

Bioarchaeology in East Asia

Edited by

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and Kyungcheol Choy

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Bioarchaeology in East Asia

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Editorial: Overview and progress in bioarchaeological research in East Asia

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KEYWORDS

bioarchaeology, East Asia, overview, progress, prospect

Editorial on the Research Topic
[Overview and progress in bioarchaeological research in East Asia](#)

1 Introduction

Bioarchaeology applies the methods and techniques from the geological, biological, and environmental sciences to analyze biological remains at archaeological sites. This interdisciplinary approach addresses important archaeological questions relevant to past human behaviors and society. Bioarchaeological research in East Asia has accelerated since the 21st century given the application of new analytical techniques and access to biological remains at important archaeological sites. This Research Topic, *Bioarchaeology in East Asia*, presents the latest research and systematic review of bioarchaeology in East Asia. It provides a platform to communicate among scholars who are interested in East Asia and better understand the biocultural evolution of human ancestors in this region.

A total of 12 articles contributed by Japanese and Chinese scholars have been published in this Research Topic. They can be divided into two categories according to article styles, *i.e.*, reviews and original research. The reviews offer a systematic overview and perspectives on radiocarbon dating, ancient DNA, rice domestication, and bioavailable Sr isoscapes in China and bioarchaeology in Japan. The original research presents analytical results from animal assemblages, human skeletons, and red substances covering chank-made shell beads and discusses the archaeological implications in depth.

2 Contributions to this research topic

2.1 Review articles

Chen summarized the process of establishing ¹⁴C dating laboratories and analytical techniques since the 1960s in China and proposed that the golden age for ¹⁴C dating arrived at the beginning of the 2000s. Chen pointed out that the technical improvement from β -decay counting to accelerator mass spectrometry had great impacts on Chinese

archaeology. [Chen](#) introduced several case studies to set up chronological frameworks for discussing the origin of early *Homo Sapiens*, the neolithization processes, and the Xia–Shang–Zhou dynasties. Lastly, [Chen](#) proposed four future directions to guide research in China, such as the establishment of more radiocarbon dating laboratories, further development of protocols for sample preparation and radiocarbon measurements, close cooperation between dating specialists and field archaeologists, and the need to establish a chronology of the historical era.

[Gao and Cui](#) summarized the chronological improvement of ancient DNA extraction techniques since the 1980s and emphasized the importance of next-generation sequencing (NGS) for ancient DNA research, indicating the arrival of the paleogenomic era. Using the accumulated paleogenomic data from China in recent years, they extensively summarized the migration and admixture histories of humans during the periods of the Upper Paleolithic, the Neolithic, and the Bronze Age. They also presented some unresolved questions to be investigated in the future, such as the Tibetan genetic history and the southward expansion of farmers in the Yangtze River region.

[Pan](#) re-evaluated the concepts of domestication in detail and drew a schematic paradigm to show the relationship of changing human behavior and variations of rice phenotypes and genotypes with the process of rice domestication. Based on the systematic review of archaeobotanical data (spikelet base, seed size, double-peaked tubercle phytolith, bulliform phytolith, and phytolith in spikelet base), four criteria were forwarded as empirical evidence to indicate the status of rice cultivation in either wild or domesticated variants. Subsequently, the spatial and temporal framework of rice domestication and dispersals was discussed according to different regions in the human behavioral perspective.

[Tang and Wang](#) overviewed the research history of Sr isotope analysis from its initial introduction to China in 2003. They constructed the first bioavailable Sr isoscape in China by compiling Sr isotopic data to identify human migration patterns and different patterns of utilizing animal resources in Chinese prehistory. Furthermore, they updated the Sr isoscape by the addition of newly produced Sr isotopic data from Southeast and South Asia, shedding new light on human communications and connections between customs and technologies at a broader spatial scale. Lastly, they investigated new analytical techniques to reconstruct the mobility of animals and humans at a lifetime scale.

[Nagaoka](#) presented a systematic review of bioarchaeological research in Japan. Since the first work on medieval human skeletons in 2003, bioarchaeological research in Japan has developed quickly and diversified. The author performed a statistical analysis of bioarchaeological articles published in the journal *Anthropological Science* between 2003 and 2022. The results showed that the proportion of articles focusing on ancient human skeletons increased from 42% in 2003–2007 to 85.1% in 2018–2022. Additionally, the number of articles covering diverse subjects such as paleopathology, stable isotopes, and bioarchaeology increased in contrast to the decrease in the number of articles related to human morphological analyses. The author also demonstrated the challenges facing the further pursuit of bioarchaeological research in Japan, including the dearth of physical

anthropology laboratories and the disciplinary separation between archaeology and physical anthropology.

2.2 Original research articles

[Sawada et al.](#) investigated the pathological conditions of human skeletons at the Tianluoshan and Hemudu sites in China during the Hemudu culture period (7000–5500 BP) and compared the health conditions between early farmers and hunter-gatherers. They suggested that low rice production and the diverse lifestyles of Hemudu farmers did not lead to an extreme decline in human health but, rather, to a decline in oral health. However, the long-term work in rice fields and their environment caused more physical stress for farmers than hunter-gatherers.

For the first time, [Zhang et al.](#) reported the bioarchaeological data (ages, sex, stature, and pathological conditions) of 16 skeletons from the cliff necropolises on the Chengdu Plain in China dating to the Iron Age. Based on their discussion of human demography, burial practices, stature, oral health, and dietary patterns, this study provides new insights into the physiological stress and health of the humans interred in the cliff tomb.

[Hu et al.](#) collected zooarchaeological data from 26 Neolithic and Bronze Age sites in the Guangzhou region, Shaanxi, China, to reveal the diachronic change of meat procurement strategies and its association with different factors. They found that the meat procurement strategies were not linear, *i.e.*, simply from hunting to husbandry, and that different strategies existed between settlements with different ranks instead. Furthermore, they discovered more reliance on domesticated bovids during the pre-Zhou and Western Zhou periods. They argued that the meat procurement strategies in the Guanzhong Basin were influenced by population sizes, social forms, and the natural environment.

[Festa et al.](#) investigated the exploitation of animal resources during the period of the Shang–Zhou transition (11th century BCE) by examining the faunal assemblages at the Sunjia and Xitou sites in China. They found that animal husbandry was mainly composed of pig farming and supplemented by extensive herding of goats and cattle. They suggested that the diverse use of animal resources by humans could have been regarded as a response to the growing climatic deterioration and also have been caused by the increasing interaction with pastoral communities in the north.

[Eda et al.](#) examined four immature Phasianidae bones by collagen peptide fingerprinting and radiocarbon dating to investigate the origin of domestic chicken in the Japanese archipelago. The results showed that the domestication of chicken in Japan occurred in the fourth and third centuries BCE. Therefore, they have proposed that the earliest domestic chicken in Japan can be dated to the middle Yayoi period, the lower chronological limit for the introduction of chickens to Japan.

[Li et al.](#) reported a comprehensive dataset obtained by the analyses of zooarchaeology, stable isotopes, and radiocarbon dates of animal remains at the Heishuigou cemetery, encompassing the Han Dynasty (202 BCE–220 CE) in the Hexi Corridor. These results showed that humans utilized multiple animals as funerary objects and that chickens, pigs, and dogs mainly ate C₄ foods (millets or their byproducts). In combination with previously reported isotopic data of animals, they found an

increasing reliance on herbivorous livestock, with a decrease of C_4 fodders from 2300 to 200 BCE, and a counter situation during the Han Dynasty. This consequence might be probably due to the military control by the Han Empire and the massive immigration of populations from the Yellow River Valley.

Wang et al. analyzed the red substances covering shell beads made of chank from the Indian Ocean, which were unearthed at the Qulong site (c. 800–500BC) on the Tibetan Plateau, by several analytical methods (pXRF, XRD, FTIR, and laser Raman spectroscopy) to reveal the chemical properties of red pigments and binding media. The results showed that the red pigments were iron oxide and that the binding media originated from pulverized bones and other proteinaceous materials. However, different formulas of bone powders and associated binders suggested the cultural complexity and diversity of local “applying red” traditions in the Tibetan Plateau and adjacent areas in prehistoric times.

3 Concluding remarks

I am encouraged to see the 12 publications of this Research Topic in *Frontiers in Earth Science*, which enhance our understanding of bioarchaeological research in East Asia. Moreover, this is the first time that bioarchaeological studies in East Asia have been combined and published within one Research Topic. I am looking forward to seeing more scholars in East Asia becoming interested and involved in another Research Topic in the future.

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Ancient genomes reveal the origin and evolutionary history of Chinese populations

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Mitochondrial DNA was first successfully extracted from ancient remains approximately 4 decades ago. Research into ancient DNA has been revolutionized due to improvements in next-generation sequencing (NGS) technology in the early 21st century, as well as advances in the field of ancient DNA extraction and enhancement. In recent years, a large number of paleogenomic data has shed light on the origin and evolution of humans, and provided new insights into the migration and admixture events of populations, as well as the spread of languages and technologies. As China is located in the eastern part of Eurasia, it plays an integral role in exploration of the genetic history of Eurasians throughout the history of modern human habitation. Here we review recent progress deriving from paleogenomic analysis, which helps to reconstruct the prehistory of China.

KEYWORDS

paleogenomics, Chinese populations, origin, evolutionary history, ancient DNA

1 Introduction

Over the past decades, the field of ancient DNA research has witnessed a revolution. Many remarkable achievements have been made in regards to the reconstruction of the history of humans (Pickrell and Reich, 2014; Parks et al., 2015; Slatkin and Racimo, 2016; Marciniak and Perry, 2017; Skoglund and Mathieson, 2018; Yang and Fu, 2018; Racimo et al., 2020; Bergström et al., 2021; Liu et al., 2021; Liu et al., 2022). Initially, ancient DNA studies focused on highly variable and partially coding regions of mitochondrial DNA and the informative single nucleotide polymorphisms (SNPs) of the Y chromosome. After 2006, the introduction of NGS technology and the optimization of the methods on ancient DNA extraction, library construction and DNA enrichment (Meyer and Kircher, 2010; Kircher et al., 2011; Barnett and Larson, 2012; Dabney and Meyer, 2012; Carpenter et al., 2013; Gansauge and Meyer, 2013; Korlević et al., 2015; Slon et al., 2017), enabled large scale genome-wide ancient DNA investigations on prehistoric populations, which opened new windows into the past. Paleogenomic study leads to a more comprehensive understanding of patterns of human variation and mobility through time and space. Genome-wide data from archaic human, such as Neanderthals and Denisovans, provides direct insights into human evolution (Green et al., 2010; Reich et al., 2010; Fu et al., 2015; Racimo et al., 2015; Prüfer et al., 2017; Slon et al., 2018; Chen et al., 2020; Mafessoni et al.,

2020; Hajdinjak et al., 2021). Large-scale studies of continental populations on the scale of deeply sampled time transects can offer direct evidence for the genetic origin of populations, and enrich the details of human migration and interaction, both, temporally and spatially (Olalde and Posth, 2020; Vicente and Schlebusch, 2020; Zhang and Fu, 2020; Choin et al., 2021; Willerslev and Meltzer, 2021).

Situated in the eastern part of Eurasia and connecting North, Central and Southeast Asia, China serves as a vital area for the reconstruction of human history of Asia. Its vast territory and diverse topography was a cradle for a long history of human settlement and thriving ancient civilizations. The origin and evolutionary history of Chinese populations has been studied extensively within different research fields, such as archaeology, history, linguistics, anthropology, genetics, and more recently, palaeogenomics. Although paleogenomic research in China was launched relatively late, genome-wide data of ancient Chinese populations has been accumulating rapidly in recent years. The ancient DNA studies have provided valuable information on demographic history in China and East Asia, and have been pivotal in the efforts to better understand the formation and evolution of Chinese civilization.

2 The origin of Chinese populations

The origins of anatomically modern humans (AMH) in China and even East Asia have been debated at great length. There are mainly two hypotheses: multi-regional hypothesis and recent African origin hypothesis (Stringer and Andrews, 1988; Wu, 2006). Evidences collected from fossils of ancient humans unearthed in China support the multi-regional hypothesis, while genetic studies agree with the recent out-of-Africa hypothesis (Wu, 2006; López et al., 2015).

In 2017, Yang et al. sequenced the whole genome of a 40,000-year-old individual uncovered from Tianyuan Cave near Beijing, China (Yang et al., 2017). Yang et al.'s accomplishments marked the first genome-wide analysis of early modern humans in China, thus filling the sampling gap in time and space. Tianyuan individual's genome, possessed genetic characteristics similar to the one of modern Asians, who carry approximately 4%–5% Neanderthal DNA which is shared by Upper Paleolithic Eurasians. Tianyuan individual has a relatively closer relationship with present-day and ancient Asians than with Europeans. However, the genetic connection between the Tianyuan individual and the ancient European individuals of the same period (GoyetQ116-1 and Bacho Kiro) was observed, which may be related to the spread of Paleolithic Aurignacian culture (Fu et al., 2016; Hajdinjak et al., 2021). The evidence from genomic analysis showed that the Tianyuan individual was not a direct ancestor of modern Chinese populations, indicating the diversity of Asian populations 40,000 years ago. In addition, the individual dating back about 33,000 years from the Amur region

(AR33K) shared similar genetic components with the Tianyuan individual (Mao et al., 2021), which suggested that before the Last Glacial period the Tianyuan-related population was widespread in northern China.

3 Communication and integration of regional Chinese populations

Early Chinese civilizations formed in multiple regions while displaying a variety of characteristics, hence the diversity of material cultures (Wu et al., 2018). There is a plethora of topics on the genetic history of various cultural populations, their exchanges and interactions with the surrounding populations, as well as their influence on the formation of modern populations which are widely discussed. Recent paleogenomic studies have provided new insights into these issues.

3.1 The northeast region of China

The northeast region of China geographically connects North Asia and the Far East, including Liaoning, Jilin, Heilongjiang provinces, and the southeastern part of Inner Mongolia. This region has been populated since the Paleolithic Age (Pitulko, 2001). The material cultures of this area began to flourish in the Neolithic Age, and became one of the cultural and dryland agricultural centers of northern China (Liu et al., 2015). This region had a profound impact on the formation and evolution of Chinese civilization, as well as on the proliferation of agriculture in northern East Asia (Liu et al., 2015). Recently, genome-wide data from the Amur and West-Liao River regions aided researchers in the comprehension of the dynamic demographic processes of this area in China (Ning et al., 2020; Mao et al., 2021).

The Amur River (AR) region, adjacent to the Far East, is rich in natural resources. As a result, fishing, hunting and animal husbandry constitute the primary subsistence strategies in this area (Kato, 2006). The demographic history in this region was reconstructed through the genomic analysis of AR individuals dated from the Paleolithic to the Iron Age (Ning et al., 2020; Mao et al., 2021). Before the Last Glacial Maximum (LGM), the population in AR (AR33k) had similar genetic profile with the Tianyuan individual. While, at the end of the LGM, the genetic makeup (AR19k) in this area changed, and the previously widespread Tianyuan-related component may have been replaced by modern East Asian component, reflecting a genetic discontinuity from 33,000 to 19,000 years old (Mao et al., 2021). When compared to the ancient southern coastal populations of East Asia, AR19k has a closer genetic relationship with those of the ancient coastal northerners (Mao et al., 2021). Based on this observation, the genetic differences between the

northern and southern East Asians had been formed about 19,000 years ago, nearly 10,000 years earlier than previously reported potentialities. After the LGM, the populations in the AR region showed a genetic continuity, and had the closest genetic relationship with the Devil's Gate cave population (DevilsCave_N), Neolithic forager/farmers recovered from Devil's Gate cave in Far East (Sikora et al., 2019). In addition, after 14ka, although the genetic components of the population did not change, the local population size had increased, which may be related to the advent of sedentary farming in the region (Mao et al., 2021).

The West-Liao River region (WLR), an important area for the early formation and development of Chinese civilization, is one of the dryland millet agricultural centers in northern China (Teng, 2013). The people in this region had lived primarily on agriculture and fishing and hunting (Sun, 2015). In the middle of the Neolithic Age, well-developed material culture, represented by the Hongshan culture (6,500–5,000 BP), had expanded in this and neighboring regions (Teng, 2017). Researches focusing on the population origin in this area and its contacts with other millet planting regions, such as Yellow River Basin, are crucial for enlarging our knowledge on the broadcast of agriculture in China. Unlike the demographic process in the AR region, population dynamics in this area from the Middle Neolithic Age to the Bronze Age can be characterized by a series of interactions with surrounding populations (Ning et al., 2020). The populations in this region carried a mixture of ancestries from the Neolithic AR (AR_EN) and mid-Neolithic Yellow River region (YR_MN), while the proportions of these two ancestries varied with spatial and temporal scales. The mid-Neolithic Haminmangha (HMMH) population in the northern extent of the WLR, where neighbors the AR region, had more AR_EN ancestral component than contemporaneous Hongshan population from the southern part of WLR, while the late Neolithic populations had more YR_MN ancestral component than the mid-Neolithic ones. This variation of genetic makeup reflected a northward migration of the Central Plain populations, and a contact between populations from the two major dryland agricultural centers in northern China. Genetic evidence inferred that the pattern of agricultural diffusion in northern China may be driven by population movements. As a consequence of climatic changes during the Bronze Age, the region was no longer suitable for millet cultivation, the subsistence strategy transformed to nomadic pastoralism, accompanied with a genetic shift of the incremental AR_EN ancestry component (Jia et al., 2016).

3.2 The Central Plain of China

The Central Plain, with good natural conditions, is the cradle of Chinese civilization. Complex societies emerged in this region as early as 10,000 years ago, and the advent of agriculture can be

dated back to 8,700BP (Pang and Gao, 2006). The Yangshao and Longshan cultures, representatives of the Neolithic cultures of the Central Plain, are well-known for painted pottery (Wu, 2001). Archaeological evidences revealed the interactions between the Central Plain and surrounding regions (Shui, 2001). The genetic profile of populations from the Neolithic Age to the Late Bronze/Iron Age in Central Plain was similar, but the genetic components from southern China and Southeast Asia (SC-SEA) increased after the Late Neolithic Age, when rice farming intensified in this region (Ning et al., 2020). The correlation between the population's genetic change and the northward spread of rice farming technology from the Yangtze River Basin, inferred a demic diffusion pattern of agriculture in China. Compared with the ancient Central Plain populations, the modern Han has more ancestral components in common with SC-SEA, which may be a result of the continuous northward migration of rice-based agricultural populations during the formation of the Han. In addition, from the Neolithic Age to the Iron Age, the populations carried ancestral components similar with the Central Plain population were widely distributed in the surrounding areas, such as the upper Yellow River, Shaanxi, and Inner Mongolia, indicating continuous genetic exchanges between the Central Plain and the surrounding areas (Ning et al., 2020; Wang et al., 2021a).

3.3 The northwest region of China

The northwest region of China is a pathway connecting the East and the West, playing a vital role in cultural and population exchanges. The genetic history of this region has been debated for a long time.

Laying at a junction between East and Central Asia, Xinjiang has a complex demographic history. Cultural exchanges and population interactions between East and West Eurasia had occurred in this region long before the opening of the Silk Road (Shao, 2009; Damgaard et al., 2018). The peopling of Xinjiang is a highly debated topic. Mainly two hypotheses were proposed to explain the origins, the steppe hypothesis and the oasis hypothesis. The steppe hypothesis posits that the initial immigrants to Xinjiang were the Afanasievo-related populations from the Altai region north of the Tarim Basin (Mallory and Mair, 2000). In contrast, the oasis hypothesis suggests that farmers from Bactria-Margiana Archaeological Complex (BMAC) region arrived in the Tarim Basin during the Bronze Age (Chen and Hiebert, 1995). In 2021, Zhang et al. reported the findings from ancient DNA study conducted on the mummies from the Xiaohu culture in the Tarim Basin (Zhang et al., 2021). Supporting neither of the previous hypotheses, the paleogenomic analysis of the Bronze Age Tarim Basin population showed a local origin model. The genetic components of the Tarim population were neither from steppe populations nor

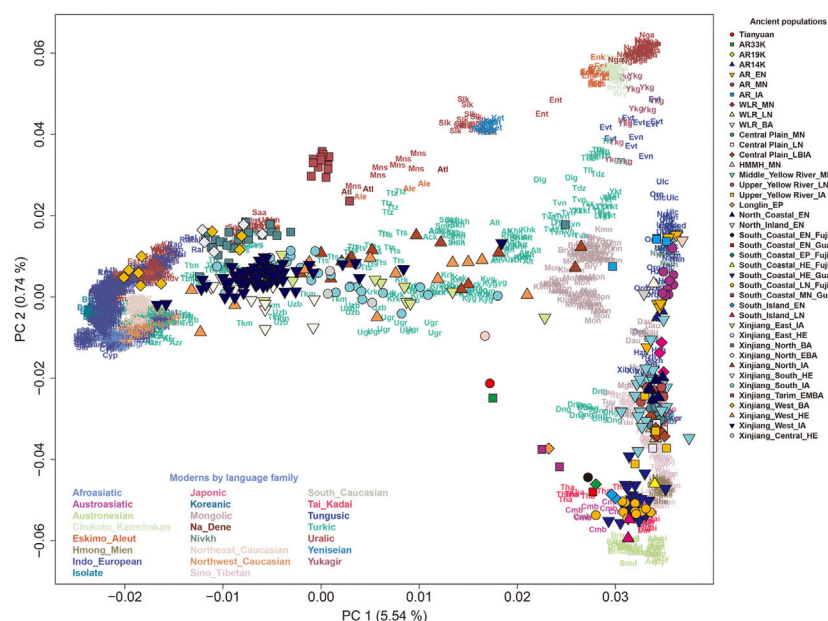


FIGURE 1

PCA Analysis for ancient Chinese populations. The PCA was constructed from present-day Eurasians in the Human Origins dataset, and the ancient individuals were projected onto the top PCs.

related to BMAC populations, but rather a mixture of ancient North Eurasians (ANE) and ancient Northeastern Asians (ANA). The date of this admixture event was estimated at 10,000–6,000 years ago. The ANE-related component, currently best represented by the Tarim mummies, may have been widespread in Eurasia during the Bronze Age and prior. Although cultural exchanges occurred between Xiaohe and the surrounding populations, Xiaohe population was genetically isolated and maintained long-term genetic stability. Unlike the Xiaohe population, the early populations from the Dzungarian Basin derived a majority of their ancestral components from the Afanasievo population, and additionally, from the local ANE ancestry.

Subsequently, a genomic study of more than 200 ancient individuals in Xinjiang also proved that the ancestral components of the early Bronze Age populations in northwestern Xinjiang derived not only from the local populations, represented by the Xiaohe population, but also from the Afanasievo, the Chemurchek, and the Shamanka populations (Kumar et al., 2022). Frequent interactions and admixtures between these populations during the Bronze Age in Xinjiang and the surrounding regions, such as the western steppe, Central Asia, and Northeast Asia, reflected a complex genetic history of the Xinjiang populations. In the middle and late Bronze Age, the ancestral component from the Andronovo emerged in Xinjiang populations, and an increasing influx of ancestry from East Asia was observed.

Compared to the Bronze Age populations, the Iron Age populations presented high influxes of Central Asian and East Asian ancestral components with more diverse origins, including Han and Xiongnu. The genetic profile of the Iron Age populations was still observed in historical and present-day Xinjiang populations. The presence of the Saka component in the Iron Age Xinjiang may be related to the spread of the Indo-Iranian languages in Xinjiang (Bailey, 1970).

These genomic studies of Xinjiang aid in efforts to comprehend the demographic history of the region and further contribute to the literature on the transmission of technology and language (Zhang et al., 2021; Kumar et al., 2022).

Located in the northwest, the middle and upper Yellow River region is one of the important birthplaces of Chinese civilization. Both archaeological and linguistic studies had indicated that early populations in this region had a substantial impact on the formation and development of Sino-Tibetan populations (Van, 2005). The genetic analysis of the genomes from a 5,000-year-old population in this region (Wang et al., 2021a) revealed that the Neolithic population in the Yellow River Basin once migrated to the Tibetan and the Central Plain, likely broadcasting Sino-Tibetan languages, and may be one of the common ancestors of Sino-Tibetan speakers. The Iron Age population in Taiwan derived their ancestry not only from farmers residing in the Yangtze River Basin, but also from

ancient people habituated northern China (Wang et al., 2021a). The trail of findings confirmed a southward migration of the northern Chinese people. The genetic composition of modern Han can be modeled as a mixture of the ancestries from the Yellow River Basin and the Iron Age Taiwan to varying degrees, which inferred the causes of the genetic differences between the northern and southern Han.

3.4 The coastal areas of China

The genetic patterns and divergent processes of the northern and southern Chinese populations are important to explore the deep population history of East Asia. To gain insights from the genomic perspective, the researchers collected Neolithic human remains spanning the coastal areas of northern China (Shandong) and Inner Mongolia (9,500–7,700 years ago), and the southern coastal areas (12,000–500 years ago) (Yang et al., 2020; Wang et al., 2021b). The genomic data indicated that the northern East Asian ancestral component, represented by the Neolithic Shandong individuals spread to the Yellow River Basin and even northward to the eastern Siberian steppes at least 9,500 years ago, whereas the populations from the southern coastal areas and Taiwan Strait island had the genetic components of the southern East Asian ancestry, represented by the Neolithic individuals from Fujian and its adjacent islands 8,400 years ago (Yang et al., 2020). These two genetic components separated into two distinct genetic lineages. Over time, the genetic differences between the northern and southern populations have gradually narrowed, suggesting frequent population migration and mixing between the north and the south since the Neolithic Age.

The southern coastal region of China, bordering Southeast Asia, has a long history of modern human occupation. It is a key area for studying the origin and evolutionary history of modern humans in eastern Eurasia and Oceania.

The paleogenomic analysis on the individuals from Fujian and the surrounding areas, dated back to 9,000 to 8,000 years ago, indicated that the southern ancestry was greatly reduced in the current southern mainland populations (Yang et al., 2020; Wang et al., 2021b). However, this ancestral component had significant influence on the present-day Austronesians, and was closely related to the Southeast Asian populations in the late Neolithic period.

The genome-wide study conducted on the human remains dating 10,686–294 years ago in Guangxi identified a previously unsampled East Asian ancestry, Longlin (10,686–10,439 cal BP, Guangxi ancestry), whose genetic profile is different from the ancient southern ancestry (Fujian ancestry) and the 8,000-year-old Hòabínhan from

Southeast Asia (Wang et al., 2021b). The interaction between populations carrying Fujian ancestry and Guangxi ancestry occurred 9,000 years ago. The Guangxi ancestry persisted until 6,400 BP in southern China. The admixture of populations between southern China and Southeast Asia occurred as early as 9,000–6,400 years ago, that is, before introducing of the agricultural technology in the region.

4 Conclusion and perspectives

The observed 5 years-long rapid development of paleogenomic research conducted in China has provided a large amount of valuable information for tracing the origin and demographic history of Chinese populations, for instance, the genetic characteristics of the 40,000-year-old Tianyuan individual and the widespread of this ancestry (Yang et al., 2017; Mao et al., 2021). China has a vast territory and diverse topography. The genetic landscape and the demographic events of prehistoric populations differ regionally (Figure 1, Supplementary Table S1). The northern and southern populations diverged as early as 19,000 years ago and the genetic differences between them reduced through time, indicating continuous population interactions. Amur region experienced genetic shift at the end of the LGM, and after that, this region exhibited long-term genetic continuity. Genetic shift and population movement accompanying with the spread of agriculture were observed in West-Liao River region. Central Plain population expanded to surrounding areas and their genetic components from SC-SEA were increased through time. The dynamic pattern of Xinjiang populations can be characterized by a series of admixture between local ancestry, represented by early Xiaohé population, and surrounding populations. The southern coastal region experienced complex interaction between different genetic ancestries before the introducing of farming.

However, our understanding of the history of Chinese populations is far from complete. Numerous questions were put forth, such as the direct ancestry of Chinese populations, when and how the northern populations interact with the southern ones, the genetic composition and the expansion of the ancient farmers from the Yangtze River region, the origin and evolution of the Tibetans, and so on. Although there has been some accumulation of ancient genomic data, there is still a long way to reconstruct the complex genetic process of the Chinese people. In the future, with more paleogenomic data from previous unsampled regions under time and space transections, for instance, the central region of China, as well as the advances in archeometry, linguistics, bioinformatics, genomics and proteomics, multidisciplinary research can provide us with extraordinary insights into the origin of Chinese populations, the pattern of population migration and interaction, as well as the adaptation to the environment through time.

Author contributions

Briefly, the contributions from the individual co-authors are: YC lead the study and the writing of the MS; SG collect the published data and participate the writhing of the 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.

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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.2022.1059196/full#supplementary-material>

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Faunal exploitation during the Proto-Zhou period in the Jing River Valley: Evidence from Sunjia and Xitou

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This study examines faunal assemblages from the Proto-Zhou sites of Sunjia and Xitou, in the Jing River Valley (Central Shaanxi Province), to address questions concerning the exploitation of different animal resources in the context of the Shang-Zhou dynastic transition in the 11th century BCE. Although the assemblages from Sunjia and Xitou were small and sub-optimally preserved, this study demonstrates that the inclusion of such assemblages is essential to building upon our understanding of the human exploitation of animal resources. Our zooarchaeological analysis shows an increase in husbandry, with pig farming being complemented by extensive caprine and cattle herding. A diversified use of animal resources, and especially the larger number of bovids, could have been prompted by the need for a wider and more efficient exploitation of the immediate environment, in response to growing climatic deterioration, in addition to an increase in interactions with northern pastoral communities. Identified patterns of livestock biometry and relative taxonomic abundance show various degrees of agricultural engagement and a relatively complex livestock economy, suggesting the development of socio-economic complexity in the Jing River Valley in the late second millennium BCE.

KEYWORDS

subsistence strategies, Jing River Valley, Sunjia, Xitou, proto-zhou, bronze age, zooarchaeology

1 Introduction

An understanding of the exploitation of animal resources is crucial to our appreciation of ancient subsistence strategies, land use, and regional social transformations (Crabtree, 1990; Zeder, 1991; Lhuillier and Mashkour, 2017). Zooarchaeology in Northern China has been consistently applied to the study of ancient remains, with the aim of understanding faunal temporal and spatial

distribution, past environments and human-animal relationships (Yuan, 2015). Systematic studies on ancient domestication (Huang, 2010), regional trajectories (Flad et al., 2007; Yuan et al., 2007), secondary products (Li et al., 2014a; Li et al., 2014b; Brunson et al., 2016) and craftsmanship (Campbell et al., 2011; Hou et al., 2018) have been largely centered in the Neolithic and dynastic periods in the Central Plain (Huang, 2000; Li, 2011a; 2011b; Li et al., 2014a). Limited work has been done in other contexts, including Bronze Age Central Shaanxi, which has to date obfuscated the economic conditions underpinning the emergence of the Zhou as a political authority in the late 11th century BCE (Yuan, 2000; Zhongguo Shehui Kaxue Xueyuan Kaogu Yanjiushuo, 2007; Li et al., 2019, Li et al., 2020).

The Proto-Zhou Culture (ca. 1400–1050 BCE) refers to the cultural complex created by the Zhou people and their alliance in Northwest China before the formal establishment of the Zhou Dynasty (1050–256 BCE). In other words, it indicates the dry-farming communities that inhabited the central Loess Plateau during the late Shang Dynasty (1600–1050 BCE) (Huang et al., 2003; Cao, 2007). Epigraphic sources have consistently been used to identify the ancestral land of Zhou, the dominant idea being that it was located in the northern and middle reaches of the Jing River (Khayutina, 2020). According to written sources and inscriptions, the main Proto-Zhou centers include the Zhouyuan region, which encompasses present-day Qishan and Fufeng Counties, and Feng and Hao, located on the western and eastern banks of the Feng River, near Xi'an. Their location is supported by archaeological evidence (Zhang and Yin, 2004; Khayutina, 2010). The earliest Zhou centers, Tai, in the valley of the Wei River, and Bin, in the middle reaches of the Jing River, have not been precisely identified, although most scholars have located the Ancient Bin Area (Gu Bin di 古邠地) in a region roughly covering the present-day counties of Binzhou, Xunyi, Changwu and Chunhua (Zhang, 2004; Li, 2006; Niu 2017; Khayutina, 2020).

“The Archaeological Investigation of the Ancient Bin Area” project has identified and excavated several Proto-Zhou sites in the Jing River Valley, including Nianzipo (Changwu County) (Zhongguo Shehui Kaxue Xueyuan Kaogu Yanjiushuo, 2007), Zaoshugou (Wang and Chen, 2010; Xibei Daxue Wenhua Yichan Xueyuan yu Kaoguxue Xueyuan Zhongxin, 2012), Zaolinheta (Dou et al., 2019) (both in Chunhua County), Xiaweiluo (Xibei Daxue Wenhua Yichan Xueyuan yu Kaoguxue Xueyuan Zhongxin et al., 2006), Sunjia and Xitou (all three in Xunyi County) (Xibei Daxue Wenhua Yichan xueyuan et al., 2020; Xibei Daxue Wenhua Yichan xueyuan et al., 2021). Taxonomic typological comparison of structures and pottery material, sometimes combined with radiocarbon dating results, has allowed the attribution of these sites to the Proto-Zhou Culture. Sites excavated in the context of this project were among the first in the region at which significant zooarchaeological research was undertaken (Zhongguo Shehui

Kaxue Xueyuan Kaogu Yanjiushuo, 2007; Li et al., 2019, 2020) (Figure 1).

The study of faunal assemblages has suggested that the ancient inhabitants of Central Shaanxi herded sheep and goats in the gullies and hills, and raised pigs on the plain and loess platforms, while also practicing some hunting and fishing (Cheng et al., 2017; Festa and Monteith, 2022). Results from the Zaoshugou and Zaolinheta assemblages have revealed that residents practiced intensive farming and animal husbandry in and around the settlements, complemented by extensive caprine herding on marginal lands (Li et al., 2019, 2020). By contrast, pigs would appear to have been important domesticated animals at Fengxi (Yuan, 2000), Nianzipo (Zhongguo Shehui Kaxue Xueyuan Kaogu Yanjiushuo, 2007) and Beicun (Cao, 2001), where relatively low ratios of bovids compared to pigs have been identified. Our analysis of the faunal assemblages from Sunjia and Xitou represents novel data on animal economies in the Jing River Valley during the second millennium BCE, which allows for a more solid understanding of the regional subsistence strategies in the context of the emergence of the Zhou authority.

1.1 Sunjia and Xitou

The Sunjia and Xitou sites are located in Zhanghong Town, in northwestern Xunyi County (Xianyang City, Shaanxi Province), along the north-western boundary of the Guanzhong Plain and the southern limits of the Loess Plateau (Shaanxi wenwuju 2012). This “transitional zone” is characterized by platforms, gullies, and hill ridges ranging from 960–1350 masl, which form a segmented natural landscape (Festa and Monteith, 2022). The local river, the Sanshui, runs diagonally across the territory from northeast to southwest before flowing into the Jing River (Figure 2). Although the hillsides have been relatively recently terraced, historically, this region would have only had limited land for cultivation.

Sunjia and Xitou are multi-phase settlements with cemeteries showing fairly constant use from the Neolithic period to historic times. The excavation report of Sunjia has not yet been published. The site is located ~8 km northeast of the Yuandi Community. Preliminary excavations in 1994 discovered a pit containing Proto-Zhou pottery sherds (Lei, 2010) and, in 2017, further excavations uncovered various residential features, such as trash pits, ditches, and houses, and a number of joined-crotch *li* vessels (Zhang, 2004). Xitou is a large site located ~1 km west of the Yuandi Community, with a total area of about 100 km². Over the course of various excavation campaigns since the 1990s, it has been revealed that the site was occupied serially over the periods of Yangshao, Longshan, Shang, Western Zhou and even later (Xibei Daxue Wenhua Yichan xueyuan et al., 2020; Xibei Daxue Wenhua Yichan xueyuan et al., 2021;



FIGURE 1
Map of the archaeological sites mentioned in this research.



FIGURE 2
The landscape of the Jing River Valley. Photo credit: Xiao Jiarui.

Shaanxi 2012; Guojia wenwuju 1998) (Table 1). In 2018, excavations were undertaken in the Yuzuipo sector of the south-eastern section of the Xitou site (hereafter abbreviated to Xitou) (Figure 3). An area of ca. 700 m² was excavated and

various structures, including houses ($n = 4$), ash pits ($n = 128$), ash ditches ($n = 9$) and tombs ($n = 14$) were uncovered. These features were found to contain a large number of ceramics, lithics and animal remains (Xibei Daxue Wenhua Yichan

TABLE 1 Chronology of Northern China associated with this study.

Period	Date (BCE)
Yangshao	5,000–3,000
Longshan	3,000–2000
Erlitou	2000–1,500
Erligang (Early Shang)	1,600–1,300
Anyang/Yinxu (Late Shang)	1,300–1,050
Western Zhou	1,050–772

xueyuan et al., 2021). These animal remains constitute the faunal evidence examined in this paper. To date no radiocarbon dates have been published for either of these sites. Chronological attribution of the remains to the Late Shang period has been inferred from the stratigraphy and taxonomic typologies of the tombs, buildings, and ceramics therein. The relevant culture levels in Sunjia and Xitou show significant parallels with artefacts and structures excavated from the Nianzipo, Zoolinhetan and Zaoshugounao sites, for which radiocarbon dates have been published (Zhang, 2004; Gong, 2018; Xibei Daxue Wenhua Yichan xueyuan et al., 2020; Xibei Daxue Wenhua Yichan xueyuan et al., 2021).

2 Materials and methods

This study is based on the faunal remains excavated from Sunjia in 2017 and Xitou in 2018. Although mostly recovered from residential contexts, a few finds were discovered in four burials in Xitou (M2, M6, M7, and M12). Hand selection was employed during excavation, but no sieving. The animal bone assemblages totaled 1,097 fragments at Sunjia and 519 fragments at Xitou.

The analysis was conducted in two different locations: the zooarchaeology laboratory of Northwest University (Xi'an), and the Northwest University archaeology base in Xunyi County. Taphonomic processes—fragmentation, sub-aerial weathering, burning, gnawing—and human modifications were quantified for all fragments to evaluate the general preservation of the assemblages. The completeness of bones was recorded differently for identified and non-identified bones: the former was measured according to proportion of the whole bone (0.25, 0.25–0.5, 0.5–0.75, ≈ 1); for the latter maximum length measures were recorded. Sub-aerial weathering and burning were also recorded according to Behrensmeier (1978) and Stiner et al. (1995), respectively. Worked bones were identified into the lowest identifiable taxonomic unit and classified, according to their possible function considering



FIGURE 3

The area of Xitou. The red triangle indicates the Yuzuipo locale, from which our faunal evidence has been excavated. Adapted from Xibei Daxue Wenhua Yichan xueyuan et al. (2020).

shape, size and (when possible) use-wear, into four main categories—tools, oracle bones, decorations, and discarded material.

Identification was attempted for every fragment using the reference collection of the zooarchaeological laboratory of Northwest University, online reference resources (e.g., archaeozoo.org) and published identification manuals (e.g., Hillson, 1992; France, 2009). All identified bones were used to calculate the number of identified specimens (NISP) and minimum number of individuals (MNI). NISP was used to quantify species abundance and was calculated using fragments identified to family and beyond following Clason (1972)'s method. By counting fragments that can be cross-mended as one, Clason's method limits the risk of counting fragments from the same element more than once and, therefore, reduces the boost from large-size bones and animals. Considering the poor preservation of the faunal remains, the limited availability of reference specimens, and the uncertainties surrounding the standards to distinguish sheep and goats (Zeder and Pilaar, 2009; Zeder and Lapham, 2010; Salvagno and Albarella, 2017), the two were combined into the "caprine" category. MNI was calculated on the basis of diagnostic zones present (Dobney and Rielly, 1988). Because of the small number of faunal remains unearthed from graves in Xitou, they were considered along with those from the residential context in the counts of NISP and MNI, in order to provide an omnicomprehensive understanding of the taxonomic abundancy at the site. However, as the inclusion of fauna in funerary contexts implies intentional deposition, the provenance of the bones was signaled in the resulting counts and considered, when necessary, during the interpretation of the results.

Mortality profiles for cattle, pigs and caprines were generated based on bones epiphyseal fusion alongside tooth eruption and wear. Considering the fragmentary evidence, cattle dental aging was attempted using Jones and Sedler (2012), which works relatively well with loose teeth. Caprine mandibles were aged using Payne (1973), which identifies tooth eruption and wear stages for the lower deciduous premolar (dp4), permanent premolar (P4) and molars (M1, M2, and M3). Available dp4 and M3 from single individuals to which age stages could be assigned were also used. The information was then converted into a survivorship curve, which expresses the percentage of animals killed at each age. For pigs, we employed Lemoine et al. (2014)'s "specific system", which allows to consider both mandibles and maxillae. Epiphyseal fusion analysis for cattle was carried out following O'Connor (2003), while for pigs Zeder et al. (2015) was employed, because it relates to Lemoine et al. (2014)'s dental aging system. Analysis on caprines was conducted by referring to Zeder (2006)'s system, which relates tooth eruption and wear to long bone fusion to refine age determination accuracy.

Measurements were taken for whole elements and undamaged parts following Von den Driesch (1976). Body-size of cattle and pigs was examined using the Log Standard Index (LSI) method. In spite of several imperfections (e.g., the assumption that all bones vary proportionally in size with the standard animal, neglecting other factors impacting the body proportions), the LSI method has the merit of increasing sample sizes, when few measurable specimens are available (Meadow, 1981, Meadow, 1984). Due to the lack of published measurement data from complete Chinese specimen skeletons, Degerbol and Fredskild (1970)'s female aurochs from Mesolithic Denmark, and Russel (1993)'s modern Hungarian female wild boar were used as standard animals for LSI analysis of cattle and pigs, respectively. Pigs' body size and upper M3 measurements were also used to investigate the domestication status (Mayer, et al., 1998).

3 Results

3.1 Taphonomy

Taphonomic processes impact the preservation of faunal remains after the animal's death and affect basic data as well as those from age profiles (Lyman, 1994; Lam et al., 2010). Table 2 Our assemblages were both fairly fragmented and extensively weathered, especially in Sunjia, where severe weathering affected more than 62% of the fragments (Behrensmeier, 1978) (Figure 4). Assuming that sub-aerial weathering is irrelevant for buried bones (Reitz and Wing, 2008), most specimens in Sunjia and Xitou must have been



FIGURE 4
Weathered cattle metacarpal from Sunjia.

TABLE 2 Quantification of the taphonomic processes affecting the faunal remains in Sunjia and Xitou.

Taphonomic process	Recording categories	Sunjia		Xitou	
		Number	%	Number	%
Fragmentation Identified specimens ^a	<0.25	192	66.67	268 (3) ^b	74.6
	0.25–0.5	27	9.38	33	10.5
	0.5–0.75	58	20.1	34(1) ^b	11.1
	≈1	11	3.82	12	3.8
Total		288	100	351	100
Fragmentation Non-Identified specimens ^a	<5 cm	461	84	116 (1) ^b	82
	5–10 cm	81	14.4	18	18
	10–15 cm	7	1.3	0	0
Total		549	100	135	100.00
Weathering (Behrensmeyer, 1978)	0	407	37.1	156 (7) ^b	30.8
	1–3	6	0.6	144	27.2
	4–5	684	62.3	222	42
Total		1,097	100	529	100
Burning (Stiner et al., 1995)	0	1,044	95.1	518 (7) ^b	99.2
	1	1	0.1	0	0
	2	6	0.6	2	0.4
	3	30	2.7	2	0.4
	4	7	0.6	0	0
	5	3	0.3	0	0
	6	6	0.6	0	0
Total		1,097	100	529	100
Animal Marks	Animal marks	24	2.2	35	6.6
	No Animal Marks	1,073	97.8	487 (7) ^b	93.4
Total		1,097	100	529	100

^a262 and 43 fragments in Sunjia and in Xitou respectively could not be measured.

^bNumber in parentheses are fragments found in funerary contexts, included in counts.

exposed on the surface, instead of being rapidly buried. The fact that the remains recovered from burials lack weathering marks further supports this conclusion. All taxa had been subjected to weathering. A low rate of gnawing marks in Sunjia (2% of the fragments) contrasted with the high degree of weathering. Elsewhere, this phenomenon has been associated with abandonment phases during which human and animal activities were absent (Madgwick and Mulville, 2015). This is plausible, as some settlements in the regions, such as Jiangzhai (Lintong, Xi'an), appear to have been abandoned for some time before being reoccupied decades or centuries later (Peterson and Shelach, 2012). Another possibility is that some areas were fenced allowing weathering to occur but preventing animals' access

(Lawson et al., 2000). Some gnawing marks may have also been erased by weathering processes, which were particularly severe in Sunjia. In Xitou, the rate of gnawing marks was higher (7% of the fragments), and this is consistent with the higher presence of carnivores at the site (see below). In both sites, the limited evidence of burning activity was evenly distributed taxonomically, and mostly included bones burned to dark-brown or black (Stiner et al. (1995)'s stage 3), with only some semi-calcined and calcined specimens (Stiner et al. (1995)'s stages 4–6). Their presence, in association with cultural features, including residential structures and bones/antlers artefacts, suggests that these burned bones were products of anthropogenic activities (David, 1990).

TABLE 3 Animal taxa by type of worked bones at Sunjia and Xitou.

Site	Taxon	Tools		Oracle bones		Decorations		Discarded material		Total	
		N°	%	N°	%	N°	%	N°	%	N°	%
Sunjia	Cattle	1	4.8	2	9.5	0	0	0	0	3	14.3
	Deer	6	28.5	0	0	0	0	0	0	6	28.5
	Caprine	1	4.8		0	0	0	0	0	1	4.8
	Large mammal	3	14.2	1	4.8	0	0	0	0	4	19
	Medium mammal	5	23.8	1	4.8	0	0	1	4.8	7	33.4
Total		16	76.1	4	19.1			1	4.8	21	100
Xitou	Cattle	2	10	6 (1) ^a	30	0	0	0	0	8	40
	Deer	1	5	0	0	(1) ^a	5	2	10	4	20
	Caprine	1	5	0	0	0	0	0	0	1	5
	Pig	1	5	0	0	0	0	0	0	1	5
	Large mammal	1	5	1	5	0	0	1	5	3	15
	Medium mammal	2	10	1	5	0	0	0	0	3	15
Total		8	40	8	40	1	5	3	15	20	100

^aNumber in parentheses are fragments found in funerary contexts, included in counts.

3.2 Worked bones

A total of 16 and 8 tools, and 4 and 8 ritual objects were recovered from Sunjia and Xitou respectively, forming ca. 2% of the fragments in each site. Human modifications were unevenly distributed across taxa, cattle and deer being the most popular. Table 3 Cattle scapulae were used to make oracle bones. Deer elements—mostly antlers, but also bones and teeth—were used as tools and decorative items. In Xitou, the assemblage of worked bones was more varied, occasionally including pig specimens. A fair number of modified bones came from large and medium mammals which could not be identified to species (Figure 5). Notably, one cattle oracle bone and one decorated deer tooth were discovered in graves M7 and M12, respectively. Discarded material found in both sites suggests local production activity.

3.3 Taxonomy

By considering fragments that could be cross-mended as one (Clason, 1972), we counted 614 and 420 specimens in Sunjia and Xitou respectively. Mammals dominated the two assemblages (98.9% and 100% in Sunjia and Xitou, respectively). The remaining finds in Sunjia consisted of

birds ($n = 2$; 0.3%) and shellfish ($n = 5$; 0.8%). The overall paucity of remains of small-sized animals and small bones in the assemblages may relate to the lack of sieving on site. Around half of the specimens could not be identified beyond family. Such identification was possible for 355 and 236 specimens in Sunjia and Xitou, respectively (Figure 6; Table 4). Among the finds in Xitou, identified faunal remains from graves were only 4 (1.6% NISP). Few wild mammal species were observed (Sunjia, 7% NISP; Xitou, 5% NISP), more than half of which were cervids. The domestic assemblage was prevalent in both sites (Sunjia, 93% NISP; Xitou, 95% NISP) and it was remarkably homogeneous, with only a few taxa represented. Domestic cattle had a significant presence (Sunjia, 30% NISP; Xitou, 41% NISP), although caprine NISP was higher in Sunjia (33% NISP). Pigs made up 17% NISP in Sunjia and 18% NISP in Xitou, respectively. A fair number of dog remains were found (Sunjia, 7% NISP; Xitou, 10% NISP). Notably, 3 fragments were from grave M2 in Xitou (1.3% MNI). The least represented taxon was horse (Sunjia, 6% NISP; Xitou, 3% NISP).

