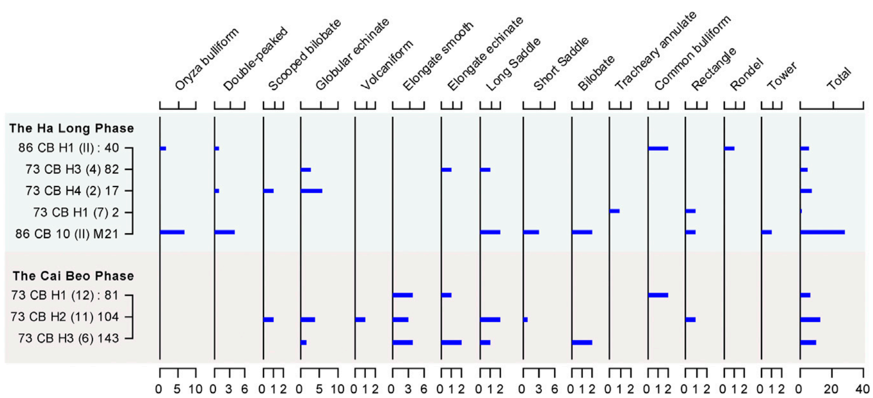

FIGURE 7 | Phytolith assemblages recovered from surface residues on stone tools.

4.2 Yams (32.04% of the Total Starches)

SEA and its adjacent areas are presumed to be one central origin and diversification center for yams (Arnau et al., 2017). In addition, several species of *Dioscorea*, such as *D. alata, D. esculenta*, *D. pentaphylla*, etc. commonly are consumed as regular dietary foods or as famine foods, fodder, and ethnomedicines in tropical Asia and the Pacific region (Maneenoon et al., 2008; Dutta, 2015; Andres et al., 2017). For example, an ethnobotany study in Peninsular Thailand reported that 15 species of *Dioscorea* were found in the living areas of the hunter-gatherer Sakai tribe at Banthad Range, in which eight species are consumed as main food sources by the Sakai people there (Maneenoon et al., 2008).

Previously, many archaeobotanical findings of yams have been reported from highland New Guinea, where modern humans possibly exploited yams around 40,000 years ago and integrated them into cultivation before 6,000 years BP (Fullagar et al., 2006; Summerhayes et al., 2010). In ISEA, related archaeobotanical evidence had been sporadic (Paz, 2001; Mijares, 2007; Oliveira, 2008). The starch granules and charred parenchyma from *Dioscorea* sp. discovered in the Niah cave presumably could date back to 40,000 years ago (Barton, 2005; Barker et al., 2007). Compounded with the lack of sufficient approaches of archaeobotanical works in ISEA, the roots and tubers naturally were preserved quite poorly in the humid tropical environments. Until now, with our new research, the evidence has been missing about *Dioscorea* consumption dating back to pre-Neolithic or even Neolithic MSEA. The study at Cai Beo has recovered a variety of yams, such as *Dioscorea alata, Dioscorea esculenta, and Dioscorea bulbifera*. These *Dioscorea* spp. plants require a set of knowledge and skill in harvesting, grinding, roasting, and processing (Maneenoon et al., 2008; Sharma and Bastakoti, 2010).

4.3 Acorns (11.61% of the Total Starches)

At least four genera of Fagaceae were recovered from Cai Beo, identified as *Quercus* sp., *Cyclobalanopsis* sp., *Lithocarpus* sp., and *Castanopsis* sp. As seen in other hunter-gatherer societies (Nguyen, 2008; Nguyen, 2014), the exploitation of acorns was an essential practice among the ancient hunter-gatherers in Ha Long Bay.

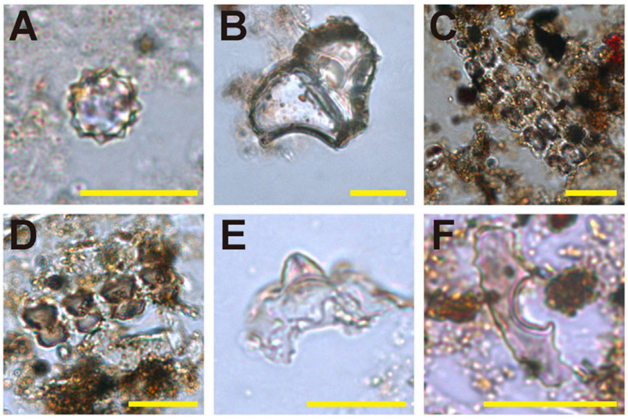

FIGURE 8 | Examples of phytoliths extracted from the Cai Beo site. **(A)** Palm spheroid echinate, **(B)** Bulliform flabellate from Oryza, **(C–D)** Bilobates parallel, **(E)** Double-peaked glume cell, **(F)** Volcaniform phytolith. Scale bar, 20 μm.

Several types of tree nuts, such as *Juglans*, *Quercus*, *Castanopsis*, *Canarium*, are found frequently at Hoabinhian sites (ca. 20,000–9,000 years BP) in Northern Vietnam (Nguyen, 2008; Nguyen, 2013), as well as in other parts of Mainland Southeast Asia (Higham, 2014). Pollen studies have shown that these trees, including Fagaceae plants, were prevalent during the middle Holocene in Northern Vietnam (Nguyen, 2014). The Fagaceae plant resources in Vietnam, rich with 45 species of the genus *Quercus*, have been identified. Among these 45 species, 38 belong to the subgenus *Cyclobalanopsis*, and seven species belong to subgenus *Quercus* (Binh et al., 2018a; Binh et al., 2018b). In addition, *Castanopsis* sp. and *Lithocarpus* sp., which belong to the evergreen forest type, can be found on rocky slopes in Cat Ba National Park on Cat Ba Island today (Thin, 1998).

The processing tasks of acorns as foods are diverse and labor intensive. Many species of acorns were used as tree crops in ancient societies across the world (Cao et al., 2007). The seeds of acorn trees could be consumed after being ground into flour and leached (Mason, 1995). Furthermore, acorn trees often were used as timber for floors, furniture, and traditional remedies (Dolai et al., 2012; Wan Omar et al., 2019).

Ancient acorns were reported from nearby coastal hunter-gatherer sites in the Pearl River Delta in Guangdong, dating to a similar age as the Cai Beo phase (Li, 2020). According to the study by Li (2020), acorns and oak-chestnuts undoubtedly were the essential starch sources for the indigenous communities in the Pearl River Delta during 6,000 through 4,500 years BP, and the use of acorns reduced after 4,500 years BP, about the time when rice farming started to appear. Similarly, our discoveries from Cai Beo suggest that acorns were part of the standard diet of ancient foragers in Ha Long Bay.

4.4 Palms

Micro-remains from Arecaceae are commonly found in ancient sites in Mainland and Island SEA (Yen, 1977; Paz, 2001; Barton, 2005; Oliveira, 2008; Castillo et al., 2020). At Cai Beo, two types of starches from palms were recovered. The Sugar Palm (*Arenga pinnata*) is an endemic plant to Southeast Asian countries (Haryoso et al., 2019). In Vietnam, it is grown on the highlands in the central or northern parts of Vietnam (Nguyen et al., 2014). It is a versatile plant, and almost all of its physical and production parts can be utilized (Ishak et al., 2013; Azhar et al., 2019).

The Kitul Palm (*Caryota urens*) naturally inhabits the understory tree stratum in tropical Asia's moist lowlands and submontane forests (Rangabhashiyam and Selvaraju, 2015). It is a multipurpose palm. The starch extracted from its pith is known as "Kithul flour", and it is claimed to have health benefits according to folklore and Ayurveda (Wimalasiri et al., 2016).

Moreover, the discovery of palm phytoliths in Cai Beo indicates that the plant naturally grew near the site. A previous study at Xincun (ca. 5,300 through 4,420 years BP) of the Guangdong coast, about 700 km to the north from the Ha Long Bay, demonstrated that the sago-type palms were a primary plant food before the rice in south China (Yang et al., 2013). Although the finding of starches from Arecaceae at Cai Beo is limited (N=15, 0.68% of the total), we cannot exclude the possibility that people had produced and consumed sago in Ha Long Bay.

4.5 Edible Ferns

The starches of a terrestrial fern (*Angiopteris* sp.) have been identified on the grinding stones excavated from Cai Beo. Edible ferns are among the most common wild food plants used by people worldwide. The stems, rhizomes, leaves, young fronds, shoots, and some whole plants of ferns can be used as food (Mannan et al., 2008). The rhizomes and stems of the *Angiopteris* sp. are rich in starch, and they are served as food in India and in China (Chen et al., 2010; Liu et al., 2012). In particular, in China, ethnic minorities in Yunnan, Guangxi, Guizhou, and Sichuan provinces of the southwest region consume much more fern species than the other parts of China (Liu et al., 2012).

The border region between the South-eastern Yunnan Province and Northern Vietnam is one of the areas with the richest biological diversity, including that of *Angiopteris* (Wang et al., 2020). During the historical period of SEA, the ferns of *Angiopteris evecta* (G. Forst.) Hoffm., *Cibotium barometz* (L.) J. Smith, *Cyathea* spp., and *Pteridium aquilinum* (L.) Kuhn were served as supplementary food sources or used for producing alcohol. These traditions have mostly been abandoned since modern times (De Winter and Amoroso, 2003). The recovered ancient starches of *Angiopteris* sp. from Cai Beo could reflect an early case of extracting and processing fern starch food in SEA more than 5,000 years ago.

4.6 Rice

Today, the findings of rice remain in MSEA date back to about 4,100–4,000 years BP (Weber et al., 2010; Barron et al., 2017; Nguyen, 2017; Castillo et al., 2018). The evidence for rice farming in the southern coastal areas of China dates back to about 5,000–4,500 years BP (Zhang and Hung, 2010; Yang et al., 2017; Li, 2020). A recent study confirms that the earliest rice and millet appeared together in the Pearl River Delta of Guangdong at 4,800–4,600 cal. years BP (Deng et al., 2022).

Sixteen rice phytoliths (N=16) were identified from the Ha Long cultural layer at Cai Beo. Although the discovered rice phytoliths from the site were too few to conclude whether they were wild or domesticated, importantly the rice phytoliths were restricted solely to the upper layer of this site within the Ha Long Culture association (**Figure 7**). These findings likely relate to the general context of agriculture that started in the northeastern coast of Vietnam around 4,500 years BP.

These findings emphasize the many new elements of cultural material remains that first appeared in the Ha Long cultural layer of this site. Indeed, further research will be needed to investigate this topic through more radiocarbon dating and a detailed chronology of the sites in this region.

4.7 *Kaempferia*

The genus of *Kaempferia* is widespread and cultivated throughout SEA (Nopporncharoenkul and Jenjittikul, 2017). Nowadays, Rhizomes and leaves of *Kaempferia galanga* often are used as a flavoring in Vietnamese cuisine. However, because the discovered number of Type VI Starch in this study is small (N=8), the ancient use of these plants is unclear at this time.

4.8 Banana

Although only one starch granule and one phytolith of bananas (*Musa* sp.) were found in Cai Beo, this finding indicates the possibility that these coastal hunter-gatherers may have known about bananas and attempted to exploit them.

5 CAI BEO IN A WIDER CONTEXT

The coastal habitations in Northeastern Vietnam and Southern China experienced a rapid development by 7,000 years BP, reflected in larger settlements and dense population. Several cultural groups contemporary with Cai Beo flourished in inland and offshore Southern China and northern Vietnam, for example as seen in the Xiantouling cultural group in Guangdong coast, the Keqiutou group in Fujian, the Dingshishan group in Guangxi, and the Da But group in Thanh Hoa and Ninh Binh. Even though they presented distinct cultural characteristics, some of the similarities in burial practices, pottery vessels, or stone artifacts indicated the cultural contacts between these hunter-gatherer communities in southern China and northern Vietnam (Zhang and Hung, 2012; Hung, 2019).

These hunter-gatherers shared similar subsistence patterns and likely consumed or utilized specific types of plants and animals. Archaeobotanical research in Guangdong and Guangxi revealed the diverse plant resources exploited by these affluent hunter-gatherers. For example, at least since 9,000 years BP or even earlier, Canarium nuts had been in long-term use by hunter-gatherers in southern China and Southeast Asia (Deng et al., 2019). The ancient settlement of Xincun in coastal Guangdong utilized a wide range of starch-rich plant foods, particularly sago palms, their dominant exploited plant (Yang et al., 2013). In addition, macro and micro remains of Acorns (*Quercus*, *Lithocarpus*, *Cyclobalanopsis*, *Castanopsis*) have been recovered from several sites in the Pearl River Delta region (Yang et al., 2017; Li, 2020). Domesticated animals such as dogs and pigs served essential roles in farming economies in East Asia. Still, a few of these animals may have been managed or domesticated in older pre-farming contexts. One of the best representatives is the domesticated dog (*Canis familiaris*) that appeared at least 9,000–7,000 years BP in the contexts of hunter-gatherer sites in Guangxi (Lu, 2010), belonging to the Dingsishan pre-farming group (Lu, 2010; Zhang and Hung, 2012; Hung et al., 2017; Matsumura et al., 2019). Some of the domestic dogs probably arrived in northern Vietnam during the pre-farming context.

Although starches and unidentified charred tubers that may come from *Dioscorea* sp. or *Colocasia* sp. were found at the Zengpiyan cave site in Guangxi (Institute of Archaeology, Chinese Academy of Social Sciences, 2003; Lu, 2003), this hypothesis of southern China origin for taro was disputable for the lack of sufficient evidence (Denham et al., 2018). Through our study, the exploitation of a broad range of plant resources by the Cai Beo people, particularly starchy root and tuber crops, including taro, can be confirmed confidently. These geophytes provided the most critical support for the coastal hunter-gatherers before rice farming in Northeastern Vietnam. As mentioned, the large subtropical and tropical area around the Himalaya region, with the high diversity of plants, has been regarded as one of the plant domestication centers for geophytes, especially taro (Matthews, 1991; Zhao, 2011; Matthews, 2014). Under such a wider cultural context, we may reconsider whether geophyte cultivation had been practiced in some ways before the arrival of rice and millet detestation in MSEA.

6 CONCLUSION

In addition to the evidence of acorns and edible ferns, the Cai Beo research validates the intensive use of taros and yams in ancient MSEA for the first time. The findings provide solid evidence to highlight the significance of tuber foods in early SEA, wherein some root crops likely were managed artificially or formally cultivated in certain degrees before rice and millet agriculture dispersed into this area around 4,500 to 4,100 years ago. Recently, a similar conclusion was proposed for other nearby coastal sites of similar age, specifically where geophyte cultivation possibly had existed in the Pearl River Delta before the time of rice farming (Li, 2020).

The first discovery of rice phytoliths in the Ha Long cultural layer is significant. The result is consistent with the findings of a recent study of ancient DNA, concluding that the Ha Long population (4,381–3,926 cal. years BP from Hon Hai Co Tien) was the admixture between the local hunter-gatherers (the Hoabinhian) and the ancestors of East Asians (McColl et al., 2018) who came to MSEA with rice and/or millet agriculture.

Further systematic research in the chronology of the Cai Beo site, as well as the cultural relation and farming dispersal between coastal southern China and the Ha Long Bay area will contribute to a complete picture of understanding the dynamic and possibly diverse transformation of human dietary habits and the early management, domestication, or translocation of certain animals and plants.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HH, WW, and KN conceived and designed the study. KN and HL provided the archaeological samples. HH, WW, KN, HL, and CZ collected the study samples. WW analyzed the data. WW and HH wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Ahmed, I., Lockhart, P. J., Agoo, E. M. G., Naing, K. W., Nguyen, D. V., Medhi, D. K., et al. (2020). Evolutionary Origins of Taro (*Colocasia esculenta*) in Southeast Asia. *Ecol. Evol.* 10 (23), 13530–13543. doi:10.1002/ece3.6958

Andres, C., AdeOluwa, O. O., and Bhullar, G. S. (2017). "Yam (*Dioscorea* spp.)," in *Encyclopedia of Applied Plant Sciences*. 2nd ed (San Diego: Academic Press), 435–441. doi:10.1016/b978-0-12-394807-6.00177-5

Arnau, G., Bhattacharjee, R., Mn, S., Chair, H., Malapa, R., Lebot, V., et al. (2017). Understanding the Genetic Diversity and Population Structure of Yam (*Dioscorea Alata* L.) Using Microsatellite Markers. *PLoS One* 12 (3), e0174150. doi:10.1371/journal.pone.0174150

Azhar, I., Risnasari, I., MuhdiSrena, M. F., and Riswan, M. F. (2019). The Utilization of Sugar Palm (*Arenga Pinnata*) by the People Around Batang Gadis Nasional Park Area. *IOP Conf. Ser. Earth Environ. Sci.* 305, 012016. doi:10.1088/1755-1315/305/1/012016

Barker, G., Barton, H., Beavitt, P., Bird, M., Daly, P., Doherty, C., et al. (2002). Prehistoric Foragers and Farmers in South-East Asia: Renewed Investigations at Niah Cave, Sarawak. *Proc. Prehist. Soc.* 68, 147–164. doi:10.1017/s0079497x00001481

Barker, G., Barton, H., Bird, M., Daly, P., Datan, I., Dykes, A., et al. (2007). The 'human Revolution' in Lowland Tropical Southeast Asia: the Antiquity and Behavior of Anatomically Modern Humans at Niah Cave (Sarawak, Borneo). *J. Hum. Evol.* 52 (3), 243–261. doi:10.1016/j.jhevol.2006.08.011

Barron, A., Turner, M., Beeching, L., Bellwood, P., Piper, P., Grono, E., et al. (2017). MicroCT Reveals Domesticated Rice (*Oryza Sativa*) within Pottery Sherds from Early Neolithic Sites (4150-3265 Cal BP) in Southeast Asia. *Sci. Rep.* 7 (1), 7410. doi:10.1038/s41598-017-04338-9

Barton, H. (2005). The Case for Rainforest Foragers: the Starch Record at Niah Cave, Sarawak. *Asian Perspect.* 44 (1), 56–72. doi:10.1353/asi.2005.0005

Barton, H. (2012). The Reversed Fortunes of Sago and Rice, *Oryza sativa*, in the Rainforests of Sarawak, Borneo. *Quat. Int.* 249, 96–104. doi:10.1016/j.quaint.2011.03.037

Barton, H., and White, J. P. (1993). Use of Stone and Shell Artifacts at Balof 2, New Ireland, Papua New Guinea. *Asian Perspect.* 32 (2), 169–181.

Bellwood, P., Oxenham, M., Hoang, B. C., Dzung, N. K., Willis, A., Sarjeant, C., et al. (2011). An Son and the Neolithic of Southern Vietnam. *Asian Perspect.* 50, 144–175. doi:10.1353/asi.2011.0007

Binh, H. T., Ngoc, N. V., Bon, T. N., Tagane, S., Suyama, Y., and Yahara, T. (2018a). A New Species and Two New Records of *Quercus* (Fagaceae) from Northern Vietnam. *PhytoKeys* 92, 1–15. doi:10.3897/phytokeys.92.21831

Binh, H. T., Ngoc, N. V., Tagane, S., Toyama, H., Mase, K., Mitsuyuki, C., et al. (2018b). A Taxonomic Study of *Quercus Langbianensis* Complex Based on Morphology and DNA Barcodes of Classic and Next Generation Sequences. *PhytoKeys* 95, 37–70. doi:10.3897/phytokeys.95.21126

Blench, R. M. (2012). Vernacular Names for Taro in the Indo-Pacific Region: Implications for Centres of Diversification and Spread in *Senri Ethnological Studies*. Editors M. Spriggs, D. Addison, and P. J. Matthews (Osaka: National Museum of Ethnology) 78, 21–43. doi:10.15021/00002508

Bowdery, D. (1999). Phytoliths from Tropical Sediments: Reports from Southeast Asia and Papua New Guinea. *Bull. Indo-Pacific Prehistory Assoc.* 18, 159–168. doi:10.7152/bippa.v18i0.11711

Cao, M., Deng, M., and Zhang, D. X. (2007). Primary Research on Resources and Exploitation of Fagaceae in Guangxi. *Guihaia* 27 (2), 170–173. (In Chinese).

Castillo, C. C., Carter, A., Kingwell-Banham, E., Zhuang, Y., Weisskopf, A., Chhay, R., et al. (2020). The Khmer Did Not Live by Rice Alone: Archaeobotanical Investigations at Angkor Wat and Ta Prohm. *Archaeol. Res. Asia* 24, 100213. doi:10.1016/j.ara.2020.100213

Castillo, C. C., Fuller, D. Q., Piper, P. J., Bellwood, P., and Oxenham, M. (2018). Hunter-gatherer Specialization in the Late Neolithic of Southern Vietnam - the Case of Rach Nui. *Quat. Int.* 489, 63–79. doi:10.1016/j.quaint.2016.11.034

Chen, W.-c. (2007). The Late Neolithic Adaptation of the Northeastern Coast of Vietnam. *Field Archaeology of Taiwan* 11, 1–26. *(In Chinese)*.

Chen, Y., Tao, Y., Lian, X., Wang, L., Zhao, Y., Jiang, J., et al. (2010). Chemical Constituents of *Angiopteris esculenta* Including Two New Natural Lactones. *Food Chem.* 122 (4), 1173–1175. doi:10.1016/j.foodchem.2010.03.111

Colani, M. (1938). *Découvertes Préhistoriques dans les parages de la Baie d'Along*. Hanoi: Dans Institut Indochinois pour l'Etude de l'Homme.

De Winter, W., and Amoroso, V. (2003). *Plant Resources of South-East Asia No 15 (2) Cryptogams: Ferns and Fern Allies*. Leiden: Backhuys Publishers.

Deng, Z., Huang, B., Zhang, Q., and Zhang, M. (2022). First Farmers in the South China Coast: New Evidence from the Gancaoling Site of Guangdong Province. *Front. Earth Sci.* 10, 858492. doi:10.3389/feart.2022.858492

Deng, Z., Hung, H.-c., Fan, X., Huang, Y., and Lu, H. (2017). The Ancient Dispersal of Millets in Southern China: New Archaeological Evidence. *Holocene* 28 (1), 34–43. doi:10.1177/0959683617714603

Deng, Z., Hung, H.-c., Li, Z., Carson, M. T., Huang, Q., Huang, Y., et al. (2019). Food and Ritual Resources in Hunter-Gatherer Societies: Canarium Nuts in Southern China and beyond. *Antiquity* 93 (372), 1460–1478. doi:10.15184/aqy.2019.173

Denham, T. P., Haberle, S. G., Lentfer, C., Fullagar, R., Field, J., Therin, M., et al. (2003). Origins of Agriculture at Kuk Swamp in the Highlands of New Guinea. *Science* 301 (5630), 189–193. doi:10.1126/science.1085255

Denham, T., Zhang, Y., and Barron, A. (2018). Is There a Centre of Early Agriculture and Plant Domestication in Southern China? *Antiquity* 92 (365), 1165–1179. doi:10.15184/aqy.2018.156

Dolai, N., Karmakar, I., Kumar, R. B., Bala, A., Mazumder, U. K., and Haldar, P. K. (2012). Antitumor Potential of *Castanopsis Indica* (Roxb. Ex Lindl.) A. DC. Leaf Extract against Ehrlich's Ascites Carcinoma Cell. *Indian J. Exp. Biol.* 50, 359–365.

Dutta, B. (2015). Food and Medicinal Values of Certain Species of *Dioscorea* with Special Reference to Assam. *J. Pharmacogn. Phytochemistry* 3 (5), 15–18.

Fullagar, R., Field, J., Denham, T., and Lentfer, C. (2006). Early and Mid Holocene Tool-Use and Processing of Taro (*Colocasia Esculenta*), Yam (*Dioscorea* sp.) and Other Plants at Kuk Swamp in the Highlands of Papua New Guinea. *J. Archaeol. Sci.* 33 (5), 595–614. doi:10.1016/j.jas.2005.07.020

Golson, J., Denham, T., Hughes, P., Swadling, P., and Muke, J. (2017). *Ten Thousand Years of Cultivation at Kuk Swamp in the Highlands of Papua New Guinea*. Canberra: ANU Press.

Golson, J. (2020). "The Origins and Development of New Guinea Agriculture," in *The Emergence of Agriculture*. Editors D. R. Harris and G. C. Hillman. 1st ed (London: Unwin Hyman), 175–186. doi:10.4324/9781003060765-9

Hang, Y., Xu, L., Shi, D., Qin, H., and Zhou, Y. (2006). Morphological Features and its Taxonomic Significance of Starch Grain in Subterranean Organ of *Dioscorea* L. In China. *J. Plant Resour. Environ.* 15 (4), 1.

Haryoso, A., Amzu, E., Hikmat, A., Sunkar, A., and Darusman, D. (2019). Ethnobotany of Sugar Palm (*Arenga Pinnata*) in the Sasak Community, Kekait Village, West Nusa Tenggara, Indonesia. *Biodiversitas* 21 (1). doi:10.13057/biodiv/d210116

Higham, C. (2013). Hunter-Gatherers in Southeast Asia: From Prehistory to the Present. *Human Biol.* 85 (1–3), 21–44. doi:10.3378/027.085.0302

Higham, C. (2014). *Early Mainland Southeast Asia: From First Humans to Angkor*. Bangkok: River Books Press.

Higham, C. F. W. (2021). "Chapter 2 The Neolithic Occupation of Southeast Asia," in *The Languages and Linguistics of Mainland Southeast Asia: A Comprehensive Guide*, 21–32. 8. doi:10.1515/9783110558142-002

Huan, X., Lu, H., Wang, C., Tang, X., Zuo, X., Ge, Y., et al. (2015). Bulliform Phytolith Research in Wild and Domesticated Rice Paddy Soil in South China. *PloS One* 10 (10), e0141255. doi:10.1371/journal.pone.0141255

Hung, H.-c. (2019). Prosperity and Complexity without Farming: the South China Coast, c. 5000-3000 BC. *Antiquity* 93 (368), 325–341. doi:10.15184/aqy.2018.188

Hung, H-c., Zhang, C., Matsumura, H., Li, Z., and Shinoda, K. (2017). "Neolithic Transition in Guangxi: A Long Development of Hunting-gathering Society in Southern China," in *Bio-Anthropological studies of Early Holocene Hunter-gatherer Sites at Huiyaotian and Liyupo in Guangxi, China*. Editors H. Matsumura, H-c. Hung, Z. Li, and K. Shinoda (Tokyo: National Museum of Nature and Science), 205–228.

Institute of Archaeology, Chinese Academy of Social Sciences (IA CASS) (2003). "Archaeological Team of Guangxi Province, Guilin Zengpiyan Site Museum, and Archaeological Team of Guilin City," in *Guilin Zengpiyan* (Beijing: Wenwu). *(In Chinese).*

Ishak, M. R., Sapuan, S. M., Leman, Z., Rahman, M. Z. A., Anwar, U. M. K., and Siregar, J. P. (2013). Sugar Palm (*Arenga pinnata*): Its Fibres, Polymers and Composites. *Carbohydr. Polym.* 91 (2), 699–710. doi:10.1016/j.carbpol.2012.07.073

Katharina, N., Ae, C., Terry, B., Maria, A. R., Luc, V., and Scott, C. L. (2019). International Code for Phytolith Nomenclature (ICPN) 2.0. *Ann. Bot.* 124 (2), 189–199. doi:10.1093/aob/mcz064

Laboratory of the Institute of Archaeology (CASS) (1977). Report of Radiocarbon Dates (4). *Archaeology* (3). 200–204.

Lentfer, C. J. (2009). "Building a Comparative Starch Reference Collection for Indonesia and its Application to Palaeoenvironmental and Archaeological Research," in *Archaeological Science Under a Microscope : Studies in Residue and Ancient DNA Analysis in Honour of Thomas H. Loy*. Editors M. Haslam, G. Robertson, A. Crowther, S. Nugent, and L. Kirkwood. 1st ed (Canberra, Australia: ANU E Press), 80–101. doi:10.22459/ta30.07.2009.07

Li, Z. (2019). "Early Maritime Subsistence and Adaptive Ocean Cultures along the Beibu Gulf Coast," in *Prehistoric Maritime Cultures and Seafaring in East Asia*. Editors C. M. Wu and B. V. Rolett (Singapore: Springer Nature Singapore Pte Ltd), 127–139. doi:10.1007/978-981-32-9256-7_7

Li, Z. (2020). *Plant-related Subsistence in the Pearl River Delta, Southern China, from 6,000 BP to 3,000 BP*. PhD Thesis. Leicester: University of Leicester.

Liu, Y., Wujisguleng, W., and Long, C. (2012). Food Uses of Ferns in China: A Review. *Acta Soc. Bot. Pol.* 81 (4), 263–270. doi:10.5586/asbp.2012.046

Loy, T. H., Spriggs, M., and Wickler, S. (2015). Direct Evidence for Human Use of Plants 28,000 Years Ago: Starch Residues on Stone Artefacts from the Northern Solomon Islands. *Antiquity* 66 (253), 898–912. doi:10.1017/s0003598x00044811

Lu, H., Liu, Z., Wu, N., Berné, S., Saito, Y., Liu, B., et al. (2008). Rice Domestication and Climatic Change: Phytolith Evidence from East China. *Boreas* 31 (4), 378–385. doi:10.1111/j.1502-3885.2002.tb01081.x

Lu, P. (2010). *Zooarchaeological Study on the Shell Middens in the Yong Valley of Guangxi*. PhD Thesis. Beijing: Chinese Academy of Social Sciences. (In Chinese).

Lu, T. L.-D. (2003). "A Preliminary Analysis for the Residues on the Surface of Unearthed Stone Tools in Zengpiyan Site," in *Zhengpiyan Site of Guilin*. Editors: Institute of Archaeology (Chinese Academy of Social Science), Antiquities Task Force in Guangxi Zhuang Autonomous Region, Museum of Zengpiyan site in Guilin, and Antiquities Task Force in Guilin. 1st ed (Beijing: Cultural Relics Press), 646–651.

Maneenoon, K., Sirirugsa, P., and Sridith, K. (2008). Ethnobotany of *Dioscorea* L. (Dioscoreaceae), a Major Food Plant of the Sakai Tribe at Banthad Range, Peninsular Thailand. *Ethnobot. Res. App.* 6, 385–394. doi:10.17348/era.6.0.385-394

Mannan, M. M., Maridass, M., and Victor, B. (2008). A Review on the Potential Uses of Ferns. *Ethnobot. Leafl.* 12, 281–285.

Mason, S. (1995). "Acornutopia? Determining the Role of Acorns in Past Human Subsistence," in *Food in Antiquity*. Editors J. Wilkins, M. Harvey, and M. Dobson. 1st ed (Exeter: University of Exeter Press), 12–24.

Matsumura, H., Hung, H.-c., Higham, C., Zhang, C., Yamagata, M., Nguyen, L. C., et al. (2019). Craniometrics Reveal "Two Layers" of Prehistoric Human Dispersal in Eastern Eurasia. *Sci. Rep.* 9 (1), 1451. doi:10.1038/s41598-018-35426-z

Matthews, P. J. (1991). A Possible Tropical Wildtype Taro: *Colocasia esculenta* Var. *Aquatilis*. *Indo-Pacific Prehistory Assoc. Bull.* 11, 69–81. doi:10.7152/bippa.v11i0.11374

Matthews, P. J., Agoo, E. M. G., Tandang, D. N., and Madulid, D. A. (2012). "Ethnobotany and Ecology of Wild Taro (*Colocasia esculenta*) in the Philippines: Implications for Domestication and Dispersal," in *Senri Ethnological Studies*. Editors M. Spriggs, D. Addison, and P. J. Matthews (Osaka: National Museum of Ethnology) 78, 307–340. doi:10.15021/00002523

Matthews, P. J. (2014). *On the Trail of Taro: An Exploration of Natural and Cultural History*. Osaka: National Museum of Ethnology.

McColl, H., Racimo, F., Vinner, L., Demeter, F., Gakuhari, T., Moreno-Mayar, J. V., et al. (2018). The Prehistoric Peopling of Southeast Asia. *Science* 361 (6397), 88–92. doi:10.1126/science.aat3628

Mijares, A. S. B. (2007). *Unearthing Prehistory: The Archaeology of Northeastern Luzon, Philippine Islands*. Oxford: British Archaeological Reports.

Nguyen, D. T., and Clarkson, C. (2013). The Organisation of Drill Production at a Neolithic Lithic Workshop Site of Bai Ben, Cat Ba Island, Vietnam. *Bull. Indo-Pacific Prehistory Assoc.* 33, 24–39. doi:10.7152/bippa.v33i0.14508

Nguyen, H. T. M. (2013). Neolithic Vegetation in Northern Vietnam: an Indication of Early Agricultural Activities. *J. Austronesian Stud.* 4, 1.

Nguyen, K. D. (2019). "The Late Neolithic to Early Bronze Age on the Northeastern Coast of Vietnam," in *Prehistoric Maritime Cultures and Seafaring in East Asia*. Editors C. M. Wu and B. V. Rolett (Singapore: Springer Nature Singapore Pte Ltd), 141–157. doi:10.1007/978-981-32-9256-7_8

Nguyen, K. S. (2009). *Prehistorical Site Cai Beo on Cat Ba Island*. Hanoi: Social Sciences. (In Vietnamese).

Nguyen, Q. M., and Tran, T. H. (2009). Geoarchaeology of The Maritime Region in Northeast Vietnam. *Bull. Indo-Pacific Prehistory Assoc.* 29, 56–60. doi:10.7152/bippa.v29i0.9478

Nguyen, T. M. H. (2014). *Holocene Vegetation and Human Activities in Northern Vietnam*. PhD Thesis. Tokyo: University of Tokyo.

Nguyen, T. M. H. (2017). Burnt Rice from Four Archaeological Sites in Northern Vietnam. *Vietnam Soc. Sci.* 179, 64–77.

Nguyen, V. (2005). The Da but Culture: Evidence for Cultural Development in Vietnam during the Middle Holocene. *Bull. Indo-Pacific Prehistory Assoc.* 25, 89–94. doi:10.7152/bippa.v25i0.11918

Nguyen, V. (2008). Hoabinhian Macrobotanical Remains from Archaeological Sites in Vietnam: Indicators of Climate Changes from the Late Pleistocene to the Early Holocene. *Bull. Indo-Pacific Prehistory Assoc.* 28, 80–83. doi:10.7152/bippa.v28i0.12019

Nishimura, M. (2006). "Chronological Framework from the Palaeolithic to Iron Age in the Red River Plain and the Surrounding," in *Prehistoric Archaeology of South China and Southeast Asia*. Editor Institute of Archaeology, Chinese Academy of Social Sciences (Beijing: Cultural Relics Publishing House), 347–373.

Nopporncharoenkul, N., and Jenjittikul, T. (2017). *Kaempferia Noctiflora* (Zingiberaceae), a New Species from Northern Thailand. *Phytotaxa* 316 (1), 67. doi:10.11646/phytotaxa.316.1.6

Oliveira, N. V. (2008). *Subsistence Archaeobotany: Food Production and the Agricultural Transition in East Timor*. PhD Thesis. Canberra: The Australian National University.

Oliveira, N. V. (2012). Recovering, Analysing and Identifying *Colocasia Esculenta* and *Dioscorea* spp. From Archaeological Contexts in Timor-Leste. *Senri Ethnol. Stud.* 78, 265–284. doi:10.15021/00002521

Opara, L. U. (2003). *Edible Aroids: Post Harvest Operations*. Rome: Food and Agriculture Organization of the United Nations.

Paz, V. J. (2001). Archaeobotany and Cultural Transformation : Patterns of Early Plant Utilisation in Northern Wallacea. *J. Acoust. Soc. Am.* 106 (4), 1538–1544. doi:10.17863/CAM.15977

Pearsall, D. (2016). *Paleoethnobotany: A Handbook of Procedures*. 3rd ed. New York: Routledge.

Peng, C. L. (2018). *The Research of Early Archaeological Cultures in Vietnam*. Nanning: Guangxi Science & Technology Publishing House. (In Chinese).

Pyramarn, K. (1989). "New Evidence on Plant Exploitation and Environment during the Hoabinhian (Later Stone Age) from Ban Kao Caves, Thailand," in *Foraging and Farming: The Evolution of Plant Exploitation*. Editors D. R. Harris and G. C. Hillman. 1st ed (London: Routledge), 282–291.

Rangabhashiyam, S., and Selvaraju, N. (2015). Evaluation of the Biosorption Potential of a Novel *Caryota Urens* Inflorescence Waste Biomass for the Removal of Hexavalent Chromium from Aqueous Solutions. *J. Taiwan Inst. Chem. Eng.* 47, 59–70. doi:10.1016/j.jtice.2014.09.034

Saxena, A., Prasad, V., Singh, I. B., Chauhan, M. S., and Hasan, R. (2006). On the Holocene Record of Phytoliths of Wild and Cultivated Rice from Ganga Plain: Evidence for Rice-Based Agriculture. *Curr. Sci.* 90, 1547–1552.

Sharma, L. N., and Bastakoti, R. (2010). Ethnobotany of *Dioscorea* L. With Emphasis on Food Value in Chepang Communities in Dhading District, Central Nepal. *Bot. Orient.* 6, 12–17. doi:10.3126/botor.v6i0.2905

Spriggs, M., and Matthews, P. J. (2012). "Irrigated Taro in the Indo-Pacific: Multiple Perspectives," in *Senri Ethnological Studies*. Editors M. Spriggs, D. Addison, and P. J. Matthews (Osaka: National Museum of Ethnology), 341–347.

Summerhayes, G. R., Leavesley, M., Fairbairn, A., Mandui, H., Field, J., Ford, A., et al. (2010). Human Adaptation and Plant Use in Highland New Guinea 49,000 to 44,000 Years Ago. *Science* 330 (6000), 78–81. doi:10.1126/science.1193130

Thin, N. N. (1998). The Vegetation of the Catba National Park in Vietnam. *Korean J. Ecol.* 21 (2), 139–149.

Wan Omar, W. B., Zakeri, H. A., Salim, J. M., Fisall, U. F. M., and Amran, M. A. (2019). "Genetic Diversity of Seed Storage Protein in Selected Melastomataceae and Fagaceae from Tasik Kenyir," in *Greater Kenyir Landscapes*. Editor M. T. Abdullah, A. Mohammad, M. N. Mohammad, and M. S. Lola (Cham: Springer), 83–90. doi:10.1007/978-3-319-92264-5_8

Wang, T., Xiao, B., Liu, E.-D., Nguyen, K. S., Duan, J.-Q., Wang, K.-L., et al. (2020). Rediscovery of *Angiopteris Tonkinensis* (Marattiaceae) after 100 years, and its Revision. *PhytoKeys* 161, 1–9. doi:10.3897/phytokeys.161.54912

Wang, Y., and Lu, H. (1993). *Research on Phytolith and its Application*. Beijing: Ocean Press. (In Chinese).

Weber, S., Lehman, H., Barela, T., Hawks, S., and Harriman, D. (2010). Rice or Millets: Early Farming Strategies in Prehistoric Central Thailand. *Archaeol. Anthropol. Sci.* 2 (2), 79–88. doi:10.1007/s12520-010-0030-3

Wimalasiri, G. E. M., Ranasinghe, P., Gunaratne, D. M. A., and Arachchi, L. P. V. (2016). Antioxidant and Anti-diabetic Properties of *Caryota urens* (Kithul) Flour. *Procedia Food Sci.* 6, 181–185. doi:10.1016/j.profoo.2016.02.044

Yang, X., Barton, H. J., Wan, Z., Li, Q., Ma, Z., Li, M., et al. (2013). Sago-type Palms Were an Important Plant Food Prior to Rice in Southern Subtropical China. *PLoS One* 8 (5), e63148. doi:10.1371/journal.pone.0063148

Yang, X., Wang, W., Zhuang, Y., Li, Z., Ma, Z., Ma, Y., et al. (2017). New Radiocarbon Evidence on Early Rice Consumption and Farming in South China. *Holocene* 27 (7), 1045–1051. doi:10.1177/0959683616678465

Yang, X., Wu, W., Perry, L., Ma, Z., Bar-Yosef, O., Cohen, D. J., et al. (2018). Critical Role of Climate Change in Plant Selection and Millet Domestication in North China. *Sci. Rep.* 8 (1), 7855. doi:10.1038/s41598-018-26218-6

Yen, D. E. (1977). "Hoabinhian Horticulture? the Evidence and the Questions from Northwest Thailand," in *Sunda Sahul: Prehistoric Studies in Southeast Asia, Melanesia Australia*. Editor J. Allen, J. Golson, and R. Jones (London: Academic Press), 567–599.

Yoshino, H. (2002). Morphological and Genetic Variation in Cultivated and Wild Taro. *Vegeculture East. Asia Ocean.* 16, 95–116.

Zhang, C., and Hung, H.-c. (2010). The Emergence of Agriculture in Southern China. *Antiquity* 84 (323), 11–25. doi:10.1017/s0003598x00099737

Zhang, C., and Hung, H.-c. (2012). Later Hunter-Gatherers in Southern China, 18 000–3000 BC. *Antiquity* 86 (331), 11–29. doi:10.1017/s0003598x00062438

Zhao, Z. (2011). New Archaeobotanic Data for the Study of the Origins of Agriculture in China. *Curr. Anthropol.* 52 (S4), S295–S306. doi:10.1086/659308

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The Agriculture and Society in the Yiluo River Basin: Archaeobotanical Evidence From the Suyang Site

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The Yiluo River Basin is located in the Central plains of China, an area commonly known as the center of origin of ancient Chinese civilization. Agriculture lays the foundation for social and economic development and triggers societal change and archaeobotany can provide important clues on this issue. archaeobotanical study is an important perspective on the relationship between agriculture and society development. However, relatively few archaeobotanical studies have been conducted in the Yiluo River basin, and the paucity of data has hindered our understanding of the relationship between agriculture and society. Therefore, the archaeobotanical analysis at the Suyang site in the middle reach of the Luo River provides an opportunity to understand how and why agriculture and society developed. Our analytical results of carbonized plant remains and phytolith, coupled with radiocarbon dating, showed that millet was the main crop in the late Yangshao culture (5,500–5000 BP), followed by rice. However, rice cultivation in the area expanded during the Longshan culture (5,000–4000 BP), and its importance as a crop possibly exceeded millet at Suyang. From the late Yangshao to Longshan, rice was cultivated in wet fields by utilizing the Luo River floodplain on a large scale. The proportion of rice at Suyang is the highest among contemporary sites in the Central Plains. It may be related to many reasons such as suitable environmental and geomorphological conditions, advanced water management, the influence of the Qujialing culture, and population growth. In the late Yangshao culture, crop processing seemed to have been concentrated in communal areas. However, during the Longshan culture, different steps of crop processing were scattered throughout the site. This change is hypothesized as a change in the family structure and economic production. After the Yangshao period, the nuclear family became the fundamental unit for social, cultural, and economic production in the Central Plains.

Keywords: agriculture, rice cultivation, people and the environment, social organization, yiluo river basin

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INTRODUCTION

As a primary center of agriculture, China developed two different farming practices around the same time during 8,000–9000 BP (Liu et al., 2009; Bettinger et al., 2010; Zhao, 2014; Yang et al., 2015; Crawford et al., 2016; Wang et al., 2018; Zhao et al., 2020). One was developed in northern China mainly for millet crops, and it spread throughout northern China (Qin, 2012). The other was developed in the Yangtze River Basin mainly for rice and was traditionally thought to be confined to southern China (Zhao and Zhang, 2009; Crawford et al., 2016). However, two sites (Jiahu and Yuezhuang) located in northern China have contradicted the traditional understanding since carbonized rice dating to 8000 BP was found, suggesting that rice had already been introduced to northern China by that time. It is believed that rice appeared in central China as earlier as the Peiligang period and became ubiquitous from the middle to late Yangshao period (Yang et al., 2015; Zhong, 2018; Wang et al., 2018). Therefore, these lines of evidence suggest that the two societies with distinctive farming systems continually interacted with each other while maintaining their separate characteristics, thus forming an important cultural landscape since the Neolithic period (Qin, 2012; Lu, 2017).

Agriculture plays an important role in the formation of Chinese civilization, and the highly developed farming system is the foundation of Chinese civilization (Qin, 2012; Lu, 2017). The Yiluo River Valley, located in central North China and with the Erlitou site as its capital from the early 2nd millennium B.C., is crucial in identifying the origins of Chinese civilization and early state formation (Han, 2015). Previous archaeobotany studies in the Luoyang Basin have provided some insights into the development of agriculture in this area. Plant remains show that dry-farming agriculture featuring millet was the principal subsistence economy of the early Yangshao period (Liu et al., 2002; Li et al., 2021). In the late Yangshao period, rice was produced in this region and became an important crop in the following Longshan period (cf. Wanggedang site). Rice generally appeared in the Central Plains of the late Yangshao and Longshan cultures. Distinguishing between wet and dry rice is key to understanding the role of rice systems in social and economic development or environmental change, given how water management is crucial to the development of a complex society. Paddy fields were found at the Huizui site in the Yiluo River basin (Rosen et al., 2015), in the sites of central Henan Province, such as Zhuhai and Yingyang sites, were considered dry rice (Wang et al., 2018, Wang et al., 2019). It is not clear which type of rice is located in the middle of the Luo River Valley.

The present archaeobotany studies in the Yiluo River Valley are mostly focused on its lower course and the Luoyang Basin and the diachronic changes in the agricultural structure (Liu et al., 2002; Zhang et al., 2019; Zhong et al., 2019). The data obtained are preliminary and use material from survey fieldwork, which lacks the application of agricultural technology and the interaction between agriculture and landscape (Lu, 2002; Lee et al., 2007; Zhang et al., 2014; Li and Zhang, 2020). The site surveyed was Suyang, located in the middle part of the Luo River Valley and surrounded by hills. Site occupation dates from the Yangshao period to the Longshan period. Comparing plant remains from Suyang with other contemporaneous sites in Central China can inform us about the subsistence and cultivation methods of crops in the area before early states appeared. In addition, analyzing the interplay between people and the environment would enhance our knowledge of the formation of the civilization of the Yiluo River Valley.

SUYANG SITE ARCHAEOLOGICAL BACKGROUND

The Suyang site is located in Suyang village in Yiyang County, Henan Province (**Figure 1**). It is situated in the middle of the Luo River Valley, to the south of the Luo River and north of Xionger Mountain. It extends from the foot of the mountain as a gentle slope covering 620,000 m^2 with an east-west width of 970 m and a north-south length of 1,040 m. The thickness of the cultural layer is 6 m. The main modern crops grown in the area are wheat and corn; according to local villagers, rice was also grown on the floodplain of the Luo River in the 1960s.

The site is divided into two areas: Suyang and Xiacun, which both have ditches that keep every area separate from the other. From 2016 to 2019, the Luoyang City Cultural Relics and Archaeology Research Institute conducted excavations in Suyang and Xiacun (**Figure 1**). The excavation areas covered 2,000 m^2, exposing buildings, pits, ditches, and other types of relics. Dating from the late Yangshao cultural to the Miaodigou II and Wangwan III periods, because Miaodigou II samples too little, this article merges Miaodigou II and Wangwan III into the Longshan period when discussing related issues (the latest excavation show that more Miaodigou Phase II remains have been found at the site, and more samples will be analyzed in the future to distinguish the agriculture of Miaodigou Phase II and Wangwan Phase III). The Suyang site was a large and important center during the late Yangshao and Longshan periods in the middle of the Luo River Valley (Ren, 2021).

MATERIALS AND METHODS

The samples analyzed in this research were obtained from excavations undertaken during the 2016 and 2017 fieldwork seasons. All samples were collected using a targeted sampling strategy (Zhao, 2010). A total of 90 flotation samples were collected in 2016 from 63 contexts, including 10 samples from pits dating to the late Yangshao period and 53 samples from pits, stratum, and ditches dating to the Longshan period. Each sample has a volume ranging from 10 to 20 liters, with an average of 16.51 liters and a total of 1,040 liters. For the phytolith analysis, 54 samples were included, 32 from Suyang excavation pits and 11 from Xiacun excavation pits, and 11 from Xiacun excavation dishes.

All flotation samples were floated using bucket flotation on-site, and the carbonized plant remains were collected in 0.85 and 0.18 mm mesh. Once dried, the light fractions were transferred to sturdy plastic containers and labeled. After being sent to the Archaeobotany Laboratory at Shandong University (ALSDU), the light fractions were sorted, identified, and photographed using a Nikon SM 100. The number of unidentifiable seeds or plant parts was not included in the total number of analyses or the discussion.

All phytolith samples were processed and analyzed using Rosen's protocol. (Rosen and Weniner, 1994; Rosen, 1999; Rosen, 2005). After sieving with a 0.3 mm mesh, 1 g of fine soil was removed and processed in the following steps: 1) 10%

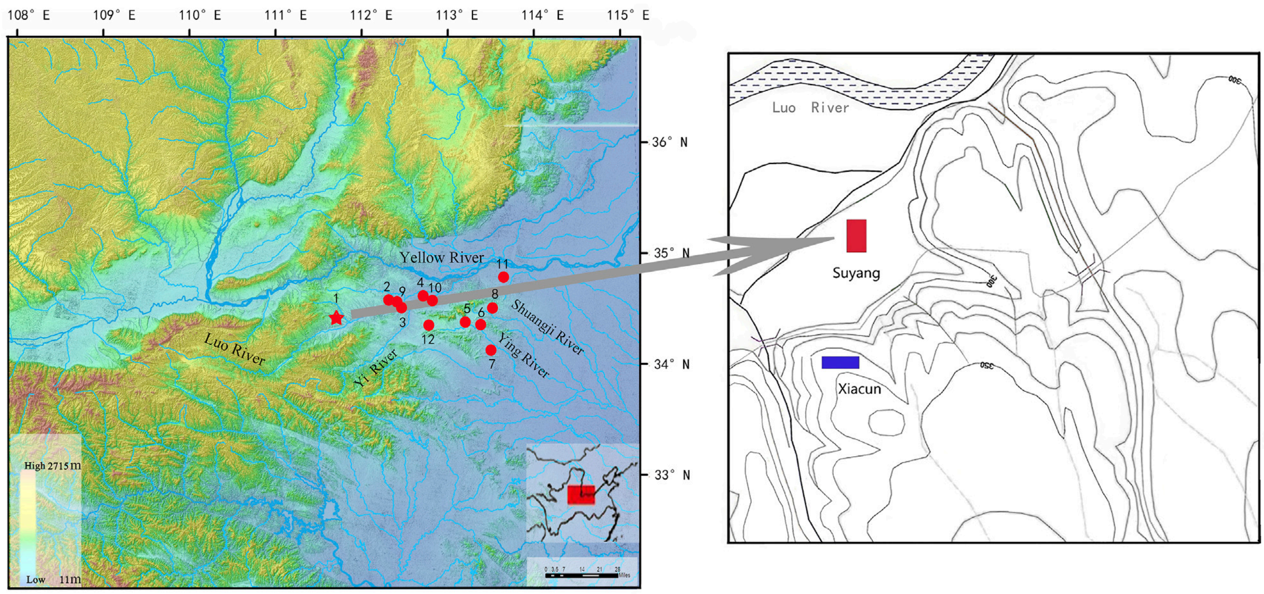

FIGURE 1 | Map showing the location of sites mentioned in the text: (1. Suyang, 2. Zhonggou, 3. Wanggedang, 4. Erlitou, 5. Wangchenggang, 6. Chengyao, 7. Wadian, 8. Xinzhai, 9. Zaojiaoshu, 10. Huizui, 11. Zhuzhai, 12. Yingyang) and the spatial distribution of excavation areas of the Suyang site.

hydrochloric acid (HCL) and hydrogen peroxide (H_2O_2) was added to each sample to remove organic matter and minerals such as calcium and ferrum; 2) HCL was centrifuged with distilled water for 3–5 times to clean the acid; 3) 5% sodium hexametaphosphate solution was added and the samples were left for 12 h to break soil particles; 4) the soil was transferred to a test tube and pure water up to 8 ml was added; the samples were then stirred and left alone for 70 min before the water containing clay was poured out; this step was repeated five times until the clay was totally removed and the samples were then parched; 5) the samples were transferred to a small crucible and heated in a muffle oven at 500°C for 2–2.5 h; 6) the soil was transferred to a test tube while cadmium iodide heavy solution was added to make the phytoliths float on the surface of the solution. The phytoliths were then collected and washed with distilled water; and 7) fixed slides were made with neutral gum.

The identification and statistics of phytoliths were identified using a Nikon Eclipse LV100P0L microscope (200×). Four hundred phytoliths were randomly chosen from each sample to identify and count; then, they were photographed with a Nikon BF53 camera. All classifications and identifications were based on the International Code for Phytoliths Nomenclature 1.0, research on phytoliths of modern plants, and published documents on phytoliths. The identification of rice (*Oryza sativa*), foxtail millet (*Setaria italica*), and broomcorn millet (*Panicum miliaceium*) were based on the standards of Pearsall and Lu (Lu et al., 2009), which meant that the phytoliths of millet were only counted if it had more than two specific figures (Fujiwara, 1995; Pearsall et al., 1995; Lu et al., 2009; Gu et al., 2013; Weisskopf, 2014; Weisskopf and Lee, 2016). Some phytoliths such as square-, oblong-, pointed-, and rod-shaped phytoliths are counted even though they cannot be classified as any species because their assemblages are useful in rebuilding the ancient environment (Wang and Lu, 1993). The quantitative statistics of phytoliths follow Albert's method, which calculates the concentration of phytoliths in 1 g soil and calculates the percentage of each kind of phytolith (Albert and Weiner, 2001). Every plant's ubiquity is calculated by the formula *the number of samples of a certain kind of plant: the total sample number ×100%.*

DATING AND ARCHAEOBOTANIC RESULTS

Dating

Thirteen cereal grain samples from the Suyang site were sent to the State Key Laboratory of Isotope Geochemistry accelerator mass spectrometry (AMS) radiocarbon dating at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences. Each date was calibrated by OxCal v4.4.2, using the IntCal20 atmospheric curve. The 13 AMS C-14 dates obtained from the Suyang site are shown in **Table 1**. Except for one abnormal date from single wheat grain, the dates are consistent with the dates proposed by ceramic typology (Ren, 2021). The available dates can be grouped into two sets. The first set contains three dates covering the time interval 2,917–2,747 cal BC (95.4% range), which falls within the late Yangshao culture age. The second set covers the period from 2,760 to 1939 cal BC (95.4% range), which corresponds to the Longshan culture.

Macro-Botanical Remains

Except for 61 stems, 47,083 seeds or fruits were recovered from flotation, of which 45,963 were identified (**Table 2** and **Figure 2**). The density was 44.2 seeds/fruits per liter of soil. Five types of

TABLE 1 | Radiocarbon dates and calibration results of the Suyang site.

Lab no.	Feature no.	Material	Period	Radiocarbon date, BP	Calibrated date (BC/AD, 2σ)
GZ8848	SH15	Foxtail millet	Late Yangshao	4,310 ± 20	2,917–2,899
GZ8849	SH18	Foxtail millet	Late Yangshao	4,310 ± 35	2,930–2,889
GZ8847	SH16	Rice	Late Yangshao	4,165 ± 20	2,811–2,747
GZ8860	XH7	Broomcorn millet	Longshan	4,145 ± 25	2,760–2,665
GZ8858	XH11	Foxtail millet	Longshan	4,145 ± 30	2,762–2,663
GZ8856	XG1-48	Foxtail millet	Longshan	4,045 ± 25	2,533–2,494
GZ8855	XG1-31	Rice	Longshan	3,880 ± 25	2,454–2,418
GZ8851	SH52	Rice	Longshan	3,900 ± 20	2,381–2,348
GZ8859	XH9	Foxtail millet	Longshan	3,745 ± 20	2,200–2,158
GZ8854	XG1-11	Foxtail millet	Longshan	3,605 ± 25	1980–1926
GZ8861	XH27	Foxtail millet	Longshan	3,610 ± 20	1980–1939
GZ8862	XH39	Foxtail millet	Longshan	3,615 ± 30	1985–1939
GZ8853	H34	Wheat	Longshan	–5,160 ± 15	1963AD

crops were found in the samples, including foxtail millet, broomcorn millet, rice, soybean (*Glycine max*), and wheat (*Triticum aestivum*). The total amount was 45,562, accounting for 99.13% of the carbonized plant remains. Every sample had all these crops, so the ubiquity of each kind was 100%. Except for some immature millets, most millets were mature and complete. Among rice, broken fragments were more abundant than complete seeds, of which 1,850 were rice spikelet bases. There were not many soybeans found in the samples, but the complete ones were six times more than the incomplete ones. The relative percentages of each crop type were as follows: foxtail millet, 73.89%; rice, 25.19%; broomcorn millet, 0.75%; and soybean, 0.16%. The ubiquity of foxtail millet is clear from all samples and periods; therefore, it seems obvious that foxtail millet was the dominant crop at the Suyang site. However, there are interesting trends; the values of relative percentage and ubiquity changed throughout the study period. The relative percentage of foxtail millet declined from the late Yangshao period to the Longshan period, while rice witnessed an increase. Simultaneously, the ubiquity of the broomcorn millet saw a slight increase, but its amount was still far below that of the others. In contrast, soybean maintained a low relative percentage throughout the entire period (**Figure 3**). Finally, one wheat seed was found in the sample TN5W9H34; however, it turned out to be an intrusion from modern times after AMS C-14 dating (**Table 1**).