MNI of domesticated animals (cattle, caprines, pig, dog, and horse) showed a general consistency across the two sites (Table 4). Caprines were the best represented category in both assemblages (Sunjia, 37.5% MNI; Xitou, 29% MNI), followed by pigs (Sunjia, 25% MNI; Xitou, 24% MNI) and cattle (19% MNI in both sites). The MNI slightly differed from the NISP, especially in Xitou, where cattle



FIGURE 5
Fragment of an oracle bone from Sunjia, made out of a large mammal's scapula.

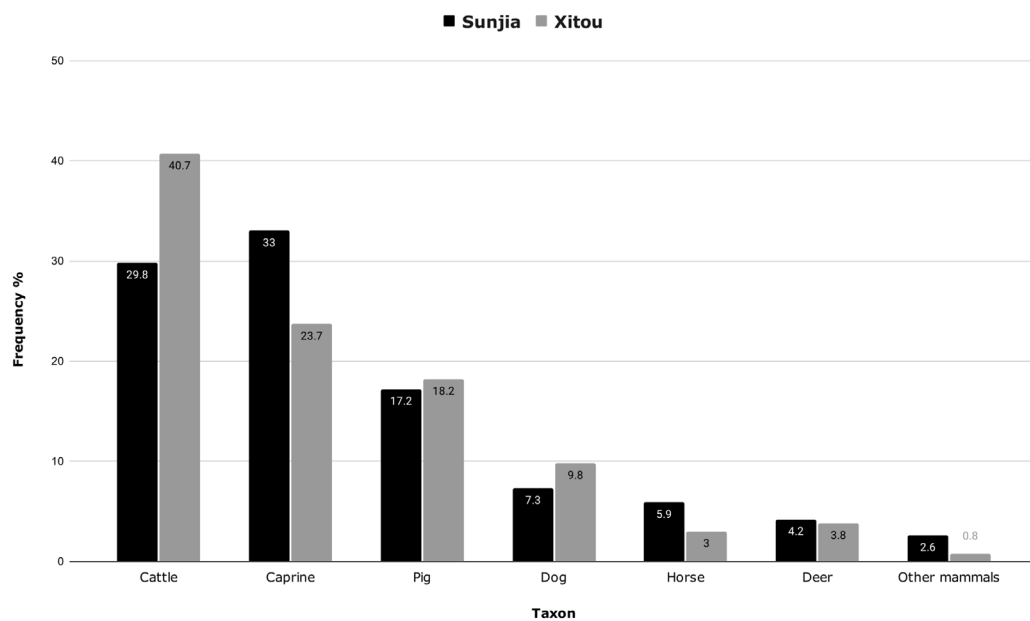


FIGURE 6
Frequency of different taxa from Sunjia and Xitou by %NISP.

elements were predominant. Breakage analysis showed that the assemblages were quite fragmented, suggesting a possible boost in the cattle NISP proportion. For dogs, a relatively high MNI in Xitou (20% MNI) was due to the presence of 4 mandibles, one of which

(alongside one cranial bone and one vertebra) came from grave M2. The MNI value for dogs in Sunjia was lower, but still significant (12.5% MNI). The horse was the least represented taxon by MNI (Sunjia, 6.2% MNI; Xitou, 5% MNI).

TABLE 4 NISP and MNI of faunal remains from Sunjia and Xitou.

Category	Taxon	Sunjia				Xitou			
		NISP	NISP %	MNI	MNI%	NISP	NISP %	MNI	MNI%
Domestic Mammals	331	93.2	16	100	225	95.4	20	100	
	Cattle, <i>Bos taurus</i>	106	29.8	3	18.8	96	40.7	4	20
	Caprine, <i>Ovis aries</i> / <i>Capra hircus</i>	117	33	6	37.5	56 (1) ^a	23.7	6	30
	Pig, <i>Sus scrofa domesticus</i>	61	17.2	4	25	43	18.2	5	25
	Dog, <i>Canis familiaris</i>	26	7.3	2	12.5	23 (3) ^a	9.8	3(1) ^a	20
	Horse, <i>Equus caballus</i>	21	5.9	1	6.2	7	3	1	5
Wild Mammals	24	6.8			11	4.6			
	Bear, Ursidae	2	0.6			1	0.4		
	Roe Deer, <i>Capreolus</i>	1 (2) ^b	0.3			2	0.8		
	Sika Deer, <i>Cervus nippon</i>	10 (1) ^b	2.8			2 (1) ^b	0.9		
	Musk Deer, <i>Moschus</i> sp		-			1	0.4		
	Unidentified Cervid	4 (20) ^b	1.1			4 (4) ^b	1.7		
	Fox, <i>Vulpes</i> sp	1	0.3						
	Hare, <i>Lepus</i> sp	2	0.6						
	Rat, <i>Rattus</i> genus	1	0.3						
	Zokor, <i>Eospalax</i> sp	3	0.8			1	0.4		
Total Identified Mammals	355	100			236	100			

^aNumber in parentheses are fragments (NISP) or individuals (MNI) found in funerary contexts, included in counts.

^bNumbers in parentheses are antler fragments, not included in counts.

3.4 Age at death

Mortality data allows the identification of unnatural mortality patterns among animal populations, which can be attributed to human decisions and related to domestication and herd management (Li et al., 2014a; Reitz and Wing, 2008). The small assemblages prevent more than a very general idea of mortality patterns for the main domestic taxa in Sunjia and Xitou.

3.4.1 Cattle

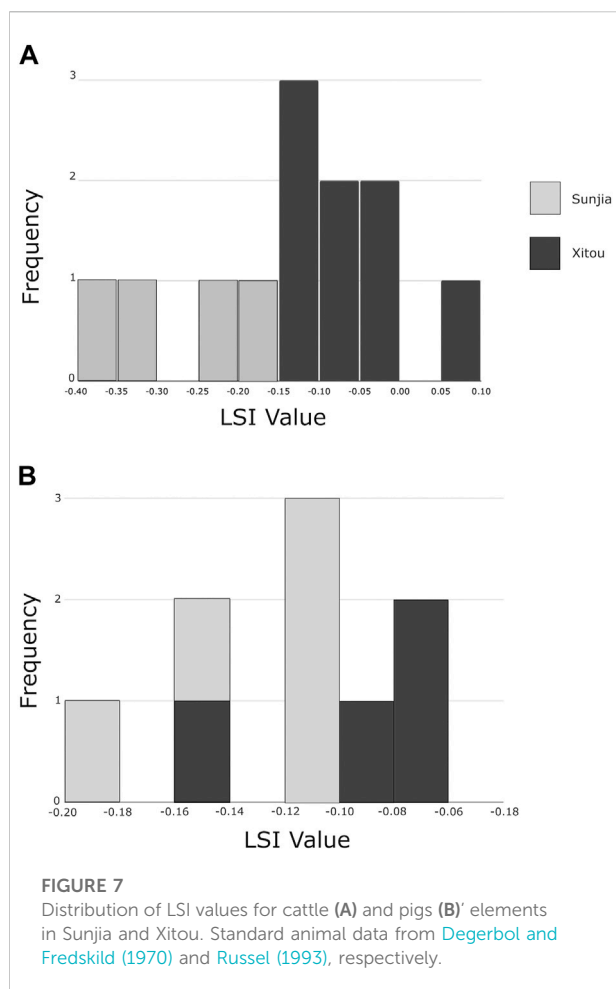
One mandible from Sunjia gave a rough estimation of death age at ca. 2 years old. 3 deciduous teeth in Xitou could be related to one or more relatively young individuals. Another 4 upper M2 and 4 lower M2 were available for examination in Sunjia and Xitou, respectively. They were all in wear, making the individuals possibly older than 18 months (Jones and Sadler, 2012). The tiny sample impacted the overall significance of the epiphyseal fusion analysis results, causing some slightly nonsensical outputs (O'Connor, 2003) (Supplementary Figure S1). Overall, mortality data showed a gradual attrition with a significant proportion of older specimens.

3.4.2 Caprine

Mandibles from Sunjia (3) and Xitou (8) were examined, along with 4 lower loose teeth—3 M3 and 1 dP4 in Sunjia; 1 dP4 and 2 M3 in Xitou (Payne, 1973). The Xitou assemblage lacked data for several age groups. However, the overall dental analysis results showed a fairly gradual killing pattern for both sites (Supplementary Figure S2). In Xitou, usable samples for epiphyseal fusion analysis were only available for Zeder (2006)'s fusion groups D and E—respectively representing fusion at 18–30 months and 30–48 months. 3/8 and 2/7 specimens were fused or fusing respectively, collectively suggesting that around a third of the individuals survived beyond these age ranges. Epiphyseal fusion analysis results for Sunjia showed an early kill-off at face value, however, they were skewed by a couple of fairly complete juveniles found in a single unit, which made up ca. 40% of the sample size.

3.4.3 Pigs

Survivorship curves for pigs show a relatively gradual attrition, insofar as the very small samples can be trusted. The examination of 3 mandibles and 3 maxillae from Sunjia and 7 mandibles and 1 maxilla from Xitou, showed a fair representation of younger (ca. 12 months) and older



individuals (16–30 months) (Lemoine et al., 2014) (Supplementary Figure S3) Epiphyseal fusion analysis was attempted using Zeder et al. (2015) but the numbers ($n = 5$ in each site) were just too small to be meaningful.

3.5 Size

Postcranial measurements of cattle bones showed that all of the specimens at Sunjia were smaller than all of those at Xitou—the LSI values ranging from -0.40 to -0.15 and from -0.15 to 0.06 respectively (Figure 7A; Supplementary Table S1). Our assemblages did not comprise a sufficient number of long bones with fused proximal and distal epiphyses, which would have been optimal specimens for measurement (Von den Driesch, 1976). The analysis was largely conducted on partial long bones shafts with one of the two epiphyses fused, phalanges, and other elements, such as astragali and one atlas. In spite of the obvious limitations, the size variation was statistically significant (Mann-Whitney U -test: $U=0$, $p=0.0040$).

A similar trend was observed for pigs, although there was some overlap, and the difference was not statistically significant ($U=3$, $p=0.1111$) (Figure 7B; Supplementary Table S2). Pigs were identified as domestic. They were invariably smaller than the female wild boar taken as reference animal. Moreover, 3 upper M3 and 2 lower M3 measurements were consistent with Mayer et al. (1998)'s values for domestic pigs. The sample was, however, very small and might not fairly represent the ancient assemblage of suines. Data for sheep were insufficient for a comparison ($n = 2$ in Xitou).

4 Discussion

This paper has demonstrated that taxonomic abundance and some information about human exploitation of animal resources can be inferred from two small and poorly preserved assemblages. Sunjia and Xitou were rather consistent in terms of taxonomic composition. Both assemblages were dominated by domestic species, though some wild fauna was also present, suggesting that the economy was based on husbandry supplemented with some hunting and fishing. Cattle and caprines dominated the assemblages in Sunjia and Xitou. Our results are consistent with those obtained by Li et al. (2019), and Li et al. (2020) for the coeval neighboring sites of Zaoshugou and Zaolinheta (Figure 8; Supplementary Table S3), suggesting a growing importance of these two taxa in the region during the Late Shang period. Increasing bovid herding marks a clear difference in comparison with previous periods, when pigs were predominant. In Central Shaanxi pigs were domesticated relatively early, probably in the Mid-Neolithic (Barton et al., 2009), and their importance grew during the Yangshao and Longshan periods (Hu, 2001; Hu et al., 2011; Wang et al., 2013). A slight, though steady, increase in bovids, at the expense of pigs, has been documented from the end of the Longshan period (Hu, 2001; Hu et al., 2008; Hu et al., 2016), and according to our results, became more dramatic in the late second millennium BCE (for a summary see Festa and Monteith (2022)).

The exploitation strategies present at the sites of Sunjia and Xitou may have been dependent on multiple simultaneous variables. The shift to bovid husbandry could be related to the increasingly cold and dry climate, which followed the Holocene Climatic Optimum, and which could have had an impact on livestock management. While caprines and cattle are fairly tolerant of arid conditions, pigs require a plentiful and dependable supply of water (Burrin, 2001). Also, under cold and arid conditions, agricultural productivity would have declined, prompting a wider and more diversified use of the landscape, including the expansion into marginal lands, which would have been easier with bovid herding (Festa and Monteith, 2022). An et al. (2005) and Huang et al. (2004) have shown that climatic deterioration in the Loess Plateau caused many rain-fed

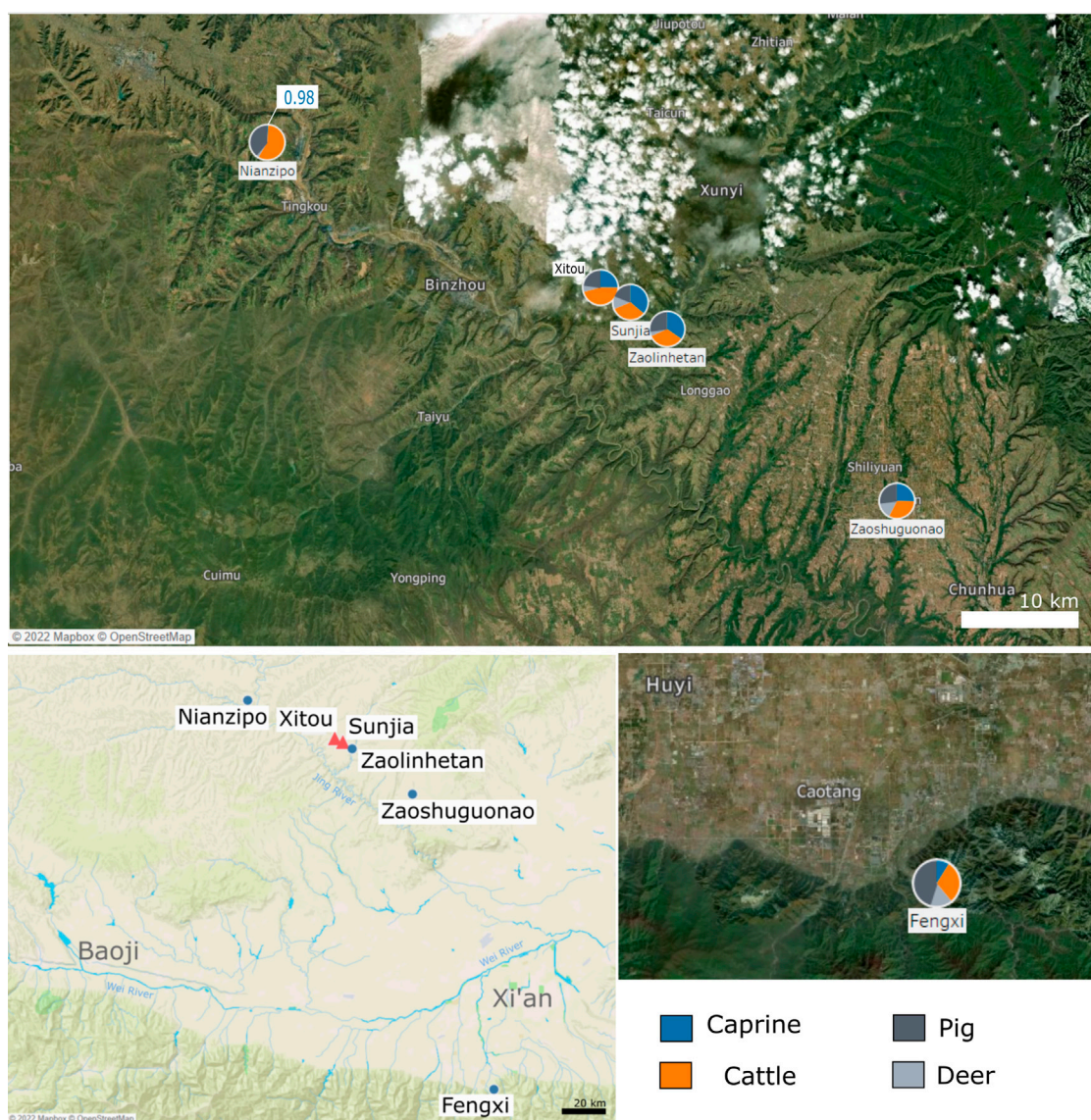
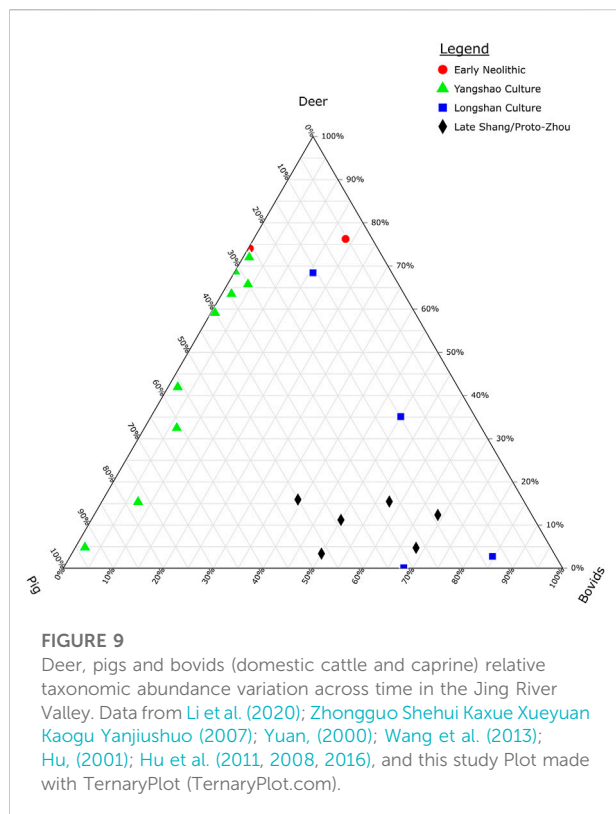


FIGURE 8

Relative taxonomic frequency for the main domestic taxa (pigs, caprine and cattle) and deer by %NISP in the sites mentioned in this study: Sunjia and Xitou (this study), Nianzipo (*Zhongguo Shehui Kaxue Xueyuan Kaogu Yanjiushuo, 2007*), Zaolinhetao, Zaoshuguonao (*Li et al., 2020*). Bottom right: Relative taxonomic frequency for the main domestic taxa (pigs, caprine and cattle) and deer by %NISP in Fengxi (*Yuan, 2000*). Data from the sites of Beicun and Laoniupo were not complete and could not be plotted with those from the other sites. Bottom left. Location of the mentioned sites (Tableau, F. Monteith)

agricultural economies to shift toward agro-pastoralism and mobile pastoralism from the beginning of the second millennium BCE. Similar conclusions were reached by research in Central Asia and Northern China (*Frachetti et al., 2017; Wang et al., 2021*). Although the residents of the Jing River Valley practiced agriculture and raised some pigs (*Lu and Zhang, 2008; Li et al., 2009; Chen et al., 2019*), the growing importance of cattle and caprine husbandry may have allowed for a more extensive exploitation of the immediate environment in response to regional climatic changes.

The Proto-Zhou faunal assemblage from the site of Fengxi (Xi'an), located in the plain of the Wei River Valley, includes a relatively larger proportion of pigs than Sunjia and Xitou (*Yuan, 2000*). Similarly, in Laoniupo (Xi'an), also located in the plain, pig remains were well represented. These two assemblages present certain limitations, one being the sample size of the former, which was very small, including less than 200 elements. Additionally, the assemblage from Fengxi was discovered over the course of 50 years of intermittent archaeological excavations, with recovery and recording protocols not followed to a modern



standard: the context in which the bones were and some relevant data are, therefore, controversial. The bones at Laoniupo were mostly found in funerary contexts, which implies intentional selection (Zhang et al., 2007). The variations in the faunal assemblages between these and our sites may, nevertheless, indicate an adaptation to distinct eco-topographic conditions—the former being on a broad flat plain and the latter in a hilly region consisting of gullies and loess platforms. The climatic deterioration following the Climatic Optimum may have affected agriculture to different degrees in the plain and the hilly transitional region to the north, resulting in a different response by the residents of the two areas. Deterioration in climatic conditions would have led to cultivation being less productive in the Jing River valley—where arable land was more limited to begin with—than on the plain. Therefore, while the communities in the plain could rely on a complementary strategy of mixed millet-rice agriculture and pig husbandry (Zhang et al., 2010), the residents of Sunjia and Xitou may have diversified the exploitation of animal resources to cope with new climatic conditions. Bovid husbandry would have been quite efficient, because these animals do not compete with humans for food, do not require copious amounts of water, and could also be used for secondary products.

It is however, noteworthy, that suines, along with deer, were well represented at the sites of Beicun (Yao County) and

Nianzipo (Changwu County), which were also located in the hilly region to the north of the Guanzhong Plain. Beicun was reported only in terms of MNI and based on a specific selection of skeletal elements, which not necessarily represent the whole assemblage (Cao, 2001). Additionally, the context in which the skeletal elements were found is controversial and it is unclear whether these bones were from a residential or a funerary context, or both. Similarly, the assemblage from Nianzipo, made up mostly of long bones shafts with traces of human modifications, was retrieved from specific contexts associated with bone production, and may not be fully representative of the whole assemblage (Zhongguo Shehui Kaxue Xueyuan Kaogu Yanjiushuo, 2007). Notwithstanding, the high proportion of pigs at these sites suggests that cultural preferences may have influenced the regional animal exploitation strategies (Figure 8; Supplementary Table S3).

Increasing caprine and cattle herding in Sunjia and Xitou may also have been due to the influence of neighboring pastoral communities in the north. In the second-first millennium BCE mobile and semi-mobile pastoralism dramatically increased, and small and large-scale population movements created a solid network of interaction across Central Asia, Northwest China and Mongolia (Frachetti et al., 2017; Høisæter, 2017). In particular, the growing importance of bovids in the Jing River Valley may be related to the southern migration of pastoralist communities from the Northern Loess Plateau and the Mongolian Plateau. The existence of relations between the residents of Central Shaanxi and those inhabiting the northern regions has been recorded in ancient texts, such as Shiji Zhou Benji (史记·周本纪) (Huang et al., 2003; Khayutina, 2020). Most importantly, such interactions are documented by archaeological evidence of steppe-type of artefacts in several Proto-Zhou sites in Central Shaanxi (Zhang, 2004; Zhang, 2016; Wang and Chen, 2010).

The relatively gradual attrition shown by survivorship curves in Sunjia and Xitou contrasts with a planned and specialized meat production and suggests that the animals were exploited also for their secondary products. However, more data will be required to confirm this supposition. Evidence of worked bones indicates that some taxa were exploited for ritual purposes. For example, cattle scapulae were used to make oracle bones. The Shang used cattle scapulae and turtle shells as divinatory tools (Hou et al., 2018), however at our Proto-Zhou cultural sites only the former was found. Similar finds were recorded at Zaoshugounao and Zaolinhetao (Li et al., 2020), signaling some difference between the Shang and the Proto-Zhou in terms of ritual practices. The percentage of oracle bones in Sunjia (0.4% of the fragments) and Xitou (1.5% of the fragments) was comparable to coeval sites at Zaoshugounao (0.5% NISP) and Zaolinhetao (1.5% NISP) (Li et al., 2020), but lower than at Nianzipo (Changwu County) (3% NISP), where specimens made of horse and other animals' scapulae

were also found (Zhongguo Shehui Kaxue Xueyuan Kaogu Yanjiushuo, 2007). This may indicate a certain degree of ritual variability in the region, and, at the same time, reflect a decline of Shang practices in some areas of the Jing River Valley, in favor of other divinatory systems, such as that by hexagrams, mentioned in written sources (Khayutina, 2020). Only one fragmented oracle bone was found in the filling of grave M7 in Xitou. This was the only cattle remain recovered from our mortuary contexts, suggesting the development of different ritual practices compared to the Shang period, when whole or dismembered cattle were placed in graves (Lu, 2015; Hou et al., 2019). This inference is not only based on faunal assemblages from the four graves examined in this study, but also on comparable finds at Zaoshuguonao (Li et al., 2020). By contrast, the discovery of three bones belonging to (likely) one young dog in grave M2 in Xitou seems to find parallels with dog mortuary depositions of the Shang (Li and Campbell, 2019), further supporting the variety of ritual traditions present in the Jing River Valley in the late 11th century BCE.

Despite the close proximity (~4 km) and the shared topography and climate, a significant variation in cattle body size was observed between Sunjia and Xitou. In spite of the small assemblage and the above-mentioned limitations concerning the bones selection, this distinction is quite remarkable and it is worthy to be discussed in terms of extensive/intensive subsistence practices. As discussed above, in the late second millennium BCE, new economic strategies developed in the Jing River valley as a response to climatic changes, which brought about a certain degree of reorganization of the rural landscape and promoted the exploitation of more marginal lands. Archaeobotanical data for Xitou and Sunjia are currently not available, however, crop cultivation and consumption in the region has been demonstrated by archaeobotanical and isotope research results from neighboring Proto-Zhou sites (Lan 2017; Chen et al., 2019), and various degrees of reliance on agriculture have been discussed by Li et al. (2020) for the Zaoshuguonao and Zaolinheta sites. If this was the case, foddering and breeding strategies would have been adjusted to different farming systems, affecting the animal size (Trentacoste, 2020). Different degrees of engagement in agricultural practice would have had a significant impact on localized animal exploitation, in particular, the use of cattle. A more intense and extensive agricultural activity would have favored animal labour in the fields, and, therefore, the investment in large specimens, potentially even oxen, for traction, which would have significantly increased land productivity; by contrast, small-scale agricultural systems would have incentivized human labor, and, therefore, the exploitation of smaller and cheaper cows for secondary products and light work (Bogaard et al., 2019; Trentacoste et al., 2021). The use of cattle for traction in Northern China during the late Shang period has been demonstrated by paleopathological studies on animal bones (Lin et al., 2018), however, the poor conditions of our

assemblages hampered such analysis. In the absence of this data, it might be possible to use the size difference in cattle to differentiate between economic structures (Trentacoste, et al., 2021): a larger, more complex agricultural center, such as Xitou, would have required sizable animals, while in smaller settlements, such as Sunjia, smaller less-demanding female specimens, would have been preferred.

A non-statistically significant, yet notable, variation in pig size was identified between the sites of Sunjia and Xitou, with the former generally presenting smaller specimens than the latter. Although the sample size used in this study was small, this variation can signal different animal husbandry practices at the two sites. Chen et al. (2014)'s isotopic analyses of pig bones from the Mid-Late Neolithic sites of Dongying and Wayaogou, in the Wei River valley, have shown various feeding strategies in the region, with some pigs kept on intensive foddering, whereas others received less millet fodder. If the occupants of Sunjia and Xitou engaged in agriculture to different degrees, this may have had an impact on pig husbandry strategies, as less productivity would have left less surplus for pigs. Different feeding strategies would, in turn, have influenced the animals' size.

The argument that the residents of the two sites may have relied on different agricultural and livestock management strategies is further suggested by the higher caprine/pig ratio by NISP in Sunjia (1.9), compared to that in Xitou (1.3), which would indicate a lower degree of agricultural engagement in the former site (Miller 1997). Considering that the two sites are only about 4 km apart and share environmental and climatic conditions, different economic strategies could have depended, at least partially, on social, cultural and political factors.

Wild animals were scarce, particularly in Xitou, where the dearth of arrowheads from the archaeological context further suggests limited hunting (Xibei Daxue Wenhua Yichan xueyuan et al., 2020). A few shell fragments constitute the only evidence of aquatic resource exploitation. This is unexpected given the large regional water system and the fair use of mollusks and fish documented from various sites in Central Shaanxi, both earlier than, and contemporary to, Sunjia and Xitou (Cheng et al., 2017; Li et al., 2020). This lack in our assemblages is presumably due to poor sieving and taphonomic agents. The relative paucity of wild taxa in our sites is in line with regional zooarchaeological and paleodietary research, which has shown that while hunting and fishing were practiced in the Neolithic, their importance declined during the Longshan period, possibly due to population growth and the increasing demand for meat, both for consumption and ritual purposes (Cheng et al., 2017). The decrease continued during the Late Shang period, when hunting and fishing may have become less related to the need for food (Li et al., 2021; Festa and Monteith, 2022) (Figure 9; Supplementary Table S3). Ancient texts and archaeological evidence indicate that hunting, in particular, was a significant social activity during the Shang and Zhou dynastic periods (Cheng et al., 2017; Fiskešjő, 2001). Most importantly, faunal evidence from Sunjia and Xitou indicates that wild taxa, especially deer, were exploited for tool-making, supporting Li et al. (2021)'s argument of a shift occurred

in the cervids regional exploitation, from meat to antlers. The discovery of some unworked post-cranial elements from deer associated with tools made of antlers—and to a lesser extent, bones and teeth—and debris suggests that local residents hunted cervids and produced artefacts locally.

5 Conclusion

Although the faunal remains from Sunjia and Xitou were sub-optimally preserved, their analysis is an important addition to zooarchaeological research as it represents a novel interrogation of micro-regional variation in the exploitation of animal resources in the Jing River valley in the Proto-Zhou period. A growing importance of bovids was identified at the sites. A diversified use of animal resources may have been prompted by the local population's need to exploit the immediate and peripheral environment more efficiently, in response to the climatic deterioration following the Climatic Optimum. Interactions with northern communities of pastoralists, which migrated southward from the northern Loess Plateau and the Mongolian Plateau, could also have promoted more intensive bovid husbandry in the region.

A diversified use of animal resources and a wider and more efficient exploitation of the immediate environment, in addition to the alliances with neighboring communities, may have underpinned the socio-economic foundation behind the emergence of the Zhou as a political power. Li et al. (2020) speculate that diverse faunal use could reflect the development of economic and social complexity in the Jing River Valley in the late second millennium BCE. Such a conclusion was supported by our research results. Patterns of livestock biometry and relative taxonomic abundance in Sunjia and Xitou indicate that the residents of the two sites may have engaged in agriculture to different degrees and relied on different livestock management strategies. Xitou would have been a larger agricultural center, while Sunjia would have been a smaller settlement. This distinction suggests the development of some degree of regional social complexity in the Jing River Valley in the late second millennium BCE. While this study has demonstrated that variations in faunal exploitation strategies can inform socio-economic developments, these should be the subjects for further investigation, when more zooarchaeological results will be considered along with relevant archaeological evidence.

Data availability statement

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

Author contributions

Conceptualization, MF and YL; methodology, MF and YL; formal analysis, MF, DO, QW, TZ, and YL; resources, HD, BL, and YL; data curation, MF; writing—original draft preparation, MF; writing—review and editing, MF, DO, FM, and YL; visualization, MF and FM; project administration, HD; funding acquisition, MF, HD, and YL. All authors have read and agreed to the published version of the manuscript.

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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.

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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.2022.1072897/full#supplementary-material>

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Transformation of animal utilization strategies from the late Neolithic to the Han Dynasty in the Hexi Corridor, northwest China: Zooarchaeological and stable isotopic evidence

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The trajectory and influencing factors for changes to ancient human livelihoods in the Hexi Corridor of northwest China have been intensively discussed. The Hexi Corridor is a key crossroads for trans-Eurasian exchange in both the prehistoric and historical periods. Although most studies have focused on the reconstruction of human paleodiet and plant subsistence, the diachronic change of animal utilization strategies spanning the prehistoric and historical periods remains unclear, due to the absence of zooarchaeological and isotopic studies, especially in Han Dynasty (202 BCE–220 CE). Here we report new zooarchaeological, stable isotope, and radiocarbon dating data from the Heishuiguo Cemetery of the Han Dynasty in the Hexi Corridor, indicating that humans mainly used domestic chickens, pigs and sheep as funerary objects, with other buried livestock including cattle, horses and dogs. Stable carbon and nitrogen isotope data suggest humans might have fed chickens, pigs and dogs more C₄ foods (likely millets or their byproducts) than herbivorous livestock in the Heishuiguo during the Han Dynasty. Compared to other prehistoric zooarchaeological and isotopic studies in the Hexi Corridor, we detected an increasing significance of herbivorous livestock in animal utilization strategies compared with omnivorous livestock, and a basic declining weight of C₄ foods in fodders from ~2,300 to 200 BCE, which was probably induced by long-distance exchange and climate fluctuation. However, the trend was reversed during the Han Dynasty in the Hexi Corridor, primarily due to the control of the area by the Han Empire and the subsequent massive immigration from the Yellow River valley of north China.

KEYWORDS

zooarchaeology, isotopic analysis, animal exploitation, Han dynasty, the late Neolithic, Hexi corridor

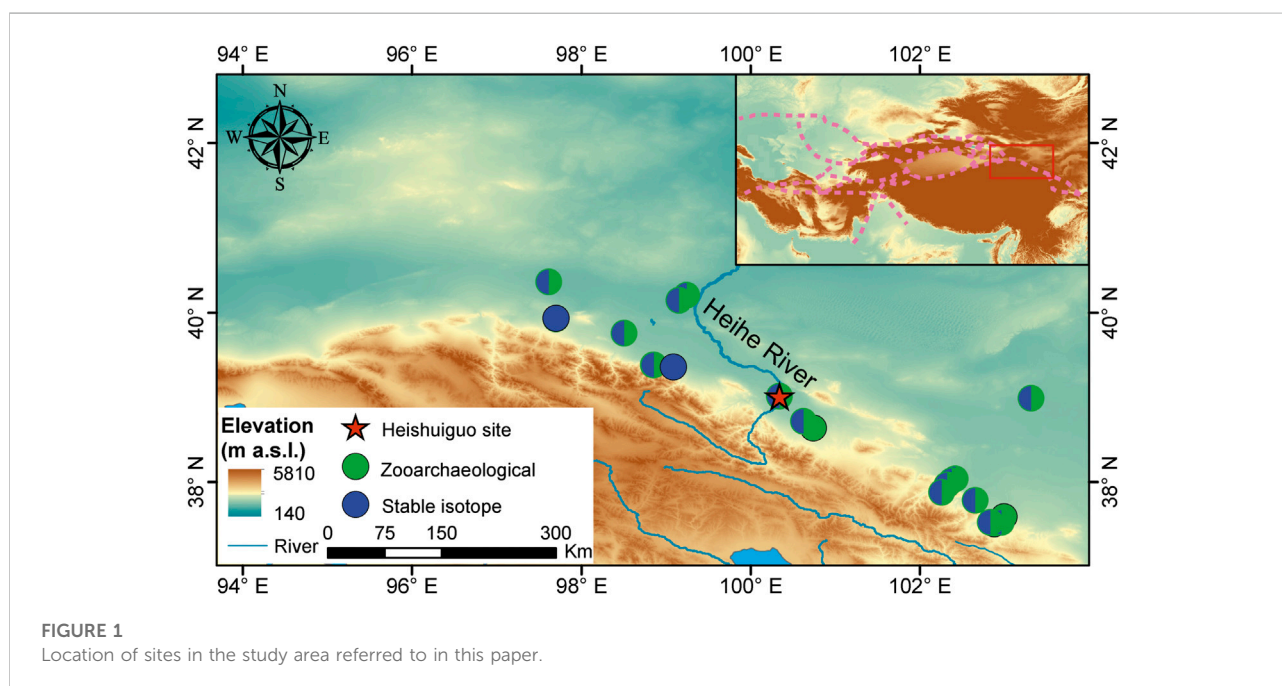
1 Introduction

With the rapid development of bioarchaeological research in recent decades, the spatio-temporal variation of human subsistence strategies in different corners of the planet since the Neolithic has been increasingly concerned (Piperno and Dillehay, 2008; Hu, 2018, 2021; Chen et al., 2020; Fernández-Crespo et al., 2020). This has been especially true of the area around the ancient Silk Road and Eurasian Steppes (Wang et al., 2019; Zhou et al., 2020; Li et al., 2021a; Librado et al., 2021), where there were major passageways for the dispersal of farming and herding groups across Eurasia during both the prehistoric and historical periods (Dong et al., 2017; Frachetti et al., 2017; Liu et al., 2019). Trans-Eurasian exchange emerged and intensified mainly through these two passageways after the third Millennium BCE (Spengler et al., 2014; Wang et al., 2019; Zhang et al., 2021), profoundly influencing human livelihoods across the Old World (Jeong et al., 2018; Ning et al., 2020; Dong et al., 2022), especially in the crossroads areas for long-distance exchange, such as the Hexi Corridor in northwest China.

The earliest human settlement in the Hexi Corridor can be traced back to ~2,800 BCE (Dong et al., 2018), thereafter human subsistence strategies in the area varied evidently before Han Dynasty. Archaeobotanical and isotopic studies reveal that humans in the Hexi Corridor mainly cultivated foxtail and broomcorn millet and consumed C_4 foods during ~2,800–2,000 BCE. They then cultivated millets, wheat and barley, consumed both C_3 and C_4 foods during ~2,000–1,300 BCE, while the significance of barley and wheat in plant subsistence far exceeded foxtail and broomcorn millet

during ~900–200 BCE (Zhou et al., 2016; Yang et al., 2019a; Dong G et al., 2020). According to isotopic evidence and historical records, the weight of millet crops in human subsistence strategies was evidently improved in the Hexi Corridor during the Han Dynasty as compared to the early Iron Age (Gao, 2014; Li, 2021).

In contrast to the rapid accumulation of archaeobotanical data, zooarchaeological studies have been conducted at just a few prehistoric sites in the Hexi Corridor (Figure 1). Current studies suggest that the primary livestock shifted from indigenous pigs and dogs to introduced sheep, cattle, and horses between ~2,800 and 2,000 BCE and ~2,000–1,300 BCE, respectively, while herbivorous livestock became the dominant subsistence animal during ~900–200 BCE in the Hexi Corridor (Yang et al., 2019a; Dong G et al., 2020; Dong G. H. et al., 2020; Ren et al., 2022). No zooarchaeological data from the Han Dynasty sites in the area has been reported, and therefore the diachronic change of animal utilization strategies spanning the late prehistoric and historical periods remains enigmatic. Recently, abundant animal bones were unearthed from the Heishuiguo (HSG) Cemetery of the Han Dynasty in the Hexi Corridor. Here we try to further the research on these issues, based on the identification assemblage, as well as carbon and nitrogen isotope analysis of buried animal remains in the Heishuiguo Cemetery, compared to published data from elsewhere in the Hexi Corridor. Moreover, we compare zooarchaeological and stable isotopic data with historical and paleoclimate studies, in order to explore the influencing factors



behind the transformation of animal utilization strategies in the Hexi Corridor.

2 Study areas

2.1 Geographical background

The Hexi Corridor (37.28°–42.80°N, 92.38°–102.20°E) is located in northwestern China (Figure 1). It is a long narrow corridor, spanning about 1,000 km from the Wushaoling Mountains in the east to the Yumenguan Pass in the west, which bordering the Tibetan Plateau in the southwest, the Mongolian Plateau in the north, the Loess Plateau in the east, and the Tarim Basin and Tianshan Mountains in the west. The study area is characterized by a semi-arid and arid climate, with mean annual temperatures varying from 4°C to 9°C and annual average precipitation of 178 mm (see <http://data.cma.cn>). From east to west, the precipitation decreases gradually. The Shule, Shiyang, and Heihe Rivers are the three largest inland rivers in the region. The natural vegetation in the study area is dominated by C₃ vegetation (Su et al., 2011) and the sedimentary $\delta^{13}\text{C}$ values demonstrate the predominance of C₃ plants during the Holocene (Jiang et al., 2019). The Hexi Corridor is the essential route from China to the Central Asia, West Asia and even Europe, and the region has been an important route for human migration and cultural diffusion from prehistoric to historical times. The study area is located at a key location on the Silk Road and has played an important role in prehistoric trans-Eurasian exchange, especially since the Han Dynasty (202 BCE–220 CE).

Heishuiguo Cemetery (36.92°N, 100.34°E) is located in the middle of the Hexi Corridor (Figure 1), in Ganzhou, Zhangye, and it is near to the Heihe River. The river valley provides a critical connection between the Hexi Corridor and the Eurasian Steppe, making these sites a hub for prehistoric and historical cultural exchange in northwestern China. In 2018, the Gansu Provincial Institute of Cultural Relics and Archaeology conducted rescue excavation of the Heishuiguo Cemetery and excavated 118 Han Dynasty tombs. Based on the cultural relics and funeral objects found, it shows that the Heishuiguo Cemetery was mainly used from the middle Western Han Dynasty to the middle Eastern Han Dynasty (GPICRA, 2019).

2.2 Archaeological background

The archeological framework of cultural evolution in the study area has been established according to previous studies (Wang, 2003; Li, 2011; Yang et al., 2019b). In the Hexi Corridor, the chronological order of archeological culture is: the Machang types of the Majiayao culture (2,300–2,000 BCE), the Qijia/Xichengyi culture (2,000–1,600 BCE), the Siba culture

(1,600–1,300 BCE), the Shanma culture (900–200 BCE), the Shajing culture (800–200 BCE), the Western Zhou Dynasty (1,046–771 BCE), the Eastern Zhou Dynasty (770–256 BCE), and the Han Dynasty (202 BCE–220 CE).

According to cultural phases and the radiocarbon dates, we divided the data into four periods: 2,300–2,000 BCE, 2,000–1,300 BCE, 900–200 BCE, and the Han Dynasty (202 BCE–220 CE).

3 Materials and methods

A total of 1,020 pieces of animal bones were unearthed from 27 Han Dynasty tombs in Heishuiguo Cemetery during the excavation in 2018, all of which could be identified to the species level. All animal remains were collected carefully by hand, any attachments on the bone surface were washed away, and then they were left to dry in the shade. Taxon identification and measurement were carried out in the MOE Key Laboratory of Western China's Environmental Systems (Ministry of Education) in Lanzhou University on the basis of the morphological characteristics of the bones. In the course of the zooarchaeological analysis, we compared the Heishuiguo Cemetery remains to ancient animal specimens kept in our lab and some atlases of modern animal bones (Schmid, 1992; France, 2008).

Thirty-three animal bones were selected for isotopic analysis. Bone collagen was extracted by the acid-alkali-acid method, which has been described in other papers (e.g. Ma et al., 2016; Hu, 2018; Li, 2021). The percentages of C and N, the atomic C/N ratios, and the isotopic values of all collagen samples were processed in an isotope mass spectrometer (IsoPrime-100 IRMS) combined with an element analyzer (Vario Pyro Cube). Carbon and nitrogen isotopic ratios were measured relative to VPDB and AIR standard samples, respectively. The analytical precision of isotopic ratios was better than 0.2‰. We used SPSS 25.0 for statistical analysis. The Mann-Whitney and Kruskal-Wallis tests were used to detect differences between different groups of samples.

In addition, two animal collagen samples were radiocarbon dated at Lanzhou University, China. All ^{14}C dates were calibrated in OxCal 4.4.4 (Ramsey, 2021) with the IntCal 20 calibration curve (Reimer et al., 2020) and reported as 'BCE or CE'.

4 Results

The number of animal remains identified to the species level is defined as NISP (the number of identified specimens) and the minimum number of individuals (MNI) of each species can be estimated based on their NISP. All the buried animal remains at Heishuiguo were identified as domestic animals, including chickens (*Gallus gallus domestica*), pigs (*Sus scrofa domestica*), cattle (*Bos taurus*), sheep (*Ovis aries*), and dogs (*Canis lupus familiaris*) (GPICRA, 2019). All animal identification data are listed in

TABLE 1 Proportion of identified animal remains from the excavation of the HSG Cemetery in 2018.

Period	Species	NISP	NISP (%)	MNI	MNI (%)
Phase 1	Gallus gallus domestica	147	35.34	8	38.10
	Sus scrofa domesticus	132	31.73	9	42.86
	Bos taurus	93	22.36	2	9.52
	Ovis aries	35	8.41	1	4.76
	Canis lupus familiaris	9	2.16	1	4.76
Total		416	100.00	21	100.00
Phase 2	Gallus gallus domestica	328	58.89	15	68.18
	Sus scrofa domesticus	125	22.44	4	18.18
	Ovis aries	103	18.49	2	9.09
	Aves	1	0.18	1	4.55
Total		592	100.00	22	100.00
undefined	Gallus gallus domestica	47	93.33	1	50.00
	Equus ferus caballus	1	6.67	1	50.00
Total		48	100.00	2	100.00

NISP: number of identified specimens; MNI: minimum number of individuals.

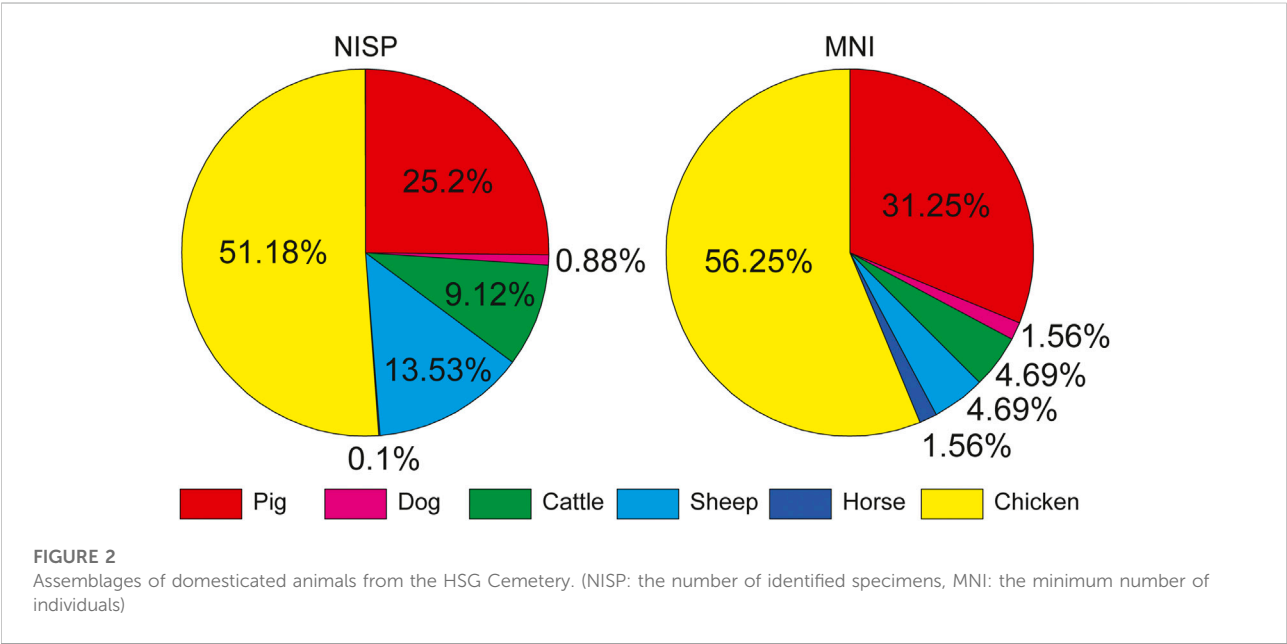
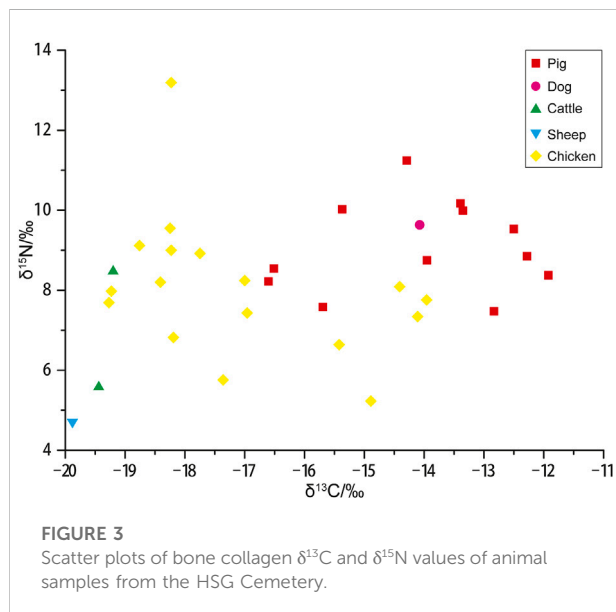


Table 1 and plotted in Figure 2. Based on the characteristics of the cemetery (GPICRA, 2019) and the results of the absolute dating, the animal samples in this study were divided into two periods: 190–50 BCE (phase 1, the middle Western Han) and 50 BCE–200 CE (phase 2, late Western Han to Eastern Han). During phase 1, both the NISP and MNI data for animal bones indicate that chickens account for more than 35 percent of the total

animal remains, with 147 fragments representing 8 individuals. The number of pig bones is similar to chicken bones, with 132 by NISP vs. 9 by MNI. The number of cattle bones is less than chickens and pigs during this phase, making up approximately one-fifth of the total animal remains by NISP and one-tenth of the total animal remains by MNI. The remaining samples contained the bones of sheep and dogs, which make up a small proportion of the total

TABLE 2 Summary of carbon and nitrogen isotope results for animal samples.

Phase	Species	Number	$\delta^{13}\text{C}(\text{‰})$			$\delta^{15}\text{N}(\text{‰})$		
			Mean	SD	Range	Mean	SD	Range
1	Chicken	5	-16.0	2.3	-19.3 ~ -14.0	6.9	1.3	5.2 ~ 8.1
	Pig and dog	6	-13.9	1.5	-15.7 ~ -11.9	8.8	1.2	7.5 ~ 10.0
	Sheep and cattle	2	-19.3	0.2	-19.4 ~ -19.2	7.0	2.0	5.6 ~ 8.5
	Total	13	-15.5	2.5	-19.4 ~ -11.9	7.8	1.6	5.2 ~ 10.0
2	Chicken	11	-17.6	1.5	-19.2 ~ -14.1	8.6	1.8	6.6 ~ 13.2
	Pig and dog	7	-14.2	1.8	-16.6 ~ -12.3	9.3	1.1	8.2 ~ 11.2
	Sheep and cattle	1	-19.9	–	–	4.7	–	–
	Total	19	-16.5	2.4	-19.9 ~ -12.3	8.6	1.8	4.7 ~ 13.2
Undefined	Chicken	1	-17.0	–	–	8.2	–	–



animal bones of the phase. During phase 2, except for one large bird bone, the samples found are domestic animal remains including chickens, pigs, and sheep. The number of chicken bones is dominant among all animal bones in phase 2, with 328 fragments representing 15 individuals (58.89% by NISP, 68.18% by MNI). Although pigs and sheep account for about 20% (NISP) of all the animal remains, the number of pigs is twice that of sheep, according to the MNI data. In addition, there were 47 chicken bone fragments and one horse (*Equus ferus caballus*) bone excavated from Heishuiguo, which cannot be classified into definite cultural stages without cultural relics and funeral objects.

Animal isotopic data (Table 2 and Supplementary Figure S1; Figure 3) are usually used to reflect the strategies of ancestors to

feed and manage animals. The $\delta^{13}\text{C}$ value for chickens, pigs, and dogs implies that their diets (mixed C_3 and C_4 foods) did not notably change during either phase (mean $\delta^{13}\text{C}$ for chickens: -16.0 vs. -17.6, $p=0.189$; for pigs and dogs: -13.9 vs. -14.2; $p=0.721$). However, pigs and dogs ate more C_4 foods than chickens in both phases. In addition, the diet of domesticated herbivores showed heavy reliance on C_3 plants and did not change in either phase (mean $\delta^{13}\text{C}$: -19.3 vs. -19.9). The $\delta^{15}\text{N}$ value for pigs and dogs during the two phases is highest, which suggests they may eat a significant amount of animal protein included in the leftovers or feces of ancestors. The $\delta^{15}\text{N}$ value for chickens is similar to that of sheep and cattle in phase 1, while the $\delta^{15}\text{N}$ value for chickens is far higher than that of sheep and cattle in phase 2 (Table 2). The reason for this phenomenon may be that the chickens in phase 2 took a certain amount of animal protein, such as worms. All analyzed domesticated herbivore individuals exhibited $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values consistent with a diet based heavily on C_3 terrestrial sources.

The ^{14}C dating results of two animal bone collagen samples from the Heishuiguo Cemetery are shown in Table 3. The direct dating result of one piece of sheep bone is 25 cal CE~203 cal CE, approximately corresponding to the Eastern Han Dynasty. Another date from one piece of pig bone ranges from 165 cal BCE to 2 cal BCE, falling within the scope of the Western Han Dynasty.

5 Discussion

5.1 Animal utilization strategy during the Han Dynasty in the Hexi Corridor

The identification results of animal remains in the Heishuiguo Cemetery show that domestic chickens, pigs and

TABLE 3 Calibrated radiocarbon dates for animals from the HSG Cemetery.

Lab number	Sample Name	Dating material	¹⁴ C age BP	Calibrated age BCE/CE (95.4% prob.)
LZU19244	2018ZHM10	Sheep bone	1930 ± 20	25 cal CE~203 cal CE
LZU19245	2018ZHM16	Pig bone	2080 ± 20	165 cal BCE~2 cal BCE

sheep were the dominant species in the funerary animals of these Han Dynasty tombs according to both the NISP and MNI data (Table 1 and Figure 2). This suggests that feeding chickens, pigs, and sheep might play an import role in human livelihoods in the Hexi Corridor during the Han Dynasty, which can also be verified from writing records in historical documents, such as “*Shiji-Huozhizhuan*” (《史记·货殖列传》) and “*Jichurbo*” (《鸡出入簿》) written on Xuanquan bamboo slips (悬泉汉简) (Wang, 2017).

Carbon isotopic evidence suggests that pigs and dogs consumed mixed C₄ and C₃ foods (Figure 3), basically corresponding to human diets in the Heishuiguo (Li, 2021). According to historical records, both C₄ crops (e.g. foxtail millet and broomcorn millet) and C₃ crops (e.g. barley, wheat, and beans) were important plant subsistence in the Hexi Corridor (Gao, 2014), suggesting humans might have fed pigs and dogs with byproducts of these crops. Carbon isotopes of chicken bones are generally lighter than omnivorous livestock, but heavier than herbivorous livestock (Figure 3), suggesting chickens were also fed with a certain amount of C₄ foods, though most of them mainly consumed C₃ foods. Carbon isotopes of sheep and cattle bones are lighter than those of pigs, dogs, and chickens, suggesting those herbivorous livestock were likely free grazing in the Hexi Corridor area during the Han Dynasty. Nitrogen isotopes of different animal remains in the Heishuiguo Cemetery mostly overlap, although the values from omnivorous livestock exceed other buried animal remains to some extent (Figure 3), probably implying that some pigs and dogs consumed more animal protein. One sample from chicken showed a significantly higher δ¹⁵N value than other individuals, probably because it ate more worms in the wild. As recorded in the historical document “*Jiazhengfa*” (《家政法》), chickens like to dig into soil with their claws to find and eat invertebrates such as earthworms and grubs.

In addition to chicken, pig, and sheep remains, a few bones of cattle, dogs, and horses were identified from the Heishuiguo Cemetery (Figure 2). While the low proportion of them in buried animal assemblage may not indicate these livestock were insignificant in human livelihoods in the Hexi Corridor during the Han Dynasty. Here we only take the record of horses in historical documents as an example. The Hexi Corridor is the main traffic route connecting the Central Plains and the Western Regions from east to west. There is no doubt that horses were one of the most common domesticated animals used for animal power on the Silk Road. In more than

60,000 Hexi Han bamboo slips, including Dunhuang Han bamboo slips (敦煌汉简), Juyan Han bamboo slips (居延汉简), Jianshui Jinguang Han bamboo slips (肩水金关汉简), Diwan Han bamboo slips (地湾汉简), and Xuanquan Han bamboo slips (悬泉汉简), there are a large number related to horses (Chen, 2006; Zhou, 2013; Xu, 2016). These bamboo slips records involve the introduction of horses, the setting of hurdles, the distribution of feed, the management of post horses, the registration of passing horses, the treatment of sick horses, and the consumption or treatment of bone and meat after the death of sick horses. Evidence from the slips enables us to better understand not only the policies concerning horses at the time, but also the development of transportation, the postal service and the social economy in Hexi Corridor.

The buried animals can reflect the regional characteristics of the domesticated animals themselves. The animal remains assemblage of the Heishuiguo Cemetery is evidently different to that of contemporaneous cemeteries in other areas of China. Pigs, dogs, and chickens are the most common animals buried in Han Dynasty tombs in Central China, followed by sheep, cattle, deer, and rabbits. For example, the unearthed animal bones in Luozhuang Han Dynasty tomb in Jinan, Shandong Province, are mainly pigs, dogs, sheep, and rabbits (Fang, 2003; Liu, 2015). The nomadic people living in grasslands and plateaus mostly choose sheep, horses, and cattle as burial animals, while aquatic products, livestock, and poultry are more common in coastal areas (Wang et al., 1988; Dodson et al., 2013). Although the humans of the Heishuiguo are most likely to move from the Central Plains or to be descendants of the Han farmers, the Heishuiguo Cemetery does not share the same animal burial customs as the Central Plains. Chickens, pigs, and sheep are the dominant species at the Heishuiguo Cemetery, which indicates that this site may have integrated influences from the Central Plains and plateau areas. This is because pigs, dogs, and chickens are the most common animals buried in the Central Plains, while sheep is the dominant specie in plateau areas. This shows that the custom of sacrificing animals was diversified in different regions of China during the Han Dynasty. The Heishuiguo Cemetery had their own uniqueness, which may imply the fusion of cultures from different regions. It also corresponds with the characteristics that males in the Heishuiguo mainly came from the Yellow River basin and females mainly came from local areas (Xiong et al., 2022).

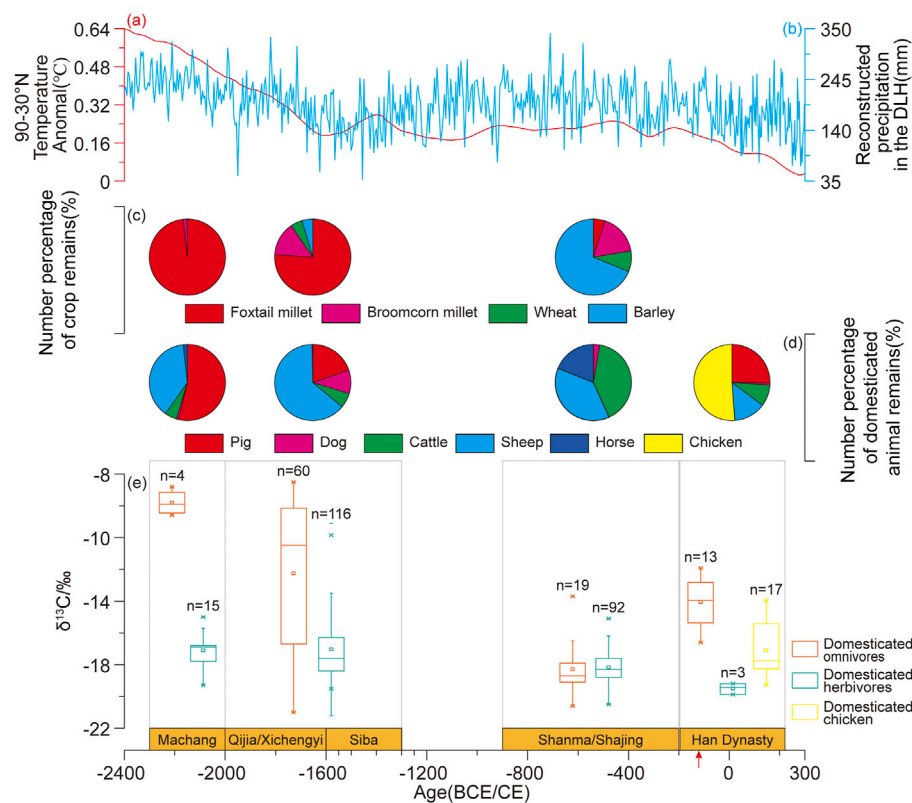


FIGURE 4

Transformation of animal utilization strategies during 2,300 BCE–220 BCE in the Hexi Corridor, compared with climate records, crop remains, domesticated animal remains and $\delta^{13}\text{C}$ values of animal samples from sites in the study area. (A) Northern Hemisphere (30°–90°) temperature record compared to 1961–1990 instrumental mean temperature (Marcott et al., 2013). (B) Reconstructed precipitation from DLH (Delingha, Yang et al., 2021). (C) Assemblages of crop remains from sites in the Hexi Corridor. (D) Assemblages of domesticated animal remains from sites in the Hexi Corridor. (E) $\delta^{13}\text{C}$ values of animal samples from sites in the Hexi Corridor. n: the number of isotopic data. Red arrow: the Han Empire controlled the Hexi corridor (121 BCE).

5.2 Diachronic change of animal utilization strategies from the late Neolithic to the Han Dynasty in the Hexi Corridor

From the late Neolithic Age to the Han Dynasty (~2,300 BCE–220 CE), the species of livestock raised by human being in the Hexi Corridor gradually diversified (Figure 4D). During the period of the Machang culture (~2,300–2,000 BCE), Qijia/Xichengyi culture and Siba cultures (~2,000–1,300 BCE), the ancestors raised only pigs, dogs, cattle, and sheep. During the Shanma/Shajing culture (~900–200 BCE), ancestors began to raise horses. In Han Dynasty (202 BCE–220 CE), domestic chickens were also added to the large family of livestock in the Hexi Corridor. Thus, it was also not until the Han Dynasty that the Hexi Corridor really saw the prosperity of the six animals (六畜兴旺) in *Guanzi · Mumin* (《管子·牧民》). Pigs and dogs originated from the Central Plains of China, together with cattle and sheep domesticated in the West Asia, first appeared

at the same time in the Mozuizi site of Machang culture in the east of the Hexi Corridor (Lv and Yuan, 2018). Their remains were unearthed in large numbers at the Lijiageleng site of the Qijia culture, the Huoshiliang and Ganggangwa sites of the Xichengyi culture, and the Xichengyi site, the Donghuishan cemetery, the Ganguya cemetery and the Sanbadongzi site of the Siba culture in the middle and west of the Hexi Corridor (Qi, 1998; Fu, 2016; Song et al., 2016; Yang et al., 2019a; Ren et al., 2022). The number of horse remains unearthed in the Hexi Corridor is small, and they were found during the archaeological investigation of the Gudongtan and Zhaojiashuimo sites of Shanma culture (Yang et al., 2019a). The results of zooarchaeological identification show that the Han tombs of the Heishuiguo Cemetery contained the remains of pigs, dogs, cattle, sheep, horses, and chickens. In addition, according to the records of preserved documents and Han bamboo slips (e.g. Juyan Han bamboo slips), camels and donkeys were also important domestic animals used by the ancestors of the

Hexi Corridor in Han Dynasty (Wang, 2017; Ge, 2018; Zhang, 2020).

From the Machang culture period to the Shajing/Shanma culture period, the proportion of pigs in the livestock combination in the Hexi Corridor gradually decreased, and the importance of cattle, sheep, and horses obviously increased (Figure 4D). However, to the Han Dynasty, the number of chickens and pigs increased rapidly, surpassing that of cattle and sheep (Figure 4D). During the Machang culture period, pigs were the main livestock, followed by sheep and cattle (Lv and Yuan, 2018). During the Qijia/Xichengyi, Siba, and Shanma/Shajing culture periods, the proportion of cattle and sheep of domestic animals gradually increased, while the proportion of pigs declined rapidly until it disappeared (Qi, 1998; Fu, 2016; Song et al., 2016; Yang et al., 2019a; Ren et al., 2022). To the Han Dynasty, the proportion of cattle and sheep in domestic animals decreased sharply, while the proportion of chickens and pigs increased. Notably, chickens and pigs accounted for more than three-quarters of the total livestock assembly. The evolution history of animal resource utilization strategies in the Hexi Corridor before the Han Dynasty reflects a process of raising pigs related to agriculture from prosperity to extinction, and the grazing of cattle and sheep from weak to strong. After the Han Dynasty, due to the strategy of the imperial government of the Han Dynasty, the utilization of animal resources was influenced by the Central Plains, and pigs reappeared and chickens dominate the whole livestock assemblage.

The research results of carbon and nitrogen stable isotopes of animal remains from the late Neolithic to the Iron Age (~2,300–202 BCE) in the Hexi Corridor show that the carbon isotope values of domestic omnivores were gradually decrease (Figure 4E). This suggests that they changed from a nearly pure C_4 diet to a mixed C_3/C_4 diet (Atahan et al., 2011; Yang et al., 2019; Ma et al., 2021; Vaiglova et al., 2021; Qiu et al., 2022), and that the consumption of C_3 crops (e.g., barley, wheat) by omnivores was gradually increasing (Dong G et al., 2020). It shows that the breeding strategies of the ancestors of Hexi Corridor for pigs and dogs have changed significantly in different periods. In addition, domestic herbivores gradually changed from a mixed C_3/C_4 diet to a primarily C_3 diet during this period (Atahan et al., 2011; Yang et al., 2019a; Ma et al., 2021; Vaiglova et al., 2021; Qiu et al., 2022), indicating that the feeding and management strategies of cattle and sheep may also have changed. To the Han Dynasty, the relatively enriched $\delta^{13}C$ values of chickens, pigs, and dogs suggested that they had a mixed C_3 and C_4 diet, so these animals may have increased consumption of C_4 crops (i.e., foxtail millet and broomcorn millet) and/or their byproducts again.

5.3 Influencing factors for the transformation of animal utilization strategies from the late Neolithic to the Han Dynasty in the Hexi Corridor

5.3.1 The impact of climate change

The increased cattle and sheep in the Hexi Corridor during the Qijia/Xichengyi, Siba (~2,000–1,300 BCE), and Shanma/Shajing (~900–200 BCE) culture periods compared to the Machang culture period (~2,300–2,000 BCE) broadly coincided with the forest degradation and grassland expansion. The tree ring-based precipitation evidence from Qilian Mountain and the oxygen isotope record of stalagmites clearly indicate a weakened Asian summer monsoon and reduced monsoonal precipitation during this period (Figure 4B, Yang et al., 2021). Furthermore, the temperature records from the Northern Hemisphere imply a cooling trend at the same time (Figure 4A, Marcott et al., 2013). The cooling and drier climate resulted in the forest degradation and grassland expansion, as suggested by pollen records (Zhao and Yu, 2012), thereby facilitating the expansion of pastoralism in this region. In addition, the southward migration of Eurasian steppe nomads (e.g., Scythians) may also have promoted the development of pastoralism in the Hexi Corridor during this period (Shao and Yang, 2006; Han, 2008; Frachetti, 2012; Han, 2012; Yang et al., 2016; Dong G et al., 2020). The increased C_3 -crops consumption by domesticated omnivores, as suggested by the depleted $\delta^{13}C$ values, coincides with the increased proportion of barley and wheat unearthed from the archaeological sites in the Hexi Corridor (Flad et al., 2010; Fan, 2016; Zhou et al., 2016; Jiang et al., 2017; Yang et al., 2019a). The expanded planting scale of barley and wheat probably happened because these crops were more tolerant to the cooling climate than millets (Chen et al., 2015; Dong G et al., 2020).

5.3.2 The impact of geopolitical change

In Han Dynasty, animal utilization strategies of people in the Hexi Corridor were significantly different from those of prehistoric times. The diverse livestock reflects that both raising domesticated omnivores (e.g., chickens and pigs) and grazing domesticated herbivores (e.g., sheep, cattle, horses) played important roles in the economic system. The increased raising of chickens and pigs in the region most likely resulted from geopolitical changes. The Han Empire governed the Hexi Corridor from ~100 BCE, and fought frequently with the Huns (GPICRA, 2019). In order to consolidate the border area of the Han Empire, the government implemented a military reclamation system and a large number of males migrated to the Hexi Corridor (Yang, 2010; GPICRA, 2019). These immigrants also brought the customs of the Central Plains into the Hexi Corridor, i.e., the raising and burial of chickens and pigs (Gao, 2010; Liu, 2010). The abundant cattle and sheep remains show that pastoralism was still a crucial economic

strategy (GPICRA, 2019). This may be related to the wide distribution of grasslands and deserts in the region. The diverse animal utilization strategies clearly reflect the integration of the farming culture from Central China and nomadic culture from Eurasian grassland into the Hexi Corridor. In addition, geological records and historical documents suggest a relatively warm and humid climate during the Han Dynasty (Ljungqvist, 2010; Ge et al., 2013; Li et al., 2021b), which was conducive to the development of agriculture and animal husbandry.

Our results suggest that changes in animal utilization were mainly passive adaptations to climate change in the Hexi Corridor during the prehistoric period, and that migration to the border was the main influencing factor on animal utilization strategy in the region after the Han Dynasty. This study is important for understanding important scientific issues such as prehistoric and Han Dynasty animal utilization strategies in the Hexi Corridor, human-environment interactions, and communication and mutual appreciation among different cultures and populations.

6 Conclusion

The study is important for understanding significant scientific issues such as animal utilization strategies and human-environment interactions during the prehistoric times and the Han Dynasty in the Hexi Corridor. Our zooarchaeological and isotopic study in the Heishuiguo Cemetery provides valuable new data to reconstruct animal utilization strategies in the Hexi Corridor during the Han Dynasty. The results suggest the significance of domestic chickens, pigs, and sheep in livelihoods was likely much higher than other livestock including cattle, horses, and dogs. Humans may have fed pigs and dogs plenty of C_4 foods (probably millets or their byproducts), fed chickens a small amount of C_4 foods with evident difference, while cattle and sheep mainly ate C_3 plants.

In contrast to previous studies in the Hexi Corridor, the trajectory of animal utilization strategies change from the late Neolithic to the Han Dynasty can be preliminarily outlined. The importance of herbivorous livestock in animal utilization strategies and C_3 foods in fodders gradually increased in the Hexi Corridor during ~2,300–200 BCE. However, the trend changed in Han Dynasty, with the chickens, pigs, and sheep becoming the most important livestock, and the significance of C_4 plants in fodders being notably enhanced. This was mainly due to the introduced customs of immigrants from the Yellow River valley of north China, occurring after the Han Empire controlled the Hexi Corridor.

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

The study was designed by XL, LR, WW, ML, and LD conducted field works and sample collection. LR, WW, and MM completed experiments and data correction. WW, XL, and LR analyzed data and designed the figures. XL, WW, MM, ML, LD, YY, GC, and LR wrote the manuscript. All authors discussed the results and commented on the manuscript.

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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.

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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.2022.1064803/full#supplementary-material>

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A score of bioavailable strontium isotope archaeology in China: Retrospective and prospective

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Bioavailable strontium isotope analysis was proposed for prehistoric human ecology almost 40 years ago and rapidly became one of the most important tools to trace past migratory behaviours. Since its first introduction to China in 2003, this method has greatly improved our understanding of migrations on individual- and community-scales over the past 20 years. This paper summarizes the current state of knowledge regarding identifying non-locals, utilizing animal resources, and strontium isoscapes for China based on empirical data. By incorporating additional baseline data, we updated and extended the current bioavailable strontium isoscape for China and Southeast and South Asia. These data will shed new light on intercultural communications and the spread of customs and technologies. In the future, strontium isotope analysis will involve an integrated provenancing system along with multiple approaches such as various isotopes and different modellings. Correspondingly, the existing isoscape needs to improve its spatial resolution and predictive accuracy to source the non-local archaeological biological remains. Furthermore, advances in analytical techniques allow us to reconstruct lifetime mobility of animals and humans at high temporal resolution.

KEYWORDS

bioavailable strontium isotope, migration, animal resource utilizations, isoscape, Chinese archaeology

Introduction

Migrations are closely related to almost all aspects of archaeology, including but not limited to the dispersals of *Homo sapiens*, the origin of civilizations, and intercultural connections. In 1985, Jonathon E. Ericson first introduced strontium isotope analysis into archaeology as a methodological approach to characterize specific catchment areas and trace the sources of various materials. He noticed that significant variation in strontium isotope depends on age and type of rock and, characteristic of local geology, strontium isotopes pass through the food chain with negligible fractionation, thus providing a promising method to reconstruct migration/residence pattern for ancient individuals (Ericson, 1985).

In the following several years, the methods for removal of non-biological strontium (Sillen, 1986, 1989; Price et al., 1992), evaluation of preservation condition (Price et al., 1992), and determination of local range (Price et al., 2002; Bentley et al., 2004), were gradually established by different groups, making strontium isotope analysis practicable in the geochemical lab around the world. Over the past several decades, strontium isotope analysis has become the most fundamental approach to understand past migratory behaviours and the connections between different communities.

In 2003, ZHANG Xuelian first introduced the principle of strontium isotope analysis in archaeology in China (Zhang, 2003). Since then, Chinese scholars have released strontium isotopic data from more than 40 sites and discussed topics from the identification of non-local individuals and subsistence strategies to the potential provenance of biomaterials in broader contexts. Partly because most results were published in our native Chinese, little international attention was paid to this rapidly developing subject in China, in contrast to the incredible achievements of strontium isotope and the promising future of Chinese archaeology.

This paper reviews the main achievements in strontium isotope archaeology across China. There are several parts to our article. The first section presents an overview of the early stage of strontium isotopic analysis in China, spanning from 2003 to 2007. The second section details the advances of bioavailable strontium isotope archaeology in three themes. A third section concludes with recommendations for applying this method in future investigations.

Pioneering introductions and pilot studies in China

It is worth mentioning that Jonathon E. Ericson reported the earliest bioavailable strontium isotopic analysis to Chinese samples. In 1986, he submitted an abstract to the Geological Society of America (Ericson, 1989), in which he compared the $^{87}\text{Sr}/^{86}\text{Sr}$ values of Pleistocene *Gigantopithecus* (extinct) tooth enamel and cave bear femur both collected from the *Gigantopithecus* Cave, Liucheng County, Guangxi, South China. He found significant differences between them and tentatively attributed the difference to diagenesis or diets. Unfortunately, this work was ignored by Chinese academic communities. Even before we wrote this, the first author personally communicated with several potentially relevant researchers and failed to get more details.

The Chinese debut of strontium isotope analysis was a review of dietary research authored by ZHANG Xuelian, who works for the Institute of Archaeology, Chinese Academy of Social Sciences (IA-CASS). In the paper on *Acta Anthropologica Sinica* 人类学学报, for the first time in China, the emerging strontium isotope

analysis was described as “a tracer of human habitats which is playing an increasing role in archaeology” (Zhang, 2003).

Later that year, QIAN Junlong translated and published an abstract of a case study (Freestone et al., 2003), in which strontium isotope was used to investigate Near East glass production. The strontium isotope technique was then applied to source traded ceramics in China (Li et al., 2005, 2006), in cooperation with geochemists from Queensland University, Australia.

Strontium isotope analysis is an instrument-dependent laboratory technology. As early as 2004, a team from the University of Science and Technology of China (USTC) established an analytical method for $^{87}\text{Sr}/^{86}\text{Sr}$ analysis to discriminate the production site of ancient pottery in their lab (Zhang et al., 2004). Slightly later, geochemists at Jilin University independently developed a pretreatment method for analyzing the strontium isotopic composition of ancient human bones (Zhou et al., 2005). These efforts demonstrated the accessibility of $^{87}\text{Sr}/^{86}\text{Sr}$ analysis for archaeological remains in China.

In 2007 a doctoral candidate YIN Ruochun from USTC made a thorough and detailed review on the application of isotopic strontium in archaeology (Yin and Zhang, 2007), covering strontium geochemistry, principles of strontium isotopes as a geologic tracer, cases for identifying the local range and for migration in Americas and European. Besides, they paid sufficient attention to sample pretreatment; for example, the chemical treatments should be processed in an ultraclean lab.

Progresses over the latest twenty years

Since YIN Ruochun reported the first bioavailable strontium isotopic data for Chinese archaeology in 2008 (Yin et al., 2008), bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$ analyses have been conducted for more than 40 sites across the country in the past 15 years (Figure 1). These works covered various topics with the foci on migratory behaviours of populations and individuals, exploitations of faunal resources, and the development of strontium isoscape.

Identification of non-local individuals

The Jiahu site, situated at the south Central Plains, is one of China's most critical early Neolithic sites. Here, YIN Ruochun opened the door of strontium isotope archaeology in China. During his doctoral studies at USTC, Yin and his supervisors first established the local range using five teeth enamel $^{87}\text{Sr}/^{86}\text{Sr}$ values of archaeological pigs. They successfully identified five non-locals from 14 human individuals (Yin et al., 2008). This is the first time for Chinese scholars to analyze strontium isotopic signals for archaeological biological remains following the protocols

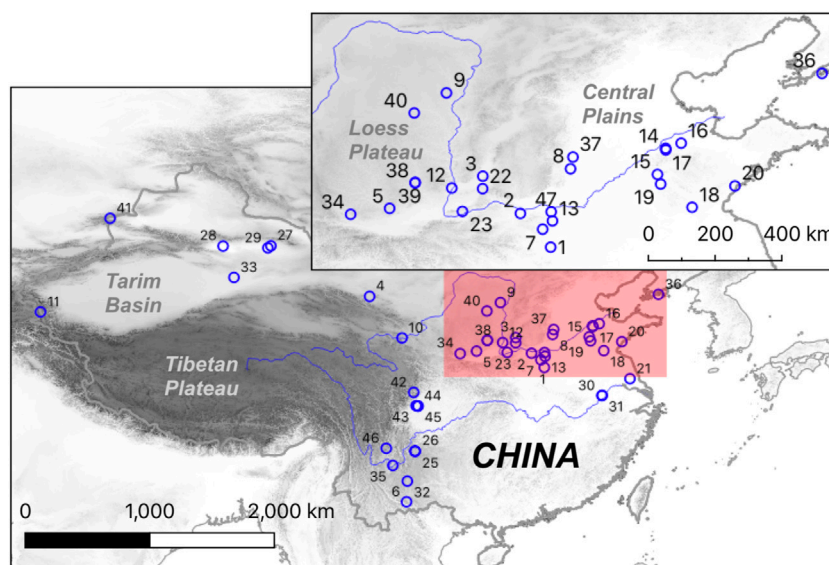


FIGURE 1

Archaeological sites with bioavailable strontium isotope data in China and the sites were presented in the order of year of publication. 1 Jiahu (Yin et al., 2008); 2 Erlitou (Zhao et al., 2011a; 2012b); 3 Taosi (Zhao et al., 2011b; Zhao and He, 2014); 4 Heishuiguo (Zhao, 2012); 5 Zaoshugou (Lan, 2017); 6 Mayutian (Zhang et al., 2014); 7 Wadian (Zhao et al., 2012a; Zhao and Fang, 2014); 8 Yinxu (Zhao et al., 2015); 9 Shimao (Zhao et al., 2016a); 10 Lajia (Zhao et al., 2016b; Zhao et al., 2018); 11 Jirzankal (Wang et al., 2016); 12 Liangdaicun (Chen, 2012); 13 Wangjinglou (Zhao et al., 2018); 14 Jiaojia; 15 Dawenkou (Fang, 2018); 16 Dinggong; 17 Chengziya; 18 Guangzhuang; 19 Yinjiacheng; 20 Liangchengzhen; 21 Jiangzhuang; 22 Zhoujiazhuang; 23 Qingliangsi (Wu, 2018; Wu et al., 2019b; Zhang et al., 2021); 24 Hongyingpan; 25 Yinzitan; 26 Jigongshan (Zhang et al., 2018; Zhang et al., 2022a); 28 Wubu; 28 Jiayi; 29 Aisikexia'ernan (Li, 2019; Wu et al., 2021); 30 Lingjiantan; 31 Weigang (Zhao et al., 2019); 32 Shamaoshan (Wu et al., 2019a); 33 Lop2015 cemetery 1 (Wang et al., 2020); 34 Xuechi (Tang et al., 2020, 2022); 35 Houzidong (Wang, 2020); 36 Xiaozhushan (Zhao et al., 2021); 37 Nancheng (Hou et al., 2021); 38 Shijiahe; 39 Zhaitouhe (Liu, 2021); 40 Jiadamao (Zhao et al., 2022); 41 Adunqiaolu (Cong et al., 2021); 42 Yingpanshan; 43 Hongqiaocun; 44 Jinsha; 45 Shi'erqiao; 46 Gujiabu (Lin et al., 2022); 47 Zhengzhou Shang City (Fang et al., 2022).

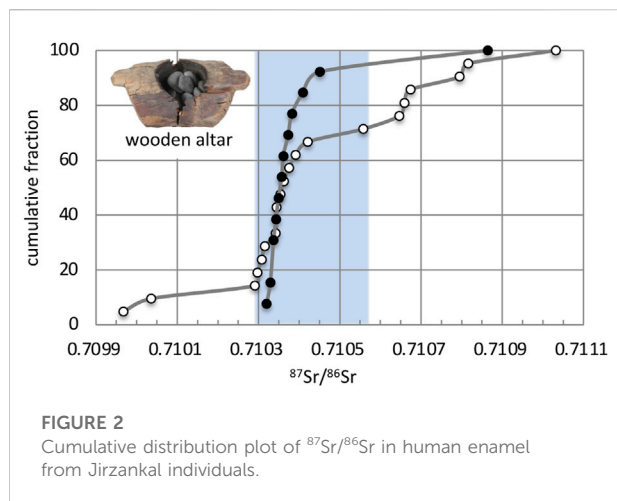
recommended by T. Douglas Price et al., 2002. These results showed the potential of strontium isotope research on migration and greatly encouraged subsequent research.

A latest Neolithic site, Taosi, was situated in south Shanxi Province. At the site, an internal rammed-earth wall separated the elite's residential and ceremonial areas from those inhabited by commoners, signifying a stratified society that occurred during the interval of 4,500–3900 BP. An archaeometry team led by ZHAO Chunyan from IA-CASS established the local $^{87}\text{Sr}/^{86}\text{Sr}$ range using the signals of archaeological domestic pigs and primarily concluded that a part of domesticated sheep (two out of five) and cattle (two out of four) were imported into the site (Zhao et al., 2011b). Similar conclusions also arrived from a contemporary Wadian site located at the upper reaches of the Huaihe River. Referring to the local range inferred from the signals of mice, Zhao et al. (2012a) identified several non-local pigs, sheep and cattle. Combined with zooarchaeological evidence, the authors argued that Wadian occupants imported sheep and cattle from outside and hunted wild pigs nearby. Based on faunal isotopic analysis, the authors also worked on the human remains and revealed high proportions of immigrants at the two sites. Specifically, 14 out of 21 analyzed Taosi human individuals (Zhao and He, 2014) and all five analyzed Wadian

individuals (Zhao and Fang, 2014) fell out of the local range determined by pig and mouse, respectively.

On the Chinese Loess Plateau, the latest Neolithic Shimao site, contemporary with the sites of Taosi and Wadian, showed a different scenario of livestock provenance. Compared with the archaeological pigs, strontium isotopic signals identified a single non-local sheep from the 19 analyzed individuals of cattle and sheep. The differences in livestock provenances between Shimao and Taosi/Wadian are partly related to their locations. The former was situated in the steppe zone, making it much easier to access herds. This explanation was reconfirmed by another slightly older site, Jiadamao (4,500–4,300 yrBP (Zhao et al., 2022), from the Loess Plateau.

The Erlitou Site marked the earliest Bronze Age urban society, and the archaeological culture existed in the Yellow River valley from approximately 3,900 to 1,500 yrBP. Zhao and her colleagues determined the local $^{87}\text{Sr}/^{86}\text{Sr}$ range of 0.71190–0.71226 using ten pigs' enamel signals, and further identified seven out of 14 sheep and two out of seven cattle non-locals (Zhao et al., 2011a). For the Wangjinglou site of the Erlitou period, $^{87}\text{Sr}/^{86}\text{Sr}$ signals identified five non-local cattle out of nine analyzed individuals (Zhao, 2018). Both Erlitou and



Wangjinglou would imply an extensive exchange network of livestock during the very early stage of Chinese Bronze Age.

Yinxu, ca. 1,300–1,046 yrBP, was the site of the first well-documented capital in ancient China, famous for the earliest records of Chinese writing systems and the earliest domestic horses in China (Kikuchi et al., 2019). Among the horses, five individuals (5/10) fall outside the local range of $^{87}\text{Sr}/^{86}\text{Sr}$ (0.71132–0.71174) defined by nine archaeological cattle bones from the cemetery, strongly suggesting imported horses to the Central Plains during the late Shang period.

Besides these critical sites mentioned above, several sites from the Chinese frontiers also revealed valuable information about the ancient migratory pattern. In the southwest corner of China, the highest-status individual from Mayutian showed a significantly lower $^{87}\text{Sr}/^{86}\text{Sr}$ value than others, suggesting a distinct geographic origin (Zhang et al., 2014). This is the very first archaeological paper about Chinese bioavailable strontium isotope in international journals.

Close to the China-Tajik border, ten immigrants out of 34 individuals from the Jirzankal cemetery, ~2,500 yrBP, were identified using strontium isotopic approach, where the local range was constrained by 12 ovicaprine bones. The immigrants were partly accompanied by exotic musical instruments, harps, and oriental silk textiles, suggesting active long-distance exchanges across the Eurasian continent (Wang et al., 2016). Besides, the Jirzankal cemetery attracted considerable attention partly due to the unique wooden altars (Wu and Tang, 2016), which were once used for inhaling cannabis fumes (Ren et al., 2019). When placing the 13 individuals buried with the altar into the context of strontium isotopic signals, we found that only one individual from the grave M50 fell out of the local range, suggesting that the practice of inhaling cannabis more likely rooted in the local settlers on the Pamir Plateau, instead of the exotic convention (Figure 2).

The shadow represents the local range of $^{87}\text{Sr}/^{86}\text{Sr}$ for the site. Black dots denote individuals buried with altars, and circles show individuals without an altar. The variance in $^{87}\text{Sr}/^{86}\text{Sr}$ is significantly larger for the individuals without altar ($n = 21$) than for individuals with altar ($n = 13$), by an F test ($p < 0.001$ without outliers M50, and $p < 0.05$ adding the outliers).

Exploitations of faunal resources

As a consistent and essential part of the subsistence of ancient society, the circulation networks of domestic animals are one of the critical aspects. Strontium isotopic analysis can identify and source non-local animals in ancient zooarchaeological assemblages, providing valuable information on mobility across China.

At the lower reaches of the Yellow River, animal remains from the Dinggong site were subjected to strontium isotope analysis to detect their potential provenances (Wu et al., 2018). The data indicated that the $^{87}\text{Sr}/^{86}\text{Sr}$ values of the animals were different on taxa, i.e., freshwater bivalves had highly variable $^{87}\text{Sr}/^{86}\text{Sr}$ values. In contrast, freshwater fishes showed more uniform $^{87}\text{Sr}/^{86}\text{Sr}$ signals, and the terrestrial mammals and reptiles had the variability in between. Taking the regional geological background, the authors argued that the residents of the Dinggong Site obtained freshwater fish nearby the site and fetched bivalves from the mountainous area.

More importantly, the authors got a similar $^{87}\text{Sr}/^{86}\text{Sr}$ value from an osteoderm of Chinese alligator found in the site and made a preliminary discussion about indigenous or extraneous alligators (Wu et al., 2018). Recently the authors conducted multiple isotope analyses of the alligator remains from three sites in the Yellow River basin (Zhang et al., 2021), including Qingliangsi, Dinggong, and Yinjiacheng sites. Both $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$ values of the alligator osteoderms were consistent with the background signals where the site was situated. These data further supported the indigenous alligators during the Late Longshan Period, appealing more attention to the paleoenvironment reconstruction and the relationship between humans and the Chinese alligator. Using a similar approach, ZHAO Chunyan worked on the livestock supply during the Erlitou period (Zhao, 2018). Based on the local range defined by pigs, high proportions of immigratory sheep were found from the Erlitou site, while more immigratory cattle were supplied to the contemporary Wangjinglou site. Those results suggested complicated livestock supply networks for different sites during the early Chinese Bronze Age.

On the Yunnan-Guizhou Plateau, the sites of Jigongshan, Hongyingpan, and Yinzitan established an integrated chronology of the Yelang, a minority monarchy in ancient southwest China, from the Bronze Age to the Early Iron Age (Zhang, 2014). Horse remains were excavated from the Jigongshan and Yinzitan sites, allowing for understanding horse trading of the Yelang

civilization using strontium isotope analysis (Zhang et al., 2018). The Jigongshan site, the earliest Bronze Age remains (1,300–800 BC) in the area, is parallel with the period of the Late Shang to the Early western Zhou dynasties in central China, when horses were just being used extensively in war and transportation. There are three out of seven horse tooth samples with the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios outside of the range of regional geological background. Among the three outliers, two values are significantly higher, possibly indicating that the horses come from places outside the karst areas. The Yinzitan site covers the Later Warring States to the former Han period when domestic horses were used extensively in China. Three out of four horses from the site showed $^{87}\text{Sr}/^{86}\text{Sr}$ ratios outside of the regional signals. The authors noted that the outlying horses from Jigongshan and Yinzitan sites shared similar $^{87}\text{Sr}/^{86}\text{Sr}$ values and proposed that the ancient people obtained the horses through trade with the Dian and Zuo tribes. This work shed lights on the interregional horse trade in Southwest China (Zhang et al., 2018; Zhang and Zhang, 2021).

From the Early Neolithic to the early historical periods, the pattern of animal exchanges evidenced by strontium isotopes offered detailed insights into circulation networks. As a preliminary analysis, Wang and her colleagues employed previously reported strontium isotopes measured in tooth enamel from domesticated animals (cattle, sheep/goats, horses, and dogs) in 13 sites to investigate animal movement and circulation across the Yellow River Basin over extended periods (Wang et al., 2021). They found that a few strontium isotope ratios outliers for animals from large Late Neolithic sites, suggesting the initial expansion of animal circulation systems and an increase in the proportion of animals originating from various regions from the Late Shang period until the Western Zhou Dynasty, accompanying an increasing degree of social complexity.

Most recently, we reported 29 sacrificial animal enamel $^{87}\text{Sr}/^{86}\text{Sr}$ values from a Chinese imperial ritual site, Xuechi, and clearly demonstrated that no sacrificial animals were raised in the vicinity of the site (Tang et al., 2022). Comparison of $^{87}\text{Sr}/^{86}\text{Sr}$ values from Xuechi with other six sites on the Chinese Loess Plateau showed an increasing trend in variation of faunal $^{87}\text{Sr}/^{86}\text{Sr}$ values from the latest Neolithic to imperial periods, in concordance with the territorial expansion model for state formation (Spencer, 1998, 2010). These tentative attempts were broadening the reach of strontium isotope archaeology.

Strontium isoscape for China

The field of isotope sourcing is rapidly expanding and forming the realm of data science and community efforts to make modelling products widely accessible (Bowen and West, 2019). To estimate the geographic origins of immigrants, a pre-requisite is a bioavailable isoscape of the region. Like the global

practices, the development of strontium isoscape in China has lagged behind hydrogen, oxygen, or carbon isotopic systems, partly due to the challenging, relatively expensive, instrument-dependent $^{87}\text{Sr}/^{86}\text{Sr}$ analysis.

Until recently, the first regional bioavailable strontium isoscape was generated for the Tarim Basin, the hub on the Silk Roads connecting eastern and western civilizations. Using 73 river samples, the bioavailable strontium isoscape across the Tarim Basin and the neighbouring area was established by the kriging interpolation method (Wang et al., 2018). The basin exhibits a strong south-north gradient in $^{87}\text{Sr}/^{86}\text{Sr}$ values. The rivers draining the northern regions are less radiogenic than the rivers south of the basin. The rivers from the north have a mean value of 0.7105 ± 0.0007 ($n=25$), whereas the rivers south of the Tarim Basin have a mean value of 0.7118 ± 0.0008 ($n = 29$). Besides, an overlap exists in between. Using this map, seven out of 27 individuals were successfully identified as the non-locals from a Han-Jin cemetery in the Lop Nur (Wang et al., 2020).

Collected from previous publications and supplemented by a targeted collection in China, the authors (Wang and Tang, 2020) compiled a bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$ dataset for China. After internal normalization for instrumental mass-dependent isotopic fractionation and removal of outliers using the Anselin's Local Moran's I statistic, the very first large-scale bioavailable strontium isotope map of China was generated using the kriging interpolation based on 1872 samples. The $^{87}\text{Sr}/^{86}\text{Sr}$ dataset shows considerable heterogeneity and has a distinct strontium isotope distribution based on the characteristics of geological and isotope data in China. Evidenced by integrated geochemical data, the biosphere $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are primarily consistent with the underlying geological bedrocks. Besides, poor matches are also observed in regions with high relief, eolian deposits and complex geology, mainly due to the insufficient sampling density. This strontium isoscape laid a solid ground for studying past migrations in China.

Based on the detailed $^{87}\text{Sr}/^{86}\text{Sr}$ baseline map, strontium isotope analysis on ancient human and animal remains will improve our understanding the role of human and animal migrations in shaping history. Reassessment of spatial variability of the bioavailable strontium isotopes for China using the geographic detector model (GDM) (Zhang et al., 2022b) revealed that the watershed factor explains 50.35% of the spatial variation of bioavailable strontium isotopes, while, in a descending order, the climate, terrain, geology, and soil in China explained much less. Furthermore, the GDM suggested that the non-linear interactions between watershed and geology explained 59.90% of spatial variation in bioavailable Sr isotopes. These results indicated that the natural processes still dominate the bioavailability of strontium isotopes in China and required a proper proxy to establish the local range of $^{87}\text{Sr}/^{86}\text{Sr}$ for a specific site.

It must be pointed out that past migratory behaviours were not limited to modern administrative boundaries. The

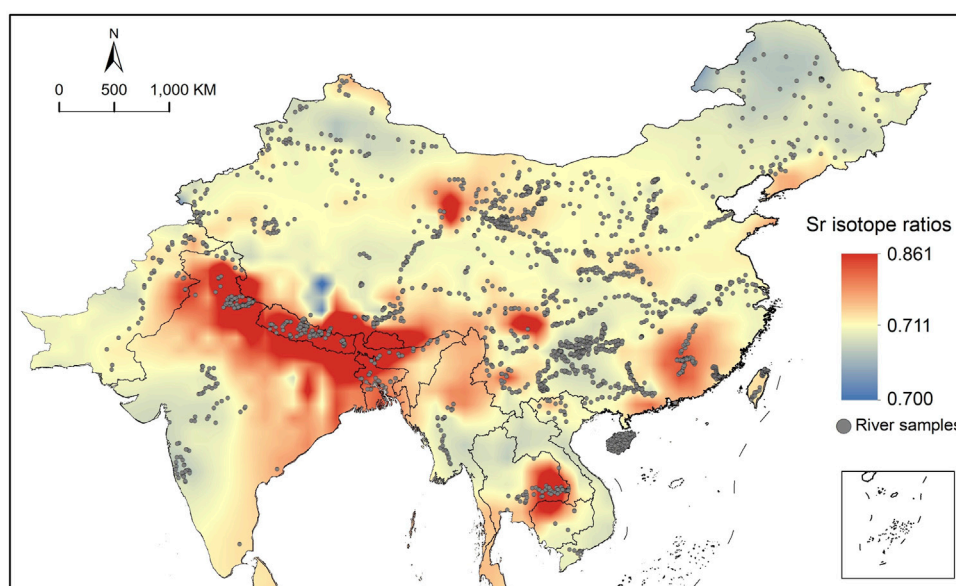


FIGURE 3

The updated bioavailable strontium isoscape for China and its neighbouring area. The dataset consists of 3,158 water samples, and details can be available from the [Supplementary File](#).

bioavailable strontium isoscape presented above mainly resulted from the ease of data collection rather than the actual ancient migration. Based on the compilation of newly released river water $^{87}\text{Sr}/^{86}\text{Sr}$ data and previous datasets (Bataille et al., 2020; Wang and Tang, 2020), we update the current bioavailable strontium isoscape for China and neighbouring regions using the kriging interpolation (Figure 3). These data will provide a helpful reference set for archaeological, forensic and environmental studies, especially for studying the interconnections among east, south, and southeast Asia.

Summary and prospects

Strontium is an element in rocks and is released into water and soils by weathering, then incorporated into the mineralized tissue of organisms as substitution of calcium. Due to the ratio $^{87}\text{Sr}/^{86}\text{Sr}$ independent to physiochemical and biological processes, this bioavailable strontium isotopic ratio reflects the local geologic background, making it the most helpful tracer of provenances. The surge in bioavailable strontium isotope research over the past 20 years is yielding new insights into Chinese archaeological and historical studies. For the first time, it enabled direct estimation of individual migration and laid the ground for evaluating mobility through geospecific isotope ranges. Our present work provides an overview of the outstanding achievements of bioavailable strontium isotopes in Chinese

archaeology, ranging from migratory individuals, and animal resource exploitation, to strontium isoscape for China.