In addition, 401 weeds with a 0.87% relative percentage and 73.02% ubiquity were found in the samples from 37 species, of which the most abundant were Leguminosae, Chenopodiaceae, Lamiaceae, Euphorbiaceae, Polygonaceae, and Cyperaceae. *Chenopodium* sp., *Digitaria* sp., *Setaria* sp., *Lespedeza bicolor*, *Melilotus* sp., *Perilla* sp., and *Polygonum aviculare. Chenopodium* sp. is a crop with a long history of cultivation in some areas, such as America and Taiwan (Smith, 1984). The seeds of *Chenopodium* sp. unearthed together with millet from the Han Yangling site might be the earliest evidence of Chenopodium's cultivation (Yang et al., 2009). While *Chenopodium sp.* remains from the Nanwa site, the Guchengzhai site and the Erlitou site recognized these seeds as a cultivar (Cheng et al., 2012; Wu et al., 2014), whether *Chenopodium sp.* is a farmed crop remains uncertain. In Suyang, it is possible that people gathered this weed to eat as a vegetable. *Lespedeza bicolor* and *Melilotus sp.* are common at sites in central China, and they are recognized as fodder or fuel; however, those from the Suyang site do not necessarily fit this hypothesis based on their scarcity. Fruits found in the samples were rare and were probably used as food to enrich their diet.

MICRO-BOTANICAL REMAINS (PHYTOLITH)

Various phytoliths were recovered from the samples, with an average density of 560,000 p/g. A total of 19,913 phytoliths from 54 samples were classified into 25 morphotypes. Densities varied significantly between positive samples, ranging from 2.10 to 22.84%. The identifiable morpho-types were from the husk or stem/leaf of rice and millet (**Figure 4**).

The relative percentages of broomcorn millet, foxtail millet, and rice from the Yangshao period were 10.99, 11.46, and 77.55%, respectively (**Figure 5**). All samples from this period had more phytoliths from the husk than the stem/leaf, regardless of whether the phytoliths belonged to millet or rice. It is worth noting that the pits H1, H5, H15, and H16③ only had phytoliths belonging to the husk of millet or rice. For the Longshan period, the relative percentages of these three crops were 18.67, 14.25, and 67.09%, respectively. Similarly, in the former period, the phytoliths of husk were more abundant than those of stem/leaf. However, the relative percentage of the latter type increased. Pits SYH41, XCH7, XCH11②, XCH12②, XCH14, XCH15, and XCH17 reverted the trend where phytoliths of stem/leaf were more abundant than husk ones. In addition to rice and millet varieties, phytoliths belonging to reed were found in 28 samples (51.85%) There is a note-worthy difference between the results of phytoliths and carbonized seeds. The relative proportion of common millet and rice is much higher in the phytolith assemblage than in the carbonized results and this could be due to the biases of charred preservation (Tanja and Manfred, 2008). The microscopic nature of the phytolith

TABLE 2 | Summary of the Suyang Site Flotation Samples (all phases combined).

Occupation	Yangshao	Longshan				
Sector	Suyang	Suyang	Xiacun	Xiacun	Xiacun	—
Context of samples	Pits	Pits	Pits	Trenches	Cultural levels	—
No. of samples	10	18	27	7	1	63
Volume (litres)	120	370.5	443.5	118	12	1,064
Cultigens						
Setaria italica	23,322	1783	7,904	523	132	33,664
Panicum miliaceum	170	61	101	12	1	345
Oryza sativa,grains	2,589	6,461	404	167	8	9,629
Oryza sativa,spikelets	1746	34	63	7	—	1850
Glycine max	37	18	9	9	—	73
Triticum aestivum	—	1	—	—	—	1
Weedy plants						
Melilotus sp	2	24	11	5	—	42
Lespedeza bicolor	—	3	12	3	—	18
Medicago sativa	—	3	6	—	—	9
Glycine soja	3	—	1	—	—	4
Digitaria sp	30	3	29	2	—	64
Setaria sp	10	14	19	—	—	44
Avena fatua	—	—	7	—	—	7
Tragus berteronianus	—	—	3	—	—	3
Eleusine indica	—	2	—	—	—	2
Echinochloa sp	—	2	—	—	—	2
Perilla sp	—	3	10	—	—	13
Amethystea caerulea	1	—	—	2	—	3
Polygonum aviculare	—	—	7	6	—	13
Rumex acetosa	1	—	—	2	—	3
Polygonum lapathifolium	1	—	1	—	—	2
Polygonum strigosum	—	1	—	—	—	1
Polygonum maackianum	—	—	1	—	—	—
Polygonum orientale	1	1	—	—	—	2
Chenopodium sp	84	14	10	10	—	118
Thladiantha dubia	1	2	1	—	—	4
Scirpus validus	4	1	—	—	1	6
Scirpus juncoides	—	1	—	2	—	3
Galium aparine	—	—	—	1	—	1
Acalypha australis	1	—	1	—	—	2
Erodicm stephaniancm	—	3	—	—	—	3
Girardinia suborbiculata	2	—	—	—	—	2
Cryptotaenia japonica	—	1	1	3	—	5
Vitex negundo	2	1	—	—	—	3
Arisaema. sp	—	—	1	—	—	1
Corydalis bungeana	—	2	—	—	—	2
Kalopanax septemlobus	—	—	1	—	—	1
Patrinia sp	—	—	—	1	—	1
Fleshy fruits and nuts						
Ziziphus sp	1	—	—	—	—	1
Diospyros sp	—	1	—	—	—	1
Chaenomeles sp	—	—	1	—	—	1
Unidentifiable nutshell	8	—	—	4	—	12
Unidentifiable	374	233	431	70	12	1,120
Total seeds	28,390	8,673	9,035	829	154	47,081
Seed Density (no./liter)	236.58	23.41	20.37	7.03	12.8	44.25

should be noticed as well. The amount of each sediment sample is so small when compared to the whole feature that a tiny difference in sampling could be magnified to a larger extent on the data. After balancing the influencing factors, the carbonized plant remains will be used as the main evidence to discuss the relevant issues of crops, and the phytolith data will be the subordinate one.

DISCUSSION

The Status of Rice

Foxtail millet seemed to be the most important crop at the Suyang site during the late Yangshao period. Rice was less present but important at the site, considering its 80% ubiquity. Broomcorn and soybean did not equal the status of

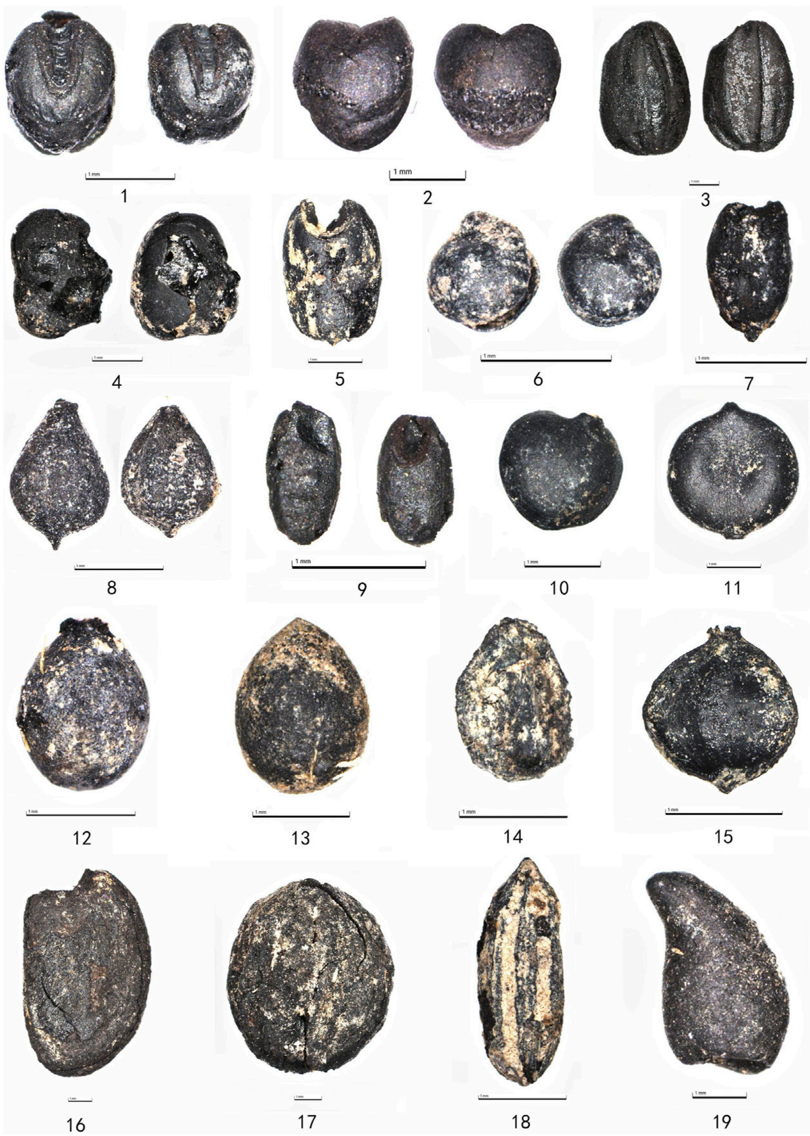

FIGURE 2 | Representative charred plant remains from the Suyang site: (1) *Setaria italica,* (2) *Panicum miliaceum,* (3) *Oryza sativa,* (4) *Glycine max,* (5) *Triticum aestivum,* (6) *Chenopodium* sp., (7) *Setaria* sp., (8) *Scirpus Validus,* (9) *Digitaria* sp., (10) *Corydalis* sp., (11) *Polygonum lapathifolium,* (12) *Acalypha australis,* (13) *Perilla* sp., (14) *Patrinia villosa,* (15) *Scirpus juncoides,* (16) *Diospyros* sp., (17) *Ziziphus* sp., (18) *Cryptotaenia japonica,* (19) *Chaenomeles* sp.

foxtail millet and rice, although broomcorn millet was slightly more important than soybean. During the Longshan period, while the status of rice seemed to improve over time.

The percentage and ubiquity of seed remains of foxtail millet would place this crop as the most important cultigen throughout both periods of occupation of the site. However, it is necessary to consider the grain size (weight) before estimating their role in the plant diet. According to the weight of carbonized seeds unearthed at the Suyang site, the results indicate that rice grew in prominence during the late Yangshao period and surpassed foxtail millet. Rice may have surpassed foxtail millet as the most important crop by the Longshan period, as shown in the graph below (**Figure 6**).

Evidence from experimental archaeology also supports our result. When comparing the number of glume phytoliths from rice and millet of equal weight, the data showed underestimated the rice. Modern experiments have shown that millet produces more phytoliths than rice; therefore, it is expected that when millet and rice are equal in weight, millet phytoliths are more likely to occur (Zhang, 2010). In our analysis, glume phytoliths of rice were more abundant than millet, suggesting that more rice might have been produced than millet at the Suyang site. Meanwhile, we also need to pay attention to the following issues. First of all, because there are only experimental data on the glume of millet and rice, the above discussion is based on the glumes of millet and rice, and the stem leaves are not discussed. Second, phytoliths from millet husk are very fragile and more

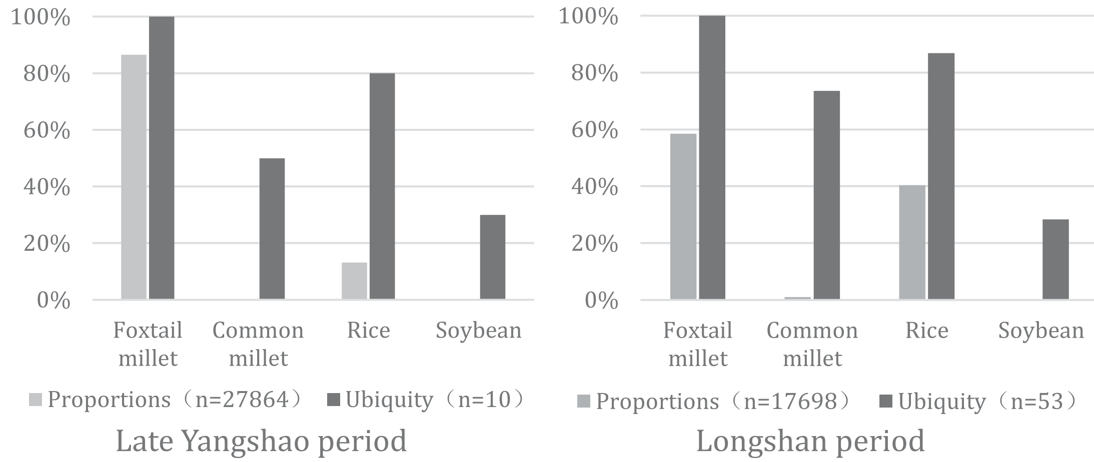

FIGURE 3 | Proportions and ubiquity of crops at the Suyang site (Late Yangshao period and Longshan period).

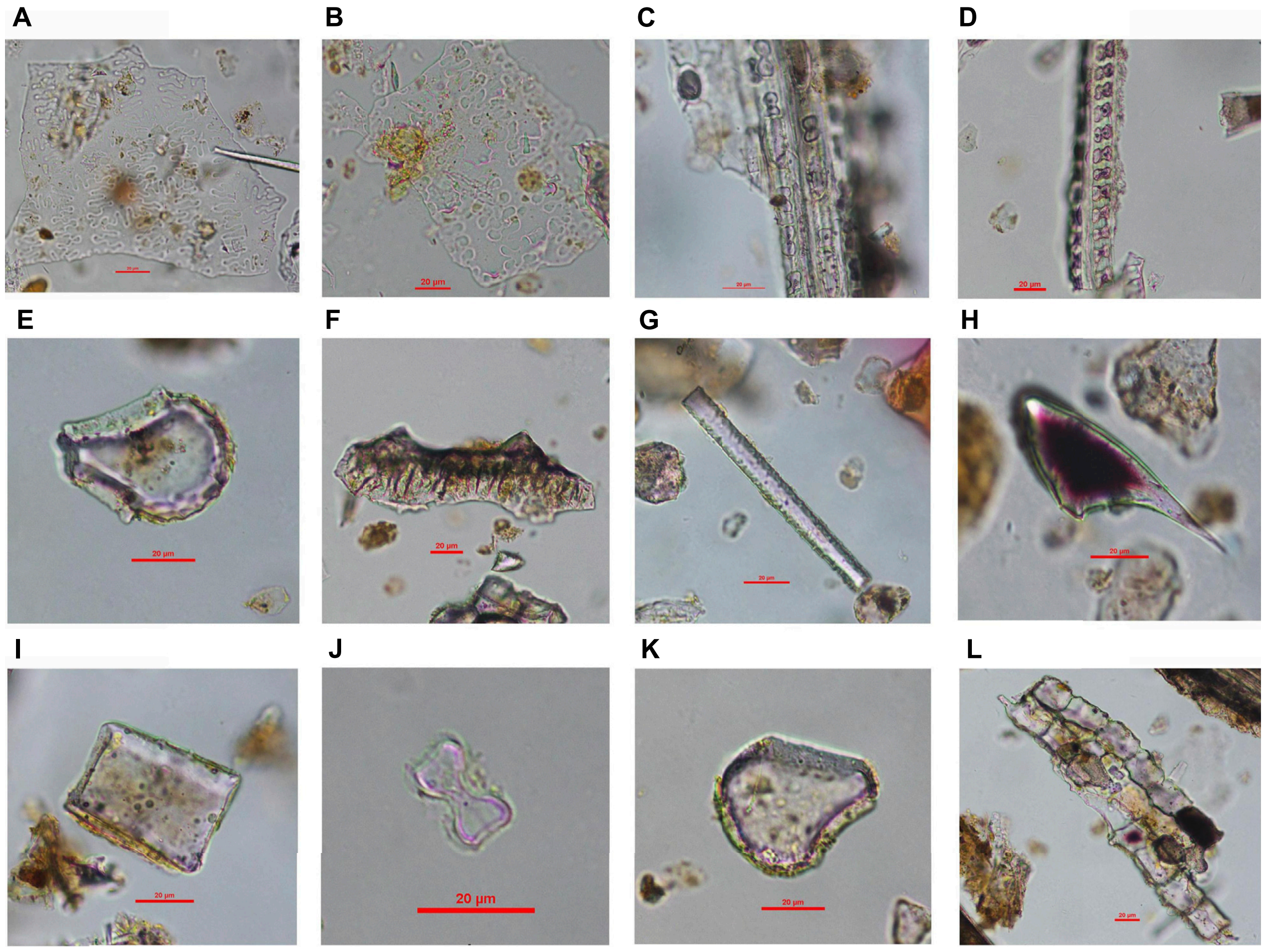

FIGURE 4 | Main phytolith types from the Suyang site. **(A)** η-type from husks of common millet, **(B)** Ω-type from husks of foxtail millet, **(C)** Vertically-bilobe from Poaceae, **(D)** parallel-bilobe from rice leaf/stem, **(E)** bulliform from rice leaf, **(F)** double-peaked from rice husk, **(G)** smooth elongate, **(H)** pointed, **(I)** square, **(J)** bilobate, **(K)** bulliform, **(L)** rectangle.

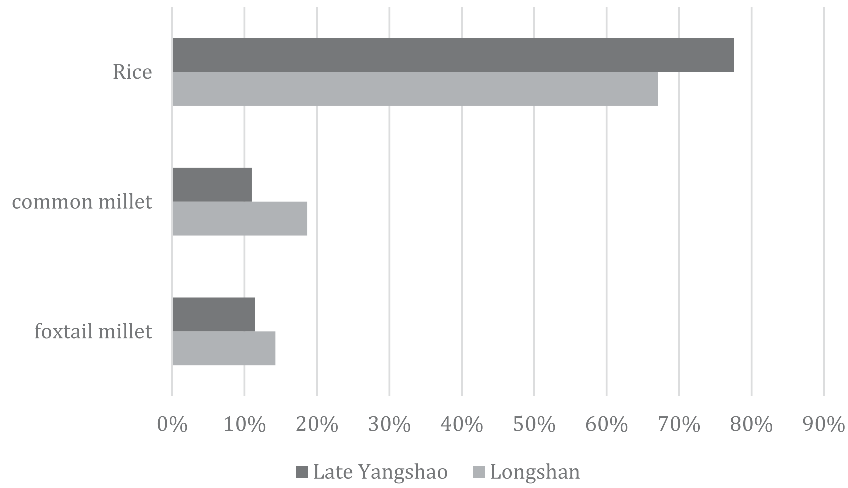

FIGURE 5 | Proportions of glume phytoliths of common millet, foxtail millet, and rice at the Suyang site.

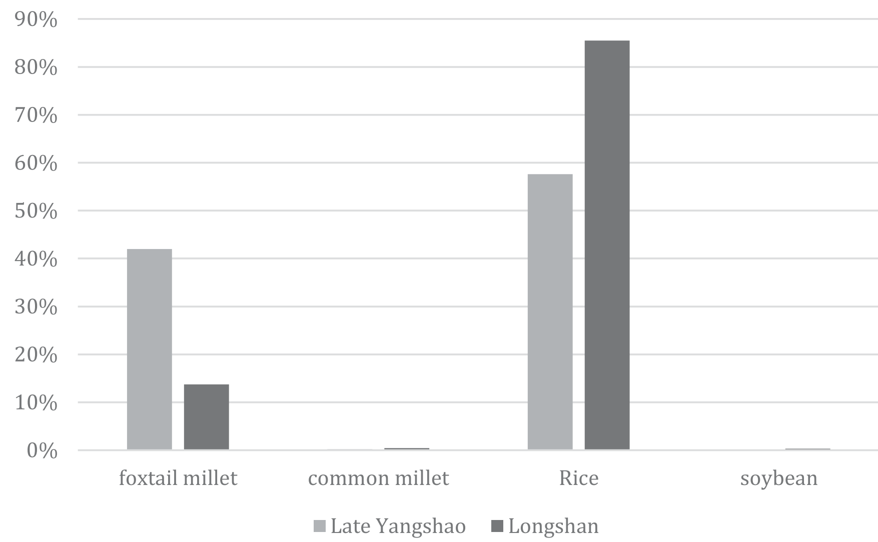

FIGURE 6 | Proportions of crops in different periods in grain weight at the Suyang site.

difficult to preserve, so the discussion based on husk is very preliminary, and more follow-up work is needed.

It is interesting to compare Suyang with other sites in Central China; as shown in **Figure 7**, the percentage and ubiquity of rice at the Suyang site are the highest. The Wanggedang site located in the Lower Luo River turned up 144 seeds of rice (4.09% of the total) recovered from systematic flotation, indicating that while rice could have been an essential part of the plant diet considering its 52% ubiquity (Zhong et al., 2019), it was still far inferior to the Suyang site. In comparison, the status of rice in the sites of Chengyao, Wangchenggang, and Xinzhai was lower than in others (Zhao, 2007; Zhong et al., 2018). In contrast, rice made a more critical contribution at the sites of Wadian in the middle of Ying River Valley, in the Mid-Holocene accumulation stage with the characteristics of minor altitude difference between terrace and riverbed, which is like a waterside village. Such a landscape made it possible for the ancients to excavate ditches and proceed with rice cultivation (Wang et al., 2015). Still, the rice in Wadian is not as crucial as that in Suyang.

The Rice Arable Systems at the Suyang Site: Wet or Dry?

The recovery of 1,850 rice spikelet bases (1746 from the late Yangshao period and 104 from the Longshan period) suggests that at least some rice was processed at the site. Phytoliths from the leaf/stem or the husk of rice spanning all kinds of contexts at the Suyang site and weeds common in paddy fields such as

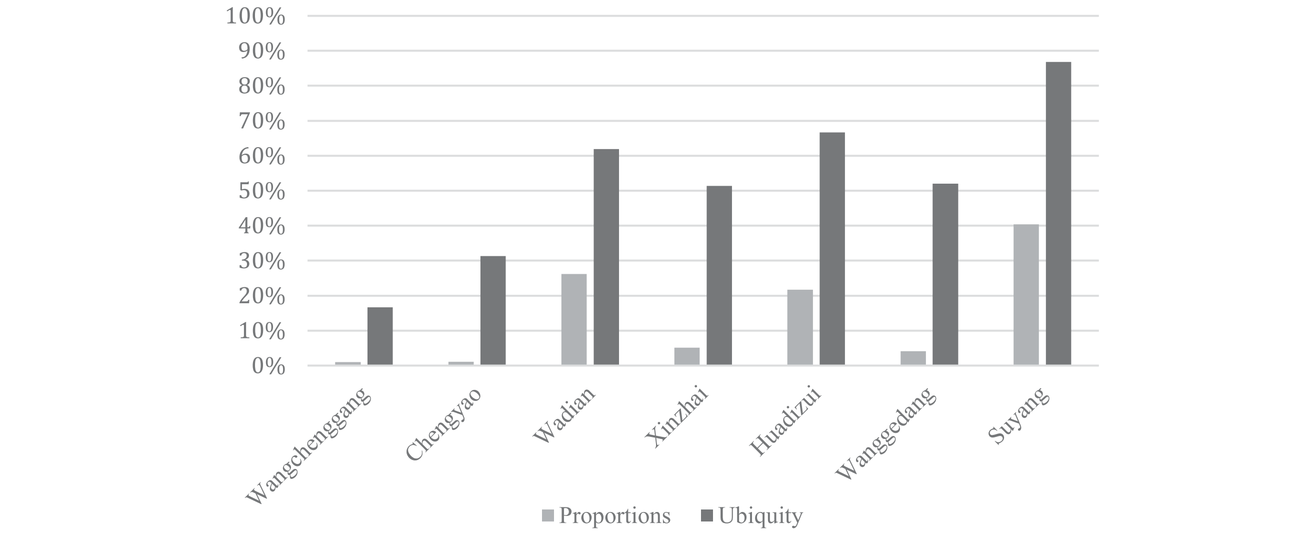

FIGURE 7 | Proportions and ubiquity of rice in the Longshan period at the Central Plain.

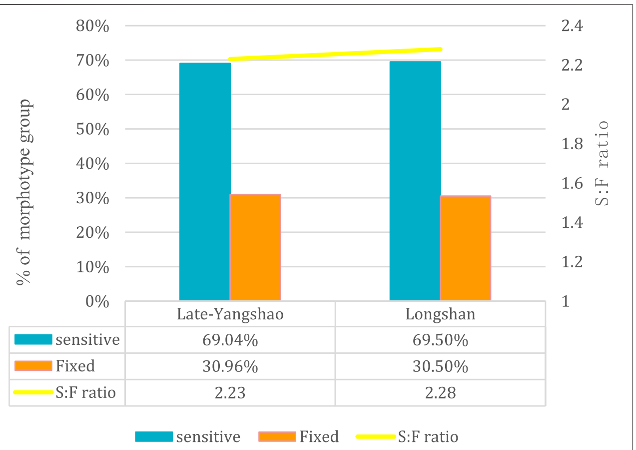

FIGURE 8 | Percentage of sensitive versus fixed phytolith morphotypes in two periods at the Suyang site; percentages exclude all phytolith types not within the sensitive/fixed classification as defined by Weisskopf et al. (2015a).

Echinochloa and Cyperaceae were recovered in flotation samples, suggesting that rice was produced locally. Given that previous research has failed to certify whether rice was cultivated by local people in the Luo River Valley (Lee et al., 2007), the Suyang site offers compelling evidence demonstrating that people during the late Yangshao period in the Luo River Valley cultivated rice.

Even though the research suggests that Central China had a favorable environment for rice cultivation in ancient times, the question remains as to what kind of rice people chose to cultivate (Wang et al., 2018). According to Weisskopf et al. (2015a), different ratios of phytolith morphotypes to produce silica in the grass (fixed) and those that need sufficient water (sensitive) could be used to rebuild rice water settings in rice cultivation. The fixed: sensitive ratio at Suyang demonstrated a substantially higher ratio of sensitivity to fixed phytoliths, indicating that the setting in the late Yangshao period was similar to that in the Tianluoshan and Shunshanji sites (Luo et al., 2020). In the case of Tianluoshan, the results suggested wet paddy agriculture (Weisskopf et al., 2015a). Therefore, it is likely that the rice remains from Suyang was cultivated in irrigated paddies (**Figure 8**). It is possible that the people living at the site might have taken advantage of the surrounding swamps to grow rice. In the following Longshan period, the setting did not seem to have changed according to the ratio.

Furthermore, four seeds of *Scirpus juncoides*, a shallow water plant, were recovered at Suyange, indicating wet conditions around the settlement. Additionally, a paddy system was found in the Huizui site located in the lower Yiluo River Valley, which led us to search for the same thing at Suyang (Rosen et al., 2017). Unfortunately, there have been no remains suggestive of a paddy field found, probably due to the small excavation areas.

Now, we answer why the status of rice in the Longshan period grew despite the lack of improvement in the cultivation method. Paleoenvironmental research suggests that Henan Province was under the influence of subtropical monsoon climate during the Yangshao period, which means that it had higher temperature and precipitation than in modern times (Li et al., 2015). For example, the deposited sediment from a profile at the Zaojiaoshu site revealed that the area had a warmer and wetter climate than the present, parallel to the modern climate in the middle-lower Yangtze Plain (Zhang and Li, 1997). The charcoal found in the Suyang site was from various species, including Aceraceae, Bambusoideae, *Pinus* sp., *Quercus* sp., *Populus* sp., *Ulmus* sp., and *Cercidiphyllum* sp. *Bambusoidea,* only grows in wet and humid environments, indicating that the area enjoyed a wetter climate in the past. Suyang Site is located on the T2 terrace of southern Luo River (Ren, 2021). About 7,200 years ago, the T1 terrace began to form along with the erosional downcutting of Luo River. The increasing sediment accumulation brought sediment-rich clay and ponding depressions which provided the appropriate setting for rice cultivation. It is credible that there are large floodplain areas suitable for rice cultivation on both sides of the Luo River near the he Suyang site (Rosen et al., 2015; Zhang et al., 2018; 2019).

In addition to environmental reasons, culture and population are also important factors affecting rice cultivation. From the late Yangshao period, the Qujialing culture showed a definite northward trend, the western Henan area and the Luoyang Basin was greatly influenced by the Qujialing culture from the Middle Yangtze Region (Dai, 1998; Meng, 2011). A few relics of Miaodigou II culture were found in the Suyang site, and many relics have Qujialing cultural characteristics. With the solid northward movement of Qujialing culture, the middle reaches of the Luo River were included in the late Yangshao period (Ren, 2021). The spread of the Qujialing culture into this area, the Suyang site would have impacted greatly on rice farming that focused Yangtze pattern.

The increase in population might have resulted from the rice agriculture in the Suyang site. According to the excavation results, there more relics of the Longshan period were recovered in the Xiacun areacomparing with the former period (Ren, 2021), which suggests the residential area of the settlement expanded to the Xiacun areas during the Longshan period. The possible reason behind this scenario could be the population growth. Rice is a higher-yielding crop with good taste than millet, and the large-scale cultivation of rice can feed more of the population. However, Rice is also a labor-intensive crop. From our data, the population growth could also mean an increased workforce used in water management and relative production activities in rice cultivation.

Crop Processing and Social Organization

Crop processing refers to the process that takes place after harvest and before consumption, which generally contains several stages such as threshing, winnowing, dehusking, and sieving (Reddy, 1997). Because the processing steps require particular assemblages, they can be identified by examining the composition of each sample. According to previous research, all processing steps belonged to either earlier stages featuring threshing reflected by many leaf/stem parts, small and light weed seeds, and immature grains in the byproducts; or to later stages featuring dehusking associated with clean grains and husks in the byproducts (Fuller and Steven, 2009; Fuller et al., 2014). Phytoliths can provide information about the crop processing style, provided that crops such as rice and millet produce different phytoliths in different parts of their body (Harvey and Fuller, 2005). For example, the ratios of the stem, husk, and weed can be used to infer the site, stages, and scale of crop processing, which can further reveal the division and organization of social labor and economy (Stevens, 2003; Fuller and Steven, 2009; Fuller et al., 2014; Weisskopf, 2014).

The phytoliths produced by the stem/leaf of millet and rice are ubiquitous at the Suyang site, indicating that the crops were harvested by reaping at the sheaf. Three samples (H1, H3, H6) of the Yangshao period only had phytoliths of husk and several mature grains, while the other samples have much the same phytoliths, which demonstrates that dehusking was a routine activity at the Suyang site. The samples of this period did not have any assemblage characteristic of the earlier stages of the crop processing, which is possible because the threshing field was not in the sampling area.

For the Longshan period samples, the amount and ubiquity of phytoliths produced by the stem/leaf of millet and rice were higher than the former, indicating continuity in the harvesting style. Some samples from the Suyang (H18, H26, H27) and Xiacun (H17, H18, G1(14)) areas have a large number of husk phytoliths, especially sample H18①-6 with a density of 830,000 particles per gram. These samples indicate that dehusking was still a routine activity at the Suyang site during the Longshan period. Meanwhile, other samples from the Suyang area (H7, H14, H41) and Xiacun area (H11②, H12②, H15) provided evidence based on stem/leaf phytoliths for threshing activity, suggesting that the whole processing activities were carried out inside the settlement in the Longshan period.

The absence of evidence suggestive of threshing activities on-site during the Yangshao period may also indicate that in earlier periods, these activities were communal and were undertaken in large open areas outside the settlement perimeter. If this is true, a change in the crop processing pattern in later periods when threshing and dehusking are observed inside the village may point to the development of nuclear families. Researchers have shown that the nuclear family as an economic unit started to show its independence and desire for wealth and status during this period, as a consequence of the development of social complexity in the area (Dai, 2013). Furthermore, evidence from the Baligang site (Weisskopf et al., 2015b), the sites in Ying River Valley (Zhao, 2007), Shu River Valley (Song et al., 2019), and the upper Hutuo River Valley (Jiang et al., 2019) all indicate the conversion of labor from the late Yangshao period to the Longshan period. It should be noted that t the number of samples analyzed in this study remains small, and this part is only a discussion based on the sampling area. When the site is re-excavated, we will supplement the sample size and discuss this again.

CONCLUSION

This research suggests that rice became a great crop opportunity produced locally. Large amounts of rice base remain in some common paddy weeds such as *Echinochloa* and Cyperaceae, and the ubiquity of phytoliths of stem/leaf all point toward this conclusion. While dry farming agriculture featuring foxtail millet was the primary subsistence economy at the Suyang site during its earlier periods, rice cultivation developed throughout the Longshan period and became a staple food. Population growth probably stimulated this change. Additionally, all lines of evidence indicate that rice played a much more critical role at the Suyang site than other sites in Central Plains; it may be related to many reasons such as suitable environmental and geomorphological conditions, the influence of the Qujialing culture, and population growth.

By combining the analyses of carbonized remains and phytoliths, we discussed two issues concerning the economic and social structure of Suyang. The dispersion of social production and the reduction of the social unit from communal to nuclear families seemed to have been widespread in northern China in later periods according to the crop processing patterns we analyzed. In future research, attention should be paid to the combination of carbonized plant remains, phytoliths, starch, and other micro-remains. The significance of the plant remains should be discussed from an interdisciplinary perspective. At the same time, multi-proxy investigations should provide a more informed understanding of the relationship between plant remains and human behavior, as well as ancient society.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

FY: Extraction and identification of carbonized plant remains and phytoliths,Writing Original Draft QD: Assist with phytolith extraction and data analysis BC: Site excavation and collation of archaeological background GR: Site excavation and collation of archaeological background YJ: Charcoal identification and analysis GJ: Provide research guidance, research funding and revise drafts.

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REFERENCES

Albert, R. M., and Weiner, S. (2001). "Study of Phytoliths in Prehistoric Ash Layers Using a Quantitative Approach," in *Phytoliths: Applications in Earth Sciences and Human History. Lisse*. Editors J. D. Meunier and F. Colin (Lisse: A.A. Balkema Publishers), 251–266.

Bettinger, R. L., Barton, L., and Morgan, C. (2010). The Origins of Food Production in North China: A Different Kind of Agricultural Revolution. *Evol. Anthropol.* 19 (1), 9–21. doi:10.1002/evan.20236

Cheng, W. W., Zhang, J. Z., and Cai, Q. F. (2012). Analysis of Plant Reminds from the Guchengzhai Site in Xinmi, Henan. *Huaxia Archaeol.* 1, 54–62. doi:10.16143/j.cnki.1001-9928.2012.01.001

Crawford, G. W., Chen, X., Luan, F., and Wang, J. (2016). People and Plant Interaction at the Houli Culture Yuezhuang Site in Shandong Province, China. *Holocene* 26 (10), 1594–1604. doi:10.1177/0959683616650269

Dai, X. M. (1998). Evolution of the Neolithic Cultural Pattern in the Yellow River Valley. *Acta Archaeol. Sin.* 4, 389–418.

Dai, X. M. (2013). "The Process of Social Complication in the Longshan Period in the Central Plains. School of Archaeology and Museology and Chinese Archaeology Research Center," in *Archaeological Research(10)*. Editor Peking University (Beijing: science press), 539–581.

Fujiwara, H. (1995). "Research into the History of Rice Cultivation Using Plant Opal Analysis," in *Current Research in Phytolith Analysis, Application in Archaeology and Paleoecology*. Editors D. M. Pearsall and D. R. Piperno (Philadelphia(PA): MASCA, University of Pennsylvania), 147–158.

Fuller, D. Q., and Steven, C. (2009). "Agriculture and the Development of Complex Societies: an Archaeobotanical Agenda," in *From Foragers to Farmers: Papers in Honour of Gordon C. Hillman*. Editors A. S. Fairbairn and E. Weisskopf (Oxford and Oakville: Oxbow Books), 37–57.

Fuller, D. Q., Stevens, C., and McClatchie, M. (2014). "Routine Activities, Tertiary Refuse and Labor Organization: Social Inferences from Everyday Archaeobotany," in *Ancient Plants and People Contemporary Trends in Archaeobotany*. Editors M. Madella, C. Lancelotti, and M. Savard (Tucson: University of Arizona Press), 174–217.

Gu, Y., Zhao, Z., and Pearsall, D. M. (2013). Phytolith Morphology Research on Wild and Domesticated Rice Species in East Asia. *Quat. Int.* 287 (FEB21), 141–148. doi:10.1016/j.quaint.2012.02.013

Han, J. Y. (2015). *Early China-the Making of the Chinese Cultural Sphere*. Shanghai: Shanghai Guji Press, 229.

Harvey, E. L., and Fuller, D. Q. (2005). Investigating Crop Processing Using Phytolith Analysis: the Example of Rice and Millets. *J. Archaeol. Sci.* 32 (5), 739–752. doi:10.1016/j.jas.2004.12.010

Jiang, Y. C., Dai, X. M., Wang, L. Z., Wang, X. Y., and Qin, L. (2019). Plant Macro Remains Reveal the Agricultural Pattern and Regional Variation of the Shanxi Plateau during the Longshan Period. *Quat. Sci.* 39 (1), 123–131. doi:10.11928/j.issn.1001-7410.2019.01.12

Lee, G.-A., Crawford, G. W., Liu, L., and Chen, X. (2007). Plants and People from the Early Neolithic to Shang Periods in North China. *Proc. Natl. Acad. Sci. U.S.A.* 104 (3), 1087–1092. doi:10.1073/pnas.0609763104

Li, K. F., Ma, C. M., Gao, W. H., Li, S. Y., Li, Z. X., and Pan, Y. F. (2015). Progress and Trend of Holocene Environmental Archaeology in Henan Province. *Prog. Geogr.* 34 (7), 883–897. doi:10.18306/dlkxjz.2015.07.010

Li, Y. P., and Zhang, J. N. (2020). The Influence of Landscape Evolution on Rice Planting in the Middle-Late Neolithic Period to Xia-Shang Dynasties in Luoyang Basin. *Quat. Sci.* 40 (2), 499–511. doi:10.11928/j.issn.10017410.2020.02.19

Li, Y., Zhang, J., Zhang, X., and Zhao, R. (2021). Agriculture, the Environment, and Social Complexity From the Early to Late Yangshao Periods (5000-3000 BC): Insights From Macro-Botanical Remains in North-Central China. *Front. Earth Sci.* 9, 662391. doi:10.3389/feart.2021.662391

Liu, L., Chen, X., Lee, Y. K., Wright, H., and Rosen, A. (2002). Settlement Patterns and Development of Social Complexity in the Yiluo Region, North China. *J. Field Archaeol.* 29 (1/2), 75–100. doi:10.2307/3181486

Liu, X., Hunt, H. V., and Jones, M. K. (2009). River Valleys and Foothills: Changing Archaeological Perceptions of North China's Earliest Farms. *Antiquity* 83 (319), 82–95. doi:10.1017/S0003598X00098100

Lu, H. (2017). New Methods and Progress in Research on the Origins and Evolution of Prehistoric Agriculture in China. *Sci. China Earth Sci.* 60, 2141–2159. doi:10.1007/s11430-017-9145-2

Lu, H., Zhang, J., Wu, N., Liu, K.-b., Xu, D., and Li, Q. (2009). Phytoliths Analysis for the Discrimination of Foxtail Millet (Setaria Italica) and Common Millet (Panicum Miliaceum). *PLoS ONE* 4 (2), e4448. doi:10.1371/journal.pone.0004448

Luo, W., Yang, Y., Zhuang, L., Gan, H., Gu, C., Huang, C., et al. (2020). Phytolith Evidence of Water Management for Rice Growing and Processing between 8,500 and 7,500 Cal Years Bp in the Middle Huai River Valley, China. *Veget Hist. Archaeobot* 30, 243–254. doi:10.1007/s00334-020-00782-2

Märkle, T., and Rösch, M. (2008). Experiments on the Effects of Carbonization on Some Cultivated Plant Seeds. *Veget Hist. Archaeobot* 17 (Suppl. l .1), 257–263. doi:10.1007/s00334-008-0165-7

Meng, Y. Z. (2011). The Process of the Qujialing Culture from South to North. *Huaxia Archaeol.* 3, 51–63. doi:10.16143/j.cnki.1001-9928.2011.03.003

Pearsall, D. M., Piperno, D. R., Dinan, E. H., Umlauf, M., Zhao, Z., and Benfer, R. A. (1995). Distinguishing Rice (Oryza Sativa Poaceae) from Wild Oryza Species through Phytolith Analysis: Results of Preliminary Research. *Econ. Bot.* 49 (2), 183–196. doi:10.1007/BF02862923

Qin, L. (2012). *Archaeobotanical Research and Expectation Chinese Agriculture Origin*. School of archaeology and museology of Peking University, center of the study of Chinese archaeology of Peking University, archaeological research(9). Beijing: Cultural relics publishing house press, 260–315.

Reddy, S. N. (1997). If the Threshing Floor Could Talk: Integration of Agriculture and Pastoralism during the Late Harappan in Gujarat, India. *J. Anthropol. Archaeol.* 16 (2), 162–187. doi:10.1006/jaar.1997.0308

Ren, G. (2021). Brief Report on the Excavation of the Xiacun Area in the Suyang Site at Yiyang, Henan Province. *Cult. Relics Central China* 5, 23–38.

Rosen, A., Macphail, R., Liu, L., Chen, X., and Weisskopf, A. (2017). Rising Social Complexity, Agricultural Intensification, and the Earliest Rice Paddies on the Loess Plateau of Northern China. *Quat. Int.* 437, 50–59. doi:10.1016/j.quaint.2015.10.013

Rosen, A. M., Lee, J., Li, M., Wright, J., Wright, H. T., and Fang, H. (2015). The Anthropocene and the Landscape of Confucius: A Historical Ecology of Landscape Changes in Northern and Eastern China during the Middle to Late-Holocene. *Holocene* 25 (10), 1640–1650. doi:10.1177/0959683615594241

Rosen, A. M. (1999). "Phytolith Analysis in Near Eastern Archaeology," in *The Practical Impact of Science on Near Eastern and Aegean Archaeology, Weiner Laboratory Monograph 3*. Editors S. Pike and S. Gitin (London: Archae type Publications), 9–15.

Rosen, A. M. (2005). "Phytolith Indicators of Plant and Land Use at Çatalhöyük," in *Inhabiting Çatalhöyük, Reports from the 1995-99 Seasons*. Editor I. Inhodder (CambridgeLondon: Mcdonald Institute for Archaeological Research, British. Institute of Archaeology at Ankara), 203–212.

Rosen, A. M., and Weiner, S. (1994). Identifying Ancient Irrigation: a New Method Using Opaline Phytoliths from Emmer Wheat. *J. Archaeol. Sci.* 21 (1), 125–132. doi:10.1006/jasc.1994.1013

Smith, B. D. (1984). Chenopodium as a Prehistoric Domesticate in Eastern North America: Evidence from Russell Cave, Alabama. *Science* 226 (4671), 165–167. doi:10.1126/science.226.4671.165

Song, J., Wang, L., and Fuller, D. Q. (2019). A Regional Case in the Development of Agriculture and Crop Processing in Northern China from the Neolithic to Bronze Age: Archaeobotanical Evidence from the Sushui River Survey, Shanxi province. *Archaeol. Anthropol. Sci.* 11, 667–682. doi:10.1007/s12520-017-0551-0

Stevens, C. J. (2003). An Investigation of Agricultural Consumption and Production Models for Prehistoric and Roman Britain. *Environ. Archaeol.* 8 (1), 61–76. doi:10.1179/env.2003.8.1.61

Wang, C., Lu, H., Gu, W., Wu, N., Zhang, J., Zuo, X., et al. (2019). The Development of Yangshao Agriculture and its Interaction with Social Dynamics in the Middle Yellow River Region, China. *Holocene* 29 (1), 173–180. doi:10.1177/0959683618804640

Wang, C., Lu, H., Gu, W., Zuo, X., Zhang, J., Liu, Y., et al. (2018). Temporal Changes of Mixed Millet and Rice Agriculture in Neolithic-Bronze Age Central Plain, China: Archaeobotanical Evidence from the Zhuzhai Site. *Holocene* 28 (5), 738–754. doi:10.1177/0959683617744269

Wang, H., Zhang, H., Zhang, J. F., and Fang, Y. M. (2015). Geomorphology Evolution Process and Related Issues at Wadian Site in Yuzhou, Henan Province. *Cult. Relics South. China* 4, 81–91. doi:10.3969/j.issn.1004-6275.2015.04.015

Wang, Y. J., and Lu, H. Y. (1993). *Research and Application of Phytolith*. Beijing, China: Ocean Press.

Weisskopf, A., Deng, Z., Qin, L., and Fuller, D. Q. (2015b). The Interplay of Millets and Rice in Neolithic Central China: Integrating Phytoliths into the Archaeobotany of Baligang. *Archaeol. Res. Asia* 4 (4), 36–45. doi:10.1016/j.ara.2015.10.002

Weisskopf, A., Qin, L., Ding, J., Ding, P., Sun, G., and Fuller, D. Q. (2015a). Phytoliths and Rice: from Wet to Dry and Back Again in the Neolithic Lower Yangtze. *Antiquity* 89 (347), 1051–1063. doi:10.15184/aqy.2015.94

Weisskopf, A. R., and Lee, G.-A. (2016). Phytolith Identification Criteria for Foxtail and Broomcorn Millets: a New Approach to Calculating Crop Ratios. *Archaeol. Anthropol. Sci.* 8 (1), 29–42. doi:10.1007/s12520-014-0190-7

Weisskopf, A. R. (2014). *Millets, Rice and Farmers: Phytoliths as Indicators of Agricultural, Social and Ecological Change in Neolithic and Bronze Age Central China*. BAR International Series. Oxford: Archaeo Press, 2589.

Wu, W. W., Zhang, J. H., and Jin, G. Y. (2014). *Archaeobotany Evidence of Agriculture from Erlitou to Han Dynasty at Nanwa Site in Dengfeng*, 1. Henan: Cultural Relics of Central China, 109–117.

Yang, X., Liu, C., Zhang, J., Yang, W., Zhang, X., and Lü, H. (2009). Plant Crop Remains from the Outer Burial Pit of the Han Yangling Mausoleum and Their Significance to Early Western Han Agriculture. *Sci. Bull.* 54 (13), 1738–1743. doi:10.1007/s11434-009-0048-z

Yang, Y. Z., Li, W. Y., YaoCheng, L. Z. J., Zhang, J. Z., and Xing, Y. J. (2015). Plant Resources Utilization at the Tanghu Site during the Peiligang Culture Period Based on Starch Grain Analysis, Henan Province. *Quat. Sci.* 35, 229–239.

Zhang, B. Y., and Li, R. Q. (1997). The Environmental Change of Luoyang Basin in Holocene. Holocene Climate in Luoyang Basin. *J. Beijing Normal Univ. Sci.* 33 (2), 275–280.

Zhang, J., Xia, Z., Zhang, X., Storozum, M. J., Huang, X., Han, J., et al. (2018). Early-middle Holocene Ecological Change and its Influence on Human Subsistence Strategies in the Luoyang Basin, North-Central China. *Quat. Res.* 89 (2), 446–458. doi:10.1017/qua.2017.104

Zhang, J., Zhang, X., and Xia, Z. (2014). Research on Charred Plant Remains from the Neolithic to the Bronze Age in Luoyang Basin. *Chin. Sci. Bull.* 59, 3388–3397. doi:10.1360/N972013-00022

Zhang, J., Zhang, X., Xia, Z., Xu, H., and Zhao, H. (2019). Geomorphic Changes along the Yiluo River Influenced the Emergence of the First Urban Center at the Erlitou Site, Central Plains of China. *Quat. Int.* 521, 90–103. doi:10.1016/j.quaint.2019.06.038

Zhao, Z. J. (2010). *Archaeobotany: Theory Methodology and Practice*. Beijing: Science Press, 38.

Zhao, Z. J. (2007). "Identification and Analysis of the Charts and Seeds by Flotation," in *Archaeological Discovery and Research at the Wangchenggang Site in Dengfeng (2002-2005)*.School of Archaeology and Museology, Peking University and Henan Provincial Institute of Cultural Relics and Archaeology(Zhengzhou: Great Elephant Publisher), 516–534.

Zhao, Z. J. (2014). The Process of Origin of Agriculture in China: Archaeological Evidence from Flotation Results. *Quat. Sci.* 34, 73–84. doi:10.3969/j.issn.1001-7410.2014.10

Zhao, Z. J., and Zhang, J. Z. (2009). Flotation Results of 2001 Excavation Season from Jiahu Site. *Archaeology* 8, 84–93.

Zhao, Z. J., ZhaoYu, C. H. J. C., Wang, T., Cui, T. X., and Guo, J. N. (2020). Analysis of Flotation Results from the Donghulin Site of Beijing. *Archaeology* 7, 99–106.

Zhong, H. (2018). The Tentative Research of the Spreading of Rice in the Central Plain during the Prehistoric Period. *West. Archaeol.* (15), 211–223.

Zhong, H., Wu, Y. H., Zhang, H. L., and Zhao, Z. J. (2019). Analysis of Flotation Results from the Wanggedang Site of Luoyang in Henan Province. *Agric. Archaeol.* 1, 7–17.

Zhong, H., Zhang, Y. Q., Wu, Q., and Zhao, Z. J. (2018). Analysis of Flotation Results from the Chengyao Site of Dengfeng in Henan Province. *Agric. Archaeol.* 6, 7–16.

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Micro Plant Remains Reveal the Function of Grooved Pottery Vessels From the Late Neolithic Meishan Site in Central China

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From 6,000 calendar years before the present (cal BP) onward, grooved pottery vessels occurred in the lower and middle reaches of the Yangtze River in succession. After a thousand years, it was introduced into central China and became popularized there around 4,000 years ago. It has been proposed that the function of the grooved vessels was to process plant foods, replacing the previously used stone slabs and rollers in terms of ethnographic investigation, but there is a lack of solid and direct evidence although little evidence of starches has been provided. Here we report our study on the sherds of grooved vessels from the Meishan site in central China (late Longshan and Erlitou period). The combined starch and phytolith analyses were first used together to examine the residues on the sherds. Starches from geophytes, millets (*Setaria italica* and *Panicum miliaceum*), wheat (*Triticum aestivum*), and phytoliths from the glumes and leaves of these crops plus rice (*Oryza sativa*), seem to suggest that the grooved pottery vessels were likely to be used to grind geophytes and dehusk grain seeds. But, incorporating the extremely low proportion of grooved vessels to entire pottery tools at the site, we hypothesize that the invention of grooved vessels may have been related to the success of rice domestication and may have been used as tools to pound by-products of crops, leaves, and husks somehow.

Keywords: starch grain analysis, phytolith analysis, food processing, agricultural society establishment, Yangtze river

INTRODUCTION

During the transition from the Paleolithic Age to the Neolithic Age, stone slabs and rollers were widely used in the world. By means of starch grain analysis, it has been demonstrated that these tools were used to process cereal grains and other edible plant foods (Liu et al., 2010a, 2010b, 2018; Piperno et al., 2004; Yang et al., 2009, 2012a). From the Neolithic Age onward, as staple crops were gradually domesticated and agricultural communities came to be established during the period of 6,000–5,000 cal BP (Zhao, 2019), the number of stone slabs and rollers decreased notably in China, and then almost disappeared by the stage of the Longshan culture around 4,000 years ago (Ding, 2007; Song, 1997; Zeng and Zhu, 2012).

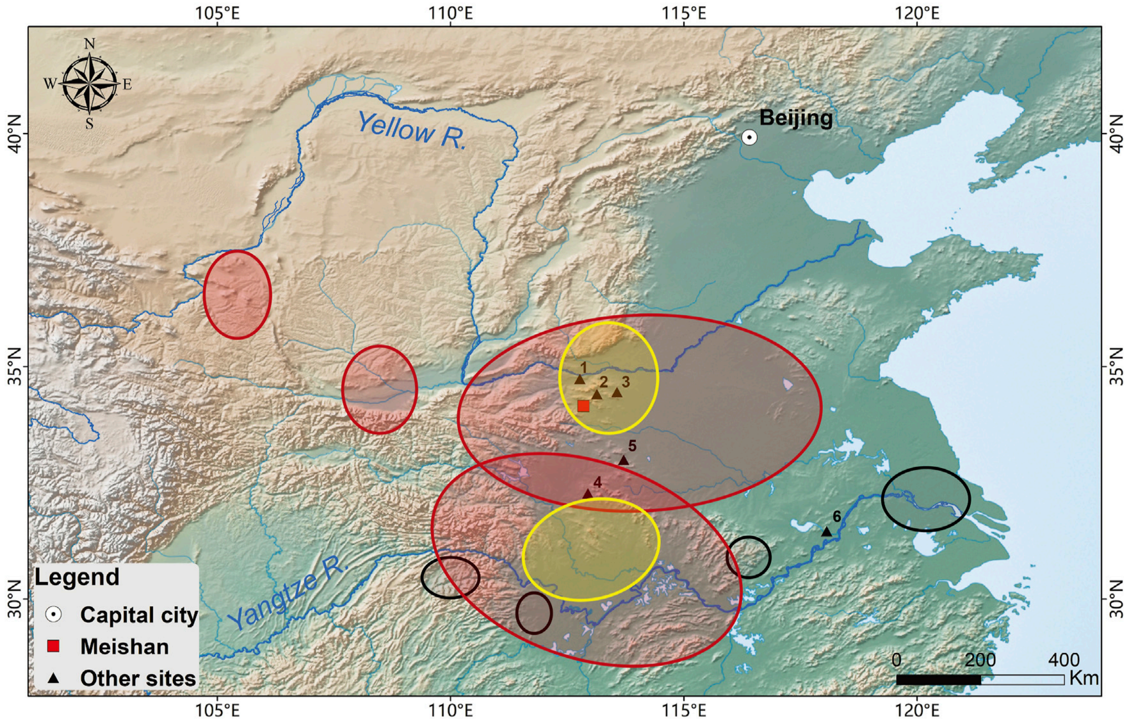

FIGURE 1 | Spatiotemporal distribution of grooved pottery vessels and location of the Meishan site and other archaeological sites mentioned in the text. Shadows with black circles indicate the initial area where the grooved pottery vessels appeared during the early phase of the Songze culture, shadows with yellow circles indicate the scenario during the mid-Songze culture, and shadows with red circles indicate the scenario at around 4,000 years ago. 1, Yanshi Shangcheng; 2, Wangchenggang; 3, Xinzhai; 4, Diaolongbei; 5, Yangzhuang; 6, Lingjiatan. The base map was obtained from the Natural Earth public domain map dataset (https://www.naturalearthdata.com/downloads/10m-raster-data/).

Since 6,000 cal BP, a type of pottery began to appear in the lower reaches of the Yangtze River, then immediately occurred in the middle Yangtze River regions and the middle reaches of the Yellow River, and was eventually popularized during the Erlitou culture (3,800–3,500 cal BP) (**Figure 1**) (Wang, 2019). After that, it showed signs of abating, although it still existed (An, 1986; Ding, 2007). This particular pottery is generally named *kecaopen* (刻槽盆in Chinese character), meaning grooved vessels. The features of this kind of ceramic are mostly like the pot from the same period but decorated with crisscross or radial grooves on the inner wall of the pots (**Figure 2**). Since the grooved vessels happened to occur as agricultural societies were established and as the number of the stone slabs and rollers decreased (Jin, 2013; Zhao, 2019), the hypothesis is that the function of grooved vessels was to replace stone slabs and rollers to grind or filter plant foods, in terms of ethnographic observation (An, 1986; Chen, 2005; Ding, 2007; Liu, 1991; Ye, 1989; Ye and Li, 1996; Zhang, 2017). Therefore, direct evidence is needed to verify this hypothesis.

As the studies on the function of stone slabs and rollers which were once suggested to be processing acorns, mineral pigments, animal skins, and so on (Xie et al., 1989; Zhao, 2005; Liu et al., 2010a), directly evidenced by the means of starch grain analysis to process cereal grains and other edible plants at least (Liu et al., 2010a, 2010b, 2018; Piperno et al., 2004; Yang et al., 2009, 2012a), starch recovered from the residues on the interior face of the grooved vessels can be tested for the aforementioned hypothesis. Previous studies on grooved vessel sherds from the Diaolongbei site and the Lingjiatan site in the middle and lower Yangtze River regions, respectively, were reported (Tao et al., 2009; Sun et al., 2019). Some unidentifiable starch grains were recovered from the residues on three 5000-year-old potsherds from the Diaolongbei site, and incorporating the evidence of the bright red color of stained starches with Congo red, it is deduced that grooved vessels at the Diaolongbei site were used for steaming or boiling some starchy foods, but the specific sources of the starches are not clear (Tao et al., 2009). Starches from the Lingjiatan site further revealed that wild resources, such as acorns and geophytes, were processed by using grooved vessels at 5,000 cal BP or so (Sun et al., 2019). A recent publication reported a similar study on 17 potsherds of grooved vessels from an early Bronze Age site, Yanshi Shangcheng, in central China, and raised a hypothesis that grooved vessels were perhaps used to dehusk seeds and make flour from dried roots and acorns, based on the morphological features of ancient starch grains from the residues on these potsherds (Reinhart, 2020).