As an emerging and promising field in China, several critical issues for bioavailable strontium isotope study remain open and need more consideration in the future.

Quality control of measurement

Thermal ionization mass spectrometry (TIMS) and multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) have been widely used to determine strontium isotopic composition in China. To determine accurate and precise $^{87}\text{Sr}/^{86}\text{Sr}$ values, utmost care must be practiced from sampling, sample pretreatment, and optimization of the instrument, to mass bias correction during measurements. Correspondingly, the protocol of pretreatment, the reference material and its reproducibility during the measurements, as well as the correction method of mass fractionation, need to be clarified in data reports. For the burgeoning laser ablation MC-ICP-MS (LA-MC-ICP-MS) technique, matrix-matched calibration standards are essential to generate results comparable to TIMS and MC-ICP-MS, especially for samples with low-strontium concentration, such as teeth enamel of humans and mammals (Wang and Tang, 2019). If effective, the LA-MC-ICP-MS method can generate $^{87}\text{Sr}/^{86}\text{Sr}$ data with a spatial resolution data better than $50\mu\text{m}$, allowing for

reconstruction of high temporal resolved life history (e.g., Wooller et al., 2021; Miller et al., 2022).

Better isoscapes

The potential for precise tracking of migratory behaviour largely depends on the accuracy of the isoscape produced using empirical data and/or geostatistical models. Like other places around the world, these models and data are always imperfect; thus, continued improvements in data and models are needed to improve the scope and quality of isoscape. Even in the updated version presented above, bioavailable strontium isoscape for China and the neighbouring areas is still to be improved continuously. Furthermore, the isoscape for China generated mainly on water samples from streams and lakes does not meet the demand for high-resolution tracking; various materials (e.g. plants and low-mobility animal skeletons) and robust models are required for an isoscape of higher spatial resolution. Recently, researchers combined the sampling data and machine learning methods such as random forest regression models to draft the large-scale bioavailable strontium isoscape for Europe (Bataille et al., 2018; Hoogewerff et al., 2019) and even the global scale (Bataille et al., 2020), which greatly improved the predictive power of isoscapes. However, the accuracy of these models is highly relied on the sampling data and the global Sr isoscape shows extremely poor performances in China due to the lack of baseline data in that study. In addition, a web-based portal to bioavailable strontium isoscape is convenient for relevant researchers.

Whether “local” or “non-local” according to strontium isotope method is a relative concept. When tracing animal and human migrations, researchers should give a clear description of the migration scale that they discuss, i.e. a local scale or a large scale (how large?). In this case, the choice of baseline types and the extent of sampling becomes particularly important. For example, the selection of the baseline samples must take into account the research questions and research objects. Deep ground water, for example, is not suitable to establish baselines as it is not likely to form a significant part of strontium intake by humans and terrestrial animals.

Individual history of migration

A great tradition of “through remains, into humans 透物见人” in Chinese archaeology calls for care to personal experiences of the past individual, besides the whole scheme of contemporary societies. Since not all teeth formed simultaneously, high-resolution isotope ratios of the enamel from different teeth allow reconstructing

different life snapshots. The combination of strontium isotopes with others of distinctly spatial heterogeneity, such as oxygen, hydrogen, and lead, will produce quantitative results at various spatial resolutions and offer the potential for understanding mobility information at individual and community scales.

Integrated sourcing methodology and beyond

There are great desires for sourcing various materials, inorganic and organic, in Chinese archaeology. For instance, archaeological plant remains, such as seeds, leaves, and stems, hold great potentials of provenancing their past movements (Styring et al., 2019) and, at this stage, practicable protocol for extracting their original $^{87}\text{Sr}/^{86}\text{Sr}$ signals are imperative, although the approach has been applied for determining geographical authenticity of modern agriculture products (e.g., Lagad et al., 2017; Liu et al., 2020). Furthermore, cross-discipline collaborations of petrology, geochemistry, immunomics, proteomics and ancient genomics, as well as statistics and modelling, are a promising approach to establish an integrated sourcing methodology for different materials. A most recent pilot study, in which strontium isotopes were used to track cocoons (Liu et al., 2022), is a positive signal for broadening the reach of strontium isotope tracer. Along with the emphasis on individual life and specific material, the structures, identities and collective action are also the foci of bioavailable strontium isotope, especially to the transition in the developments of Chinese civilization and history.

Author contributions

ZT and XW designed the research, collected and visualised the data, and wrote the manuscript.

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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.

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Supplementary material

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Investigation of red substances applied to chank shell beads from prehistoric site of Qulong in Ngari Prefecture, Tibet, China

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“Applying red” is a common phenomenon observed in Chinese archaeological sites, with the red pigments having been identified as red ochre or cinnabar if ever been scientifically analyzed. However, this is not the case for Tibet. Although a relatively large number of red-painted artifacts have been recovered in Tibet dating from the Neolithic Period to the Tubo Dynasty, little effort has been made on the pigment composition. Recently, nearly one hundred red substances covered shell beads made of the scared chank (*Turbinella pyrum*), a large conch from the Indian Ocean, were unearthed from the Qulong site (c. 800–500 BC) in the Ngari plateau, western Tibet. This shell beads assemblage represents the largest and most concentrated group of chank shell beads recovered in the Tibetan Plateau and its surrounding regions. It provides a crucial clue for exploring the local “applying red” tradition. In this study, eight shell beads excavated from the Qulong site were examined by the Portable Energy-dispersive X-ray Fluorescence Spectrometer (pXRF), X-ray diffraction (XRD), Fourier Transform infra-red spectroscopy (FTIR), and Laser Raman spectroscopy. The results are as follows: 1) the coloring agent of all red pigments on the shell bead is iron oxide, i.e., red ochre; 2) bone powder that has not been heated to high temperatures (above 600°C) and proteinaceous binders were added to the paint on the outer surface of sample QSM1-11a, but the thin layer on its interior surface was without bone powder; 3) bone powder was not added to the red residues on samples other than QSM1-11a, QSM1-13b, and QSM2-12. This research may reveal the complexity and diversity of the red substances applied to shell beads from Qulong, and shed light on our understanding of human practices and local customs in the Tibetan plateau and the surrounding areas in prehistoric times.

KEYWORDS

applying red, Tibetan plateau, prehistoric period, Qulong site, chank shell beads

1 Introduction

“Applying red” is a common phenomenon observed during archaeological excavations in China, especially in burial contexts. Red painted or stained artifacts and human skeletons were widely recovered from Chinese archaeological sites ranging in date from the Paleolithic Period to the Historical times (see details in [Gao, 2011](#); [Tian, 2018](#)). According to the scientific analyses conducted on the red paint or pigment sampled from sites, most of them were proved to be red ochre or cinnabar.

In recent decades, several burial sites in Tibet have also been reported with the discoveries of grave bottoms scattered with red powder or funerary goods painted red, dating from the late Neolithic Period to the Tubo Dynasty. However, the majority of them have simply been characterized as red substances or red residues, usually without scientific examination. A rare example of scientifically examined red paint may come from the Qugong Site (late Neolithic, c. 1750–1500 BC) in Lhasa ([IACASS & BCRTAR, 1999](#)), where around 200 red painted stone tools, elaborately manufactured and displaying clear traces of usage, were recovered ([Wang, 2014](#)). The colorant was identified as ochre by emission spectrometry analysis ([IACASS and BCRTAR, 1999](#)). It is also reported that cinnabar powder was discovered underneath the human skeleton from Tomb PGM6 (cal. 725–170 BC) at the Gelintang Locus of the Piyang-Donggar Site in Zanda County, Ngari Prefecture ([CTSSU et al., 2001a](#)). However, no related scientific analysis has been published yet. In addition, although archaeological pigments are often mixed with other materials, little attention has been paid to the organic binders that could have been added to the pigments from Tibetan “applying red” contexts and therefore no relevant results have been published. Consequently, more research needs to be done on these red substances, if available.

The 2019 excavation season at the Early Metal Age (c. 1000 BC–AD 600, see definition in [Tong, 1985](#)) site of Qulong in western Tibet yielded 96 shell beads made of the sacred chank shell, which were covered or stained by red substances in varying degrees. At the same time, beside them were also discovered red residues on and around the human remains. Although the red traces remaining on human skeletons and grave bottoms cannot be analyzed due to belated sampling and contamination, the shell beads could provide an excellent opportunity to examine the “applying red” phenomenon in Tibetan archaeology.

In this study, various non- and micro-invasive analytical techniques were adopted on eight samples of the chank shell objects. The goal is to investigate the material composition of the red substances applied to these shell beads, and then unveil the hidden human behaviors behind them to shed new light on the early indigenous culture and ancient tradition on the world’s highest plateau.

2 Materials and methods

2.1 Archaeological background and chank shell beads

The Ngari plateau, covering western Tibet with an average elevation of over 4,500 m MSL ([Ye et al., 2016](#)), is reputed as “the top of the Roof of the World” ([Tang et al., 2018](#)). This region was once the heart of the ancient Zhang Zhung Culture till the seventh century and the Guge kingdom in the later historical period (10th–17th century) ([Huo, 1997](#); [Li, 2017](#)). In the last two decades, a series of archaeological researches, including excavations and surveys, have been conducted in Ngari, mainly concentrated in the Zanda Basin, the cradle of the ancient cultures that originated in the Ngari area.

Qulong, a site from the Early Metal Age, is located around Qulong Village in Zanda County, Ngari Prefecture, Tibet. The site contains nine scattered loci (4,200–1,600 m MSL) on both banks of the mainstream of the upper Langqen Zagbo (Sutlej) River, encompassing an area over 100,000 m². A team of archaeologists from Shaanxi Provincial Institute of Cultural Heritage and Archaeology and Northwest University in China has carried out a long-term project at the Qulong Site beginning in 2018. Numerous archaeological remains, including petroglyphs, cave dwellings, tombs, and stone house foundations, have been uncovered (The archaeological report of the Qulong site has not been published. The archaeological data from Qulong presented in this paper were provided by co-author Lin Xi, the team leader of the Qulong excavation for the 2019 season).

During the 2019 excavation season, 96 perforated shell beads were unearthed in three tombs at two loci of Qulong: Tomb 2019QSZM1 at Sazha Locus and Tombs 2019QSM1 and 2019QSM2 at Sailaquinbopu Locus. In these tombs, human skeletons were buried with numerous animal bones and shell objects. Pottery and artifacts made of stone, wood, bronze and iron were also recovered. Although no radiocarbon dates of the three tombs are available yet, they could be typologically dated to around 2,800–2,500 years ago. All these beads are made of the shell of sacred chank, a large Indian Ocean conch *Turbinella pyrum* ([Subba Rao, 2003](#)) which has been intensively exploited as raw materials for ornaments production in South Asia since the Harappan period (e.g. [Kenoyer, 1991](#)). More than half of them are roughly disc-shaped, while around a quarter show profiles as flat collared beads (e.g., [Figure 1C](#), QSZM1-26), followed by a few triangular, rounded square, and irregular shell pieces. The shell beads vary in size between 17 and 68 mm along the long axis. Most beads have a single central perforation, whereas others bear two or more, apparently used as pendants and/or buttons.

Although all the beads exhibit varying degrees of weathering, red substances remained on one or both sides of the beads. It is

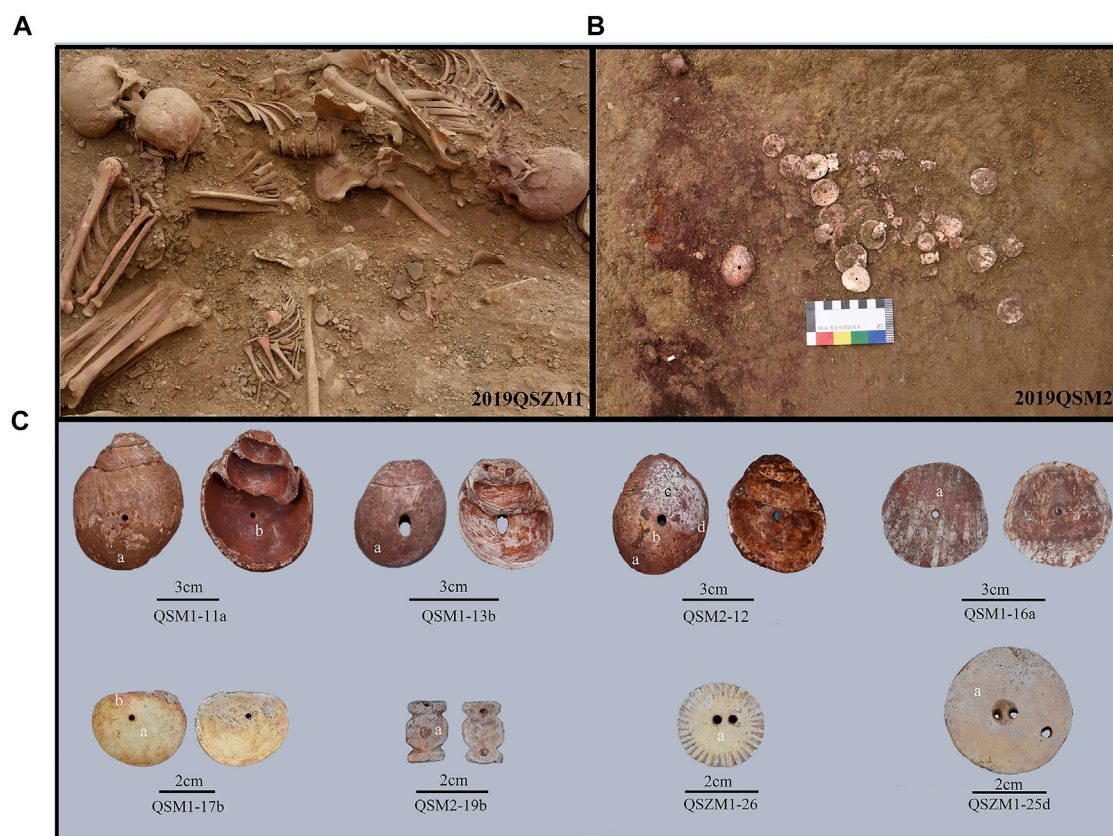


FIGURE 1
(A) Tomb 2019QSZM1 at Sazha Locus of Qulong Site; (B) Tombs 2019QSM2 at Sailaqqinbopu Locus of Qulong Site; (C) Photos of samples in this study.

worth noting that about one-tenth of the samples exhibit a relatively thick red layer, such as QSM1-11a, QSM1-13b, and QSM2-12 (see Figure 1C). At the same time, other shell artifacts only show red traces that could barely be detectable with the naked eye (e.g., Figure 1C, second row). As Figures 1A,B show, a red substance, unclearly paint coat or powder, was found covering the bodies of the tomb owners and the shell beads nearby. Thus, it could be reasonable that the red residue attached to the shell surface was the red substance intentionally splashed onto the deceased during the burial process, signifying an indigenous funeral practice. It may explain the situation of the shell beads with faint red traces (e.g., sample QSM1-17b, Figure 1C), but not all the objects. Consequently, if the composition of this thick layer differs from that of the weak residues that remained on other shell decorations, it is possible that these thick red layers were not left behind due to the burial practice. If the result is opposite, we cannot rule out the possibility that the thick layer was also formed during funeral rites. In addition, take QSM1-11a as an example. It shows different textures of the pigment layers on two sides: the inner surface (of the original conch) displays a thin layer of red paint

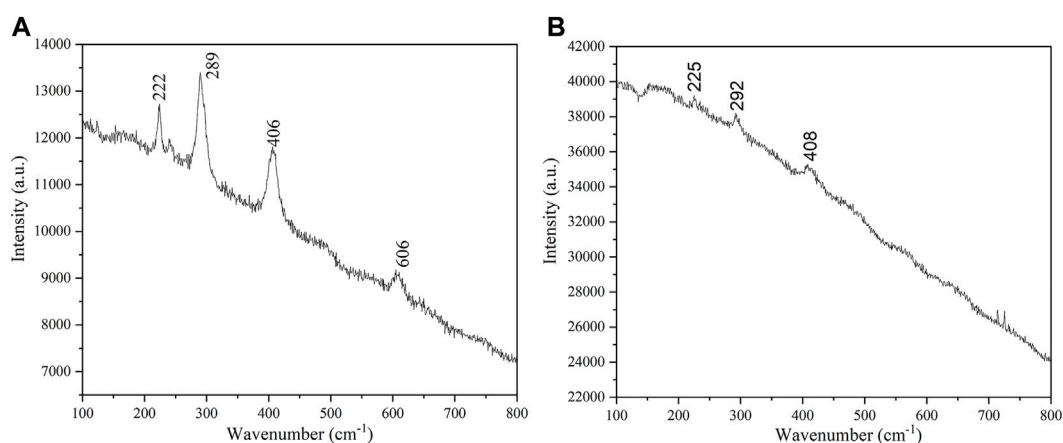
that is homogenous and bright; the outside, on the contrary, is crusted by a much thicker and darker layer, although part of it has flaked away. The contrast in texture of the substances may suggest a discrepancy in their composition. If it is the case, the formation of the red layers on QSM1-11a could not all have resulted from the red pigment splashing behavior. In short, two questions arise: 1) Are the thick coatings on some of the shell beads comprised of the same material as the faint red pigment remnant on the remaining shell objects? 2) Are the compositions of the red layers on the inner and outer surfaces of sample QSM1-11a different?

2.2 Methods

To answer these questions, non-destructive methods of pXRF and Raman spectrum were used to analyze the chemical composition and phase composition of pigments on the inner and outer surfaces of eight shell beads. In addition, the powder scraped from the outer surface of sample QSM1-11a was detected by micro-invasive methods including XRD and FTIR.

TABLE 1 The chemical composition of the shell beads from the Qulong Site (wt%).

Sample number	Test point	Na ₂ O	MgO	Al ₂ O ₃	SiO ₂	P ₂ O ₅	K ₂ O	CaO	Fe ₂ O ₃	SrO
QSM1-11a	a	n.d	n.d	4.03	14.98	30.01	0.52	48.71	1.52	0.23
	b	n.d	n.d	n.d	17.25	0.00	n.d	56.12	25.95	0.69
QSM1-13b	a	n.d	n.d	n.d	5.30	9.49	n.d	82.17	2.80	0.25
QSM2-12	a	n.d	n.d	n.d	5.55	38.27	n.d	55.01	1.02	0.15
	b	n.d	n.d	1.31	9.84	37.11	0.21	49.39	1.99	0.15
	c	n.d	n.d	n.d	6.59	1.70	0.34	90.40	0.73	0.24
	d	n.d	n.d	n.d	6.36	32.10	0.10	60.24	1.03	0.18
QSM1-16a	a	0.22	n.d	n.d	3.89	1.56	0.11	93.23	0.81	0.19
QSM1-17b-1	a	n.d	n.d	n.d	1.52	0.18	n.d	98.07	0.03	0.20
	b	n.d	n.d	n.d	3.43	0.18	n.d	95.81	0.36	0.21
QSM2-19b	a	n.d	n.d	1.89	9.60	1.50	0.50	85.74	0.52	0.25
QSZM1-26	a	n.d	n.d	n.d	3.25	0.23	n.d	96.18	0.05	0.28
QSZM1-25d	b	n.d	n.d	n.d	4.29	0.21	0.06	95.10	0.10	0.24

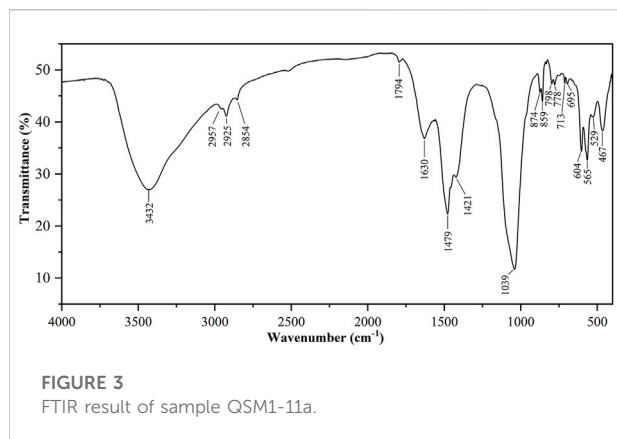
**FIGURE 2**
Raman results of sample QSM2-12 (A) and QSM1-16a (B).

2.2.1 Portable energy-dispersive X-ray fluorescence spectrometer (pXRF)

The pXRF test was performed on all eight shell beads, and the test points are marked in [Figure 1C](#). The chemical compositions of the samples were analyzed *via* pXRF (OURSTEX 100FA) at the Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences. The target material of this pXRF spectrometer is palladium (Pd). The excitation voltage of the X-ray tube is 40 or 15 kV, the current is 0.5 or 1.0 mA, the maximum power is 50 W, and the diameter of the X-ray focal spot is about 2.5 mm.

2.3.2 X-ray diffraction (XRD)

About 10 mg of pigment powder was scraped from the outer surface of sample QSM1-11a for XRD analysis. XRD analyses were performed on a Rigaku TTR-III X-ray diffractometer using Cu K α irradiation ($\lambda = 0.154056$ nm) at the University of Science and Technology of China. A small amount of pigment powder was scraped from the sample for analysis. The 2θ range was 10–70. The crystal phase of the samples was determined by comparison with standard spectra in the Jade software.



2.3.3 Laser Raman spectroscopy (Raman)

Raman spectroscopic analysis of samples QSM2-12 and QSM1-16a was performed with a LabRAM HR Evolution (HORIBA JY) at the Instruments Center for Physical Science, USTC. The equipment's parameters for this analysis were set up as follows: excitation light sources, 785 nm; the detection range, 100–800 cm^{-1} ; power, 17 mW; and common objective lens, $\times 50$.

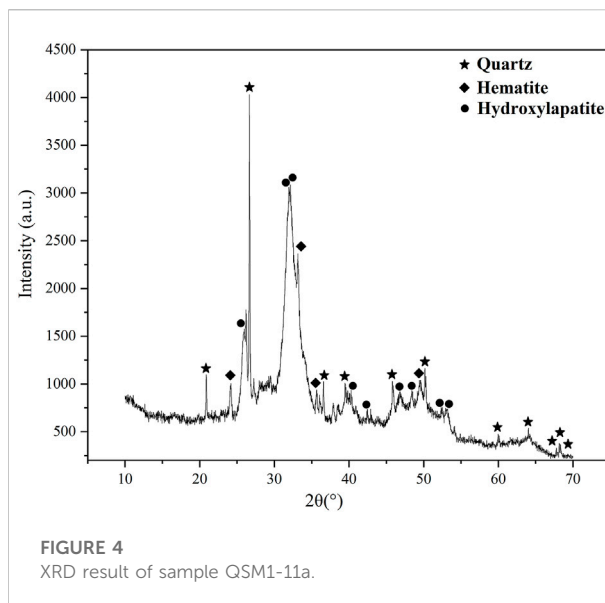
2.3.4 Fourier transform infra-red spectroscopy (FTIR)

The sample used for FTIR analysis was from the powder remaining after the XRD analysis. FTIR analysis was performed at the Instruments Center for Physical Science, USTC. KBr tablet method was used for the FTIR test. The model of the instrument is Nicolet 8700, with a spectral range of 4000–400 cm^{-1} and a resolution of 0.1 cm^{-1} .

3 Results

3.1 Pigment composition

The red pigment of eight samples contains Fe, indicating that the coloration may be Fe_2O_3 (Table 1). To further confirm the composition of the pigment, samples QSM2-12 and QSM1-16a were analyzed by Raman spectroscopy, and the external surface of QSM1-11a was analyzed by XRD and FTIR. The Raman spectrum of QSM2-12 and QSM1-16a (Figure 2) shows bands at 222 cm^{-1} , 289 cm^{-1} , 406 cm^{-1} , and 606 cm^{-1} , which are entirely characteristic of hematite (Guglielmi et al., 2022). The infrared spectrum of QSM1-11a (Figure 3; Table 1) shows bands at 467 cm^{-1} and 529 cm^{-1} which may be assigned to hematite (Bikiaris, et al., 2000), while there are also obvious diffraction peaks of hematite in the XRD spectrum (Figure 4). Therefore, the chemical composition and phase analysis show that the red pigment used in the shell beads unearthed at the Qulong Site



is hematite. In addition, XRD results show the presence of quartz, which may be derived from the soil on the surface of the sample.

Interestingly, Ca and P were detected in the outer surface pigments of samples QSM1-11a, QSM2-12, and QSM1-13b. The shell beads matrix is CaCO_3 , which will have a certain impact on pXRF analysis. But theoretically, the influence of the matrix on the compositional analysis is relatively small, given the thick coating layer. Therefore, the possibility that a kind of substance rich in Ca and P, such as bone ash, was mixed into the pigment should be considered. The XRD result of samples QSM1-11a indicated that hydroxyapatite was indeed present in the red pigment. The FTIR spectra recorded on the same samples also show the peaks of hydroxyapatite. A strong peak at 1,039 cm^{-1} is due to the asymmetric ν_3 stretching modes of $(\text{PO}_4)^{3-}$, the 562 and 598 cm^{-1} peaks are due to the asymmetric bending ν_4 mode of $(\text{PO}_4)^{3-}$ groups (Opris et al., 2022). Besides the phosphate groups peaks, we observed weaker peaks due to the carbonate groups as follows: the 875 cm^{-1} peak is due to the symmetric ν_2 mode of $(\text{CO}_3)^{2-}$ groups, and the 1,421 and 1,479 cm^{-1} are due to the asymmetric ν_3 stretching modes of $(\text{CO}_3)^{2-}$ groups (Opris et al., 2022). The results showed that the red pigment was indeed doped with bone powder. However, the inner surface of the sample QSM1-11a lacks P, suggesting that no bone meal was added during the production of the outer surface pigments.

To further understand the production process of the pigments, the SF value of the infrared spectrum were calculated to evaluate whether the bone meal was heated. The FTIR SF calculation for bioapatite is based on the degree of peak splitting in the PO_4^{3-} antisymmetric bending mode (Andrew and John, 1996), which is an important index when discussing bone crystallinity after long-term depositing or burning. First, a local

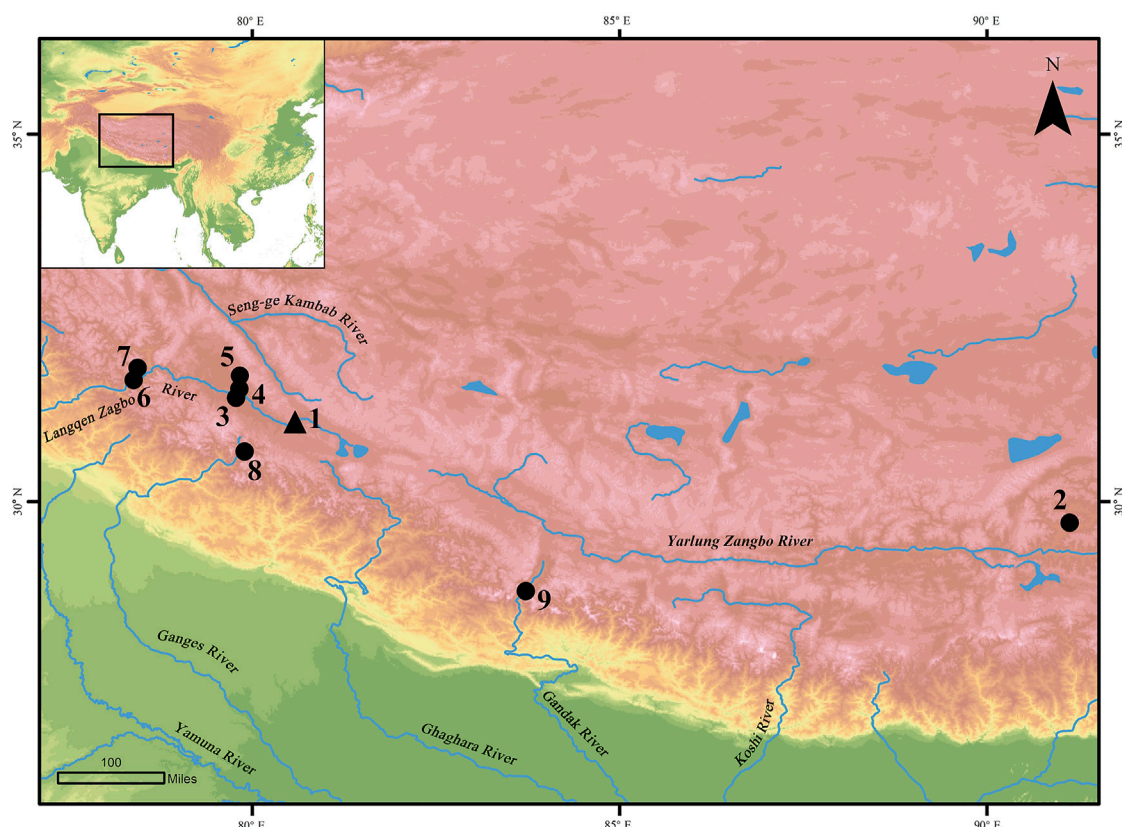


FIGURE 5

Location of the sites mentioned in the article. 1. Qulong; 2. Qugong; 3. Chuvthag; 4. Gepa Serul; 5. Gelingtang; 6. Lippa; 7. Ropa; 8. Malari; 9. Chokhopani.

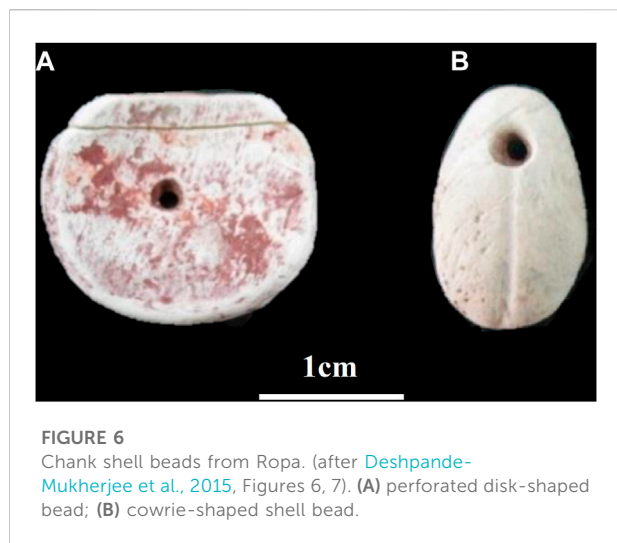
baseline was drawn between 750 and 495 cm^{-1} . Then the heights of the $\nu_3\text{PO}_4^{3-}$ antisymmetric bending frequencies at approximately 565 cm^{-1} (A) and 604 cm^{-1} (B) above this local baseline were measured and divided by the height of the enclosed valley (C) (Weiner and Bar-Yosef, 1990; Todd and Mary, 2001). Finally, the SF value of the bone meal in the samples QSM1-11a was calculated to be 3.7 by the formula $\text{SF} = (\text{A} + \text{B})/\text{C}$. The SF index of modern bones is about 2.8–3.0, and that of archaeological samples is about 3.5–4.8 (Guo et al., 2017). SF values are relatively stable until 600°C, then strongly increase to reach values around 8–10 for samples heated from 700 to 900°C (Lebon et al., 2008). The SF of the bone meal in the QSM1-11a pigment is within the range of the SF value of the archaeological sample, indicating that it has not been heated at a high temperature above 600°C.

In general, the red pigments of the shell beads of the Qulong site are all hematite, and most of the samples are doped with bone powder that has not been heated at a high temperature (600°C). Unlike the outer surface, the pigment on the inner surface of sample QSM1-11a does not contain bone powder, which may indicate the different processes of pigment production.

3.2 Glue identification

Binding media is an important part of ancient painted cultural relics. In the process of making a color painting, mixing glue and mineral pigment can improve the force between pigment particles and strengthen the adhesion between the pigment and the painting matrix (Yang et al., 2011a). The study on the existence and types of binding media will help us to understand the production technology of the surface pigments of the shell beads at the Qulong site and the utilization of animal and plant resources by the ancients.

The binders in ancient painted cultural relics include proteins, lipids, polysaccharides, resins, etc. For QSM1-11a, the strong absorption bands in the 1,628–1,636 cm^{-1} were attributed to amide I (C=O stretching), a characteristic of proteinaceous binders (Pellegrini et al., 2016). Additionally, the asymmetrical N-H stretching vibration occurring near 3,300 cm^{-1} , the methyl (CH_3) and methylene (CH_2) groups produce asymmetric stretching vibrations respectively at 2957 and 2925 cm^{-1} and small symmetric stretching bands at 2,854 cm^{-1} (CH_2) (Invernizzi et al., 2018). Common ancient



proteinaceous binders include animal glue (bone glue, skin glue, fish bladder glue), eggs (whole egg, egg yolk, egg white), and milk. Among them, the whole egg, egg yolk, skin glue, and milk are rich in lipids, and there are C=O stretching bands near 1745 cm^{-1} of their infrared spectra ([Ma et al., 2020](#)). There is no band near 1745 cm^{-1} in the infrared spectrum of sample QSM1-11a, which may indicate that the proteinaceous binders were not from the whole egg, egg yolk, skin glue, milk, and other lipids rich materials, but the degradation of lipids during the burial process increases the uncertainty of the deduction. Previous studies showed that during the evolution of organisms from the sea to the land, some amino acids in collagen, such as serine (Ser), were gradually converted into hydroxyproline (Hyp) to adapt to the new higher temperature living environment ([Song et al., 2008](#)). Therefore, the content of hydroxyproline (Hyp) in the collagen of terrestrial mammals is significantly higher than that of fish, while the content of serine (SER) is lower than that of fish. It is shown that there is an absorption peak generated by the stretching vibration of the C-O on the serine side chain in the $1,076\text{ cm}^{-1}$ of fish bladder gum, which is not significant in the infrared spectrum of terrestrial mammalian gum ([Yang et al., 2011b](#)). There is no peak around $1,076\text{ cm}^{-1}$ in the infrared spectrum of sample QSM1-11a, which may indicate that the proteinaceous binders are not fish bladder glue, but the effect of protein degradation cannot be excluded.

In addition, due to the presence of bone powder that has not been heated at a high temperature, it is still necessary to further explore whether the protein detected on the surface of the shell beads was attributed to bone powder or intentionally added glue. The amide-to-phosphate ratio (amide I/ PO_4) has been used to evaluate the preservation of collagen in bone ([Trueman et al., 2004](#); [Fredericks et al., 2012](#); [Kontopoulos et al., 2020](#)). [LeBon et al. \(2016\)](#) studied 42 bone samples from the Pleistocene to modern times. The results showed that the

amide I/ PO_4 ratio had a good correlation with the nitrogen content, and thus established the relationship between the amide I/ PO_4 ratio and N wt% and Collagen wt%:

$$\text{Nwt\%} = 20.6 \text{ amide I/PO}_4 + 0.31 \quad (1)$$

$$\text{Collagen wt\%} = 113.13 \text{ amide I/PO}_4 + 1.69 \quad (2)$$

According to the guidance of [Bouchard et al. \(2019\)](#), the ratio of the area under the Amide I peak to the area under the phosphate ν_3 peak was calculated, and the amide I/ PO_4 value of QSM1-11a was about 0.23. Thus, the nitrogen content is about 5.05% and the collagen content is about 27.71wt%. Depending on the species and type of bone, fresh bone contains 20–35% organic matrix, of which ~90% is collagen, giving bone a %N of ~3.5–4.5% ([Brock et al., 2012](#); [Currey 2012](#); [Richter et al., 2022](#)). [Kontopoulos et al. \(2020\)](#) tested 266 human and animal bone samples from 10,000 BC to 1850 AD, and found that the amide I/ PO_4 values were lower than 0.2 and the collagen content was lower than 25%. Meanwhile, the mean value of amide I/ PO_4 value in 42 bones was measured by [LeBon et al. \(2016\)](#) was 0.08, the highest value was 0.18, the mean value of N content was 2.29%, and the highest value was 4.25%. In the case of the degradation of ancient proteins and the reduction of the thermal stability of collagen by hematite ([Pellegrini et al., 2016](#)), proteinaceous binders should be added to the surface of shell beads at the Qulong Site, resulting in such high nitrogen content and collagen content, even higher than that of fresh bones.

Therefore, FTIR results showed that the outer pigment of sample QSM1-11a was doped with bone powder and then added with proteinaceous binders. There are no characteristic peaks of the whole egg, egg yolk, skin glue, milk, and other lipids-rich binders and fish bladder glue in the spectrum. However, the degradation limits the exploration of the specific ancient binder.

4 Discussion and conclusion

The scientific analyses show that the red pigment applied to chank shell beads included in this paper is red ochre, one of the most important pigments found at archaeological sites around the globe ([Langley and O'Connor, 2018](#); [Siddall, 2018](#)). Nevertheless, different types of shell objects are treated differently. For example, phosphorus-rich substances were found in the pigments on the outer surfaces of samples QSM1-11a, QSM1-13b, and QSM2-12. XRD and FTIR results suggest that they were bone powder. However, phosphorus did not exist on the inner surfaces of these samples and both sides of the other analyzed shell beads, indicating the absence of bone powder. Moreover, proteinaceous binders were detected in the powder scraped from the outer paint of sample QSM1-11a,

which are necessary for bonding thick pigments. Therefore, we believe that the shell beads with thick pigments on the outside were already coated with a red pigment mixed with ochre, bone powder and proteinaceous binders before burial. Although it is still challenging to determine the specific types of binders, anthropological evidence may provide a clue. Modern Tibetan herders often boil the milk dregs separated from the butter making them into a thick, dark brown paste, that is used as a binder to apply red paint on their faces. The glue can be immediately used to smear the face or stored for later use. Only a tiny amount of water must be added, and then it can be used after being heated to a thick paste (Melvyn and Cynnthia, 1990). The ethnographic record may shed light on future research.

For the other shell samples from Qulong, the situation is different. The photographs taken during fieldworks (Figures 1A, B) exhibit red pigments scattered around the shell beads and the human skeletons, suggesting a burial practice after the beads were placed in the tombs. Thus, for the shell objects as samples QSZM1-25d, QSM1-17b, QSM1-16a, and QSM2-19b, they might not have been painted before interment but stained red due to the ochre powder splashing activity in the funeral. In addition, samples QSM1-11a, QSM1-13b, and QSM2-12, showing similarities in shape and bearing a thick red layer on the outside, have a thin and brighter layer on the inner surface, apparent in sample QSM1-11a. Although the composition of the thin layer could not be distinguished from other weak red traces, its distinct texture and appearance may indicate that the three samples' inner surfaces had also been painted red before the funeral, despite a pigment difference in composition from that of the outer surfaces. This may indicate an elaborate treatment of this type of shell ornaments. In brief, the diversity of material compositions of the red substances may indicate that the ancient people at Qulong treated a variety of shell bead types in different ways according to their distinct functions, or that the source of these shell objects had defined their forms and colors before being imported into Qulong.

Similar perforated chank shell beads, ranging in number from one to 17, were also sporadically recovered from a few sites in the Himalayan region, including Chuvthag (c. 2,400–2200 BP) (IACASS et al., 2015; Tong, 2021, 539–540), Gepa Serul (c. 3500–2200 BP) (CTSSU et al., 2001b) (The shell beads information from Gepa Serul as well as the archaeological background were provided by co-author Songmei Hu who participated the excavation at Gepa Serul in 2018.) and Piyang-Donggar (Gelintang Locus, c. 2700–2200 BP) (CTSSU et al., 2001a) in western Tibet, Malari (c. 2400–2100 BP) (Deshpande-Mukherjee et al., 2015; Bhatt and Nautiyal, 1987–88), Lippa (c. 2600–2300 BP) (Nautiyal et al., 2014; Deshpande-Mukherjee et al., 2015) and Ropa (probably close to the period of Malari and Lippa) (Deshpande-Mukherjee et al., 2015) in northwestern Indian, and Chokhopani (c. 2800 BP) (Simons et al., 1994; Tiwari, 1984/85) in north Nepal (see

locations in Figure 5). Most of these shells are disk-shaped and show faint or no traces of red pigment, except for two beads from Ropa in Himachal Pradesh, India (Figure 6A). As shown in Figure 6A, the photographed shell bead from Ropa is similar in shape and color to samples QSM1-11a, QSM1-13b and QSM2-12 from Qulong, demonstrating solid connections between these artifacts from the two sites. It is worth noting that besides these two red shell objects, the other 13 cowrie-shaped shell beads, which were stored together with the red ones in a large vessel when unearthed, are purely white (Figure 6B). Thus, the red beads should have been painted red before being buried with other white shell beads, just as the three samples from Qulong.

Most of the archaeological data from the Qulong site has yet to be analyzed and published. Based on the limited information, only three gemstone beads of carnelian or agate and a handful of small bronze tools were recovered with the chank shell beads at Qulong, indicating a not very prominent social status of the tomb owners. Besides, although the red pigment splashing activity during the funeral ceremony might suggest a religious behavior, there is no evidence to support that the tomb owners were associated with indigenous shamanism. So far, we could assume that these shell beads were used as ornaments for the Qulong people, such as headdresses, necklaces, and buttons sewn onto clothing, in the same manner as in today's traditional Tibetan dress. Moreover, the red-painted ones might have been the key design in a set of shell beads since the Tibetan people have been fond of red since ancient times. For example, as the Old Book of Tang (Jiutangshu) records, in the time of the Tubo King Songtsen Gampo's reign (seventh century), there was a popular custom of painting faces red in Tibet (Liu, 1974; Li, 2006). Therefore, although it is difficult to determine whether the shell ornaments were manufactured and/or painted red locally, the affection for the color red by the Qulong people is perceptible.

The present study was designed to examine the material composition of the red substances adhering to eight chank shell beads recovered from the 2019 season excavation at Qulong in western Tibet. The results are as follows: 1) the coloring agent of all red pigments on the shell bead is iron oxide, i.e., red ochre; 2) bone powder that has not been heated to high temperatures (above 600°C) and proteinaceous binders were added to the paint on the outer surface of sample QSM1-11a, but the thin layer on its interior surface was without bone powder or organic binders; (3) neither bone powder nor proteinaceous binders were added to the red residues on samples other than QSM1-11a, QSM1-13b, and QSM2-12. The results of this study suggest the complexity and diversity of the red substances applied to shell beads from Qulong and clarify our understanding of human practices and local customs in the Tibetan plateau and the surrounding areas in prehistoric times. More research is required to determine the specific kind of protein glue mixed with ochre in the future.

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

JW, BZ, and AF conceived the study and designed the experiments; BZ and YZ performed the experiments; LX and SH provided the samples; all the authors discussed the data and contributed to writing the manuscript.

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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.

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Radiocarbon dating and its applications in Chinese archeology: An overview

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Radiocarbon dating is a well-established chronometric technique that has been widely employed in Chinese archeology since the first radiocarbon laboratory started operating in the Institute of Archaeology at the Chinese Academy of Sciences in 1965. In the three decades of studies that followed, achievements were made in radiocarbon dating, especially in measurement techniques, sample preparation, and the establishment of regional chronological frameworks. There is no doubt that Chinese archeology entered a golden age with the assistance of radiocarbon dating techniques at the beginning of the 2000s. It is, however, also true that compared to Western countries, China has reported far fewer radiocarbon dates than expected. This paper presents an overview of the history of the radiocarbon dating technique and its significant applications in Chinese archeology, focusing on the transition from β -decay counting to accelerator mass spectrometry. Some of the breakthroughs in studies of the Upper Paleolithic, early *Homo sapiens*, neolithization, and the Xia and Shang dynasties are highlighted. We conclude the paper with a brief discussion of future work and research directions that need to be explored.

KEYWORDS

radiocarbon dating, archeology, China, chronology, methodology, applications

1 Introduction

In 1949, the first set of radiocarbon dates was published by Willard Frank Libby and colleagues (Libby et al., 1949). Shortly afterward, radiocarbon dating laboratories were established in many Western countries (Deevey et al., 1965). Radiocarbon dating was considered to be a great revolution in prehistoric archeology in the 20th century (Daniel, 1959), and it “came as a godsend to archeology” (Renfrew, 1976: 53–75). This new technique allowed archeologists to examine archeological remains and associated events with well-delineated chronological frameworks. It contributed significantly to addressing critical concerns in archeological studies such as the chronologies of specific events and the formation of new hypotheses and research paradigms (Kuzmin, 2009).

Radiocarbon (^{14}C) is a radioactive isotope of carbon with a half-life of 5730 years. It is continuously generated in the upper atmosphere by the interaction of ^{14}N with cosmic ray-induced neutrons. Rapidly oxidized in the air, ^{14}C atoms enter the global carbon cycle in the form of $^{14}\text{CO}_2$, which plants absorb during photosynthesis. Animals ingest the carbon by eating plants and release a portion through respiration and excretion. When animals die, their exchange of carbon with the biosphere (and eventually the atmosphere) ceases, and the decay of radiocarbon causes the level to decrease with time. When the remaining radiocarbon in a plant or animal sample is determined, the age of the sample can be calculated. Radiocarbon ratios of the analyzed samples indicate the elapsed time since the last exchange of carbon between the sample and the environment (Libby, 1955). Radiocarbon dates are usually reported in uncalibrated years BP (before the present),

where BP refers to 1950 AD. Calendar years can be calculated from the uncalibrated year BP using the calibration curve.

China was among the first Asian countries to adopt radiocarbon dating for archeological research. Xia Nai, Director of the Institute of Archaeology of the Chinese Academy of Science (IA-CAS, which in 1977 became the Institute of Archaeology of the Chinese Academy of Social Sciences, or IA-CASS), introduced the radiocarbon dating technique to Chinese archeologists in 1955 (Xia, 1955). In 1965, the first radiocarbon dating laboratory was set up in the IA-CAS, a groundbreaking event in Chinese radiocarbon chronology (Lab of IA-CAS, 1972). More than half a century has passed since the first group of radiocarbon dates was reported in China, and significant progress has been made in the construction of testing facilities and in radiocarbon dating methods, which continued to improve the precision and accuracy of measurements. Tens of thousands of radiocarbon readings have been published in the Old and the New World. The present paper reviews the development of radiocarbon dating methods and their applications in Chinese archeology, especially studies on the Upper Paleolithic and early modern human origins, Neolithization, Neolithic chronology, and the Xia–Shang–Zhou Chronology Project.

2 Developments in radiocarbon dating methods in China

2.1 β -decay counting and advances in radiocarbon dating

After World War II, the application of radioisotopes became widespread in natural sciences, and ^{14}C was used for archeological dating in this context. Prior to the 1960s, Chinese archeology, especially of prehistoric periods, relied strongly on the stratigraphy of archeological sites and the typology of artifacts in inferring the dates. However, the low precision and unreliability of this method often led to controversial conclusions. Realizing the great potential of radiocarbon dating for establishment of more solid and finer chronological frameworks, the IA-CAS hired two nuclear physicists, Qiu Shihua and Cai Lianzhen, and supported them in setting up a radiocarbon dating laboratory at the CAS. The construction began in 1959 and was completed in 1965. Qiu and Cai first ran tests on samples of known ages dating to the Neolithic and Bronze Age in China, and the ^{14}C dates were compatible with the dating results inferred from archeological stratigraphy and pottery typology. The first radiocarbon dating laboratory in China started to produce radiocarbon dates soon afterward (Lab of IA-CAS, 1972; Rudolph, 1973). For the first time, “Chinese prehistory can be placed on an absolute-chronological basis” (Chang, 1973). Other radiocarbon dating laboratories were set up in China in the 1970s (Radiocarbon Lab of the Institute of Geochemistry of the CAS, 1973; Radiocarbon Lab of the Institute of Geology of the CAS, 1974). In 1981, about fifty ^{14}C labs were registered for the first National Conference on ^{14}C ; however, most of them were built for geoscience studies (Editorial Group of the First National Conference on ^{14}C , 1984).

The first radiocarbon dating laboratory at the IA-CAS adopted the gas counting technology, which could generate dates up to 40,000 years BP. Over the years, Qiu and others made significant efforts to improve the quality of measurements and achieved this goal by enhancing the facilities, especially the gas-proportional counters, the electronic detectors, high-voltage power supplies, shielding

devices, and vacuum systems for sample preparation (Lab of IA-CAS, 1972). In the 1960s, the application of liquid scintillation counting (LSC) technology for measuring ^{14}C was proven successful in radiocarbon dating laboratories of the West (Tamers, 1960). Compared to the gas method, LSC simplified the sample preparation procedures, required a smaller sample size, and ran tests with shorter times and lower background. In 1975, Yuan Sixun and others set up the first LSC radiocarbon dating laboratory at Peking University (Radiocarbon Dating Lab of Archaeology of Peking University, 1976). Subsequently, more radiocarbon dating laboratories in China began using the LSC method, including both new laboratories and ones that had previously used gas-proportional counting facilities (Lab of IA-CASS, 1978; Radiocarbon Dating Lab of Institute of Science and Technology for Cultural Relics Preservation 1978).