Starches are usually contained in caryopses, geophytes, and palm piths, while phytoliths are usually produced in leaves and glumes. In fact, all human behaviors of dehusking, grinding, or cooking could cause starch residues to stick to the surface of the ware. Although the survival chances of phytoliths are much less

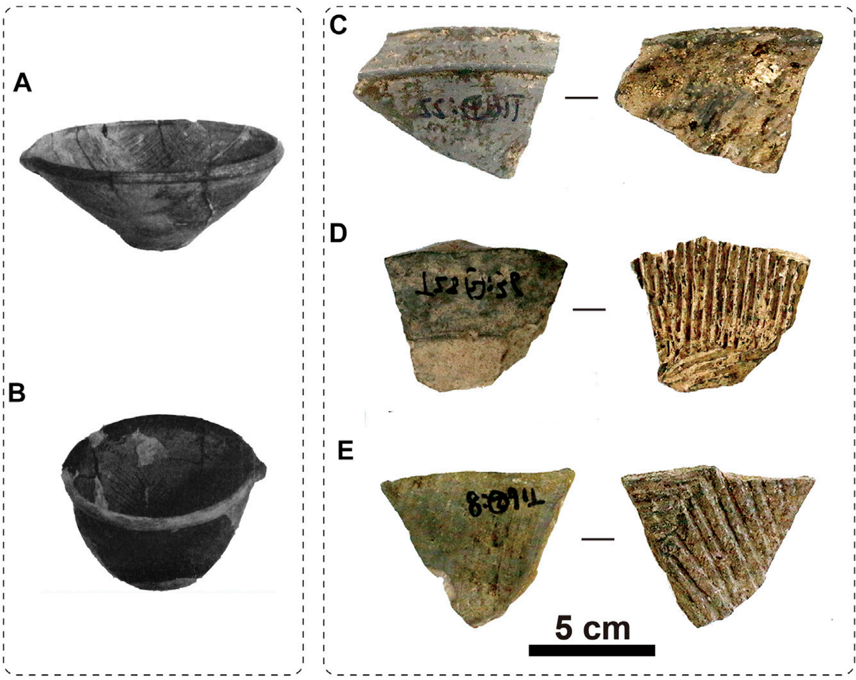

FIGURE 2 | Typical grooved pottery vessels and potsherds from the Meishan site. **(A,B)** Two types of the grooved vessels at the Meishan site, taken in the 1970s, without a scale bar (Fang, 1975). **(C,D)** Images for exterior (left) and interior (right) faces of three sampled potsherds in this study. **(C)** Potsherd with very shallow grooves. **(D)** Potsherd with deep grooves, ~0.2 cm in depth. **(E)** Potsherd bearing cross grooves, ~0.1 cm in depth.

than those of starches on the surface of the wares because of their non-sticky property, phytoliths from the glumes fortunately recovered from the residues would be a definite indicator for dehusking seeds.

Here we report our study on the residues adhering to the interior faces of grooved pottery vessels excavated from the Neolithic-Bronze Meishan site in central China using the analyses of both starch and phytoliths, to provide direct evidence for the function of this type of vessels.

MATERIALS AND METHODS

Meishan

The Meishan site (112.85°E, 34.15°N) is located in Ruzhou City of Henan Province in central China (**Figure 1**). It was found in 1958 and excavated during several seasons in 1970, 1975, 1987–1988, 1995, and 1997. Dwells, buries, pottery kilns, wells, pits, and other ruins were revealed, and stone tools of an axe, sickle, chisel, and knife, bone tools of arrowhead, awl, and hairpin, and pottery vessels of pots, steamer, plate, spinning wheels etc., were discovered, recording the evolution track of societies from the Longshan culture to the Erlitou culture in central China (Henan No. 2 team, Institute of Archaeology, Academy of Social Sciences, 1982; Yuan, 1991). Four AMS ^{14}C dating data obtained from bones put the occupation during the period of 4,000–3,500 cal BP (You et al., 2017).

Pottery Sherds of Grooved Vessels for Study

There are two types of grooved vessels excavated from the Meishan site. One is gray sand-tempered pottery with an inverted rim, whose upper part is trumpet-shaped and the lower part is cylindrical-shaped, with radiating grooves on the interior face (**Figure 2A**). The other type is gray clay pottery in the shape of a basin with an inverted rim and arc wall, of which the interior face is incised with oblique grooves and the exterior face is decorated with chopped basket patterns (**Figure 2B**). The sherds from the grooved vessels take up 3.3% of excavated potsherd assemblage at the Meishan site.

A total of 20 sherds of grooved potteries from the Meishan site are selected for this study (**Figures 2C–E**), that is, 14 sherds from the late Longshan period (~4,000 cal BP) and six from the early Erlitou period (~3,800 cal BP), which were excavated during the excavation seasons of 1995 and 1997 conducted by the Henan Provincial Institute of Archaeology. Half of the sherds are from gray sandy pottery, and the other half are from gray clay pottery. Of them, only one sherd, No. T19②b: 17, of the early Erlitou period, can be discerned from a vessel with a spout. Ancient starches from residues on both interior and exterior faces of the sherds were recovered, and then ancient phytoliths were recovered after starch extraction from six residue samples of the late Longshan and two residue samples from the early Erlitou (**Supplementary Tables S1, S2**).

Analyses of Starch and Phytolith

When we discuss the functions of a specific type of artifact, sources of residues on the surfaces are crucial. Usually, phytoliths and starches recovered from the residues on artifacts will be compared with those from sediments to confirm whether the artifacts were the primary source of these micro plant remains or not (Hart, 2011). However, the potsherds in our study were excavated more than 20 years ago, and contemporaneous sediments which are often used as contamination control samples are not available. Therefore, residues from the interior face can be compared with those from the exterior face of each potsherd based on an assumption that in the same taphonomic conditions, the contamination chances of both faces of the sherds are equal (Barton et al., 1998). If the quantity of micro plant remains on the exterior face of a sherd is more than or equal to that on the interior face, we think the recovered plant remains are sourced from the secondary deposition from the site's sediments after the tools were discarded, although we cannot rule out the possibility that plant residues on the exterior face are derived from the same plant-processing activity as the manipulator's hands touched both the foods and vessels at the same time, and the vessels must have been surrounded by the foods as they were processed. As a matter of fact, previous studies have demonstrated that few starches could be retrieved from sediment and soil (Li et al., 2010; Yang et al., 2012a; Ma et al., 2017).

To dislodge adhering residues, the interior face of the potsherds was submerged in ultrapure water and shaken in an ultrasonic water bath for 5–10 min, then the starches were isolated with heavy liquid flotation using a solution of CsCl at a density of 1.8 g/cm^3. The recovered residues were mounted in 10% glycerine and 90% water on a slide and examined with both white and cross-polarized light at a magnification of × 400. Starch grains were counted, analyzed for morphological features, and then recorded and compared with those from the modern reference collection. For a detailed protocol, refer to previous publications (Yang et al., 2009; 2012a, 2015). To identify the recovered starches, we compiled a modern reference collection of more than 260 species from 20 families of plants that are common in China, including crops and their wild related species, roots and tubers, tree seeds, and peas and beans (**Supplementary Figure S1**) (Wang et al., 2013; Wang et al., 2018; Yang and Perry, 2013; Yang et al., 2012b, 2013). Other published collections are consulted as well (Reichert, 1913; Piperno et al., 2000, 2004; Liu et al., 2010a, 2013, 2014).

Recovery of residues for the phytolith analysis followed that of sampling for starches. A heavy liquid of zinc bromide ($ZnBr_2$) at a density of 2.35 g/cm^3 was used to isolate phytoliths which were then mounted on a slide with Canada Balsam. Phytolith identification and counting were performed at × 400 magnification. Descriptions and nomenclature of the phytoliths followed the International Code for Phytolith Nomenclature (ICPN) 2.0 (Neumann et al., 2019). To identify phytoliths in the archaeological samples, published modern reference collections were consulted (Rosen, 1992; Wang and Lu, 1993; Piperno, 2006; Lu et al., 2009; Zhang et al., 2011; Gu et al., 2013; Ball et al., 2016; Ge et al., 2018).

RESULTS

Ancient Starches Recovered From the Residues

In total, 380 starch grains were recovered from all samples that can be classified into four groups (**Figures 3**, **4** and **Supplementary Table S1**).

Group A includes 238 starch grains in total, dominating the recovered starch assemblage. The grains are characterized by a polyhedral shape with central and open hila, and by Y-shaped or stellated fissures on occasion (**Figures 3A,B**). Sometimes, short lines radiating from the center to the edges on the surfaces of grains can be observed. The size of starch grains ranges from 7.2– 26.0 μm. Both the sizes and the morphological features of this group of starches are consistent with those of foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum miliaceum*) (**Supplementary Figures 1A,B**) (Ge et al., 2010; Yang et al., 2012b). Some granules larger than those from modern reference collections might be the result of grinding (Ma et al., 2019).

Group B includes 24 starch grains that are characterized by distinctive lenticular shapes and larger sizes between the range of 15.67~33.3 μm (**Figure 3C**). Pressure craters on the surface are apparent. They are identical to starch grains from the modern tribe Triticeae in which wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) are included (**Supplementary Figures 1C,D**) (Yang and Perry, 2013).

Group C includes 26 starch grains that have the shape of a partial sphere, hemisphere, or sphere, with facets (**Figures 3D,E**). The hilum is eccentric. Starch grains in this group are similar to geophytes (**Supplementary Figures 1E,F**), in which Chinese yams (*Dioscorea* spp.) could be identified at least.

Group D comprises unidentifiable starch grains because of their lack of typical morphological features or severe damage.

Ancient Phytoliths Recovered From the Residues

More than 7,000 phytoliths were recovered from the potsherds after the recovery of starches (**Supplementary Table S2**). A total of 265 typical bulliform phytoliths from rice leaves (**Figure 3F**), 253 double-peaked phytoliths from the glume of rice grains (**Figure 3G**), 89 Ω-type and 136 η-type phytoliths from glumes of foxtail and broomcorn millets (**Figures 3H,I**), and 34 phytoliths with wave patterns in conjunctions from glumes of wheat or barley are observed (**Figure 3J**), in addition to bilobate-scooped phytoliths usually extracted from the leaves of Paniceae and Oryzoideae (**Figures 3K,L**) (Piperno, 2006; Lu et al., 2009, 2017; Ball et al, 2016). The percentage of each type of phytolith is shown in **Figure 5**. It should be noted that the phytoliths from leaves, such as bulliform, bilobate, saddle, blocky, and rondel phytoliths, can reach up to half of the phytolith assemblage.

DISCUSSION

Plant Subsistence at the Meishan Site

From other contemporaneous sites of the Longshan culture in central China, such as the sites of Yangzhuang, Wangchenggang,

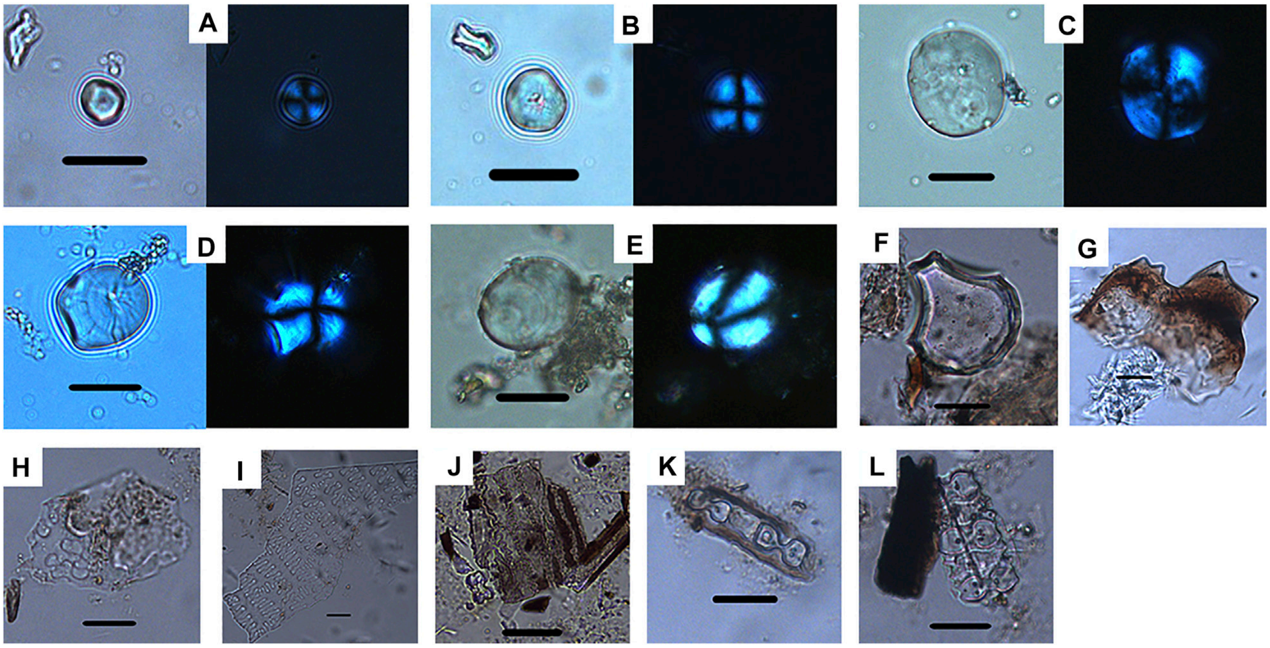

FIGURE 3 | Starch grains **(A–E)** and phytoliths **(F–L)** recovered from the residues on the grooved vessels from the Meishan Site. Each starch grain is shown in brightfield and polarized views. **(A,B)** Foxtail millet or broomcorn millets; **(C)** Triticeae; **(D)** geophytes; **(E)** possibly Chinese yam; **(F)** rice bulliform phytoliths from rice leaves with increased scale numbers; **(G)** double-peaked phytolith from rice husk; **(H)** Ω-type phytolith from foxtail millet husk; **(I)** η-type phytolith from broomcorn millet husk; **(J)** dendritic epidermal phytolith possibly from wheat or barley glumes; **(K)** bilobate-scooped phytolith from Oryzoideae; and **(L)** bilobate-scooped phytolith from Paniceae. Scale bar, 20 μm.

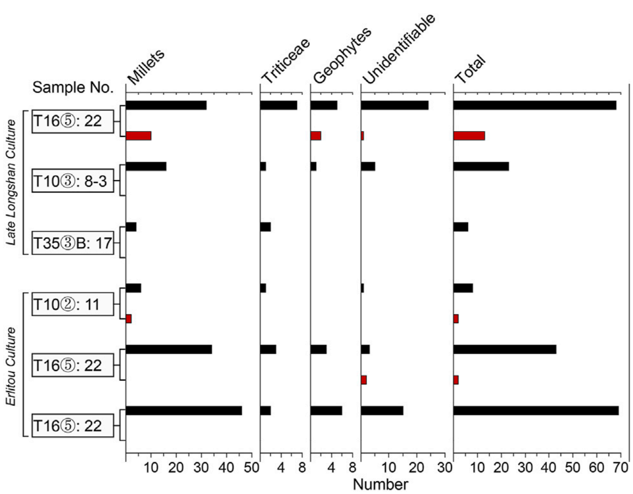

FIGURE 4 | Number of starch grains recovered from the residues on the grooved pottery vessels at the Meishan site. Black and red bars represent the samples from the interior face and exterior face, respectively. The results from possible contaminated potsherds have been excluded.

Xinzhai, etc., charred seeds of five grains which were staple crops in historical North China (Zhao, 2011), foxtail and broomcorn millets, rice, wheat, and soybean (*Glycine max*), were recovered by floatation (Jiang et al., 1998; Zhao and Fang, 2007; Zhong et al., 2016). Since barley was introduced in central China by the Shang and Zhou Dynasties (Guo and Jin, 2019), and the Meishan site should have the same plant subsistence as these contemporaneous sites, the phytoliths with wave patterns in the conjunctions and the starches identified to the tribe Triticeae recovered from the Meishan site should be from wheat. Therefore, multiple lines of evidence of both phytoliths and starches from the residues on the grooved vessels from the Meishan site comprise four grains except soybean which does not produce both phytoliths and characteristic starch grains. However, starches of geophytes from the Meishan site supplement archaeobotanical data from other contemporaneous sites of the late Longshan culture in central China.

Function of the Grooved Pottery Vessels

Phytoliths recovered from eight sherds show that the number of phytoliths on the interior face of each sherd is much higher than that on the exterior face, indicating phytoliths sourced from the use of vessels. But starch data are not ideal. The number of recovered starches is so small that there are 380 from 20 sherds in total for statistical analysis. Excluding the sherds of which both faces yield equal or approximately equal numbers of starches or those that their exterior faces yield more starches than the interior faces, in other words, excluding those sherds that might have a secondary deposition from contemporaneous sediments, only three sherds, T16⑤: 22, T10③: 8-3, and T35③B: 17 of the Longshan culture, and three sherds, T10②: 11, T16②: 8, and T10③: 8-1 of the Erlitou culture

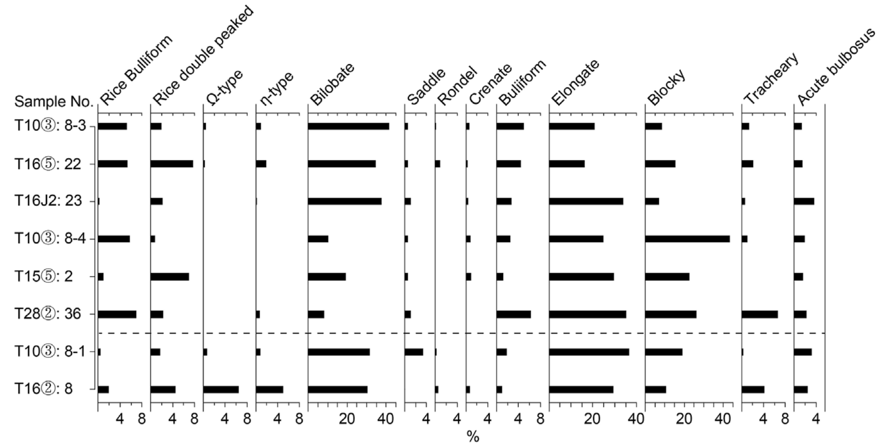

FIGURE 5 | Percentage of each type of phytolith recovered from the residues on the grooved pottery vessels at the Meishan site. The samples above the dashed line are from the late Longshan period, while the samples below the dashed line are from the Erlitou period.

remain useful for the discussion about the function of grooved vessels (**Figure 4**).

Both the phytoliths and starches from the residues confirm that grooved vessels were indeed used to process plants, as shown by previous studies at the sites of Lingjiatan and Yanshi Shangcheng (Sun et al., 2019; Reinhart, 2020). Wang (2019) summarized the studies on the grooved vessels in China and tends to agree that the principal function of the vessels was grinding geophytes. In our study, starch grains from Chinese yams and other geophytes were observed from residues on the interior faces of four sherds, providing evidence for the function of grinding geophytes again.

We recovered 7,595 phytoliths and 380 starch grains from the residues on 20 potsherds (**Supplementary Tables S1, S2**). The pattern of abundant phytoliths with a few starches from glumes and/or caryopsis of rice, foxtail millet, broomcorn millet, and wheat in this study seem to suggest that the grooved vessels were once used to dehusk seeds. But, two factors should be taken into consideration. One is the large number of phytoliths from leaves, taking up 54% of the phytolith population (**Figure 5**); the other is the small number of sherds from grooved vessels, taking up 3.3% of the pottery ware at the Meishan site. If the grooved vessels were used as principal tools to dehusk seeds, it could be difficult to cope with the work in an agricultural community using 3.3% pottery tools. The phytoliths from leaves may have been occasionally brought in when harvesting ears, so we cannot exclude the function of dehusking seeds. However, we prefer to hypothesize that they are the result of intentionally pounding leaves and husks for some reason. A few starch grains recovered from the potsherds might source the husks on which some starches had been left as dehusking. Rice leaves were once proved to be tempered in the ceramic mold for casting bronze ware during history (Lu et al., 1996). The reason for processing leaves and husks by the grooved vessels at the Meishan site needs further study.

We also noted that the function of grooved vessels is not related to the materials used for pottery manufacturing, because either sand-tempered sherds or clay sherds have the same starch and phytolith patterns in their residues. Since only one sherd is apparently from the vessel with a spout, we cannot distinguish the difference of functions between vessels with or without a spout, though we think the spout is a crucial factor in understanding the function.

Pottery Function and Agricultural Development

The function of early potteries from China, Japan, and Russia in East Asia has been proved to be used for cooking food by the residue analysis (Craig et al., 2013; Shoda et al., 2020; Yang et al., 2012a, 2014, 2015). Furthermore, millet starches recovered from charred residues on early potteries excavated from the sites of Nanzhuangtou, Donghulin, and Zhuannian indicated that the function of cooking plant food and that the invention of pottery manufacturing in the North China Plain may have been related to early farming activities around 10,000 years ago (Yang et al., 2012a, 2014, 2015). By the mid-Neolithic Age, the functions of pottery began to diversify with the establishment of the agricultural societies, and a variety of cooking utensils and containers were often discovered (Wang, 2005).

It is accepted that the middle and lower reaches of the Yangtze River are two centers of rice domestication. Rice was fully domesticated by around 8,000 cal BP in the middle regions after more than 2000 years of cultivation (Deng et al., 2015); however, in the lower regions, the process of rice domestication lasted for even 4,000 years and ended by around 6,000–5,500 cal BP (Fuller et al., 2009; Fuller, 2011; Gao, 2012; Ma et al., 2016, 2018). During the Yangshao Period, rice agriculture spread to central China, joining the millet agriculture (Qin, 2012; Zhang et al., 2012). It seems that the grooved vessel is highly relevant to rice agriculture. The earliest grooved pottery vessels were invented at the archaeological sites of the early stage of the Songze culture (6,000–5,300 cal BP) in the lower reaches of the Yangtze River (Wang, 2019), which happened to be consistent with the end of the process of rice domestication (Ma et al., 2018), and the technics first spread among the rice agricultural societies in the Middle Yangtze River regions during the mid-Songze culture, then was gradually introduced in central China by around 5,300 years ago where rice had been introduced, and local crops, two millets and wheat, were added to be processed as well.

Incorporating the rice phytoliths in a large number on the interior face of grooved vessels at the Meishan site (**Figure 5** and **Supplementary Table S2**), we hypothesize that the grooved pottery vessel might be invented to process by-products of rice for some reason, and to dehusk seeds and grind geophytes, probably. The hypothesis needs systematic residue analysis on the grooved pottery vessels from the lower Yangtze River regions to central China, from the earliest ones to the latest ones, in future studies.

CONCLUSION

Different from the previous studies based on the ethnographic investigation or single ancient starch evidence, we combine phytolith evidence in the study methods to conclude the function of the grooved pottery vessels, taking an example of the study on sherds excavated from the Meishan site, in central China. We studied the residues on the potsherds of grooved vessels at the Meishan site occupied during the period of 4,000–3,500 cal BP. In terms of starches from geophytes including Chinese yam and starches/phytoliths from glumes and leaves of rice, foxtail and broomcorn millets, and wheat, we demonstrate that the grooved pottery vessels were once principally used to pound leaves and husks for some reason, and to grind geophytes and dehusk crop seeds, possibly. In terms of the timing of rice domestication, we raised a hypothesis that the invention of grooved pottery vessels may have been related to the end of rice domestication in the lower reaches of the Yangtze River. This hypothesis needs further study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XY and JY designed the study. YZ, ZM, and XY performed microfossil analysis. TW, JY, YZ, XY, and GY analyzed the data. TW, JY, and XY wrote the manuscript with contributions from all authors.

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SUPPLEMENTARY MATERIAL

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REFERENCES

An, J. (1986). The Discussion of the Grinding Bowl (In Chinese). *Archaeology* 4, 344–347.

Ball, T., Chandler-Ezell, K., Dickau, R., Duncan, N., Hart, T. C., Iriarte, J., et al. (2016). Phytoliths as a Tool for Investigations of Agricultural Origins and Dispersals Around the World. *J. Archaeol. Sci.* 68, 32–45. doi:10.1016/j.jas.2015.08.010

Barton, H., Torrence, R., and Fullagar, R. (1998). Clues to Stone Tool Function Re-examined: Comparing Starch Grain Frequencies on Used and Unused Obsidian Artefacts. *J. Archaeol. Sci.* 25, 1231–1238. doi:10.1006/jasc.1998.0300

Chen, Z. (2005). Talk about the Origin and Development of Kejia Leicha and Leicha Porridge (In Chinese). Guangdong Tea 6, 19–21.

Craig, O. E., Saul, H., Lucquin, A., Nishida, Y., Taché, K., Clarke, L., et al. (2013). Earliest Evidence for the Use of Pottery. *Nature* 496, 351–354. doi:10.1038/nature12109

Deng, Z., Qin, L., Gao, Y., Weisskopf, A. R., Zhang, C., and Fuller, D. Q. (2015). From Early Domesticated Rice of the Middle Yangtze Basin to Millet, Rice and Wheat Agriculture: Archaeobotanical Macro-Remains from Baligang, Nanyang Basin, Central China (6700-500 BC). *PLoS One* 10, e0139885. doi:10.1371/journal.pone.0139885

Ding, L. (2007). The Investigation of the Function of the Grooved Pottery in Prehistory (In Chinese). *Relics Museology* 6, 45–51.

Fang, X. (1975). Investigation and Trial Excavation of Meishan Site in Linru City Henan Province (In Chinese). *Archaeology* 5, 285–294.

Fuller, D. Q. (2011). Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice* 4, 78–92. doi:10.1007/s12284-011-9078-7

Fuller, D. Q., Qin, L., Zheng, Y., Zhao, Z., Chen, X., Hosoya, L. A., et al. (2009). The Domestication Process and Domestication Rate in Rice: Spikelet Bases from the Lower Yangtze. *Science* 323, 1607–1610. doi:10.1126/science.1166605

Gao, Y. (2012). "A Study of Plant Remains and Neolithic Subsistence in Tai Lake Region," in *Chinese (Dissertation)* (Beijing, China: Peking University).

Ge, W., Liu, L., and Jin, Z. (2010). Morphological Analyses on Starch Grains of Five Grass Species and Their Significance for Archaeology (In Chinese). *Quat. Sci.* 32, 269–281.

Ge, Y., Lu, H., Zhang, J., Wang, C., He, K., and Huan, X. (2018). Phytolith Analysis for the Identification of Barnyard Millet (*Echinochloa* sp.) and its Implications. *Archaeol. Anthropol. Sci.* 10, 61–73. doi:10.1007/s12520-016-0341-0

Gu, Y., Zhao, Z., and Pearsall, D. M. (2013). Phytolith Morphology Research on Wild and Domesticated Rice Species in East Asia. *Quat. Int.* 287, 141–148. doi:10.1016/j.quaint.2012.02.013

Guo, R., and Jin, G. (2019). Study on the Wheat Agriculture in the Pre-qin Period of Haidai Region (In Chinese). *Quat. Sci.* 39, 144–160. doi:10.11928/j.issn.1001-7410.2019.01.14

Hart, T. C. (2011). Evaluating the Usefulness of Phytoliths and Starch Grains Found on Survey Artifacts. *J. Archaeol. Sci.* 38, 3244–3253. doi:10.1016/j.jas.2011.06.034

Henan, H. (1982). Excavation of the Meishan Site in Linru, Henan Province (In Chinese). *Acta Archaeol. Sin.* 4, 427–476.

Jiang, Q., Song, Y., Li, Y., and Han, J. (1998). Environmental Archaeology of Longshan Period of the Yangzhuang Site in Zhumadian, Henan Province (In Chinese). *Archaeol. Cult. Relics* 2, 34–40.

Jin, G. (2013). Study on Food Structure of Residents in Longshan Culture (In Chinese). *J. Chin. Humanit.* 2, 99–111.

Li, M., Ge, Q., Wang, Q., Cai, L., and Ren, X. (2010). Ancient Starch Grains from Ash Pits of Fengtai Site, Qinghai Province and Their Application in Archaeology (In Chinese). *Quat. Sci.* 30, 372–376. doi:10.3969/j.issn.1001-7410.2010.02.14

Liu, L., Bestel, S., Shi, J., Song, Y., and Chen, X. (2013). Paleolithic Human Exploitation of Plant Foods during the Last Glacial Maximum in North China. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5380–5385. doi:10.1073/pnas.1217864110

Liu, L., Field, J., Fullagar, R., Bestel, S., Chen, X., and Ma, X. (2010a). What Did Grinding Stones Grind? New Light on Early Neolithic Subsistence Economy in the Middle Yellow River Valley, China. *Antiquity* 84, 816–833. doi:10.1017/s0003598x00100249

Liu, L., Field, J., Fullagar, R., Zhao, C., Chen, X., and Yu, J. (2010b). A Functional Analysis of Grinding Stones from an Early Holocene Site at Donghulin, North China. *J. Archaeol. Sci.* 37, 2630–2639. doi:10.1016/j.jas.2010.05.023

Liu, L., Levin, M. J., Bonomo, M. F., Wang, J., Shi, J., Chen, X., et al. (2018). Harvesting and Processing Wild Cereals in the Upper Palaeolithic Yellow River Valley, China. *Antiquity* 92, 603–619. doi:10.15184/aqy.2018.36

Liu, L., Ma, S., and Cui, J. (2014). Identification of Starch Granules Using a Two-step Identification Method. *J. Archaeol. Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

Liu, Z. (1991). The Uses and the Proper Name of the Burnisher (In Chinese). *Agric. Archaeol.* 1, 283–294.

Lu, H. (2017). New Methods and Progress in Research on the Origins and Evolution of Prehistoric Agriculture in China. *Sci. China Earth Sci.* 60, 2141–2159. doi:10.1007/s11430-017-9145-2

Lu, H., Wu, N., and Wang, Y. (1996). The Identification of Fan-Shaped Phytolith of Rice and its Application in Archaeology (In Chinese). *Archaeology* 4, 84–88.

Lu, H., Zhang, J., Wu, N., Liu, K.-b., Xu, D., and Li, Q. (2009). Phytoliths Analysis for the Discrimination of Foxtail Millet (*Setaria Italica*) and Common Millet (*Panicum Miliaceum*). *PLoS One* 4, e4448. doi:10.1371/journal.pone.0004448

Ma, Y., Yang, X., Huan, X., Gao, Y., Wang, W., Li, Z., et al. (2018). Multiple Indicators of Rice Remains and the Process of Rice Domestication: A Case Study in the Lower Yangtze River Region, China. *PLoS One* 13, e0208104. doi:10.1371/journal.pone.0208104

Ma, Y., Yang, X., Huan, X., Wang, W., Ma, Z., Li, Z., et al. (2016). Rice Bulliform Phytoliths Reveal the Process of Rice Domestication in the Neolithic Lower Yangtze River Region. *Quat. Int.* 426, 126–132. doi:10.1016/j.quaint.2016.02.030

Ma, Z., Perry, L., Li, Q., and Yang, X. (2019). Morphological Changes in Starch Grains after Dehusking and Grinding with Stone Tools. *Sci. Rep.* 9, 2355–2413. doi:10.1038/s41598-019-38758-6

Ma, Z., Zhang, C., Li, Q., Perry, L., and Yang, X. (2017). Understanding the Possible Contamination of Ancient Starch Residues by Adjacent Sediments and Modern Plants in Northern China. *Sustainability* 9, 752. doi:10.3390/su9050752

Madella, M., Alexandre, A., and Ball, T. (2005). International Code for Phytolith Nomenclature 1.0. *Ann. Bot.* 96, 253–260. doi:10.1093/aob/mci172

Neumann, K, Strömberg, C. A. E., Ball, T, Albert, R. M, Vrydaghs, L, and Cummings, L. S. (2019). International Code for Phytolith Nomenclature (ICPN) 2.0. *Ann. Bot.* 124, 189–199.doi:10.1093/aob/mcz064

Piperno, D. (2006). *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists*. New York: AltaMira Press.

Piperno, D. R., Ranere, A. J., Holst, I., and Hansell, P. (2000). Starch Grains Reveal Early Root Crop Horticulture in the Panamanian Tropical Forest. *Nature* 407, 894–897. doi:10.1038/35038055

Piperno, D. R., Weiss, E., Holst, I., and Nadel, D. (2004). Processing of Wild Cereal Grains in the Upper Palaeolithic Revealed by Starch Grain Analysis. *Nature* 430, 670–673. doi:10.1038/nature02734

Qin, L. (2012). The Research and Prospect of the Agricultural Origin of Chinese Agriculture (In Chinese). *Archaeol. Res.* 9, 260–315.

Reichert, E. (1913). *The Differentiation and Specificity of Starches in Relation to Genera, Species*. etc. Washington, DC: Carnegie institution of Washington, 1–97.

Reinhart, K. (2020). Analysis of Grooved Ceramic Vessels from the Early Chinese Bronze Age Site of Yanshi Shangcheng: Preliminary Evidence of Food Preparation Methods through Starch Analysis. *J. Archaeol. Sci. Rep.* 29, 101852. doi:10.1016/j.jasrep.2019.05.017

Rosen, A. M. (1992). Preliminary Identification of Silica Skeletons from Near Eastern Archaeological Sites: an Anatomical Approach. In: G. R. Rapp Jr, and S. C Mulholland (ed) *Phytolith Systematics: Emerging Issues*. Plenum, New York, pp. 129–147.doi:10.1007/978-1-4899-1155-1_7

Shoda, S., Lucquin, A., Yanshina, O., Kuzmin, Y., Shevkomud, I., Medvedev, V., et al. (2020). Late Glacial Hunter-Gatherer Pottery in the Russian Far East: Indications of Diversity in Origins and Use. *Quat. Sci. Rev.* 229, 106124. doi:10.1016/j.quascirev.2019.106124

Song, Z. (1997). Prehistoric Food Processing Technology: On the Origin of Grinding Tools and Pestle (In Chinese). *Agric. Archaeol.* 3, 187–195. doi:10.32028/9781789697926-4

Sun, Q., Shuo, Z., Wu, Y., and Yang, Y. (2019). Starch Grain Analysis of the Grooved Basin from the Lingjiatan Site, Hanshan County, Anhui Province (In Chinese). *Acta Anthropol. Sin.* 38, 132–147. doi:10.1007/s13131-019-1460-6

Tao, D., Yang, Y., Huang, W., Wu, Y., and Wu, Y. (2009). Residue Analysis for the Artifacts from Diaolongbei Site (In Chinese). *Archaeology* 9, 94–98. doi:10.1007/s11434-009-0545-0

Wang, C., and Lu, H. (2012). Research Progress of Fan-Shaped Phytolith of Rice and Relative Issues (In Chinese). *Quat. Sci.* 32, 269–281.

Wang, N. (2019). "The Study of Grooved Basin in Prehistoric," in *Chinese) (Dissertation)* (Xi'an, China: Northwest University).

Wang, Q., Jia, X., Li, M., and Yang, X. (2013). Morphological Analysis of Starch Grains from Common Food Legumes in China and its Application in Agricultural Archaeology (In Chinese). *Wenwuchunqiu* 3, 8–14.

Wang, T. (2005). *A Study on Early Pottery (In Chinese) (Dissertation)*. Beijing, China: Peking University.

Wang, W., Ma, Y., Li, Z., Ma, Z., and Yang, X. (2018). Morphological Analysis of Modern Starch Grains of Underground Storage Organs in China (In Chinese). *Quat. Sci.* 38, 1409–1423.

Wang, Y., and Lu, H. (1993). *The Study of Phytolith and its Application (In Chinese)*. Beijing: China Ocean Press.

Xie, X., Yan, J., and Tao, F. (1989). The Shizitan Site of Epipaleolithic Age in Shanxi, China. *Archaeol. J.* 3, 305–323.

Yang, X., Ma, Z., Li, J., Yu, J., Stevens, C., and Zhuang, Y. (2015). Comparing Subsistence Strategies in Different Landscapes of North China 10,000 Years Ago. *Holocene* 25, 1957–1964. doi:10.1177/0959683615596833

Yang, X., Ma, Z., Wang, T., Perry, L., Li, Q., Huan, X., et al. (2014). Starch Grain Evidence Reveals Early Pottery Function Cooking Plant Foods in North China. *Chin. Sci. Bull.* 59, 4352–4358. doi:10.1007/s11434-014-0500-6

Yang, X., and Perry, L. (2013). Identification of Ancient Starch Grains from the Tribe Triticeae in the North China Plain. *J. Archaeol. Sci.* 40, 3170–3177. doi:10.1016/j.jas.2013.04.004

Yang, X., Wan, Z., Perry, L., Lu, H., Wang, Q., Zhao, C., et al. (2012a). Early Millet Use in Northern China. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3726–3730. doi:10.1073/pnas.1115430109

Yang, X., Yu, J., Lü, H., Cui, T., Guo, J., and Ge, Q. (2009). Starch Grain Analysis Reveals Function of Grinding Stone Tools at Shangzhai Site, Beijing, *Sci. China Ser. D-Earth Sci.*, 52. Beijing, 1164–1171. doi:10.1007/s11430-009-0089-9

Yang, X., Zhang, J., Perry, L., Ma, Z., Wan, Z., Li, M., et al. (2012b). From the Modern to the Archaeological: Starch Grains from Millets and Their Wild Relatives in China. *J. Archaeol. Sci.* 39, 247–254. doi:10.1016/j.jas.2011.09.001

Ye, W. (1989). An Examination of the Origin and Development of the Grinding Bowl (In Chinese). *Archaeology* 5, 456–462.

Ye, W., and Li, D. (1996). "The Archeological Research of the Ancient Societies in Yiluo Region in the Past 40 Years," in Forty Years of Archaeology Study in Luoyang: Proceedings of the Seminar on Archaeological Research in Luoyang in 1992 (In Chinese) (Beijing: China Science Press), 11.

You, Y., Yuan, G., Zhao, Y., and Jin, S. (2017). Zooarchaeological Research on the Meishan Site in Linru, Henan Province. *South. Relics* 3, 165–176.

Yuan, G. (1991). Excavation Report of the Meishan Site in Linru in 1987-1988 (In Chinese). *Huaxia Archaeol.* 3, 5–23.

Zeng, H., and Zhu, H. (2012). Some Thoughts on the Use of Stone Millstone in Neolithic Period in China (In Chinese). *Agric. Archaeol.* 3, 137–140.

Zhang, J., Lu, H., Gu, W., Wu, N., Zhou, K., Hu, Y., et al. (2012). Early Mixed Farming of Millet and Rice 7800 Years Ago in the Middle Yellow

River Region, China. *PLoS One* 7, e52146. doi:10.1371/journal.pone.0052146

Zhang, J., Lu, H., Wu, N., Yang, X., and Diao, X. (2011). Phytolith Analysis for Differentiating between Foxtail Millet (*Setaria Italica*) and Green Foxtail (*Setaria Viridis*). *PLoS One* 6, e19726. doi:10.1371/journal.pone.0019726

Zhang, X. (2017). A Preliminary Research on the Pottery Wine Vessels of the Songze Culture (In Chinese). *Archaeology* 12, 69–81.

Zhao, S. (2005). Are Stone Slabs and Rollers for Grain Processing? *Agric. Archaeol.* 3, 134–147.

Zhao, Z. (2011). Characteristics of Agricultural Economic Development during the Formation of Chinese Civilization (In Chinese). *J. Natl. Mus. China* 1, 27–39.

Zhao, Z., and Fang, Y. (2007). Flotation Results from the Wangchenggang Site in Dengfeng, Henan Province (In Chinese). *Huaxia Archaeol.* 2, 78–89.

Zhao, Z. (2019). Introduction of the Origin of Agriculture in China (In Chinese). *Res. Heritages Preserv.* 4, 5–11.

Zhong, H., Zhao, C., Wei, J., and Zhao, Z. (2016). Flotation Results from the Xinzhai Site in Xinmi, Henan Province in 2014 (In Chinese). *Agric. Archaeol.* 1, 21–29.

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ORIGINAL RESEARCH
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Investigating Biases Associated With Dietary Starch Incorporation and Retention With an Oral Biofilm Model

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Dental calculus has proven to contain a wealth of information on the dietary habits of past populations. These insights have, to a large extent, been obtained by the extraction and identification of starch granules contained within the mineralised dental plaque from a wide range of regions and time periods. The scope of previous studies have been limited to microfossil extraction and identification to reconstruct dietary preferences from the archaeological record, and few studies have attempted to address the biases of starch retention in dental calculus. Those that have considered this problem have been limited to *in vivo* studies on modern humans and non-human primates. Here, we present a multispecies oral biofilm model, which allows experimental research on starch incorporation and retention to be conducted on *in vitro* dental calculus in a controlled laboratory setting. The biofilms were exposed to treatment solutions with known quantities of dietary starches (wheat and potato) during the 25 days growth period. After this, the starch granules were extracted from the mature biofilm (by dissolution in EDTA), and counted. We show that the granule counts extracted from the model dental calculus represented a low proportion (ranging from 0.06% to 0.16%) of the total number of granules exposed to the biofilms throughout the experiment. Additionally, we found that the ratios of granule sizes from the extracted starch granules differed from the original treatment solutions, with large granules (>20 μm) consistently being under-represented. We also found a positive correlation between the absolute granule counts and dry-weight of the biofilm (r = 0.659, 90%CI[0.463, 0.794]), meaning the absolute quantity of starch granules will increase as the size of the calculus deposit increases. A similar, but weaker correlation was found between the concentration (count per mg) of granules and dry-weight (r = 0.3, 90%CI[0.0618, 0.506]). Our results complement and reinforce previous *in vivo* studies suggesting that dental calculus presents a very small, and partly biased picture of the original dietary intake of starches, with an over-representation of plants producing granules smaller than 20 μm in size. The experimental model presented here is well-suited to address the need for further validation of methods and biases associated with dietary research on dental calculus.

Keywords: oral biofilm models, starch, alpha-Amylase, dental calculus, palaeodiet reconstruction

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1 INTRODUCTION

Dental calculus has proven to contain a wealth of dietary information in the form of plant microfossils (Henry and Piperno, 2008; Hardy et al., 2009), proteins (Warinner et al., 2014a; Hendy et al., 2018), and other organic residues (Buckley et al., 2014). This dietary information can be preserved within the mineralised dental plaque over many millennia, providing a unique window into the food-related behaviours of past populations (Henry and Piperno, 2008; Tao et al., 2020; Jovanović et al., 2021) and extinct species (Hardy et al., 2012; Henry et al., 2014).

Until recently, only a few studies directly investigated the presence of plant microremains in the dental calculus of archaeological remains. The ability to extract phytoliths from the dental calculus of archaeological fauna to investigate diet was first noted by Armitage (1975), and later by Middleton and Rovner (1994), and Fox et al. (1996). Starches and phytoliths were extracted from human dental calculus by Cummings and Magennis (1997).

In more recent years, the study of dental calculus has increased exponentially, and the wealth of information contained within the mineralised matrix has largely been acknowledged. The use of dental calculus spans a wide variety of archaeological research areas, such as oral microbiome characterisation (including pathogens) through the analysis of DNA and proteins (Adler et al., 2013; Warinner et al., 2014b), microbotanical remains (Henry and Piperno, 2008; Hardy et al., 2009; Mickleburgh and Pagán-Jiménez, 2012), other organic residues and proteins from dietary compounds (Buckley et al., 2014; Hendy et al., 2018), and nicotine use (Eerkens et al., 2018). Especially the extraction of starch granules has become a rich source of dietary information, as starch granules have proven to preserve well within dental calculus over a variety of geographical and temporal ranges (Piperno and Dillehay, 2008; Henry et al., 2014; Tao et al., 2020; Jovanović et al., 2021).

Despite this, our knowledge of dental calculus and the incorporation pathways of the various markers is limited (Radini et al., 2017), as is our knowledge of information-loss caused by these pathways. Additionally, the methods we use to extract and analyse dental calculus, and make inferences on past diets represent another potential source of bias. Studies on both archaeological and modern individuals have explored these biases in more detail. Extraction methods were tested by Tromp and colleagues (2017), specifically regarding decalcification using HCl or EDTA. The authors found significantly more starches with the EDTA extraction method than the HCl extraction method; however, as noted by the authors, comparisons involving archaeological calculus are problematic due to variability between and within individuals. Studies conducted on modern humans (Leonard et al., 2015) and non-human primates (Power et al., 2015; Power et al., 2021) have explored how well microremains (phytoliths and starches) extracted from dental calculus represent the actual dietary intake. These studies are justifiably limited, despite meticulous documentation and observation, due to unknown variables and uncertainty involved in this kind of *in vivo* research. Dental calculus is a complex oral biofilm with a multifactorial aetiology and variable formation rates both within and between individuals (Haffajee et al., 2009; Jepsen et al., 2011), contributing to the stochasticity of starch representation being observed in numerous studies. Additionally, the concentration of oral α-amylase differs both between and within individuals (Froehlich et al., 1987; Nater et al., 2005), causing different rates of hydrolysis of the starch granules present in the oral cavity. Add to this the effects of the many different methods of starch processing (Hardy et al., 2018), as well as post-depositional processes that are still being explored (Mercader et al., 2018; García-Granero, 2020), and it becomes clear that using dental calculus to reconstruct diet is a highly unpredictable process.

In this exploratory study, we use an oral biofilm model to investigate the retention of starch granules within dental calculus in a controlled laboratory setting, allowing us full control over dietary input. Our main questions concern the representation of granules extracted from the calculus compared to the actual intake. How much of the original diet is incorporated into the calculus, and how much is recovered? Is there differential loss of information from specific dietary markers that affects the obtained dietary information, and how does this affect the representation of diet from extracted microremains?

We find that, despite the absence of α-amylase in the model, a limited proportion of the starch input is actually retained in the calculus. We also observed a shift in the size ratios of individual starch granules that are incorporated into the calculus, and that the number of incorporated starch granules increases as the size of the calculus deposit increases.

2 MATERIALS AND METHODS

2.1 Biofilm Formation

In this study we use a multispecies oral biofilm model following a modified protocol from Sissons and colleagues (1991) and Shellis (1978). In brief, a biofilm inoculated with whole saliva was grown on a substrate suspended in artificial saliva, and fed with sugar (sucrose). After several days of growth, the biofilm was exposed to starch solutions. Mineralisation of the biofilm was aided by exposure to a calcium phosphate solution. After 25 days of growth, the mineralised biofilm was collected for further analysis. The setup comprises a polypropylene 24 deepwell PCR plate (KingFisher 97003510) with a lid containing 24 pegs, which is autoclaved at 120°C, 1 bar overpressure, for 20 min. The individual pegs were the substrata on which the calculus grew. Using this system allowed for easy transfer of the growing biofilm between saliva, feeding solutions, and mineral solutions.

The artificial saliva (AS) is a modified version of the basal medium mucin (BMM) described by Sissons and colleagues (1991). It contains 2.5 g/L partially purified mucin from porcine stomach (Type III, Sigma M1778), 5 g/L trypticase peptone (Roth 2363.1), 10 g/L proteose peptone (Oxoid LP0085), 5 g/L yeast extract (BD 211921), 2.5 g/L KCl, 0.35 g/L NaCl, 1.8 mmol/L $CaCl_2$, 5.2 mmol/L Na_2HPO_4 (Sissons et al., 1991), 6.4 mmol/L $NaHCO_3$ (Shellis, 1978), 2.5 mg/L haemin.

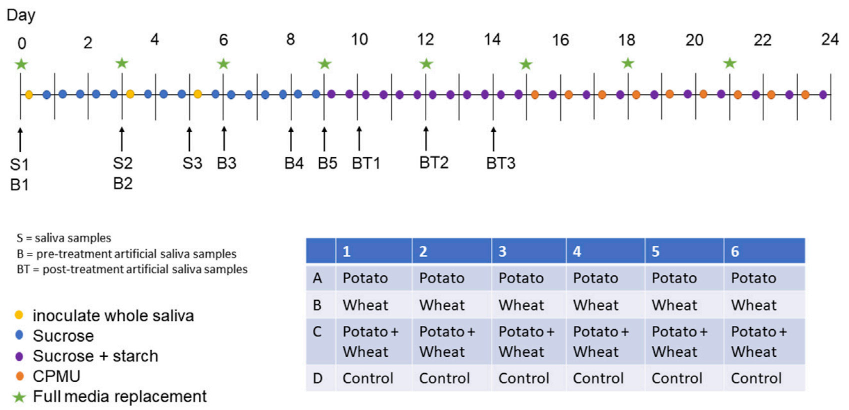

FIGURE 1 | Overview of experiment protocol including the plate setup.

This is subsequently adjusted to pH 7 with NaOH pellets and stirring, autoclaved (15 min, 120°C, 1 bar overpressure), and supplemented with 5.8 μmol/L menadione, 5 mmol/L urea, and 1 mmol/L arginine (Sissons et al., 1991).

Fresh whole saliva (WS) for inoculation was provided by a 31-year-old male donor with no history of caries, who abstained from oral hygiene for 24 h. No food was consumed 2 hours prior to donation and no antibiotics were taken up to 6 months prior to donation. The saliva was filtered through a sterilised (with sodium hypochlorite, 10–15% active chlorine) nylon cloth to remove particulates. Substrata were inoculated with 1 ml/well of a two-fold dilution of WS in sterilised 20% (v/v) glycerine for 4 h at 36°C, to allow attachment of the salivary pellicle and plaque-forming bacteria. After initial inoculation, the substrata were transferred to a new plate containing 1 ml/well AS and incubated in a shaking incubator (Infors HT Ecotron) at 36°C, 30 rpm. The inoculation process was repeated on days 3 and 5. AS was partially refreshed once per day and fully refreshed every 3 days, throughout the experiment, by transferring the substrata to a new plate containing stock AS. To feed the bacteria, the substrata were transferred to a new plate, containing 5% (w/v) sucrose, for 6 minutes twice daily, except on inoculation days (days 0, 3, and 5), where the samples only received one sucrose treatment after inoculation.

Starch treatments were initiated on day 9 to avoid starch granule counts being affected by α-amylase hydrolysis from saliva inoculation days. An α-amylase (EC 3.2.1.1) activity assay was conducted to confirm that no amylase was present in the model before starch treatments started. Starch treatments replaced sucrose treatments, occurring twice per day for 6 minutes. The starch treatments involved transferring the substrata to a new plate containing a 0.25% (w/v) starch from potato (Roth 9441.1) solution, a 0.25% (w/v) starch from wheat (Sigma S5127) solution, and a 0.5% (w/v) mixture of equal concentrations (w/v) wheat and potato. All starch treatments were created in dH_2O with 5% (w/v) sucrose. Before transferring biofilm samples to the starch treatment plate, the plates were agitated to keep the starches in suspension in the solutions. During treatments, the rpm was increased to 60 to facilitate contact between starch granules and biofilms.

After 15 days, mineralisation was encouraged with a calcium phosphate monofluorophosphate urea (CPMU) solution containing 20 mmol/L $CaCl_2$, 12 mmol/L NaH_2PO_4, 5 mmol/L Na_2PO_3F, 500 mmol/L urea, and (0.04 g/L MgCl) (Pearce and Sissons, 1987; Sissons et al., 1991). The substrata were submerged in 1 ml/well CPMU for 6 minutes, five times daily, in a 2 h cycle. During the mineralisation period, starch treatments were reduced to once per day after the five CPMU treatments. This process was repeated for 10 days until the end of the experiment on day 24 (see **Figure 1** for an overview of the protocol).

All laboratory work was conducted in sterile conditions under a laminar flow hood to prevent starch and bacterial contamination. Control samples that only received sucrose as a treatment were included to detect starch contamination from the environment or cross-contamination from other wells in the same plate.

2.2 Amylase Activity Detection

An α-amylase (EC 3.2.1.1) activity assay was conducted on artificial saliva samples collected from the plate wells on days 3, 6, 8, 9, 10, 12, and 14. Whole saliva samples were collected on days 0, 3, and 5 as positive controls. Collected samples were stored at 4°C until the assay was conducted on day 18. All samples and standard curves were run in triplicates on two separate plates. Positive control saliva samples were compared against a standard curve containing H_2O, while artificial saliva samples were compared against a standard curve containing stock AS (due to the colour of artificial saliva). Two photometric readings were conducted for each plate with a 540 nm filter on a Multiskan FC Microplate Photometer (Thermo Scientific 51119000). The protocol is a modified version of an Enzymatic Assay of α-Amylase (https://www.sigmaaldrich.com/NL/en/technical-documents/protocol/protein-biology/enzyme-activity-assays/enzymatic-assay-of-a-amylase) (Bernfeld, 1955), which measures the amount of maltose released from starch by α-amylase activity. Results are reported in units (U) per mL enzyme, where 1 U releases 1 μmole of maltose in 6 min. The detailed protocol can be found here: https://www.protocols.io/view/amylase-activity-bw8jphun.

2.3 Treatment Solutions

A 1 ml aliquot of each starch solution was taken, from which 10 μL was mounted on a microscope slide with an 18 x 18 mm

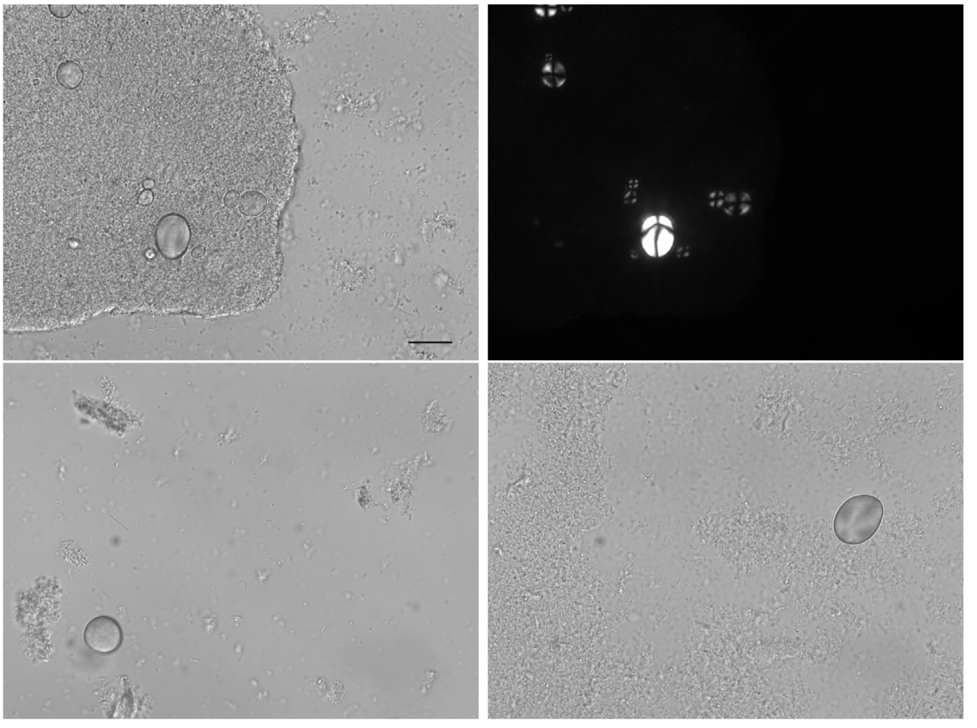

FIGURE 2 | Microscope images of biofilm samples that were exposed to the starch solutions. Starch granules can be seen within bacterial communities and isolated. Scale bar = 20 μm.

coverslip, and counted under a light microscope (Zeiss Axioscope A1). For wheat and mixed treatment samples, we counted three slide transects (at ca. 1/4, 1/2, and 3/4 of the slide), and the sample counts were extrapolated to the total number of granules exposed to the samples over 16 days of treatments (see Supplementary Material for more details). For potato treatment samples, the whole slide was counted.

2.4 Extraction Method

Extraction of starches from the calculus samples was performed by dissolving the calculus in 0.5 M ethylenediaminetetraacetic acid (EDTA) (Tromp et al., 2017; Modi et al., 2020; Le Moyne and Crowther, 2021), and vortexing for 3 days until the sample was completely dissolved. Twenty μl of sample was mounted onto a slide with an 18 × 18 mm coverslip. When transferring the sample to the slide, the sample was homogenised using the pipette to ensure that the counted transects were representative of the whole slide. The count from the slide was extrapolated to the whole sample (see Supplementary Material for more detail).

Both wheat and potato granules were divided into three size categories: small (<10 μm), medium (10–20 μm), and large (>20 μm).

2.5 Statistical Analysis

Statistical analysis was conducted in R version 4.2.0 (2022-04-22) (R Core Team, 2020) and the following packages: tidyverse (Wickham et al., 2019), broom (Robinson et al., 2021), here (Müller, 2020), and patchwork (Pedersen, 2020).

To see if biofilm growth was differently affected by starch treatments, a one-way ANOVA with sample weight as the dependent variable (DV) and treatment as the grouping variable (GV) was conducted. To analyse granule counts and calculate size proportions, mean counts for each treatment were taken across both experimental plates, resulting in a mean count for each granule size category within each treatment.

Pearson's r was conducted on sample weight and total starch count, as well as sample weight and starch count per mg calculus. The total count for each sample within a treatment was standardised by z-score to account for the differences in magnitude between the potato and wheat counts. This was applied to total biofilm weight and starch count per mg calculus (also z-score standardised) to account for differences in starch concentration in the calculus (as per Wesolowski et al., 2010).

3 RESULTS

All samples yielded sufficient biofilm growth and starch incorporation to be included in the analysis (**Figure 2**), resulting in a total of 48 biofilm samples (two plates of 24), 45 of which were used for analysis (three samples were set aside for later analysis). Most control samples contained no starch granules, while some contained negligible quantities (see Supplementary Material).

TABLE 1 | Summary statistics for biofilm dry-weights (in mg) by treatment.

Treatment	Mean	SD	Min	Max
control	5.44	2.45	1.67	11.20
mix	4.28	1.95	1.50	8.44
potato	6.25	2.07	2.54	8.92
wheat	5.53	3.45	0.56	9.80

3.1 No Amylase Activity Detected in the Model

No α-amylase activity was detected in any of the artificial saliva samples from any of the days that were sampled. Only positive controls (saliva) contained amylase activity that could be detected in the assay, ranging from 9.93 to 30.2 U/mL enzyme (full results can be found in the Supplementary Material). The results are not comparable to other studies presenting α-amylase activity levels in humans, as the unit definition may differ; however, they are sufficient to show that there is no activity in the model.