Sample preparation was significantly improved in the 1970s, along with the associated devices. In 1965, when acetylene gas was prepared, calcium was used to absorb carbon dioxide extracted from the samples; calcium was replaced by magnesium in the 1970s and by lithium in the early 1980s (Huang et al., 1981). At about the same time, automated testing techniques were used to measure ^{14}C and computers were used for processing the data, which significantly improved the efficiency, the data quality, storage, and retrieval (Lab of IA-CASS, 1983). In the 1990s, small sample sizes (100–250 mg) could be run for radiocarbon dating on LSC devices, which was a great advantage for dating archeological samples where only a small amount of material was available (Zhou et al., 1994).

Beginning in the 1950s, the A.D.1890 tree rings and other early 20th-century wood samples were used as ‘modern’ reference materials for calibrating radiocarbon dating results around the world. Oxalic acid was adopted internationally as the carbon standard at the 4th International Radiocarbon Conference (Godwin, 1959), and at the 8th International Radiocarbon Conference, sucrose ANU was recognized as the international sub-standard (Polach and Krueger, 1972). As the number of radiocarbon laboratories increased in China, so did the desire for reference materials that could be used to cross-check and validate the radiocarbon dates between different laboratories. A consensus was reached at the National Isotopic Geology Conference held in Guiyang that a modern carbon standard for radiocarbon studies should be used in China. Qiu, the principal investigator, and specialists from Peking University and the Chinese Academy of Sciences, were engaged in the project. In 1977, sucrose refined from beets harvested in Inner Mongolia was announced as the raw material for producing charred sucrose as the modern carbon standard. The $\delta^{13}\text{C}$ of charred sucrose is $-19.32\text{‰} \pm 0.56$ relative to PDB, which is very close to the -19‰ value of oxalic acid II. The averaged FM (fraction of the modern standard) was $1.362\text{‰} \pm 0.003$, suggesting that sucrose and oxalic acid II were very similar (1.3407 ± 0.001) (Mann, 1983; Qiu et al., 1983; Polach, 1989). Charred sucrose was thus approved as the modern carbon reference and national standard, known as the Chinese sugar carbon standard (CSC). Employing the new standard, radiocarbon dating was significantly improved, with simple chemical preparation, no more than 1% residue after burning, and no significant isotopic fractionation (Qiu et al., 1983). Recently, researchers started a project that aimed to make CSC a secondary material for accelerator mass spectrometry (AMS) radiocarbon dating. To achieve this goal, CSC particles were ground into a finer powder that ensured data homogeneity for AMS dating (Xu et al., 2013).



FIGURE 1
First national conference on ^{14}C in China (1981).

On September 15–18, 1981, the First National Conference on ^{14}C dating was held in Beijing. Over 70 participants attended the conference, and more than 40 reports were submitted (Figure 1). The presentations not only focused on archeology and geosciences but also on the most recent advances in radiocarbon dating techniques of China and abroad, especially in sample pretreatment, LSC facilities, error analysis of radiocarbon data, $\delta^{13}\text{C}$ measurement, and the application of AMS in radiocarbon dating (Editorial Group of the First National Conference on ^{14}C , 1984). In 1982, the 2nd National Conference on ^{14}C dating was held in Nanjing University. The Radiocarbon Chronology Group supervised by the Chinese Association for Quaternary Research was approved and an official announcement was made that the national radiocarbon dating conference would be organized on a regular basis (every 3 years) together with summer schools on radiocarbon dating methodologies. Radiocarbon dating results from different laboratories were also reported at this conference (Li, 2009). The workshops and national conferences significantly improved the theoretical and methodological background of specialists and technicians from different Chinese dating laboratories, which made a significant contribution to China's radiocarbon chronology in the decade that followed.

2.2 Accelerator mass spectrometry (AMS) radiocarbon dating

AMS is a method for measuring radioactive nuclides with long half-lives and to measure ^{14}C as early as in 1977 (Bennett et al., 1977; Muller, 1977; Nelson et al., 1977; Berger, 1979). AMS can directly measure the relative abundance of ^{14}C in a sample, a feature that distinguishes it from conventional methods based on β -decay counting. It not only reduces the test time from several hours to a few minutes but also enables measurements of specimens in small volumes and even at the molecular level, namely, ~ 1 mg carbon (1/1000 of the β -decay counting method). The successful application of the AMS radiocarbon dating technique was enthusiastically welcomed by researchers in archeology, earth sciences, and biological pharmacy. In 1979, Chen Tiemei introduced this newly developed dating technique to China (Chen, 1979). At the First National

Radiocarbon Conference, Cai Lianzhen (Cai, 1984) and Shen Chengde (Shen, 1984) presented an overview of AMS radiocarbon dating and its global applications. At the Fourth National Conference on Radiocarbon, on November 5–9, 1988, five of the 29 papers reported on the AMS radiocarbon dating technique, target preparation, and its applications in archeology (Radiocarbon Dating Society of Chinese Quaternary Research Association, 1990).

The first set of AMS radiocarbon data for Chinese archeological samples was reported in 1989 to support a discussion on the chronology of Shandingdong (Upper Cave) of the Zhoukoudian locality in Beijing (Chen et al., 1989). Several institutions began to set up AMS facilities at about the same time (Zhou and Chen, 2009). Peking University completed a dedicated tandem-based AMS facility in 1992, the Peking University accelerator mass spectroscopy (PKUAMS) laboratory with the financial support of the National Natural Science Foundation of China. The PKUAMS laboratory's spectrometer consisted of an ion source with 20-position sample trays, a fast-switching injection line, a 6-MV EN-tandem accelerator, and a post-acceleration analysis and detection system. The first batch of AMS data, reported in 1993, showed a sensitivity of 10^{-14} with a precision better than 1.7% for a modern sample (Chen et al., 1993). A precision of about 1% and a blank sample background lower than 0.006 MC or 43 Ka were achieved for the graphitization technique with the updated power supply, data acquisition and control system, and the highly intensified Cs sputtering ion source (Guo et al., 1995).

From 1996 to 2000, radiocarbon dating was incorporated into the Xia-Shang-Zhou Chronology project. To achieve a high precision, several key facilities were upgraded to the AMS system of Peking University, including replacing the ion source with a 40-sample NEC MC-SNICS device, a pneumatic sample change system, updated vacuum, injector, computer control and data acquisition systems. The new PKUAMS obtained ^{14}C measurements with a precision of $\sim 0.5\%$ and provided over 200 dates for the Xia-Shang-Zhou archeological chronology project (Guo et al., 2000; Liu et al., 2000). While thousands of samples have been dated using the PKUAMS system, there are many more radiocarbon dates needed in the fields of archeology and earth sciences. To meet this need, a new compact AMS system based on the model 1.5SDH-1 Pelletron accelerator with a maximum terminal voltage of 0.6 MV was purchased from the

National Electrostatics Corporation (NEC), installed in Peking University, and used exclusively for radiocarbon dating. The new system obtained ^{14}C measurements at a precision $<0.4\%$ and a background <0.03 pMC, or 65 Ka (Liu et al., 2007). Shortly afterward in 2006, another 3 MV Tandem-based AMS system, manufactured by High-Voltage Engineering Europe (HVEE), was installed at the Xi'an Accelerator Mass Spectrometry Center (XAAMS), the joint AMS laboratory of the Institute of Earth Environment, and Xi'an Jiaotong University. The XAAMS is a multi-element system for radiocarbon measurement with a precision of $\sim 0.2\%$ (Zhou et al., 2006). Recently, dozens of AMS facilities were purchased overseas to serve the needs of radiocarbon dating (Wu, 2021), including another 0.6 MV ^{14}C AMS facility manufactured by NEC and installed at the Guangzhou Institute of Geochemistry (CAS) in 2014 (Zhu et al., 2015), and a MICADAS type AMS by ETH Zurich at Lanzhou University.

In China, AMS radiocarbon dating has been applied directly not only on carbonized grains such as rice and soybean but also on plant microfossils such as pollen (Zhang et al., 2021) and phytoliths (Wang and Lu, 1997; Jin et al., 2014; Zuo et al., 2017; Zuo and Wu, 2019). In addition, soil sediments and monomer compounds in pottery residues were also collected for dating (Yuan et al., 1997). The widespread application of AMS radiocarbon dating has significantly revolutionized our understanding of archeological events.

3 Radiocarbon dating applications in Chinese archaeology

3.1 Chronology of the upper Paleolithic and the origin of early *Homo sapiens*

More than 2000 Paleolithic sites have been reported in China, and human fossils have been unearthed from about 80 of them (Ge et al., 2021). Most human fossils from Paleolithic sites were from the late-Middle to the Late Pleistocene, which makes China a core region for studying the evolution of early modern humans (EMHs) and their adaptations to diverse environments (Liu et al., 2016; Gao et al., 2019). Archaeology is a science of time, so dating is one of the most important fulcrums in archeological research. Chronology is the key to understanding human origins and evolution as well as shifting cultural materials. Radiocarbon dating is a well-accepted, scientifically based chronometric method for determining specimen ages up to $\sim 55,000$ years old (Hajdas et al., 2021), and it was thus essential for studying the evolution of EMHs and anatomically modern humans (AMH) and related archeological materials during the Upper Paleolithic.

Prior to the introduction of radiocarbon dating techniques, the ages of Paleolithic sites and hominin fossils were largely inferred from the stratigraphy and associated faunal assemblages, which often resulted in disagreements over the chronologies. The situation started to change in 1972, when the radiocarbon laboratory of the IA-CAS (Lab of IA-CAS, 1972) published the first set of ^{14}C data. It included a sample of wood associated with Ziyang No. 1, an EMH skull dated to 7500 ± 130 years BP, using the gas proportional counting method. In 1976, bone collagen was extracted from animal fossil samples at late Pleistocene sites, including Zhiyu in Shanxi, dated to $28,135 \pm 1,330$ ^{14}C BP, and the Upper Cave of Zhoukoudian in Beijing, dated to $18,340 \pm 410$ ^{14}C BP, to shed light on the chronology of the

Upper Paleolithic sites and EMH fossils (Lab of IA-CASS and Lab of Institute of Vertebrate Paleontology and Paleoanthropology of CAS, 1976).

In the early 1980s, radiocarbon data from more than ten Paleolithic sites were published (IA-CASS, 1983). Although most were consistent with the dates inferred from fossilized faunal assemblages, some remained controversial and were very likely questionable. An Zhimin argued that the problematic dates may have resulted from obscure archeological contexts and taphonomy, although the large error in the β -decay counting method could also be the cause. He suggested that, whenever possible, a single radiocarbon date should not be used because "one date is no date" (An, 1983). This called for greater attention to the sampling strategy and a full understanding of the depositional process. In 1988, a preliminary chronological framework was constructed for the Upper Paleolithic period based on paleoanthropological data including radiocarbon dating, K-Ar dating, uranium series, thermoluminescence (TL), and paleomagnetism (Chen, 1988).

In the late 1980s and early 1990s, the AMS technique was adopted to date Upper Paleolithic samples, which refined the chronological frameworks for archeological and paleoanthropological studies (Yuan et al., 1995). The calculated ages of the Zhoukoudian Upper Cave in Beijing can be used as an example to show how the advances in radiocarbon dating over the decades contributed to a better understanding of the Upper Paleolithic. The Zhoukoudian Upper Cave is well-known for EMH fossils, first unearthed in 1933 and 1934. Three human skulls and fragmented human fossils were discovered in layer 4 in the lower room (Pei, 1933; 1939; Norton and Gao, 2008). Some articulated human fossils colored with red ochre were found, leading to the assumption that the cave was a burial site. No chronometric methods were available at the time the human fossils were discovered, but the excavator suggested an age of Late Pleistocene based on faunal assemblages (Pei, 1940). In the late 1970s, two animal bones from the Upper Cave were dated using LSC, generating radiocarbon dates of $18,340 \pm 410$ ^{14}C BP (ZK-136-0) and $10,180 \pm 360$ ^{14}C BP (ZK-136-01). As sodium hydroxide was not used in collagen extraction, humic acid and other organic carbon compounds may have contributed to the apparently younger ages (Lab of IA-CASS, 1980). In 1989 and 1992, Chen and others prepared 12 bone samples using the acid-alkali-acid (AAA) procedure suggested by the Radiocarbon Accelerator Unit at the University of Oxford and published two groups of AMS ^{14}C data on the Zhoukoudian Upper Cave. Most of the sampled bones from the Lower Room were dated between $29,100 \pm 520$ ^{14}C BP and $23,700 \pm 350$ ^{14}C BP, much older than the two LSC ^{14}C dates, (Chen et al., 1989; Chen et al., 1992).

Recently, Li and others reanalyzed the taphonomy and stratigraphic contexts (Li et al., 2018a) and selected 11 bones from the Upper Cave for AMS ^{14}C dating at the Oxford Accelerator Unit, where ultrafiltration was added to the AAA procedure to collect large molecular weight proteins (Bronk Ramsey et al., 2004). Eight of the 11 bone samples yielded dates from 50 ka BP to 34 ka BP. Two samples with clear archeological contexts produced dates of $30,010 \pm 360$ ^{14}C BP ($34,744$ cal BP– $33,551$ cal BP) and $32,800 \pm 500$ ^{14}C BP ($38,376$ cal BP– $35,825$ cal BP). Since the artifacts and ornaments found at the Upper Cave showed similarities to those at Denisova in Siberia, the chronology led some archeologists to argue that the Upper Cave EMHs had made contact with other hominin populations further to the north some 35.0 ka BP (Li et al., 2018a). The hypothesis is consistent with the Tianyuan EMH fossils at the Zhoukoudian

Tianyuan Cave, which were dated to $34,430 \pm 510$ ^{14}C BP ($40,328 \pm 816$ cal BP) using AMS ^{14}C measurements (Shang et al., 2007; Fu et al., 2013; Yang et al., 2017).

To date, the progress in radiocarbon dating has refreshed our understanding of lithic technology and human behavior during the Upper Paleolithic as well as the interaction among hominin groups at Shuidonggou (Li et al., 2019) and Jinsitai (Li et al., 2018b), for example, and the Paleolithic sites around the Songshan Mountain (Wang and Wang, 2014). A large number of radiocarbon dates have been reported from more than 300 sites, which supports new discussions of EMH evolution and migrations (Wu, 2018; Zhang et al., 2018; Gao et al., 2019), the diversity of lithic industry (Wang, 2019), the introduction and routes of spreading Levallois (Hu et al., 2019), microblade technologies (Yi et al., 2016), and the broad spectrum revolution (Janz, 2016).

3.2 Radiocarbon dating and neolithization

The Neolithic is characterized by some fundamental changes including the use of pottery, food production, and settled village life. These changes are now regarded as long-term processes, to which the term “Neolithization” is often applied. In the Near East, food production preceded the use of pottery. However, the opposite was true in China, where Neolithization occurred in the vast, geographically diverse landscapes (Kuzmin et al., 2009). The extensive radiocarbon dating of prehistoric materials has allowed archeologists to construct a chronological framework for significant changes (e.g., the use of pottery and the shift from hunting–gathering–fishing to subsistence farming) that initiated Neolithization in several regions of mainland China.

Since the 1970s, radiocarbon dating has also been used to investigate the origin of pottery making in China. Sherds of very early pottery vessels have been unearthed from the sites of Xianrendong and Diaotonghuan in Jiangxi Province, Zengpiyan and Miaoyan in Guangxi province (Zhang and Hung, 2012), Yujiagou (Gai and Wei, 1977) and Nanzhuangtou (Baoding Institute of Cultural Relic Management et al., 2010) in Hebei Province, Donghulin (School of Archaeology and Museology of Peking University, et al., 2006) in Beijing City, Houtaomuga (Wang, 2018) and Shuangta (Wang and Duan, 2013) in Jilin Province, and Xiaonanshan (Heilongjiang Institute of Cultural Relics and Archaeology and Raohe County Office of Cultural Relics, 2019) in Heilongjiang Province. It was judged from archeological stratigraphy and pottery typology that many of these sherds arguably dated to the early Neolithic Age (Jiangxi Provincial Committee of Cultural Relics, 1963; Guangxi Team of Cultural Relics and Guilin Management Committee of Cultural Relics, 1976; Jiangxi Provincial Museum, 1976). In the 1970s and 1980s, LSC radiocarbon dating applied to freshwater shells and animal bones associated with sherds reported dates of 8000 ^{14}C BP or earlier. However, contradictory results do sometimes exist between stratigraphy and radiocarbon data. In Xianrendong, for example, freshwater shells (ZK-39) sampled from the upper layer were dated to $10,870 \pm 240$ ^{14}C BP (Lab of IA-CAS, 1974), which was older than the animal bone (ZK-92-0) from the lower layer with an age of 8575 ± 235 ^{14}C BP (Lab of IA-CAS, 1977). This may be due to the effect of dead carbon in the limestone region. Modern archeological samples including wood, charcoal, grain seeds, shells, and bones have been collected for

radiocarbon dating and ^{14}C ages of 1000–2000 years were suggested to offset the freshwater reservoir effect (Radiocarbon Lab of Peking University and Radiocarbon Lab of Institute of Archaeology of Chinese Academy of Social Sciences, 1982).

Beginning in the 1990s, the AMS ^{14}C facility made radiocarbon tests with small sample sizes possible. In 1997, Yuan and colleagues successfully extracted absorbed humic acids and intrinsic carbon of pottery clay from sampled sherds from the Miaoyan and Yuchanyan sites in southern China for AMS radiocarbon dating and published the results. Combined with the radiocarbon dates obtained from charcoal and animal bones, it was confirmed that pottery was used in southern China as early as 16.0 cal ka BP (Yuan et al., 1997). Further radiocarbon dating studies refined the earliest use of pottery at Yuchanyan to ca. 18,300 cal BP to 15,430 cal BP (Boaretto et al., 2009) and up to ca. 20,000 to 19,000 cal BP for Xianrendong (Wu et al., 2012). These dates indicate the widespread use of pottery in southern, northern, and northeastern China from 15,000 to 10,000 BP.

China was also one of the centers for agriculture and animal husbandry. While the middle and lower Yangtze River valley of southern China is home to rice cultivation, northern China is well-known for the domestication of foxtail and common millets (Zhao, 2011). Radiocarbon dating results have suggested that plant cultivation occurred thousands of years after the introduction of pottery. Only a very few early Neolithic sites used pottery as well as cultivated plants and practiced animal husbandry: Donghulin (Zhao et al., 2020), Nanzhuangtou (Hou et al., 2021), and Shangshan (Zhao, 2010). Pre-domesticated rice remains were found at sites such as Xianrendong during the late phase, around 10,000 BP or earlier (Zhang, 2000). Radiocarbon dates and micro- and macro-plant fossils have suggested that rice and millets were cultivated separately in southern and northern China some 10,000 years ago. Rice domestication could have begun. 9400 to 9000 BP at the Shangshan and Hehuashan sites in the lower Yangtze River valley (Zuo et al., 2017), while millet cultivation was securely dated to around 10,000 BP at the Donghulin (Zhao et al., 2020) and Cishan (Lu et al., 2009) sites.

In summary, radiocarbon dating has provided important clues to the Neolithic transition in China. Pottery making began around 20,000 BP in China, preceding agriculture by thousands of years. This sharply distinguishes China from the Near East (Makibayashi, 2014; Kuzmin, 2016), and future investigations including radiocarbon dating studies are necessary to confirm this distinction.

3.3 Chinese Neolithic chronology

The construction of Neolithic chronological frameworks is fundamental to understanding the archeology of cultural systems and their interactions. This has remained a key topic in Chinese archeology since the 1970s. In 1977, Xia Nai proposed a tentative chronological framework for Neolithic China using 129 published radiocarbon data sets (Xia, 1977). By the early 1990s, over two thousand radiocarbon dates had been published, about half of which dealt with the Neolithic Age or before 4,000 BP (IA-CASS, 1992). This substantial amount of radiocarbon data made possible the construction of several regional chronological frameworks (Chang, 1987; An, 1991), which continue to be refined using new radiocarbon dates.

The published radiocarbon data have allowed archeologists to divide Neolithic China into the following proposed categories: early

Neolithic (ca. 15,000 BP - 8500 BP or earlier); middle Neolithic, characterized by the pre-Yangshao cultures (ca. 8500 BP - 6900 BP); late Neolithic, represented by the Yangshao cultures (ca. 6900 BP - 4500 BP); and final Neolithic, represented by the Longshan cultures (ca. 4500 BP-3800 BP) (IA-CASS, 2012; Liu and Chen, 2012). The refinement of chronological frameworks has been carried out for the Yangshao (Zhang et al., 2013) and Longshan cultures in the Central Plains (Zhang, 2021), the Lower Haidai region and the lower Yangtze River valley (Long and Taylor, 2015; Long et al., 2017), as well as the Bronze Age Hexi Corridor (Yang et al., 2019). Chronological studies have also been conducted at the Xichengyi (Zhang et al., 2015) and Xiaozhushan (Zhang et al., 2016) sites.

Radiocarbon dating has substantially contributed to the long-term transdisciplinary project, the Origins of Chinese Civilization. Joint efforts of archeologists and radiocarbon experts over 20 years have revised the Neolithic to early Bronze Age chronology in the Yellow River and Yangtze River valleys. Large settlements and urban centers dating from 5500 BP to 3500 BP, such as Liangzhu, Shijiahe, Taosi, Shimao, and Erlitou, have received close attention in radiocarbon dating projects because they are crucial for the understanding of emergent social complexity as well as the formation of Chinese Civilization (Wang and Zhao, 2022). It is widely agreed that early Chinese civilization can be traced back to 6000 BP to 5500 BP in the Yellow, Yangtze, and West Liao River valleys (Wang and Zhao, 2022). Liangzhu, the earliest capital-level settlement in the lower Yangtze River valley and the earliest state in prehistoric southern China (Renfrew and Liu, 2018), had developed a unique hydraulic system around 5100 years ago (Liu et al., 2017).

The Chinese Civilization Exploration Project entered its fifth phase beginning in November 2020. From 2020 to 2024, radiocarbon dating projects will focus on 13 centralized centers in the Western Liao River, Yellow River, and Yangtze River valleys in the hope of offering a refined chronological foundation for research on the origin of Chinese civilization.

3.4 The Xia-Shang-Zhou chronology project

The Xia-Shang-Zhou Chronology project was commissioned in 1996 and completed in 2000 (Li, 2002). The project aimed to provide a reliable chronology of the Xia, Shang, and Western Zhou dynasties of ancient China, through the collaboration of specialists in archeology, history, astronomy, and radiocarbon dating, which played a decisive role in this project. To achieve high-precision radiocarbon data, the dating team optimized the preparation method for charcoal and bone samples and also improved the analytical precision of LSC to 2-3% and of AMS to 5% by upgrading the associated facilities (Qiu and Cai, 1997; Liu et al., 2000; Yuan et al., 2007). These achievements, combined with sequential sampling and 'wiggle-matching' of tree ring dates, allowed researchers to obtain several reliable chronologies.

Combining radiocarbon dates with the records of key events in literature, the project proposed that the reigning years of Western Zhou kings could be placed before the first year of the Gonghe Era (共和元年) or 841 BC and the late Shang kings from Wuding (武丁) to Zhou (纣). A relatively detailed chronology of the early Shang period was proposed along with a chronological framework of the legendary Xia Dynasty (Li, 2002). The project leaders combined radiocarbon dating with research in archeology, history, epigraphy, and astronomy. Data on related dates of historical records, bronze vessel inscriptions,

and astronomical phenomena were collected and cross-checked for authenticity before use. Sequential samples from several critical sites, especially burials of the Xia, Shang, and Western Zhou, were obtained for radiocarbon dating by LSC and AMS before adopting Bayesian models (Xia-Shang-Zhou Chronology Project Expert Group, 2022). Taking the Western Zhou period as an example, sequential samples were collected for radiocarbon dating from the cemeteries of Marquis Yan at Lulihe and the Jin State at Qucun of Tianma (天马曲村), which laid the foundation for constructing the chronology of the Western Zhou. While the boundaries were delineated in the Bayesian model, information from bronze inscriptions, historical documents, and astronomical calendars was also considered (Guo Z. et al., 2016). A detailed chronology of the Western Zhou kings before 841 BC in this context was given.

In this way, the project obtained sequential dates and established a final chronological framework of the Xia, Shang, and Western Zhou dynasties. The Xia dynasty was dated from ca. 2070 BC to 1600 BC and the Shang from ca. 1600 BC to 1046 BC, aligned with the same reigns of nine kings and the reign years of ten Western Zhou kings from Wu (武) to Li (厉) (Xia-Shang-Zhou Chronology Project Expert Group, 2022). Despite some debate and criticism, the project significantly promoted the development of China's radiocarbon dating technology, especially the AMS facility of Peking University, and profoundly changed the research paradigm of Chinese archeology. Soon after the completion of the Xia-Shang-Zhou Chronology project, another national project, "The Origins of Chinese Civilization", was put on the agenda, which has adopted the two fundamental principles of "government support and specialist responsibility" and "multidisciplinary research" since its inception in 2001.

4 Concluding remarks

Over half a century has passed since the first radiocarbon dating laboratory was set up and implemented in the winter of 1965, and significant achievements have been made as described previously. In the 1970s and 1980s, radiocarbon dating became the primary method for dating Chinese archeological discoveries to coordinate them with the infrastructure constructions across mainland China. The advent of radiocarbon dating laboratories occurred at just the right time when Chinese archeology was at the stage of constructing chronologies related to cultural development. Published radiocarbon data helped archeologists establish reliable chronicles of archeological cultures, especially the Neolithic and Bronze Age archeology, in different regions of mainland China. In this case, it would seem that the impact of radiocarbon dating on Chinese archeology was somewhat different from that of the West, where significant changes—the so-called radiocarbon revolution—were triggered by advances in the knowledge of antiquity and study of prehistory (Deniel, 1959).

Since the late 20th century, radiocarbon dating has continuously developed and undergone many revolutions. Advances in the precision of radiocarbon dating measurements and refinements in processing methods for separating and preparing carbon with regard to the specific deposit of interest have made it possible to date a variety of materials in samples weighing as little as tens of micrograms (Bronk Ramsey, 2008; Brock et al., 2010; Synal, 2013; Walker and Xu, 2018). Recent achievements in extracting specific biomarkers from absorbed food residues in pottery vessels have proven to be very helpful in providing clues to the absolute ages of the consumption of specific

foodstuffs, pottery utilization, and typochronologies (Casanova et al., 2020). In this case, improvements in sampling calibration methods and programs, as well as new calibration curves, confirm the increased potential for high-resolution chronological studies of archeological events (Reimer et al., 2020; Bronk Ramsey et al., 2021; Reimer, 2021). When comparing advances in radiocarbon dating between China and the West in the past decades, however, we have to recognize that there is a long way to go not only in the methodology of radiocarbon dating but also the breadth and depth of its applications in archeology. In the following section, some potentially fruitful research directions are recommended for future studies.

First, more radiocarbon dating laboratories are needed for archeological research. In recent years, over ten laboratories have been established by universities and institutions for radiocarbon dating, some of which have also purchased AMSs (Wu, 2021). However, most of these laboratories are targeted to the geosciences for measurement services. In contrast, only two laboratories are directly related to archeology, namely, the laboratories of the Institute of Archaeology of the Chinese Academy of Social Sciences and Peking University. This limited number of laboratories and specialists needs to be increased to meet the demands of archeological research for radiocarbon dating in China. Therefore, it is necessary to increase the number of laboratories for archeology, especially graphitization labs, the associated facilities, and trained technicians.

Second, there is still a big gap between China and the West in methodology. Radiocarbon dating in China has gradually developed by learning from the West since the 1960s. It is considered a tool for dating antiquities, and there needs to be more exploration in sample preparation and radiocarbon measurement. Therefore, it is necessary to carry out research on isolating and purifying carbon from traditional archeological materials such as bones, mollusks, charred seeds, wood, and charcoal but also from specific compounds and biomarkers. To constantly improve the quality of graphitization and efficiency and precision of testing, it will be necessary to maintain frequent exchanges with the international radiocarbon dating communities.

Third, close cooperation is desirable between specialists in radiocarbon dating and field archeology to address archeological questions. The success of radiocarbon dating studies depends on the optimization of material sampling. Sampling strategies should be designed to answer specific questions, which calls for cooperation of researchers in laboratories and archeologists in the field to discuss the expected results and possible limitations of the methodology of the dating project. To obtain a refined dataset, Bayesian modeling is often applied with the help of independently available information as the

preconditions for the chronology model. As a result, instead of waiting in the laboratories, radiocarbon specialists must be engaged in field work for a deeper understanding of the taphonomy of sampled archeological materials to establish a proper chronological model.

Last, radiocarbon dating research projects of Chinese archeology mainly focus on prehistory and Bronze Age, while only a few publications (e.g., Guo Q. et al., 2016) pay attention to the chronological studies of historical archeological issues. Therefore, well-designed and planned investigations are needed for more detailed specific issues of historic archeology besides prehistoric chronicles.

Author contributions

CX conceived the project, collected and analyzed the literature, and wrote the manuscript.

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Conflict of interest

The author declares 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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The earliest evidence of domestic chickens in the Japanese Archipelago

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The chicken (*Gallus gallus domesticus*) is the most conventional domestic animal whose main ancestor is the red junglefowl, found in Southeastern Asia and the southern part of China. Chickens were believed to have been brought to the Japanese Archipelago through the Korean Peninsula during the Yayoi period, but its exact age is unknown. Based on the sexual dimorphism of morphology, we pointed out that most chickens in the Yayoi period were males and that they were rarely bred in Japanese Archipelago. During the 58th survey of the Karako-Kagi site (Tawaramoto Town, Nara Prefecture), four pieces of immature Phasianidae bone were excavated from a division groove dating from the early middle Yayoi period. In this study, we performed collagen peptide fingerprinting identification and radiocarbon dating of immature Phasianidae bones from the Karako-Kagi site. Consequently, two peptide mass peaks unique to chickens were observed in samples from the immature bones, which were revealed to be derived from immature chickens. The calibrated age of the sample was confirmed to be between the fourth and third century BCE, which coincided with the opening age of the division groove. These results suggest that chickens have been successively bred since the beginning of the middle Yayoi period, at least in the Karako-Kagi village. The date was regarded as the lower limit for the introduction of chickens into the Japanese Archipelago, Korean Peninsula, and East Asia.

KEYWORDS

collagen peptide fingerprinting, domestic chicken, Japan, radiocarbon dating, ZooMS

1 Introduction

Estimated at more than 33 billion birds, the domestic chicken (*Gallus gallus domesticus*) is the most common livestock in the world (Robinson et al., 2014; Lawler, 2015; FAO, 2022). It is bred in all continents except Antarctica and in all countries except the Vatican City. According to the latest genome research, its main ancestor is a subspecies of the red junglefowl (*G. g. spadiceus*), which is distributed in Northern Thailand, Myanmar, and Southwestern China and is thought to have interbred with other subspecies of the red junglefowl and other *Gallus* fowls in the process of domestication (Wang et al., 2020). The common ancestor of the subspecies of the red junglefowl and domestic chickens is estimated to have diverged approximately 12,800 to 6,200 years ago (Wang et al., 2020). It has been clarified that chicken bones reported from Chinese and European sites during the early and middle Holocene are often misidentified or belong to a later age (Eda et al., 2016b; Peters et al., 2016; Best et al., 2022; Peters et al., 2022). How chickens have spread around the world is still being debated (Zeuner, 1963; Xiang et al., 2014; Eda et al., 2016b; Peters et al., 2016; Eda, 2021; Peters et al., 2022).

Historical records indicate that chicken exploitation in ancient China had begun by 641 BCE at the latest (Yuan, 2010). This is based on the mention of “six livestock” including horses, cattle, sheep, pigs, dogs, and chickens in an ancient Chinese historical narrative history, “Zuo Zhuan, 19th year of Xi Gong” (=641 BCE). Meanwhile, 14 sites (seven in China, six in Japan, and one in Mongolia) were shown as early sites where red junglefowl/domestic chicken existed in East Asia in the recent review by Peters et al. (2022). Among these, the oldest bones were found in Yinxu and Dasikongcun (Henan Province, Late Shang Dynasty, 1,320–1046 BCE) (Ma et al., 1955; Hou, 1989). However, both were evidently older than 641 BCE. Although Peters et al. (2022) regarded those records as acceptable, none of the bones were directly dated and could have belonged to a later age. The two chicken skeletons found in Dasikongcun were chronologically reliable as they were found in royal tombs of the late Shang dynasty (Ma et al., 1955). However, there was no explanation for the bone identification and no figures of bones. As far as we know, no reliably identified domestic chicken bones have been directly dated in the East Asia outside of these two sites, and the date of chicken introduction to the eastern regions, such as the Korean Peninsula and Japanese Archipelago, is not well known.

Chickens are thought to have been introduced to Japanese Archipelago from the Chinese continent and the Korean peninsula (Nishimoto, 1993; Eda, 2018). In Japan, Peters et al. (2022) identified six sites in the Yayoi period as early habitats of the red junglefowl/domestic chicken. Additionally, chicken and candidate chicken bones were found in the Karako-Kagi site, Tawaramoto Town, Nara Prefecture, Japan (Eda et al., 2016a). These bones were dated to be from the beginning of the middle Yayoi period (the late fourth to early third century BCE) and are regarded as the oldest in the Japanese Archipelago (Eda, 2018). On the site, one reliably identified chicken tarsometatarsus and four pieces (a femur and three elements of the pelvis) of immature Phasianidae bones were found (Eda et al., 2016a). As the successive breeding of chickens was questioned by the male-biased sex ratio in the Yayoi Period (Eda, 2016; Eda, 2018), the identification of immature Phasianidae bones was required. However, morphological discrimination criteria for immature chicken bones from Japanese wild indigenous pheasants (green pheasant (*Phasianus versicolor*) and copper pheasants (*Syrnaticus soemmerringii*)) have not been established.

Zooarchaeology by mass spectrometry (ZooMS) (Buckley et al., 2010) has been a rapidly evolving approach in the last decade (Richter et al., 2022). Some of the advantages of using bone collagen instead of DNA for analyzing archaeological samples include a higher extraction rate, lower risk of contamination, smaller sample, and lower cost (Buckley et al., 2010), although the risk of contamination is currently widely recognized (Hendy et al., 2018). The ZooMS approach allows the identification of zooarchaeological Phasianidae bones from Japanese archaeological sites (Eda et al., 2020). Eda et al. (2020) revealed that modern chickens and Japanese wild indigenous pheasants showed different peptide mass peaks and that these were useful to the differentiation of chicken bones from those of wild pheasants.

To determine the timing of chicken dispersal in the Japanese Archipelago, this study conducted direct radiocarbon dating of a chicken bone from the Karako-Kagi site. Prior to the dating, we used the ZooMS approach to identify immature Phasianidae bones and explore the possibility of successive breeding during the Yayoi period. The date was regarded as the lower limit for the introduction of chickens into the Japanese Archipelago, Korean Peninsula, and East Asia.

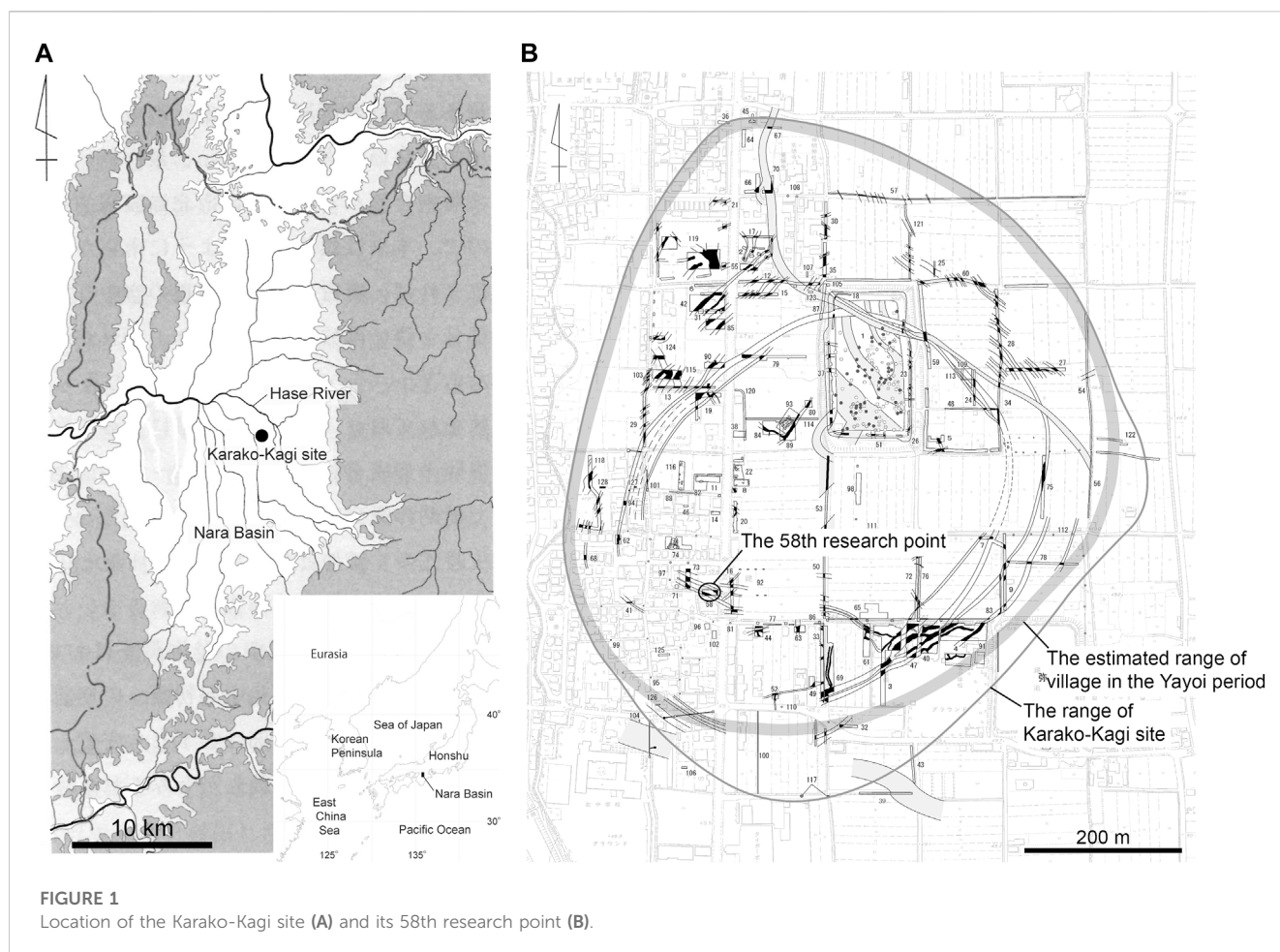
2 Materials and methods

2.1 Archaeological samples

The Karako-Kagi site is a settlement from the Yayoi period (from ~fifth century BCE to ~second century CE), surrounded by multiple moats and located at the center of the Nara Basin, on an alluvial area at 48–50 m altitude (Fujita, 2019) (Figure 1). The area of the site is estimated to be ~420,000 m² (650 m in the east and west, 750 m in the north and south). It is presumed that the mainstream and tributaries of the Hase River flowed northeast and southwest of the village and that branched-out smaller rivers flowed near the village and into moats. Many artifacts, including earthenware, woodenware, and metalware, as well as the remains of large buildings, have been excavated. Some of the artifacts were brought in from remote regions (more than 500 km away), suggesting that a wide range of exchanges had already occurred. Based on these characteristics, it is considered that the Karako-Kagi village was a settlement that played the role of a leader of the Kinki region (Fujita, 2019).

The 58th research point of the Karako-Kagi site is located in the western part of the site (Eda et al., 2016a). The research area of the point was 138 m² (20 m in the east and west, 6.6–7.6 m in the north and south) and provided 180 containers of artifacts and natural remains from the early Yayoi period and the Middle Age. Most of the animal remains were found in a division groove (SD-106 and SD-106 B) and were assumed to date back to the Yayoi period, based on the accompanying pottery that belonged to the same period. Carp (*Cyprinus carpio*), frog (*Anura*), pheasant/fowl (Phasianidae) including domestic chicken and green pheasant, duck (*Anatidae*), passerine (*Passeriformes*), Japanese hare (*Lepus brachyurus*), rodent (*Muridae*) including large Japanese field mouse (*Apodemus speciosus*), red fox (*Vulpes*), marten (*Martes melampus*), boar (*Sus scrofa*), and sika deer (*Cervus nippon*) were identified based on 194 bones (Eda et al., 2016a). Of these animals, mammals were predominant—especially boars, which may have included domestic ones—and most were considered for meat.

In total, 10 bones of Phasianidae were found at the point: nine from a division groove, SD-106, and one from waste soil (Eda et al., 2016a). The 15 m-long groove, confirmed at the southwest corner of the 58th research point, is presumed to extend outside the excavation area. It is interpreted that the groove was dug up twice: the first (SD-106 B) being ~4.0 m wide and 1.3 m deep, and the second (SD-106) being ~3.0 m wide and 0.9 m deep. The opening age of the groove was estimated based on the accompanying pottery: mainly Yamato II-1 and II-2 types for the former, and mainly Yamato II-3 type for the latter. According to Eda and Inoué (2011), three of the Phasianidae bones were identified as belonging to a lower taxonomic level: a tarsometatarsus with the medial plantar crest from SD-106 was identified as domestic chicken, a femur with grater trochanter foramina from waste soil was recognized as green pheasant, and an immature femur without grater trochanter foramina (Figure 2.1) from SD-106 was confirmed as chicken/copper pheasant. Other seven Phasianidae bones, including three immature unfused bone elements of Phasianidae pelvis (Figure 2.2), were also found, but it was impossible to ascertain whether they were derived from chickens or wild pheasants. The immature femur and a piece of the pelvis were collected for analysis. For the ZooMS analysis, bone powder (~1 mg) was sampled in an ancient biomolecule laboratory at the Hokkaido University Museum using sterilized powder-free nitrile gloves, and dental drills cleaned with hydrochloric acid (HCl) and distilled water. Radiocarbon



dating was conducted on the immature femur by taking ~100 mg of bone powder to a clean room at the University Museum of the University of Tokyo.

2.2 ZooMS analysis

Isolation and digestion of collagen peptides were conducted as described in [Eda et al. \(2020\)](#) in an ancient biomolecule laboratory at the Hokkaido University Museum. In brief, the bone powder was demineralized with HCl, the acid-insoluble pellet was gelatinized by heating in ammonium bicarbonate, and the gelatinized sample was digested with sequencing-grade trypsin. Following the digestion, the supernatant was acidified with trifluoroacetic acid and desalinated using a C18 ZipTip. The sample solution was spotted onto a target plate and mixed with α -cyano-4-hydroxycinnamic acid matrix solution. Fractions of each collagen digest were analyzed using an UltrafleXtreme mass spectrometer (Bruker, Billerica, MA, United States) at the Central Institute of Isotope Science, Hokkaido University. [Eda et al. \(2020\)](#) revealed that chickens and red junglefowls have peaks of 1604.8 m/z (1+; GDPGPVGPVGPAGAFGPR) and frequently 1620.8 m/z (1+; GDP*GPVGPVGPAGAFGPR, in which * shows *postmortem* oxidation), while green and copper

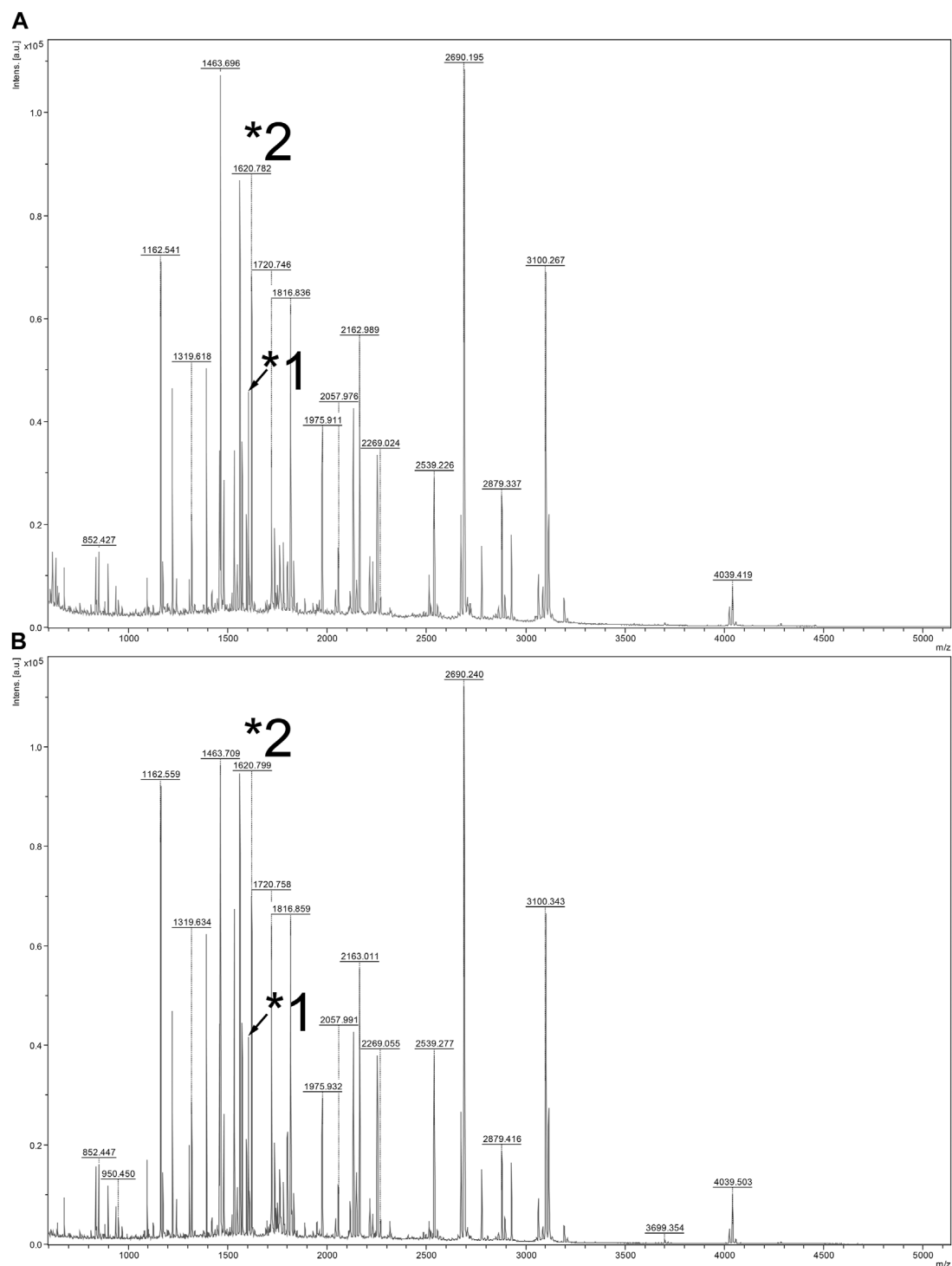


FIGURE 3
Time-of-flight mass spectrometry profile of two samples from the Karako-Kagi site [(A): left femur and (B) right pelvis]. Note that both samples were identified as chicken due to the presence of two peaks (*1 and *2).

pheasants have a peak of 1578.8 m/z (1+; GDPGPVGAVGPAGAFGPR) because of an amino acid substitution. Discrimination of domestic chicken/red junglefowl and Japanese wild pheasant was conducted based on the presence or absence of these biomarkers. Peaks within ± 0.2 m/z were considered to be the same.

2.3 Radiocarbon dating

To measure ^{14}C , collagen was prepared using a modified Longin's method (Longin, 1971; Yoneda et al., 2002) and graphitized using the methods described by Omori et al. (2017). An elemental analyzer (Vario

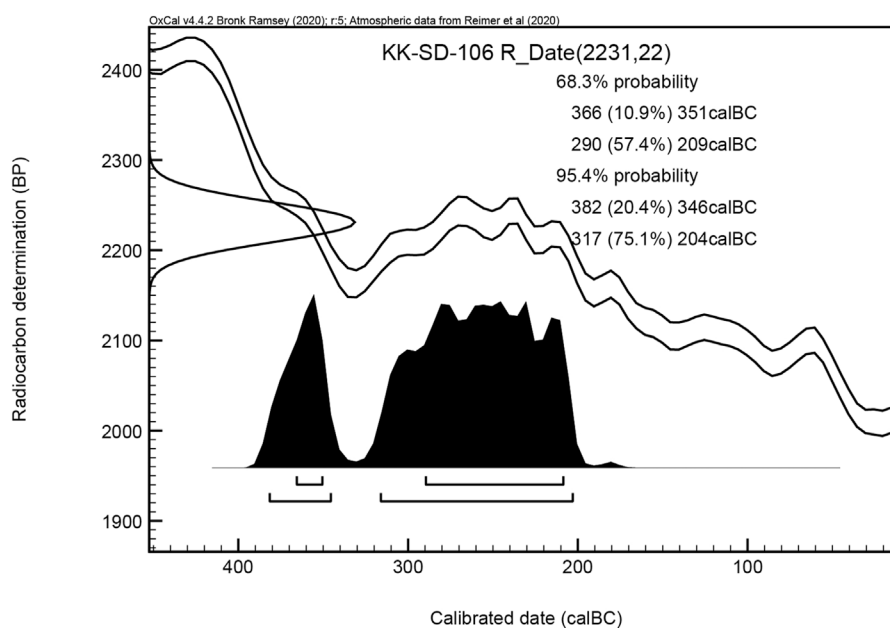


FIGURE 4
Calibrated ^{14}C date of collagen from immature chicken bone collected from the Karako-Kagi site.

ISOTOPE select, Elementar Analysensysteme GmbH) was used to combust the samples and isolate pure CO_2 from the combusted gas (Omori et al., 2017). Graphite was then produced by the catalytic reduction of the sample CO_2 with H_2 gas and Fe powder. The radiocarbon content of the graphite was measured using an accelerator mass spectrometer (AMS) at the University Museum of the University of Tokyo. The radiocarbon dates were calibrated using OxCal4.2 software (Bronk Ramsey, 2009) and IntCal20 calibration curves (Reimer et al., 2020).

3 Results

3.1 ZooMS analysis

Peaks of 1604.8 and 1620.8 rather than 1578.8 were observed in both the immature femur and pelvis (Figure 3, Supporting Material). A comparison of these peaks with those from reference modern Phasianidae specimens showed that these immature samples from the Karako-Kagi site were derived from chickens.

3.2 Radiocarbon dating

Carbon and nitrogen concentrations (weight %) of extracted collagen were 42.4% and 14.6%, respectively, showing a good agreement of its atomic C/N ratio of 3.4 with a biological range between 2.9 and 3.6 (DeNiro, 1985). The ^{14}C age of the immature femur was determined to be $2,231 \pm 22$ BP. After calibration, the age was calculated at 381–204 BCE (95.4%) (Figure 4), corresponding to the middle Yayoi period as assigned to its archaeological context (Fujio, 2013).

4 Discussion

The ZooMS analysis identified two immature Phasianidae bones from the Karako-Kagi site as chicken/red junglefowl based on two biomarkers. The radiocarbon dating clearly showed that the immature femur dated between the fourth and third century BCE, which is consistent with the chronological feature of the accompanied pottery (the late fourth to early third century BCE) (Fujita, 2019). This suggests that the bone is not intrusive from a later age. To the best of our knowledge, this is the first case of direct radiocarbon dating of a chicken bone from a Japanese and East Asian archaeological site. Furthermore, the immature chicken femur was found in a stratigraphic layer from the middle Yayoi period, which is the oldest stratigraphic context for chicken bone findings (Eda, 2018). As mentioned in the Introduction, chickens were perhaps introduced to Japanese Archipelago from the Chinese continent and the Korean peninsula (Nishimoto, 1993; Eda, 2018). Therefore, the estimated age, fourth to third century BCE, is regarded as the lower limit for their introduction onto the Japanese Archipelago and East Asia, especially the Korean Peninsula.

Thus far, most of the confirmed or candidate chicken (10 out of 11) bones in the Yayoi period were identified as male parts (Eda et al., 2016a; Eda, 2016), suggesting that chickens could not have been reproduced in most of the Japanese Archipelago (Eda, 2018). However, the existence of an immature chicken at the Karako-Kagi site during the middle Yayoi period (fourth to third century BCE) suggests that domestic chickens were successively bred and not only male but also female chickens were brought into the Karako-Kagi village. The finding seems insufficient to consider that the successive breeding of domestic chickens was popular during the Yayoi period because of the unique characteristics of the Karako-Kagi site. The village is considered to have been one of the largest during the Yayoi period, and it flourished the most in the middle of this era (Fujita, 2019). At the Karako-Kagi site, various local pottery objects were found, such as

from the Totoumi (current Shizuoka Prefecture) and Shinano regions (current Nagano Prefecture) in the east and northern Kyushu in the west, which cover an area of 700 km. There were few central-hub village sites where a large number of such pottery artifacts have been found, suggesting that a distribution network to Karako-Kagi village had been established (Fujita, 2019). The successive breeding of domestic chickens during the Yayoi period could have only been possible in a powerful central-hub village such as the Karako-Kagi.

Hitherto, no morphological criteria have been established for distinguishing domestic chicken/red junglefowl bones from indigenous wild pheasants in East Asia. As the ZooMS approach used in this study was created to identify chickens from the middle size wild pheasant species in Japan (Eda et al., 2020), further studies of modern osteological specimens are necessary to identify materials from East Asia, excluding Japan. However, the approach is still effective in distinguishing between candidate chicken bones and non-chicken bones. Applying ancient DNA analysis to bones in which chicken-specific peaks were observed, it was possible to reliably identify chicken bones in East Asia.

Recent critical reviews of studies on the westward expansion of the domestic chicken (Best et al., 2022; Peters et al., 2022) revealed some intriguing patterns of the human-chicken relationship that differed from those observed for the Yayoi period. In many areas of Europe, such as Britain, Italy, and the Czech Republic, chickens appeared initially as skeletons buried individually, and then along with humans (Best et al., 2022). There is a consistent time lag between the introduction of chickens and their consumption by humans, suggesting that they were initially regarded as exotica and recognized as a source of food only several centuries later (Best et al., 2022). Chicken bones have been reported as burial goods in tombs or materials excavated from cemeteries at Dasikongcun (Late Shang Dynasty), Maojiaping (Gansu Province, spring and autumn Period, 770–476 BCE and Warring States Period, 476–221 BCE), Jiuliandun (Hubei Province, Warring States Period) and Shenmingpu (Henan Province, Early Han Dynasty, 202–141 BCE) in China (Peters et al., 2022). Further, a chicken skull was reported from an ash pit in Yinxu while six chicken bones were reported from settlement layers at the capital city of Zhu (Shandong Province, spring and autumn Period and Warring States Period). There are no records of chickens being buried alone in East Asia (Peters et al., 2022).

Although chickens in the Japanese Archipelago do not seem to have been recognized as a source of food in the Yayoi period (Nishimoto, 1993; Niimi, 2009), there are no reports of chickens that were individually buried or buried with humans, and none of the bones were found in any special context (Eda, 2018). Female chickens with medullary bone have been found from the early times in England, while juvenile bones have been found in Italy (Best et al., 2022). Therefore, the male-biased appearance, similar to the Japanese Yayoi period, has not been recognized in other parts of the world. It has also been pointed out that the introduction of chickens to the Western region happened around the same time as that of rice (*Oryza sativa*) (indica) and Chinese millets (*Panicum miliaceum* and *Setaria italica*) (Peters et al., 2022). However, the domestication or introduction of rice and Chinese millets in East Asia, including the Japanese Archipelago, would have occurred much earlier than that of the chicken. Rice (*japonica*) domestication was established by the fourth millennium BCE in

southern China while Chinese millets domestication was established by the sixth millennium BCE in northern China (Larson et al., 2014). These cereals were introduced to the Korean Peninsula in the middle second millennium BCE (Shoda, 2009), then to the Japanese Archipelago in the later Final Jomon Period (~10th century BCE) (Nakazawa, 2009). The difference between the introduction of chickens to the Western region and that to the Japanese Archipelago and East Asia is a topic for future research.

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.

Author contributions

ME designed the research; SF organized the samples; ME and HI performed the ZooMS analysis; MY performed the radiocarbon dating; ME wrote the draft, and all the authors approved the final version of the article.

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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.

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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.2023.1104535/full#supplementary-material>

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Bioarchaeological analysis of the human skeletal remains from cliff tomb burial of the Wangyuancun site in Leshan, Chengdu Plain, Southwest China

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Though archaeological and historical-document evidences for the cliff necropolises in ancient China were reported in literature, the bioarchaeological analysis for them is still absent. It is therefore the aim of this paper presents the first bioarchaeological analysis for the skeletal samples of cliff necropolises from the Iron Age in China. In this work, skeletal remains of 16 individuals (two males, three females, seven unsexed adults, one subadult, and three indeterminate sex and age individuals) of Wangyuancun site (on the border of the Chengdu Plain, China) were examined for the stature, dental pathologies, linear enamel hypoplasia, cribra orbitalia, degenerative osteoarthritis of the vertebrae and major joints, periostitis, trauma and other evidence of disease. The demographic structure of Wangyuancun site is characterized by a younger mean age at death and a low mortality rate for infants and children. The stature estimates show that these Iron Age people were similar in size compared with the people live in contemporary southwestern region of China. Most of the samples lacked indications of stress, but a few had cribra orbitalia, osteoarthritis, osteophytosis and various dental pathologies. Periosteal reactions were common, but the lesions are generally moderate or mild. These bioarchaeological results broaden our understanding of the health and lifestyle of the cliff tomb population of Chengdu Plain in the Iron Age. Our bioarchaeological examination of the skeletal samples of Wangyuancun site provides a rare opportunity to address questions concerning ancient people's diet, health, disease and stress of population in the Iron Age of Chengdu Plain, Southwest China.

KEYWORDS

bioarchaeology, palaeopathology, dental pathology, health, lifestyle, cliff tomb burial, Chengdu plain

1 Introduction

Bioarchaeological investigations provide important means to inform on the life experiences of past people and understand past population demographics, health and certain aspects of culture. (Katzenberg, 2008; Larsen, 2015). Bioarchaeology is currently experiencing growth in many regions of China, but there is still a big gap in the south, especially the southwest of China, where human bones are poorly preserved due to hot and humid climate and acidic soil conditions.

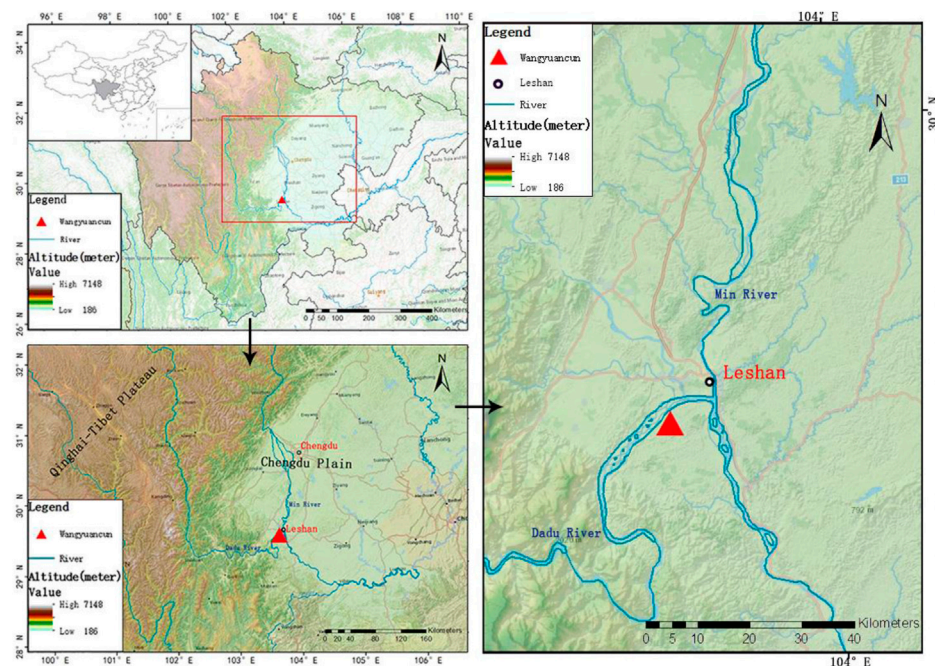


FIGURE 1
Map showing the location of Wangyuancun.

The Chengdu Plain is an alluvial plain located between the western Sichuan Basin and the southeastern edge of the Qinghai-Tibet Plateau, with an altitude of 400–750 m and an area of about 8,500 km² (Liang, et al., 2014), which is the largest plain in southwest China. This region has been the cultural and political center of the Sichuan Basin from prehistory to the present. In the pre-Qin period, Baodun culture in the late Neolithic age, Sanxingdui culture and Shierqiao culture in the Bronze age, etc. were born in this relatively independent geographical unit, which historians and archaeologists call ancient Shu civilization (Duan, 2011; Dianzeng, 2005; Xiang, 2018). In 316 B.C., Qin annexed Ba Shu (Qu et al., 1987), and since then the Chengdu Plain has been under the effective control of the central government. During the Han Dynasty, with the influx of immigrants, many cultural factors merged here and the burial system of the Chengdu Plain took on a complex and diverse appearance. It includes both traditional vertical shaft pits, which have been popular since the Warring States, and two new forms of burial, namely, brick chamber tombs distributed on flat dams and cliff tombs cut into the cliffs and slopes on both sides of rivers (Elias, 2019; Dehao, 2022). Thus far, only Zhang Jun, Yuan Haibing and other scholars have done research and reports related to physical anthropology on a few sites in this area (Zhang and Zhangyi, 2006; Yuan, 2016; Yuan et al., 2017). There have been few isotope studies that focus on archaeologically derived materials from the Chengdu Plain. Yi Bing and others (Yi, B., et al., 2018) conducted carbon and nitrogen isotopic analysis of human bone collagen (ribs and femora) as well as dentin serial sections from Gaoshan Ancient City site. Up to now, the general situation of the physical characteristics and health status of the ancient people in the Chengdu Plain is still unclear.

The cliff tomb is a kind of tomb with local characteristics that was widely popular in southwest China from the Han Dynasty to the Sixth Dynasty in about five hundred years, featuring multiple chambers buried deeply into the cliffs, along the Yangtze River and its larger tributaries, such as the Fu, Tuo and Min Rivers (Erhu, 1988). This type of tombs is distributed in a large number in Sichuan Province, especially in the Chengdu Plain. Only some sporadic historical documents have records about the cliff tomb, it is generally believed that this is the so-called “immortal cave” where ancient immortals practiced alchemy (Liu, 815 AD; Du, 933 AD). Ming et al., local chronicles believe that the caves were the residence of local barbarians, and it is called the Barbarian Cave (Cao, 1614 AD; Chang and Yang, 1816 AD; Gong).

The first Western explorer to draw attention to the caves was Alexander Wylie. He traveled through Sichuan in 1868, and subsequently reported on the caves to the Royal Geographical Society in London (Alexander, 1869). Subsequent Western explorers followed Wylie. Thus it was that by the turn of the 20th century, the cliff-side caves in Sichuan were almost unanimously understood to have been the residences of barbarians. Japanese anthropologist Torii Ryuzō was the first professional archaeologist who visit Sichuan in 1902. After investigation, Torii believed that these caves were not the residences of barbarians, but ancient Han tombs (Ryuzo, 1926), which aroused the attention of academic circles. Subsequently, many Chinese and foreign scholars conducted investigations and researches on the caves one after another. It is well-known since they were reported by many researchers in China and abroad in the first half of the early 20th century (Torrance, 1910; Ségalen, 1915; Bishop, 1916; Ségalen et al., 1923; Bedford, 1937; Shang, 1940; Moruo, 1941; Zhigao, 1942; Dekun, 1946).



FIGURE 2

(A), Site view; (B) Interior of the cliff tomb (M38); (C) Sarcophagi in the coffin chamber (M52).

The archaeological excavation of the cliff tombs can be traced back to 1941, when the Academia Sinica's Institute of History and Philology and the Construction Society excavated dozens of cliff tombs in Pengshan (Nanjing Museum, 1991). After the founding of New China, as a large number of cliff tombs were investigated and excavated, the scholars' understanding of cliff tombs gradually became clear. Researchers generally agreed that cliff tomb is distinct to the southwestern region of China, and that this type of burial practice became a custom form of burial in the region by the period of Eastern Han dynasty (Liu, et al., 2014; Cheng, 1957; Liu, 1958; Kyong-McClain, J. 2010). Each tomb is usually used as a whole functioned as a shared burial space for the family or lineage and may have been employed over several generations, and some of which were reused during Tang-Song Period and even later (Xiaoliang Ma, 2012; Fei et al., 2016; Jiang, et al., 2022). These cliff tombs provided invaluable materials for the reconstruction of the tomb system, ideology, social economy, art, architecture and history in the region during this period (Erhu, 1988).

Numerous tombs of this type across the Leshan area on the southwestern edge of the Chengdu Plain (in the lower valley of the Min River), which is the most densely distributed, largest and most representative area in Sichuan have been published in architectural and archaeological surveys (Fuhua and Dan, 1956; Xiang, 2003; National Cultural Heritage Administration, 2009; Sichuan Province Institute of Cultural Relics and Archaeology, Sichuan Grotto Temple Conservation Research Institute, and Leshan Giant Buddha Grottoes Research Institute, 2022). However, there have been no study of bioarchaeology based on skeletal analysis, and we even

know nothing about the people buried here themselves. Because graves were often (re-)used over long periods, coupled with the hot and humid climate and acidic soil in the Sichuan Basin, most of the human bones in the cliff tombs are difficult to preserve completely. The skeletal samples were very scarce, there was little opportunity to directly investigate the physical characteristics, health, and lifestyle of the people in cliff tombs. In order to get a clear picture of life quality and amount of physical stress of people in cliff tombs, in this work a comprehensive bioarchaeological study was conducted on the skeletal remains that were excavated from the Wangyuancun site near Leshan in the confluence of the Minjiang River and the Dadu River (Figure 1).

In the rest of this article, Section 2 will describe materials and methods used in this study. In Section 3, bioarchaeological results for Wangyuancun site will be shown in detail. And based on these results, a comparative study of demography, stature, oral health and dietary patterns, as well as physiological stress and health of ancient population in Wangyuancun site will be discussed in Section 4. Finally, concluding remarks will be made in Section 5.

2 Materials and methods

2.1 The site and skeletal samples

The cliff tomb group of Wangyuancun site (abbreviation as WYC) is located in the north of a village with the same name, Angu

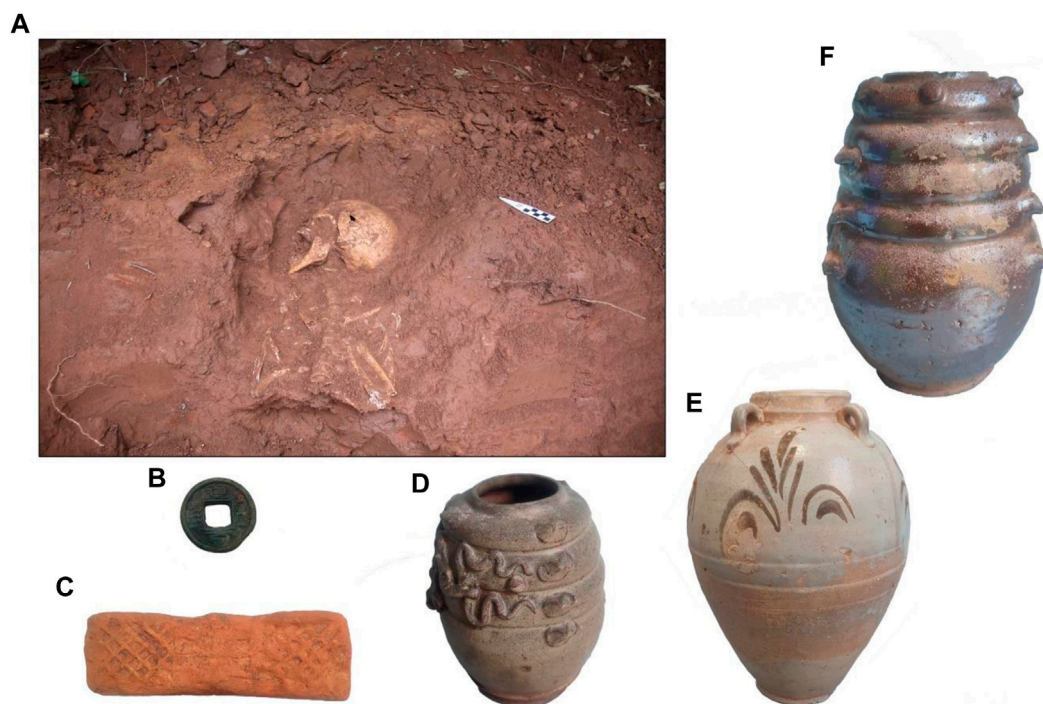


FIGURE 3

(A) Burial of 47 showing the human bone preservation; (B)–(F) Some artifacts from Cliff Tombs.

Town, Leshan City, Sichuan province (Figure 1). It is situated in a typical hilly terrain, at an average altitude of 351 m. Since the 1950s, multiple institutions carried out a number of fieldworks on it. This cemetery contains 136 independent tombs carved into the side of a hill covering a range of approximately 580 m (National Cultural Heritage Administration, 2009). As at other sites in Sichuan province, these tombs are organized as groups laid out in horizontal layers along the upper reaches of the hills. The first excavation of this site was undertaken under the direction of one of the authors (Guolin JIN) in 2021, to cooperate with the construction of the Leshan-Xichang Expressway project. A total of 29 cliff tombs were cleared in this excavation, most of which were multi-chamber tombs of medium scale. The structure of the tomb is generally composed of tomb passages, tomb doors, corridors, front halls, main chamber or sacrificial chamber, coffin chambers, niches, etc. Three types of coffins, including stone sarcophagus, clay coffin, and wooden coffin, were found in different combinations in different tombs. Because most of the tombs were disturbed, the clay and wooden coffins were incomplete and human bones were scattered throughout the chambers (Figures 2, 3).

No radiocarbon dates have been carried out at this site, however, the burial objects showed that the tombs were erected around the East Han dynasty and then used for burial roughly more than one thousand years. Artifacts found with the WYC burials included coins from the Han Dynasty and Shu-Han Dynasty, potteries from the Han Dynasty, and porcelains from the Tang and Song Dynasties. Most tombs were empty, with no traces of skeletal materials, four graves (M54, M68, M71, and M73) contained at least one skeleton, and three graves (M38, M47, and M52) contained remains of more

than three individuals. The skeletons were studied in the bioarchaeological laboratory at the Sichuan Province Institute of Cultural Relics and Archaeology in 2022.

The samples used consisted of at least 16 individuals (Table 1). During the excavation and retrieval of the commingled remains, we created secondary context by labeling skeletal elements according to the position in which they were discovered, thus allows as many skeletal elements as possible to be assigned to possible individuals. The skeletons in the burial were mostly disturbed, most of the human bones in the cliff tombs are difficult to preserve completely. Overall, the preservation condition of skeletons was not very good, some of them were fragmentary or incomplete. All human remains were cleaned with soft brushes and in some cases also with wooden or metal sticks to minimize post-excavation damages. Preservation and reconstruction were performed whenever necessary.

2.2 Methods

To gain an understanding of the health and living conditions of the WYC population, data on age, sex, osteometrics, and pathological conditions were collected on each burial. (Buikstra and Ubelaker, 1994).

Because the skeletons in the burial were mostly disturbed, the techniques used to sort the commingled remains were also used to assign these remains to the primary context skeletons. These techniques included a combination of the physical anthropological analysis and visual pair matching on the basis of bone morphology and taphonomy. The analysis of the remains

TABLE 1 Skeletal samples used in this study.

Grave	MNI	MNI based on	Specimen no	Description	Sex	Age	Stature	Pathological condition
M38	4	femur	M38:R1	relatively complete skull; few broken vertebrae and ribs; incomplete limb bones	M	30–35	Yes	carious lesion, periodontal disease, calculus, periapical abscesses, AMTL, cribra orbitalia, maxillary sinusitis
			M38:R2	Clavicles, scapulas; incomplete limb bones; bilateral -calcaneus; bilateral -talus	Ind	Adult	—	PR (bilateral-tibiae)
			M38:R3	a few fragments of limb bones	Ind	Adult	—	—
			M38:R4	Only one teeth and a few tiny bone fragments	Ind	Adult	—	—
M47	5	femur	M47:R1	relatively complete skull; incomplete postcranial bones	F	30±	Yes	calculus, periodontal disease, mild OA (R scapulae, bilateral-auricular surface)
			M47:R2	Incomplete skull and postcranial bones	M?	30–40	—	calculus, periodontal disease, AMTL, PR (bilateral-femur, L ulna), OA (R mandibular condyle), fracture (R radius)
			M47:R3	Incomplete bilateral hip bones and bilateral femurs	F?	Adult	—	PR (bilateral-femur), OA (bilateral hips, R knee)
			M47:R4	A few tiny bone fragments	Ind	Ind	—	—
			M47:R5	A few tiny bone fragments	Ind	Ind	—	—
M52	3	Teeth and wear	M52:R1	13 teeth only	Ind	20–25	—	—
			M52:R2	4 teeth only	-	7–10	—	—
			M52:R3	Only one tooth	Ind	25–30	—	—
M54	1		M54	Right tibial stump and few fragments	Ind	Ind	—	—
M68	1		M68	Incomplete skull; pelvis and clavicle; fragment of limb bones	F?	28–36	—	carious lesion, calculus, periodontal disease, AMTL, PR (bilateral-femur, bilateral-tibia)
M71	1		M71	Fragment of hips and limb bones	Ind	Adult	—	PR (bilateral-tibia, L fibula), OA (L hip)
M73	1		M73	Small fragment of femur	Ind	Adult	—	—

F, female; M, male; Ind, indeterminate sex or age; PR, periosteal reaction; OA, osteoarthritis; AMTL, antemortem tooth loss.

TABLE 2 Stature estimation for adults from WYC.

Individual (sex)	Side/bone	Measurement (mm)	Formula	Stature (cm)	Stature method
M38:R1(M)	Right humerus	309	$751.77 + 2.88 (309) \pm 44.24$	164.2	Xiangqing (1984)
M47:R1(F)	Left radius	196	$65.720 + 4.721 (196)$	149.1	Zhang, (2001)

entailed a full standard physical anthropological analysis, and the “Standards for Data Collection from Human Skeletal Remains” by Buikstra and Ubelaker (1994) was used as a basis to document this information.

The age-at-death of adults was estimated using the symphysis pubis, auricular surface of the ilium, sternal end of the rib, cranial suture closure, and (when necessary) tooth wear (Brothwell, 1981; Buikstra and Ubelaker, 1994). For immature individuals, approximate age was estimated based on dental development and epiphyseal closure (Scheuer and Black, 2004). The sex estimation of the individuals was carried out by macroscopic assessments of the pelvis and skull (Buikstra and Ubelaker, 1994; Ubelaker, 1999). As

the estimation of sex is known to be somewhat unreliable in the immature skeleton, no attempt was made to estimate sex for the subadult individuals.

Preservation of the long bones dictated which bone was used to estimate stature. Measurement maximum length of complete long bones after Martin (1914) was taken, and the stature was calculated following the formulas proposed for “Chinese” by Shao Xiangqing and Zhang jizong (xiangqing SHAO, 1984; ZHANG, 2001).

Each bone or tooth from the WYC sample was examined for the presence of pathological conditions, using macroscopic and non-destructive techniques. Special attention was paid to the identification of dental diseases, periostitis, degenerative joint

TABLE 3 Demography and dental sample summary for the WYC skeletal assemblage.

	Females	Males	Indeterminate sex	Total
N	2	2	0	4
Alveoli	46	42	0	88
Alveoli/N	23	21	—	22
N	2	2	4	8
Teeth	31	28	15	74
Teeth/N	15.5	14	3.75	9.125
Teeth/Alveoli	0.652	0.333	—	0.830

N, total number of individuals assessed.

diseases, cribra orbitalia, porotic hyperostosis, and traumatic injuries. Diagnoses were made according to the criteria outlined by Ortner (2003), Mann and Hunt (2013).

For the assessments of the oral conditions, the teeth were observed macroscopically with both fluorescent and incandescent lighting and a magnifying light when necessary. All pathologies were analyzed and presented by teeth and alveoli. The following variables were focused on: caries, calculus, antemortem tooth loss (AMTL), periodontal disease, abscesses and Dental enamel hypoplasia (DEH).

Periostitis, a non-specific indicator of infectious diseases, though it becomes increasingly rare in the present day, is one of the most commonly encountered abnormalities in archaeological samples, prior to the discovery of antibiotics and their use as a medical treatment modality (Ortner, 2003). The presence of periostitis has been reported worldwide as an important health marker of archaeological skeletons. Periostitis typically manifests as “fine pitting, longitudinal striations, and eventual formation of plaque-like new bone” (Roberts and Manchester, 2007; Larsen, 2015). We report frequencies of infection for the following postcranial bones: humerus, ulna, radius, femur, tibia, and fibula.

Degenerative joint disease (DJD), or osteoarthritis, is characterized by the progressive formation of bone spurs or lipping of the articular joint surfaces (Buikstra and Ubelaker, 1994;

Ortner, 2003). These changes are associated with the normal aging process. Identified osteoarthritis through the presence of exophytic growth on the joint margins and erosion on joint surfaces (Larsen, 2015; Waldron 2009). A positive diagnosis of osteoarthritis was given based on the presence of porosities, lipping, and eburnation. Analysis of degenerative joint disease within the WYC population focused on the six major appendicular joints (shoulder, elbow, hip, knee, wrist and ankle) implementing recommendations from the “Standards for Data Collection from Human Skeletal Remains” (Buikstra and Ubelaker, 1994) for arthritis pathology. DJD of the temporomandibular articular surface (TMAS) and sacroiliac joint was also recorded in this study.

Because sample sizes were generally small, Fisher’s Exact Test (FET) was used to test for statistical significance of categorical (discontinuous) data (Thomas 1986). All FET statistics were calculated with a significance level of $\delta = 0.05$ (two-tailed).

3 Results

3.1 Demography

The minimum number of individuals was determined by the visual pair matching of skeletal elements on the basis of similarities observed in bone morphology, age and sex, trauma and pathology and taphonomic alterations. Based on counts of skeletal elements, there is a minimum of 16 individuals. The age and sex distributions of the WYC burials are provided in Table 1. Three individuals are too fragmentary for precise age and sex estimation. Of the individuals over 18 years of age for whom sex could be determined, two are males or probable males, three are females or probable female, and seven lacked skeletal markers useful for sex estimation.

3.2 Estimated stature

Due to poor preservation of the skeleton, only two adult individuals of definable sex have well-preserved limb bones. The

TABLE 4 Dental pathology in the WYC collection.

	Tooth count					Individual count			
	A/O (%)					A/O (%)			
	Male	Female	Sex diff. <i>p</i> -value	Indeterminate Sex	Total	Male	Female	Indeterminate Sex	Total
Dental caries	2/28 (7.14)	2/31 (6.45)	1.000	0/15 (0.00)	4/74 (5.41)	1/2 (50.00)	1/2 (50.00)	0/4	2/8 (25.00)
Periodontal disease	3/28 (10.71)	7/31 (22.58)	0.306	0/0 (-)	10/59 (16.95)	2/2 (100.00)	2/2 (100.00)	0/0	4/4 (100.00)
Calculus	27/28 (96.43)	31/31 (100)	0.475	0/15 (0.00)	58/74 (78.38)	2/2 (100.00)	2/2 (100.00)	0/4	4/8 (50.00)
AMTL	5/42 (11.91)	2/46 (4.35)	0.251	0/0 (-)	7/88 (7.95)	2/2 (100.00)	1/2 (100.00)	0/0	3/4 (75.00)
Alveolar abscess	1/42 (2.38)	0/46 (0.00)	0.477	0/0 (-)	1/88 (1.14)	1/2 (50.00)	0/2 (0.00)	0/0	1/4 (25.00)
DEH	0/28 (0.00)	0/31 (0.00)		0/15 (0.00)	0/74 (0.00)	0/2 (0.00)	0/2 (0.00)	0/4	0/8 (0.00)

A/O, affected/observed; AMTL, antemortem tooth loss; DEH, dental enamel hypoplasia.
p is the probability of Fisher’s exact test difference from sex.

TABLE 5 Counts and prevalence rates by element of periosteal reaction in the WYC collection.

	Male		Female		Indeterminate sex		Total	
	A/O	Freq	A/O	Freq	A/O	Freq	A/O	Freq
Femur	2/4	50%	4/5	80%	0/7	0%	6/16	37.5%
Tibia	0/0	—	2/2	100%	4/8	50%	6/10	60%
Fibula	0/0	—	0/1	0	1/3	33.33%	1/4	25%
Humerus	0/4	0	0/4	0	0/2	0	0/10	0
Ulna	1/4	25%	0/4	0	0/0	0	1/8	12.5%
Radius	0/3	0	0/3	0	0/	0	0/6	0
Total	3/15	20%	6/19	31.58%	5/20	25%	14/54	25.93%

A, number of affected long bones; O, number of observed long bones; Freq, frequency (%).

stature of the WYC people was estimated from the maximum length of the long bones, as summarized in Table 2. Stature of male (M38:R1) is assessed as approximately 164.2 cm from the maximum length of the right humerus, using Shao Xiangqing’s stature formulae (Xiangqing, 1984), and the stature of female (M47:R1) is 149.1 cm from the maximum length of the left radius, using Zhang Jizong’s stature formulae (Zhang, 2001). Male stature was about 15 cm higher than female stature.

3.3 Paleopathology

3.3.1 Dental pathologies

Teeth generally survive well and provide an important source of information about dental diseases. A summary of the demographic information, the number of individuals, and the tooth/alveoli analysis, is presented for the samples in Table 3, and the frequencies of oral diseases are shown in Table 4. Some photos for examples of dental diseases are shown in Figure 4.

The prevalence of caries is based on both the *insitu* teeth in the four skulls and the loose teeth from four individuals without skulls. The prevalence rate of WYC is 25% (2/8), and the frequency of caries is present in 5.41% (4/74) of the analyzed teeth (Figure 2). Carious lesions are not present among subadults while the frequency is similar for both sexes (7.14% vs. 6.45%), the difference is not statistically significant ($p = 1$). All the 4 affected teeth were mandibular molars, including 2 first molars, 1 s M and 1 third molar. Molars have significantly more caries than non-molars. From the location of lesions, the diseased parts are all at the cemento-enamel junction (CEJ), and the degree is relatively mild (Grade 1–2).

AMTL is present in 3 individuals (M38:R1, M47:R2, M68) out of 4 observables, or 7 of 88 teeth (8%). Among the 7 lost teeth, 3 were the mandibular middle incisors, 2 were the first premolars, and one each was the second M and the third molar. AMTL is present in 11.91% of the male and 4.35% of the female - the difference is not statistically significant ($p = 0.251$). It is worth noting that one of the AMTL incisor teeth (M38:R1) that had no sign of lesions around it, which is speculated to be caused by intentional tooth extraction or long-term use of the tooth as a tool.

Of the 4 individuals available for observation, all had significant periodontal disease. Considering only the adults, 59 teeth were examined, and 16.95% (10/59) had periodontal disease. Periodontal disease is present in 10.71% of the male and 22.58% of the females, and the difference is not statistically significant ($p = 0.306$).

Calculus is relatively common and is present in four out of eight individuals, or 50%. Calculus is present in 96.43% of the male and 100% of the female—the difference is not statistically significant ($p = 0.475$). All cases are of the supragingival type.

Furthermore, the series exhibit a low prevalence of periapical abscesses. 88 teeth and alveoli were available for analyzing, only one maxillary premolar (from an adult male, M38:R1) exhibits periapical abscesses. And there is no enamel hypoplasia on the 72 examinable permanent teeth.

Overall, the oral health condition of WYC people is characterized by higher incidence of calculus (78.38%) and periodontitis (16.95%), lower incidence of dental caries (5.41%), AMTL (7.95%), alveolar abscess (1.14%), and absence of enamel hypoplasia.

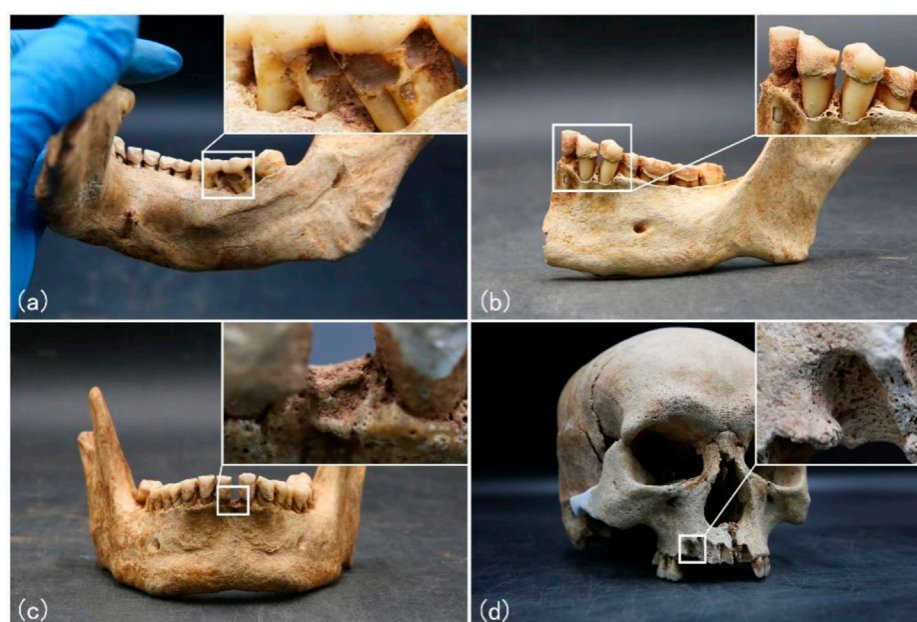


FIGURE 4

(A) Lingual views of mandible from M38:R1 showing carious lesions (arrow) at the cemento-enamel junction (CEJ) of RM₁ and RM₂; (B) left lateral views of the mandible of M47:R1, exhibiting periodontal disease. (C) Anterior view of mandible from M38:R1 showing dental calculus deposits affecting all teeth, AMTL (arrow) of LI₁; (D) Probable periodontitis and associated alveolar inflammation, M38:R1.

3.3.2 Periostitis

Periosteal reactions were common in WYC population. Of 10 individuals able to be scored, 5 showing signs of osteoperiostitis on one or more skeletal elements. 25.93% long bones (14/54) displayed periosteal lesions unrelated to traumatic events. All of the recorded cases of this pathology represent mild to moderate lesions. The lower limbs were the most commonly affected area, 60% (6/10) had tibial lesions and 37.5% (6/16) had tibial lesions. Periostitis is present in 31.58% of the female long bone and 20% of the male long bone. No statistically significant sex difference is present in the frequencies of these conditions (Table 5; Figure 5).

3.3.3 Degenerative joint disease (DJD)

DJD is present in four out of nine individuals, or 44.44% of the sample. The main infection joint involves the sacroiliac joint, hips, shoulders, knees, and temporomandibular joint. The individuals with osteoarthritis of major appendicular joints were a young female and two adults of indeterminate sex, M47:R1, M47:R3 and M71. M47:R1, a female of age 30, exhibits mild degeneration in the right glenoid fossa of scapula. But the corresponding humeral head does not exist. M47:R3, an adult of indeterminate sex, bilateral hips joint present with mild to moderate osteoarthritis. M71, an adult of indeterminate sex, shows more severe arthritis on the right hip. The vertebrae were generally poorly preserved, precluding many observations including identification to individual elements in many cases. Only one individual (M47:R1, a female of 30 age) had evidence of degeneration of the spine (cervical was affected). The right shoulder joint shows a slight inflammatory response. M47:R1 was the only one of the concerned individuals to exhibit some

osteophytes and erosive lesion of the auricular surface of bilateral ilium, which suggests signs of sacroiliitis. In addition to the above pathological conditions, we also found evidence of temporomandibular arthritis in one individual (M47:R2, a male of age 30–40).

The total frequency of DJD in major joints in the analyzed sample is 20.51%, with a higher frequency in females (27.78%) compared to males (9.09%), but without statistical significance. (Table 6; Figure 6).

3.3.4 Traumatic injuries

Traumatic injury is not explicitly presented in this sample. There was only one individual, M47:R2, a middle-aged male, whose right distal radius with proliferative bone formation may exhibit evidence of suspected Corley's fracture.

3.3.5 Cribra orbitalia and porotic hyperostosis

A total of 4 individuals with complete or fragments of the cranium were assessed for porotic hyperostosis. Only one individual (M38:R1) had lesions, which was located on the frontal bone. The individual was a male of age 30–35, have both porotic hyperostosis and cribra orbitalia (Figure 7).

4 Discussion

4.1 Demography and burial practice

The WYC skeletal series are characterized by an evident under-representation of sub-adults, and lack of infants. There was only one



FIGURE 5
Non-specific periosteal reaction widespread periosteal reaction of the lower limbs (M68, left tibia).

TABLE 6 Counts and prevalence rates by surface of DJD in the WYC collection.

	Male		Female		Indeterminate sex		Total	
	A/O	Freq	A/O	Freq	A/O	Freq	A/O	Freq
DJD (TMAS)	1/3	33.33%	0/3	0.00%	0/0	0.00%	1/6	16.67%
Shoulder	0/2	0.00%	1/2	50.00%	0/0	0.00%	1/4	25.00%
Elbow	0/4	0.00%	0/3	0.00%	0/1	0.00%	0/8	0.00%
Wrist	0/3	0.00%	0/2	0.00%	0/0	—	0/5	0.00%
Sacroiliitis	0/2	0.00%	2/2	100.00%	0/0	—	2/4	50%
Hip	0/0	—	2/4	50%	1/1	100%	3/5	60%
Knee	0/0	—	1/2	0	0/2	0%	1/4	25.00%
ankle	0/0	—	0/0	—	0/3	0%	0/3	0.00%
Total	1/11	9.09%	5/18	27.78%	1/9	11.11%	8/39	20.51%

A, number of affected articular surface; O, number of observed articular surface; Freq, frequency (%); TMAS, temporomandibular articulating surface of temporal bone.

subadult identified, comprising 6.25% of the total analyzed samples. In most of populations from the prehistorical period of China these values are between 15% and 30% (XIN, 2004; Wang, 2012), while in some exceptional cases these values can rise to as high as 61% (Xi'an Banpo Museum. 1988). However, in samples of Iron Age, the percentage of sub-adult skeletons is reduced to 2%–6% (Liang,

2008; Sun and Zhu, 2014; Dongyue et al., 2022). A review of the available literature implies that this observation is a common phenomenon that under-representation of sub-adults exists in the youngest age group during the late Iron Age of China (Hou, 2013; ZHOU, 2014). The following conjectures may be for the lack of young children in WYC. Animal activity and flooding should be

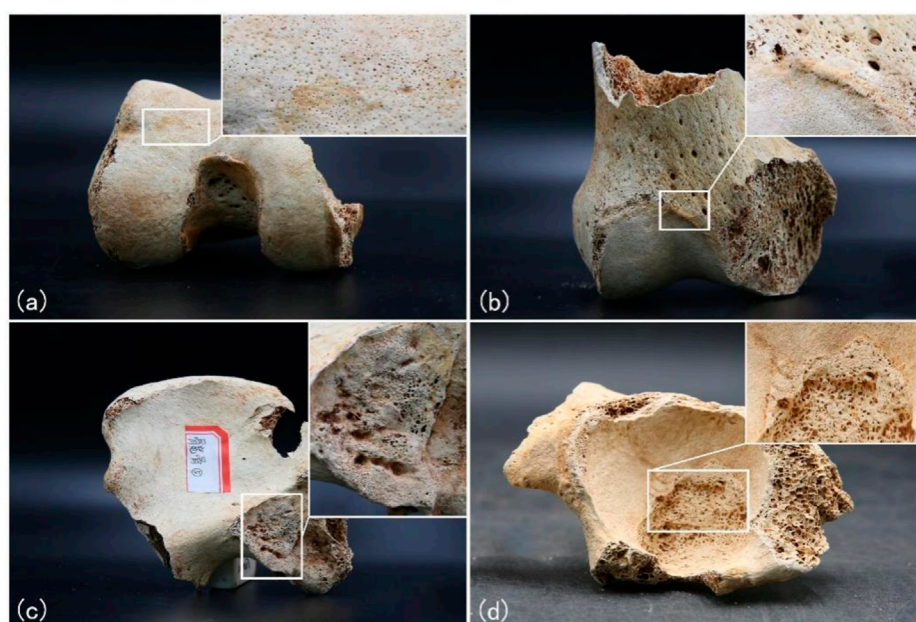


FIGURE 6

(A): Porosity on the Patellar Surface of Femur (M38:R1); (B): Localized Osteophytes on patellar surface of femora; (C): the auricular surface of ilium from M47:R1 showing some osteophytes and erosive lesion, which suggest signs of sacroiliitis. (D): M47:R3.

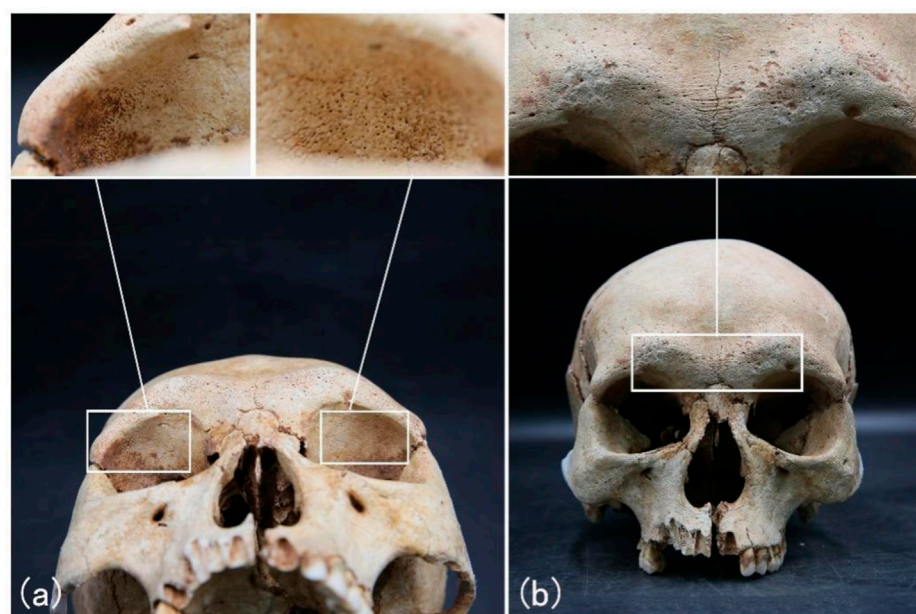


FIGURE 7

healed cribra orbitalia (A) and porotic hyperostosis (B) were found on the skull of M38:R1.

considered as potential causes of young children's bone loss in our case, as young children's bones are smaller and lighter and therefore probably more prone to those influences. Another probable reason may be that (very) young children received different burials, because cliff tomb is used as a family cemetery, and younger children may not be eligible to be buried in.

In tomb 47 (M47), at least one adult male and two adult females were identified. In tomb 52 (M52), at least two adults and a child aged 7–10 were identified. The demographic profile of the individuals in the cliff tomb with multiple burials therefore reveals that the choice of corporeal treatment was not determined solely by age or sex: males, females and children were both inhumed. In addition, except that

TABLE 7 Comparison of prevalence rates and dental caries between WYC and other ancient groups.

Population	Location	Period	Economy	Individual number/teeth number	Caries prevalence of individual (%)	Caries prevalence of tooth (%)	References
Qinglongquan	Yunxian, Hubei Province	Late Neolithic	Agriculture	87/1,075	55.2	11.9	ZHOU, et al., (2013)
Baojialiangzi	Chengdu, Sichuan Province	The Warring States—Han Dynasty	Agriculture	57/457	15.79	2.84	YUAN., et al., (2018)
Xitun	Beijing	Western Han Dynasty	Agriculture and Livestock	201/3,784	39.30	6.77	ZHOU, et al., (2017)
Wangyuancun	Leshan, Sichuan Province	Eastern Han—Song Dynasty	Agriculture	8/74	25	5.41	This study
Changan group	Changan county, Shaanxi Province	Tang Dynasty	Agriculture	62/632	62.90	14.58	Yong (2008)
Zhongnanshijicheng	Guangrao, Shandong Province	Ming Dynasty	Agriculture	17/248	82.35	15.73	Yuni et al. (2021)

M71 has only one main tomb, the internal structures of the other tombs include multiple coffin chambers in addition to the main tomb, implying that multiple individuals were buried in it. Multiple individuals buried in one tomb plausibly represents several generations of a lineage or household. The demographic profile supports the interpretation of multiple individuals in one tomb as parents/children or spouses.