3.2 Treatment Type had Minimal Effect on Biofilm Growth

A one-way ANOVA suggests that the type of starch used during the biofilm growth period had a minimal effect on the growth of the biofilm (expressed as total dry weight of the sample), $F(3, 43) = 1.16$, $p = 0.335$. A summary of sample weights is available in **Table 1**.

3.3 Starch Counts

It was not possible to differentiate between potato and wheat starches smaller than ca. 10 μm. These were counted as wheat, as we assumed that the majority of the small granules were wheat. We make this assumption based on the counts of small starches in the wheat-only and potato-only solutions. Of the combined amount of small starches in these two solutions, 99.2% are from wheat.

The separate wheat and potato solutions were made with a 0.25% (w/v) starch concentration, while the mixed-starch solution was made with 0.25% (w/v) of each starch, with a total concentration of 0.50% (w/v). The mixed treatment had the highest absolute count of starch granules in solution (mean = 2.9×10^7), while the biofilms exposed to the wheat solution preserved the greatest number of granules (mean = 2.77×10^4). The potato treatment had the lowest absolute counts in both the solution (3.02×10^6) and in the biofilm samples (4,850) (**Tables 2**, **3**).

3.3.1 Proportion of Available Starches Incorporated in Samples

The proportion of total starches from the solutions that were incorporated into the samples ranged from 0.06% to 0.16%, with potato granules being more readily incorporated than wheat in both the separated- and mixed-treatment samples (**Table 4**). There is an inverse relationship between the absolute starch count in the solutions and the proportional incorporation of starches in the biofilm samples, i.e., potato had the lowest absolute count in solutions, but the highest proportional incorporation, and vice versa for the mixed treatment.

Wheat incorporation was most affected in the mixed-treatment samples, with only 0.06% of the total available starches being incorporated into the sample, compared to 0.16% in the separated wheat treatment.

3.3.2 Size Ratios Differ Between Solutions and Samples

Overall, medium starch granules had a higher mean rate of incorporation (0.171%) than small (0.120%) and large (0.066%) starch granules across all treatments, while large potato starches had the lowest rate of incorporation across all treatments.

The difference in incorporation between the size categories resulted in a change in size ratios between the original starch solutions and the extracted samples. Large potato granules (>20 μm) were most affected, with a 32.3% decrease in relative abundance in the potato-only treatment, and a 26.5% decrease in mixed treatments. Medium granules increased in relative abundance across all samples, while small granules decreased in wheat treatments and increased in potato treatments (**Figure 3**).

3.3.3 Biofilm Weight Correlated Positively With Extracted Starch Counts

Pearson's r suggests a strong positive correlation between the total weight of the biofilms and the total starch count (standardised by z-score) extracted from the samples across treatments, $r = 0.659$, 90%CI[0.463, 0.794], $p < 0.001$ (**Figure 4A**).

The same test was applied to total biofilm weight and starch count per mg calculus (also standardised by z-score), resulting in a weak positive correlation, $r = 0.3$, 90%CI[0.0618, 0.506], $p = 0.0403$ (**Figure 4B**).

TABLE 2 | Mean starch counts from solutions, including the proportional makeup of the different sizes of granules.

Solution	Starch	Small (%)	Medium (%)	Large (%)	Total (%)
mix	potato		1,051,733 (53.1%)	928,000 (46.9%)	1,979,733 (100.0%)
mix	wheat	18,838,400 (69.7%)	6,403,200 (23.7%)	1,794,133 (6.6%)	27,035,733 (100.0%)
mix	both	18,838,400 (64.9%)	7,454,933 (25.7%)	2,722,133 (9.4%)	29,015,467 (100.0%)
potato	potato	123,733 (4.1%)	1,337,867 (44.4%)	1,554,400 (51.5%)	3,016,000 (100.0%)
wheat	wheat	16,139,467 (63.5%)	6,434,133 (25.3%)	2,830,400 (11.1%)	25,404,000 (100.0%)

TABLE 3 | Mean starch counts extracted from samples with standard deviation (SD), including the proportion of granule sizes of the total count.

Treatment	Starch	Small (%)	SD	Medium (%)	SD	Large (%)	SD	Total (%)	SD
mix	potato			1959 (79.6%)	1801	501 (20.40%)	446	2460 (100%)	2189
mix	wheat	9515 (54.60%)	8,860	6,522 (37.4%)	6,026	1,381 (7.93%)	1,196	17,417 (100%)	15,878
mix	both	9515 (47.90%)	8,860	8,480 (42.7%)	7,653	1882 (9.47%)	1,596	19,877 (100%)	17,768
potato	potato	351 (7.24%)	297	3,565 (73.6%)	2402	930 (19.20%)	929	4,846 (100%)	3,316
wheat	wheat	15,235 (55.00%)	11,944	12,148 (43.9%)	11,052	1953 (7.06%)	2016	27,680 (100%)	23,554

TABLE 4 | The mean percentage of starches from the solutions that were incorporated into the samples.

Treatment	Starch	Small (%)	Medium (%)	Large (%)	Total (%)
mix	potato		0.19	0.05	0.12
mix	wheat	0.05	0.10	0.08	0.06
mix	both	0.05	0.11	0.07	0.07
potato	potato	0.28	0.27	0.06	0.16
wheat	wheat	0.09	0.19	0.07	0.12

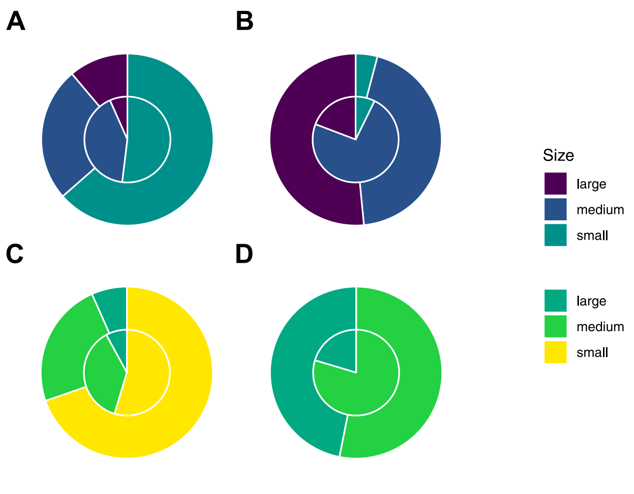

FIGURE 3 | Proportion of sizes of starch granules from solutions (outer ring) and treatment samples (inner ring) in separated wheat **(A)** and potato **(B)** treatments, and mixed wheat **(C)** and potato **(D)** treatments.

4 DISCUSSION

Here, we have provided a method for exploring the incorporation of dietary starches into the mineral matrix of a dental calculus biofilm model. Our results show that a very low proportion of the starches exposed to the biofilm during growth are retained in the mineral matrix, and that the size of the starch granules may affect the likelihood of incorporation. The proportions of starch granules (of all sizes) present in the extracted samples were similar across all treatments (0.06% to 0.16%), despite large differences in absolute granule counts between wheat (mean = 25,404,000) and potato (mean = 3,016,000) solutions.

The absolute counts, however, differed more visibly between treatments and was proportional with the total count of granules in the treatment solutions. Wheat and mixed solutions had the highest absolute mean count of starch granules, and also had the highest absolute mean count of starch granules extracted from the dental calculus (**Tables 2**, **3**). This suggests that the starches that are more frequently consumed will be present in higher quantities in the dental calculus, at least prior to inhumation and degradation in the burial environment. Despite the low proportion of granules recovered from the model calculus (0.06% to 0.16%), the absolute counts were still substantially greater than counts recovered from archaeological remains (Wesolowski et al., 2010; Tromp and Dudgeon, 2015; Tromp et al., 2017), which could in part be due to the lack of oral amylase activity in our model. Previous research conducted on dental calculus from contemporary humans and non-human primates suggest a high level of stochasticity involved in the retention of starch granules in dental calculus, and that starch granules extracted from dental calculus are underrepresented with regard to actual starch intake, which is consistent with our findings (illustrated by high standard deviations and low proportional incorporation). Leonard and colleagues (2015) found individual calculus samples to be a poor predictor of diet in a population, as many of the consumed plants were missing from some individual samples, but were present in others.

Power and colleagues (2015) presented similar findings in non-human primates, where phytoliths were more representative of individual diets than starch granules. The size bias is also consistent with the findings by Power and colleagues (2015), who found that plants producing starches 10–20 μm in size were over-represented; however, the representation of granules larger than 20 μm in their study is unclear.

We have also shown that the size of the starch granules influences the likelihood of incorporation into the calculus. Starch granules larger than 20 μm in maximum length were underrepresented in the calculus samples compared to the original starch solutions, an effect that was consistent across all three treatments. Medium granules (10–20 μm) were often over-represented (**Table 4** and **Figure 3**). Large potato granules were most affected, potentially because of the greater size-range. They can reach up to 100 μm in maximum length, whereas wheat granules generally only reach up to 35 μm (Gismondi et al., 2019; Haslam, 2004; Seidemann, 1966, 174–176). Granule morphology may also play a role. Large wheat granules are lenticular and have a larger surface area compared to volume, whereas large potato granules are ovoid and have a larger volume compared to surface area (van de Velde et al., 2002; Jane et al., 1994; Reichert, 1913,

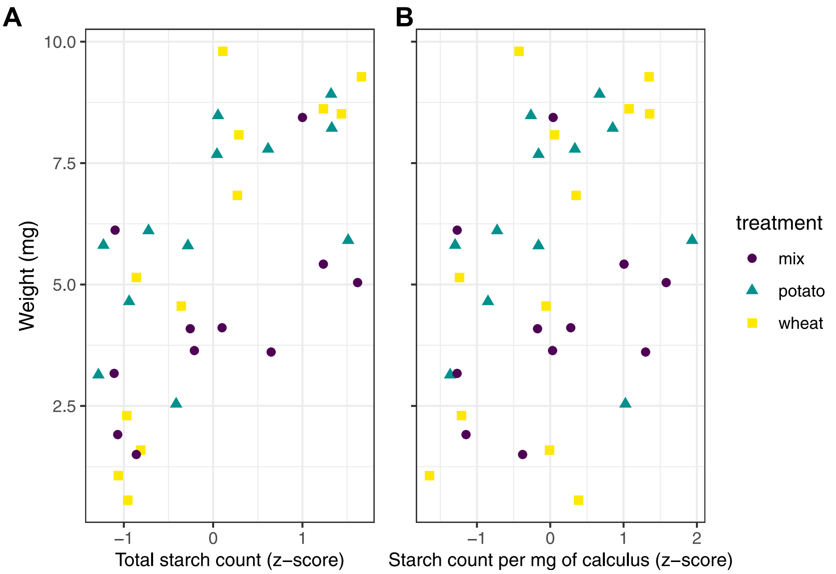

FIGURE 4 | Scatter plots of **(A)** sample weight in mg and standardised starch count by z-score for separated treatments, and **(B)** sample weight in mg and standardised count of starch grains per mg calculus.

364–365; Seidemann, 1966, 174–176). Another potentially important factor from our results is the size of the calculus deposit. We found a strong positive correlation between size of biofilm deposit and retained starch granules (**Figure 4A**), meaning larger calculus deposits contain a higher quantity of granules; a result that contradicts findings from archaeological contexts (Wesolowski et al., 2010; Dudgeon and Tromp, 2014). When the concentration of starch granules per mg calculus is considered, the correlation is weaker, but still present (**Figure 4B**). While the larger deposits contain a higher absolute count, our findings also suggest that they contain a slightly higher concentration of starches. This may also explain the lower mean retention of starch granules in mixed treatments compared to wheat treatments. Wheat treatment samples (mean = 5.53 mg) were on average larger than mixed treatment samples (mean = 4.28 mg) (**Table 1**); and while mixed treatment solutions contained the highest mean overall granule counts, wheat treatment samples had the highest mean starch retention. Further research is needed to determine why this differs from previous archaeological findings.

The mechanism by which starch granules are incorporated into plaque and calculus remains largely unknown, and few studies have directly investigated potential mechanisms. We know that a proportion of the starch granules entering the mouth can become trapped in the plaque/calculus, and can be recovered from archaeological samples of considerable age (Buckley et al., 2014; Henry et al., 2014; Wu et al., 2021). Studies have also shown that not all starch granules come from a dietary source. Other pathways include cross-contamination from plant interactions in soil, such as palm phytoliths adhering to the skin of sweet potatoes (Tromp and Dudgeon, 2015), or accidental ingestion not related to food consumption (Radini et al., 2017; Radini et al., 2019).

When starch granules enter the mouth, whether through ingestion of food or accidental intake, they immediately encounter multiple obstacles. It is likely that the bulk of starch granules are swallowed along with the food, and are only briefly present in the oral cavity. Other granules that are broken off during mastication may be retained in the dentition through attachment to tooth surfaces (including plaque and dental calculus) and mucous membranes (Dodds and Edgar, 1988; Kashket et al., 1991). Bacteria also have the ability to adhere to starch granules (Topping et al., 2003), which would allow starches to attach to bacterial communities within the biofilm. These granules are then susceptible to mechanical removal by the tongue, salivary clearance, and hydrolysis by α-amylase (Kashket et al., 1996). The susceptibility of granules to hydrolysis depends on the crystallinity and size of the starch granule, as well as the mode of processing. Smaller and pre-processed (e.g., cooked) starch granules are more susceptible to enzymatic degradation, while dehydrated starches will have a reduced susceptibility (Björck et al., 1984; Franco et al., 1992; Lingstrom et al., 1994; Haslam, 2004; Henry et al., 2009). Cracks on the surface of the dental calculus, as well as unmineralised islands and channels may also be able to contain starch granules (Tan B. et al., 2004; Charlier et al., 2010; Power et al., 2014). Starch granules that are trapped in these pockets are (at least to some extent) protected from aforementioned clearance mechanisms, especially once a new layer of plaque has covered the surface of the plaque/calculus. The size bias against large granules (>20 μm) from both wheat and potato (**Table 4**) may give further credence to this incorporation pathway, as the smaller starch granules have an advantage over larger granules, and can be stored in larger quantities. This was also suggested by Power and colleagues (2014), who observed clusters of starches within dental calculus, rather than an even distribution across the surface of the dental calculus. Granules trapped in plaque/calculus may still be susceptible to hydrolysis, as α-amylase has the ability to bind to both tooth enamel and bacteria within a biofilm and retain a portion of its hydrolytic activity (Scannapieco et al., 1993; Nikitkova et al., 2013; Tan B. T. K. et al., 2004, Tan et al., 2004 B.). After the death of an individual, starches within dental calculus are susceptible to further degradation by post-depositional processes, depending on burial environment (pH, temperature, moisture content, microorganisms) (Franco et al., 1992; Haslam, 2004; Henry et al., 2009; García-Granero, 2020). Future study should explore how burial affects the recovery of starch from the biofilm model.

The absence of α-amylase in the model is a limitation of this study, as the total granule counts were not subject to hydrolysis. This would likely have reduced and affected the size ratios, as smaller starches may be more susceptible to hydrolysis (Franco et al., 1992; Haslam, 2004). The absence may also affect biofilm growth due to the lack of amylase-bacterium interactions (Nikitkova et al., 2013). Conversely, the model may benefit from the absence of α-amylase, because it can allow us to directly explore its effect on starch counts in future experiments, where α-amylase can be added to the model in concentrations similar to those found in the oral cavity (Scannapieco et al., 1993). We are able to show how absolute counts in the treatments cause a difference in incorporation. However, this was merely a side-effect of the difference in the number of granules in potato and wheat solutions of the same concentration (w/v). Further research should test multiple differing concentrations of the same starch type. The use of EDTA may also have affected counts. While previous studies have shown negligible morphological changes caused by exposure to EDTA (Tromp et al., 2017; Modi et al., 2020; Le Moyne and Crowther, 2021), these studies have not considered changes to separate size categories within starch types, and whether shifts in size ratios occur due to exposure to the pre-treatment chemicals. The total number of granules on a slide often exceeded a number that was feasible to count in a reasonable time period, so we calculated the total counts by extrapolating from three slide transects. Thus, we reasonably assume that the three transects are a good representation of the entire slide, and that the distribution of all granules on the slide is relatively homogeneous.

Finally, we only used native starches in the experimental procedure and the results will likely differ for processed starches (García-Granero, 2020). Based on the comparatively low counts obtained by Leonard and colleagues (2015, Supplement 2), processing and amylase may have a substantial effect on starch granule retention in the oral cavity.

While we are unable to sufficiently address the mechanism(s) of starch incorporation with the data obtained in this study, the dental calculus model presented here is uniquely suited to explore these questions and may improve interpretations of dietary practices in past populations. Further analyses using this model can address the call for more baseline testing of biases associated with dietary research conducted on dental calculus (Le Moyne and Crowther, 2021). Our high-throughput experimental setup allows us a higher degree of control over the factors that influence starch incorporation and retention, such as dietary intake, differential survivability of starches, and inter- and intra-individual variation in plaque accumulation and mineralisation. The latter is especially difficult to control *in vivo* as it is influenced by numerous factors including genetics, diet, salivary flow, and tooth position and morphology (Haffajee et al., 2009; Jepsen et al., 2011; Simón-Soro et al., 2013; Proctor et al., 2018; Fagernäs et al., 2021), as well as evolutionary differences (Fellows Yates et al., 2021). The set of limitations for our model differ from *in vivo* methods and, as such, we expect our model to complement the results and interpretations of existing and new *in vivo* studies. It can also facilitate training of students and researchers on methods of dental calculus analysis, such as starch and phytolith extraction and identification, where it can replace the use of finite archaeological resources.

5 CONCLUSION

This preliminary study shows that a very small proportion of the input starch granules are retained in a dental calculus model. This and previous studies have shown that calculus has a low capacity for retention of starch granules, an effect that is compounded by diagenetic effects in archaeological remains, resulting in low overall counts of extracted granules. The proportion of starches consumed will in many cases be reflected in the quantity of starches extracted from the dental calculus—i.e., the more starch granules entering the oral cavity, the more will be recovered from extraction—at least in modern calculus samples unaffected by diagenesis and hydrolysis. Whether or not this also applies to archaeological samples remains to be tested. Additionally, we have shown that the size of granules will influence the likelihood of incorporation, as large (>20 μm) starches have a decreased incorporation rate, medium (10–20 μm) starches an increased rate, and small (<10 μm) granules remained somewhat constant. The size of calculus deposit also seems to influence the capacity of granule incorporation; as the size of the deposit increases, so does the absolute count of incorporated granules.

While we have shown multiple factors that influence the likelihood of incorporation, the process still appears to be somewhat stochastic. Further research is needed to make sense of the contributing factors, and to explore the mechanisms of intra-oral starch incorporation and retention in dental calculus. The oral biofilm model described in this study provides a method to explore the incorporation and extraction of dietary compounds from dental calculus in a controlled laboratory setting. We do not expect our model to replace *in vivo* methods; instead, it can provide a complementary means to address the limitations of *in vivo* studies, and unearth the potential biases associated with dietary research conducted on archaeological dental calculus.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

All scripts and data used in the analysis are available on OSF (https://osf.io/uc5qy/) and Github (https://github.com/bbartholdy/byoc-starch), following the format provided by the rrtools package (Marwick, 2019). More detailed protocols are available on OSF (https://osf.io/akevs/) and protocols.io (https://www.protocols.io/workspaces/byoc). Additional tables and figures are available in the Supplementary Material (https://osf.io/ucxsv/).

AUTHOR CONTRIBUTIONS

AGH acquired the funding for the analysis. BB and AGH conceptualised and designed the study. BB conducted the experiments and analysed the data. BB wrote the manuscript. AGH reviewed and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.886512/full#supplementary-material

REFERENCES

Adler, C. J., Dobney, K., Weyrich, L. S., Kaidonis, J., Walker, A. W., Haak, W., et al. (2013). Sequencing Ancient Calcified Dental Plaque Shows Changes in Oral Microbiota with Dietary Shifts of the Neolithic and Industrial Revolutions. *Nat. Genet.* 45, 450–455. e1. doi:10.1038/ng.2536

Armitage, P. L. (1975). The Extraction and Identification of Opal Phytoliths from the Teeth of Ungulates. *J. Archaeol. Sci.* 2, 187–197. doi:10.1016/0305-4403(75)90056-4

Bernfeld, P. (1955). "[17] Amylases, α and β," in *Methods in Enzymology* (Cambridge, MA, USA: Academic Press), 149–158. doi:10.1016/0076-6879(55)01021-5

Björck, I., Asp, N. G., Birkhed, D., Eliasson, A. C., Sjöberg, L. B., and Lundquist, I. (1984). Effects of Processing on Starch Availability *In Vitro* and *In Vivo*. II. Drum-Drying of Wheat Flour. *J. Cereal Sci.* 2, 165–178. doi:10.1016/S0733-5210(84)80030-2

Buckley, S., Usai, D., Jakob, T., Radini, A., and Hardy, K. (2014). Dental Calculus Reveals Unique Insights into Food Items, Cooking and Plant Processing in Prehistoric Central Sudan. *PLOS ONE* 9, e100808. doi:10.1371/journal.pone.0100808

Charlier, P., Huynh-Charlier, I., Munoz, O., Billard, M., Brun, L., and Grandmaison, G. L. d. l. (2010). The Microscopic (Optical and SEM) Examination of Dental Calculus Deposits (DCD). Potential Interest in Forensic Anthropology of a Bio-Archaeological Method. *Leg. Med.* 12, 163–171. doi:10.1016/j.legalmed.2010.03.003

Cummings, L. S., and Magennis, A. (1997). "A Phytolith and Starch Record of Food and Grit in Mayan Human Tooth Tartar," in *The State-Of-The-Art of Phytoliths in Soils and Plants*. Editors A. Pinilla, J. Juan-Tresserras, and M. J. Machado (Spain: CSIC Press).

Dodds, M. W. J., and Edgar, W. M. (1988). The Relationship between Plaque pH, Plaque Acid Anion Profiles, and Oral Carbohydrate Retention after Ingestion of Several 'Reference Foods' by Human Subjects. *J. Dent. Res.* 67, 861–865. doi:10.1177/00220345880670051301

Dudgeon, J. V., and Tromp, M. (2014). Diet, Geography and Drinking Water in Polynesia: Microfossil Research from Archaeological Human Dental Calculus, Rapa Nui (Easter Island). *Int. J. Osteoarchaeol.* 24, 634–648. doi:10.1002/oa.2249

Eerkens, J. W., Tushingham, S., Brownstein, K. J., Garibay, R., Perez, K., Murga, E., et al. (2018). Dental Calculus as a Source of Ancient Alkaloids: Detection of Nicotine by LC-MS in Calculus Samples from the Americas. *J. Archaeol. Sci. Rep.* 18, 509–515. doi:10.1016/j.jasrep.2018.02.004

Fagernäs, Z., Salazar-García, D. C., Avilés, A., Haber, M., Henry, A., Maurandi, J. L., et al. (2021). Understanding the Microbial Biogeography of Ancient Human Dentitions to Guide Study Design and Interpretation. *bioRxiv, 2021.08.16.456492.* doi:10.1101/2021.08.16.456492

Fellows Yates, J. A., Velsko, I. M., Aron, F., Posth, C., Hofman, C. A., Austin, R. M., et al. (2021). The Evolution and Changing Ecology of the African Hominid Oral Microbiome. *Proc. Natl. Acad. Sci. U.S.A.* 118. doi:10.1073/pnas.2021655118

Fox, C. L., Juan, J., and Albert, R. M. (1996). Phytolith Analysis on Dental Calculus, Enamel Surface, and Burial Soil: Information about Diet and Paleoenvironment. *Am. J. Phys. Anthropol.* 101, 101–113. doi:10.1002/(SICI)1096-8644(199609)101:1<101:AID-AJPA7>3.0.CO;2-Y

Franco, C. M. L., do Rio Preto, S. J., and Ciacco, C. F. (1992). Factors that Affect the Enzymatic Degradation of Natural Starch Granules -Effect of the Size of the Granules. *Starch/Stärke* 44, 422–426. doi:10.1002/star.19920441106

Froehlich, D. A., Pangborn, R. M., and Whitaker, J. R. (1987). The Effect of Oral Stimulation on Human Parotid Salivary Flow Rate and Alpha-Amylase Secretion. *Physiology Behav.* 41, 209–217. doi:10.1016/0031-9384(87)90355-6

García-Granero, J. J. (2020). Starch Taphonomy, Equifinality and the Importance of Context: Some Notes on the Identification of Food Processing through Starch Grain Analysis. *J. Archaeol. Sci.* 124, 105267. doi:10.1016/j.jas.2020.105267

Gismondi, A., D'Agostino, A., Canuti, L., Di Marco, G., Basoli, F., and Canini, A. (2019). Starch Granules: A Data Collection of 40 Food Species. *Plant Biosyst. - Int. J. Deal. all Aspects Plant Biol.* 153, 273–279. doi:10.1080/11263504.2018.1473523

Haffajee, A. D., Teles, R. P., Patel, M. R., Song, X., Yaskell, T., and Socransky, S. S. (2009). Factors Affecting Human Supragingival Biofilm Composition. II. Tooth Position. *J. Periodontal Res.* 44, 520–528. doi:10.1111/j.1600-0765.2008.01155.x

Hardy, K., Blakeney, T., Copeland, L., Kirkham, J., Wrangham, R., and Collins, M. (2009). Starch Granules, Dental Calculus and New Perspectives on Ancient Diet. *J. Archaeol. Sci.* 36, 248–255. doi:10.1016/j.jas.2008.09.015

Hardy, K., Buckley, S., Collins, M. J., Estalrrich, A., Brothwell, D., Copeland, L., et al. (2012). Neanderthal Medics? Evidence for Food, Cooking, and Medicinal Plants Entrapped in Dental Calculus. *Naturwissenschaften* 99, 617–626. doi:10.1007/s00114-012-0942-0

Hardy, K., Buckley, S., and Copeland, L. (2018). Pleistocene Dental Calculus: Recovering Information on Paleolithic Food Items, Medicines, Paleoenvironment and Microbes. *Evol. Anthropol.* 27, 234–246. doi:10.1002/evan.21718

Haslam, M. (2004). The Decomposition of Starch Grains in Soils: Implications for Archaeological Residue Analyses. *J. Archaeol. Sci.* 31, 1715–1734. doi:10.1016/j.jas.2004.05.006

Hendy, J., Warinner, C., Bouwman, A., Collins, M. J., Fiddyment, S., Fischer, R., et al. (2018). Proteomic Evidence of Dietary Sources in Ancient Dental Calculus. *Proc. R. Soc. B* 285, 20180977. doi:10.1098/rspb.2018.0977

Henry, A. G., Brooks, A. S., and Piperno, D. R. (2014). Plant Foods and the Dietary Ecology of Neanderthals and Early Modern Humans. *J. Hum. Evol.* 69, 44–54. doi:10.1016/j.jhevol.2013.12.014

Henry, A. G., Hudson, H. F., and Piperno, D. R. (2009). Changes in Starch Grain Morphologies from Cooking. *J. Archaeol. Sci.* 36, 915–922. doi:10.1016/j.jas.2008.11.008

Henry, A. G., and Piperno, D. R. (2008). Using Plant Microfossils from Dental Calculus to Recover Human Diet: a Case Study from Tell Al-Raqā'i, Syria. *J. Archaeol. Sci.* 35, 1943–1950. doi:10.1016/j.jas.2007.12.005

Jane, J.-L., Kasemsuwan, T., Leas, S., Zobel, H., and Robyt, J. F. (1994). Anthology of Starch Granule Morphology by Scanning Electron Microscopy. *Starch/Stärke* 46, 121–129. doi:10.1002/star.19940460402

Jepsen, S., Deschner, J., Braun, A., Schwarz, F., and Eberhard, J. (2011). Calculus Removal and the Prevention of its Formation. *Periodontology* 55, 167–188. doi:10.1111/j.1600-0757.2010.00382.x

Jovanović, J., Power, R. C., de Becdelièvre, C., Goude, G., and Stefanović, S. (2021). Microbotanical Evidence for the Spread of Cereal Use during the Mesolithic-Neolithic Transition in the Southeastern Europe (Danube Gorges): Data from Dental Calculus Analysis. *J. Archaeol. Sci.* 125, 105288. doi:10.1016/j.jas.2020.105288

Kashket, S., Zhang, J., and Van Houte, J. (1996). Accumulation of Fermentable Sugars and Metabolic Acids in Food Particles that Become Entrapped on the Dentition. *J. Dent. Res.* 75, 1885–1891. doi:10.1177/00220345960750111101

Kashket, S., Van Houte, J., Lopez, L. R., and Stocks, S. (1991). Lack of Correlation between Food Retention on the Human Dentition and Consumer Perception of Food Stickiness. *J. Dent. Res.* 70, 1314–1319. doi:10.1177/00220345910700100101

Le Moyne, C., and Crowther, A. (2021). Effects of Chemical Pre-treatments on Modified Starch Granules: Recommendations for Dental Calculus Decalcification for Ancient Starch Research. *J. Archaeol. Sci. Rep.* 35, 102762. doi:10.1016/j.jasrep.2020.102762

Leonard, C., Vashro, L., O'Connell, J. F., and Henry, A. G. (2015). Plant Microremains in Dental Calculus as a Record of Plant Consumption: A Test with Twe Forager-Horticulturalists. *J. Archaeol. Sci. Rep.* 2, 449–457. doi:10.1016/j.jasrep.2015.03.009

Lingstrom, P., Birkhed, D., Ruben, J., and Arends, J. (1994). Effect of Frequent Consumption of Starchy Food Items on Enamel and Dentin Demineralization and on Plaque pH *In Situ*. *J. Dent. Res.* 73, 652–660. doi:10.1177/00220345940730031101

Marwick, B. (2019). *Rrtools: Creates a Reproducible Research Compendium*.

Mercader, J., Akeju, T., Brown, M., Bundala, M., Collins, M. J., Copeland, L., et al. (2018). Exaggerated Expectations in Ancient Starch Research and the Need for New Taphonomic and Authenticity Criteria. *FACETS* 3, 777–798. doi:10.1139/facets-2017-0126

Mickleburgh, H. L., and Pagán-Jiménez, J. R. (2012). New Insights into the Consumption of Maize and Other Food Plants in the Pre-columbian Caribbean from Starch Grains Trapped in Human Dental Calculus. *J. Archaeol. Sci.* 39, 2468–2478. doi:10.1016/j.jas.2012.02.020

Middleton, W. D., and Rovner, I. (1994). Extraction of Opal Phytoliths from Herbivore Dental Calculus. *J. Archaeol. Sci.* 21, 469–473. doi:10.1006/jasc.1994.1046

Modi, A., Pisaneschi, L., Zaro, V., Vai, S., Vergata, C., Casalone, E., et al. (2020). Combined Methodologies for Gaining Much Information from Ancient Dental Calculus: Testing Experimental Strategies for Simultaneously Analysing DNA and Food Residues. *Archaeol. Anthropol. Sci.* 12, 10. doi:10.1007/s12520-019-00983-5

Müller, K. (2020). *Here: A Simpler Way to Find Your Files*.

Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., et al. (2005). Human Salivary Alpha-Amylase Reactivity in a Psychosocial Stress Paradigm. *Int. J. Psychophysiol.* 55, 333–342. doi:10.1016/j.ijpsycho.2004.09.009

Nikitkova, A. E., Haase, E. M., and Scannapieco, F. A. (2013). Taking the Starch Out of Oral Biofilm Formation: Molecular Basis and Functional Significance of Salivary α-Amylase Binding to Oral Streptococci. *Appl. Environ. Microbiol.* 79, 416–423. doi:10.1128/AEM.02581-12

Pearce, E. I. F., and Sissons, C. H. (1987). The Concomitant Deposition of Strontium and Fluoride in Dental Plaque. *J. Dent. Res.* 66, 1518–1522. doi:10.1177/00220345870660100101

Pedersen, T. L. (2020). *Patchwork: The Composer of Plots*.

Piperno, D. R., and Dillehay, T. D. (2008). Starch Grains on Human Teeth Reveal Early Broad Crop Diet in Northern Peru. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19622–19627. doi:10.1073/pnas.0808752105

Power, R. C., Salazar-García, D. C., Wittig, R. M., Freiberg, M., and Henry, A. G. (2015). Dental Calculus Evidence of Taï Forest Chimpanzee Plant Consumption and Life History Transitions. *Sci. Rep.* 5, 15161. doi:10.1038/srep15161

Power, R. C., Salazar-García, D. C., Wittig, R. M., and Henry, A. G. (2014). Assessing Use and Suitability of Scanning Electron Microscopy in the Analysis of Micro Remains in Dental Calculus. *J. Archaeol. Sci.* 49, 160–169. doi:10.1016/j.jas.2014.04.016

Power, R. C., Wittig, R. M., Stone, J. R., Kupczik, K., and Schulz-Kornas, E. (2021). The Representativeness of the Dental Calculus Dietary Record: Insights from Taï Chimpanzee Faecal Phytoliths. *Archaeol. Anthropol. Sci.* 13, 104. doi:10.1007/s12520-021-01342-z

Proctor, D. M., Fukuyama, J. A., Loomer, P. M., Armitage, G. C., Lee, S. A., Davis, N. M., et al. (2018). A Spatial Gradient of Bacterial Diversity in the Human Oral Cavity Shaped by Salivary Flow. *Nat. Commun.* 9, 681. doi:10.1038/s41467-018-02900-1

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Radini, A., Nikita, E., Buckley, S., Copeland, L., and Hardy, K. (2017). Beyond Food: The Multiple Pathways for Inclusion of Materials into Ancient Dental Calculus. *Am. J. Phys. Anthropol.* 162, 71–83. doi:10.1002/ajpa.23147

Radini, A., Tromp, M., Beach, A., Tong, E., Speller, C., McCormick, M., et al. (2019). Medieval Women's Early Involvement in Manuscript Production Suggested by Lapis Lazuli Identification in Dental Calculus. *Sci. Adv.* 5, eaau7126. doi:10.1126/sciadv.aau7126

Reichert, E. T. (1913). *The Differentiation and Specificity of Starches in Relation to Genera, Species, Etc: Stereochemistry Applied to Protoplasmic Processes and Products, and as a Strictly Scientific Basis for the Classification of Plants and Animals*. Washington, D.C.: Carnegie institution of Washington.

Robinson, D., Hayes, A., and Couch, S. (2021). *Broom: Convert Statistical Objects into Tidy Tibbles*.

Scannapieco, F. A., Torres, G., and Levine, M. J. (1993). Salivary α-Amylase: Role in Dental Plaque and Caries Formation. *Crit. Rev. Oral Biol. Med.* 4, 301–307. doi:10.1177/10454411930040030701

Seidemann, J. (1966). *Stärke-Atlas: Grundlagen der Stärke-Mikroskopie und Beschreibung der wichtigsten Stärkearten*. Berlin: Parey.

Shellis, R. P. (1978). A Synthetic Saliva for Cultural Studies of Dental Plaque. *Archives Oral Biol.* 23, 485–489. doi:10.1016/0003-9969(78)90081-X

Simón-Soro, Á., Tomás, I., Cabrera-Rubio, R., Catalan, M. D., Nyvad, B., and Mira, A. (2013). Microbial Geography of the Oral Cavity. *J. Dent. Res.* 92, 616–621. doi:10.1177/0022034513488119

Sissons, C. H., Cutress, T. W., Hoffman, M. P., and Wakefield, J. S. J. (1991). A Multi-Station Dental Plaque Microcosm (Artificial Mouth) for the Study of Plaque Growth, Metabolism, pH, and Mineralization. *J. Dent. Res.* 70, 1409–1416. doi:10.1177/00220345910700110301

Tan, B., Gillam, D. G., Mordan, N. J., and Galgut, P. N. (2004a). A Preliminary Investigation into the Ultrastructure of Dental Calculus and Associated Bacteria. *J. Clin. Periodontol.* 31, 364–369. doi:10.1111/j.1600-051X.2004.00484.x

Tan, B. T. K., Mordan, N. J., Embleton, J., Pratten, J., and Galgut, P. N. (2004b). Study of Bacterial Viability within Human Supragingival Dental Calculus. *J. Periodontology* 75, 23–29. doi:10.1902/jop.2004.75.1.23

Tao, D., Zhang, G., Zhou, Y., and Zhao, H. (2020). Investigating Wheat Consumption Based on Multiple Evidences: Stable Isotope Analysis on Human Bone and Starch Grain Analysis on Dental Calculus of Humans from the Laodaojing Cemetery, Central Plains, China. *Int. J. Osteoarchaeol* 30, 594–606. doi:10.1002/oa.2884

Topping, D. L., Fukushima, M., and Bird, A. R. (2003). Resistant Starch as a Prebiotic and Synbiotic: State of the Art. *Proc. Nutr. Soc.* 62, 171–176. doi:10.1079/PNS2002224

Tromp, M., Buckley, H., Geber, J., and Matisoo-Smith, E. (2017). EDTA Decalcification of Dental Calculus as an Alternate Means of Microparticle Extraction from Archaeological Samples. *J. Archaeol. Sci. Rep.* 14, 461–466. doi:10.1016/j.jasrep.2017.06.035

Tromp, M., and Dudgeon, J. V. (2015). Differentiating Dietary and Non-dietary Microfossils Extracted from Human Dental Calculus: The Importance of Sweet Potato to Ancient Diet on Rapa Nui. *J. Archaeol. Sci.* 54, 54–63. doi:10.1016/j.jas.2014.11.024

van de Velde, F., van Riel, J., and Tromp, R. H. (2002). Visualisation of Starch Granule Morphologies Using Confocal Scanning Laser Microscopy (CSLM). *J. Sci. Food Agric.* 82, 1528–1536. doi:10.1002/jsfa.1165

Warinner, C., Hendy, J., Speller, C., Cappellini, E., Fischer, R., Trachsel, C., et al. (2014a). Direct Evidence of Milk Consumption from Ancient Human Dental Calculus. *Sci. Rep.* 4, 7104. doi:10.1038/srep07104

Warinner, C., Rodrigues, J. F. M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., et al. (2014b). Pathogens and Host Immunity in the Ancient Human Oral Cavity. *Nat. Genet.* 46, 336–344. doi:10.1038/ng.2906

Wesolowski, V., Ferraz Mendonça de Souza, S. M., Reinhard, K. J., and Ceccantini, G. (2010). Evaluating Microfossil Content of Dental Calculus from Brazilian Sambaquis. *J. Archaeol. Sci.* 37, 1326–1338. doi:10.1016/j.jas.2009.12.037

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., et al. (2019). Welcome to the Tidyverse. *Joss* 4, 1686. doi:10.21105/joss.01686

Wu, Y., Tao, D., Wu, X., and Liu, W. (2021). Diet of the Earliest Modern Humans in East Asia. *Rev.* doi:10.21203/rs.3.rs-442096/v1

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Human Diet Patterns During the Qijia Cultural Period: Integrated Evidence of Stable Isotopes and Plant Micro-remains From the Lajia Site, Northwest China

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The diet of prehistoric humans in the Qijia period (4,400–3,500 BP) was significantly changed by the advent of dry agriculture and food globalization. However, it is yet to be proven whether wild plants were exploited despite the cultivation of millet, and whether wheat crops, cattle, and sheep originating from southwestern Asia were adopted into the regional human diet. This study presents stable isotope, starch grain, and phytolith analyses of 24 human teeth from the Lajia site in Qinghai, which is a representative Qijia culture settlement site. The carbon and nitrogen isotope results show that the subjects primarily ate C4 plants and had a high protein diet. Starch grain and phytolith results showed that the inhabitants consumed broomcorn millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), Triticeae, tubers and roots, along with other Pooideae and Poaceae plants. This data proves that although broomcorn and foxtail millet formed the mainstay of the Qijia diet, it also included a wide range of plants, such as the tribe Triticeae, tubers and roots, which would have been foraged rather than grown. Compared with the other three contemporaneous sites in Northern China, the proportion of millet starches was highest at the Lajia site, while the type and proportion of foraged plants were the lowest. This was probably because of the arid environment in the region, which could not have supported sufficient plant resources for foraging, which in turn might have led to enhanced millet cultivation and/or a greater reliance on hunting. No wheat or barley traces were found in human teeth in Lajia, and the high proportion of nitrogen was possibly related to the consumption of sheep because sheep bones were found in a zooarchaeological study. This study enhances our understanding of the subsistence strategies present in Qijia culture and of prehistoric food globalization, which is of pivotal significance for a deeper understanding of interactions between east and west Asia during the Neolithic and Bronze ages.

Keywords: prehistoric diet, the lajia site, dental calculus, carbon and nitrogen isotopes, starch grain, phytolith

1 INTRODUCTION

Marking a major evolutionary episode in human history, the transition from hunter/gatherer to agricultural economies spanned several millennia and occurred independently in 10 or more different world regions, including western and eastern Asia (Harris, 1972; Rindos, 1986; Lev-Yadun et al., 2000; Yang et al., 2012a). In these independent regions, a number of indigenous wild progenitor species from wheat and millet plants were domesticated in the Fertile Crescent of western Asia and northern China in east Asia, respectively, commencing approximately 10,000 BP (Lev-Yadun et al., 2000; Riehl et al., 2013; Lu et al., 2009a; Yang et al., 2012a; Wang et al., 2016). Thereafter, a new era in the use and creation of food resources by humans saw the rise of intensive activities around plant cultivation and animal husbandry. This led to a gradual decline in the proportion of hunter/gatherer economies and a reduction in the diversity of the human diet. This then significantly changed the subsistence economic pattern of ancient humans (Yan, 1982; Crawford et al., 2005; Crawford, 2006; Gignoux et al., 2011; Lu, 2017). Over time, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), which were first domesticated in western Asia, spread to Europe and western Central Asia by 8,000 BP, reaching eastern Central Asia and northwestern China between 4,500 and 4,000 BP (Zhao, 2015; Liu et al., 2017; Liu et al., 2019). Broomcorn (*Panicum miliaceum*) and foxtail millet (*Setaria italica*), domesticated in eastern Asia, spread into eastern central Asia between 4,500 and 4,000 BP and into western Asia and Europe prior to 3,500 BP (Frachetti et al., 2010; Liu et al., 2016a; Stevens et al., 2016; Dong et al., 2017; Lu, 2017). In other words, transcontinental cultural exchange at this time was correlated; wheat and millet crops as well as sheep, cattle, bronze and gold vessels, lapis lazuli, bead decorations, and jade ware were used mainly in eastern central Asia and Northwest China between 4,500 and 3,500 BP with the development of different technologies and the emergence of modes of transport, including domesticated horses and vehicles (Kuz'mina, 2008; Anthony, 2010; Spengler and Willcox, 2013; Spengler et al., 2014). The exchange of food globalization in prehistory between 4,500 and 3,500 BP (**Figure 1A**) brought important changes to the structure and diversity of the human diet in eastern Central Asia and Northwest China (Frachetti et al., 2010; Jones et al., 2011; Spengler and Willcox, 2013; Spengler et al., 2014; Jones et al., 2016; Dong et al., 2017). In this case, hunter/gatherers were gradually marginalized in the system of the human food acquisition between 4,500 and 3,500 BP, due to the emergence and development of agriculture and transcontinental cultural and technological exchanges in Eurasia. However, hunter/gatherers have always been associated with different human economic systems; for example, the economy of hunter/gatherers is still an important part of the local economic system in some ethnic groups, which survives to this day (Du and Chen, 2016).

Qijia culture (4,400–3,500 BP) is in the ecotone of agriculture and animal husbandry between the Qinghai Tibet Plateau and Loess Plateau in northwest China (**Figure 1B**) and plays an important role in cultural communication between the eastern and western parts of Eurasia (Han, 2019). Previous studies have suggested that millet cultivation became well established during the Qijia cultural period (Zhang, 2013; Ma et al., 2014; Wang et al., 2015; Zhang and Ye, 2016; Wang and Zhen, 2021), however, only a few carbonized wheat traces were recorded for this time period. For example, carbonized wheat remains represent less than three percent of the whole plants, while the proportion of wheat remains in the Lajia and Changning sites is less than 0.01% (Zhang, 2013; Wang and Zhen, 2021). Although the carbonized plant remains from the cultural layers of the archaeological sites can reflect the agricultural structure of the Qijia culture, these results cannot supply direct evidence of the human diet. However, isotopic analysis of human skeletal remains provides a rough outline of the likely food sources and serves as a reflection of the human diet at that time. Values of $\delta^{13}C$ from human bones dated

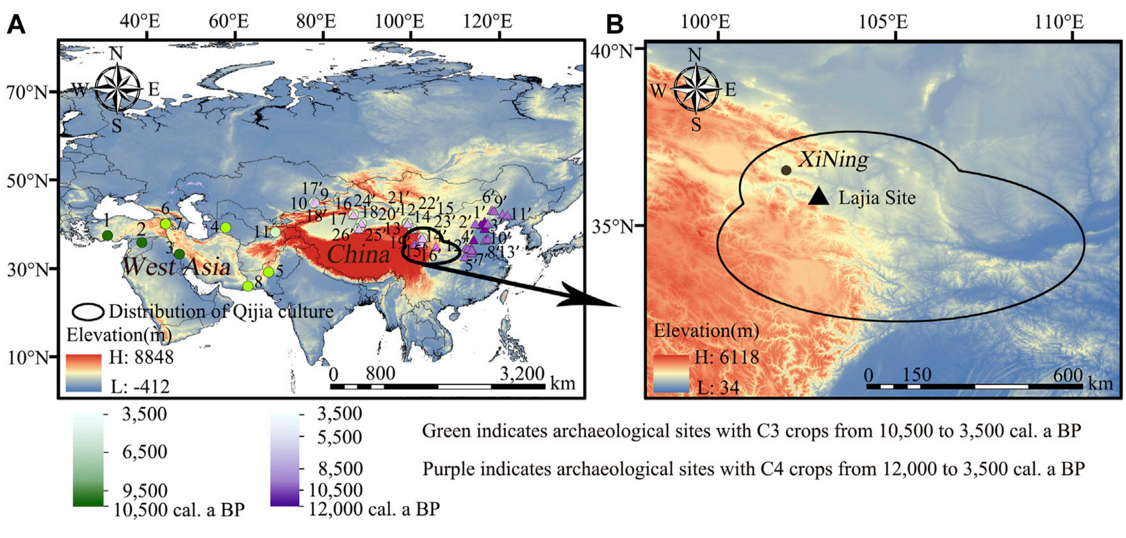

FIGURE 1 | (A) Location of archaeological sites with C3 and C4 crops from 12,000 to 3,500 cal a BP (Modified from Dong et al., 2017), **(B)** Location of the Lajia site.

to between 4,500 and 4,000 BP from northwestern China were dominated by C4 signals (**Figure 1A**), suggesting that the extent of mixing between western and eastern crops was very limited, but the C3 signals (**Figure 1A**) increased between 4,000 and 3,500 BP (Liu et al., 2014; Zhang and Ye., 2016; Dong et al., 2017). However, these results do not allow for the identification of specific plant species present in human diet during the Qijia period.

Recently, human teeth and dental calculus are being increasingly used as target sample types for the analysis of human diet (Beaumont et al., 2012; Henry et al., 2012; Chen et al., 2021). Stable carbon and nitrogen isotopic (δ13C and δ15N) analyses of bone collagen from human teeth remains is a conventional method of exploring the subsistence strategy of ancient humans (Beaumont et al., 2012). Dental calculus, a biomineral that entraps food particles, including some plant micro-remains as it forms, provides a direct record of plant consumption (Henry et al., 2012; Chen et al., 2021). Earlier research indicates that plant micro-remains, including starch grains and phytoliths, are well preserved in the calcification of dental calculus (Lieverse, 1999), and provide direct evidence of the human diet (Henry et al., 2012; Chen et al., 2021).

In the present study, human teeth and dental calculus unearthed from the Lajia site were selected and investigated using isotopic analysis, starch grain, and phytolith analyses to build a more comprehensive view of the human diet and subsistence strategy, in addition to transcontinental cultural and technological exchanges during the Qijia period. As a part of starch grain analysis, we also explored the extent to which different processing methods may have led to morphological changes in Poaceae food plants, to understand if and how the foods consumed by people in the Qijia culture were produced.

2 MATERIALS AND METHODS

2.1 Materials

The Lajia site (**Figure 1B**; 102°49′40″E, 35°51′15″N) covers an area of 200,000 m^2 and is located on a terrace on the north bank of the Yellow River in the Guanting Basin near Xialajia Village, Minhe County, Qinghai Province. The Lajia site is a large Qijia culture settlement situated in the transition zone between the eastern foot of the Qinghai Tibet Plateau and western Loess Plateau. Three excavation seasons in 2000, 2002–2003, and 2014–2017 uncovered house foundations and tombs in addition to ceramics, stone tools, jade objects, bone artifacts, and human bones (Ye, 2002; Du et al., 2019). Radiocarbon dating of human bones collected from house foundations in F3 and F4 (Nos. 2000QMLF3Ⅰ and 2000QMLF4IV) revealed that the site was occupied around 3,565 ± 25 and 3,580 ± 25 cal a BP (Zhang et al., 2014). When corrected by IntCal 20 (Ramsey, 2009; Reimer et al., 2020), the dates were adjusted to 3,860 ± 48 cal a BP and 3,882 ± 44 cal a BP.

Location of archaeological sites with C3 crops from 10,000 to 3,500 cal a BP: 1) Nevali Cori site (10,557–10,259 BP; Lösch et al., 2006), 2) Abu Hureyra site (10,117–9,021 BP; Moors, 2016), 3) Chogha Golan site (9,800 BP; Riehl et al., 2013), 4) Jietun site (8,328–7,712 BP; Harris et al., 1993), 5) Mehrgarh site (8,000–7,000 BP; Costantini, 2008), 6) Aratashen site (6,948–6,660 BP; Hovsepyan and Willcox, 2008), 7) Aknashen site (7,035–6,350 BP; Hovsepyan and Willcox, 2008), 8) Miri Qalat site (6,000–4,000 BP; Tengberg, 1999), 9) Tasbas site Phase 1 (4,655–4,423 BP; Spengler et al., 2014), 10) Begash site Phase 1a (4,407–4,148 BP; Spengler et al., 2014), 11) Sarazm site (5,000–4,000 BP; Spengler and Willcox, 2013), 12) Huoshiliang site (3,979–3,819 BP; Sheng et al., 2020), 13) Ganggangwa site (3,974–3,710 BP; Dodson et al., 2013), 14) Xichengyi site (3,972–3,848 BP; Zhang X. L. et al., 2015), 15) Jinchankou site (3,976–3,846 BP; Chen et al., 2015), 16) Xintala site (3,921–3,580 BP; Zhao et al., 2013), 17) Gumugou cemetery (3,834–3,695 BP; Zhang G. L. et al., 2015), 18) Xiaohe cemetery (3,707–3,478 BP; Flad et al., 2010).

Location of archaeological sites with C4 crops from 12,000 to 3,500 cal a BP: 1') Nanzhuangtou site (12,408–11,000 BP; Yang et al., 2012a), 2') Zhuannian site (11,000–9,700 BP; Yang et al., 2014), 3') Donghulin site (11,000–9,500 BP; Yang et al., 2012a), 4') Cishan site (10,300–7,000 BP; Lu et al., 2009a), 5') Baligang site (8,700–5,000 BP; Deng et al., 2015), 6') Baiyinchanghan site (8,500–7,400 BP; Tao et al., 2011), 7') Egou site (8,500–7,000 BP; Liu et al., 2015), 8') Shawoli site (8,500–7,000 BP; Zhang et al., 2011), 9') Xinglonggou Loc.1 (8,000–7,500 BP; Zhao, 2014), 10') Yuezhuang site (8,000–7,800 BP; Wu et al., 2011), 11') Chahai site (8,000–7,500 BP; Wu, 2015), 12') Bangou site (8,000–7,000 BP; Liu et al., 2013), 13') Xihe site (7,660–7,000 BP; Wu, 2014), 14') Heibiya site (4,760–4,330 BP; Jia, 2012), 15') Hurere site (5,290–4,620 BP; Jia, 2012), 16') Hongtujiaozi site (4,960–4520 BP; Jia, 2012), 17') Tasbas site Phase 1 (4,655–4,423 BP; Spengler et al., 2014), 18') Begash site Phase 1a (4,407–4,148 BP; Spengler et al., 2014), 19') Gongshijia site (4,210–3,670 BP; Jia, 2012), 20') Huoshiliang site (3,979–3,819 BP; Sheng et al., 2020), 21') Ganggangwa site (3,974–3,710 BP; Dodson et al., 2013), 22') Xichengyi site (3,972–3,848 BP; Zhang X. L. et al., 2015), 23') Jinchankou site (3,976–3,846 BP; Chen et al., 2015), 24') Xintala site (3,921–3,580 BP; Zhao et al., 2013), 25') Gumugou cemetery (3,834–3,695 BP; Zhang G. L. et al., 2015), 26') Xiaohe cemetery (3,707–3,478 BP; Flad et al., 2010).

Twenty-four teeth belonging to seven individuals (numbered F4Ⅱ, F4Ⅲ, F4Ⅴ, F4Ⅹ, F7Ⅰ, M8, and M11) from two house foundations and two tombs at the Lajia site were selected as samples (**Table 1** and **Figure 2**), seven human teeth from each individual were chosen for carbon stable isotope measurements, twenty-four dental calculi were analyzed using starch grain and phytolith analyses. The human teeth from the Lajia site used in this study were dated to between 3,900 and 3,800 BP (Zhang et al., 2014).

2.2 Methods

2.2.1 Analysis of Carbon and Nitrogen Stable Isotope

Seven of the 24 human teeth were selected for carbon and nitrogen stable isotope measurements. The δ13C and δ15N values were measured at Beta Analytic laboratories in Miami, United States, and the analytical precisions were ±0.3% and ±0.5%, respectively. The test results are listed in **Table 1**. The data were analyzed and processed using Origin 9.1 software.

TABLE 1 | Information on human tooth samples at the Lajia site and results of carbon and nitrogen isotopic testing.

Sample No.		Tooth	δ13C (%)	δ15N (%)	C/N	%C	%N
F4 II Skull	1	Molar					
	2	Molar	-5.42	13.76	3.2	36.41	13.41
F4 III Skull	3	Upper left canine					
	4	Upper right second molar	−6.13	10.33	3.1	39.4	14.7
	5	Maxillary premolar					
F4 V Skull	6	Upper right second molar	−7.52	10.93	3.1	39.08	14.49
	7	Upper left first premolar					
	8	Lower left first molar tooth					
	9	Lower left second milk molar					
F4 X Skull	10	Upper left first molar					
	11	Molar					
	12	Molar	−6.43	13.85	3.2	38.87	14.34
F7 I Skull	13	Lower left first molar					
	14	Lower right second milk molar					
	15	Right upper second molar	−7.93	12.54	3.1	39.9	14.94
	16	Upper left permanent molar					
	17	Lower right second milk molar					
M8 Skull	18	Upper left first premolar					
	19	Upper left second premolar					
	20	Right upper first premolar	−8.17	10.92	3.1	40.64	15.08
	21	Lower left second premolar					
M11 Skull	22	Upper Left Lateral Incisor					
	23	Upper right second molar					
	24	Lower right first premolar	−5.19	9.71	3.2	38.05	14.09

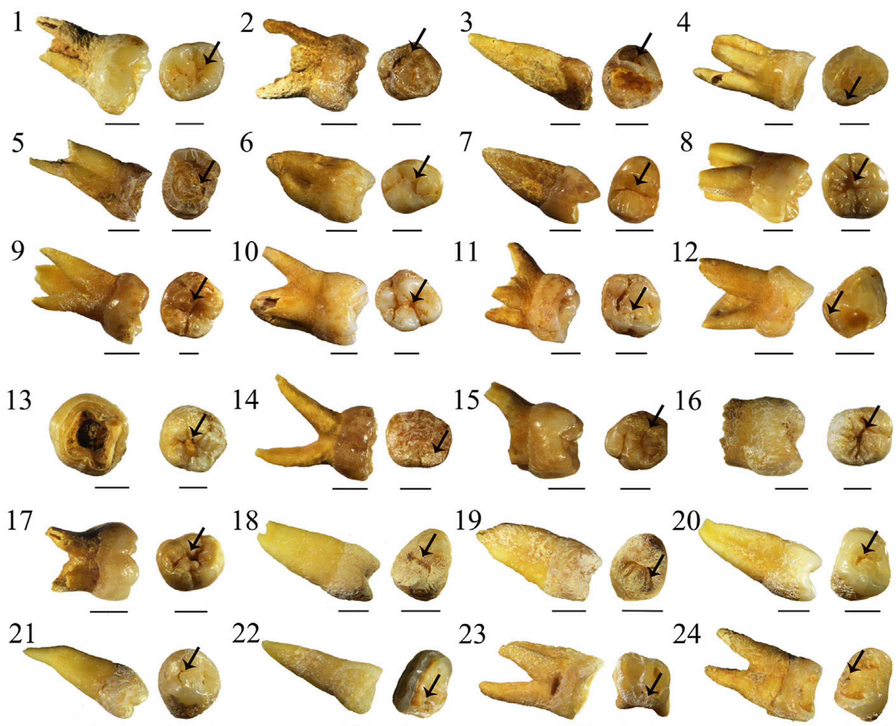

FIGURE 2 | Twenty-four human teeth from seven individuals from the house foundations and tombs of the Lajia site in Qinghai Province. **(1,2)** belong to the F4 II human skull; **(3-5)** belong to the F4 III human skull; **(6-9)** belong to the F4 V human skull; **(10–12)** belong to the F4 X human skull; **(13–17)** belong to the F7 I human skull; **(18–21)**, belong to the M8 human skull; **(22–24)** belong to the M11 human skull. **(1–24)** lateral and frontal views of each tooth. Black arrows show the sampling locations. (Scale bar: 5 mm).