4.2 Stature

Adult stature is influenced by genetics, environment, diet and other factors, it is considered a broad, non-specific indicator of the relationship between humans and their social, economic, and cultural contexts (Larsen, 2002). The stature of the male individual (M38:R1) is similar to that of modern Sichuan rural men (165.39 cm) (Xilin et al., 1994), but is shorter than the average stature of prehistoric Yingpanshan men (168.33 cm) (Yuan et al., 2018) who lived in the upper reaches of the Min River. However, the stature of the female individual (M47:R1) is significantly shorter than that of modern Sichuan rural women (154.65 cm) (Xilin et al., 1994) and also shorter than that of Tang Dynasty women in Chengdu Plain, of 156.01 cm (Yuan, 2016). Due to differences in genetics, environment, and dietary levels, populations in various parts of China have different physical characteristics. Previous studies showed that the height of population in southwest region, where WYC is located, is the shortest region in modern China (Zhang, 1988; Lei et al., 2004; Liguang Ma, et al., 2008), and the limited two stature data of WYC are consistent with this.

4.3 Oral health and dietary patterns

Dental caries is the most commonly reported oral paleopathological condition in the osteological literature. Its etiology is complex, but diet is an essential factor in the process (Hillson, 2008a). The carbohydrate

content of the diet is generally considered to be the most important factor affecting the prevalence of dental caries in ancient humans, because the presence of carbohydrate-rich cereals in the oral cavity can easily lead to the growth of bacteria that cause dental caries (Arens, 1999; Hillson, 2008b; Larsen, 2015).

The WYC sample has a lower caries frequency (25% of individuals; 5.41% of teeth) than most other historic populations in China, but slightly higher than that of the Bronze-Early Iron Age Baojialiangzi site (15.79% of individuals; 2.84% of teeth) in the Chengdu Plain (Table 7). This result may be related to rice farming in the Chengdu Plain. Previous stable isotopic and phytoarchaeological studies suggest that the Chengdu Plain began to plant rice in the late Neolithic Age, and a large number of rice was planted in the Sanxingdui culture (d'Alpoim Guedes, 2011; Guedes et al., 2013; Yu and Jiao, 2015; Yi, B. et al., 2018; Ying et al., 2021). Rice became the main source of ancient food for people, and this situation continued until the Ming Dynasty (Sun, 2009). Although there is currently no data on dental caries in the rice farming population in China, however, based on evidence from prehistoric bone samples from a series of prehistoric sites in Southeast Asia suggest that there is a limited relationship between rice agriculture and caries (Tayles et al., 2000; Willis, A., & Oxenham, M. F., 2013).

Dental calculus is another oral paleopathological condition that is highly related to diet. Although oral hygiene, salivary flow and other non-dietary factors can influence calculus rates (Lieverse, 1999), diet is the main factor affecting frequencies on a population level (Novak, 2015). The relationship between diet and the formation of dental calculus is not straightforward (Hillson, 2001), and high calculus rates have been associated with both high protein and high carbohydrate diets (Meiklejohn and Zvelebil, 1991; Lieverse, 1999). The presence of large amounts of calculus on ancient teeth is by itself not necessarily an indicator of specific dietary components but does supply evidence that assist in determining dietary patterns.

Few studies of skeletal remains in this region reported the prevalence of dental calculus. Compared with a small number of sites reported in other parts of China, the high rate and severity of dental calculus in WYC sample is similar to Xiaohe Cemetery which is a Bronze Age site

located in the Lop Nur area of Xinjiang. The Xiaohe people ate beef, mutton, milk and other carnivorous foods, as well as plant foods with relatively high carbohydrate content such as wheat and millet.

Analysis of oral diseases, especially dental caries and calculus, suggests a high-carbohydrate and high-protein dietary pattern among WYC population. Cliff tomb burials developed during a period in the southwest's history when it experienced relative social and political stability and great economic prosperity. Since the Qin and Han Dynasties, the level of agricultural production in the Chengdu Plain has developed rapidly and it has become a well-known grain-producing area in the country (Gu, 111 AD). People eat rice and other grains as their staple food, and pigs, cattle, sheep, chickens, dogs, and aquatic fish are their main sources of meat, forming a stable and rich food structure, and points to a reliance on a high carbohydrate and high protein diet.

4.4 Physiological stress and health

Generic measures of health are usually drawn from evidence of metabolic diseases like anaemia, in which frequencies of porotic hyperostosis, and childhood stress is recoverable through evidence of dental enamel hypoplasias. The sample used represents a population with relatively low levels of stress and disease. The frequencies of the non-specific markers of stress in the WYC series are slightly low. There is only one individual cribra orbitalia and no enamel hypoplasia. Previous studies suggest that the low frequency of cribra orbitalia may be a result of sufficient levels of vitamins B12 and C in food (Walker et al., 2009). Studies of living children have documented association between higher frequencies of hypoplastic defects and poor nutrition and low socioeconomic status (Goodman et al., 1991; Goodman et al., 1992). In addition, periosteal reactions were common in WYC population, but the lesions are generally moderate or mild. No signs of violence-related trauma were found in any of the bone samples (only one suspected Kresh fracture was found), suggesting that WYC residents lived in a relatively peaceful society. All the evidences indicate a relatively good quality of life for the majority of WYC population and can therefore be argued that environmental hardships were not excessive in the area.

Results of disease and health analyzes are consistent with the documented. According to ancient documents, during the Warring States Period, the economic production of the Chengdu Plain, including agriculture, had reached a relatively high level (Qian, 91 BC; Qu, 1984, Jin dynasty). After the Han Dynasty, with the influx of immigrants from the north, the productivity level of Chengdu Plain increased significantly, and agriculture developed rapidly, gradually becoming a "Land of Abundance".

5 Conclusion

In this work, a comprehensive bioarchaeological study of iron age skeletal samples from the cliff tomb group of Wangyuancun (WYC) site was carried out. The minimum number of individuals was determined by the visual pair matching of skeletal elements based on similarities observed in bone morphology, age and sex, trauma and pathology and taphonomic alterations. Bioarchaeological techniques were used, and the following results were obtained.

Of the 16 individuals, two individuals were identified to be males, three possibly female and one juvenile. Death age of 7 individuals was determined, mostly in youth-middle age, and no infant individuals identified. The state of preservation of the bones is not very good. Nonetheless, the remains furnish considerable information about the mortuary practices of residents buried in the cliff tombs of Southwest China during the Iron Age. The identification of males, females and subadults among the bones and teeth in the same tomb suggests that spouses, parents and children probably from several generations of the same lineage or household were buried there. Reconstruction of the height of an adult male and an adult female, respectively, reveals that WYC was shorter in stature compared with other ancient Chinese populations. Each bone or tooth was examined for the presence of pathological conditions, and special attention was paid to dental diseases, periostitis, degenerative joint diseases, cribra orbitalia, porotic hyperostosis, and traumatic injuries. 5.41% of teeth have at least one carious lesion, and the presence of calculus was recorded in 78.38% of teeth within the sample. This sample showed a lower level of carious lesions and a higher frequency of calculus, suggests a high-carbohydrate and high-protein dietary pattern among WYC population. Most of the samples lacked indications of stress, but a few had cribra orbitalia, osteoarthritis, osteophytosis and various dental pathologies. Periosteal reactions were common, but the lesions are generally moderate or mild. The analyzed pathological changes indicate a relatively good quality of life for the majority of the population and can therefore be argued that environmental hardships were not excessive in the area.

These bioarchaeological results broaden our understanding of the health and lifestyle of the cliff tomb population of Chengdu Plain in the Iron Age. Our bioarchaeological examination of the skeletal samples of WYC site provides a rare opportunity to address questions concerning ancient people's diet, health, disease and stress of population in the Iron Age of Chengdu Plain, Southwest China. The biological and pathological data collected in this work provide an important data base for future anthropological research.

Nevertheless, it is important to point out that the evidence of palaeopathology in the ancient residents of Wangyuancun site may be under-represented due to the fragmented nature of the skeletal assemblage. The sample of 16 individuals in this work is certainly a very small one from which to draw general conclusions reflecting the entire living population from which it came. Certainly biological, cultural, and other selective biases are present in this skeletal series as in almost any archaeological sample. Due to the scarcity of supporting material many questions about cliff burials remain difficult to answer. For example, the identity of the majority of those buried in cliff tombs is unknown, nor do we know how the funeral protocol for cliff tomb burial was conducted, and whether there was practice of secondary burials. We hope to expand the sample size at Wangyuancun site and from other related sites, and combined with isotope and other related studies in future, to better understand the life history of these ancient people and their social conditions.

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

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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.

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Supplementary material

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Paleopathological characteristics of Neolithic early rice farmers in the lower reaches of the Yangtze river

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Paleopathological investigations of human remains from the Neolithic Hemudu culture in the lower reaches of the Yangtze River in southern East Asia were conducted to clarify the health status of people in early rice-farming societies. Our results show that the occurrence ratios of cribra orbitalia and enamel hypoplasia did not differ significantly between early rice farmers and hunter-gatherers. By contrast, the occurrence ratios of periosteal reactions, dental caries, and antemortem tooth loss in adults were higher among the early rice farmers. Based on these findings and the results of archaeological research on the Hemudu culture, it was suggested that: 1) the Hemudu culture adopted a diversified livelihood strategy that was not overly dependent on rice as a food resource, which did not lead to an extreme decline in health status, 2) the work in the rice fields or the working environment caused stress to the workers, and 3) the rice-farming society's dietary habits led to a decline in oral health. Our results provide new paleopathological insights into the health status of early rice farmers in East Asia. However, the sample size of early rice farmers used in this study was small, and more data are needed to verify the validity of the views presented here.

KEYWORDS

palaeopathology, stress marker, oral health, early rice farmers, Hemudu culture, East Asia

1 Introduction

The beginning of agriculture was a significant event in human history as it brought about a means of food production under human control. Among the various agricultural plants used as food, rice is undoubtedly one of the most important, along with wheat. The lower reaches of the Yangtze River in southern East Asia is considered one of the origins of rice agriculture (Fuller et al., 2009; Fuller, 2011; Larson et al., 2014). Rice cultivation and utilization were initiated during the early Holocene period (Shangshan culture, 11,000–8,600 years ago). Subsequently, during the Hemudu culture around 7,000–5,500 years ago, systematic rice cultivation began with the establishment of rice paddies (Zhejiang, 2003; Nakamura S. 2010; Zhao, 2011; Liu and Chen, 2012; Zuo et al., 2017; Crawford, 2022). Large quantities of carbonized rice, farming tools, animal remains, and stilt-house ruins were found at the Hemudu and Tianluoshan sites of the Hemudu culture period, revealing the specific aspects of life and society of early rice-farming people.

TABLE 1 Human skeletal materials (number of individuals).

	Adult female	Adult male	Adult of unknown sex	Juvenile	Total
Early rice farmers (Hemudu site)	1	2		5	8
Early rice farmers (Tianluoshan site)	4	5		5	14
Hunter-gatherers (Huiyaotian and Liyupo sites)	38	36	8	9	91



FIGURE 1
Map showing the location of the sites discussed. Map by C.F.W. Higham courtesy GeoMapApp (www.geomapapp.org), CC by Ryan et al. (2009).

Paleopathological studies examining changes in human health status during the adoption of agriculture as a new means of livelihood can suggest important implications for exploring the development of human societies. For instance, when Native American populations began cultivating corn in North America before the arrival of Europeans, linear enamel hypoplasia (LEH), a stress marker, became more frequent than that during the preceding hunter-gatherer period. This might have resulted from the fact that the early agricultural food production system was immature and reliance on a single crop may have led to nutritional deficiencies, or

changes in population density and habitat at the beginning of agriculture made the spread of infectious diseases more likely (Goodman et al., 1980; Larsen, 1995; Larsen, 1999; Hillson, 1996). An increased frequency of stress markers at the onset of agriculture has also been reported in the Eastern Mediterranean (Angel, 1984; Smith et al., 1984) and Western Asia (Rathbun, 1984). Also regarding prehistoric Asia, where rice farming spread, there has been discussion about the impact of the introduction of agriculture on the health status of the people. For example, in Vietnam and Thailand in continental Southeast Asia, the

TABLE 2 Frequency of cribra orbitalia (CO).

	Early rice farmers						Hunter-gatherers		Fisher's exact test
	Hemudu		Tianluoshan		Subtotal				Farmers' subtotal vs. Hunter-gatherers
	N	%	N	%	N	%	N	%	<i>p</i> -value
Juvenile	1/3	33.3	0/1	0.0	1/4	25.0	-	-	-
Adult	0/2	0.0	0/5	0.0	0/7	0.0	3/24	12.5	1.000
Total	1/5	20.0	0/6	0.0	1/11	9.1	3/24	12.5	1.000

N: Number of skulls with CO/Total number of skulls with orbital roof.

adoption of agriculture has been speculated to be accompanied by a decline in oral health, as evidenced by the increased frequency of dental caries and antemortem tooth loss (AMTL) during the Neolithic period (Willis and Oxenham, 2013; Oxenham et al., 2018). Even in the Far Eastern Japanese Archipelago, the frequency of dental caries increased in the Yayoi period, when rice farming was introduced, than in the earlier hunter-gatherer society of the Jomon period (Fujita, 1995; Fujita, 2009). Additionally, the frequency of LEH decreased or was similar in the Yayoi and subsequent Kofun periods compared to the Jomon period (Yamamoto, 1988; Temple, 2010). However, continental Southeast Asia and the Japanese Archipelago are areas where the established rice-farming culture spread from continental East Asia, and not the places of origin of rice farming. The paleopathological characteristics of early farming groups have not been elucidated in the lower reaches of the Yangtze River, where rice farming originated.

This study aims to clarify the health status of Neolithic early rice farmers in areas of rice farming origin. To this end, we investigated the occurrence of stress markers such as cribra orbitalia (CO), LEH, periosteal reaction, and indicators of oral health such as dental caries and AMTL in the human remains from the Neolithic Hemudu Culture excavated from the Hemudu and Tianluoshan sites in the lower reaches of the Yangtze River. This study provides new insights into the health status of early rice-farming populations in East Asia based on paleopathological studies.

2 Materials and methods

2.1 Early rice farmers' remains from the Neolithic Hemudu culture

The human remains of early rice farmers used as materials in this study included eight burial human skeletons from the Hemudu site and 14 from the Tianluoshan site, with a total of 22 burial skeletons (Table 1). In addition, 12 scattered human remains excavated from the Tianluoshan site were used as materials (mentioned later). The Hemudu and Tianluoshan sites are located in Zhejiang Province in southern China, and both are from the Hemudu culture—7,000–5,500 years ago (Figure 1).

The Hemudu site was discovered in 1973 and excavations were conducted between 1973 and 1978 (Zhejiang, 2003). Eight human skeletons (one adult female, two adult males, and five juveniles of unknown sex) were found during the excavations and stored at the Hemudu Site Museum. The skulls of one adult male and one adult female were cleaned, and the results of the morphological research have been reported (Zhejiang, 2003). However, the post-cranial trunk and limb parts of these two skeletons were not exhumed, and the entire grave pits were preserved in the museum. The other six whole skeletons were not exhumed; all grave pits were preserved in the museum, and their anthropological reports have not been published. In this study, we examined eight human skeletons for CO, LEH, dental caries, and AMTL. However, some parts of the skull that were in an earth-encased state could not be adequately observed. In addition, the postcranial parts were not investigated for periosteal reactions because it was impossible to remove them from the soil of the grave pit.

The Tianluoshan site, located 7 km from the Hemudu site, was discovered in 2001 and has been continuously excavated since 2004. Its archaeological results have been reported by Nakamura (2010b) and Matsui and Kikuchi (2016); however, no anthropological reports of excavated human remains have been published. In this study, 14 buried human skeletons (four adult females, five adult males, and five juveniles of unknown sex) stored at the Tianluoshan site museum, and 12 scattered human remains (one adult mandible, two adult right femurs, one adult left femur, one adult left tibia, and seven permanent teeth) that were considered to have originated from individuals other than those in these burials, were examined for the presence of CO, LEH, periosteal reaction, dental caries, and AMTL.

2.2 Hunter-gatherers' remains from the early holocene southern East Asia

There were no hunter-gatherer sites in the lower reaches of the Yangtze River, from which many human remains were excavated. Therefore, the human skeletons of non-agricultural hunter-gatherers in the Early Holocene, approximately 9,000 to 7,000 years ago, from the Huiyaotian and Liyupo sites in Guangxi Province, Southern China (Matsumura et al., 2017), were used as comparative materials for early farmers in this study (Figure 1). The Huiyaotian and Liyupo sites, as well as the

TABLE 3 Frequency of linear enamel hypoplasia (LEH).

	Early rice farmers						Hunter-gatherers		Fisher's exact test
	Hemudu		Tianluoshan		Subtotal				Farmers' subtotal vs. Hunter-gatherers
	N	%	N	%	N	%	N	%	<i>p</i> -value
LEH in UI1	2/3	66.7	2/2	100.0	4/5	80.0	14/19	73.7	1.000
LEH in LC	2/3	66.7	4/4	100.0	6/7	85.7	20/40	50.0	0.112

N: Number of teeth with LEH/Total number of teeth remains, UI1: Upper first incisor, LC: Lower canine.

Hemudu and Tianluoshan sites, belong to the warm humid climate (Cfa) category of southern East Asia in the Köppen climate classification. They share similarities with the Hemudu cultural sites in being located in a plain area along the lower reaches of a large river. Paleopathological investigations of 91 hunter-gatherer human skeletons from the Huiyaotian and Liyupo sites were conducted by one of the authors of this study (JS) using the same methods as those used in this study (Sawada, 2017a; Sawada, 2017b; Sawada et al., 2017).

2.3 Age and sex estimation

The age of juvenile human remains was estimated based on the tooth formation status (Smith, 1991), eruption status (Ubelaker, 1999), and the fusion status of the epiphyseal ends of the limb bones (Maresh, 1970; Fazekas and Kosa, 1978; Cunningham et al., 2016). The age of adult remains was estimated based on the closure status of the cranial sutures (White et al., 2012) and the metamorphosis of the pubic symphysis surface (Brooks and Suchey, 1990).

The sex of the human remains was estimated from the shape of the adult coxal bone and skull, based on the sex determination method in White et al. (2012).

2.4 Paleopathological investigation

2.4.1 Cribra orbitalia (CO)

CO is a pathological condition characterized by porotic or sieve-like lesions in the supraorbital bone. CO is caused by nutrient deficiencies, such as vitamin B12, vitamin C, or iron-deficiency, anemia, mainly during growth (Walker et al., 2009; Oxenham and Cavill, 2010), and has been used in studies of ancient human populations (Larsen, 1995). The identification criteria for CO were based on those described by Nathan and Haas (1966) and Hirata (1988). The presence of CO was confirmed by naked-eye observations, and individuals with CO in either or both orbits were considered to have CO.

2.4.2 Linear enamel hypoplasia (LEH)

LEH is an enamel thickness defect that results from physiological stress during crown formation. This disease is regarded as a relatively sensitive and nonspecific indicator of stress (Goodman et al., 1980; Nikiforuk G and Fraser, 1981; Goodman and Rose, 1990). The upper first incisors (UI1) and lower canines (LC) were observed using a 10x magnifying lens under an LED light. According to Goodman and Rose (1990) and

Hillson (1996), a groove or row of small pits parallel to the perikymata on the dental crown surface is considered LEH. To align the conditions for observation of the crowns, teeth with more than half of their original crown height were investigated, and LEHs that appeared below half of their original crown height were recorded.

2.4.3 Periosteal reaction

Adult femoral and tibial diaphyses remaining over half of the original length were used for the investigation or periosteal reaction. The criteria summarized by Weston (2008) were used to determine the presence of a periosteal reaction in the diaphysis.

2.4.4 Dental caries

The presence or absence of dental caries was investigated in all the erupted permanent teeth. Caries were identified according to the classification criteria commonly used in dentistry (C1: lesions mainly confined to the crown enamel; C2: dentin affected, but not the pulp cavity; C3: lesions reaching the pulp cavity; and C4: only the tooth root remained). Since it is sometimes difficult to identify C1 when observing archaeological human remains, C2 or higher, which can be determined with certainty as caries, was recorded as caries. The presence of dental caries was confirmed by observation using a 10x magnifying lens.

2.4.5 Antemortem tooth loss (AMTL)

The alveoli of all permanent teeth were examined for each tooth socket to determine whether the tooth had lost while alive. AMTL was determined based on alveolar conditions according to Ortner (2003).

One of the authors (JS) collected all paleopathological data to prevent inter-observer errors.

2.5 Statistical analyses

Fisher's exact test was used to examine any statistically significant difference in the frequency of occurrence of each paleopathological characteristic between early farmers and hunter-gatherers. The significance level was set at $p < 0.05$. Statistical analyses were conducted using the SPSS software package for Macintosh, version 25.0.0 (IBM).

3 Results

Because of the small number of human remains of both sexes identified in the materials of the early rice farmers of the

TABLE 4 Frequency of occurrence of periosteal reaction.

	Tianluoshan early rice farmers		Hunter-gatherers		Fisher's exact test
	N	%	N	%	Farmers vs. Hunter-gatherers <i>p</i> -value
Femur	1/12	8.3	0/76	0.0	0.136
Tibia	3/9	33.3	4/71	5.6	0.028
Total	4/21	19.0	4/147	2.7	0.009

N: Number of adult bones with periosteal reaction/Total number of adult bones.
The left and right were combined.

TABLE 5 Frequency of dental caries.

	Early rice farmers						Hunter-gatherers		Fisher's exact test
	Hemudu		Tianluoshan		Subtotal		N	%	Farmers' subtotal vs. Hunter-gatherers
	N	%	N	%	N	%			<i>p</i> -value
Permanent tooth	3/20	15.0	4/36	11.1	7/56	12.5	45/820	5.5	0.041

N: Number of erupted permanent teeth with caries/Total number of erupted permanent teeth.

Hemudu culture, the sexes were combined and analyzed as a whole. Fisher's exact test of sex composition showed no statistically significant difference between early farmers (five females and seven males) and hunter-gatherers (38 females and 36 males).

3.1 CO

The occurrence ratio of CO in juveniles of early rice farmers was 25.0%, and that in adults was 0.0% (Table 2). By contrast, the incidence of CO among hunter-gatherers was 12.5% in adults (no juveniles with preserved supraorbital walls). Fisher's exact test was used to examine any statistical difference in the frequency of CO occurrence between farmers and hunter-gatherers. No significant difference was found at the 0.05 level.

3.2 LEH

The occurrence rates of LEH in early rice farmers in UI1 and LC were 80.0% and 85.7%, respectively. The occurrence rates of LEH in the hunter-gatherer population were 73.7% and 50% in the UI1 and LC, respectively (Table 3). Fisher's exact test showed no statistically significant differences between farmers and hunter-gatherers at the 0.05 level.

3.3 Periosteal reaction

As mentioned above, examining the limb bones from the Hemudu site was impossible; therefore, only the bones from the Tianluoshan site were used to analyze the periosteal reaction. Periosteal reactions observed in these materials were mild. The

occurrence ratio of periosteal reactions in the adult lower limb long bones of the farmers (19.0%) tended to be higher than that of the hunter-gatherers (2.7%), and Fisher's exact test showed a statistically significant difference (Table 4).

3.4 Dental caries

The percentage of dental caries in the total number of erupted permanent teeth was 12.5% in early rice farmers compared to 5.5% in hunter-gatherers, a statistically significant difference at the 0.05 level in Fisher's exact test (Table 5). The dental caries ratio of the Huiyaotian and Liyupo hunter-gatherers used for comparison in this study tended to be similar to the low caries rates of modern hunter-gatherer groups (Turner, 1979).

3.5 AMTL

The occurrence rate of AMTL in the early rice farmers, calculated based on the number of permanent tooth sockets, was 7.8%. This tended to be higher than that in hunter-gatherers (4.8%), although no statistically significant difference was detected (Table 6).

4 Discussion

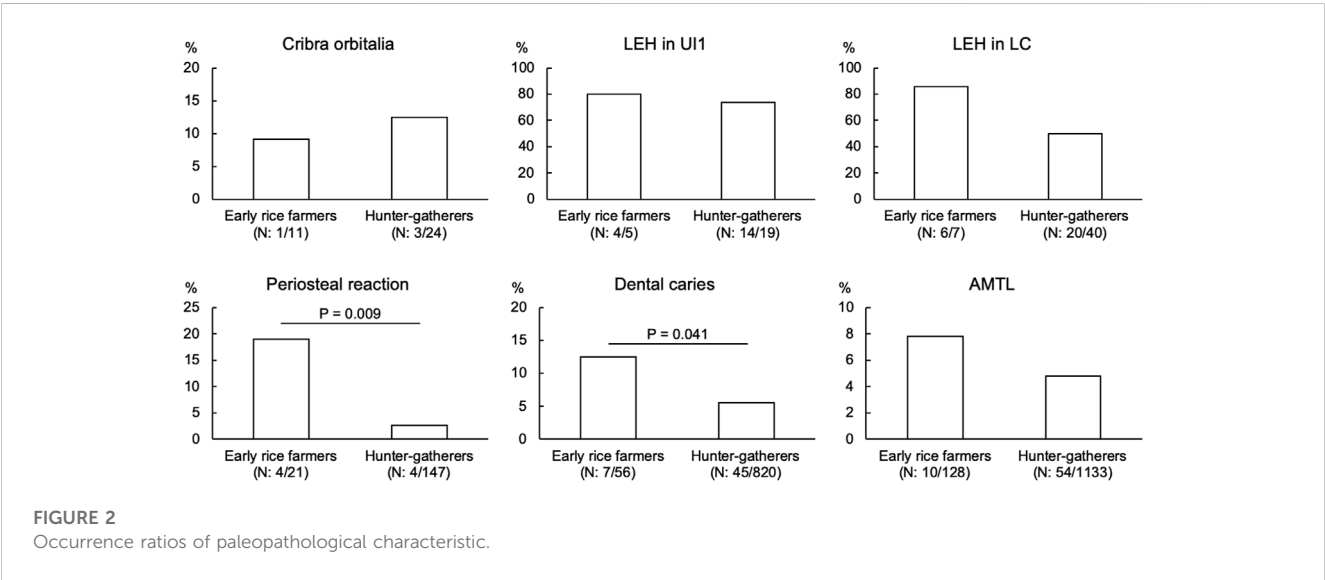
4.1 Stress markers: CO, LEH and periosteal reaction

No statistically significant difference in the frequencies of CO and LEH were found between early rice farmers of the Hemudu culture and hunter-gatherer groups in southern China. Although our findings are preliminary owing to the small sample size of early rice farmers,

TABLE 6 Frequency of AMTL.

	Early rice farmers						Hunter-gatherers		Fisher's exact test
	Hemudu		Tianluoshan		Subtotal				Farmers' subtotal vs. Hunter-gatherers
	N	%	N	%	N	%	N	%	p-value
Socket for AMTL	0/62	0.0	10/66	15.2	10/128	7.8	54/1133	4.8	0.138

N: Number of sockets for AMTL/Total number of sockets in adult maxilla and mandible remains.



there was no evidence that the health status of early rice farmers was appreciably different from that of hunter-gatherers. While it is thought that health declined after the introduction of agriculture in North America and the Mediterranean (Goodman et al., 1980; Angel, 1984; Smith et al., 1984; Larsen, 1995), such changes may not have occurred in the Neolithic societies of East Asia that began rice farming. In this regard, examining the characteristics of people's livelihoods during Hemudu culture may be necessary. Zooarchaeological studies suggest that the people of the Hemudu culture consumed a variety of mammals and fish acquired through hunting and fishing, as well as domestic animals, such as pigs and geese (Matsui and Kikuchi 2003; Eda et al., 2022). A diversified food acquisition strategy that did not rely heavily on rice farming may have contributed to a stable food supply and prevented a significant decline in the health status of the people during the Hemudu culture period.

However, for periosteal reactions in adult bone, the occurrence ratio in the early rice farmers was significantly higher than that in the hunter-gatherer group (Figure 2). The periosteal reaction is an early response of the bone to the effects of disease or trauma, and is considered an indicator of non-specific stress (Larsen, 1999; Waldron, 2021). Although it is difficult to extrapolate a specific causative lesion from the periosteal reaction alone (Weston, 2008), unlike LEH, which is derived from stress in infancy, the periosteal reaction in adult bones is probably derived from stress at a later age. The high incidence of periosteal reactions in early rice farmers may be related to infections caused by long hours of farming in rice fields with high mechanical stress, or noxious insects such as schistosomes (Ross et al., 2013) and

mosquitoes that are easily contacted in the aquatic environment. Future studies on human bones for paleopathological features associated with infection and whether DNA from infection remains in human bones will allow for a more specific discussion of the etiology of periosteal reactions.

4.2 Oral health: dental caries and AMTL

The occurrence ratios of dental caries and AMTL in people of the Hemudu culture tended to be higher than those in the hunter-gatherer group (Figure 2). These results suggest that early rice-farming societies in the lower reaches of the Yangtze River had factors that contributed to oral hygiene deterioration. In general, the occurrence of dental caries is related to factors such as caries pathogens (e.g., mutans), dietary characteristics (sugar and sticky foods), dental habits, and lifestyle (Hillson, 1996; Hillson, 2008; Waldron, 2021). Temple and Larsen (2007) reported that a rice-based diet is responsible for dental caries in rice-farming populations. However, Willis and Oxenham (2013), who presented data on the decline in oral health during the introduction of agriculture in Southeast Asia, are critical of the view that rice is the main cause of dental caries, based on a review by WHO (2003), which stated that "rice has low potential cariogenicity." Willis and Oxenham (2013) highlighted sexual differences in the quality of oral hygiene and physiological factors that lead to poor oral hygiene in females (especially during pregnancy).

We could not examine sexual differences because the sample size was too small to allow sex determination owing to the large number of juvenile and fragmentary human bone materials that are difficult to determine. We hope that future excavations will increase the amount of human material from early farming groups in the lower reaches of the Yangtze River and advance the study of sexual differences in the occurrence ratios of paleopathological features.

5 Conclusion and future direction

The frequencies of occurrence of stress markers and oral pathological features in early rice farmers in the lower reaches of the Yangtze River and hunter-gatherers in the early Holocene in southern China were examined. The results showed that: 1) the occurrence ratios of CO and LEH were not significantly different between early rice farmers and hunter-gatherers, and 2) the occurrence ratios of dental caries, AMTL, and adult periosteal reactions were higher in early rice farmers. Based on these findings, we hypothesized that early rice-farming societies during the Hemudu culture had a diversified subsistence strategy that did not rely too heavily on rice as a food resource, that work in rice fields and its environment brought new stressors that were different from those of hunter-gatherer subsistence, and that the diet of rice-farming societies led to a decline in oral health. However, the sample size of early rice farmers used in this study was small; therefore, additional data will be needed in the future to verify the validity of the views presented here.

Problematised as an “Osteological Paradox” (Wood et al., 1992; Wright and Yoder, 2003; DeWitte and Stojanowski, 2015; Pilloud and Schwitalla, 2020), if one died at the time of stress, the stress could not leave traces in the bones. Therefore, a group’s high or low frequency of stress markers may not simply reflect the society’s low or high health status. For example, a high occurrence of stress markers but a low percentage of severe stress markers might indicate that severely stressed individuals died, rather than the environment being less susceptible to severe stress. Although the sample size was small, and the proportion of severe cases could not be examined in this study, such an examination is necessary when additional materials are available. In addition, it is expected that a combination of paleopathological analyses and paleo-demographic studies of life expectancy will provide valuable insights. The frequency of CO, periosteal reactions, and dental caries, which have been reported to change with age (Larsen, 1995; Waldron, 2021), were only discussed in two categories, adults and juveniles, owing to the scarcity of materials. Future studies on the human remains of rice farmers, including those from the lower reaches of the Yangtze River after the Hemudu culture (e.g., Okazaki and Takamuku, 2019; Okazaki et al., 2021), will help resolve these issues and clarify people’s health status in the development of rice-farming societies.

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.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JS, GS, WH, SK, MY, and SN contributed to the conception and design of the study. JS, SK, and FS contributed to the organization and analyses of the materials. JS wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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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.2023.1225928/full#supplementary-material>.

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Meat procurement strategy from the Neolithic to the Bronze Age in the Guanzhong region of Shaanxi Province, China

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Meat procurement strategies, displaying a great variety world-widely, are ideal for examining how geography and culture shape the subsistence. We collate zooarchaeological data from 26 Neolithic and Bronze Age sites/locales in the Guanzhong region (Shaanxi Province, China), a key region of early Chinese civilization, to demonstrate the changing process of meat procurement and its associations with environmental, demographic and societal factors. Comparing the proportion of the domesticates versus wild animals, along with the proportion of the domesticated pigs versus bovids (i.e., sheep, goat, and cattle), we summarize three characteristics of the meat procurement strategy at Guanzhong. Firstly, the changing pattern of meat procurement did not follow a linear progression of shifting subsistence from hunting to husbandry, albeit involving acceleration and regression from the pre-Yangshao period to the Western Zhou dynasty. Secondly, contrasting meat procurement strategies might have been employed between central settlements and lower-ranking settlements. Thirdly, an increasing importance of domesticated bovids was evident during the pre-Zhou and Western Zhou periods. We therefore argue population size and societal form might have shaped meat procurement strategies, in addition to natural environments. Subsistence, in turn, also lays the economic foundation for social development. It was not until the pre-Zhou and Western Zhou periods, when a more stable meat procurement strategy involving utilizing a variety of the domesticates was pervasively employed in this region, that Guanzhong had gradually gained its prominence in Chinese civilization.

KEYWORDS

Guanzhong region, Neolithic, Bronze Age, meat procurement strategy, zooarchaeology

Introduction

Food serves a vital lens through which various aspects of past and present societies could be understood (Twiss, 2015). In China, a growing body of most recent research has indicated that the diversity of culinary traditions and subsistence strategies, particularly those related to food production and consumption, can be traced back to as early as prehistoric times (Liu and Reid, 2020; Taché et al., 2021), which were closely associated with regional differences in resource availability, technical tradition, economic mode,

and even attitudes toward food (Fuller and Rowlands, 2011; Liu et al., 2016; Liu and Reid, 2020). Meat procurement strategy forms a critical part of culinary traditions and subsistence strategies. Zooarchaeologists have drawn a general picture of prehistoric meat procurement strategies in China (Yuan, 1999). Zooming in on this picture, we can clearly see a growing regional diversity entangled with environmental and cultural complexity, and intertwined with different trajectories of societal development. It is therefore necessary to select key regions for study, hence rendering insights into the causes and consequences of meat procurement, and its role in the development of social complexity in various regions.

Studies in the Central Plains and the Lower Yangtze Valley provide two examples. The Central Plains was the cradle of China's first dynasties. A productive and stable agricultural system involving husbandry diversification and intensification laid the foundations for the birth of the early civilization (Yuan, 2016; Jing et al., 2020), while the collapse of the Liangzhu culture in the lower Yangtze Valley demonstrates that the imbalanced husbandry over-relying on homogenous domesticated species would have restrained population growth and prevented the formation of the multi-central political structure, making it incapable to withstand the risks imposed by natural disasters and social conflicts, and finally leading to the collapse of the Liangzhu society (Yuan et al., 2020). It is apparently seen in these two examples that there was a complicated relationship between meat procurement strategies and societal development.

Guanzhong is another vital region for the discussion of the formation and development of Chinese early civilization (Zhang, 2014; Wang, 2021). The Guanzhong region, located in the Shaanxi Province, has long been a center of ancient human activities, with Palaeolithic sites dating to at least 750,000 years ago, and 13 dynasties having built their capitals here from the West Zhou to Tang dynasties (1046 BCE–907 CE), making it an irreplaceable role in the history of Chinese civilization (National Cultural Heritage Administration, 1998). It is also the core area of the Yangshao culture, one of the peaks of Chinese Neolithic cultures. Yangshao, known for its well-developed painted pottery, was one of the longest-continued (about 5000 BCE–3000 BCE), most widely distributed, and most far-reaching archaeological cultures in Chinese Neolithic (Liu and Chen, 2012; Wei, 2021). Thirdly, the Guanzhong region is of imperative importance in terms of cultural exchange. The Loess Plateau where the Guanzhong region is located had been a key passage for the prehistoric spread of several important crops (millet, rice and wheat) and domesticated animals (pig, cattle, goat and sheep) both across Eurasia and within East Asia. Zooarchaeological research on this region has been carried out most fully in China (Yuan, 2015), with over 20 sites have been systematically studied, providing rich datasets for us to discuss meat procurement strategies. Recognizing the importance of this region, researchers have recently shifted their focuses towards subsistence economy in Guanzhong (e.g., Zhao and Chen, 2011; Qu et al., 2018; Zong et al., 2021; Festa and Monteith, 2022). However, there is still a number of the sites that have not been covered by their datasets and a lack of discussion on the relationship between the regional subsistence economy and the corresponding societal development. In this study, we collate zooarchaeological data from 26 sites/locales in the Guanzhong region and summarize the characteristics, causes and consequences

of the meat procurement strategy, thus attempting to explore the links between meat procurement strategies and the regional trajectory of societal development.

Guanzhong: geographical and archaeological background

The Guanzhong region is a basin plain located in the central part of Shaanxi Province (Figure 1). The Wei River and its tributaries flow through this area, carrying a large amount of sediment and nutrients, gradually forming the flat and fertile Guanzhong Plain. The surrounding mountains prevent it from cold air currents, maintaining this region in a relatively warm and humid climate which is ideal for agricultural development (Shi, 1963; Compilation group of Shaanxi agricultural geography, Department of Geography and Northwest University, 1979; Nie, 1981; Jin et al., 2002).

The chronology of the regional archaeological culture from the Neolithic to the Bronze Age in Guanzhong has been clearly depicted (Institute of Archaeology, Chinese Academy of Social Sciences, 2010; Gong, 2018) (Table 1). The Laoguantai culture (5900 BCE–5000 BCE) is the earliest Neolithic culture in Guanzhong (National Cultural Heritage Administration, 1998), since which sedentary lifeway and food production had begun (Barton et al., 2009; Atahan et al., 2011). During the Laoguantai period, dogs (*Canis lupus familiaris*) and pigs (*Sus scrofa domestica*) were domesticated. The subsequent Yangshao culture was a key period for the establishment of the agricultural economy and the emergence of social complexity (Liu, 2005; Dai, 2012). The Yangshao culture was comprised of three phases: the Banpo culture (4900 BCE–3800 BCE), the Miaodigou culture (3900 BCE–3600 BCE) and the Xiwangcun culture (3600 BCE–2900 BCE). The Yangshao culture was replaced by the Longshan culture around 2900 BCE. The early Longshan period is the Miaodigou II culture (2900 BCE–2600 BCE) and the late Longshan period is the Keshengzhuang culture (2600 BCE–2000 BCE). The number of sites and settlement size generally declined compared with those of the Yangshao period and large-scaled settlements or luxurious burials were hardly found (Liu, 2005; Zhang, 2017). New domesticated species, such as cattle (*Bos taurus*), sheep (*Ovis aries*) and goat (*Capra hircus*), were introduced during the Keshengzhuang culture period (Liu et al., 2001). Xia (2070 BCE–1600 BCE), Shang (1600 BCE–1046 BCE), pre-Zhou (1300 BCE–1046 BCE), and Western Zhou (1046 BCE–771 BCE) comprise the Bronze Age culture (Expert Group of the Xia-Shang-Zhou Project, 2000; Yuan, 2019a). The Guanzhong region, being a peripheral area to the great polities in the Central Plains during the Xia-Shang period, had not become the political and economic center until the Western Zhou dynasty (National Cultural Heritage Administration, 1998; Shang-Zhou Archaeology Research Office of Shaanxi Provincial Institute of Archaeology, 2018). During the Bronze Age, domesticated horses and chicken firstly emerged in this region (Yuan, 2015).

Materials and methods

We collate published data from 26 sites/locales in the Guanzhong region. Only mammals are considered in this study,

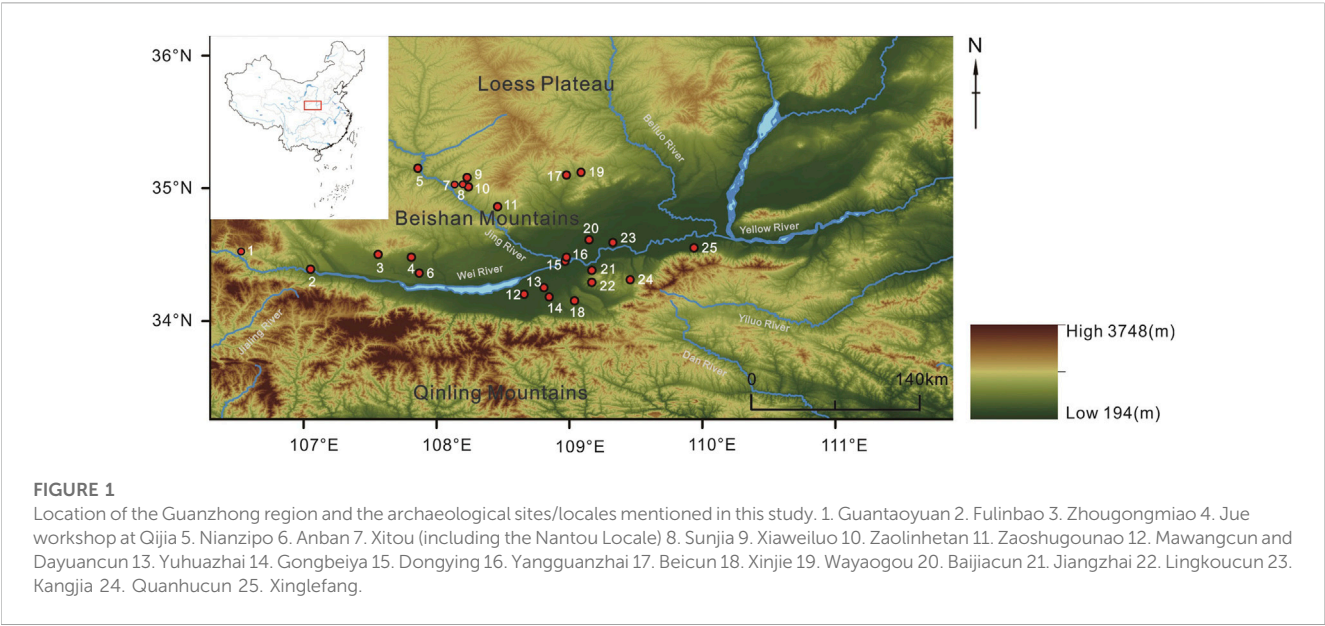


TABLE 1 The chronology of archaeological culture from the Neolithic to the Bronze Age in the Guanzhong region.

Bronze Age	—	Western Zhou	1,046–771 BCE
		Pre-Zhou	1,300–1,046 BCE
		Shang	1,600–1,046 BCE
		Xia	2,070–1,600 BCE
Neolithic Age	Longshan	Keshengzhuang culture	2,600–2,000 BCE
		Miaodigou II culture	2,900–2,600 BCE
	Yangshao	Xiwangcun culture	3,600–2,900 BCE
		Miaodigou culture	3,900–3,600 BCE
		Banpo culture	4,900–3,800 BCE
	Pre-Yangshao	Laoguantai culture	5,900–5,000 BCE

as they are better representations of meat procurement strategies considering their contribution in human diet. Number of Identified Specimens (NISP) is the primary option for quantitative analysis in this study as it is a more direct reflection of the quantity of the faunal assemblage compared with Minimum Number of Individual (MNI) which is derived from NISP and influenced by aggregation method (Lyman, 2008). Only for sites where NISP data is unavailable, we alternatively use MNI. Taking the sample size into consideration, faunal assemblages with NISP over 100 or MNI over 10 are included (Figure 1; Table 2).

Previous studies have suggested that hunting and husbandry were two major forms of meat procurement strategies in China (Yuan, 1999). We therefore calculate the proportion of the domesticated versus wild animals for each site to assess the relative reliant on hunting and husbandry in regional subsistence.

We further compare the relative abundance of the domesticated pigs and bovids (i.e., sheep, goat and cattle) in order to explore the changing husbandry practices. Pig was one of the earliest domesticates original in China while the domesticated bovids were introduced by the third

millennium BC (Yuan, 2015). The introduction of these west-Asian original domesticates would have re-shape the local subsistence to various extents (Zhang, 2017). As seen in the Gansu-Qinghai region where a transformation from agricultural towards a mixed agropastoral economy was evident (Chen, 2018). At the scenario in Guanzhong remains unclear. As a result, by calculating the proportion of the domesticated pigs versus bovids, we attempt to capture the changing progress in subsistence economy and its influence on societal development.

Studies focusing other regions have shown that settlements of different scales may use different subsistence strategies (Price et al., 2017; Yuan et al., 2020; Tao et al., 2022a; Itahashi, 2022). The settlement hierarchy in the Guanzhong region appeared at least during the middle Yangshao period (Dai, 2012). We group them into two categories: the central urban settlements over 20 ha or with walls, and those lower ranking ordinary settlements under 20 ha according to Liu’s criteria (2005) (Table 3). The patterning of zooarchaeological data from the two settlement categories are compared.

Results

The results of our analysis are summarized in Table 2; Figure 2.

During the pre-Yangshao period, all sites at Guangzhong procured meat resources mainly by hunting wild animals, supplemented by raising livestock. During the early Yangshao period, most sites still relied heavily on hunting wild animals and supplemented by raising livestock. However, there are a few sites where raising livestock was the major way to procure meat resources, suggesting a growing reliance on husbandry. During the middle Yangshao period, husbandry became the major meat procurement strategy despite the coexistence of the wild-resources exploitation. During the late Yangshao period, the level of livestock husbandry significantly declined in most sites except for the Xinjie site and a simultaneous intensification of wild animal utilization is found. Pigs and dogs were the only two domesticates in the pre-Yangshao and Yangshao periods. Husbandry and hunting co-existed during the Longshan period. During the early Longshan period, faunal assemblages

TABLE 2 Summary of the zooarchaeological data from the Neolithic to the Bronze Age sites in the Guanzhong region.

Period	Site	Archaeological culture	Species of domesticated animals	Approaches to counting	Number	Proportion of domesticated animals (%)	Proportion of pigs in domesticated animals	Proportion of domesticated bovids (sheep, goat, and cattle) in domesticated animals	Proportion of the rest domesticated animals	References
Pre-Yangshao	Baijiacun	Laoguantai culture	Pig, dog	NISP	706	37	97%	0%	3%	Zhou (1994)
	GuantaoyuanII	Laoguantai culture	Pig	NISP	148	6	100%	0%	0%	Hu (2007)
	GuantaoyuanIII	Laoguantai culture	Pig	NISP	267	3	100%	0%	0%	Hu (2007)
Early Yangshao	LingkoucunII	Lingkoucun culture	Pig	NISP	315	57	100%	0%	0%	Zhang et al. (2004)
	Jiangzhail	Banpo culture	Pig, dog	NISP	2,203	24	99%	0%	1%	Qi (1988)
	Lingkoucun III	Banpo culture	Pig	NISP	116	52	100%	0%	0%	Zhang et al. (2004)
	Wayagou	Banpo culture	Pig, dog	NISP	6,094	40	96%	0%	4%	Wang H. (2011)
	YuhuazhailI	Banpo culture	Pig	MNI	28	39	100%	0%	0%	Hu and Yang (2017)
	JiangzhailI	Shijia culture	Pig	NISP	342	13	100%	0%	0%	Qi (1988)
	YuhuazhailII	Shijia culture	Pig, dog	MNI	34	12	75%	0%	25%	Hu and Yang (2017)
Middle Yangshao	Dongying (early phase)	Miaodigou culture	Pig	NISP	205	20	100%	0%	0%	Hu (2010)
	Yangguanzhai	Miaodigou culture	Pig, dog	NISP	375	94	82%	0%	18%	Hu et al. (2011)
	Quanhucun	Miaodigou culture	Pig, dog	NISP	2,693	83	90%	0%	10%	Hu (2014)
	Xinglefang	Miaodigou culture	Pig, dog	NISP	155	95	97%	0%	3%	Hu and Yang (2019)
Middle and late Yangshao	Fulinbao	Miaodigou and Xiwangcun culture	—	NISP	180	0	—	—	—	Wu, 1993
	Gongbeiya	Miaodigou and Xiwangcun culture	Pig, dog	NISP	220	44	77%	0%	23%	Zong et al. (2021)
Late Yangshao	Jiangzhai IV	Xiwangcun culture	Pig, dog	NISP	588	16	95%	0%	5%	Qi (1988)
	Yuhuazhai IV	Xiwangcun culture	Pig	MNI	20	45	100%	0%	0%	Hu and Yang (2019)

(Continued on following page)

TABLE 2 (Continued) Summary of the zooarchaeological data from the Neolithic to the Bronze Age sites in the Guanzhong region.