2.2.2 Extraction Experiments of Starch Grains and Phytoliths From Dental Calculus

A dental pick was used to detach the visible calculus from all the teeth. Calculus fragments from each individual sample were collected in a piece of aluminum foil and immediately transferred into 1.5-ml centrifuge tubes. Dental calculus from each tooth was then treated using an ultrasonic cleaner according to the following steps (Lu et al., 2009a; Lu et al., 2009b; Yang et al., 2012a; Yang et al., 2012b; Chen et al., 2021):

Dental calculus was first cleaned gently with ultrapure water to remove adhesive particles. After washing, the samples were placed in a centrifuge tube and 20 ml of ultrapure water was added before the tube was placed in an ultrasonic cleaner for 10 min. A solution of 6% H_2O_2 was added to release starch grains that were potentially trapped within the dental calculus and let it stand for 1 h. Ultrapure water was then added, and the sample was centrifuged and cleaned at 2,500 rpm for 5 min. Excess liquid was poured off and only the residue was retained. This step was repeated three times. Then, 10% HCl was added to remove calcium impurities and the mixture was allowed to stand for 20 min. The solution was washed until a neutral pH was reached. A CsCl heavy liquid with a specific gravity of 1.8 g/cm^3 was added and the solution was centrifuged. The supernatant containing the starches was decanted into a fresh tube. Ultrapure water was then used to clean the floating substances four times for the preparation of starch granule slides. The lower residual liquid after a flotation selection of starch to extract phytoliths was then added to the 2.35 g/cm^3 $ZnBr_2$ heavy liquid for another floating selection. Starch granule slides were prepared by mounting the recovered residue onto a slide in a solution of 25% glycerin and 75% ultrapure water. The slides were then sealed with neutral gum. Phytolith slides were prepared by mounting the recovered residue in a neutral resin and fixing them using a cover glass.

2.2.3 Modern Simulation Experiments

Prehistoric foods required preparation prior to human consumption, and the main methods included physical methods, such as shelling and grinding (Wright, 1994), as well as heat treatment, such as boiling (Barton and Torrence, 2015). Therefore, the starch grains and phytoliths were manually prepared and/or boiled prior to the formation of dental calculus. Earlier research has shown that morphological changes in ancient starches taken from dental calculus were evident, but had little effect on phytoliths (Wang and Lu, 1993; Wu and Wang, 2012). To accurately identify ancient starches, we performed a simulation experiment of grinding and boiling starch grains from millet and wheat crops from dry farming. The experimental procedure was as follows:

The seeds of modern foxtail and broomcorn millets, and common wheat were selected as samples. The seeds were then cleaned and dried to remove any impurities. To observe the morphological change after grinding the starches, each sample (0.5 g) was placed in a mortar and ground for 2–5 min to obtain three samples for the grinding simulation experiment. The ground samples were then added to a centrifuge tube containing 5 ml of ultrapure water and placed in a low-temperature environment for 1 h. To observe the morphological changes after boiling the starches, each sample (0.5 g) was added to a centrifuge tube with 5 ml of ultrapure water and boiled for 30 min in a water bath. A timer was started after boiling the water. Samples were taken at 5-min intervals, and 18 samples (six groups) were obtained for the cooking simulation experiment. After heating, the samples were cooled for 1 h. The starch suspension generated during the grinding and cooking simulation experiments was extracted using a disposable pipette, a few drops were then added to a glass slide before 25% glycerin was added, and the slide was then sealed with neutral gum.

After being dried at ≤40°C, the slide was examined using a Leica DM 750 microscope to observe starch grains and phytoliths at ×200 magnification, once identified they were photographed at ×400 magnification. The size and morphological characteristics of the starch grains were measured and recorded. The analysis was performed at the Environmental Archaeology Laboratory at Northwest University. The morphological identification and classification of starch grains were based on the modern starch grain database and related research results, which were accumulated in this laboratory (Yang et al., 2010; Wan et al., 2012; Yang and Perry, 2013; Ma et al., 2014). A comparison with published pictures and materials has been made for the identification and description of phytoliths (Wang and Lu, 1993; Lu and Liu, 2003; Piperno, 2006; Lu et al., 2009a; Lu et al., 2009b; ICPT, 2019; Ge et al., 2020). We counted and identified the starch grains and phytoliths found on the glass slide from each ancient tooth sample, and 200 grains from each sample from the simulation experiment were analyzed to obtain data on the morphological characteristics and length of the starch grains.

All the utensils used in the above experiments were cleaned in ultrapure water, and then subjected to high-temperature steaming-boiling and ultrasonic cleaning before use. In addition, all utensils for each sample were treated in the same way before extraction of starch grains to prevent external pollution or cross-contamination between samples. All toothpicks, pipettes, centrifuge tubes, and powder-free gloves used in this study were disposable, to prevent cross-contamination.

3 RESULTS

3.1 Results of Carbon and Nitrogen Stable Isotope Analysis

According to the results from Beta Laboratory, United States, the mole ratios of the seven human teeth from Lajia site range from 3.1 to 3.2, and the C and N content was 36.41–40.64% and 13.41–15.08% (**Table 1**), respectively, which is consistent with the values of modern bone collagen. The range of δ^{13}C was −5.42–−8.17, and the average value was −6.68 ± 1.11 (n = 7); the average value of δ15N was 11.72 ± 1.54 (n = 7; **Table 1** and **Figure 3**). Previous studies have shown that collagen with a C and N content of bone collagen falls in the range of 15.3–47.0% and 5.5–17.3%, respectively, and a C/N ratio range of 2.9%–3.6 can be considered as non-contaminated (DeNiro, 1985). Therefore, seven samples from the Lajia site were valid and could be used for stable isotope analysis (**Table 1**).

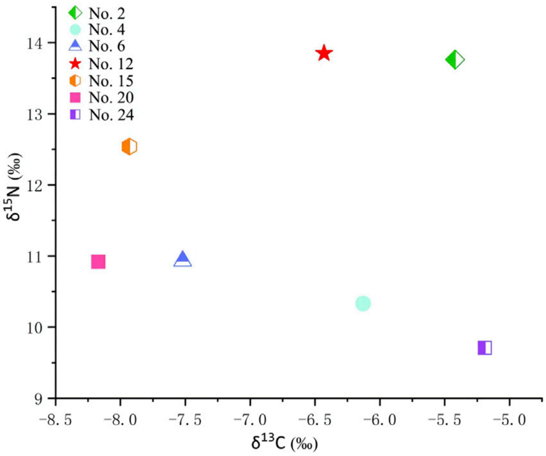

FIGURE 3 | Scatter diagram for $\delta^{13}C$ and $\delta^{15}N$ values of the seven human tooth samples from the Lajia site.

3.2 Results of Starch Grain Analysis From Dental Calculus

A total of 611 starch grains were extracted from 24 teeth taken from seven ancient human individuals and divided into Types I–IV (**Figure 4**; **Table 2**) according to morphology and granule size.

Type I: 277 granules were observed. They were polyhedral, free of layered texture with centric hila, and most had Y- and star-shaped fissures through the hila (**Figures 4A–C′**). This class of starch grains can be further divided into Type I-A intact starch grains ($n = 226$) with the long-axis particle size ranges from 8.0 to 27.0 μm and an average particle size of 14.9 ± 3.8 μm and Type I–B damaged starch grains ($n = 31$; **Figures 4A–B′**) with long-axis particle size ranging from 11.7 to 30.2 μm and an average particle size of 21.3 ± 5.2 μm. These are characterized by surface collapse, blurred extinction cross under polarized light, and cracks on some of the surfaces (**Figures 4C, C′**). Such starch grains were most likely from dry crops of foxtail and broomcorn millet.

Type II: 54 granules were observed. These granules have a biconvex shape and are mostly almost circular with a centric hilum. The extinction cross is seen to be X-shaped under polarized light, and after a slight knock to twist it around, the starch grains were oval shaped and possessed a longitudinal dent (**Figures 4D,F′**). This class of starch grains can be further divided into Type II-A ($n = 25$) intact starch grains with a long-axis particle size range of 12.9–37.7 μm and an average particle size of 26.3 ± 6.5 μm; and Type II-B ($n = 29$) damaged starch grains with a long-axis particle size range of 18.8–48.6 μm and an average particle size of 32.4 ± 8.2 μm. These starch molecules appeared transparent,

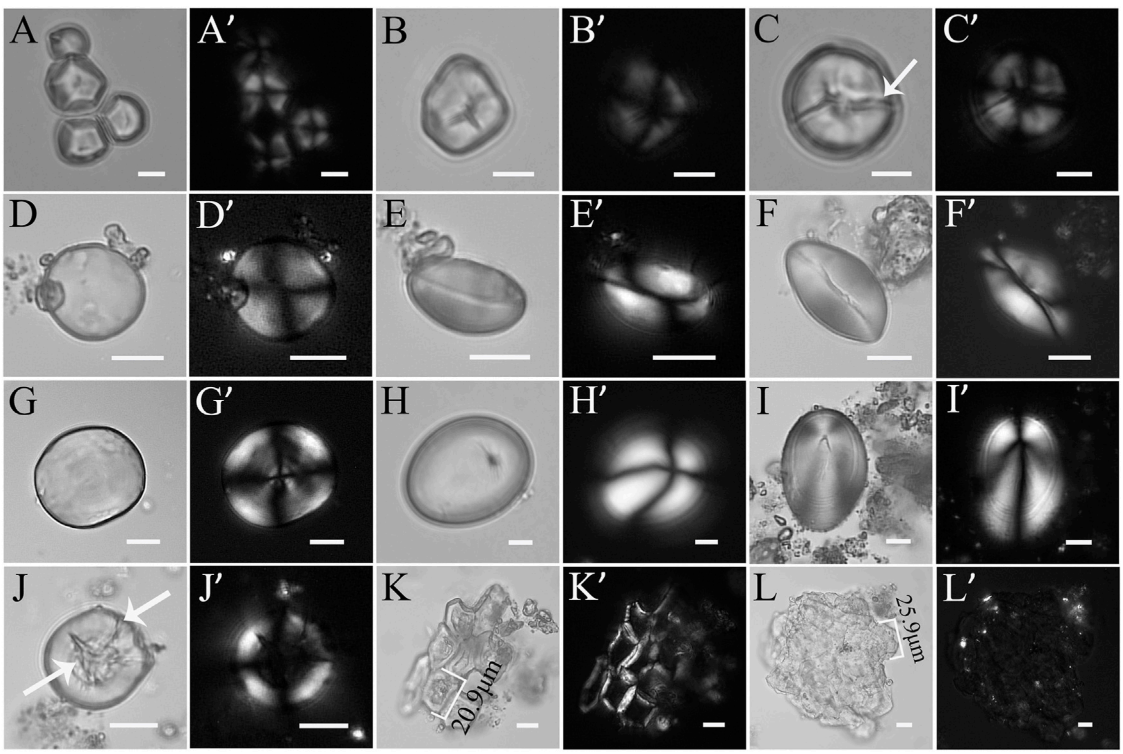

FIGURE 4 | Types of starch grains extracted from 24 human dental calculus at the Lajia site. **(A-C′)**, Type I: **(A-B′)**, Type I intact starch grains; **(C,C′)**, Type I damaged starch grains. **(D-G′)**, Type II: **(D-F′)**, Type II-A intact starch grains; **(G,G′)**, Type II-B damaged starch grains. **(H-I′)**, Type III; **(J-L′)**, Type IV: **(J,J′)**, Type IV-A; **(K,K′)**, Type IV-B; **(L,L′)**, Type IV-C. **(A, A′-L, L′)**, Photos were taken under bright field (left) and polarized light (right). (Arrow indicates damage in starch grains. Scale bar: 10 μm).

TABLE 2 | Classification, quantity and percentage statistics of starch grains from human dental calculus at the Lajia site.

Sample No.		Type-I		Type-II		Type-III	Type-IV			Percent of Total (%)
		Intact	Damaged	Intact	Damaged		IV-A	IV-B	IV-C	
F4II human teeth	1	19					1			3.27
	2	67	4		1	2	1			12.27
F4III human teeth	3	3	10	2		1	2		6	3.93
	4	10							20	4.91
	5	1	1	1						0.49
F4V human teeth	6	15	3	7	11		1			6.06
	7	4	1			1				0.98
	8	3		3	4					1.64
	9								6	0.98
F4X human teeth	10	26	1							4.42
	11	34	4				4			6.87
	12	2							15	2.78
F7I human teeth	13	4		2	1	1	1	8		2.78
	14	2					3			0.82
	15	3				1		48		8.51
	16	5						21	50	12.44
	17			2				12		2.29
M8 human teeth	18	3	1	1	1	1				1.15
	19	10					1		4	2.45
	20	3	1	3	9		2		4	3.60
	21		1	2					5	1.31
M11 human teeth	22	10	1		2	1	1			2.45
	23	20	1			1	4		30	9.17
	24	2	2	2					21	4.42
Percent of total (%)		40.26	5.07	4.09	4.75	1.47	3.44	14.57	26.35	100
				45.34		8.84			44.35	

and their layered texture is clear, with some having lost their extinction cross or on which the extinction cross is blurred under polarized light (**Figures 4G, G′**). These starch grains are likely derived from the Triticeae tribe.

Type III: A total of nine granules were extracted. They were oval, with an extremely unusual hilum, and no cracks were identified. After rotation, they were still oval, and the arms of the extinction crosses were slightly bent (**Figures 4H–I′**). Their long-axis particle size ranges from 19.2 to 55.6 μm, and their average particle size is 38.5 ± 11.1 μm. Type III starch grains originate from roots and tubers.

Type IV: A total of 271 granules are classified as unidentified, but can be further subdivided into three types: IV-A, IV-B, and IV-C. Type Ⅳ-A (n = 21) are mainly the independent starch grains that are damaged and unidentified, with the middle part sunken or hollow, the extinction blurred or lost, and the basic outline identifiable. Their long-axis particle size ranged from 13.6 to 49.6 μm, and their average particle size is 25.9 ± 9.8 μm (**Figures 4J, J′**). Type Ⅳ-B (n = 89) starch grains were in an aggregated state, with a residual edge outline and collapsed internal structure. Their long-axis particle size ranged from 13.1 to 31.0 μm and their average particle size is 21.3 ± 3.4 μm (**Figures 4K, K′**). Type Ⅳ-C (n = 161) starch grains, overlapped with each other, had completely lost their independent outline, with no extinction characteristics under polarized light. Their long-axis particle size ranged from 22.8 to 37.6 μm and their average particle size is 31.1 ± 3.4 μm (**Figures 4L, L′**).

3.3 Results of Phytolith Analysis of Dental Calculus

A total of 1,085 phytoliths were extracted from 24 ancient human teeth, of which 1,019 were identifiable and included 13 types. It was observed that INTERDIGITATING η-type phytoliths from the husks of broomcorn millet (n = 7, 0.65%; **Figure 5B**), INTERDIGITATING Ω-type phytoliths from the husks of foxtail millet (n = 2, 0.18%; **Figure 5A**), and INTERDIGITATING phytoliths from the husks of the family Poaceae (n = 5, 0.46%; **Figures 5C, D**). Besides, a total of 495 ACUTE BULBOSUS phytoliths were found, which accounted for the highest proportion (45.62%; **Figure 5K**); there were also BLOCKY phytoliths (25.44%; **Figure 5M**), ELONGATE ENTIRE (8.29%; **Figure 5I**), RONDEL phytoliths (5.90%; **Figure 5E**), ELONGATE DENTATE (3.50%; **Figure 5J**), BILOBATE phytoliths of Panicoideae (1.84%; **Figure 5H**), BULLIFORM FLABELLATE phytoliths (1.20%; **Figure 5M**), CRENATE phytoliths (0.37%; **Figure 5F**), ELONGATE DENDRITIC of Pooideae (0.37%; **Figure 5G**), and TRACHEARY phytoliths (0.09%; **Figure 5N**).

3.4 Results of Starch Grain Analysis in Grinding and Cooking Experiments

The results of the grinding simulation experiments for modern foxtail and broomcorn millets, and common wheat are consistent with those of our earlier study (Ma et al., 2019). The observations and statistical results suggest that: 1) grinding causes physical

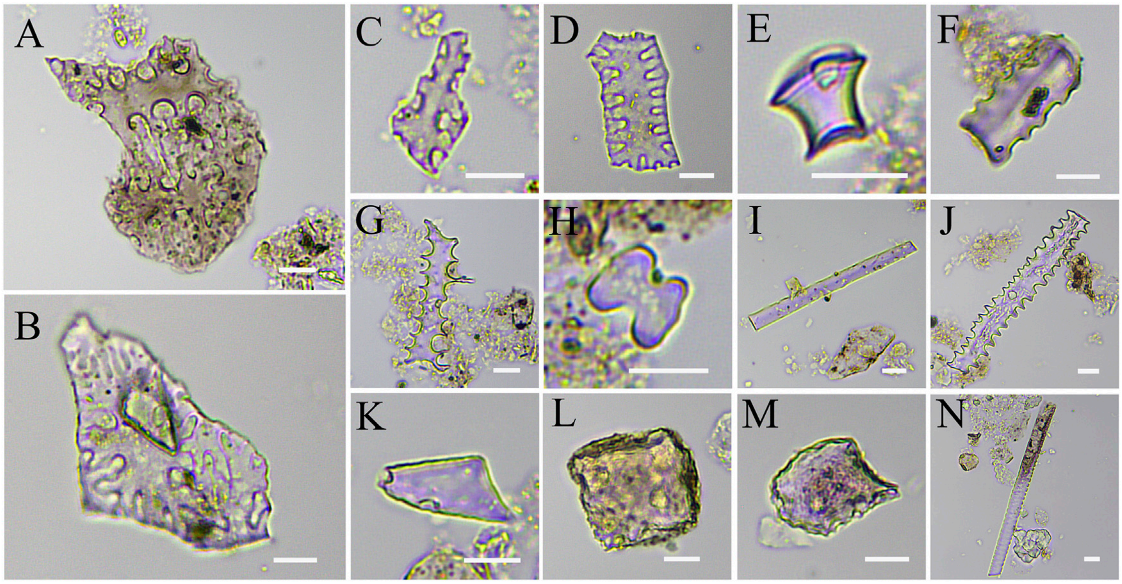

FIGURE 5 | Types of phytoliths extracted from 24 human dental calculus at the Lajia site. **(A)–(D)**, Epidermal silica layer: **(A)** INTERDIGITATING Ω-type **(B)** INTERDIGITATING η-type, **(C,D)** INTERDIGITATING; **(E)** RONDEL, **(F)** CRENATE, **(G)** ELONGATE DENDRITIC, **(H)** BILOBATE, **(I)** ELONGATE ENTIRE, **(J)** ELONGATE DENTATE, **(K)** ACUTE BULBOSUS, **(L)** BLOCKY, **(M)** BULLIFORM FLABELLATE, **(N)** TRACHEARY (Scale bar: 10 μm).

damage to some starch grains and results in changes in their appearance and shape, which is mainly reflected by the roughened surface and the edges becoming cracked and/or broken. 2) A positive correlation between the grinding strength and the content of damaged starch grains was observed (**Figures 7V–X'**). 3) Grinding causes deformation of the extinction cross of starch grains under polarized light, but the position of the hilum is generally not influenced.

The starch grain analysis results in the cooking simulation experiments of modern foxtail and common millets, and common wheat were consistent with those of earlier studies (Ge et al., 2010; Hong et al., 2013; Lu et al., 2014). It is suggested that: 1) crystal structure of starch grains becomes damaged during heating, and their central parts become sunken. Starch molecules became transparent with gradual expansion and deformation. With increased heating duration, fewer small grains were visible, and the content of large grains increased. Upon determination, the long-axis particle size of modern foxtail millet starch grains increased to 14.1, 21.3, and 31.1 μm after being heated for 5, 15, and 30 min, respectively (**Figures 7D, G, J, M, P, S**). This indicates that the grain size of both foxtail and broomcorn millet doubled throughout the cooking procedure (**Figures 7E, H, K, N, Q, T**). The starch grain size of common wheat increased to 25.44 μm and 38.91–59.36 μm after being heated for 5 and 30 min, respectively (**Figures 7F, I, L, O, R, U**). 2) Upon observation, the starch grains became transparent from the center of the hilum, and the extinction crosses were unclear after heating for 5 min, but remained mostly as independent scattered individual grains (**Figures 7D, D'–F, F′**). After 5–15 min of heating, the starch grains started to aggregate with increased transparency, and the extinction cross had completely disappeared, but the basic outline was still intact (**Figures 7G, G'–L, L′**). After heating for 15–30 min, the independent outline was lost, and the starch grains were almost completely transparent without extinction characteristics (**Figures 7M, M'–U, U′**). 3) Grinding damage and cracks were observed in a small number of starch grains (**Figures 7: Y, Y'–AA, AA'**).

4 DISCUSSION

4.1 Human Diet at the Lajia Site

Using carbon and nitrogen isotope analysis of bone collagen, it is possible to trace the absorption of food by humans or animals and back trace the ratios to the food types. Earlier studies have shown that the carbon isotope ratios of bone collagen shifted by approximately 6‰ between dietary protein and consumer bone collagen (Zhang et al., 2003). Generally, the average δ13C values of C3 and C4 plants are −26% and −13% (Zhang et al., 2003), respectively. The enrichment of δ13C values between trophic levels is very low (approximately 1%–1.5%), which is often negligible. Therefore, the average δ13C values of C3 and C4 types of food are preserved in bone collagen with an approximate 6% offset, namely −20% and 7%, respectively (Merwe, 1982). The δ13C values of seven samples from the Lajia site were relatively similar (**Table 1**). The range of $\delta^{13}C$ was −5.42 to −8.17, and the average value is −6.68 ± 1.11% (n = 7). According to a formula that converts the results of $\delta^{13}C$ determination into the percentage of C4 plants in the diet (Cai and Qiu, 1984; Zhang et al., 2003; Zhang et al., 2017), the proportion of C4 plants was calculated to be 91%. This suggests that the people of the Lajia site mainly consumed C4-based foods.

Unlike the carbon stable isotope value, there is a 3–5% enrichment between consumers for the nitrogen isotope value. It is possible to use nitrogen isotopes to determine the trophic levels of the main dietary sources (Bocherens et al., 1994). Under the condition of a lack of δ15N value of each trophic level in archaeological sites, the δ15N value of herbivores is usually around 3–7%, that of omnivores is often between 7 and 9%, and that of carnivores is over 9% (Ambrose and Katzenberg, 2000). Accordingly, the δ15N values of human samples can be assessed and the relative proportions of plants and animals can be analyzed. The average δ15N above 9.7% in the Lajia site falls in the δ15N range of carnivores, showing that animal food resources were dominant. Faunal remains, including fragmented bones from pigs, sheep, and deer, were also found at the Lajia site (Ye, 2015), indicating that humans probably consumed meat from these animals, with the influence of food globalization needing to be recognized too.

In addition, compared to the stable isotopes of four human bones from the Lajia site (range of δ13C value between −7.57% and −8.4%, average δ13C value of −7.90 ± 0.35%; range of δ15N value between 9.77 and 10.25%, average δ15N value of 9.99 ± 0.17%) (Zhang and Ye, 2016), the range and average δ13C and δ15N values of this study obtained from human teeth were almost the same. As mentioned above, the δ13C and δ15N values of seven human teeth in Lajia suggest that human diets mainly included C4-based food resources supplemented with large quantities of meat.

4.2 Type and Proportion of Plant-Based Diet at the Lajia Site

The δ13C and δ15N values of human teeth at the Lajia site suggest that human diets mainly included C4-based food resources, but they do not inform us of the type and proportion of plant-based diet. Fortunately, plant residues remain embedded in dental calculus, and studies on human teeth have provided valuable data for reconstructing a comprehensive view of human diets in the past (Henry and Piperno, 2008). More than 1,600 plant remains were recovered from 24 human teeth in this study, including an assemblage of 611 starch grains (**Table 2**), representing grains from foxtail and broomcorn millets; the tribe Triticeae, roots and tubers; and an assemblage of 1,085 phytoliths (**Figures 5**, **6**), the majority of which were from the husks of the foxtail and broomcorn millets; and stems and leaves of Pooideae and Panicoideae. Of these plant micro-remains, ~54% (n = 331) of the total starches showed damage and were large in size (**Table 2**), which is different from the characteristics of over 200 Asian species housed in modern reference collections (Yang et al., 2010; Wan et al., 2012; Yang and Perry, 2013). The combination of the damage condition of extinction characteristics and contour decoration of the ancient starches suggests that the starches extracted from human dental calculus have undergone a process of alteration.

Based on the morphological characteristics identified as resulting from grinding and boiling grass grains (foxtail and broomcorn millets, and wheat) (**Figure 7**), the grains from Lajia appear to have been processed by cooking, and there is limited evidence of grinding. First, owing to the influence of factors such as the evenness of heating and water absorption, the starch grains of the same samples showed different gelatinization during boiling. Some of the starch grains only exhibited mild gelatinization, indicating that their identification features were still present (**Figures 4C, C′, G, G'**), which were identified as being from foxtail and broomcorn millets, and the tribe Triticeae. Those starch grains which exhibited serious gelatinization (**Figures 4K, K′, I, I′**), were found upon comparison of size and shape, to be closest to those of the foxtail and broomcorn millets from the cooking simulation specimens. Additionally, in the cooking simulation experiments without grinding, a handful of starch grains exhibited signs of mechanical damage (**Figures 4J, J′**), which may have been caused by stirring and squeezing during cooking, from smashing grains during hulling, or from chewing.

Except for the phytoliths from the husks of foxtail and broomcorn millets and those from the husks of Gramineae, the phytoliths of the stems and leaves of Poaceae (98.71% of the total) extracted from the dental calculus are quite likely to represent the limited grain processing techniques of the Qijia period. The stems, leaves, and husks of foxtail and broomcorn millet, and other food plants require separation before grinding and cooking (Wang and Yang, 2015). An earlier simulation study indicated that using mortars, pestles, slabs, and mullers as dehusking and grinding tools does not completely hull the grains; the highest dehusking rates achieved in the experiments were 99 and 97.9%, respectively, whereas the husked grains were not 100% intact (Wang et al., 2013). Stone knives in the Lajia site were mainly used to harvest millet crops, but they also cut the stems or leaves of plants such as Panicoideae and related taxa during use (Ma et al., 2014). During the process of harvesting and dehusking, husks, stems, or leaves of millet and other plants were combined by ancient humans; therefore, these phytoliths were commonly found in the dental calculus of the Lajia site.

During the data analysis of plant micro-remains extracted from 24 human teeth at the Lajia site, the analytical results of starch grains and phytoliths were relatively consistent in foxtail and broomcorn millets. In addition, starch grains and phytoliths from the Poaceae subfamily were similar. The tribe Triticeae belongs to the Pooideae of the Poaceae subfamily. Furthermore, the phytoliths included BILOBATE phytoliths from the leaves of Panicoideae, Elongate Dendritic, Crenate, and Rondel phytoliths from Pooideae leaves, and Elongate Dentate phytoliths from the family Poaceae (Ge et al., 2020). In summary, the plant resources of ancient people in the Qijia cultural period included foxtail and broomcorn millets, plants from the tribe Triticeae, roots, tubers, Pooideae, and Panicoideae.

With respect to the absolute quantities and appearance frequencies of the identified plant micro-remains extracted from the dental calculus, the starch grains were mainly millet belonging to C4 plants (n = 277; 81.4%); phytoliths from the husks of millet crops were also recovered from the dental calculus (n = 9, 0.82%). These results are consistent with those of carbon isotopes (91% C4-based food resources) in this study and plant macro-remains in previous research, suggesting that the dry

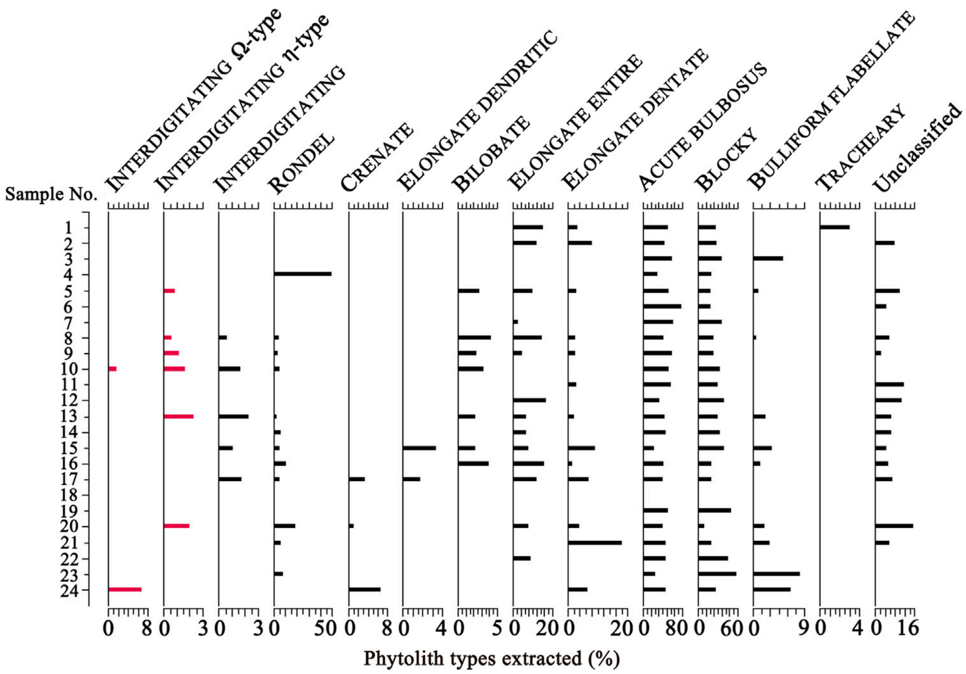

FIGURE 6 | Changes in percentages of phytolith types extracted from the 24 human dental calculus at the Lajia site.

farming structure during the Qijia cultural period was also dominated by foxtail and broomcorn millets. However, no wheat or barley was recovered from the dental calculus of the Lajia site, which is consistent with the phytolith results of earlier research on the cultural sediments of the Lajia site (Wang et al., 2015). However, two carbonized wheat remains (<0.01% of the total) were recovered from the 193 flotation assemblages of Lajia (Zhang, 2013), but were not dated using AMS (radiocarbon dating). On the one hand, these wheat remains have to be treated with caution because the quantities are small. On the other hand, we do not deny the existence of wheat crops in Lajia, but this needs to be confirmed by future research. Nevertheless, evidence from plant micro and macro-remains, and carbon isotopes suggests that wheat crops played a considerably lesser role in the human diet patterns of the inhabitants of the Lajia site during the Qijia period; that is, the degree of influence of wheat crop consumption during the spread of foods around the globe is quite limited. One of the reasons why ancient humans did not consume wheat crops is probably that the cultivation and use of wheat crops was limited by environmental factors around the Lajia site. The climate of northwest China around 4,000 years ago was different to that of the wheat origin area (Yang et al., 2021), which possibly affected its growth and cultivation. Another possible reason is due to the context of culinary choices; in West and Central Asia, there was a grinding and bread-baking tradition, while in East Asia steaming and boiling of whole grains was practiced (Liu et al., 2016b). Therefore, the adoption rate of wheat crops with hard seed coats might have been slower in this region. Wheat crops were widely used with the appearance of flour grinding technology in Northern China until the Han Dynasty (Zeng, 2005).

Notably, 9% of C3-based food resources were found in the food structures during the carbon isotope analysis of human teeth in Lajia. Meanwhile, the tribe Triticeae (n = 54; 15.9%), roots and tubers (n = 9; 2.6%) belonging to C3 plants was common in the dental calculus from human teeth (**Table 2**). Phytoliths from Panicoideae and Pooideae, belonging to C3 plants, were also found. Based on the results of the current analysis, we can confirm that the ancient inhabitants of the Lajia site ate foxtail and broomcorn millets belonging to the C4 plants of the Panicoideae subfamily. However, we cannot completely rule out the possibility that ancient people of the Lajia site ate C3 plants belonging to Panicoideae, although it is still uncertain which C3 plants of Panicoideae they may have eaten. The results show that the human diet at the Lajia site is dominated by C4-based food resources (broomcorn and foxtail millets), but they were not the only plant resources consumed. The presence of a few C3 plants from the tribe Triticeae, roots and tubers, Panicoideae, and Pooideae in this study, along with some other C3 species of *Poa* and *Oats* in an earlier flotation study (Zhang, 2013; Ye, 2015) suggest that gathering was still an important supplementary resource for the occupants of the Lajia site.

Finally, millet starch grains represented 81.4% (n = 277) of the total identified starches taken from the teeth of the human remains at this site. Four plant species were identified in the present study. The results were compared with those from the Xinzhang (4,500–4,000 cal a BP, >50%, 6), Mogou (4,000 cal a BP, 14.3%, 7), and Nancheng sites (4,000–3,600 cal a BP, 50.6%, 4) (Li et al., 2010; Ren et al., 2017; Chen et al., 2021), and the proportion of millet starch grains in Lajia was significantly higher than that at the other sites;

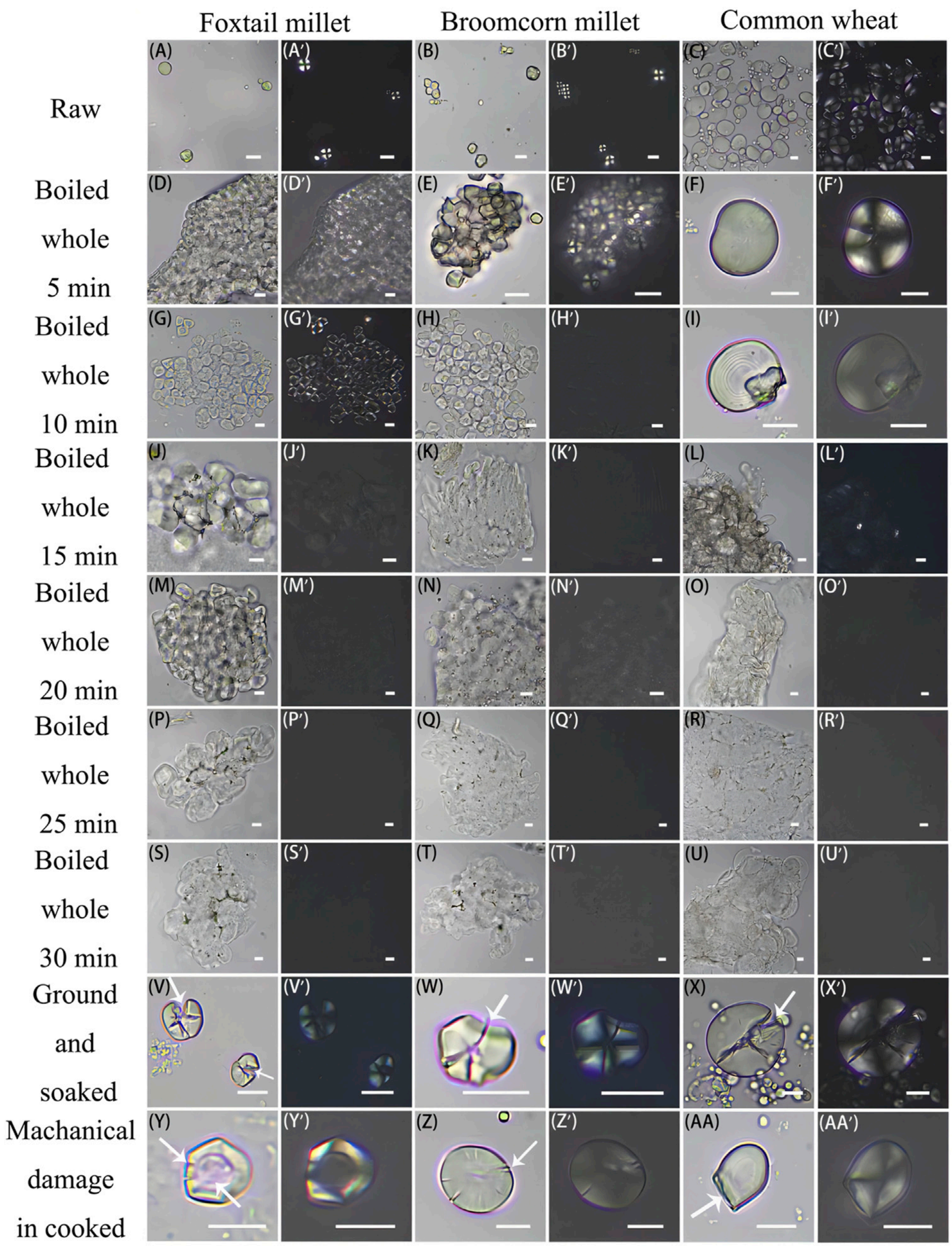

FIGURE 7 | Starch grain specimens for simulation experiments of cooking and grinding. Different characteristics of starch grains from three Poaceae plants, both raw and after processing. **(A, A' - AA, AA′)** Photos were taken under bright field (left) and polarized light (right); Arrow indicates the damage characteristics of starch grains; Scale bar: 10 μm.

simultaneously, the number and proportion of plant species was lower. The composition of the plant resources may have been driven by a relatively extreme local environment. The Lajia site is located on the eastern edge of the Qinghai-Tibet Plateau climatic zone, with a low annual average temperature and fragile agricultural ecological environment. In addition, the climate tended to be dry and cold after 4,500 cal. BP in this region (Gong et al., 2011). This means that the number of plant resources that were collected in the surrounding areas and animal resources available for hunting would be further reduced from their currently sparse modern-day levels. In addition, the Lajia site was a settlement society, observed from the rich remains including house foundations, ash pits, altars, and community squares excavated from the site (Ye, 2002), which restricted the scope of activities of the ancient humans. Therefore, this region did not have sufficient plant resources to allow a community to survive through foraging alone. This might explain the enhanced millet cultivation and/or a greater reliance on hunting and sheep rearing.

5 CONCLUSION

The results of δ13C and δ15N values in the seven human teeth and plant micro-remains from the 24 dental calculus at the Lajia site indicate that: 1) human diets mainly consisted of C4-based food with a few C3-based foods originating from the combination of millet and plants gathered, and these ancient humans had a high-protein diet possible due to their hunting activities. Based on other archaeological evidence at the site, it is probable that pigs, sheep, and deer were the main sources of this protein. 2) Based on the analysis of ancient starch grains and phytoliths, the diet of the occupants of the Lajia site included foxtail and broomcorn millet, the tribe Triticeae, roots and tubers, and plants from Pooideae of the Poaceae subfamily. Combined with data from human teeth and dental calculus, a predominantly millet-based lifestyle occurred at the Lajia site, but gathering factors are still evident. Regarding the impact of food globalization, the dietary patterns of ancient humans at the Lajia site were probably affected by the introduction of sheep, but no direct evidence of wheat crops or their consumption was observed. Finally, the results of our simulation experiment highlight the need to consider the enlargement of size when identifying different species of cooked or processed starch grains.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZM and XH designed the study and prepared the manuscript. ZM, ZL, and SL collected the samples and conducted the experiments. ZM, XH, and SL analyzed the data. ZM, XH, ZL, and MY contributed to the discussion and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.884856/full#supplementary-material

REFERENCES

Ambrose, S. H., and Katzenberg, M. A. (2000). *Biogeochemical Approaches to Paleodietary Analysis*. New York: Kluwer Academic/Plenum Publisher.

Anthony, D. W. (2010). *The Horse, the Wheel, and Language: How Bronze Age Riders from the Eurasian Steppes Shaped the Modern World*. Princeton: Princeton University Press.

Barton, H., and Torrence, R. (2015). Cooking up Recipes for Ancient Starch: Assessing Current Methodologies and Looking to the Future. *J. Archaeol. Sci.* 56, 194–201. doi:10.1016/j.jas.2015.02.031

Beaumont, J., Gledhill, A., Lee-Thorp, J., and Montgomery, J. (2012). Childhood Diet: a Closer Examination of the Evidence from Dental Tissues Using Stable Isotope Analysis of Incremental Human Dentine*. *Archaeometry* 55 (2), 277–295. doi:10.1111/j.1475-4754.2012.00682.x

Bocherens, H., Fizet, M., and Mariotti, A. (1994). Diet, Physiology and Ecology of Fossil Mammals as Inferred from Stable Carbon and Nitrogen Isotope Biogeochemistry: Implications for Pleistocene Bears. *Palaeogeogrn Palaeocl* 107 (3-4), 213–225. doi:10.1016/0031-0182(94)90095-7

Bronk Ramsey, C. (2009). Bayesian Analysis of Radiocarbon Dates. *Radiocarbon* 51 (1), 337–360. doi:10.1017/S0033822200033865

Cai, L. Z., and Qiu, S. L. (1984). Determination of Carbon-13 and Study of Ancient Recipes. *Archaeol* 10, 949

Chen, F. H., Dong, G. H., Zhang, D. J., Liu, X. Y., Jia, X., An, C. B., et al. (2015). Agriculture Facilitated Permanent Human Occupation of the Tibetan Plateau after 3600 B.P. *Science* 347 (6219), 248–250. doi:10.1126/science.1259172

Chen, T., Hou, L., Jiang, H., Wu, Y., and Henry, A. G. (2021). Starch Grains from Human Teeth Reveal the Plant Consumption of Proto-Shang People (C. 2000-1600 BC) from Nancheng Site, Hebei, China. *Archaeol. Anthropol. Sci.* 13, 153. doi:10.1007/s12520-021-01416-y

Costantini, L. (2008). The First Farmers in Western Pakistan: The Evidence of the Neolithic Agro-Pastoral Settlement of Mehrgarh. *Pragdhara* 18, 167

Crawford, G., Underhill, A., Zhao, Z., Lee, G. A., Feinman, G., Nicholas, L., et al. (2005). Late Neolithic Plant Remains from Northern China: Preliminary Results from Liangchengzhen, Shandong. *Curr. Anthropol.* 46 (2), 309–317. doi:10.1086/428788

Crawford, G. W. (2006). "East Asian Plant Domestication," in *In Archaeology of Asia*. Editor Y. Miriam (Malden: Blackwell publishing).

Deng, Z., Qin, L., Gao, Y., Weisskopf, A. R., Zhang, C., and Fuller, D. Q. (2015). From Early Domesticated Rice of the Middle Yangtze Basin to Millet, Rice and Wheat Agriculture: Archaeobotanical Macro-Remains from Baligang, Nanyang Basin, Central China (6700-5000 BC). *PloS ONE* 10 (10), e0139885. doi:10.1371/journal.pone.0139885

DeNiro, M. J. (1985). Postmortem Preservation and Alteration of *In Vivo* Bone Collagen Isotope Ratios in Relation to Palaeodietary Reconstruction. *Nature* 317 (6040), 806–809. doi:10.1038/317806a0

Dodson, J. R., Li, X., Zhou, X., Zhao, K., Sun, N., and Atahan, P. (2013). Origin and Spread of Wheat in China. *Quat. Sci. Rev.* 72, 108–111. doi:10.1016/j.quascirev.2013.04.021

Dong, G., Yang, Y., Han, J., Wang, H., and Chen, F. (2017). Exploring the History of Cultural Exchange in Prehistoric Eurasia from the Perspectives of Crop Diffusion and Consumption. *Sci. China Earth Sci.* 60 (5), 1110–1123. doi:10.1007/s11430-016-9037-x

Du, X. M., and Chen, Q. D. (2016). The Gathering Economies from the Perspective of Economic Anthropology——Investigation and Analysis from the Dulong River Canyon. *ethno-national Stud.* 1, 75

Du, Z. W., Wang, Q. Q., Yu, M. Z., Du, W., Zhen, Q., Ma, Q., et al. (2019). A preliminary report on the excavation of Xindian culture remains from 2015 to 2016 at the Lajia site in Minhe County, Qinghai. *Archaeol* 6, 3

Flad, R., Li Shuicheng, S. C., Wu Xiaohong, X. H., and Zhao Zhijun, Z. J. (2010). Early wheat in China: Results from new studies at Donghuishan in the Hexi Corridor. *Holocene* 20 (6), 955–965. doi:10.1177/0959683609358914

Frachetti, M. D., Spengler, R. N., Fritz, G. J., and Mar'yashev, A. N. (2010). Earliest direct evidence for broomcorn millet and wheat in the central eurasian steppe region. *Antiquity* 84 (326), 993–1010. doi:10.1017/S0003598X0006703X

Ge, W., Liu, L., Chen, X. C., and Jin, Z. Y. (2010). An experimental study on starch grain damage during food processing and the application in archaeology. *Archaelogy* 7, 77

Ge, Y., Lu, H., Zhang, J., Wang, C., and Gao, X. (2020). Phytoliths in Inflorescence Bracts: Preliminary Results of an Investigation on Common Panicoideae Plants in China. *Front. Plant Sci.* 10, 1736. doi:10.3389/fpls.2019.01736

Gignoux, C. R., Henn, B. M., and Mountain, J. L. (2011). Rapid, global demographic expansions after the origins of agriculture. *Proc. Natl. Acad. Sci. U.S.A.* 108, 6044–6049. doi:10.1073/pnas.0914274108

Gong, Y., Yang, Y., Ferguson, D. K., Tao, D., Li, W., Wang, C., et al. (2011). Investigation of ancient noodles, cakes, and millet at the Subeixi Site, Xinjiang, China. *J. Archaeol. Sci.* 38 (2), 470–479. doi:10.1016/j.jas.2010.10.006

Han, J. Y. (2019). The Development and Evolution of Qijia Culture: Cultural Interaction and Eurasian Context. *Cult. Relics* 7, 60–65. (in Chinese). doi:10.13619/j.cnki.cn11-1532/k.2019.07.004

Harris, D. R., Masson, V. M., Berezkin, Y. E., Charles, M. P., Gosden, C., Hillman, G. C., et al. (1993). Investigating early agriculture in Central Asia: new research at Jeitun, Turkmenistan. *Antiquity* 67 (225), 324–338. doi:10.1017/S0003598X00045385

Harris, D. R. (1972). The origins of agriculture in the tropics. *Am. Sci.* 60 (2), 180

Henry, A. G., and Piperno, D. R. (2008). Using plant microfossils from dental calculus to recover human diet: a case study from Tell al-Raqā'i, Syria. *J. Archaeol. Sci.* 35 (7), 1943–1950. doi:10.1016/j.jas.2007.12.005

Henry, A. G., Ungar, P. S., Passey, B. H., Sponheimer, M., Rossouw, L., Bamford, M., et al. (2012). The diet of australopithecus sediba. *Nature* 487 (7405), 90–93. doi:10.1038/nature11185

Hong, J., Zheng, X. L., and Liu, C. (2013). Study on particle and gel properties of total,A-type,B-type starch with different thermal damage degrees. *Cereals & Oils* 26 (8), 8

Hovsepyan, R., and Willcox, G. (2008). The earliest finds of cultivated plants in Armenia: evidence from charred remains and crop processing residues in pisé from the Neolithic settlements of Aratashen and Aknashen. *Veget Hist. Archaeobot* 17, 63–71. doi:10.1007/s00334-008-0158-6

Jia, X. (2012). *Cultural Evolution Process and Plant Remains during Neolithic-Bronze Age in Northeast Qinghai Province*. Lanzhou (Chinese): Lanzhou University. [Ph.D. dissertation].

Jones, M., Hunt, H., Kneale, C., Lightfoot, E., Lister, D., Liu, X., et al. (2016). Food globalisation in prehistory: The agrarian foundations of an interconnected continent. *Jba* 4, 73–87. doi:10.5871/jba/004.073

Jones, M., Hunt, H., Lightfoot, E., Lister, D., Liu, X., and Motuzaite-Matuzeviciute, G. (2011). Food globalization in prehistory. *World Archaeol.* 43 (3), 665–675. doi:10.1080/00438243.2011.624764

Kuzmina, E. E. (2008). *The Prehistory of the Silk Road*. Philadelphia: University of Pennsylvania Press.

Lev-Yadun, S., Gopher, A., and Abbo, S. (2000). The Cradle of Agriculture. *Science* 288 (5471), 1602–1603. doi:10.1126/science.288.5471.1602

Li, M., Yang, X., Wang, H., Wang, Q., Jia, X., and Ge, Q. (2010). Starch grains from dental calculus reveal ancient plant foodstuffs at Chenqimogou site, Gansu Province. *Sci. China Earth Sci.* 53 (5), 694–699. doi:10.1007/s11430-010-0052-9

Lieverse, A. R. (1999). Diet and the aetiology of dental calculus *Int. J. Osteoarchaeol.* 9, 2192–2232. doi:10.1002/(SICI)1099-1212(199907/08)9:4%3C219::AID-OA475%3E3.0

Liu, L., Chen, X. C., and Zhao, H. (2013). Analysis on the function of the grinding stone unearthed from the Zhaigen and Bangou sites during the late Peiligang culture period in Mengjin, Henan. *Cult. Relics. Cent. China.* 5, 76

Liu, L., Field, J., Fullagar, R., Bestel, S., Chen, X., and Ma, X. (2015). What did grinding stones grind? New light on Early Neolithic subsistence economy in the Middle Yellow River Valley, China. *Antiquity* 84 (325), 816–833. doi:10.1017/S0003598X00100249

Liu, X., Jones, P. J., Motuzaite Matuzeviciute, G., Hunt, H. V., Lister, D. L., An, T., et al. (2019). From ecological opportunism to multi-cropping: Mapping food globalisation in prehistory. *Quat. Sci. Rev.* 206, 21–28. doi:10.1016/j.quascirev.2018.12.017

Liu, X., Lightfoot, E., O'Connell, T. C., Wang, H., Li, S., Zhou, L., et al. (2014). From necessity to choice: dietary revolutions in west China in the second millennium BC. *World Archaeol.* 46 (5), 661–680. doi:10.1080/00438243.2014.953706

Liu, X., Lister, D. L., Zhao, Z., Petrie, C. A., Zeng, X., Jones, P. J., et al. (2017). Journey to the east: Diverse routes and variable flowering times for wheat and barley en route to prehistoric China. *PLoS ONE* 12 (11), e0187405. doi:10.1371/journal.pone.0187405

Liu, X., Lister, D. L., Zhao, Z., Staff, R. A., Jones, P. J., Zhou, L., et al. (2016a). The virtues of small grain size: Potential pathways to a distinguishing feature of Asian wheats. *Quat. Int.* 426, 107–119. doi:10.1016/j.quaint.2016.02.059

Liu, X., Reid, R. E., Lightfoot, E., Matuzeviciute, G. M., and Jones, M. K. (2016b). Radical change and dietary conservatism: Mixing model estimates of human diets along the Inner Asia and China's mountain corridors. *Holocene* 26, 1556–1565. doi:10.1177/0959683616646842

Lösch, S., Grupe, G., and Peters, J. (2006). Stable isotopes and dietary adaptations in humans and animals at pre-pottery Neolithic Nevallı Çori, southeast Anatolia. *Am. J. Phys. Anthropol.* 131 (2), 181–193. doi:10.1002/ajpa.20395

Lü, H., Li, Y., Zhang, J., Yang, X., Ye, M., Li, Q., et al. (2014). Component and simulation of the 4,000-year-old noodles excavated from the archaeological site of Lajia in Qinghai, China. *Chin. Sci. Bull.* 59 (35), 5136–5152. doi:10.1007/s11434-014-0663-1

Lu, H., and Liu, K.-B. (2003). Morphological variations of lobate phytoliths from grasses in China and the south-eastern United States. *Divers Distrib.* 9 (1), 73–87. doi:10.1046/j.1472-4642.2003.00166.x

Lu, H. (2017). New methods and progress in research on the origins and evolution of prehistoric agriculture in China. *Sci. China Earth Sci.* 60, 2141–2159. doi:10.1007/s11430-017-9145-2

Lu, H., Zhang, J., Liu, K.-b., Wu, N., Li, Y., Zhou, K., et al. (2009a). Earliest domestication of common millet (Panicum miliaceum) in East Asia extended to 10,000 years ago. *Proc. Natl. Acad. Sci. U.S.A.* 106 (18), 7367–7372. doi:10.1073pnas.090015810610.1073/pnas.0900158106

Lu, H., Zhang, J., Wu, N., Liu, K.-b., Xu, D., and Li, Q. (2009b). Phytoliths analysis for the discrimination of foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*). *PLoS ONE* 4 (2), e4448. doi:10.1371/journal.pone.0004448

Ma, Z., Li, Q., Huan, X., Yang, X., Zheng, J., and Ye, M. (2014). Plant microremains provide direct evidence for the functions of stone knives from the Lajia site, northwestern China. *Chin. Sci. Bull.* 59, 1151–1158. doi:10.1007/s11434-014-0174-0

Ma, Z., Perry, L., Li, Q., and Yang, X. (2019). Morphological changes in starch grains after dehusking and grinding with stone tools. *Sci. Rep.* 9 (1), 2355. doi:10.1038/s41598-019-38758-6

Merwe, N. J. (1982). Carbon Isotopes, Photosynthesis, and Archaeology: Different pathways of photosynthesis cause characteristic changes in carbon isotope ratios that make possible the study of prehistoric human diets. *Am. Sci.* 70 (6), 596–606. doi:10.2307/27851731

Moors, A. M. T. (2016). The impact of accelerator dating at the early village of Abu Hureyra on the Euphrates. *Radiocarbon* 34 (3), 850

International Committee for Phytolith Taxonomy (ICPT)Neumann, K., Neumann, K., Strömberg, C. A. E., Ball, T., Albert, R. M., Vrydaghs, L., et al. (2019). International Code for Phytolith Nomenclature (ICPN) 2.0. *Ann. Bot.* 124, 189–199. doi:10.1093/aob/mcz064

Piperno, D. R. (2006). *Phytolith: A Comprehensive Guide for Archaeologist and Paleoecologists*. Lanham MD: Altamira Press.

Reimer, P. J., Austin, W. E. N., Bard, E., Bayliss, A., Blackwell, P. G., Bronk Ramsey, C., et al. (2020). The IntCal20 Northern Hemisphere Radiocarbon Age Calibration Curve (0-55 cal kBP). *Radiocarbon* 62 (4), 725–757. doi:10.1017/RDC.2020.41

Ren, G. Y., Zhao, J., and Hou, L. L. (2017). Analysis of starch granules in human dental calculus at the Xinzhang site in Yuanping. *World. Antiq.* 2, 20

Riehl, S., Zeidi, M., and Conard, N. J. (2013). Emergence of agriculture in the foothills of the Zagros Mountains of Iran. *Science* 341 (6141), 65–67. doi:10.1126/science.1236743

Rindos, D. (1986). *The Origins of Agriculture: An Evolutionary Perspective*. Academic Press.

Sheng, P. F., Shang, X., and Zhang, P. C. (2020). A preliminary study on crop selection of ancestors from late Longshan to early Xia Dynasty in Yulin area. *Archaeol. Cult. Relic.* 2, 114

Spengler, R., Frachetti, M., Doumani, P., Rouse, L., Cerasetti, B., Bullion, E., et al. (2014). Early agriculture and crop transmission among Bronze Age mobile pastoralists of Central Eurasia. *Proc. R. Soc. B* 281 (1783), 20133382. doi:10.1098/rspb.2013.3382

Spengler, R. N., and Willcox, G. (2013). Archaeobotanical results from Sarazm, Tajikistan, an Early Bronze Age Settlement on the edge: Agriculture and exchange. *Environ. Archaeol.* 18 (3), 211–221. doi:10.1179/1749631413Y.0000000008

Stevens, C. J., Murphy, C., Roberts, R., Lucas, L., Silva, F., and Fuller, D. Q. (2016). Between China and South Asia: A middle Asian corridor of crop dispersal and agricultural innovation in the Bronze Age. *Holocene* 26 (10), 1541–1555. doi:10.1177/0959683616650268

Tao, D., Wu, Y., Guo, Z., Hill, D. V., and Wang, C. (2011). Starch grain analysis for groundstone tools from Neolithic Baiyinchanghan site: implications for their function in Northeast China. *J. Archaeol. Sci.* 38 (12), 3577–3583. doi:10.1016/j.jas.2011.08.028

Tengberg, M. (1999). Crop husbandry at Miri Qalat Makran, SW Pakistan (4000-2000 B.C.). *Veget Hist. Archaebot* 8 (1), 3–12. doi:10.1007/BF02042836

Wan, Z. W., Yang, X. Y., Li, M. Q., Ma, Z. K., and Ge, Q. S. (2012). China modern starch grain database. *Quat. Sci.* 32 (2), 371–372. doi:10.3969/j.issn.1001-7410.2012.02.24

Wang, C., Lu, H. Y., Zhang, J. P., Ye, M. L., and Cai, L. H. (2015). Phytolith evidence of millet agriculture in the late Neolithic archaeological site of Lajia, Northwestern China. *Quat. Sci.* 35 (1), 1–9. doi:10.3969/j.issn.1001-7410.2015.01

Wang, C., Lu, H., Zhang, J., He, K., and Huan, X. (2016). Macro-process of past plant subsistence from the Upper Paleolithic to Middle Neolithic in China: a quantitative analysis of multi-archaeobotanical data. *PLoS ONE* 11 (2), e0148136. doi:10.1371/journal.pone.0148136

Wang, Q., Kamijyou, O., and Luan, F. S. (2013). The Study of Stone Saddle Querns and Rollers at Beiqian Site, Jimo, Shandong Province. *Dongfang Archaeol.* 1, 154

Wang, Q. Q., and Zhen, Q. (2021). Analysis on subsistence composition and structure of Qijia culture in Qinghai province, China. *J. Qinghai Norm. Univ. Soc. Sci.* 43 (2), 77

Wang, Q., and Yang, H. Y. (2015). Several problems in the study of grinding stone tools. *Wenwu Chunqiu* 2, 3

Wang, Y. J., and Lu, H. Y. (1993). *The Study of Phytolith and its Application*. Beijing: China Ocean Press

Wright, K. I. (1994). Ground-Stone Tools and Hunter-Gatherer Subsistence in Southwest Asia: Implications for the Transition to Farming. *Am. Antiq.* 59 (2), 238–263. doi:10.2307/281929

Wu, W. W. (2015). Preliminary analysis of the economics of production at the Chahai site in Fuxin, Liaoning: Indications from the results of the analysis of stone tools starch granules. *Agricul Archaeol.* 3, 1

Wu, W. W. (2014). *Research on the Subsistence of Peiligang Period in North China*. Jinan (Chinese): Shandong University. [Ph.D. dissertation].

Wu, W. W., Yang, X. Y., and Jin, G. Y. (2011). The application of starch analysis in archaeology: Case studies on Yuezhuang and other sites. *Dongfang Archaeol.* 1, 330

Wu, Y., Wang, C., and Hill, D. V. (2012). The transformation of phytolith morphology as the result of their exposure to high temperature. *Microsc. Res. Tech.* 75 (7), 852–855. doi:10.1002/jemt.22004

Yan, W. M. (1982). The origin of rice farming in China. *Agricul Archaeol.* 1, 19

Yang, B., Qin, C., Bräuning, A., Osborn, T. J., Trouet, V., Ljungqvist, F. C., et al. (2021). Long-Term Decrease in Asian Monsoon Rainfall and Abrupt Climate Change Events Over the Past 6,700 Years. *Proc. Natl. Acad. Sci. U.S.A.* 118 (30), e2102007118. doi:10.1073/pnas.2102007118

Yang, X., Ma, Z., Wang, T., Perry, L., Li, Q., Huan, X., et al. (2014). Starch grain evidence reveals early pottery function cooking plant foods in North China. *Chin. Sci. Bull.* 59 (32), 4352–4358. doi:10.1007/s11434-014-0500-6

Yang, X., and Perry, L. (2013). Identification of ancient starch grains from the tribe Triticeae in the North China Plain. *J. Archaeol. Sci.* 40 (8), 3170–3177. doi:10.1016/j.jas.2013.04.004

Yang, X., Wan, Z., Perry, L., Lu, H., Wang, Q., Zhao, C., et al. (2012a). Early millet use in northern China. *Proc. Natl. Acad. Sci. U.S.A.* 109 (10), 3726–3730. doi:10.1073/pnas.1115430109

Yang, X. Y., Kong, Z. C., Liu, C. J., and Ge, Q. S. (2010). Morphological characteristics of starch grains of millets and their wild relatives in North China. *Quat. Sci.* 30 (2), 364–371. doi:10.3969/j.issn.1001-7410.2010.02.13

Yang, X., Zhang, J., Perry, L., Ma, Z., Wan, Z., Li, M., et al. (2012b). From the modern to the archaeological: Starch grains from millets and their wild relatives in China. *J. Archaeol. Sci.* 39 (2), 247–254. doi:10.1016/j.jas.2011.09.001

Ye, M. L. (2015). Ecological adaptation of agricultural development in Qijia culture: A preliminary study on primitive grass farming—Taking the Lajia site in Qinghai as an example. *Agricul Archaeol.* 6, 19

Ye, M. L. (2002). The excavation of the Lajia Site in Minhe , Qinghai. *Archaeol* 7, 3

Zeng, X. S. (2005). On the spread of wheat in ancient China. *J. Chin. Diet. Cul.* 1 (1), 99

Zhang, C. (2013). *Floating Selection Carbonized Plants Result and Analysis at the Lajia Site in Minhe County, Qinghai Province*. Xi'an (Chinese): Northwest University. [Master's thesis].

Zhang, G. L., Wang, S. Z., Ferguson, D. K., Yang, Y. M., Liu, X. Y., and Jiang, H. E. (2015a). Ancient plant use and palaeoenvironmental analysis at the Gumugou Cemetery, Xinjiang, China: Implication from desiccated plantremains. *Archaeol. Anthropol. Sci.* 9 (2), 145–152. doi:10.1007/s12520-015-0246-3

Zhang, X. L., Qiu, S. H., Zhong, J., Huang, Y., Lu, X. F., and Du, H. (2015b). A report on the dating of radiocarbon (41). *Archaeol* 7, 107.

Zhang, X. L., Wang, J. X., Xian, Z. Q., and Qiu, S. H. (2003). Studies on ancient human diet. *Archaeol* 2, 62

Zhang, X. L., Xu, D. G., He, Y. L., and Qiu, S. H. (2017). Analysis of Carbon and Nitrogen Stable Isotopes of Human Bones unearthed from M54 at Yinxu Site. *Archaeol* 3, 100

Zhang, X. L., and Ye, M. L. (2016). Preliminary discussion on the diet of ancestors at the Lajia site: carbon and nitrogen stable isotope analysis of human bones unearthed from the disaster site of the Lajia site. *Cult Relics South. China* 4, 197–202. doi:10.3969/j.issn.1004-6275.2016.04.027

Zhang, X. L., Ye, M. L., Qiu, S. L., and Zhong, J. (2014). ^{14}C dating and Preliminary Analysis of Lajia Site in Minhe Country. *Archaeol* 11, 91–104. (in Chinese).

Zhang, Y. H., Weng, Y., Yao, L., Zhang, J. Z., Zhou, Y. J., Fang, F., et al. (2011). Identification and analysis of starch granules on the surface of the slabs from peiligang site. *Quat. Sci.* 31 (5), 891–899. doi:10.3969/j.issn.1001-7410.2011.05.14

Zhao, K., Li, X., Zhou, X., Dodson, J., and Ji, M. (2013). Impact of agriculture on an oasis landscape during the late Holocene: Palynological evidence from the Xintala site in Xinjiang, NW China. *Quat. Int.* 311, 81–86. doi:10.1016/j.quaint.2013.06.035

Zhao, Z. J. (2015). Research on the introduction of wheat into China--archaeobotany data. *Cult Relics South. China* 3, 44

Zhao, Z. J. (2014). The process of origin of agriculture in China: Archaeological evidence from flotation results. *Quat. Sci.* 34 (1), 73–84. doi:10.3969/j.issn.1001-7410.2014.05.14

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Pottery Use and Starchy Foods During the Shuangdun Culture (ca.7.3–6.8 Ka BP) in the Middle Catchment of the Huai River, China

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The use of rice and millet has been uncovered at a few archaeological sites associated with the Shuangdun Culture (ca. 7.3–6.8 ka BP) in the middle catchment of the Huai River, China. Nevertheless, the consumption of rice, millet, and other types of plant foods at other contemporaneous sites in the same region still needs supporting information from more case studies. This article examines pottery sherds (n = 21) excavated from another representative Shuangdun Culture site at Houjiazhai with starch grain analysis. Varied types of pottery vessels contain starch remains from rice (*Oryza sativa*), foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*), Job's tears (*Coix lacryma-jobi*), Triticeae, roots of snake gourd (*Trichosanthes kirilowii*), lotus root (*Nelumbo nucifera*), Chinese yam (*Dioscorea panthainca*), lily bulbs (*Lilium* sp.), acorns (*Quercus* sp.), and beans (*Vigna* sp. or/and *Vicia* sp.). Further quantitative analysis of the starch data indicates that cereals, including rice and millet, were predominantly consumed in the pottery vessels. Changes and continuities of culinary practices are also present at Houjiazhai, which are reflected in the different pottery assemblages as well as the utilized plant species in different occupation phases at the site. Combining previous studies, this article also reveals the differences and similarities of the past population in choosing their plant food resources during the period of Shuangdun Culture in the middle catchment of the Huai River, China.

Keywords: starch grain, plant food, Shuangdun Culture, Huai River, Houjiazhai

INTRODUCTION

China was one of the world's primary centers of independent agricultural development. The most thoroughly studied early agricultural societies in China are located along the Yangtze and Yellow River valleys, which provide some of the oldest solid evidence for formalized rice (*Oryza sativa*) and millet (*Setaria italica* and *Panicum miliaceum*) farming, respectively (Jiang and Liu, 2006; Yunfei and Jiang, 2009; Yang et al., 2012). Even though it is commonly acknowledged that rice and millet farming had spread northward and southward afterward in China, the exact routes regarding the diffusions of these crops over time and space remain an area of active research (Zhang, et al., 2010; Fuller, 2011; Ma, et al., 2016; Yang, et al., 2018; Huan, et al., 2022; Long, et al., 2022).

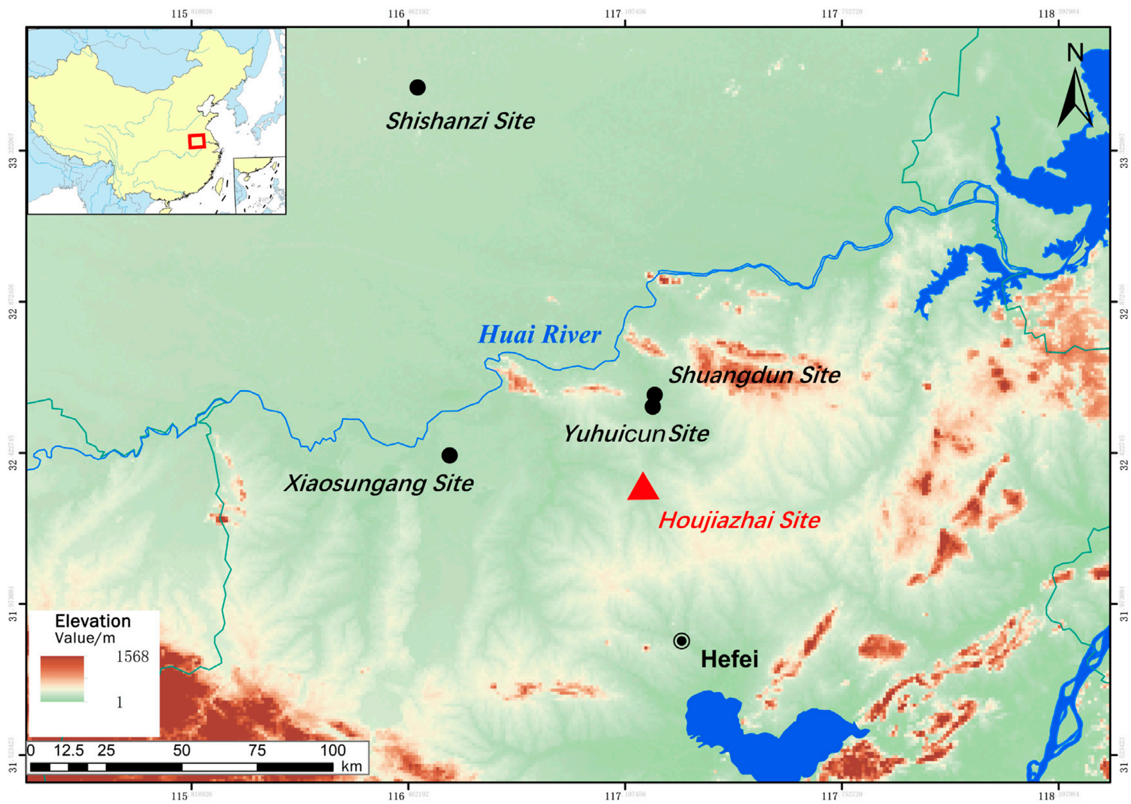

FIGURE 1 | The locations of the site of Houjiazhai and other sites associated with the Shuangdun Culture in the Middle Huai river valley.

The Huai River drains the plain between the Yangtze and Yellow Rivers and has formed a transitional climatic zone between northern and southern China (**Figure 1**). Archaeobotanists have a continuing interest in the catchment of the Huai River (CHR) not only due to its diverse prehistoric cultures but also due to some of the early significant occurrences of rice and millet observed, starting from the early to middle Neolithic period. In the upper CHR, for instance, macrobotanical remains of rice were found at the site of Jiahu (7000–5500 BC) (Zhang and Wang, 1998). In the same region, phytolith and macrobotanical remains from the sites of Tanghu and Zhuzhai revealed that mixed farming of rice and millet had started there during the middle Peiligang Culture period (c. 7,924 ± 41 to 7,640 ± 45 cal. BP) (Zhang, et al., 2012; Bestel, et al., 2018; Wang, et al., 2018). In the middle and lower CHR, several recent studies also yielded remains from either rice or millet (Luo, et al., 2016; Yang, et al., 2016; Luo, et al., 2019; Qiu, et al., 2022). These findings together make the CHR a crucial region for investigating the dispersal of rice and millet in Neolithic central-eastern China.

The present study focuses on the Shuangdun Culture (ca. 7.3–6.8 ka BP) developed in the middle CHR. This culture was named after the site of Shuangdun, which was first excavated in the year 1986 (Kan and Zhou, 2007). In addition to Shuangdun, several other excavated archaeological sites attributed to the same Shuangdun Culture comprise Shishanzi (Jia, 1992), Yuhui (Zhang et al., 2020), and Xiaosungang (Yang et al., 2015). Many of the Shuangdun Culture sites are characterized by their carved symbols on pottery vessels, which are valuable for investigating Neolithic ways of life and the origin of Chinese characters (Huang, 2012; Xu, 2007, 2008).

To understand the prehistoric use of plants during the Shuangdun Culture period, researchers have studied soil samples, stone tools, and potsherds from the site of Shuangdun through phytolith analysis or starch grain analysis (Dong, 2013; Dong, et al., 2014; Cheng, et al., 2016; Luo, et al., 2016; Yao, 2016; Xuan, 2017). The results from these studies indicate that rice and millet had already appeared at some of the Shuangdun Culture sites (**Supplementary Table S1**). Nevertheless, whether rice and millet were commonly cultivated in the entire middle catchment of the Huai River still needs supporting data from more case studies, especially taking into account that some of the previous archaeobotanical work carried out at the Shuangdun Culture sites were either based on a limited number of artefacts or a single analytical method. For instance, only 10 grinding tools were chosen for starch grain analysis at the site of Shishanzi (Dong, et al., 2014). The site of Xiaosungang was studied from the perspective of macrobotanical plant remains (Cheng, et al., 2016). Under such circumstances, potsherds from another Shuangdun Culture site of Houjiazhai were selected in the present study for starch grain analysis. The main objective of the present study is to add more data to enrich the discussion regarding plant foods consumed by the early farming groups associated with the Shuangdun Culture.

TABLE 1 | Carbon-14 dates and dendrochronologically corrected dates of bone samples excavated at Houjiazhai.

Code of laboratory	Sample provenance	Sample material dated	^{14}C date (BP)	Calibrated dates (BC)	
				1σ (68.2%)	2σ (95.4%)
ZK-2185	④	bone	6,350±110	5,467–5,402 (0.26) 5,388–5,225 (0.74)	5,517–5,046 (1)
ZK-2184	③	bone	6,260±90	5,322–5,201 (0.61) 5,175–5,071 (0.39)	5,466–5,434 (0.03) 5,429–5,405 (0.02) 5,385–4,998 (0.95)

The site of Houjiazhai is in the Village of Yuanzhuang, Dingyuan City, Anhui Province, about 60 km south of the Huai River (**Figure 1**). It is a Neolithic platform–shaped site with an area of more than 30,000 square meters. The site was discovered in the spring of 1977 and went through two excavation seasons in the spring of 1985 and the autumn of 1986 (Tang, et al., 2019). An area of 375 square meters has been revealed so far. Based on the material culture and radiocarbon dating of two bone samples unearthed at Houjiazhai (**Table 1**), it has been proposed that the site occupation lasted through two different phases: Phase I (ca. 7.3–6.8 ka BP) and Phase II (ca. 6.2–5.6 ka BP) (Tang, et al., 2019; Luo, et al., 2020).

The pottery assemblage retrieved from the excavations has been carefully classified according to their shapes, sizes, and tempered materials (Tang, et al., 2019). The changes in the pottery assemblages in Phase I and Phase II include the following: 1) cauldron vessels (*fu* in Chinese, a type of cooking vessel with fat bellies and without standing feet), which exclusively appeared in Phase I (n = 28); 2) the number of tripods (*ding* in Chinese, a type of cooking vessel with standing feet) and *dou* vessels (a type of serving vessel with a base) increased dramatically in Phase II, from 7 to 39 and 9 to 45, respectively; 3) the pottery vessels were decorated with simple carved signs in Phase I compared to those elaborately painted with geometric patterns in Phase II; and 4) the technological changes in terms of materials used for pottery tempering. For instance, *ding* vessels from Phase I were shell tempered, while they were shell or sand tempered in Phase II; *dou* vessels were tempered with plants (unidentified) in Phase I but were not tempered in Phase II. Apart from these differences, bowls unearthed from Phase I and Phase II periods were both shell or plant tempered, and their number only shows slight growth in Phase II, from 15 to 18, giving the best example for showing continuity in the pottery assemblages.

Different from the previously studied grinding tools in China and elsewhere that were mainly used for plant processing (Hamon, 2009; Li, et al., 2019; Li et al., 2020a; Chondrou, et al., 2021), different types of pottery vessels hold the unique potential to offer more information regarding the storing, cooking, and serving of plant foods (Craig, et al., 2013; Nieuwenhuyse, et al., 2015; Wang, et al., 2021). Furthermore, our previous research analyzed potsherds from the Phase II of Houjiazhai with starch grain analysis (Luo, et al., 2020). The yielded data, together with the results from the present study, allow a comparison of how different types of pottery vessels were used in different occupation periods at the same site.

MATERIALS AND METHODS

The Pottery Sherds From the Site of Houjiazhai

In the present study, pottery sherds (n = 21) unearthed from the Phase I contexts of the site of Houjiazhai were sampled for starch grain analysis (**Figure 2**). These pottery sherds were chosen because their morphological features still allow us to determine their original typologies. Potsherds that were likely involved in various food-related activities were selected to understand the use of different types of pottery vessels (**Supplementary Table S2**). Two of the samples from jars and two from an urn were selected because they were potentially used for storing foods. To investigate ceramics that were used for cooking, seven *fu* vessels and one *ding* vessel were chosen. Moreover, eight pottery sherds from bowls and one from a spoon were selected because they were likely used to serve food.

All these potsherds were briefly washed after excavation and were kept in the local museum. Although washing may have removed some valuable information on the samples, several studies have successfully extracted starch grains from washed artefacts or those stored in museums (Barton, 2007; Ciofalo, et al., 2020). More importantly, we have presented positive results for the study of the pottery sherds from Phase II of Houjiazhai (Luo, et al., 2020); thus, we believe the sampled pottery sherds retrieved from Phase I of Houjiazhai are promising to provide more valuable information regarding plant use in the earlier stage of the site.

Extraction and Recovery of Starch Grains

The published protocols for extracting starch grains from washed artefacts were consulted and slightly modified for this study (Ciofalo, et al., 2020). First, a wash bottle with ultra-purified water with significant water pressure was used to rinse the artefacts, which removed the majority of additional soil matrix that loosely adhered to and was not a part of the artefacts' use-history (Barton and Torrence, 2015). This type of washing was also intended to remove some possible modern contaminations (Chandler-Ezell and Pearsall, 2003). Second, an ultrasonic toothbrush was used on the internal surfaces of each artefact for 2 min each, followed by rinsing, and the aqueous samples were gathered from different surfaces in different 50-ml plastic tubes for further analysis. Control samples were taken from the soil attached to pottery surfaces, the local museum, and the lab where starch grain analysis was conducted. Then, these samples were processed in the lab for the recovery of starch grains using a

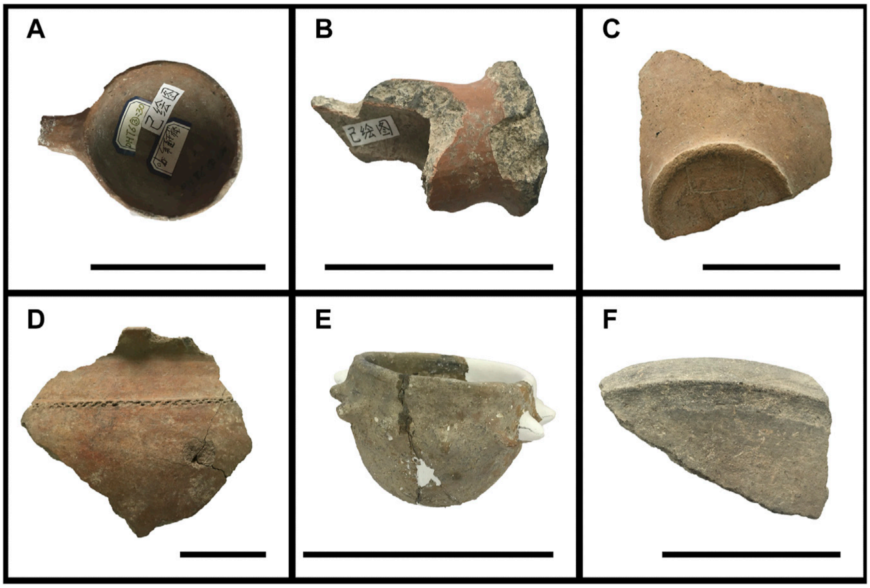

FIGURE 2 | Examples of pottery sherds subjected to starch grain analysis in this study—**(A)** DHT6③:3A, spoon; **(B)** DHT3④:274, a fragment from a bowl (*dou* type); **(C)** DHT6④:99, a bowl fragment; **(D)** DHT3③:53, jar; **(E)** DHT3③: 209, *fu*; and **(F)** DHT3③:183, a *fu* fragment (scale bar: 10 cm).

heavy-liquid solution of CsCl, the steps of which have been adopted and introduced in our previous publications (Yang et al., 2016; Luo et al., 2020).

For comparison of taxonomic ascription, we used an assembled reference collection of starch grains obtained from recent economically useful and edible plants that were collected in the archaeobotany lab at the University of Science and Technology of China (**Figure 3**). We also consulted the published data on modern starch grains (Liu et al., 2014; Wan et al., 2011; Wan et al., 2012).

RESULTS

The starch grains from this study were recovered from all the pottery sherds only, rather than the control samples taken from the museum, the lab, and the soil attached to the surfaces of the pottery sherds (**Supplementary Table S2**). Thus, we postulate the most likely cause for entrapping the discovered starch grains was through intense or prolonged use of the pottery vessels as food-related implements in the past. Overall, the starch grains identified provide insights into the consumption of diverse plant remains at the site of Houjiazhai during Phase I, including cereals, tubers, nuts, and beans (**Supplementary Table S2**).

Cereals

A total of 1,038 recovered starch grains could be identified as cereals. Type A was identified as starch grains from rice, without fissures and lamellae on their surfaces (**Figures 4a, a’, b, b’**). The grains were either singular or compound with clear extinction crosses on some larger singular grains. The size range of the singular starch grains was 2.29–9.91 μm.

Starch grains from type B (**Figures 4c, c’, d, d’, e, e’, f, f’**) were identified as Panicoideae from foxtail millet, broomcorn millet, and probably small grains from Job’s tears (*Coix lacryma-jobi*). Studies have compared starch grains from these three species and found it is difficult to separate them precisely because of their common morphological features in size and shape (Liu, et al., 2014). Starch grains from type B were singular with centric hilum, and their size range was 8.04–29.25 μm. The shapes of type B starch grains were near-circular or polygonal. Lineal or “Y”-shaped fissures were often present on type B starch grains.

Type C was identified as Job’s tears (**Figures 4g, g’, h, h’**). Although the shape of starch grains from type C shares some similarities with those from type B, starch grains from type C were characterized by eccentric hilum and extinction cross with zig-zag arms. The size range was 6.93–30.10 μm, and the average size was 19.71 μm. It should be noted that the largest grain sizes of the identified Job’s tears, foxtail millet, or broomcorn millet in the present study were slightly bigger than the data reported in a previous study (Liu, et al., 2014), in which the largest grain sizes of the modern starch grains from foxtail millet, broomcorn millet, and Job’s tears were 21.17, 12.80, and 29.20 μm, respectively. One of the possible reasons causing a larger size range in the present study could be related to the prehistoric pre-treatments (e.g., cooking and grinding) of the plants because experiments have demonstrated that various processing methods could enlarge the sizes of starch grains (Henry, et al., 2009; Wang, et al., 2017; Li et al., 2020a).

Type D starch grains were identified as Triticeae, with their size ranging from 2.598 to 47.60 μm, and the average size is 18.37 μm. Starch grains from type D were circular or lenticular, with hilum in the center. Fissures were often absent, but lamellae were present occasionally. The extinction cross is “X”-shaped and

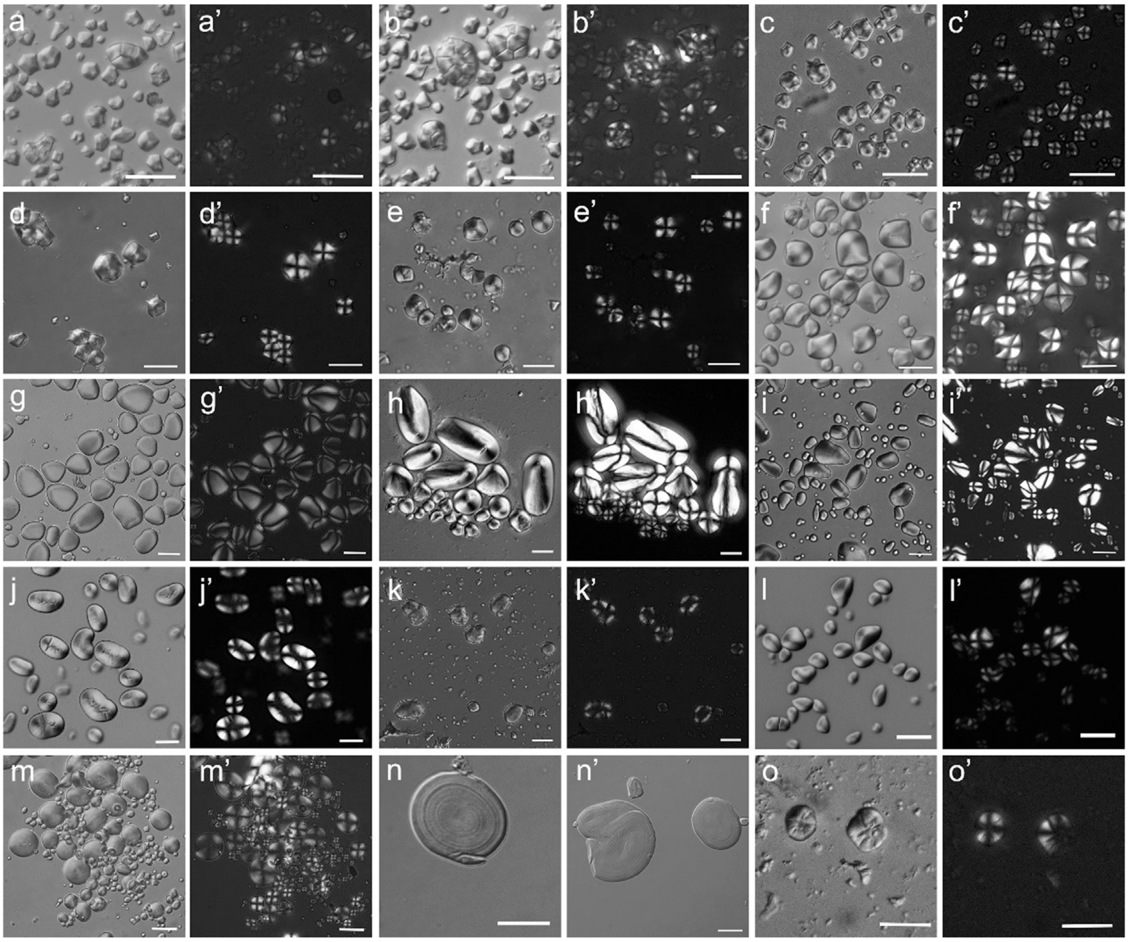

FIGURE 3 | Relevant reference collection of modern starch grains at the University of Science and Technology of China—**(a,a')** rice from Hunan, China; **(b,b')** rice from Fujian, China; **(c,c')** broomcorn millet from Shaanxi, China; **(d,d')** foxtail millet from Shaanxi, China; **(e,e')** Job's tears from Anhui, China; **(f,f')** roots of snake gourd from Anhui, China; **(g,g')** Chinese yam from Henan, China; **(h,h')** lotus root from Henan, China; **(i,i')** lily bulbs from Gansu, China; **(j,j')** mung bean from Henan, China; **(k,k')** sweet pea from Anhui, China; **(l,l')** acorns (*Quercus acutissima*) from Anhui, China; **(m,m')** wheat from Anhui, China; **(n,n')** wheat after boiling; and **(o,o')** Job's tears after grinding (scale bar: 20 μm).

vague in a few cases. Interestingly, a few starch grains from type D were discovered on cooking vessels showing more pronounced lamellae (e.g., **Figures 4q, q'**), a type of damage feature perhaps caused by boiling, according to the previous experimental studies (Henry, et al., 2009; Wang, et al., 2017).

Underground Storage Organs

A total of 129 starch grains could be identified as USOs. These starch grains were characterized by their eccentric hilum and "X"-shaped extinction cross with curved arms.

Among the starch grains from USOs, type E (**Figures 4i, i', j, j'**) were identified as snake gourd (*Trichosanthes kirilowii*), with their size ranging from 16.00 to 30.37 μm, and their average size was 21.26 μm. The forms of starch grains from type E include round, semi-circular, and bell-shaped. This type of plant has been widely discovered in prehistoric China and is traditionally used as a type of famine food (Zhu, 1406).

Starch grains from type F (**Figures 4l, l'**) were identified as lotus root (*Nelumbo nucifera*), with their size ranging from 9.43 to 47.70 μm, and their average size was 17.85 μm. The shapes of starch grains from type F include semi-circular, bell-shaped, and oblong. Different from type E, starch grains from type F often presented clear lamellae, linear fissure, and more extreme eccentric hilum on oblong starch grains.

Starch grains from type G (**Figures 4k, k'**) were identified as Chinese yam (*Dioscorea panthainca*). The starch grains differ from type E and type F in terms of their shapes, which are triangular or quadrilateral ovate. The size range of starch grains from type G was 11.84–35.91 μm, and their average size was 23.01 μm. Type G starch grains also showed extreme eccentric hilum and clear lamellae on their surfaces.

Starch grains from type H (**Figures 4m, m'**) were identified as lily bulbs (*Lilium* sp.) Similar to starch grains from types F and G, starch grains classified into type H also process eccentric hilum

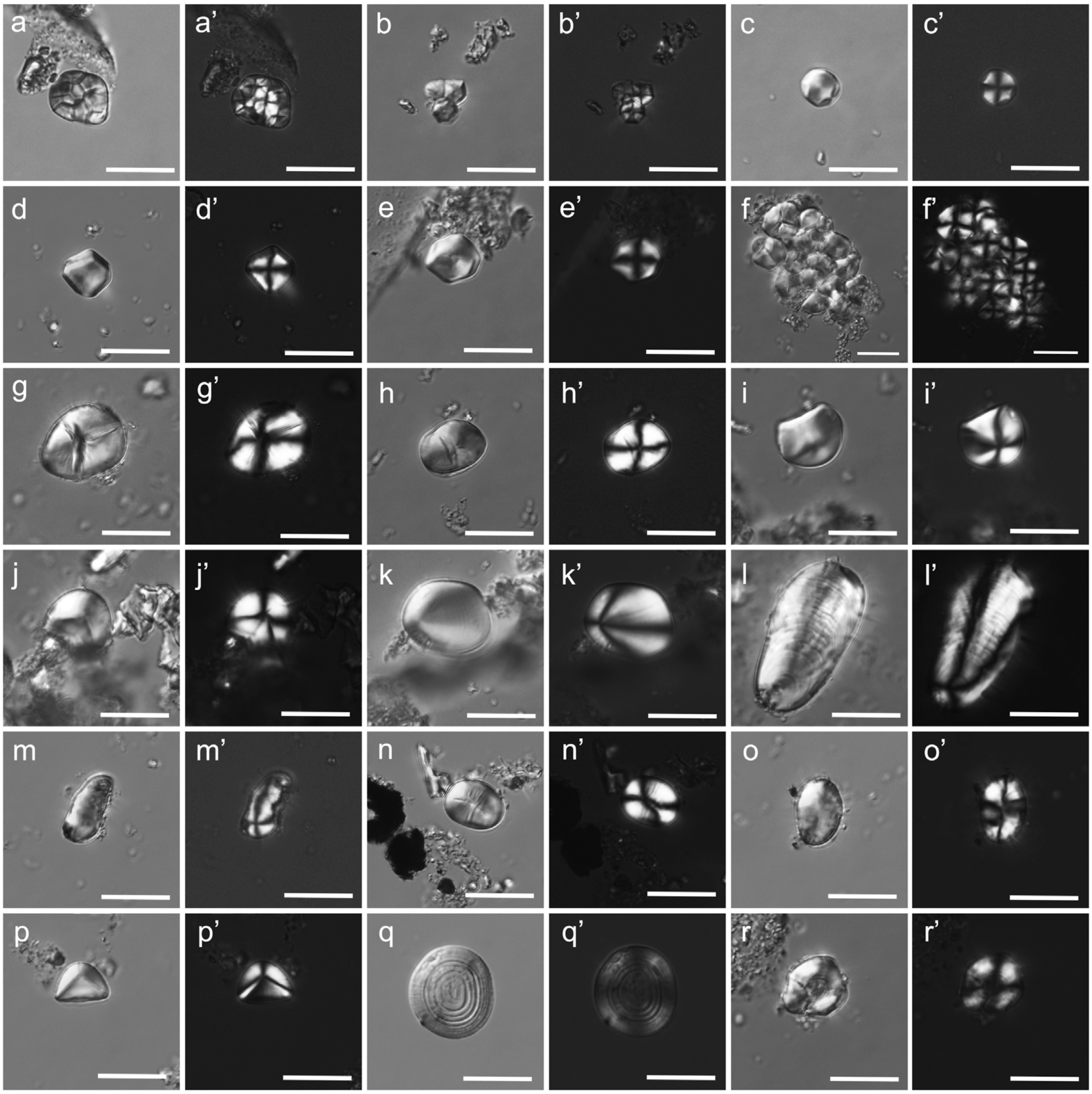

FIGURE 4 | Ancient starch grains identified on potsherds from the site of Houjiazhai—**(a,a',b,b')** rice; **(c,c',d,d',e,e',f,f')** foxtail millet, broomcorn millet, and probably small grains from Job's tears; **(g,g',h,h')** Job's tears; **(i,i',j,j')** root of snake gourd; **(k,k')** Chinese yam; **(l,l')** lotus root; **(m,m')** lily bulbs; **(n,n',o,o')** beans that may include *Vigna* sp. or/and *Vicia* sp.;**(p,p')** acorns; **(q,q')** seeds from Triticeae showing more pronounce lamellae; and **(r,r')** an example of a damaged starch grain (scale bar: 20 μm).

but were thinner in their shapes. The size of type H ranged from 15.79 to 22.39 μm, and their average size was 26.99 μm.

Acorns

A total of 11 starch grains were identified as type I (**Figures 4p, p'**) from acorns (*Quercus* sp.), the size range was 9.91–19.92 μm, and the average size was 17.85 μm. The grains were singular with "X"-shaped extinction crosses. The forms of type I included triangular-ovate and drop-shaped, with centered or slightly eccentric hilum.

Beans

A total number of 14 starch grains were classified into beans (type J) that may include *Vigna* sp. or/and *Vicia* sp. (**Figures 4n, n', o, o'**). The size range of type J was 13.81–34.25 μm, and the average size was 18.78 μm. The grains were singular and oval- or kidney-shaped. The extinction cross of type J resembled two tangent curves. On the surface of starch grains from type J, lineal fissures vertical to the longer axis of the grains were often present near the hilum.

Damaged Starch Grains

Starch grains (*n* = 117) were classified into the damaged group of type K (**Figures 4r, r'**) based on their incomplete forms and faint extinction cross. These damaged grains were identified only if they possessed typical morphological features that matched our modern reference collections (e.g., **Figures 3n, n', o, o'**). Notably,

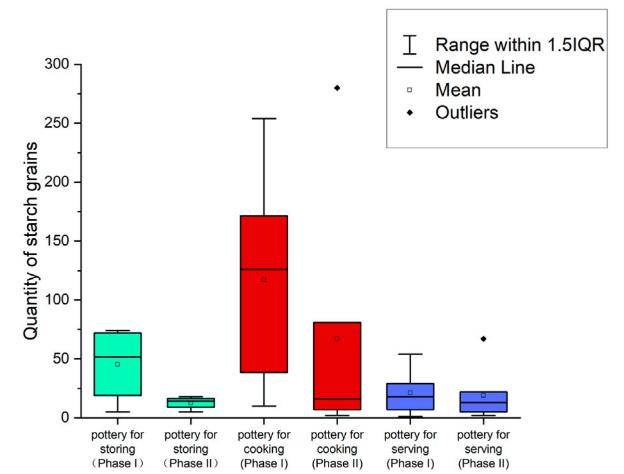

FIGURE 5 | Quantities of starch grains detected on potsherds attributed to Phase I and Phase II of Houjiazhai.

apart from potential prehistoric treatments (e.g., cooking, pounding, grinding, and fermenting) (Henry, et al., 2009; Mickleburgh and Pagán-Jiménez, 2012; Wang, et al., 2017; Li et al., 2020b), the post-depositional process and use of modern chemicals during the stage of starch extraction could have damaged starch grains (García-Granero, 2020). It is thus challenging for us to further interpret what may have caused damage to starch grains on each artefact at the current stage.

DISCUSSION

Preservation of Starch Grains on Different Types of Pottery Vessels

Because starch grains subjected to high temperature would be substantially damaged according to previous experiments (Henry, et al., 2009), it was thus predicted that starch grains, especially the intact ones, would be rare on vessels used for cooking and serving. Interestingly, both intact and damaged starch grains were found on all types of pottery vessels in the present study. Similarly, complete starch grains were also detected on cooking and serving pottery from other archaeological sites in China and elsewhere (Perry, 2004; Yang, et al., 2014). These findings suggest that starch grains would be preserved on multiple types of pottery vessels.

The number of starch grains on pottery vessels used for storing (i.e., urns and jars), cooking (i.e., *fu* and *ding*), and serving (i.e., bowls and *dou*) was also counted: Cooking vessels yielded the maximum starch grains in general (but with a few exceptions, see the sample of TQ2 in **Supplementary Table S2**), followed by vessels used for storing and serving (**Figure 5**). This phenomenon is consistent with the result in our previous study at Houjiazhai (**Figure 5**, Luo et al., 2020), in which the pottery vessels from Phase II were subjected to starch grain analysis (**Figure 5**). These findings tend to suggest that the chance of detecting starch grains can be even higher on pottery vessels used for cooking. However, these propositions still need to be tested in more case studies since the complex life histories of pottery vessels, post-depositional processes of food residues, and different sampling protocols could all potentially affect the chances of discovering food remains (Henry, et al., 2016; Hutschenreuther, et al., 2017; Li et al., 2020b).

Here, we propose two possible factors that might result in the different numbers of starch grains on pottery vessels from Houjiazhai: techniques adopted in pottery making and pottery functions. The cooking vessels from Houjiazhai were all tempered with sand or shell, with rough surfaces (Tang et al., 2019). In contrast, serving vessels (i.e., bowls) from Houjiazhai are compact because they were made without tempering materials. It has been proposed that starch grains can easily become trapped or embedded in areas of an artefact where they are protected from degradation, such as pores, micro-fractures, cracks, holes, and micro-striations on the surface of an artefact (Torrence and Barton, 2006). From this point of view, tempered pottery vessels with relatively loose structures may possess stronger abilities in capturing plant remains and provide more starch grains. Nevertheless, storage vessels from Houjiazhai were also shell-tempered, but these vessels yielded fewer starch grains than cooking pots. Thus, different uses of pottery vessels could also affect the preservation of starch grains considering the contacts of plants with storage vessels were more likely static and superficial, while cooking vessels were probably frequently adopted for boiling plant foods.

Changes and Continuities of Starchy Foods at Houjiazhai

In Phase I, starch grains from beans, Chinese yam, and lily bulbs were discovered, while none of these starch grains appeared in potsherds attributed to Phase II. The other plant species, including rice, millet, seeds from Triticeae, acorns, and lotus root, were utilized in both Phase I and Phase II. The comparison of plant species found on pottery vessels from Phase I and Phase II of Houjiazhai thus infers that people utilized more diverse plant food resources at the earlier stage.

Apart from the quantities, the ubiquities of each identified plant species in both Phase I and Phase II are calculated and compared (**Figures 6A,B**). Ubiquity here refers to the occurrence of identified plant taxa among pottery vessels associated with storing, cooking, and serving. Compared to starch grains from USOs and acorns, the ubiquities of cereals are relatively higher on all types of pottery vessels (**Figures 6A,B**), implying that cereals were mostly consumed with the pottery vessels during the periods of Phase I and Phase II at Houjiazhai. Notably, the ubiquities of certain types of plants vary in different types of pottery containers (**Figure 6**). The striking examples are lily bulbs and wild beans in Phase I, whose starch grains only appear on cooking vessels, but none of their starch grains were found on vessels used for storing and serving. Lily bulbs and wild beans were not likely stored in pots after harvesting; their low ubiquities on cooking vessels also suggest these two types of plants were not the primary food resources at Houjiazhai during the period of Phase I. In Phase II, starch remains from lily bulbs and beans were not even discovered in the pottery assemblages (**Figure 6B**).

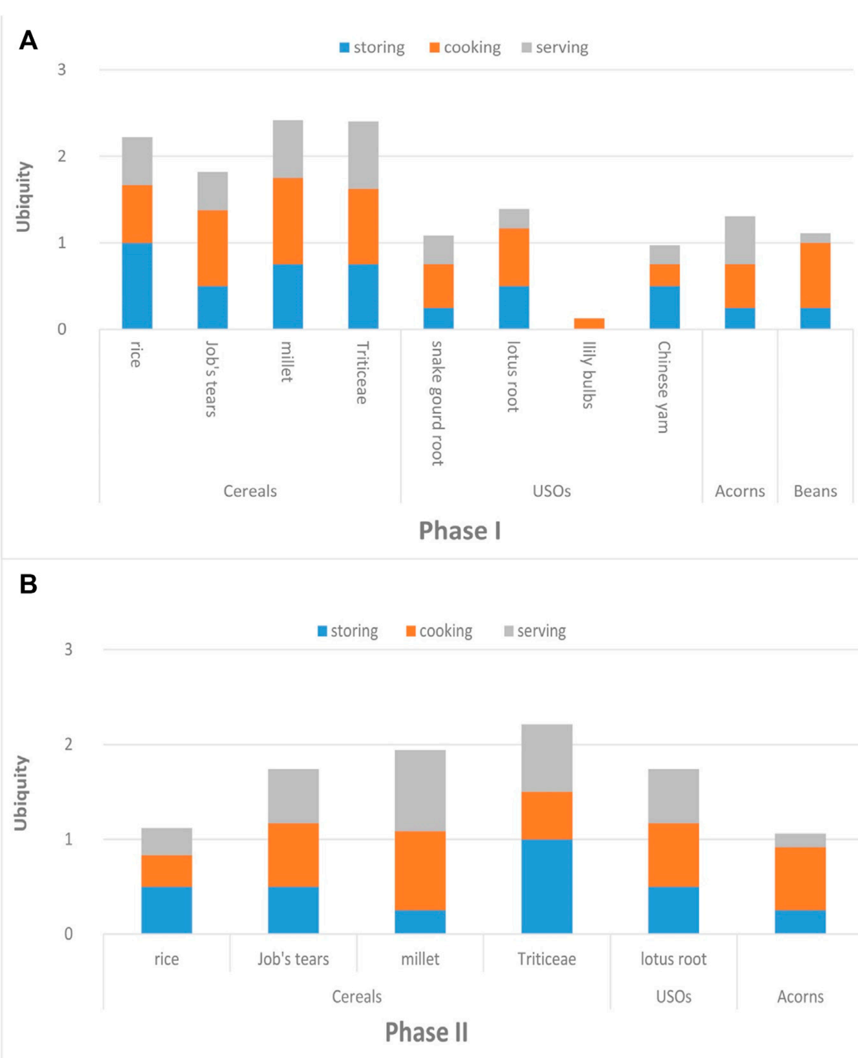

FIGURE 6 | (A) Ubiquities of different types of starch grains on Houjiazhai pottery vessels attributed to Phase I; **(B)** ubiquities of different types of starch grains on Houjiazhai pottery vessels attributed to the Phase II (note: ubiquity refers to the occurrence of identified plant taxa amongst the certain type of pottery vessels: storing, cooking, and serving, see also the **Supplementary Tables S3, S4**.).

The ubiquities of millet, Job's tears, seeds from Triticeae, lotus root, and acorns are relatively the same in Phases I and II (**Figures 6A,B**); only the ubiquity of rice starch grains decreased slightly in Phase II. These results tend to suggest that the past Houjiazhai people probably consumed less rice in Phase II. Yet, limitations of starch grain analysis have been recently discussed by a group of researchers (Langejans, 2012; Mercader, et al., 2018; García-Granero, 2020), and it has been proven that different environments affect the preservation of starch grains on artefacts, with certain starch grains being more resistant than others to amylolysis during their deposition into soils (Haslam, 2004; Hutschenreuther, et al., 2017). Another experimental research study also found a bias in the preservation of the starch grains on ancient grinding tools because starch grains from rice experienced the most morphological changes during dry-grinding processes (Li et al., 2020a). So far, the preservations of starch grains from different plant species on daily-use pottery vessels, which usually have complex life histories, have not been systematically studied. Bearing these factors in mind, we propose to consider whether rice was less intensively used at the site of Houjiazhai with other lines of evidence from macro plant remains or phytolith analysis. Nevertheless, the changes and continuities of foodways at Houjiazhai can still be reflected in the changing pottery assemblage as well as the different types of plant foods consumed in the two different occupation stages.

Diverse Plant Foods at the Shuangdun Culture Sites

Overall, the results in our study are consistent with the previous archaeobotanical work in the research region (**Supplementary Table S1**), suggesting people at the Shuangdun Culture sites utilized various plant species. Starch grains from cereals, USOs, and beans were commonly discovered on artefacts excavated at the sites of Shuangdun, Shishanzi, and Houjiazhai. These findings are supported by the macrobotanical remains identified at the site of Xiaosungang in the same period, where 40 soil samples taken from 6 ash pits and 14 cultural layers were subjected to floatation (Cheng et al., 2016). Acorns, however, were only detected at the sites of Houjiazhai and Xiaosungang (**Supplementary Table S1**).

Among the group of cereals, rice remains appeared at most of the studied Shuangdun Culture sites (3 out of 4), except for the site of Shishanzi. It is worth noting that the reason for not recovering starch grains from rice at the site of Shishanzi could be related to its small sampling size or the difficulties of detecting small starch grains from rice (Yang, et al., 2015). In terms of millet, our study at Houjiazhai and the previous phytolith analysis carried out at Shuangdun indicate millet appeared at both sites (Luo, et al., 2019). Moreover, Zhang et al. (2020) recently found evidence of millet consumption through pollen and lipid analysis of coprolites from another site of Yuhuicun that is associated with the Shuangdun Culture. The multiple lines of evidence thus confirm millet had already reached the sites located in the south of the Huai River during the Shuangdun Culture period.

CONCLUSION

The identification of starch grains discovered on potsherds attributed to the periods of Phase I of Houjiazhai indicates vessels involved in storing, cooking, and serving could provide valuable information regarding plant foods during the Shuangdun Culture period. Further quantitative analysis of the yielded data found that cooking vessels provided the highest quantity of starch grains in both Phases, which is vital for understanding the preservation of plant remains in different tempered pottery vessels. The comparison of results of starch grain analyses on potsherds attributed to Phase I and Phase II at Houjiazhai also demonstrates that prehistoric culinary practices could be studied not only in the pottery assemblages but also in the utilized plant species. Overall, the holistic approach used here, considering the classification of different types of pottery vessels as well as the published data on archaeobotany in the research region, shows a more detailed understanding of which types of starch foods were consumed and then preserved on various Neolithic pottery vessels. The findings of both rice and millet starch grains at the site of Houjiazhai (Phase I) are also valuable clues for mapping the spatiotemporal route for the spread of rice and millets in central-eastern China during the Neolithic age.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WAL and WGL designed the research study and wrote the article. HX, WY, WT, DZ, SY, XK, and ZJ conducted the sampling and analysis. All the authors read and edited the paper.

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REFERENCES

Barton, H. (2007). Starch Residues on Museum Artefacts: Implications for Determining Tool Use. *J. Archaeol. Sci.* 34 (10), 1752–1762. doi:10.1016/j.jas.2007.01.007

Barton, H., and Torrence, R. (2015). Cooking up Recipes for Ancient Starch: Assessing Current Methodologies and Looking to the Future. *J. Archaeol. Sci.* 56, 194–201. doi:10.1016/j.jas.2015.02.031

Bestel, S., Bao, Y., Zhong, H., Chen, X., and Liu, L. (2018). Wild Plant Use and Multi-Cropping at the Early Neolithic Zhuzhai Site in the Middle Yellow River Region, China. *Holocene* 28 (2), 195–207. doi:10.1177/0959683617721328

Chandler-Ezell, K., and Pearsall, D. M. (2003). Piggyback Microfossil Processing: Joint Starch and Phytolith Sampling from Stone Tools. *Phytolith. (Raleigh NC)* 15 (3), 2–8.

Cheng, Z., Yang, Y., Zhang, J., Fang, F., Xu, J., Chen, B., et al. (2016). Flotation of Plant Remains at the Site of Xiaosungang. *Disijiyanjiu (in Chin.* 36, 302–311.

Chondrou, D., Bofill, M., Procopiou, H., Vargiolu, R., Zahouani, H., and Valamoti, S. M. (2021). How Do You like Your Cereal? A Qualitative and Quantitative Use-Wear Analysis on Archaeological Grinding Tools from Prehistoric Greek Sites. *Wear* 476, 203636. doi:10.1016/j.wear.2021.203636

Ciofalo, A. J., Donner, N. R., Hofman, C. L., and Geurds, A. (2020). Uses of Pre-Hispanic Kitchenware from Central Nicaragua: Implications for Understanding Botanical Foodways. *Archaeol. Anthropol. Sci.* 12 (1), 1–14. published online first. doi:10.1007/s12520-019-00955-9

Craig, O. E., Saul, H., Lucquin, A., Nishida, Y., Taché, K., Clarke, L., et al. (2013). Earliest Evidence for the Use of Pottery. *Nature* 496 (7445), 351–354. doi:10.1038/nature12109

Dong, Z., Zhang, J., Yan, Y., Yao, L., Li, W., and Jia, Q. (2014). Starch Grain Analysis Reveals the Plant Exploitation at the Site of Shishanzi. *Disijiyanjiu* 34, 114–125. (in Chinese). doi:10.3969/j.issn.1001-7410.2014.01.14

Dong, Z. (2013). Starch Grain Analysis on Stone Tools from the Sites of Shuangdun and Shishanzi. *Master thesis A. T. Univ. Sci. Technol. China*. Hefei: China. (in Chinese).

Fuller, D. Q. (2011). Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice* 4 (3-4), 78–92. doi:10.1007/s12284-011-9078-7

García-Granero, J. J. (2020). Starch Taphonomy, Equifinality and the Importance of Context: Some Notes on the Identification of Food Processing through Starch Grain Analysis. *J. Archaeol. Sci.* 124, 105267. published online first. doi:10.1016/j.jas.2020.105267

Hamon, C. (2009). in *Lifecycle of a Neolithic Quern: Limits and Contribution of a Combined Technical and Functional Analysis of Grinding Tools*. Editors C. Hamon and J. Graefe (Bonn: Deutsche Gesellschaft für Ur-und Früh-Geschichte), 45–54.

Haslam, M. (2004). The Decomposition of Starch Grains in Soils: Implications for Archaeological Residue Analyses. *J. Archaeol. Sci.* 31 (12), 1715–1734. doi:10.1016/j.jas.2004.05.006

Henry, A. G., Debono Spiteri, C., Büdel, T., Hutschenreuther, A., Schmidt, S., and Watzke, J. (2016). Methods to Isolate and Quantify Damaged and Gelatinized Starch Grains. *J. Archaeol. Sci. Rep.* 10, 142–146. doi:10.1016/j.jasrep.2016.09.003

Henry, A. G., Hudson, H. F., and Piperno, D. R. (2009). Changes in Starch Grain Morphologies from Cooking. *J. Archaeol. Sci.* 36 (3), 915–922. doi:10.1016/j.jas.2008.11.008

Huan, X., Deng, Z., Zhou, Z., Yan, X., Hao, X., Bu, Q., et al. (2022). The Emergence of Rice and Millet Farming in the Zang-Yi Corridor of Southwest china Dates Back to 5000 Years Ago. *Front. Earth Sci.* 10, 874649. published online first. doi:10.3389/feart.2022.874649

Huang, D. (2012). Interpretation of Signs on Pottery Vessels from the Site of Shuangdun. *Dongnanwenhua*, 86–91. (in Chinese).

Hutschenreuther, A., Watzke, J., Schmidt, S., Büdel, T., and Henry, A. G. (2017). Archaeological Implications of the Digestion of Starches by Soil Bacteria: Interaction Among Starches Leads to Differential Preservation. *J. Archaeol. Sci. Rep.* 15, 95–108. doi:10.1016/j.jasrep.2017.07.006

Jia, Q. (1992). The Site Report of the Site of Shishanzi in Anhui Province. *Kaogu* 03, 193–203. (in Chinese).

Jiang, L., and Liu, L. (2006). New Evidence for the Origins of Sedentism and Rice Domestication in the Lower Yangzi River, China. *Antiquity* 80 (308), 355–361. doi:10.1017/s0003598x00093674

Kan, X., and Zhou, Q. (2007). The Site Report of Neolithic Site of Shuangdun in Bengbu. *Anhui, Kaoguxuebao*, 97–13. (in Chinese).

Langejans, G. H. J. (2012). Middle Stone Age Pièces Esquillées from Sibudu Cave, South Africa: an Initial Micro-residue Study. *J. Archaeol. Sci.* 39 (6), 1694–1704. doi:10.1016/j.jas.2011.12.036

Li, W., Pagán-Jiménez, J. R., Tsoraki, C., Yao, L., and Van Gijn, A. (2020a). Influence of Grinding on the Preservation of Starch Grains from Rice. *Archaeometry* 62 (1), 157–171. doi:10.1111/arcm.12510

Li, W., Tsoraki, C., Lan, W., Yang, Y., Zhang, J., and Van Gijn, A. (2019). New Insights into the Grinding Tools Used by the Earliest Farmers in the Central Plain of China. *Quat. Int.* 529 (Sep.20), 10–17. doi:10.1016/j.quaint.2018.10.005

Li, W., Tsoraki, C., Yang, Y., Xin, Y., and van Gijn, A. (2020b). Plant Foods and Different Uses of Grinding Tools at the Neolithic Site of Tanghu in Central china. *Lithic Technol.* 45 (3), 154–164. doi:10.1080/01977261.2020.1755789

Liu, L., Ma, S., and Cui, J. (2014). Identification of Starch Granules Using a Two-step Identification Method. *J. Archaeol. Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

Long, T., Chen, H., Leipe, C., Wagner, M., and Tarasov, P. E. (2022). Modelling the Chronology and Dynamics of the Spread of Asian Rice from Ca. 8000 BCE to 1000 CE. *Quat. Int.* 623, 101–109. doi:10.1016/j.quaint.2021.11.016

Luo, W., Gu, C., Yang, Y., Zhang, D., Liang, Z., Li, J., et al. (2019). Phytoliths Reveal the Earliest Interplay of Rice and Broomcorn Millet at the Site of Shuangdun (Ca. 7.3-6.8 Ka BP) in the Middle Huai River Valley, China. *J. Archaeol. Sci.* 102, 26–34. doi:10.1016/j.jas.2018.12.004

Luo, W., Xuan, H., Yao, L., Yang, Y., Yi, W., Kan, X., et al. (2020). Starch Grain Analysis Reveals the Plant Foods at the Site of Houjiazhai (Phase II). *Renleixuexuebao* 39, 292–305. (in Chinese).

Luo, W., Yang, Y., Yao, L., Chen, Z., Li, J., Yin, C., et al. (2016). Phytolith Records of Rice Agriculture during the Middle Neolithic in the Middle Reaches of Huai River Region, China. *Quat. Int.* 426, 133–140. doi:10.1016/j.quaint.2016.03.010

Ma, Y., Yang, X., Huan, X., Wang, W., Ma, Z., Li, Z., et al. (2016). Rice Bulliform Phytoliths Reveal the Process of Rice Domestication in the Neolithic Lower Yangtze River Region. *Quat. Int.* 426, 126–132. doi:10.1016/j.quaint.2016.02.030

Mercader, J., Abtosway, M., Bird, R., Bundala, M., Clarke, S., Favreau, J., et al. (2018). Morphometrics of Starch Granules from Sub-Saharan Plants and the Taxonomic Identification of Ancient Starch. *Front. Earth Sci.* 6, 146. doi:10.3389/feart.2018.00146

Mickleburgh, H. L., and Pagán-Jiménez, J. R. (2012). New Insights into the Consumption of Maize and Other Food Plants in the Pre-Columbian Caribbean from Starch Grains Trapped in Human Dental Calculus. *J. Archaeol. Sci.* 39 (7), 2468–2478. doi:10.1016/j.jas.2012.02.020

Nieuwenhuyse, O. P., Roffet-Salque, M., Evershed, R. P., Akkermans, P. M. M. G., and Russell, A. (2015). Tracing Pottery Use and the Emergence of Secondary Product Exploitation through Lipid Residue Analysis at Late Neolithic Tell Sabi Abyad (Syria). *J. Archaeol. Sci.* 64, 54–66. doi:10.1016/j.jas.2015.10.002

Perry, L. (2004). Starch Analyses Reveal the Relationship between Tool Type and Function: An Example from the Orinoco Valley of Venezuela. *J. Archaeol. Sci.* 31 (8), 1069–1081. doi:10.1016/j.jas.2004.01.002

Qiu, Z., Zhuang, L., Rao, H., Lin, L., and Zhuang, Y. (2022). Excavation at Hanjing Site Yields Evidence of Early Rice Cultivation in the Huai River More Than 8000 Years Ago. *Sci. China Earth Sci.* 65 (5), 910–920. doi:10.1007/s11430-021-9885-x

Tang, G., Chen, Y., and Kan, W. (2019). The Site Report of Houjiazhai in Dingyuan, Anhui Province. *Kaoguxuebao* 01, 63–112. (in Chinese).

Torrence, R., and Barton, H. (2006). *Ancient Starch Research*. Walnut Creek, Calif: Left Coast Press.

Wang, C., Lu, H., Gu, W., Zuo, X., Zhang, J., Liu, Y., et al. (2018). Temporal Changes of Mixed Millet and Rice Agriculture in Neolithic-Bronze Age Central Plain, China: Archaeobotanical Evidence from the Zhuzhai Site. *Holocene* 28 (5), 738–754. doi:10.1177/0959683617744269

Wang, J., Jiang, L., and Sun, H. (2021). Early Evidence for Beer Drinking in a 9000-Year-Old Platform Mound in Southern China. *PLoS One* 16 (8), e255833. doi:10.1371/journal.pone.0255833

Wang, J., Liu, L., Georgescu, A., Le, V. V., Ota, M. H., Tang, S., et al. (2017). Identifying Ancient Beer Brewing through Starch Analysis: A Methodology. *J. Archaeol. Sci. Rep.* 15, 150–160. doi:10.1016/j.jasrep.2017.07.016

Xu, D. (2008). A Brief Description of the Signs Discovered on Artefacts at the Site of Shuangdun. *Zhongyuanwenwu* 03, 75–79. (in Chinese).

Xu, D. (2007). The Interpretation of the Sign "■" at the Site of Shuangdun. *Dongnanwenhua* 06, 77–79. (in Chinese).

Xu, J., Zhang, H., and Chen, B. (2015). The Site Report of Xiaosungang. *Nanchengzi, Yangpu. Kaogu* 02, 3–18. (in Chinese).

Xuan, H. (2017). *Plant Food Utilization and Regional Differences between Southern and Northern Anhui*. Master Thesisa. University of Science and Technology of China, Hefei, China.

Yang, X., Fuller, D. Q., Huan, X., Perry, L., Li, Q., Li, Z., et al. (2015). Barnyard Grasses Were Processed with Rice Around 10000 Years Ago. *Sci. Rep.* 5, 16251–16257. doi:10.1038/srep16251

Yang, X., Chen, Q., Ma, Y., Li, Z., Hung, H.-c., Zhang, Q., et al. (2018). New Radiocarbon and Archaeobotanical Evidence Reveal the Timing and Route of Southward Dispersal of Rice Farming in South China. *Sci. Bull.* 63 (22), 1495–1501. doi:10.1016/j.scib.2018.10.011

Yang, X., Ma, Z., Wang, T., Perry, L., Li, Q., Huan, X., et al. (2014). Starch Grain Evidence Reveals Early Pottery Function Cooking Plant Foods in North China. *Chin. Sci. Bull.* 59 (32), 4352–4358. doi:10.1007/s11434-014-0500-6

Yang, X., Wan, Z., Perry, L., Lu, H., Wang, Q., Zhao, C., et al. (2012). Early Millet Use in Northern China. *Proc. Natl. Acad. Sci. U.S.A.* 109 (10), 3726–3730. doi:10.1073/pnas.1115430109

Yang, Y., Li, W., Yao, L., Cheng, Z., Luo, W., Zhang, J., et al. (2016). Plant Food Sources and Stone Tools' Function at the Site of Shunshanji Based on Starch Grain Analysis. *Sci. China Earth Sci.* 59 (8), 1574–1582. doi:10.1007/s11430-016-5321-9

Yao, L. (2016). *An Exploration of Ancient Starch Analysis Method and Practice in Chinese Neolithic Relics*. Hefei, China: Doctoral thesis at the University of Science and Technology of China.

Yunfei, Z., and Jiang, L. (2009). Remains of Ancient Rice Unearthed From the Shangshan Site and Their Significance. *Chinese Archaeol.* 9 (1), 159–163.

Zhang, J., Lu, H., Wu, N., Li, F., Yang, X., Wang, W., et al. (2010). Phytolith Evidence for Rice Cultivation and Spread in Mid-Late Neolithic Archaeological Sites in Central North China. *Boreas* 39 (3), 592–602. doi:10.1111/j.1502-3885.2010.00145.x

Zhang, J., and Wang, X. (1998). Notes on the Recent Discovery of Ancient Cultivated Rice at Jiahu, Henan Province: A New Theory Concerning the Origin of Oryza Japonica in China. *Antiquity* 72 (278), 897–901.

Zhang, J., Lu, H., Gu, W., Wu, N., Zhou, K., Hu, Y., et al. (2012). Early Mixed Farming of Millet and Rice 7800 Years Ago in the Middle Yellow River Region, China. *PLoS One* 7 (12), e52146. doi:10.1371/journal.pone.0052146

Zhang, Y., Zhang, D., Yang, Y., and Wu, X. (2020). Pollen and Lipid Analysis of Coprolites from Yuhuicun and Houtieying, China: Implications for Human Habitats and Diets. *J. Archaeol. Sci.* 29, 102135. reportspublished online first. doi:10.1016/j.jasrep.2019.102135

Zhu, S. (1406). *Roots and Herbs for Disaster Relief (Jiuhuangbencao in Chinese)*. Taipei: Shangwu Press.

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Morphometric Identification of Starch Granules From Archaeological Contexts: Diagnostic Characteristics of Seven Major North American Plant Families

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Starch-rich plants have played an important role in human evolution and societal development. Collected, grown, and consumed to support ever-increasing populations, such plants are integral to understanding past human diets. With the advent of starch granule analysis, plant resources that were invisible in the archaeological record can now be revealed in the cracks and crevices of artifacts. Widespread application of this technique, however, has stalled due to a lack of rigorous and standardized protocols. For example, taxonomic identification of starch granules using consistent diagnostic characteristics is still a challenge as there are no comprehensive surveys across important (i.e., dietary) plant taxa, especially at the levels of families, genera, and species. This study provides characteristics for identifying starch granules of seven major North American plant families (Amaranthaceae, Apiaceae, Fagaceae, Liliaceae, Pinaceae, Poaceae, and Solanaceae) based on systematic, morphometric studies of modern reference materials. A dichotomous key to starch granules of the seven families was also generated to aid in identification of those from archaeological contexts. Although we have focused on plants from western North America, these families occur across the globe and have had dietary significance throughout prehistory.

Keywords: starch granule analysis, taxonomic identification, western North America, human evolution, starch-rich foods

INTRODUCTION

The study of starch granules preserved in archaeological contexts, such as from dental calculus and crevices on stone tools, can indicate, often to species-level, the identity of plant foods that had been consumed. Starch granules are the energy storage structures of plants, abundantly found within seeds, fruits, and tubers. They are produced by most vascular plants and the morphology of granules is genetically controlled. Thus, the size, shape and surface features of the granules can indicate which plant taxon (e.g., family, genus, and species) produced them (Nägeli, 1858; Reichert, 1913; Shannon

TABLE 1 | Plant taxa and tissue sampled for starch.

Family	Species	Plant tissue
Amaranthaceae	*Chenopodium berlandieri*	seed/fruit
Apiaceae	*Cymopterus bulbosus*	taproot
	Lomatium donnellii	
	Lomatium macrocarpum	
	Lomatium triternatum	
Fagaceae	*Quercus agrifolia*	acorn
	Quercus douglasii	
	Quercus lobata	
Liliaceae	*Calochortus nuttallii*	bulb
	Erythronium grandiflorum	
	Fritillaria pudica	
Pinaceae	*Pinus edulis*	seed
	Pinus monophylla	
Poaceae	*Achnatherum hymenoides*	endosperm
	Sporobolus airoides	
	Elymus elymoides	
	Zea mays	
Solanaceae	*Solanum jamesii*	tuber

et al., 2009). Although this technique has shed light on the importance of plants in ancient human diets, starch granule research is still underdeveloped and faces several significant issues (Mercader et al., 2018), including rigorous methods for taxonomic identification.

The analysis of starch granules relies on careful measurements of dimensions and accurate descriptions of morphological characteristics, while taxonomic identification is based on comparison to modern reference material. Diagnostic keys for identification are especially useful and have been produced for plant taxa from China (Yang and Perry 2013), eastern North America (Messner 2011), and eastern Mediterranean (Ahituv and Henry 2022). Developing standards that increase the quality and replicability of measurements, descriptions, keys, and documentation is the next necessary step in advancing this technique. This study provides a standardized, systematic approach to defining characteristics that identify the granules of seven major plant families (Amaranthaceae, Apiaceae, Fagaceae, Liliaceae, Pinaceae, Poaceae, and Solanaceae) using reference materials of genera from western North America. A dichotomous key to starch granules of the seven plant families is also presented.

METHODS AND MATERIALS

Reference Materials

Reference materials for this study includes eighteen species from thirteen genera and seven major plant families having regional and global dietary significance (**Table 1**). Not only have these taxa been documented in ethnographic literature, the majority have also been recovered from archaeological sites across western North America. Some of these materials were collected from preserved herbarium specimens while others were collected from live plants in the field. To capture variation within a species, starch granules were extracted from three geographically dispersed individuals (replicate samples) and described using an array of morphometric characteristics (e.g., size, shape, surface features, response to polarized light). Source and collection data for can be found in **Supplementary Table S1**. Plant nomenclature follows USDA GRIN http://www.ars-grin.gov/~sbmljw/johnindex.html.

Starch Extraction

Different plant parts that most likely contain starch (seeds, fruits, nuts, endosperm, bulbs, tubers, caryopses, and taproots) were processed according to standard protocols (e.g., Torrence and Barton 2006) in the Natural History Museum of Utah (NHMU) Archaeobotany Lab. Material was ground using a sterile mortar and pestle and was sieved through a 125 µm mesh Endecott screen into a beaker using DH_20. Sample material <125 µm was transferred to a sterile 50 ml test tube and each sample was centrifuged for 3 minutes at 3000 RPM. The supernatant was discarded, and the sample pellet was transferred to a sterile 15 ml test tube. Each test tube was re-suspended with a vortex mixer, adding 7 ml of lithium heteropolytungstate (LST; specific gravity 2.00–2.35), and then centrifuged for 15 min at 1000 RPM. The sample was extracted from the heavy liquid using a pipette, carefully removing the top 1–2 mm layer of organics containing starch and placed into new 15 ml test tubes. Each sample was rinsed three times until all residual heavy liquid was removed. Samples were then rinsed with acetone, mixed with a vortex, and centrifuged for 3 min at 3000 RPM. The acetone was decanted, and samples were covered and left to dry overnight. Once dried, the samples were mixed with 50% DH_20 and 50% glycerol solution and then mounted on microscope slides.

Microscopy

Each slide was scanned using a transmitted brightfield microscope fitted with polarizing filters and Nomarski optics (Zeiss Axioscope 2, Zeiss International, Göttingen, Germany). A digital camera (Zeiss HRc) with imaging and measurement software (Zeiss Zen) were used to capture images of and measure starch granules. For each reference sample, randomly generated X-Y coordinates were used to measure and photograph approximately 100 granules from each individual plant (n=~300 from each species). All starch granules present in these photographs were measured and examined for morphological characteristics.

Granule Size and Morphology

Under ×400 magnification, the sizes, shapes, surface features and responses to polarized light of the starch granules were observed. Once identified and photographed, starch granules were described according to an established set of structural and surface characteristics (Reichert, 1913; ICSN, 2011) (**Table 2**). The size of each starch granule was measured as the maximum length through the hilum. Granules were also examined for the presence of morphological characteristics including 2-D shape, hilum position, central cavity, extinction cross, fissures, lamellae, pressure facets, and depressions along the margins (Reichert, 1913; ICSN, 2011). Starch granule size tends to be non-normally distributed and, therefore, relying on mean granule size is not appropriate for identification purposes (Louderback et al., 2017). Boxplots showing the distribution of starch granule size for each

TABLE 2 | Description of starch granule morphological characteristics adapted from Reichert (1913) and ICSN (2011).

Morphological characteristic	Description
Size	Maximum length through the hilum. Small (1–10 μm), medium (11–24 μm), and large (>25 μm)
Shape (2-D)	Circular-oval: appearing circular, rounded, and/or somewhat elongated
	Trapezoidal-triangular: either clearly trapezoidal (four-sided with two sides parallel) or somewhere in between trapezoidal and triangular (having three sides) with rounded corners
	Irregular: geometrically uneven margins
	Angular: having acute angles along margins
	Elongated: lanceolate to conical, rod-shaped
Hilum position	The point from which the layers of a starch granule forms
	Centric: within the geometric center of the granule
	Slightly eccentric: slightly off from the center of the granule
	Eccentric: well outside the center of the granule, at the proximal end
Central cavity	A surface depression or open area centered around the hilum; could be oblong or round
Extinction cross	Caused by optical interference of the layers of starch, the cross is only visible in cross-polarized light; An indistinct cross lacks clarity. A confused cross is distorted from a straight X form
Fissures	Cracks originating from the hilum of the granule
	Transverse-Stellate: extending at a right angle to the long axis of granule and/or star-shaped
	Longitudinal: extending along the long axis of the granule
	Mesial longitudinal cleft: large, deep, clean-cut crack, running through the middle of the long axis
Lamellae	Concentric growth rings; Lamellae can be lamellated (clearly visible) or non-lamellated (not visible)
Pressure facets	Indentations caused by the formation of compound granules
Depressions	Small, subtle indentations that may or may not be the result of compound granule formation

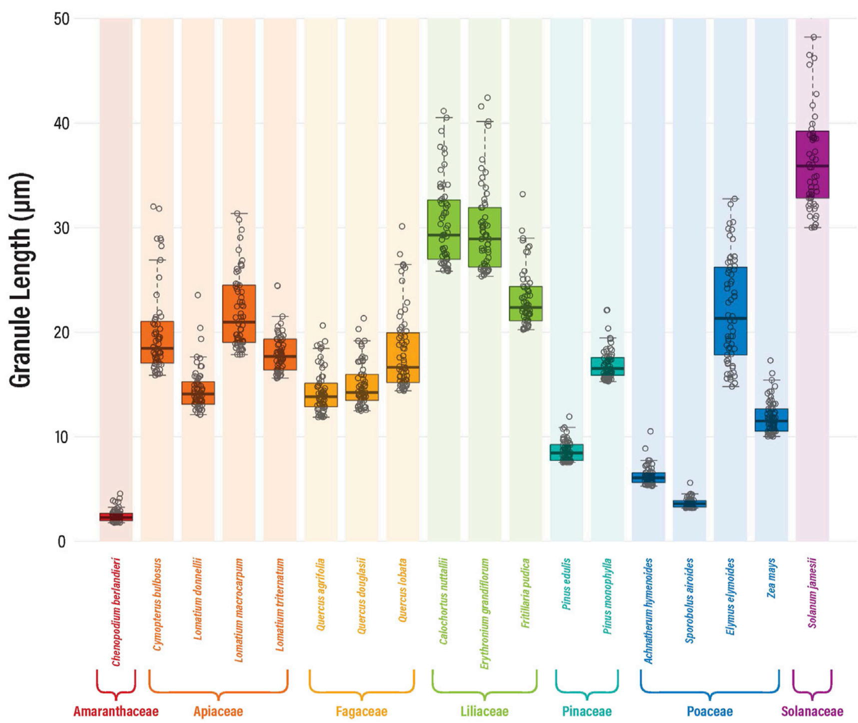

FIGURE 1 | Starch granule size distributions for the upper 20% granule lengths. All comparisons were significantly different ($p < 0.05$) except between *Cymopterus bulbosus* and *Lomatium triternatum*, *Lomatium donnellii* and *Quercus agrifolia*, *Lomatium donnellii* and *Quercus douglasii*, *Quercus douglasii* and *Quercus agrifolia*, and *Calochortus nuttallii* and *Erythronium grandiflorum*.

species was generated using the boxplot function in the "graphics" package for R v. 3.6.2 (Chambers et al., 1983; Becker et al., 1988; Murrell, 2005; R Core Team, 2019). To overlay individual granule size data, we used the jitter method within the stripchart function in the "graphics" package for R v.3.6.2.

Size distributions among all possible pairs of the study species were statistically compared using the non-parametric Kolmogorov-Smirnov

FIGURE 2 | (A,B) Frequency of morphological characteristics observed and documented on the upper 20% sizes of starch granules from each species.

(K-S) test. Applying a statistical analysis to starch granule identification promotes reproducibility and an overall increase in confidence (Louderback et al., 2017; Gao et al., 2021). It is becoming standard practice to measure the size, shape and morphological characteristics of reference starch granules from multiple populations so that variation and statistical significance can be assessed (Liu et al., 2014a; Brown and Louderback, 2020; Wilks et al., 2021).

RESULTS

Granule Size

Starch granule measurements from individual species were pooled for overall size and morphological analyses (**Supplementary Data S1**). Smaller granules tend to exhibit few diagnostic characteristics, therefore, relying on the top

FIGURE 2 | (Continued).

20% granule lengths allowed for greater differentiation between taxa because morphological characteristics occur more frequently in larger granules (Liu et al., 2014a; Louderback et al., 2017). Granule size distributions of all plant taxa are plotted in **Figure 1**. All comparisons were significantly different ($p < 0.05$), except between *Cymopterus bulbosus* and *Lomatium triternatum* (p = 0.08), *Lomatium donnellii* and *Quercus agrifolia* (p = 0.70), *Lomatium donnellii* and *Quercus douglasii* (p = 0.35), *Quercus douglasii* and *Quercus agrifolia* (p = 0.28), and *Calochortus nuttallii* and *Erythronium grandiflorum* (p = 0.38).

Granule Morphology

Morphological characteristics, including 2D shape, hilum position, central cavity, extinction cross, fissures, lamellae,

BOX 1 | Key to starch granules of seven vascular plant families.

A.	Hilum clearly eccentric	B
AA.	Hilum centric or nearly so	C
B.	Granule shape circular/oval	Liliaceae
BB.	Granule shape elongated	Solanaceae
C.	Shape uniformly circular/oval	D
CC.	Shape 3, 4, or >4 sides or irregular, with sharp or slightly rounded corners	H
D.	Granule length of top 20% less than 5 μm	Amaranthaceae
DD.	Granule length of top 20% greater than 7 μm	E
E.	Extinction cross distinct	F
EE.	Extinction cross indistinct or confused	G
F.	Granule with round central cavity lacking pressure facets	Pinaceae
FF.	Granule lacking central cavity but with pressure facets	Apiaceae
G.	Granule with oblong central cavity and lamellae	Poaceae *(Elymus elymoides)*
GG.	Granule lacking oblong central cavity and lamellae	H
H.	Granule triangular (3 sides) or trapezoidal (4 sides), slightly rounded corners	Fagaceae
HH.	Granule angular (>4 sides) or irregular	I (Poaceae)
I.	Granule length of top 20% > 10 μm and/or with transverse/stellate fissures	*Zea mays*
II.	Granule length of top 20% < 10 μm and lacking fissures	*Achnatherum hymenoides, Sporobolus airoides*, etc.

pressure facets, and depressions were documented for each granule for all 18 taxa (**Supplementary Data S1**). The frequency of those characteristics occurring on granules was calculated and expressed as a number between 0.0 and 1.0. (**Figures 2A,B**). Definitions for these characteristics were compiled primarily from ICSN (2011) and Reichert (1913), but some definitions were refined for this particular study based on previous work (Piperno et al., 2004, 2009; Holst et al., 2007; Perry and Quigg 2011; Musaubach et al., 2013; Yang and Perry 2013; Louderback et al., 2017; Brown and Louderback 2020; Joyce et al., 2021). All characteristics described above occur more frequently in the top 20% size range of starch granules and, therefore, we report frequencies for those granules (**Figures 2A,B**).

A dichotomous key for identifying starch granules of the seven plant families analyzed in this study was generated (Box 1). Because it is based only on a few species from each family, the key will undoubtedly be revised as more families, genera, and species are systematically analyzed using the characteristics presented herein. Furthermore, new techniques will lead to new diagnostic characteristics resulting in further revision and greater application of new diagnostic keys.

Different characteristics dominate the array of plant families that we have examined (**Figure 3**). For example, Amaranthaceae produces miniscule granules (<5 μm) with centric hila and a circular/oval shape. Because they are so small, it is difficult to visually discern additional morphological characteristics. Furthermore, Amaranthaceae starch are often formed in dense clusters of amyloplasts (sheets) bounded by cell walls, so it is rare to see isolated granules (even in reference material, **Figure 3**) (Reichert, 1913; Louderback, 2014; Capparelli et al., 2015; López et al., 2015). Apiaceae also produces starch granules with centric hila, circular/oval shape, and are frequently observed with transverse or stellate fissures, visible lamellae, and pressure facets (Herzog, 2014; Joyce et al., 2021). Another family that produces circular/oval granules with centric hila is Pinaceae. What distinguishes these granules from those in other families is the obvious presence of a round central cavity (Tinsley et al., 2021). Fagaceae, on the other hand, have starch granules with slightly eccentric hila and are characterized by triangular-trapezoidal shape with confused crosses, mesial longitudinal clefts, and visible lamellae (Messner 2011; Liu et al., 2014b; Brown and Louderback 2020). Liliaceae and Solanaceae produce starch granules with clearly eccentric hila, longitudinal fissures, and visible lamellae, but Liliaceae granules tend to be more circular/oval while Solanaceae are almost always elongated (Messner 2011; Louderback et al., 2017; Ahituv and Henry 2022).

Of the plant families examined by this study, Poaceae exhibits the most variation in starch granule size, shape, surface structures, and responses to polarized light. For example, *Achnatherum hymenoides* and *Sporobolus airoides* produce very small granules (<10 μm) with centric hila, but their shape is often angular, thus distinguishing them from Amaranthaceae. *Elymus elymoides* (and most Triticeae grasses—barley, wheat, rye) produce distinctive starch granules that can be quite large (up to ~30 μm), are circular/oval with oblong central cavities, indistinct crosses, and very visible lamellae. Additional characteristics (e.g., pits) have also been observed on these granules (Perry and Quigg, 2011; Yang and Perry 2013; Brown and

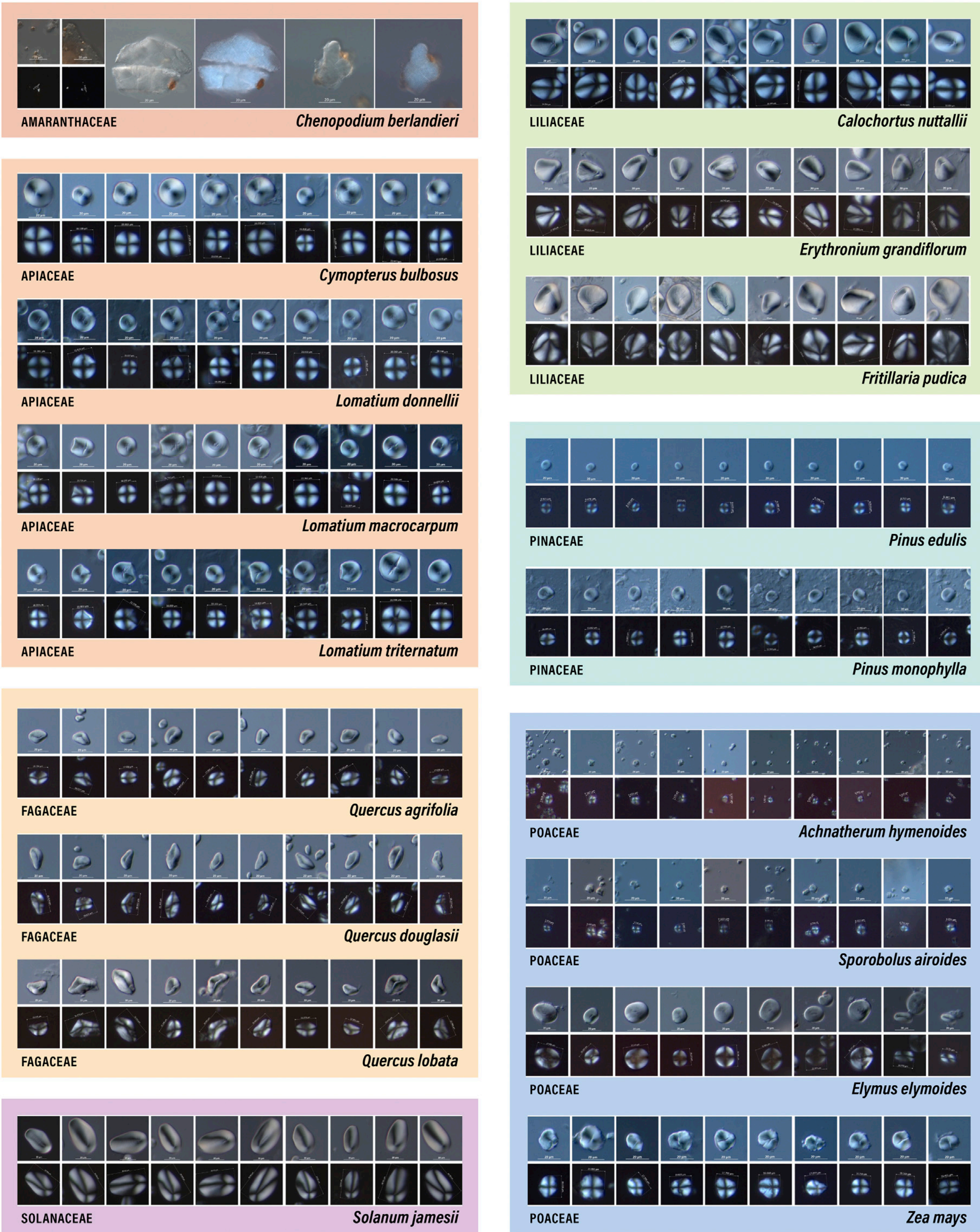

FIGURE 3 | Images of starch granules from each study species (*n*=18), shown in both differential interference contrast (DIC) (Nomarski) (top rows) and polarized (bottom rows) views.

Louderback, 2020). Finally, *Zea mays* is another grass genus that produces unique granules. These have a significantly different size range from *Elymus*, *Achnatherum*, and *Sporobolus* (**Figure 1**) and they also have an irregular shape with transeverse/stellate fissures that are not observed in other Poaceae taxa (Musaubach et al., 2013; Wilks et al., 2021).

DISCUSSION

The current study provides a standardized, systematic approach to defining characteristics that identify starch granules of seven major North American plant families (Amaranthaceae, Apiaceae, Fagaceae, Liliaceae, Pinaceae, Poaceae, and Solanaceae) that have dietary importance to humans. This approach was based on large numbers of reference starch granules from multiple plant populations of different genera. Our results revealed distinctive and, therefore, diagnostic characteristics of starch granules among the different families. A dichotomous key was developed based on these characteristics to aid in the identification of archaeological starches. Although the focus was on plant taxa from western North America, we find that our descriptions for size and morphological characteristics of starch granules are generally consistent with other studies at the genus and family levels. This includes plants from regions across the globe, such as Central and South America (e.g., Holst et al., 2007; Piperno et al., 2009; Musaubach et al., 2013; Capparelli et al., 2015; Lopez et al., 2015), China (e.g., Yang et al., 2012; Yang and Perry, 2013; Wang et al., 2019), the Mediterrean (Ahituv and Henry 2022), and eastern North America (Messner 2011).

Characteristics highlighted in our diagnostic key, however, will probably require revision once more genera are systematically analyzed. For example, the size, shape, and surface features of Poaceae starch are known to vary significantly among genera (e.g., Yang and Perry, 2013). It may be more important, therefore, to focus systematic surveys and identifications at the level of subfamilies or tribes in particularly diverse plant families.

Other intrinsic (genetic) and extrinsic (environmental) variables need to be analyzed with respect to starch granule characteristics and dynamics. Greater attention should be paid to sources of variation, particularly at the population level, and within large and diverse taxa, such as Poaceae. Furthermore, physiological variables, such as phenology, plant developmental stages, and seed maturation and fruit ripening processes should be addressed to understand how granule characteristics are affected. The taphonomy of starch granules and how that affects preservation, and ultimately the reliability of the identifications based upon subtle and microscopic characteristics, should be further assessed. However, this and many other studies to date continue to improve reliability of starch granule analysis in the understanding of plant foods in ancient human diets.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LL: conceptualization, formal analysis, methodology, writing and editing of original draft; SW: data analysis, figures, review and editing; NH: data analysis, figures, review and editing; GB: data analysis, review and editing; KJ: data analysis, review and editing; BP: botanical expertise, methodology, writing and editing of original draft.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.897183/full#supplementary-material

REFERENCES

Ahituv, H., and Henry, A. G. (2022). An Initial Key of Starch Grains from Edible Plants of the Eastern Mediterranean for Use in Identifying Archaeological Starches. *J. Archaeol. Sci. Rep.* 42, 103396. doi:10.1016/j.jasrep.2022.103396

Becker, R. A., Wilks, A. R., and Chambers, J. M. (1988). *The New S Language: A Programming Environment for Data Analysis and Graphics*. Belmont, California: Taylor Francis.

Brown, G. H., and Louderback, L. A. (2020). Identification of Starch Granules from Oak and Grass Species in the Central Coast of California. *J. Archaeol. Sci. Rep.* 33, 102549. doi:10.1016/j.jasrep.2020.102549

Capparelli, A., Pochettino, M. L., Lema, V., López, M. L., Andreoni, D., Ciampagna, M. L., et al. (2015). The Contribution of Ethnobotany and Experimental Archaeology to Interpretation of Ancient Food Processing: Methodological Proposals Based on the Discussion of Several Case Studies on Prosopis Spp., Chenopodium Spp. and Cucurbita Spp. from Argentina. *Veget. Hist. Archaeobot.* 24, 151–163. doi:10.1007/s00334-014-0497-4

Chambers, J. M., Cleveland, W. S., Kleiner, B., and Tukey, P. A. (1983). *Graphical Methods for Data Analysis*. United Kingdom: Wadsworth Brooks/Cole Advanced Books Software.

Gao, M., Moussavi, M., and Myers, D. (2021). Analysis and Specification of Starch Granule Size Distributions. *J. Vis. Exp.* 169, 61586. doi:10.3791/61586

Herzog, N. M. (2014). Starch Grain Analysis in California and the Great Basin. *Calif. Archaeol.* 6, 171–189. doi:10.1179/1947461x14z.00000000039

Holst, I., Moreno, J. E., and Piperno, D. R. (2007). Identification of Teosinte, Maize, and *Tripsacum* in Mesoamerica by using Pollen, Starch Grains, and Phytoliths. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17608–17613. doi:10.1073/pnas.0708736104

ICSN (2011). The International Code of Starch Nomenclature. Available at: http://www.fossilfarm.org/ICSN/Code.html (Accessed 10 12, 2021).

Joyce, K. T., Louderback, L. A., and Robinson, E. (2021). Direct Evidence for Geophyte Exploitation in the Wyoming Basin. *Am. Antiq.* 64, 659–674. doi:10.1017/aaq.2021.115

Liu, L., Kealhofer, L., Chen, X., and Ji, P. (2014b). A Broad-Spectrum Subsistence Economy in Neolithic Inner Mongolia, China: Evidence from Grinding Stones. *Holocene* 24 (6), 726–742. doi:10.1177/0959683614526938

Liu, L., Ma, S., and Cui, J. (2014a). Identification of Starch Granules Using a Two-Step Identification Method. *J. Archaeol. Sci.* 52, 421–427. doi:10.1016/j.jas.2014.09.008

López, M. L., Medina, M. E., and Rivero, D. E. (2015). First Records of Chenopodium spp./Amaranthus spp. Starch Grains and their Relevance to the Study of the late Holocene Human Subsistence in Central Argentina. *Holocene* 25 (2), 288–295. doi:10.1177/0959683614558652

Louderback, L. A., Herzog, N. M., and Pavlik, B. M. (2017). A new approach for identifying starch granules of wild food plants from arid western North America. *Starch - Stärke* 69 (5–6), 1600167. doi:10.1002/star.201600167

Louderback, L. A. (2014). *The Ecology of Human Diets during the Holocene at North Creek Shelter, Utah*. PhD dissertation. Seattle, WA: University of Washington. https://digital.lib.washington.edu/researchworks/handle/1773/25980.

Mercader, J., Akeju, T., Brown, M., Bundala, M., Collins, M. J., Copeland, L., et al. (2018). Exaggerated expectations in ancient starch research and the need for new taphonomic and authenticity criteria. *Facets* 3, 777–798. doi:10.1139/facets-2017-0126

Messner, T. C. (2011). *Acorns and Bitter Roots: Starch Grain Research in the Prehistoric Eastern Woodlands*. Tuscaloosa: University Alabama Press.

Murrell, P. (2005). *R Graphics*. London: Chapman Hall/CRC Press.

Musaubach, M. G., Plos, A., and Babot, M. D. P. (2013). Differentiation of Archaeological Maize (*Zea Mays L.*) from Native Wild Grasses Based on Starch Grain Morphology. Cases from the Central Pampas of Argentina. *J. Archaeol. Sci.* 40, 1186–1193. doi:10.1016/j.jas.2012.09.026

Nägeli, C. (1858). *Die Stärkekörner. Morphologishe, Physiologishe, Chemisch-Physicalisch und Systematisch-Botansiche*. Zurich: Verlag F. Schulthess.

Perry, L., and Michael Quigg, J. (2011). Starch Remains and Stone Boiling in the Texas Panhandle Part II: Identifying Wildrye (Elymusspp.). *Plains Anthropol.* 56, 109–119. doi:10.1179/pan.2011.011

Piperno, D. R., Ranere, A. J., Holst, I., Iriarte, J., and Dickau, R. (2009). Starch Grain and Phytolith Evidence for Early Ninth Millennium B.P. Maize from the Central Balsas River Valley, Mexico. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5019–5024. doi:10.1073/pnas.0812525106

Piperno, D. R., Weiss, E., Holst, I., and Nadel, D. (2004). Processing of Wild Cereal Grains in the Upper Palaeolithic Revealed by Starch Grain Analysis. *Nature* 430, 670–673. doi:10.1038/nature02734

R Core Team (2019). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Reichert, E. T. (1913). *The Differentiation and Specificity of Starches in Relation to Genera, Species, Etc.: Stereochemistry Applied to Protoplasmic Processes and Products, and as a Strictly Scientific Basis for the Classification of Plants and Animals*. Washington, DC: Carnegie Institution of Washington.

Shannon, J. C., Garwood, D. L., and Boyer, C. D. (2009). "Genetics and Physiology of Starch Development," in *Genetics and Physiology of Starch Development" in: Starch: Chemistry and Technology*. Editors J. BeMiller and R. Whistler (New York: Academic Press), 23–82. doi:10.1016/b978-0-12-746275-2.00003-3

Tinsley, D., Louderback, L., Pavlik, B., Baker, M., Townsend, T., Tucker, K., et al. (2021). *Testing the Pinyon Premise: Archaeobotanical Analyses of Sediments, Bedrock Milling Features, and Ground Stone Artifacts from West-Central Lincoln County, Nevada*. Ely, Caliente, NV: Prepared for BLM Ely District and Caliente Field Offices.

Wang, J., Zhao, X., Wang, H., and Liu, L. (2019). Plant Exploitation of the First Farmers in Northwest China: Microbotanical Evidence from Dadiwan. *Quat. Int.* 529, 3–9. doi:10.1016/j.quaint.2018.10.019

Wilks, S., Louderback, L. A., and Boomgarden, S. (2021). Starch Granule Size and Morphology as a Proxy for Water Regime Influence on Zea mays. *EblEthnobio. Lett.* 12 (1), 35–43. doi:10.14237/ebl.12.1.2021.1725

Yang, X., and Perry, L. (2013). Identification of Ancient Starch Grains from the Tribe Triticeae in the North China Plain. *J. Archaeol. Sci.* 40 (8), 3170–3177. doi:10.1016/j.jas.2013.04.004

Yang, X., Zhang, J., Perry, L., Ma, Z., Wan, Z., Li, M., et al. (2012). From the Modern to the Archaeological: Starch Grains From Millets and their Wild Relatives in China. *J. Archaeol. Sci.* 39, 247–254. doi:10.1016/j.jas.2011.09.001

Conflict of Interest: Author GHB is currently employed by Pacific Legacy, Inc., but was a graduate student at California State University, Sacramento when research was conducted for this study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Diet at the onset of the Neolithic in northeastern Iberia: An isotope–plant microremain combined study from Cova Bonica (Vallirana, Catalonia)

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The emergence of Neolithic societies was transformative, impacting many aspects of life, particularly diet. The process of Neolithization in Iberia is increasingly understood as the arrival of new people from the Central Mediterranean, who dispersed along the Iberian coasts introducing cereal production, herding, and Cardial pottery and associated material culture. Although research has clarified aspects of the cultigen-dominated economy of these new people, questions remain due to the limitations of conventional archaeobotanical and archaeozoological methods that tend to produce indirect evidence. The extent to which these early farmers adopted Mesolithic staples, which are often difficult to detect with other methods, remains unclear. Furthermore, questions surround the nature of methods of food preparation Cardial Neolithic people used when incorporating grains into their diet. In this study, we examined direct evidence of the diet from the Iberian Cardial Neolithic site of Cova Bonica (Vallirana, Baix Llobregat, Catalonia) using CN stable isotopes on bone and plant microremains trapped in dental calculus from six human individuals and associated fauna. Isotopes show a diet based on terrestrial C_3 resources, with no isotopic evidence of aquatic or C_4 resource consumption. Plant microremains (starches and phytoliths) provide evidence of cereal use, as well as of other plant foods. However, perhaps due to Bonica's early farmers' choice of grain variety, their grain processing methods, or due to specific dental calculus formation factors, the grain assemblages are rather limited and provide scarce information on food preparation.

KEYWORDS

Neolithic, diet, stable isotopes, plant microremains, Western Mediterranean

1 Introduction

1.1 Combining stable isotope and plant microremain analysis for dietary studies

The study of stable isotopes on both bone collagen and plant microremains in dental calculus is useful for recovering information on past diets and has been used individually as a direct means to trace critical aspects of prehistoric populations such as dietary patterns. While carbon and nitrogen stable isotope analysis is a well-established and frequently used method to obtain information on average protein consumption (Lee-Thorp, 2008), the study of plant microremains on dental calculus to recover information on plant food consumption is still not as frequently used as the latter (Power et al., 2015); the combination of both proxies is even scarcer. However, by combining these two techniques, there is a potential for achieving a wider picture of dietary subsistence patterns during prehistoric times (Goude et al., 2019; Goude et al., 2020; Salazar-García et al., 2021).

Isotopic diet reconstruction is based on the principle that as animal and human tissue grows, it records the isotopic composition of the diet through predictable isotope fractionation (Schoeller, 1999). For this analysis, bone collagen is usually the preferred substrate because it is both the only considerable nitrogen source from skeletal remains (Salazar-García et al., 2014a) and has accepted quality indicators to assess its isotopic integrity (De Niro, 1985; Van Klinken, 1999). However, it is necessary to consider when interpreting results that stable isotope ratios from bone collagen reflect only the main dietary protein sources consumed several years prior to death (Hedges et al., 2007; Katzenberg, 2012) rather than that of a diet as a whole, especially for nitrogen (Ambrose and Norr, 1993) since carbon may be derived from other dietary macronutrients like sugars and fats (Howland et al., 2003; Jim et al., 2004).

On the other hand, dental calculus is an oral plaque mineralized by salivary calcium. During the accumulation process, dietary and environmental information from plant and animal foods, including starch grains, phytoliths, lipids, proteins, and DNA, can become trapped and embedded. Dietary remains retrieved from dental calculus are a useful additional source of information on human diet and behavior. Amongst dietary reconstruction methods, it is a helpful complement to other methods because it offers direct information on food consumed and substances that enter the mouth (Armitage 1975; Henry and Piperno 2008; Power et al., 2014; Warinner et al., 2014; Leonard et al., 2015; Power et al., 2015). In this sense, while isotope analysis gives a more quantitative approach on the origin of consumed protein, plant microremains on dental calculus give more qualitative data on specific foods consumed. Likewise, while stable isotope analysis gives long-term averaged dietary information on protein consumption (Lee-Thorp, 2008), plant microremains can give information on other types of consumed non-protein food resources, even if less frequently eaten (Leonard et al., 2015; Power et al., 2015). Therefore, combining these two techniques provides complementary information on past diets and should be implemented in dietary studies when possible (Salazar-García et al., 2021).

The application of these analytical techniques in Mediterranean Iberia is, however, unequal between them. While studies of stable isotopes have significantly increased during the past decade in prehistoric periods (Salazar-García et al., 2018) creating an important corpus of prehistoric dietary data from the Mesolithic onward in the region and its hinterland, plant microremain studies on dental calculus are almost non-existent. Isotope studies from the Mesolithic (García-Guixé et al., 2006; Fernández-López de Pablo et al., 2013; Salazar-García et al., 2014b), Neolithic–Chalcolithic (Fontanals-Coll et al., 2015, Fontanals-Coll et al., 2017; García-Borja et al., 2013; Gibaja et al., 2016; McClure et al., 2011; Salazar-García, 2009, Salazar-García, 2011, Salazar-García, 2014; Salazar-García et al., 2013, Salazar-García et al., 2016, Salazar-García et al., 2017; Villalba-Mouco et al., 2018a; Villalba-Mouco et al., 2018b; Villalba-Mouco et al., 2019), Bronze Age (McClure et al., 2011; García-Borja et al., 2013; Salazar-García et al., 2017), and Iron Age (Salazar-García et al., 2010) periods have been performed in Mediterranean Iberia. On the other hand, plant microremain studies on dental calculus have only been performed so far on one Paleolithic site (Salazar-García et al., 2013) and one Chalcolithic site (Power et al., 2014) in prehistoric Mediterranean Iberia.

1.2 The site studied: Cova Bonica

Cova Bonica (41°22′10.29″N, 1°53′38.64″E) is located in the vicinity of Vallirana, near Barcelona (northeast of the Iberian Peninsula). The site was discovered and first excavated in 1936; however, the archaeological artifacts were not published until the 70s (Baldellou, 1974). Current archaeological excavations are conducted by Grup de Recerca del Quaternari (Universitat de Barcelona) and produced genetic (Olalde et al., 2015) and archaeological data (Oms et al., 2017; Daura et al., 2019). Amongst the archaeological data published by Daura et al., 2019, macrobotanical evidence of diet from flotation consists only of the remains of *Arbutus unedo* charcoal, which produces edible fruits, and some taxa charcoal with aromatic and medicinal properties such as *Laurus nobilis* or *Rosmarinus officinalis*.

The cave consists of a principal chamber (SP) and two lateral chambers, one next to the entrance (SL1) and another in the inner area (SL2). The cave and sediments were partially destroyed by a sparry calcite mine, but intact Cardial infillings have been preserved in two separated layers: IV_2 preserved in SP-Grao 1 and IV in SP (Figure 1).

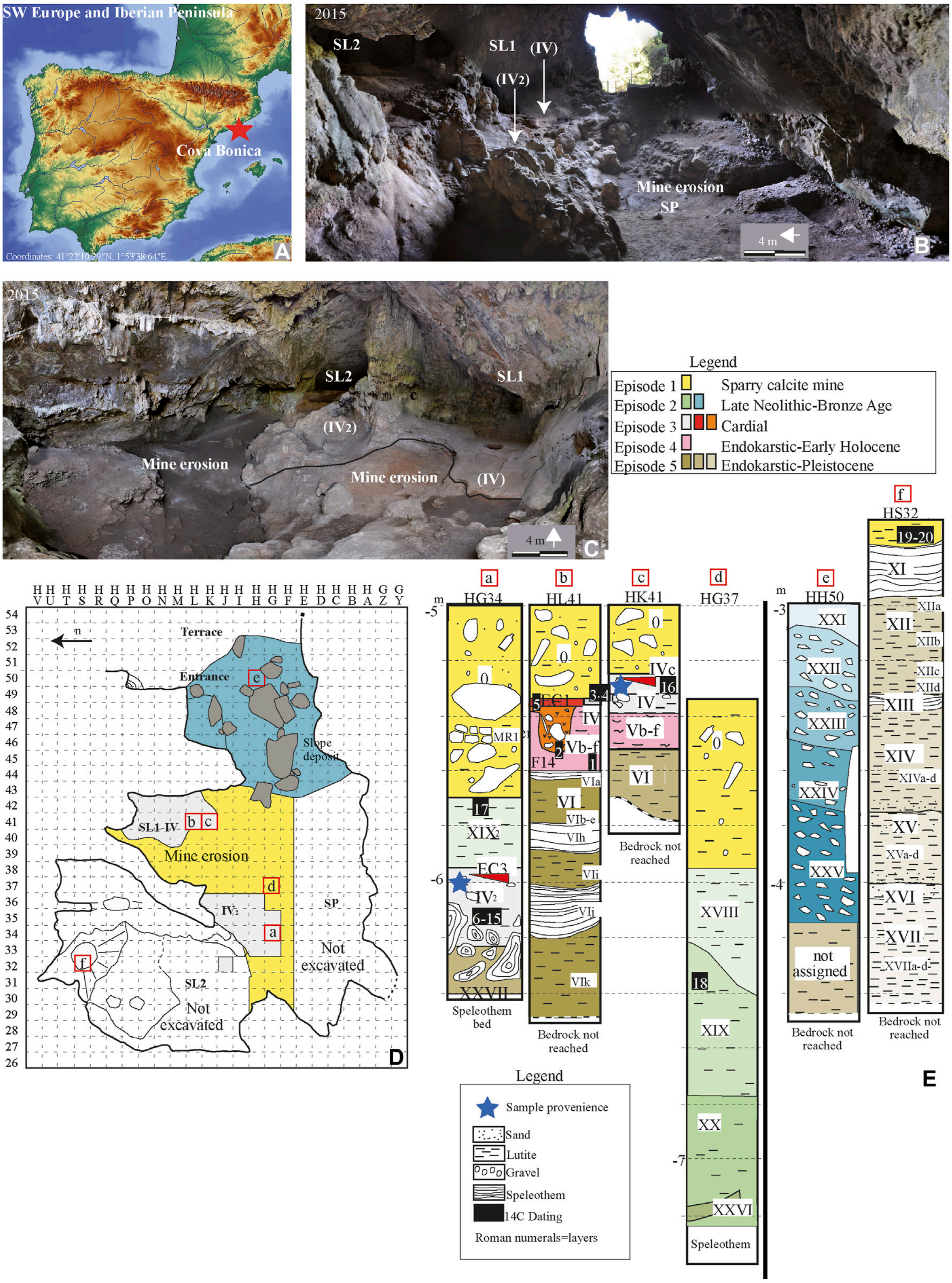

FIGURE 1
Cova Bonica. **(A)** Location of Cova Bonica in the NE of the Iberian Peninsula. **(B)** Current view of the cave entrance and main areas. **(C)** Neolithic area excavated. **(D)** Plan of the site indicating the excavated area. **(E)** Stratigraphy of the site's profiles (cave -a to d-, terrace -e-, and SL2 -f-).

The stratigraphic succession of Cova Bonica is complex and can be grouped into five episodes (1–5). Episodes 5 and 4 represent endokarstic deposits and a ceiling collapse with a restricted entrance and scarce archaeological materials. Episode 3 corresponds to the Cardial Neolithic horizon and is formed by layers IV and IV_2. Layer IV is located in SL1, laying above the stalagmitic flowstone of layer V and contains a thermoaltered sediment labeled as EC1 and 14 post-holes, one of them yielding a radiocarbon age of 6,340 ± 34 BP. Layer IV contains scarce archaeological artifacts and human remains probably displaced from layer IV_2; a sheep bone shows a ^{14}C age of 6,158 ± 32 BP, and one human individual shows an age of 6,395 ± 39 BP. Layer IV_2 lies in a depression in the cave's bedrock and between large collapsed speleothems and contains a minimum number of individuals (MNI) of 7 (ca. 315 bones): 6 individuals defined osteologically, and a seventh one defined by aDNA. The archaeological remains also include personal ornaments, lithics, bone tools, Cardial Neolithic pottery, faunal remains, and charcoal. Radiocarbon dating of the human remains recovered from layers IV and IV_2 indicates a homogeneous assemblage and a short or synchronic time span for the deposition of the human corpses around ca. 6400 BP (5,470–5,320 ka cal. BP). Episode 2, recorded in several areas of the cave, represents archaeological occupation spanning from the late Neolithic to the Bronze Age.

2 Materials and methods

For this study, we analyzed for stable isotopes 12 faunal and 6 human remains and assessed 5 samples of human dental calculus, one sediment and two associated blank controls. Human and faunal remains analyzed in the present work were recovered during the 2008–2017 field seasons conducted by Grup de Recerca del Quaternari (University of Barcelona). Materials were previously identified and studied in the Guixera laboratory (Castelldefels City Council) (Figures 2, 3). Human remains were scattered and often it was not possible to associate elements (e.g., long bones, ribs, teeth) as pertaining to the same individual with certainty.

2.1 CN stable isotope analysis

Bone samples from 6 human individuals and 12 faunal specimens from five species were sampled from Cova Bonica's Early Neolithic layer (IV_2) (Table 1) to obtain information on their diet by carrying out C and N stable isotope ratio analysis. The consumption of C_3 and C_4 terrestrial resources is distinguishable by the $\delta^{13}C$ stable isotope ratio (Van der Merwe and Vogel, 1978). Isotopic signals also help define the input in the diet of terrestrial and marine foods (Chisholm et al., 1982), although if freshwater or estuarine fish are involved, the interpretation of $\delta^{13}C$ values becomes more complex (Salazar-García et al., 2014b). The $\delta^{15}N$ stable isotope ratio increases by 3–5 ‰ up the food chain with each trophic level and is usually used to indicate the position of an organism in the food chain (Minagawa and Wada, 1984). Even if this quantification is less straightforward than previously thought (Hedges and Reynard, 2007), based on the exact values of the nitrogen ratio, it is potentially possible to differentiate between individuals that consumed more animal resources from those who consumed very little animal proteins (Fahy et al., 2013). Furthermore, the fact that aquatic food chains tend to contain more trophic levels than terrestrial ones, and therefore, show an increase in $\delta^{15}N$, helps to discriminate between the consumption of marine or C_4 terrestrial foods when samples are ^{13}C enriched (Schoeninger and DeNiro, 1984).

Prior to analysis, visible contaminants were removed with aluminum oxide powder abrasion. Methods outlined by Richards and Hedges (1999) were followed to extract collagen for the C and N isotope ratio analysis at the Stable Isotope Facilities of the University of Cape Town (Cape Town, South Africa). Whole bone fragments weighing ca. 300 mg obtained from each of the specimens were demineralized in a 0.5 M HCl solution at 5°C. They were then rinsed three times with deionized water until the pH became neutral and gelatinized over 48 h at 70°C before being filtered and ultra-filtered using 9-ml EZEE© filters to remove small particles (<8 μm) and >30 kDa Amicon© ultrafilters, respectively. Finally, the purified solutions were frozen and lyophilized before being weighed into tin capsules and loaded into mass spectrometers.

The resultant collagen product was combusted to N_2 and CO_2. The carbon and nitrogen isotope ratios in collagen were measured in duplicate (reproducibility better than 0.1 ‰) using a Finnigan Delta plus XP continuous-flow isotope ratio mass spectrometer (Thermo Fisher Scientific, United States) after being combusted in an elemental analyzer Flash EA 1112 interfaced with it (Thermo Fisher Scientific, United States), at the Isotope Facilities of the University of Cape Town (South Africa). Stable carbon isotope ratios were expressed relative to the VPDB scale (Vienna PeeDee Belemnite) and stable nitrogen isotope ratios were measured relative to the AIR scale (atmospheric N_2), using the delta notation (δ) in parts per thousand (‰). Repeated analysis of internal and international standards (methionine, Merk Gelatin, Valine, and seal bone) determined an analytical error <0.1 ‰ (1σ) for $\delta^{13}C$ and $\delta^{15}N$.

2.2 Dental calculus microremains

For samples prepared for this study, teeth were evaluated and sampled for dental calculus with a scalar in the field (Table 2).

FIGURE 2
Faunal remains from layer IV_2. **(A)** (Site # 3475) equid lower deciduous tooth (dp3). **(B)** (Site # 2969) brown bear metatarsal. **(C)** (Site # 3166) bovid calcaneum. **(D)** (Site # 3148) ovicaprid humerus. **(E)** (Site # 3147) ovicaprid humerus. **(F)** (Site # 2760) ovicaprid humerus. **(G)** (Site # 2444) bovid calcaneum. **(H)** (Site # 3167) ovicaprid humerus. **(I)** (Site # 2872) ovicaprid humerus. **(J)** (Site # 3279) ovicaprid humerus.

FIGURE 3
Human remains from layer IV_2. **(A)** (Site # 3029) humerus. **(B)** (Site # 2996) humerus. **(C)** (Site # 3121) humerus. **(D)** (Site # 3066) frontal bone. **(E)** (Site # 3075) mandible. **(F)** (Site # 2517) scapula. **(G)** (Site # 2777) vertebra. **(H)** (Site # 2303) lower canine. **(I)** (Site # 2793) lower incisor. **(J)** (Site # 2595) upper premolar. **(K)** (Site # 3260) canine. **(L)** (Site # 3155) canine. **(M)** (Site # 2618) upper molar. **(N)** (Site # 2583) upper premolar. **(O)** (Site # 3334) upper incisor. **(P)** (Site # 2406) upper canine. **(Q)** (Site # 3439) premolar. **(R)** (Site # 3343) incisor. **(S)** (Site # 3158) upper incisor.

TABLE 1 Cova Bonica $\delta^{13}C$ and $\delta^{15}N$ values from fauna and humans, human sex and age, chronology, collagen control indicators (yield, % C, % N, and C:N), S-UCT number, sampled bone, and archaeological context.

S-UCT	Site #	Archaeological context	Species	Element	Sex	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	% col.	% C	% N	C:N
19257	2777	Layer IV_2	Human	Vertebra	Indet.	8–9 yo	−19.4	8.5	1.7	39.6	14.1	3.3
19258	3066	Layer IV_2	Human	Frontal	Indet.	2.5–3 yo	−19.1	11.0	1.5	41.7	14.8	3.3
19259	2517	Layer IV_2	Human	Scapula	Indet.	4–5 yo	−19.7	8.8	4.4	42.0	14.9	3.3
19260	3121	Layer IV_2	Human	Humerus	Indet.	12–13 yo	−19.8	8.7	1.6	39.9	14.0	3.3
19261	2996	Layer IV_2	Human	Humerus	Indet.	Adult	−19.5	9.3	3.6	41.7	14.8	3.3
19262	3029	Layer IV_2	Human	Humerus	Indet.	Adult	−19.6	9.2	1.0	41.4	14.4	3.4
19263	3100	Layer IV_2	Ovicaprid	Humerus	*	Adult	−20.9	4.5	0.8	33.4	11.5	3.4
19264	2970	Layer IV_2	*Ovis aries*	Humerus	*	Adult	−19.7	4.0	1.3	42.4	15.1	3.3
19265	3328	Layer IV_2	Ovicaprid	Humerus	*	*	−20.0	3.6	1.7	42.1	14.9	3.3
19266	3148	Layer IV_2	Ovicaprid	Humerus	*	Perinatal/ fetal	−20.1	5.0	2.7	42.5	14.7	3.4
19267	3167	Layer IV_2	Ovicaprid	Humerus	*	Immature	−19.5	4.7	1.9	42.7	14.2	3.5
19268	3279	Layer IV_2	*Capra hircus*	Humerus	*	Adult	−19.1	3.7	1.0	42.4	14.7	3.4
19269	3147	Layer IV_2	Ovicaprid	Humerus	*	Fetal	−20.0	5.8	1.0	38.5	12.9	3.5
19270	2872	Layer IV_2	Ovicaprid	Humerus	*	Neonatal	−19.6	5.1	0.7	41.4	14.4	3.4
19271	2444	Layer IV_2	*Bos taurus*	Calcaneus	*	Immature	−21.0	6.4	4.1	42.7	15.2	3.3
19272	3166	Layer IV_2	*Bos taurus*	Calcaneus	*	*	−19.7	5.0	4.6	42.5	15.1	3.3
19273	3475	Layer IV_{2b}	Equid	Deciduous tooth	*	Immature	−21.0	4.2	1.7	32.1	11.3	3.3
19274	2969	Layer IV_2	*Ursus arctos*	Metatarsal	*	Adult	−19.8	8.8	3.0	42.4	14.9	3.3

Samples were subsampled in the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). When possible, we sampled chunks of supragingival dental calculus by prying them off, rather than scraping off a powder but a significant number of samples were of powder. The samples were then processed in a Heraeus safety-controlled airflow cabinet at the labs of the Department of Primatology in the Max Planck Institute for Evolutionary Anthropology. We used EDTA to decalcify the sample (Power et al., 2015; Tromp et al., 2017).

After the decalcification, the sample was rinsed twice with distilled water after centrifugation at 2,000×g for 10 min (Roth Mini-Centrifuge). Then, after the first decanting, the tubes were refilled with a 25% glycerin solution. Then we mounted 20 µL of the solution on a slide with an 18 × 18 mm or a 24 × 24 mm coverslip. During the preparation of samples, we prepared slides using an identical mounting procedure using 25% glycerin solution but without any dental calculus, to serve as a control blank to expose environmental contamination. We counted starches, phytoliths, and all other microremains irrespective of the type at 200–400x magnification using an absolute counting method with a light microscope under brightfield and cross-polarized light on an Axio Scope A1, Zeiss microscope with AxioVs40 V 4.8.2.0 software. Microremains were classified according to standard conventions (ICPN 2.0, Neumann et al., 2019).

3 Results

3.1 CN stable isotope analysis

Samples from six humans and 12 faunal specimens from five different species (*Ovis aries*, *Capra hircus*— ovicaprid when not assigned to species taxon—, *Bos taurus*, equid, and *Ursus arctos*) were taken for stable isotope analysis. All samples yielded sufficient collagen in the >30 kDa fraction for the $\delta^{13}C$ and $\delta^{15}N$ analysis in duplicate. All of them met published collagen quality controls: appropriate CN elemental percentages together with C:N ratios between 2.9 and 3.6 (De Niro, 1985; Ambrose, 1990; Van Klinken, 1999). All isotope ratio results from Cova Bonica are given in Table 1 and shown in Figure 4.

Analyzing the carbon values of the 11 terrestrial herbivores, it can be seen that their $\delta^{13}C$ mean value is -20.1 ± 0.6 (1σ) ‰ and its minimum and maximum values are -21.0 ‰ and -19.1 ‰, respectively. This herbivore value is compatible with a typical C_3 terrestrial ecosystem. Analyzing the nitrogen values, the herbivore mean $\delta^{15}N$ value is 4.7 ± 0.9 (1σ) ‰ and has minimum and maximum values of 3.6 and 6.4 ‰, respectively, establishing the trophic baseline for human subsistence. The only available omnivore carbon value is -19.8 ‰, which is also consistent with the general herbivore values and a terrestrial C_3 food ecosystem. The $\delta^{15}N$ value of the omnivore specimen analyzed is 8.8 ‰, which is 4.1 ‰ higher than the

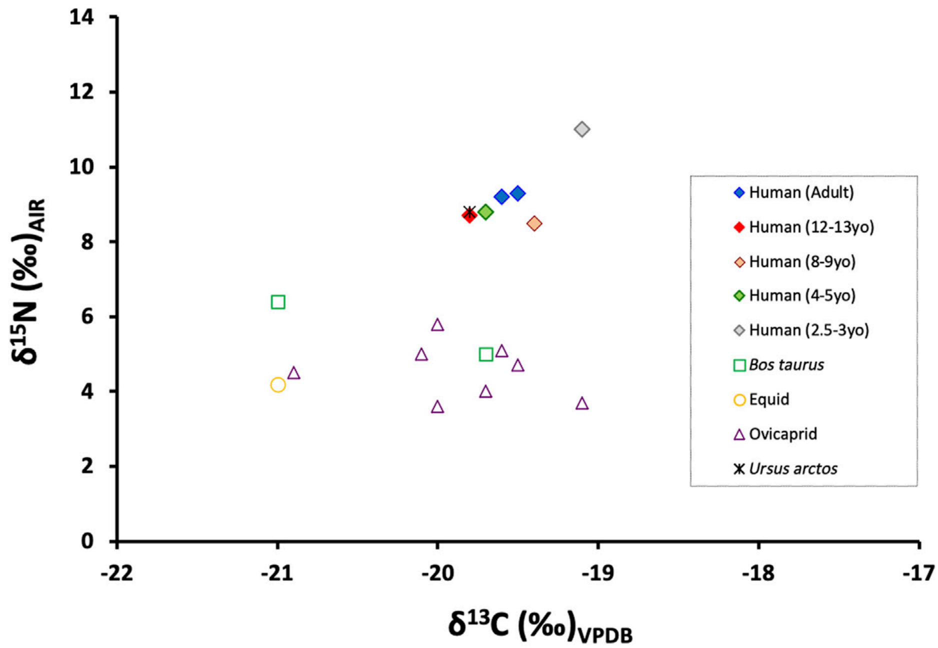

FIGURE 4
Plot of human and animal bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from Cova Bonica.

TABLE 2 Cova Bonica dental calculus samples taken for microremain microscopic analysis.

Sample code	Material	Dental piece code	Archaeological context	Species	Sex	Age	Tooth	Position	Weight (mg)
CB002	Dental calculus	3155	Layer IV_2	Human	Female	Adult	Canine	Supragingival	4.139
CB003	Dental calculus	3260	Layer IV_2	Human	Indet.	Adult	Canine	Supragingival	4.347
CB012	Dental calculus	3075	Layer IV_2	Human	Indet.	Adult	Incisor	Supragingival	5.916
CB014	Dental calculus	3334	Layer IV_2	Human	Indet.	8–9 yo	Incisor	Supragingival	7.137
CB019	Dental calculus	2618	Layer IV_2	Human	Indet.	12–13 yo	Molar	Supragingival	2.114
CB008	Sediment	*	Layer IV_2	*	*	*	*	*	0.987
CB002 b	Blank	*	*	*	*	*	*	*	*
CB012 b	Blank	*	*	*	*	*	*	*	*

herbivore mean value and situates the omnivore (*Ursus arctos*) almost one complete step higher than the herbivores in the food chain. Unfortunately, no aquatic resources are available for this site, and thus, the marine and freshwater-specific "baseline" is lacking for Cova Bonica.

All humans from the Early Neolithic period (n=6) have $\delta^{13}C$ and $\delta^{15}N$ mean values of −19.5 ± 0.2 (1σ) ‰ (min: −19.8 ‰ and max: −19.1 ‰) and 9.3 ± 0.9 (1σ) ‰ (min: 8.5 ‰ and max: 11.0 ‰), respectively. These $\delta^{13}C$ values portray a diet based on terrestrial C_3 resources, while the $\delta^{15}N$ values place humans at almost one full trophic level over herbivores (4.6 ‰ higher) and only slightly higher than the one omnivore analyzed (0.5 ‰ higher). There is no isotopic evidence of marine protein consumption.

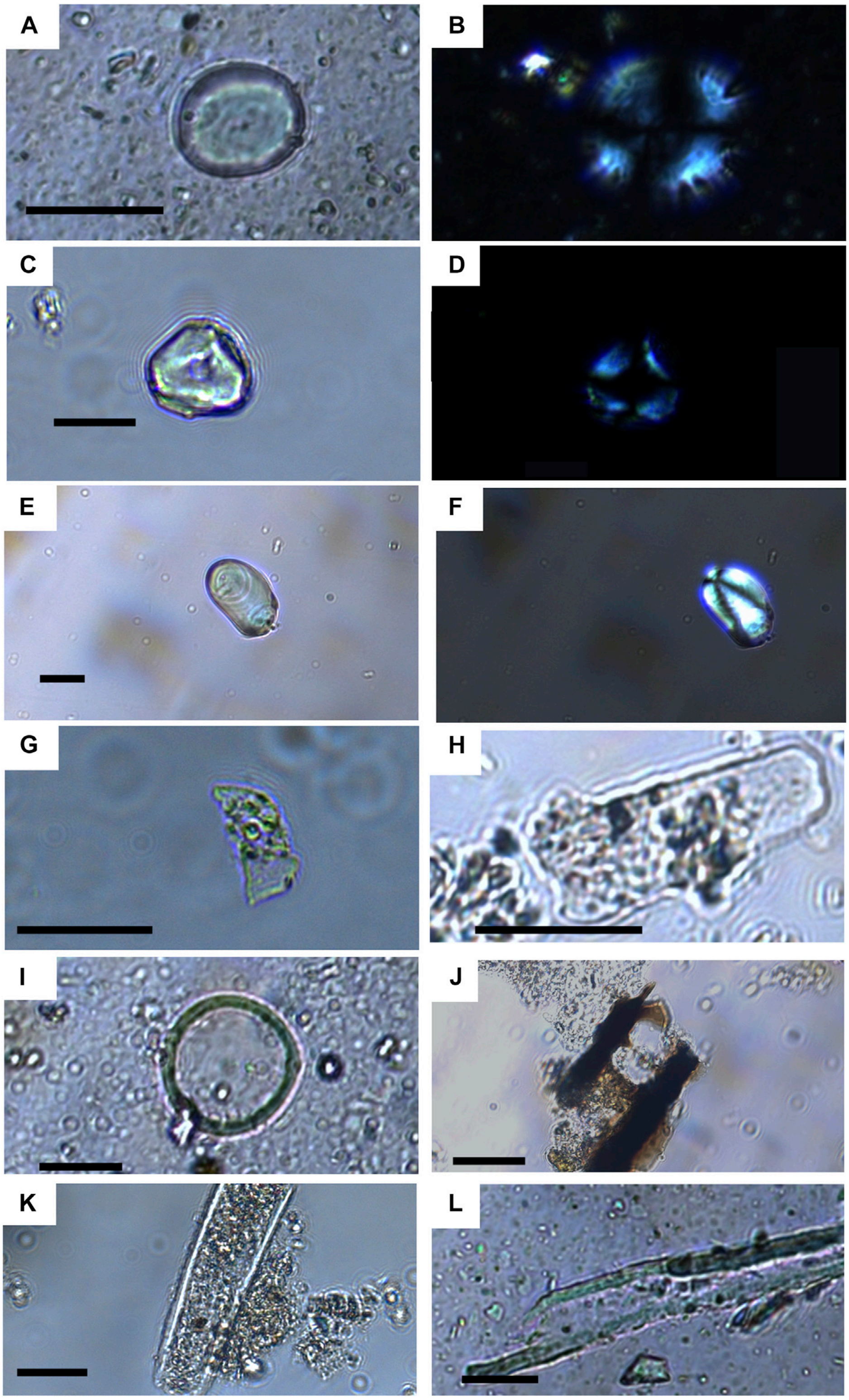

FIGURE 5
Micrographs of a selection of microremains. All scale bars represent 20 microns, **(A)** brightfield image of a lenticular starch in CB003, **(B)** cross-polarized image of the same starch, **(C)** brightfield image of faceted starch in CB003, **(D)** cross-polarized image of the same starch, **(E)** brightfield image of an eccentric starch in CB008, **(F)** cross-polarized image of the same starch, **(G)** trapezoidal (grass short-cell) phytolith in CB012, **(H)** elongate psilate thin phytolith, **(I)** indeterminate pollen from CB003, **(J)** charcoal particle in CB019, **(K)** broken probable non-human mammalian hair in CB003, and **(L)** plant fiber in CM003.

TABLE 3 Chart of total microremains. B samples represent controls. Eccentric starch variant 1: a slightly eccentric starch of variable sizes. Eccentric starch variant 2: a highly eccentric starch of variable sizes. Eccentric starch variant 3: an eccentric faceted starch over 20 microns in diameter.

	Starches										Phytoliths												
Identifier (sample code)	Lenticular starch, thick lamellae	Circular starch	Variant 1 eccentric starch	Variant 2 eccentric starch	Variant 3 eccentric starch	Small, oval, below 15 µm	Small, polyhedral	Triangular starch, faceted, no lamellae, 10–20 µm	Degraded starch	Indeterminate starch	Rondel	Trapezoid	Short-cell indeterminate	Elongate thin	Elongate rounded tip parallelepipedal with barb	Elongate curved thin	Acute bulbosus	Indeterminate hair	Spheroid echinate/ ornate	Spheroid ornate sub-ovoid	Jigsaw	Plate	Indeterminate phytolith
CB002									1	1	1	1	1		1					1	1		1
CB003	1								1					1				1					
CB012												2					1						
CB014		1	1											2									1
CB019								1		1	1	1		2			1		2			1	
CB008				17	1	2	1			1						1							
CB002 b																							
CB012 b																							

	Others																Charcoals				
Identifier (sample code)	Transparent spore	Chain spore	Brown fungal spore	Pollen indeterminate	Spore/ pollen/egg	Blue mineral	Mammal hair	Mammal skin scale	Vessel	Mineral quartz	Prismatic calcium oxalate	Annual form	Blue particle fiber	Textile fiber red	Textile fiber blue	Unknown fiber	Grass type charcoal	Indeterminate carbon	Grass charcoal	Charcoal	Unidentified general
CB002		1		2	1						4		1	1		5	1		1	37	5
CB003				1												5	2		2	90	8
CB012					1						2			2	2	1	1		1	32	5
CB014	1		2			1										1	8	13	8	86	18
CB019	2		1	2			1								1	6	2	3	2	100	3
CB008								2	1	1		1			1	3	3	1	3	56	3
CB002 b																3					2
CB012 b																4					

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3.2 Microremain analysis

Microremains were limited in the control samples, and their assemblages were entirely composed of cellulose-type fibers. Plant remains were present in all the archaeological dental calculus samples (Table 2). They occur in significant numbers in most samples. Of the microremains, charcoal was the dominant group but starches and phytoliths were also common. Fibers, pollen, fungal spores, calcium oxalates, and a variety of other unknown particles were also present. Their abundance varied considerably between samples (Figure 5).

Phytoliths are well represented in the dental calculus samples, forming the most diverse category in the assemblage by occurring in five of the samples, totaling 23 phytoliths. Phytoliths from monocotyledon plants (wild grasses, domestic cereals, sedges, etc.) were the largest group, totaling 15 phytoliths (Table 3). Of these, Poaceae was dominant, comprising six phytoliths, present in five samples. A single Poaceae type was found in sediment (CB008). It cannot be determined if the assemblage is predominantly derived from the leaves/stems of wild or domestic. Grass short-cells (rondels and trapezoids) show grasses from the pooid subtribe which are consistent with, but not exclusive of, barleys and wheat (n =4). Spheroid ornate/echinate is present in two individuals. Eudicots are represented in only two samples and represent leafy matter.

Starches are widespread in the assemblages, being present in four dental calculus samples totaling six grains. They occur in small numbers (5 or fewer starch grains). In contrast, in sediment sample CB008, they occur in a large concentration. These in CB008 are largely comprised of a highly eccentric simple starch, sub-spherical sub-ovoid types, typically with a birefringence, representing a different origin than the dental calculus starches. Lenticular starch indicating Triticeae that could derive from cultivated wheat or barley was only found in CB003. Polyhedral-type starches also occurred in CB008, and a related form is found in CB019.

Pieces of combustion products are present in all five dental calculus samples, totaling 389. They include long thin pieces of charcoal, indicative of charred grasses, rather than wood or other materials (Crawford and Belcher, 2014). They also include unidentified combustion products and the largest category, nonspecific charcoal, which is probably mostly wood charcoal in origin.

A total of five grains of pollen were found in four samples. Grains of pollen could not be identified. Calcium oxalates (6) were found in CB002 and CB012. Fungal spores totaling 8 of an unknown family were found in three individuals. A non-silica plant vessel was found in CB008, but is non-diagnostic. A mixture of cellulose-type fibers is present in the samples, totaling 32, and 4 in the sediment samples. These included colored fibers dyed red and blue (Table 3). A probable mammalian hair, identified due to its birefringence, "surface scales," and animal cell structure, was found in CB018. The medullar index indicates it is not human (Kshirsagar et al., 2009).

4 Discussion

Stable isotope and plant microremain studies are commonly used for past human dietary reconstructions. Until now, only few isotopic data of material directly dated to the earlier stages of the Neolithic are available in Eastern Iberia, unlike what is observed in Western Iberia (Guiry et al., 2016). No information on the diet inferred from plant microremains from dental calculus is available from the Early Neolithic. However, the Early Neolithic is a key period for understanding the process of Neolithization in Iberia, including dietary patterns and ecology. Mediterranean Iberia is also characterized by the low presence of human remains from this period compared with other regions, which makes the data from this study on the site of Cova Bonica even more valuable.

Previous paleogenetic data obtained from Cova Bonica (Olalde et al., 2015) support the evidence that new migrant populations introduced farming into western and central Europe. The presence of Cardial pottery, as documented in Cova Bonica, shows that the northeastern Iberian Neolithic is linked to the Italian Peninsula. It is in these regions that we see the earliest Cardial pottery and ovicaprid husbandry (Tresset and Vigne 2007; Vigne 2007) together with wheat and barley (Zapata et al., 2004; Antolín et al., 2015). Accordingly, the archaeological record (stratigraphy, radiocarbon dating, and artifacts) along the Mediterranean shoreline suggests a maritime colonization process (Zilhão 2001) that bypassed geographic obstacles using enclaves to rapidly settle lands optimal for farming (Bernabéu et al., 2015) (Figure 6A). Further insights into dietary patterns from these earlier stages of the Neolithic can help clarify the subsistence strategies of these first farming societies in the region.

The Early Neolithic individuals from Cova Bonica show a tight range of carbon isotope ratios, portraying a homogeneous protein diet based on C_3 terrestrial resources. While this is not unexpected for a Neolithic population, what is surprising is that there is no isotopic evidence whatsoever of marine protein consumption by any of the individuals. Taking into consideration that Cova Bonica is only 12 km away from the coastline (Figures 6.B,C), there is evidence at the same layers of the site of ornaments crafted from marine resources (Figures 6.H–J), and that there is jasper from the coast as well on the site (Figures 6.D–G), this population was aware of the marine environment. Of course, that there is no isotopic evidence of marine consumption does not mean necessarily that they did not eat any sea resources but that, if they ate them, they did so in such low amounts as to not be indicated by the collagen signal of their bones.

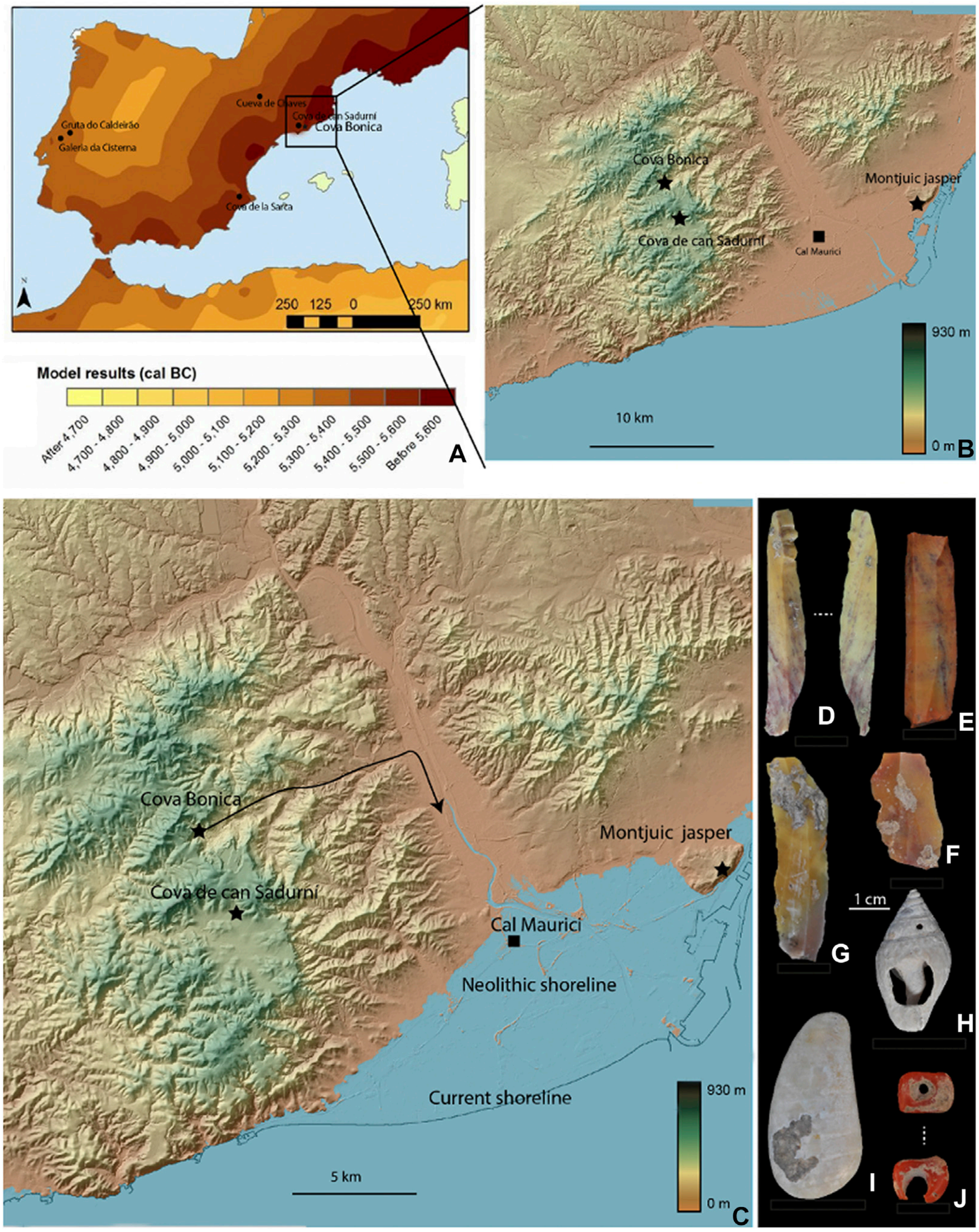

FIGURE 6
Cova Bonica in the context of regional settings. **(A)**: Model predictions for the Neolithic expansion: leapfrog voyaging with jumps of about 350 km (Isern et al., 2017). **(B–C)**: Digital elevation model (DEM) constructed using GIS version 3.24.2 and signaling current coastline **(B)** and neolithic shoreline **(C)**. Neolithic shoreline has been established using data provided from the Cal Maurici site (Daura et al., 2016). **(D–G)**: Jasper blades and flakes recovered at layer IV_2 coming from Montjuic (Barcelona). **(H–J)**: Marine ornaments recovered at layer IV_2 elaborated from *Columbella rustica* **(H)**, polished shell **(I)**, and red coral **(J)**.

Having discarded any marine signal from the $\delta^{13}C$ values, the differences observed in the $\delta^{15}N$ values (range 8.5–11.0 ‰) must be considered under another light. This difference cannot be, at this point, attributed to sex, as all human individuals are of indeterminate sex. However, the age of the different individuals analyzed should be considered, especially since four of the six total human individuals are not adults. As can be seen in Figure 3, the youngest individual analyzed (2.5–3 yo) is the one with the

TABLE 4 List of all Mediterranean Iberia sites with published carbon and nitrogen isotopic ratio analysis on human bone collagen from the Mesolithic up to the Late Neolithic–Chalcolithic period. Only samples from human adults for which both C and N results are available which meet collagen quality controls have been considered here.

Site (ca. distance from the coast on a straight line)	Archaeological chronological attribution	*n*	$\delta^{13}C$ m ±1σ (‰)	$\delta^{13}C$ max (‰)	$\delta^{13}C$ min (‰)	$\delta^{15}N$ m ±1σ (‰)	$\delta^{15}N$ max (‰)	$\delta^{15}N$ min (‰)	References
El Collado (7 km)	Mesolithic	9	−18.4 ± 0.7	−17.6	−19.5	10.3 ± 1.2	12.8	8.9	García-Guixé et al. (2006)
Santa Maira (30 km)	Mesolithic	2	−18.1	−18.0	−18.1	9.1	9.4	8.8	Salazar-García et al. (2014b)
Penya del Comptador (50 km)	Mesolithic	3	−18.5 ± 0.3	−18.2	−18.7	7.7 ± 0.1	7.8	7.6	Salazar-García et al. (2014b)
Casa Corona (80 km)	Mesolithic	1	−19.3	*	*	8.4	*	*	Fernández-López de Pablo et al. (2013)
Cingle del Mas Nou (70 km)	Mesolithic	4	−18.5 ± 0.1	−18.4	−18.6	8.8 ± 0.8	9.8	7.9	Salazar-García et al. (2014b)
Cova Bonica (12 km)	**Early Neolithic**	**3**	**−19.6 ± 0.5**	**−19.5**	**−19.8**	**9.1 ± 0.3**	**9.3**	**8.7**	**This study**
Costamar (0–1 km)	Early Neolithic	2	−19.1	−19.0	−19.1	8.2	8.5	7.9	Salazar-García (2009)
Cueva de Nerja (0–1 km)	Early–Middle Neolithic	3	−19.1 ± 0.5	−18.5	−19.4	9.0 ± 0.2	10.3	8.2	Salazar-García et al. (2017)
Feixa del Moro (200 km)	Early–Middle Neolithic	2	−20.3	−19.8	−20.7	8.7	9.1	8.2	Remolins et al. (2016)
Tossal de les Basses (0–1 km)	Middle Neolithic	11	−18.1 ± 0.6	−17.5	−19.1	11.2 ± 1.2	13.1	9.0	Salazar-García et al. (2016)
Pujolet de Moja (20 km)	Middle Neolithic	3	−19.3 ± 0.2	−19.2	−19.5	10.0 ± 0.5	10.3	9.4	Fontanals-Coll et al. (2017)
Hort d'en Grimau (27 km)	Middle Neolithic	3	−19.8 ± 0.3	−19.5	−20.1	9.2 ± 0.9	10.2	8.6	Fontanals-Coll et al. (2017)
Can Roqueta–Can Revella (15 km)	Middle Neolithic	6	−19.9 ± 0.5	−19.4	−20.4	8.4 ± 1.2	9.7	6.3	Fontanals-Coll et al. (2017)
Horts de Can Torras (27 km)	Middle Neolithic	2	−19.7	−19.6	−19.7	8.8	9.0	8.5	Fontanals-Coll et al. (2017)
Ceuró (125 km)	Middle Neolithic	3	−19.9 ± 0.2	−19.6	−20.1	8.9 ± 0.3	9.3	8.6	Fontanals-Coll et al. (2017)
Costa dels Garrics (120 km)	Middle Neolithic	2	−20.0	−19.7	−20.2	8.3	8.5	8.1	Fontanals-Coll et al. (2017)
El Llord (125 km)	Middle Neolithic	2	−19.8	−19.8	−19.8	9.9	10.2	9.6	Fontanals-Coll et al. (2017)
Puig d'en Roca (110 km)	Middle Neolithic	5	−20.3 ± 0.4	−20.0	−21.0	8.0 ± 1.2	9.1	6.1	Gibaja et al. (2016)
Can Gelats (35 km)	Middle Neolithic	1	−20.7	*	*	9.2	*	*	Gibaja et al. (2016)
Bòbila Madurell (25 km)	Middle Neolithic	36	−19.7 ± 0.3	−19.0	−20.6	9.2 ± 0.6	10.5	8.1	Fontanals-Coll et al. (2015)
Can Gambús (20 km)	Middle Neolithic	38	−19.6 ± 0.6	−16.8	−20.7	9.0 ± 0.5	10.2	7.9	Fontanals-Coll et al. (2015)
Costamar (0–1 km)	Late Neolithic	2	−18.0	−17.8	−18.2	10.1	10.4	9.8	Salazar-García (2009)
Cova dels Diablets (5 km)	Late Neo-Chalcolithic	8	−18.9 ± 0.3	−18.7	−19.5	10.0 ± 0.6	10.8	8.8	Salazar-García (2014)
Cova de la Pastora (40 km)	Late Neo-Chalcolithic	7	−19.3 ± 0.2	−19.0	−19.6	9.5 ± 0.8	10.6	8.1	McClure et al. (2011)
Avenc dels Dos Forats (40 km)	Late Neo-Chalcolithic	2	−19.1	−19.1	−19.1	10.2	10.4	10.2	McClure et al. (2011)
Coveta del Frare (75 km)	Late Neo-Chalcolithic	2	−19.0	−19.0	−19.0	9.7	9.8	9.6	García-Borja et al. (2013)
La Vital (0–1 km)	Late Neo-Chalcolithic	3	−18.7 ± 0.5	−18.3	−19.3	9.5 ± 0.8	10.3	9.0	Salazar-García (2011)
Cova del Rectoret (15 km)	Late Neo-Chalcolithic	1	−18.9	*	*	7.7	*	*	Miret Estruch et al. (2021)

highest $\delta^{15}N$ value (ca. 2 ‰ higher than the adults) as well as a slightly higher $\delta^{13}C$ value. All other sub-adult individuals (ages ranging from 4–13 yo) have isotopic values similar to those of the adults analyzed. This pattern could suggest that at the age of 2.5–3 yo, the individuals were still breastfed and in the process of being weaned (Fogel et al., 1989; Fuller et al., 2006; Herring et al., 1998). Although the sample is small, there is scarce information about these social practices from Early Neolithic people which highlights the relevance of this finding.

For all individuals from 4 years old onward, nitrogen isotope ratios show that humans possess a higher trophic level than herbivores, but have similar $\delta^{15}N$ values to the omnivore (brown bear). The archaeological record of Cova Bonica identified butchery activities mainly in adult ovicaprines, but also in other taxa, such as large bovids (Daura et al., 2019), demonstrating meat processing of domesticates. Moreover, the cave was used as a sheepfold indicating the relevance of husbandry practices for these individuals. All of this suggests that they consumed herbivore animal protein (possibly mainly ovicaprids), but also that they consumed plant foods (enough as to have similar values to the omnivore analyzed). While isotope analysis cannot shed further light on the type of plant resources consumed, the study of plant microremains trapped in dental calculus can.

The dental calculus assemblages show a distinct pattern that contrasts with the sparse remains in the control samples. This indicates the substantial integrity of the finds and that the microremains are archaeological with few phytoliths in the sediment sample. Poaceae is the dominant group of the assemblage, but counts are low relative to studies of many comparable grain-based economies (Scott et al., 2021). The only other Late prehistoric site from Mediterranean Iberia from which microremains from dental calculus have been studied is Camino del Molino (Murcia, Spain), although the site is from the Chalcolithic period (Power et al., 2014). The example of Camino del Molino does show a parallel with a relatively low-density assemblage missing expected morphotypes such as dendritics. At Camino del Molino, we do find some individuals with relatively higher numbers, as well as rare non-phytolith microremains such as sponge spicules and others. Given the purported grain-based Neolithic economy, the microremain counts in the Cova Bonica dental calculus are less than expected. They also lack unique husk types. The low Poaceae microremains count can be considered cryptic, but it could relate to limited cereal cultivation, free threshing versus non-free threshing varieties, or growing conditions that did not encourage phytolith formation in plant cells (Jenkins et al., 2011). Alternatively, it may be a product of specific grain processing methods that minimized phytolith intake.

There is evidence of eudicots, but they are rare and it is unclear if they had dietary significance. The occasional calcium oxalates we found may have also been derived from these eudicots, but they also could have originated from angiosperms. The starch assemblage is comparatively more numerous than the phytolith assemblage, but the non-dietary starches in CB008 drive this. Unidentified eccentric starches are predominant across the assemblage, representing plant storage organs, presumably from the C_3 pathway. Triticeae starch occurs, reinforcing the data from phytoliths of cereal use. The rich presence of charcoal indicates close contact with fires (Hardy et al., 2012; Buckley et al., 2021). It is unclear how close this contact was and whether these particles represent airborne charcoal inhaled, water-carried charcoal that was drunk, or the consumption of charcoal-covered or other sources.

The Cova Bonica isotope values complement the picture that the isotope analysis has already portrayed for Mediterranean Iberia from the Mesolithic up to the Chalcolithic (Table 4). Especially useful for this are the Early Neolithic individuals, as only very few have been published before for this chronology in the region. As we can see in the table, the values from all chronological periods (Mesolithic, Early Neolithic, Middle Neolithic, Late Neolithic, and Chalcolithic) are partially overlapping. Regarding marine protein, Mesolithic hunter-gatherers show intermixed isotopic values whether from coastal or inland sites, which is expected from a population with high mobility through different environments. Examples of this are the inland sites of Santa Maira and Cingle del Mas Nou and the coastal site of El Collado, as previous studies from all three reported individuals with both a full terrestrial isotopic signature and others with enough marine protein consumption to have it recorded in the collagen isotopic signal. However, during the Early Neolithic, not even individuals from coastal sites have a clear marine isotopic signal. All Early Neolithic individuals from the region studied, whether from the coastal (Cova Bonica, Costamar, and Cueva de Nerja) or inland sites (Feixa del Moro), show a terrestrial isotopic signature. It is from the Middle Neolithic onward, and especially during the last stages of the Neolithic and the Chalcolithic periods, when people start to live in more stable settlements and we could consider that the amount of marine resource consumption is many times linked to the proximity to the sea rather than to chronology or archaeological culture. Suggestive of this pattern is that most individuals reported with marine protein input in the diet are from sites directly located on the coastline (e.g., Tossal de les Basses). This same diachronic pattern is observed even in individuals from different time periods of a single same coastal site, Costamar, where its Early Neolithic individuals have a full terrestrial isotopic signal while its Late Neolithic individuals show isotopic evidence of marine protein consumption.

We must keep in mind that some of the differences observed between chronological periods and even sites of the same period could be due to non-dietary influences that modify the isotopic composition of resources consumed regardless of the resource type (i.e., factors that influence the food chain values themselves). For example, different environmental conditions between contemporary sites (e.g., coastal vs. inland; mountain vs. plain), or even between sites of a single geographic area but of different chronology, could have different isotopic food chain baselines (Goude and Fontugne, 2016). The use of complex husbandry practices and associated manuring in the region from the Late Neolithic onward could also influence crops, as well as domestic animals that feed on them, and, in turn, humans who consume the domestic crops or animals (Bogaard et al., 2007). Physiological traits of both the animals consumed (e.g. infantile vs adult specimens) and humans (age, nutritional status, and others), as well as the types of bones sampled (e.g., high vs.

low collagen turnover skeletal elements), also influence the isotopic values obtained from human bone collagen. Some of these "non-dietary" influencing factors are difficult to assess, as the archaeological assemblages provide limited information. These issues caution us away from rigid quantified interpretations when interpreting isotopic studies applied to archaeological material.

Conclusion

The combination of stable isotopes and plant microremains trapped in dental calculus in Cova Bonica brings, for the first time, the combined direct evidence of the diet of these Early Neolithic farmers in the NE Mediterranean area. The human stable isotope analysis and plant microremains from the Cardial Neolithic of Cova Bonica document a diet based on terrestrial C_3 resources. Although Cova Bonica is a mere 12 km from the current coastline, there is no isotopic evidence of marine protein consumption. The use of cereal and other plant foods is supported by plant microremains trapped in dental calculus.

The absence of marine and freshwater foods in the diet of Cova Bonica humans suggests that newcomers, at least initially, did not adapt to the fresh resources in the new land. In addition, the absence of a marine diet in people who moved through sea transport is, according to the coastal spread of the first farmers' model, remarkable. Archaeological data from the early Neolithic suggest that farming people expand hand-in-hand with this "package:" pottery, domesticated plants, livestock, polished stones, and other archaeological materials. Thus, the Cova Bonica results support the notion that newcomers expanded bearing their own subsistence strategy, focused on terrestrial resources, such as husbandry of ovicaprines and domesticated plants, instead of exploring the alternatives that became available in new territories. This lifestyle is implemented in the regions that they arrived at with suitable lands (i.e., deltaic, estuarine, and lagoonal environments) for agriculture and animal husbandry.

Data availability statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Author contributions

DS initiated and coordinated the study. JD and MS conducted the archaeological/anthropological work and the curation of the archeological material. DS, RP, JD, and MS participated in the sample management and gathering of contextual information. DS and RP organized the sample collection for analyses. DS and RP performed the laboratory work. All authors contributed to the interpretation of the data. DS wrote the first version of the manuscript, which was edited by RP, JD, and MS.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ambrose, S. H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *J. Archaeol. Sci.* 17 (4), 431–451. doi:10.1016/0305-4403(90)90007-r

Ambrose, S. H., and Norr, L. (1993). “Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate,” in *Prehistoric human bone* (Berlin, Heidelberg: Springer), 1–37.

Antolín, F., Jacoment, S., and Buxó, R. (2015). The hard knock life: Archeobotanical data on farming practices during the Neolithic (5400–300 CAL B.C.) in the NE of Iberian Peninsula. *J. Archaeol. Sci.* 61, 90–104. doi:10.1016/j.jas.2015.05.007

Armitage, P. L. (1975). The extraction and identification of opal phytoliths from the teeth of ungulates. *J. Archaeol. Sci.* 2 (3), 187–197. doi:10.1016/0305-4403(75)90056-4

Baldellou, V. (1974). Los materiales arqueológicos de la «Cova Bonica» de Vallirana (Barcelona). *Empúries Rev. món clàssic Antig. tardana* 36, 1–19.

Bernabéu, J., Barton, M., Pardo, S., and Bergin, S. M. (2015). Modeling initial neolithic dispersal. The first agricultural groups in west mediterranean. *Ecol. Model.* 307, 22–31. doi:10.1016/j.ecolmodel.2015.03.015

Bogaard, A., Heaton, T. H. E., Poulton, P., and Merbach, I. (2007). The impact of manuring on nitrogen isotope ratios in cereals: archaeological implications for reconstruction of diet and crop management practices. *J. Archaeol. Sci.* 34, 335–343.

Buckley, S., Power, R. C., Andreadaki-Vlazaki, M., Akar, M., Becher, J., Belser, M., et al. (2021). Archaeometric evidence for the earliest exploitation of lignite from the bronze age Eastern Mediterranean. *Sci. Rep.* 11 (1), 24185. doi:10.1038/s41598-021-03544-w

Chisholm, B. S., Erle Nelson, D., and Schwarcz, H. P. (1982). Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. *Science* 216 (4550), 1131–1132. doi:10.1126/science.216.4550.1131

Crawford, A. J., and Belcher, C. M. (2014). Charcoal morphometry for paleoecological analysis: The effects of fuel type and transportation on morphological parameters. *Appl. Plant Sci.* 2 (8), 1400004. doi:10.3732/apps.1400004

Daura, J., Sanz, M., Ramos, J., Riera, S., Miras, Y., Allué, E., et al. (2016). Palaeoenvironmental record of the Cal Maurici wetland sediment archive in Barcelona (NE Iberian Peninsula) between c. 6000 and 4000 cal. yr BP. *Holocene* 26, 1020–1039. doi:10.1177/0959683616632891

Daura, J., Sanz, M., Oms, F. X., Pedro, M., Martínez, P., Mendiela, S., et al. (2019). Deciphering neolithic activities from a cardial burial site (cova Bonica) on the Western mediterranean coast. *J. Archaeol. Sci. Rep.* 23, 324–347. doi:10.1016/j.jasrep.2018.10.036

De Niro, M. J. (1985). Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 3317, 806–809. doi:10.1038/317806a0

Fahy, G. E., Richards, M., Riedel, J., Hublin, J. J., and Boesch, C. (2013). Stable isotope evidence of meat eating and hunting specialization in adult male chimpanzees. *Proc. Natl. Acad. Sci. U. S. A.* 110 (15), 5829–5833. doi:10.1073/pnas.1221991110

Fernández López de Pablo, J., Salazar-García, D. C., Subirà, M. E., Roca-Togores, C., Gómez Puche, M., Richards, M. P., et al. (2013). Late mesolithic burials at casa corona (villena, Spain): Direct radiocarbon and palaeodietary evidence of the last forager populations in eastern Iberia. *J. Archaeol. Sci.* 40, 671–680. doi:10.1016/j.jas.2012.09.005

Fogel, M., Tuross, N., and Owsley, D. W. (1989). *Nitrogen isotope tracers of human lactation in modern and archaeological populations*. Washington, DC: Geophysical Laboratory-Carnegie Institution.

Fontanals–Coll, M., Subirà, M. E., Díaz-Zorita Bonilla, M., Duboscq, S., and Gibaja, J. F. (2015). Investigating palaeodietary and social differences between two differentiated sectors of a Neolithic community, La Bòbila Madurell-Can Gambús (north–east Iberian Peninsula). *J. Archaeol. Sci. Rep.* 3, 160–170. doi:10.1016/j.jasrep.2015.06.013

Fontanals-Coll, M., Subirà, M. E., Díaz-Zorita, M., and Gibaja, J. F. (2017). First insight into the neolithic subsistence economy in the north-east iberian Peninsula: Paleodietary reconstruction through stable isotopes. *Am. J. Phys. Anthropol.* 162 (1), 36–50. doi:10.1002/ajpa.23083

Fuller, B. T., Fuller, J. L., Harris, D. A., and Hedges, R. E. M. (2006). Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. *Am. J. Phys. Anthropol.* 129, 279–293.

García Borja, P., Pérez Fernández, A., Biosca Cirujeda, V., Ribera i Gomes, A., and Salazar-García, D. C. (2013). “Los restos humanos de la Coveta del Frare (La Font de la Figuera, València),” in El Naiximent d’un Poble. Història i Arqueologia de la Font de la Figuera, *Ajuntament de la Font de la Figuera*. Editors P. García Borja, E. Revert Francés, A. Y. Ribera i Gomes, and V. Biosca Cirujeda, 47–60.

Garcia-Guixé, E., Richards, M. P., and Subirà, M. E. (2006). Palaeodiets of humans and fauna at the Spanish mesolithic site of El Collado. *Curr. Anthropol.* 47, 549–557. doi:10.1086/504170

Gibaja, J. F., Fontanals-Coll, M. F., Subosqu, S., Oms, F. X., Augés, A., Javier Santos, F., et al. (2016). Human diet and the chronology of neolithic societies in the north-east of the iberian Peninsula: The necropolises of puig d’en roca and can gelats (girona, Spain). *Archaeol. Anthropol. Sci.* 9, 903–913. doi:10.1007/s12520-015-0311-y

Goude, G., and Fontugne, M. (2016). Carbon and nitrogen isotopic variability in bone collagen during the Neolithic period: Influence of environmental factors and diet. *J. Archaeol. Sci.* 70, 117–131.

Goude, G., Salazar-García, D. C., Power, R. C., Terrom, J., Rivollat, M., Deguilloux, M. F., et al. (2019). A multidisciplinary approach to Neolithic life reconstruction. *J. Archaeol. Method Theory* 26 (2), 537–560. doi:10.1007/s10816-018-9379-x

Goude, G., Salazar-García, D. C., Power, R. C., Rivollat, M., Gourichon, L., Deguilloux, M. F., et al. (2020). New insights on Neolithic food and mobility patterns in Mediterranean coastal populations. *Am. J. Phys. Anthropol.* 173, 218–235. doi:10.1002/ajpa.24089

Guiry, E. J., Hillier, M., Boaventura, R., Silva, A. M., Oosterbeek, L., Tiago, T., et al. (2016). The transition to agriculture in south-western Europe: new isotopic insights from Portugal’s Atlantic coast. *Antiquity* 90, 604–616.

Hardy, K., Buckley, S., Collins, M. J., Estalrrich, A., Brothwell, D., Copeland, L., et al. (2012). Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus. *Naturwissenschaften* 99 (8), 617–626. doi:10.1007/s00114-012-0942-0

Hedges, R. E. M., and Reynard., L. M. (2007). Nitrogen isotopes and the trophic level of humans in archaeology. *J. Archaeol. Sci.* 34 (8), 1240–1251. doi:10.1016/j.jas.2006.10.015

Hedges, R. E. M., Clement, J. G., Thomas, C. D. L., and O’Connell, T. C. (2007). Collagen turnover in the adult femoral mid-shaft: Modeled from anthropogenic radiocarbon tracer measurements. *Am. J. Phys. Anthropol.* 133, 808–816. doi:10.1002/ajpa.20598

Herring, D. A., Saunders, S. R., and Katzenberg, M. A. (1998). Investigating the weaning process in past populations. *Am. J. Phys. Anthropol.* 105, 425–439.

Henry, Amanda G., and Piperno, Dolores R. (2008). Using plant microfossils from dental calculus to recover human diet: A case study from tell al-raqā’i, Syria. *J. Archaeol. Sci.* 35, 1943–1950. doi:10.1016/j.jas.2007.12.005

Howland, M. R., Corr, L. T., Young, S. M. M., Jones, V., Jim, S., Van Der Merwe, N. J., et al. (2003). Expression of the dietary isotope signal in the compound-specific δ13C values of pig bone lipids and amino acids. *Int. J. Osteoarchaeol.* 13 (1–2), 54–65. doi:10.1002/oa.658

Isern, N., Zilhão, J., Fort, J., and Ammerman, A. J. (2017). Modeling the role of voyaging in the coastal spread of the early neolithic in the west mediterranean. *Proc. Natl. Acad. Sci. U. S. A.* 114, 897–902. doi:10.1073/pnas.1613413114

Jenkins, E., Jamjoum, K., and Nuimat, S. Al. (2011). “Irrigation and phytolith formation: An experimental study,” in *Water, life and civilisation: Climate, environment and society in the Jordan valley*. Editors S. Mithen and E. Black (Cambridge University Press), 347–372.

Jim, S., Ambrose, S. H., and Evershed, R. P. (2004). Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: Implications for their use in dietary reconstruction. *Geochim. Cosmochim. Acta.* 68, 61–72.

Katzenberg, M. A. (2012). “The ecological approach: Understanding past diet and the relationship between diet and disease,” in *A companion to paleopathology*. Editor A. L. Grauer (New Jersey: John Wiley & Sons), 97–113.

Kshirsagar, S. V., Singh, B., and Fulari, S. P. (2009). Comparative study of human and animal hair in relation with diameter and medullary index. *Indian J. Forensic Med. Pathology* 2 (3), 105–108.

Lee-Thorp, J. A. (2008). On isotopes and old bones. *Archaeometry* 50, 925–950. doi:10.1111/j.1475-4754.2008.00441.x

Leonard, C. A., Vashro, L., O’Connell, J. F., and Henry, A. G. (2015). Plant microremains in dental calculus as a record of plant consumption: A test with twe forager-horticulturalists. *J. Archaeol. Sci. Rep.* 2, 449–457. doi:10.1016/j.jasrep.2015.03.009

McClure, S., García-Puchol, O., Roca, C., Culleton, B., and Kennett, D. (2011). Osteological and paleodietary investigation of burials from Cova de la Pastora, Alicante, Spain. *J. Archaeol. Sci.* 38, 420–428. doi:10.1016/j.jas.2010.09.023

Minagawa, Masao, and Wada, Eitaro (1984). Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. *Geochimica Cosmochimica Acta* 48 (5), 1135–1140. doi:10.1016/0016-7037(84)90204-7

Miret Estruch, C., Cardona Escrivà, J., Salazar-García, D. C., García Borja, P., Pérez Fernández, A., and Guerrero Blázquez, M. (2021). La col·lecció arqueològica preshitòrica de la cova del Rectoret (Gandia, la Safor, País Valencià). *Recer. del Mus. d'Alcoi* 30, 43–62.

Neumann, K., Stromberg, C. A. E., Ball, T., Albert, R. M., Vrydaghs, L., and Cummings, L. S. (2019). International code for phytolith nomenclature (ICPN) 2.0. *Ann. Bot.* 124 (2), 189–199. doi:10.1093/aob/mcz064

Olalde, I., Schroeder, H., Sandoval-Velasco, M., Vinner, L., Lobón, I., Ramirez, O., et al. (2015). A common genetic origin for early farmers from mediterranean cardial and central European LBK cultures. *Mol. Biol. Evol.* 32, 3132–3142. doi:10.1093/molbev/msv181

Oms, F. X., Daura, J., Sanz, M., Mendiela, S., Pedro, M., and Martínez, P. (2017). First evidence of collective human inhumation from the cardial neolithic (cova Bonica, Barcelona, NE iberian Peninsula). *J. Field Archaeol.* 42, 43–53. doi:10.1080/00934690.2016.1260407

Power, R. C., Salazar-García, D. C., Wittig, R. M., and Henry, A. G. (2014). Assessing use and suitability of scanning electron microscopy in the analysis of micro remains in dental calculus. *J. Archaeol. Sci.* 49, 160–169. doi:10.1016/j.jas.2014.04.016

Power, R. C., Salazar-García, D. C., Wittig, R. M., Freiberg, M., and Henry, A. G. (2015). Dental calculus evidence of Taï Forest Chimpanzee plant consumption and life history transitions. *Sci. Rep.* 5, 15161. doi:10.1038/srep15161

Remolins, G., Gibaja, J. F., Allièse, F., Fontanals-Coll, M., Martin, P., Masclans, A., et al. (2016). The neolithic necropolis of La feixa del Moro (juberri, Andorra): New data on the first farming communities in the pyrenees. *Comptes Rendus Palevol* 15 (5), 537–554. doi:10.1016/j.crpv.2015.11.005

Richards, M. P., and Hedges, R. E. M. (1999). Stable isotope evidence for similarities in the types of marine foods used by Late Mesolithic humans at sites along the Atlantic coast of Europe. *J. Archaeol. Sci.* 26 (6), 717–722. doi:10.1006/jasc.1998.0387

Salazar-García, D. C. (2009). "Estudio de la dieta en la población neolítica de Costamar. Resultados preliminares de análisis de isótopos estables de carbono y nitrógeno," in *Flors Ureña E. (Coord.)* Torre la Sal (Ribera de Cabanes, Castellón). Evolución del paisaje antrópico desde la prehistoria hasta el medioevo. M.P.A.C. *8* (Castellón: Servicio de Investigaciones Arqueológicas y Prehistóricas-Diputación de Castellón), 411–418.

Salazar-García, D. C., Vives-Ferrándiz, J., Fuller, B., and Richards, M. P. (2010). Alimentación estimada de la población del Castellet de Bernabé (ss. V-III a.C.) mediante el uso de ratios de isótopos estables de C y N. *Saguntum Extra* 9, 313–322.

Salazar-García, D. C. (2011). "Aproximación a la dieta de la población calcolítica de La Vital a través del análisis de isótopos estables del carbono y del nitrógeno sobre restos óseos," in *La Vital (Gandia, Valencia). Vida y muerte en la desembocadura del Serpis durante el III y el I milenio a.C. T.V. 113*. Editors G. Perez Jordá, J. Bernabeu Aubán, Y. Carrión Marco, O. García-Puchol, L. L. Molina Balaguer, and M. Gómez Puche (València: Museu de Prehistòria de València-Diputación de Valencia), 139–143.

Salazar-García, D. C. (2014). "Estudi de la dieta en la població de Cova dels Diablets mitjançant anàlisi d'isòtops estables del carboni i del nitrogen en collàgen ossi. Resultats preliminars," in La Cova dels Diablets (Alcalà de Xivert, Castelló). Prehistòria a la Serra d'Irta. *Castellón, Diputació de Castelló*. Editors G. Aguilella Arzo, D. Roman i Monroig, and P. García Borja, 67–78.

Salazar-García, D. C., Power, R. C., Sanchis-Serra, A., Villaverde, V., Walker, M. J., and Henry, A. G. (2013). Neanderthal diets in central and southeastern Mediterranean Iberia. *Quat. Int.* 318, 3–18. doi:10.1016/j.quaint.2013.06.007

Salazar-García, D. C., Richards, M. P., Nehlich, O., and Henry, A. G. (2014a). Dental calculus is not equivalent to bone collagen for isotope analysis: A comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the medieval site of El raval (alicante, Spain). *J. Archaeol. Sci.* 47, 70–77. doi:10.1016/j.jas.2014.03.026

Salazar-García, D. C., Aura, E., Olària, C., Talamo, S., Morales, J. V., and Richards, M. P. (2014b). Isotope evidence for the use of marine resources in the Eastern Iberian Mesolithic. *J. Archaeol. Sci.* 42, 231–240. doi:10.1016/j.jas.2013.11.006

Salazar-García, D. C., Romero, A., García-Borja, P., Subirà, E., and Richards, M. P. (2016). A combined dietary approach using isotope and dental buccal-microwear analysis of human remains from the Neolithic, Roman and Medieval periods from the archaeological site of Tossal de les Basses (Alicante, Spain). *J. Archaeol. Sci. Rep.* 6, 610–619. doi:10.1016/j.jasrep.2016.03.002

Salazar-García, D. C., Pérez-Ripoll, M., García-Borja, P., Jordá Pardo, J. F., and Aura Tortosa, J. E. (2017). "A terrestrial diet close to the coast: A case study from the neolithic levels of Nerja cave (málaga, Spain)," in *Times of neolithic transition along the western mediterranean*. Editors D. C. Salazar-García and O. García-Puchol (Springer), 281–307.

Salazar-García, D. C., Fontanals-Coll, M., Goude, G., and Subirà, E. (2018). To 'seafood' or not to 'seafood'?" an isotopic perspective on dietary preferences at the Mesolithic-Neolithic transition in the Western Mediterranean. *Quat. Int.* 470, 497–510. doi:10.1016/j.quaint.2017.12.039

Salazar-García, D. C., Power, R. C., Rudaya, N., Kolobova, K., Markin, S., Krivoshapkin, A., et al. (2021). Dietary evidence from central asian neanderthals: A combined isotope and plant microremains approach at chagyrskaya cave (altai, Russia). *J. Hum. Evol.* 156, 102985. doi:10.1016/j.jhevol.2021.102985

Schoeller, D. A. (1999). Isotope fractionation: Why aren't we what we eat? *J. Archaeol. Sci.* 26 (6), 667–673. doi:10.1006/jasc.1998.0391

Schoeninger, M. J., and DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica Cosmochimica Acta* 48 (4), 625–639. doi:10.1016/0016-7037(84)90091-7

Scott, A., Power, R. C., Altmann-Wendling, V., Artzy, M., Martin, M. A. S., Eisenmann, S., et al. (2021). Exotic foods reveal contact between South Asia and the Near East during the second millennium BCE. *Proc. Natl. Acad. Sci. U. S. A.* 118 (2), e2014956117. doi:10.1073/pnas.2014956117

Tresset, A., and Vigne, J. D. (2007). "Substitution of species, techniques and symbols at the mesolithic-neolithic transition in western europe," in Going over: The mesolithic-neolithic Transition in north-west europe, *proceedings of the British academy 144*. Editors Wittle and V. Cummings (Oxford: Oxford University Press), 189–201.

Tromp, M., Buckley, H., Geber, J., and Matisoo-Smith, E. (2017). EDTA decalcification of dental calculus as an alternate means of microparticle extraction from archaeological samples. *J. Archaeol. Sci. Rep.* 14, 461–466. doi:10.1016/j.jasrep.2017.06.035

Van der Merwe, N. J., and Vogel, J. C. (1978). 13C Content of human collagen as a measure of prehistoric diet in Woodland North America. *Nature* 276, 815–816. doi:10.1038/276815a0

Van Klinken, G. J. (1999). Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *J. Archaeol. Sci.* 26 (6), 687–695. doi:10.1006/jasc.1998.0385

Vigne, J-D. (2007). "Exploitation des animaux et néolithisation en Méditerranée nord-occidentale," in *Pont de Roque-Haute. Nouveaux Regards sur la Néolithisation de la France Méditerranéenne*. Editors J. Guilaine, C. Manen, and J. D. Vigne (Toulouse: Archives d'Écologie Préhistorique), 221–301.

Villalba-Mouco, V., Utrilla, P., Laborda, R., Lorenzo, J. I., Martínez-Labarga, C., and Salazar-García, D. C. (2018a). Reconstruction of human subsistence and husbandry strategies from the Iberian Early Neolithic using carbon and nitrogen stable isotope ratio analysis. *Am. J. Phys. Anthropol.* 167, 257–271.

Villalba-Mouco, V., Sauqué, V., Sarasketa-Gartzia, I., Pastor, M. V., le Roux, P. J., Vicente, D., et al. (2018b). Territorial mobility and subsistence strategies during the Ebro Basin Late Neolithic-Chalcolithic: A multi-isotope approach from San Juan cave (Loarre, Spain). *Quat. Int.* 481, 28–41.

Villalba-Mouco, V., Sarasketa-Gartzia, I., Utrilla, P., Oms, F. X., Mendiela, S., Lorenzo, J. I., et al. (2019). Stable isotope ratio analysis of bone collagen as indicator of different environments conditions and dietary habits in Northeastern Iberia during the 4th and 3rd millenium calBC. *Archaeol. Anthropol. Sci.* 11, 3931–3947.

Warinner, C., Rodrigues, J. F. M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., et al. (2014). Pathogens and host immunity in the ancient human oral cavity. *Nat. Genet.* 46 (4), 336–344. doi:10.1038/ng.2906

Zapata, L., Peña-Chocarro, L., Pérez-Jordà, G., and Stika, H. (2004). Early neolithic agriculture in the iberian Peninsula. *J. World Prehist.* 18 (4), 283–325. doi:10.1007/s10963-004-5621-4

Zilhão, J. (2001). Radiocarbon evidence for maritime pioneer colonization at the origins of farming in west Mediterranean Europe. *Proc. Natl. Acad. Sci. U. S. A.* 98, 14180–14185. doi:10.1073/pnas.241522898

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