Period	Site	Archaeological culture	Species of domesticated animals	Approaches to counting	Number	Proportion of domesticated animals (%)	Proportion of pigs in domesticated animals	Proportion of domesticated bovids (sheep, goat, and cattle) in domesticated animals	Proportion of the rest domesticated animals	References
	Xinjie (early phase)	Xiawangcun culture	Pig, dog	NISP	1,609	81	92%	0%	8%	Hu (2020)
Early Longshan	AnbanIII	Miaodigou IIculture	Pig, dog	NISP	1,293	73	98%	0%	2%	Hou (2016)
	Xinjie (late phase)	Miaodigou IIculture	Pig, dog	NISP	632	63	92%	0%	8%	Hu (2020)
Longshan	Xiaweiluo	Miaodigou Iland Keshengzhuang culture	Pig, dog, goat	MNI	96	70	83%	9%	8%	Zhang (2006)
	The Nantou Locale of Xitou	Miaodigou Iland Keshengzhuang culture	Pig, dog, sheep, goat, cattle	NISP	225	89	79%	17%	5%	Wang et al. (2023)
Late Longshan	JiangzhaiV	Keshengzhuang culture	Pig, dog	NISP	334	12	51%	0%	49%	Qi (1988)
	Dongying (late phase)	Keshengzhuang culture	Pig, dog, sheep, cattle	NISP	291	57	54%	39%	7%	Hu (2010)
	Kangjia	Keshengzhuang culture	Pig, dog, sheep, goat, cattle	NISP	6,585	36	55%	33%	12%	Hu (2023)
Shang	Beicun	Shang	Pig	MNI	175	27	100%	0%	0%	Cao (2001)
Pre-Zhou	Mawangcun and Dayuancun	pre-Zhou	Pig, dog, sheep/goat, cattle, chicken, horse	MNI	20	75	60%	27%	13%	Yuan and Xu (2000)
	Nianzipo	Pre-Zhou	Pig, dog, goat, cattle, chicken, horse	NISP	9,070	97	37%	55%	8%	Zhou (2007)
	Zhougongmiao	pre-Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	3,881	89	49%	48%	3%	Zhang (2012)
	Zaoshugounao	pre-Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	9,301	84	27%	62%	10%	Li (2015)
	Zaolinhetan	pre-Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	1,305	95	21%	52%	27%	Li et al. (2019)
	Sunjia	pre-Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	355	93	18%	67%	15%	Festa et al. (2023)
	Xitou	Pre-Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	236	95	19%	68%	13%	Festa et al. (2023)

(Continued on following page)

TABLE 2 (Continued) Summary of the zooarchaeological data from the Neolithic to the Bronze Age sites in the Guanzhong region.

Period	Site	Archaeological culture	Species of domesticated animals	Approaches to counting	Number	Proportion of domesticated animals (%)	Proportion of pigs in domesticated animals	Proportion of domesticated bovids (sheep, goat, and cattle) in domesticated animals	Proportion of the rest domesticated animals	References
Western Zhou	Mawangcun and Dayuancun	Western Zhou	Pig, dog, sheep/goat, cattle, chicken, horse	MNI	44	86	47%	40%	13%	Yuan and Xu (2000)
	Jue workshop at Qijia	Western Zhou	Pig, dog, sheep, goat, cattle	NISP	1,344	92	21%	65%	15%	Ma and Hou (2010)
	Zhougongmiao	Western Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	3,787	87	22%	69%	9%	Zhang (2012)
	Zaoshugounao	Western Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	331	88	41%	42%	17%	Li (2015)
	The Nantou Locale of Xitou	Western Zhou	Pig, dog, sheep, goat, cattle, horse	NISP	1,119	86	18%	72%	9%	Wang et al. (2023)

from two sites were dominated by domesticated animals, while in most late Longshan sites faunal assemblages were predominated by wild animals, indicating a declining trend in livestock raising. Pigs were still the most abundant domesticated species despite the introduction of cattle, sheep and goats. During the Shang period, the result from Beicun suggests that wild animals were predominant and pigs were the only domesticated species whereas at Laoniupo the proportion of pigs is over 60% (Zhang et al., 2007). This case indicates that there were also two meat procurement strategies during the Shang period. A remarkable change in the meat procurement strategy has been found during the pre-Zhou and Western Zhou periods when husbandry became the major way of meat procurement in all sites. Horses and chickens have been introduced by then. The domesticated bovids for the first time outnumbered pigs, indicating their growing importance.

Taking the settlement hierarchy into account, the changing of the meat procurement strategy in Guanzhong seemed to follow two intertwined clues: the chronological process and settlement hierarchy. The change of the meat procurement strategy can be roughly divided into three stages chronologically. The first stage is from the pre-Yangshao to early Yangshao periods, when no apparent settlement hierarchy appeared and most of the sites heavily relied on hunting wild animals. The second stage is the middle Yangshao period to the Shang dynasty when a contrasting pattern was observed: a heavy reliance upon husbandry among central urban settlements and exploitation of wild animals among lower ranking settlements¹. The third stage is the pre-Zhou and Western Zhou periods, during which husbandry had become the major subsistence strategy regardless of the settlement rank. The predominant domesticated taxa shifted from pigs in the first and second stages to bovids by the third stage.

The second clue is the scale of the settlement. Pearson correlation coefficient ($r = 0.3$) showed a positive correlation between the proportion of the domesticated animals and the site size, implying a potential link between husbandry intensification and urbanization.

Discussion

Characteristics of the meat procurement strategy in the Guanzhong region

Two characteristics of the meat procurement strategy in the Guanzhong region can be summarized. Firstly, from Yangshao to Shang, the two meat procurement strategies—animal husbandry and exploitation of wild resources—seemed to have coexisted for a long time, and the meat procurement strategy was closely related to the hierarchy of settlements. While central urban settlements opted to animal husbandry, residents in lower ranking settlements mainly relied on hunting wild animals. Secondly, the change of meat procurement strategy unlike followed a progressive trajectory from hunting to husbandry. Instead, it took a winding route involving husbandry intensification alternating with regression to wild resource utilization.

1 The area of the Guantaoyuan site could not be confirmed, while the Lingkoucun site and the Dongying site were severely damaged and only a small area remains, so these sites were excluded from the discussion.

TABLE 3 Settlement hierarchy from the Neolithic to the Bronze Age in the Guanzhong region².

Site	Area (hectare)	Rank	References
Baijiacun	12	Ordinary	Institute of Archaeology, Chinese Academy of Social Sciences (1994)
Guantaoyuan	Unknown	—	
Lingkoucun	Remain 2 (seriously ruined)	—	Shaanxi Provincial Institute of Archaeology (2004)
Jiangzhai	5	Ordinary	Xi'an Banpo Museum et al. (1988)
Wayagou	5	Ordinary	Wang et al. (2014)
Yuhuazhai	7.5	Ordinary	Xi'an Institute for the Preservation of Cultural Heritage and Archaeology (2012)
Dongying	Remain 0.9 (seriously ruined)	—	Shaanxi Provincial Institute of Archaeology et al. (2010a)
Yangguanzhai	Over 100	Central	Yang (2018)
Quanhucun	60	Central	Shaanxi Provincial Institute of Archaeology et al. (2014)
Xinglefang	28.5	Central	Shaanxi Provincial Institute of Archaeology (2019)
Fulinbao	18	Ordinary	Baoji Archaeological Team (1993)
Gongbeiya	14	Ordinary	Tang et al. (2020)
Xinjie	30	Central	Shaanxi Provincial Institute of Archaeology (2020)
Anban	70	Central	Archaeology major of School of Cultural Relics and Museum, Northwest University (2000)
Xiaweiluo	Unknown	Central	Cultural heritage and Archaeology Research Center of Northwest University et al. (2006)
Xitou	100	Central	School of Cultural Heritage et al. (2020)
Kangjia	19	Ordinary	Kangjia Archaeological Team of Shaanxi Provincial Institute of Archaeology (1992)
Beicun	18	Ordinary	Shang-Zhou Group et al. (1994)
Laoniupo	140	Central	Shaanxi Provincial Institute of Archaeology (2021)
Mawangcun and Dayuancun (Fengjing)	1,000	Central	National Cultural Heritage Administration (1998)
Nianzipo	16	Ordinary	National Cultural Heritage Administration (1998)
Zaoshugounao	300	Central	Cultural heritage and Archaeology Research Center of Northwest University et al. (2012)
Zaolinhetan	8	Ordinary	School of Cultural Heritage et al. (2019)
Jue workshop at Qijia (Zhouyuan)	3,000	Central	Shaanxi Provincial Institute of Archaeology et al. (2010b)
Zhougongmiao	130	Central	Zhouyuan Archaeological Team (2006)
Sunjia	20	Central	Li and Dou (2022)

Factors influencing the changing meat procurement pattern

The relationship between the change in the meat procurement strategy at Guanzhong and environmental variation and climate fluctuation has been much discussed (e.g., [Qu et al., 2018](#); [Festa and Monteith, 2022](#)). Environments indeed provide a general context to explore changes of subsistence economy. There are many more

factors worthy of further consideration to decipher the interplay of subsistence and societal development.

First of all, the regional environment in Guanzhong, which was suitable for agriculture and rich in wild resources, would have supported the long-term coexistence of the two meat procurement strategies. On one hand, its flat terrain, the abundance of fertile loess, and a warm-humid climate have provided ideal conditions for agriculture. Isotope analyses have demonstrated that pig husbandry was closely related to millet cultivation in the Guanzhong region ([Pechenkina et al., 2005](#); [Chen et al., 2016a](#)). Agricultural intensification thus seemed a twin process with pig husbandry in central China. Consequently mild environment is key to the development of husbandry. On the other hand, wild animals were abundant in Guanzhong during the prehistoric periods ([Lander, 2020](#)), among which sika deer (*Cervus nippon*) were widely utilized as evidenced by the frequent recovery of

² Mawangcun and Dayuancun is a part of Fengjing site, so we used the area data of Fengjing site; similarly, Jue workshop at Qijia is a part of Zhouyuan site, so we used the area data of Zhouyuan site; researchers did not publish the area data of Xiaweiluo site, but they defined it as a "large site" in the report, so we marked it as a "central settlement" here.

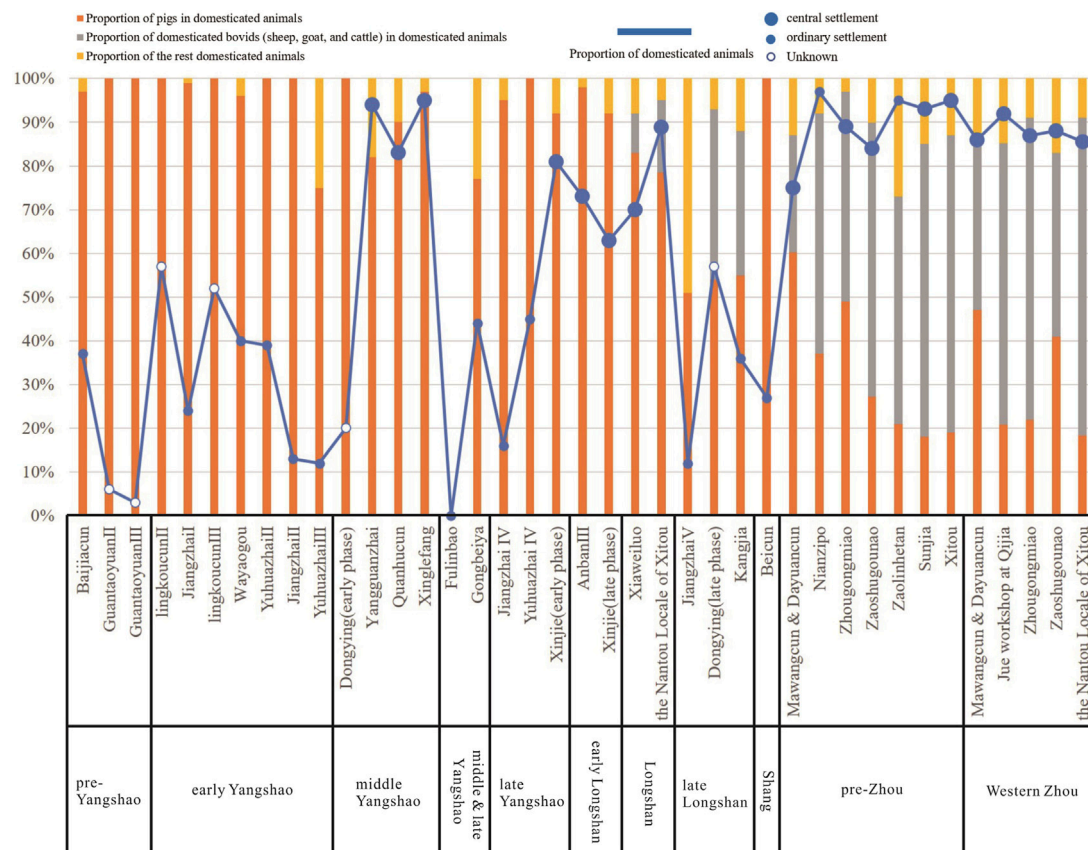


FIGURE 2

The proportion of domesticated animals in all animals, and the proportion of each kind of domesticated animals (pigs, domesticated bovids and others) in all domesticated animals in each site from the Neolithic to the Bronze Age in the Guanzhong region.

their remains in many archaeological sites during the Neolithic and the Bronze Age (Li Y. et al., 2021). It is plausible to envisage that the abundance of wild animal resources made it a reasonable choice to hunt wild animals for meat.

Population size might have influenced the change of the meat procurement strategy. Yuan (2015) proposes a “passive development theory” to explain the transformation of the meat procurement strategy in prehistoric China where two influential factors are considered: the demand for meat resources and the availability of wild animal resources. Demographic factor plays a crucial role in the adoption of a particular meat procurement strategy. This is not only because the population size affects the amount of meat that is consumed, the relationship between human settlement expansion and wildlife habitat shrinkage but also counts. The decrease in wildlife population would have increased the difficulty of hunting. The number of the Miaodigou culture sites (middle Yangshao period) in Shaanxi is more than twice that of Banpo culture (early Yangshao period) (National Cultural Heritage Administration, 1998), and the average size of the Miaodigou culture sites is almost twice that of Banpo culture, whereas the number of those large sites is more than three times that of Banpo culture (Liu, 2009). The population size can be estimated by the settlement number and size (Liu, 2005; Wang J. H., 2011). That says, during the Miaodigou culture, the population size increased

corresponding to the dramatic growth in the proportion of the domesticated animals. During the late Yangshao, Longshan, and Xia-Shang periods, when the number of sites decreased and the average site area shrunk (Weishui Team of Shaanxi Archaeological Institute, 1960; Wang and Qian, 1996; National Cultural Heritage Administration, 1998; Zhouyuan Archaeological Team, 2005; Liu, 2009; Shao, 2009; Ma, 2010; Zhouyuan Archaeological Team, 2010; Zhai, 2013; Ge, 2017; Jiang and Cui, 2017; Zhou and Cui, 2022), the proportion of the domesticated animals also declined. Guanzhong, being the political and economic center, reflourished once again during the pre-Zhou and Western Zhou periods. Not only did the number of the sites increase dramatically to over 800 (National Cultural Heritage Administration, 1998), but also the settlement density was more than twice that of the Longshan period. The settlement size also increased significantly, with the largest one exceeding 10 million square meters, forming an extremely colossal central settlement (Zhou and Cui, 2022). During this period, the meat procurement strategy shifted towards a heavy reliance on livestock. It is evident that the demographic factor had significant influences on the change of the meat procurement strategy.

The settlement hierarchy-related difference in meat procurement strategy is observed during the middle Yangshao to Shang periods, which is in agreement with the climax of the social complexity process.

In the pre-Yangshao period, the number of sites being small and most of the site sizes are between 10,000–20,000 m² (National Cultural Heritage Administration, 1998), no social differentiation is observed. Differences in the meat procurement strategy did not emerge yet. The significant disparity in settlement size began during the Miaodigou culture of the middle Yangshao period (Dai, 2012). The difference in the meat procurement strategy between central and ordinary settlements is also obvious. After the Miaodigou culture, although the growing process of social complexity dropped out in Guanzhong, a three- or four-tiered settlement hierarchy still existed (Liu, 2009). Correspondingly, two meat procurement strategies coexisted.

The dimorphic pattern in meat procurement strategy between central and ordinary settlements in Guanzhong region might have been related to the demographic factor as well. In general, large sites have a larger population and therefore greater demand for meat. Urban dwellers who were not engaged in agricultural production requires more food supplied for cities. In response to the pressure of the increasing population, some central settlements in the middle Yellow River region during the late Yangshao period increased land use efficiency and agricultural yield through different means of agricultural intensification such as mixed cropping, manuring, and expansion of farmland (Tao et al., 2022b; Yang et al., 2022). There was a simultaneous intensification of animal husbandry. Inferring from isotope results, the average $\delta^{13}\text{C}$ value of pigs from central urban settlements is $-8.6\text{‰} \pm 1.3\text{‰}$ at Xinglefang, and $-8.9\text{‰} \pm 1.3\text{‰}$ at Quanhucun respectively (Hu et al., 2014; Hu et al., 2020), which are significantly higher than that of pigs from the ordinary settlement Kangjia, which is $-10.3\text{‰} \pm 2.4\text{‰}$ (Pechenkina et al., 2005). The domesticated pigs at urban settlements apparently consumed more C₄ plants, which in the Guanzhong region were mainly millet and its by-products. Provisioned by fodder from millet cultivation, pigs could have been managed in a more enclosed and intensive way. This intensified husbandry, capable to feed more people, adapted well to the growing urbanism in Guanzhong.

The urbanization process could have brought in the shrinkage of the wildlife habitat, resulting in the decline of wildlife resources. At the same time, intensified agricultural practices left farmers less time for hunting. Residents in smaller sites, on contrary, still could have relied on hunting wild animals in the surrounding environment due to a low population density, as seen in examples of Kangjia and Jiangzhai where a variety of wild animals were utilized (Qi, 1988; Liu et al., 2001).

In the pre-Zhou and Western Zhou periods, the overall subsistence shifting to husbandry may be also attributed to some social factors. In many historical documents such as *Shi Ji Zhou Ben Ji*, *Shi Da Ya Sheng Min*, and *Shi Da Ya Gong Liu*, it is recorded that ancestors of the Zhou community specialized in husbandry, and rulers attached great importance to husbandry, encouraged people to develop husbandry (Liu, 1985). This may be an important reason for the increase in the proportion of domesticated animals in the pre-Zhou period. The husbandry intensification in the Western Zhou period may also be related to the change of social organization. The Guanzhong region was marginal to the great polities in the Central Plains during the Xia and Shang periods while in the Western Zhou period, it became

the capital of the state where agriculture production was under unified planning and strict organization by the rulers. Inferring from several historical texts, officers were assigned in charge of husbandry (Liu, 1985; Si, 2008; Geng, 2012; Lu, 2013; Zhao, 2018), decrees to plan specialized grazing land and restrict the pastoralists from changing their professions at will were issued to ensure that there were sufficient labor for animal husbandry (Liu, 1985). The inscriptions on the unearthed bronzes also record that Zhou elites controlled both the land and the labour for animal husbandry (Chen, 2022).

Changes in the livestock composition

Another issue worthy of attention is the time lag between the introduction and the intensive utilization of the domesticated bovids in Guanzhong. Unlike the rapid replacement by the bovids for the pigs in the Gansu-Qinghai region and northern Shaanxi after their introduction (Zhang, 2017), the domesticated bovids remained under-exploited until the Zhou dynasty. Apart from the fact that the mild condition at Guanzhong rendered rich wild resources and residents there experienced lower population pressure during the Longshan periods, it is also plausible that the innovative technologies (milking, processing wool) and husbandry skills (killing cycle, breeding, etc.) tailored for the utilization of the bovids' secondary products in West and Central Asia were not readily accepted by local people, and failed to be integrated into the existing local subsistence system in the Guanzhong region. As a result, the meat procurement strategy maintained the long tradition of the combination of pig husbandry and wildlife hunting.

During the pre-Zhou and Western Zhou periods, domesticated bovids replaced pig as the main livestock species. Festa and her colleagues suggest that it may be related to the increasingly cold and dry climate and the contact with northern pastoral community (Festa et al., 2023). We believe that in addition to these factors, the adjustment of livestock structure during this period may also be related to their biological natures. As the dramatic increase in the population likely led to increasing demand for meat, it is necessary to improve the utilization efficiency of land within a limited area. The biological natures of domesticated bovids make them a better choice than pigs. The first advantage is their fodder. Since cattle mainly fed on millet straw, sheep and goat mainly fed on wild plants (Chen, 2021), they could transform shrubs and grasses that were not directly edible to humans into meat. Isotopic studies on animals from the Zaoshugou and Zhougongmiao sites during the pre-Zhou and Western Zhou periods indicate a combination of penned feeding and grazing (Lan, 2017; Li N. et al., 2021). In this sense, the husbandry of the domesticated bovids developed a new way of land use out of the farmlands, significantly improving the efficiency of land use. Secondly, because of its gregarious nature, the state-organized and institutionalized ranch management formed in the Western Zhou dynasty (Okamura, 2020; Chen, 2022) made it more efficient to raise domesticated bovids than to raise pigs. In addition, the amount of meat provided by cattle is about three times that of pigs (Luo, 2012), feeding cattle is an effective way to increase the amount of meat. Besides, the age structure of the herds indicates that the acquisition of meat resources was the main aim of husbandry while secondary products were utilized though to a limited extent (Li, 2015; Li et al., 2019). The diverse utilization of domesticated

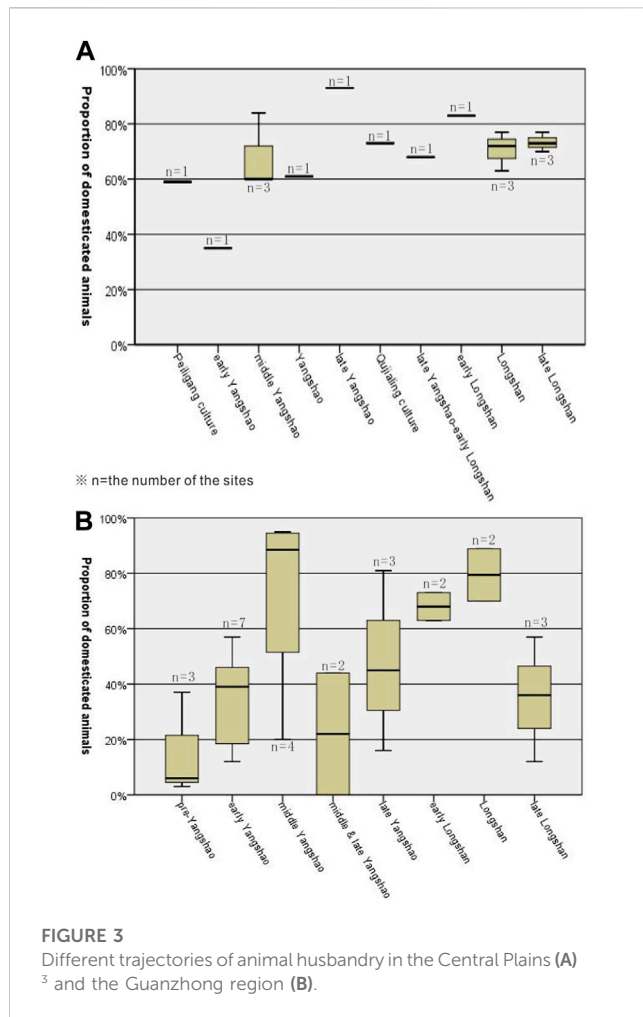


FIGURE 3
Different trajectories of animal husbandry in the Central Plains (A)
and the Guanzhong region (B).

bovids elevated their importance in the animal economy. Meanwhile, there was a rising importance of the bovids in ritual contexts during the Shang and Zhou periods (Liu et al., 2020), which could have also intensified husbandry. All of those factors have contributed to the improvement of the status of domesticated bovids in livestock during this period.

Subsistence and societies

It is noted that the Guanzhong region has the potential for the development of civilization, as evidenced by the prosperity of the Miaodigou culture and the establishment of the Western Zhou dynasty. However, the earliest civilization and complex polity did not occur there. There was a general decline of archaeological cultures in China during 1900 BCE-1500 BCE, except for the Central Plains where the Erlitou culture emerged around 1800 BCE and bred the earliest state in China (Yuan, 2016). The zooarchaeological data there shows that the proportion of livestock in the Central Plains increased from the middle Neolithic (about 7000 BCE-5000 BCE) to the terminal Neolithic (namely, the Longshan period, about 3000 BCE-2000 BCE), at a steady and continuous pace (Bai, 2020; Figure 3A). The characteristics of subsistence economy in the Central Plains may be related to the favorable natural environment for agriculture (Yuan,

2019b) and a constantly growing population size (Chen et al., 2003; Erlitou Archaeological Team, Institute of Archaeology, Chinese Academy of Social Sciences, 2005). Besides, a diversified livestock regime including pigs, dogs, sheep, and cattle had been formed rapidly during the Longshan period, securing stable meat provision for the rapid growth of the population (Yuan, 2016). This progressive development of the subsistence economy would have laid a solid economic foundation for the emergence of China's first dynasties.

On contrary, although new productivity elements such as sheep, goat and cattle were introduced in the Guanzhong region during the Longshan period, they were not widely utilized. The subsistence economy in the Guanzhong region maintained unstable and imbalanced and had not been well developed for thousands of years (Figure 3B), making it unsuitable for the formation of early civilization. During the pre-Zhou period, the meat procurement strategy changed dramatically with husbandry intensified and the livestock diversified. Consequently this development of the subsistence economy, contributing to the population growth and growing social complexity, laid a solid economic foundation for the Zhou polity to defeat the Shang and establish a new political authority.

Subsistence economy, determining food availability, and shaping the pattern of land use and labor organization, plays an imperative role in social development (Weiskopf, 2010; Barton and An, 2014). It is very likely that agricultural productivity and the invention/adoption of military technologies could have played two strong causal roles in increasing the scale and complexity of human societies (Turchin et al., 2022). In China, the establishment and expansion of agricultural complexity seemed a key force driving economic, cultural and social changes including the formation of state-level societies (Chen et al., 2016b), which has been archaeologically verified in the cases of the Central Plains and the lower Yangtze valley (Yuan, 2016; Jing et al., 2020; Yuan et al., 2020). In this study, faunal remains from the Guanzhong region support the close association between subsistence and societal development.

Conclusion

In this paper, we summarize the characteristics of the meat procurement strategy from the Neolithic to the Bronze Age in the Guanzhong region. Firstly, the development of husbandry was discontinuous and unstable, following a winding trajectory involving acceleration and regression. Secondly, there was a mosaic meat procurement pattern where heavy reliance on husbandry was more frequently found in central urban settlements and exploitation of wild animals in settlements of lower ranking throughout the Yangshao to Shang periods.

Environmental, demographic and societal factors would have shaped the meat procurement pattern. The subsistence, in turn, could have influenced the development of society. The Guanzhong region failed to evolve into a more complex great polity earlier partly due to the unstable and imbalanced subsistence during the Longshan and Shang periods. The formation of the intensified and diversified meat procurement strategy during the pre-Zhou and Western Zhou periods, would have supported the rise of the Zhou authority originating from Guanzhong.

We hope that future research can be expanded into untapped regions, such as the Sichuan Basin and Northeast China, with the

aids of isotope and ancient DNA analyses. A closer look into a particular region would certainly help contextualize our comprehension of the complex interplay of subsistence economy, culinary tradition and societal development.

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

The study was designed by QH and JY. The data was collected by QH and analyzed by QH. QH and ND wrote the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Historical overview and challenges in the development of bioarchaeology in Japan

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Archaeological human skeletons provide direct evidence of the physical features, lifestyle, diseases, mortality, and health of our ancestors. Bioarchaeology explores population-based trends that vary according to subsistence, social stratification, urbanization, and industrial development. The first systematic bioarchaeological studies in Japan were those on medieval human skeletons in Kamakura City, Japan, in 2003. However, most anthropological studies have focused on the origin and population history of the Japanese since the end of 19th century. Moreover, the number of bioarchaeological studies in Japan is far lower than that in North America, Europe, and Latin America. This paper reviews the history of bioarchaeological research in Japan and discusses the problems associated with its development.

KEYWORDS

bioarchaeology, osteoarchaeology, research history, human skeletons, Japan

1 Introduction

Archaeological human skeletons provide direct evidence of the physical features, lifestyle, diseases, mortality, and health of our ancestors. The research field of archaeological human skeletons is categorized into osteoarchaeology and bioarchaeology, which are part of physical anthropology. The lifestyle, mortality, diseases, and health of human remains have been explored by many physical anthropologists (such as Koganei, 1894; Sakura, 1964; Kobayashi, 1967; Suzuki, 1984) until the 1990s. Most physical anthropologists in Japan have actively examined the origin and population history of the Japanese since the end of the 19th century (Koganei, 1894), though some have travelled overseas to explore the population history of the Asian peoples (Matsumura et al., 2019).

2 Origins of osteoarchaeology in Japan

Kazumichi Katayama of Kyoto University wrote the book, *Ancient bones talk: Beginning of Osteoarchaeology* (1990) which introduced osteoarchaeology to Japan. In this book, Katayama defines osteoarchaeology as a study that explores lifestyles (such as, subsistence, societies, and habitual postures) from archaeological human remains using the methods of physical anthropology. He related archaeological human skeletons to the lives of ancient humans: the skeletal data could thus be related to and reveal information about the habitual postures, lifestyle, diets, diseases, mortality, and health of an ancient population. Though prior paleopathological and paleodemographic studies have explored ancient lives and diseases to yield significant results (Kobayashi, 1967; Suzuki, 1998), Katayama (1990)

proposed a new research area and tried to integrate these studies. Since then, osteoarchaeology has developed as a branch of physical anthropology in Japan. The Osteoarchaeology subcommittee of the Anthropological Society of Nippon was established in 1997, and the Japanese Society of Paleopathology was established in 2016. The spread of osteoarchaeology in Japan never lagged behind that in the United States and Europe. This can be understood from the fact that the *International Journal of Osteoarchaeology* started publication in 1991. Osteoarchaeology combines osteology and archaeology and targets both human and animal bones. However, the current study only concerns human bones, based on to Katayama (1990), who related osteoarchaeology to ancient human skeletons. In the 1990s, osteoarchaeological studies of human remains in Japan emphasized the diagnoses and interpretations of skeletal disorders (Inoue et al., 1999). Most of these studies were just case reports. The findings from these case reports are important; however, I wonder whether an accumulation of such studies could lead to a breakthrough in physical anthropology. To compensate for the lack of a grand theory, researchers in Japan prefer to use a message, “Bones talk.” In the 1990s, when *Ancient bones talk: Beginning of osteoarchaeology* was first published, this message was novel and thus attracted young researchers. However, this message has been used repeatedly, in both research and outreach programs, and has thus lost its attractiveness and novelty over the past 30 years. The message “Bones talk.” reflects a passive attitude, but researchers should be actively extracting data.

3 Transition to bioarchaeology in Japan

In the 2000s and later, research activity in osteoarchaeology in Japan decreased because the existing researchers were aging and there were very few young researchers. Since the end of the 20th century, the research area relating human skeletal remains to human lives has changed from osteoarchaeology to bioarchaeology in North America. Clark Spenser Larsen of Ohio State University published the book, *Bioarchaeology: Interpreting behavior from the human skeleton* (1997) and bioarchaeology spread worldwide. Bioarchaeology explores population-based trends that vary according to subsistence, social stratification, urbanization, industrial development, and climate change. It overcomes the limitations of osteoarchaeology which primarily focused on case reports and diagnoses of skeletal disorders. The accumulation of skeletons and the employment of new methods, such as stable isotope ecology and molecular biology, have contributed to the development of bioarchaeology.

Both osteoarchaeology and bioarchaeology target skeletal remains. However, bioarchaeology is different from osteoarchaeology in that it includes broad perspectives of regions and time periods to obtain population-based trends. The difference between osteoarchaeology and bioarchaeology is not just the difference in names, but also in the sets of concepts that determine a scientific discipline or “paradigm” as defined by Kuhn (1970).

The bioarchaeological study in Japan was first organized as a Grant-in-Aid for Scientific Research for a team with members from St. Marianna University School of Medicine (Kawasaki, Japan) and the University of Ryukyus (Nishihara, Japan). This research project

examined dental diseases, degenerative diseases of joints (Shimoda et al., 2012) and trauma (Nagaoka et al., 2009) of medieval human skeletons in Japan, but these bioarchaeological studies overlapped with osteoarchaeology and paleopathology. Most achievements that have influenced bioarchaeology in Japan have been made by foreign researchers. For example, Daniel Temple of Ohio State University examined linear enamel hypoplasia and dental caries in the Jomon and Yayoi people and showed detailed regional and temporal variations that Japanese anthropologists have not dealt with (Temple, 2007; Temple et al., 2008). His studies utilized Larsen’s (1997) concept of bioarchaeology which includes broad perspectives of regions and time periods to obtain population-based trends.

However, there are far fewer bioarchaeological studies in Japan than in North America, Europe, or Latin America. Since Suzuki (1969), the majority of the anthropological studies in Japan have focused on the origin and population history of the Japanese, and most of these studies lack a global perspective on the common causes that influence living conditions worldwide.

Fortunately, thousands of human remains are housed in physical anthropology laboratories in Japan. These materials are from various time periods and from various regions within the Japanese archipelagos and are thus ideal for elucidating whether epochal events such as subsistence changes, social stratification, climate change, and industrial revolution have affected health status. These materials can contribute to the development of bioarchaeology if more physical anthropologists become involved in this research field. Some skeletons that have been repeatedly observed by researchers over the past 100 years could still provide new insights. For example, the oldest recorded shark attack in the fisher-hunter-gatherer Jomon period was detected in such a housed skeleton in 2021 (White et al., 2021). The application of new methods to these materials has revealed the weaning age of the Jomon people (Tsutaya et al., 2014). The quality and quantity of information extracted from human remains depend on the researchers’ knowledge and analytical techniques used, although all physical anthropologists are confident in their appraisals.

4 Materials and methods

This study examined papers published in *Anthropological Science*, (2023), the official journal of the Anthropological Society of Nippon, to identify the trends in paper types, regions of research, materials, and methods and to understand the contribution of anthropology in Japan to the development of bioarchaeology. A total of 381 papers were published during 2003–2022 in *Anthropological Science*. These were categorized into four time periods: 2003–2007, 2008–2012, 2013–2017, and 2018–2022.

This study searched for certain terms in the full texts of papers using websites of *Anthropological Science*, (2023), counted the number of papers that contained these terms, and compared them with the data from the *American Journal of Biological Anthropology*, (2023) to understand the prevalence of bioarchaeology in human osteology and physical anthropology in Japan. Larsen (1997) edited a textbook on bioarchaeology that had chapters on stress and deprivation, infectious diseases, injuries and violence, activity patterns, masticatory and non-masticatory functions, isotopic analyses, biological distance, and

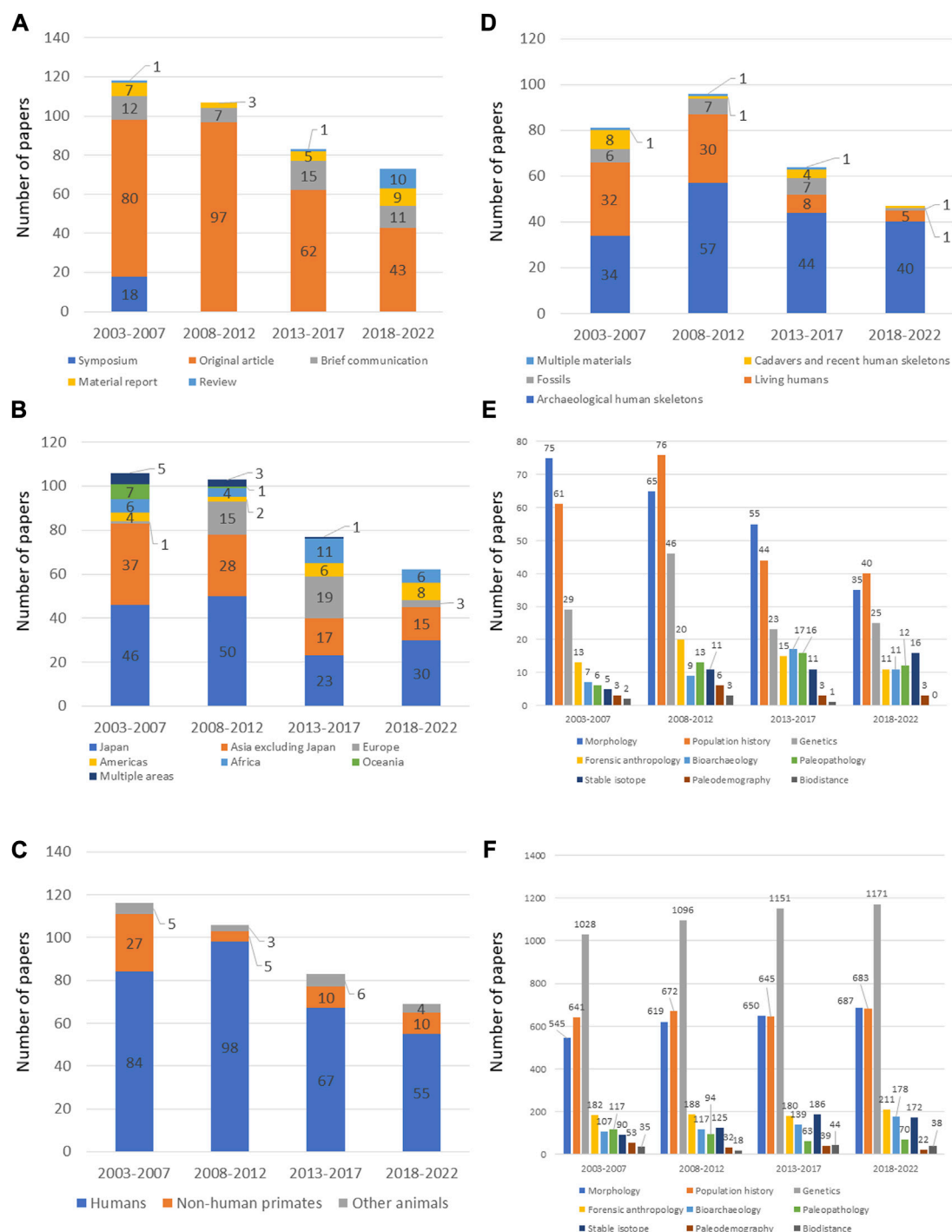


FIGURE 1

Temporal trends in the number of papers submitted to *Anthropological Science* from 2003 to 2022 (A–D) and comparison in the number of papers that contain the terms “morphology, population history, genetics, forensic anthropology, bioarchaeology, paleopathology, stable isotope, paleodemography, and biodistance” between *Anthropological Science* and the *American Journal of Biological Anthropology* (E–F). (A) paper types (symposiums, original articles, brief communications, material reports, and review articles); (B) regions of the research (Japan, Asia, Europe, America, Africa, Oceania, and multiple regions); (C) materials (humans, non-human primates, and other animals); (D) classification of human materials (archaeological human skeletons, living humans, fossils, cadavers and recent human remains, and multiple materials); (E–F) Number of papers that contain the terms “morphology, population history, genetics, forensic anthropology, bioarchaeology, paleopathology, stable isotope, paleodemography, and biodistance” in full texts from *Anthropological Science* (E) and the *American Journal of Biological Anthropology* (F).

paleodemography. White et al. (2011) edited a textbook on human osteology and its application to physical anthropology, whose chapters encompassed anatomy; assessment of age, sex, stature,

ancestry, and identity of the individual; osteological and dental pathology; postmortem skeletal modification; the biology of skeletal populations; and case studies in molecular osteology,

forensics, bioarchaeological, and paleontology. Referring to these textbooks, this study selected several terms that represent the relevant research fields: morphology, population history, genetics, forensic anthropology, bioarchaeology, paleopathology, stable isotope, paleodemography, and biodistance. Even though some bioarchaeological studies overlaps with the research in other fields, such as paleopathology, this study counted the number of papers separately for each shortlisted term.

5 Results and discussion

Several important findings were obtained in this study. The number of papers with the relevant terms published in *Anthropological Science* decreased over time (from 118 in 2003–2007 to 73 in 2018–2022) (Figure 1A; Supplementary Appendix S1). The proportion of original research articles decreased from 90.7% in 2008–2012 to 58.9% in 2018–2022 (Figure 1A; Supplementary Appendix S1). In these articles, the primary research region in all time periods was Japan (Figure 1B; Supplementary Appendix S1). The proportion of papers on non-human primates accounted for 23.3% in 2003–2007, while it decreased in the subsequent time periods (Figure 1C; Supplementary Appendix S1). The proportion of papers on ancient human skeletons among papers on all human materials increased monotonously from 42.0% in 2003–2007 to 85.1% in 2018–2022, while the proportion of papers on living humans decreased from 39.5% to 10.6% (Figure 1D; Supplementary Appendix S1).

Morphology was the most commonly used term in *Anthropological Science*, but its proportion has decreased radically from 66.3% in 2013–2022 to 47.9% in 2018–2022 (Figure 1E; Supplementary Appendix S1). However, the proportions of papers with various research terms such as paleopathology, stable isotope, and bioarchaeology has increased over time: the proportion of papers with the term “bioarchaeology” increased from 5.9% in 2003–2007 to 15.1% in 2018–2022 (Figure 1E; Supplementary Appendix S1). In contrast, the proportion of papers with the term “genetics” accounted for almost 100% in the total number of papers and the proportion of the term “bioarchaeology” was present in 10.4%, 10.7%, 12.0%, and 15.2% of the papers in the four time periods in the *American Journal of Biological Anthropology*. Comparison between the two journals shows that in the beginning of bioarchaeology, Japan lagged behind, but the proportion of papers with the term “bioarchaeology” was almost the same between the two journals in 2018–2022.

In summary, the momentum of research in physical anthropology in Japan has reduced over the past 20 years as seen by the decrease in the number of papers. Some anthropologists in Japan have moved overseas, but most have focused on population history. The number of papers on population history was 61, 76, 44, and 40 in 2003–2007, 2008–2012, 2013–2017, and 2018–2022, respectively, which was far more than those on bioarchaeology. Larsen published his textbook on bioarchaeology in 1997, but since then, there are only a small number of anthropologists in Japan who have learned the new discipline.

6 How to learn bioarchaeology in Japan

Archaeologists in Japan are worried when human remains are excavated from the site. In Japan, most students learn archaeology in

the Faculty of Letters and physical anthropology in the Faculty of Science. Students cannot learn both archaeology and anthropology. There are only three physical anthropology laboratories at the University of Tokyo, Kyoto University, and Osaka University and only a few researchers to teach bioarchaeology.

When archaeologists or students study osteology and physical anthropology from books, they often accept the idea “Bones talk” without question, and this creates a misunderstanding that bones are almighty in this field. Students must also learn about the limitations of these methods. In the estimation of sex from skeletons, the accuracy of sex classification is almost 90% based on the os coxae; however, these osteological methods cannot be applied to non-adults (White et al., 2011). Bone injuries are not always human-induced cutmarks, and are often confused by taphonomic factors (White et al., 2011). If we do not lend an ear to the skeleton, the bones will not talk. It is difficult for archaeologists or students to learn bioarchaeology only from books. Today, students have opportunities to also learn from the osteological seminars held by physical anthropologists at Nihon University (2009–2012), Nippon Dental University (2013), and Niigata University of Health and Welfare (2014 to present). It is ideal that students can learn about bioarchaeology in archaeology courses, in future.

Another important issue for researchers in bioarchaeology is finding a job. Owing to the division between archaeology and physical anthropology in Japan, researchers are caught in a dilemma between the two research fields. Archaeologists rejected these researchers stating that bioarchaeology is a science, whereas physical anthropologists reject them stating that bioarchaeology belongs to archaeology. Most physical anthropologists in Japan cannot judge bioarchaeological studies unless the origin and population history are the target of the study. Because it is almost impossible for students to learn bioarchaeology, obtain jobs, and be evaluated in academic jobs, there are only a few bioarchaeology researchers in Japan. To increase job opportunities, students should acquire interdisciplinary knowledge in a wide range of research areas, such as anatomy, forensic anthropology, genetics, archaeology, and ethnology.

However, bioarchaeology is advantageous for exploring the living conditions of ancient people using direct evidence. Bioarchaeology provides a global perspective that correlates human life with epochal events (such as climate change and social stratification). The gate of bioarchaeology in Japan is narrow; however, if students understand the situation, the opportunities beyond the gate are still open.

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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.

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Supplementary material

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Revisiting the archaeological investigations of rice domestication in China during 10,000–7,000 BP in a human behavioral context

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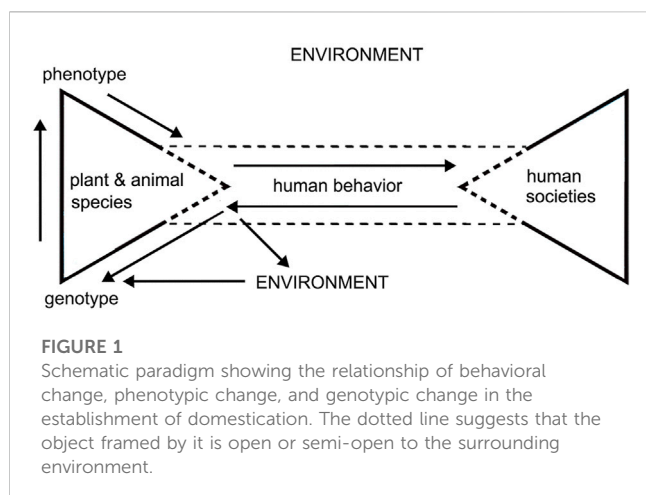
In East Asian archaeology, initial domestication and early dispersal of rice have continuously attracted scholarly interest in the recent decade, which has generated abundant new materials and revised opinions. This paper starts with a refreshed understanding of the domestication concept that emphasizes the dominant role of human behavior in the mutualistic relationship. A thorough review of the approaches to and data on reconstructing the rice story during 10,000–7,000 BP demonstrates the causally chained changes in phenotype, genotype, and human behavior in the establishment of domestication. Future studies will benefit from the revised paradigm, which has great potential to extract archaeological information to explain multiple mechanisms in rice domestication.

KEYWORDS

mutualism, human behavior, initial rice domestication, dispersal, macro-plant remains, rice phytolith

1 Introduction

Revealing rice domestication and dispersal of rice cultivation is crucial for understanding the social evolution in East Asian prehistory. Research progress in the recent decade has provided new ideas, approaches, and data for the exploration of the issue. First of all, the criterion for identifying domestication has gradually shifted from a phenotypic-trait-centered to a human-behavior-centered paradigm. It emphasizes the dominant role of humans in the interactive relationship between humans and domesticates. The refreshed theoretical perspective has pushed researchers to revise the existing methodologies and also develop new ones that are capable of reflecting human behaviors evidently associated with the establishment of the relationship. In this article, we intend to identify the appropriate methods of distinguishing wild and domesticated rice by conducting a thorough review of the methods proposed in the past 5 decades. This will then provide us a reliable foundation for critically reassessing the quality and validity of the archaeobotanical data of 10,000–7,000 BP in China that have been proposed to evidence the emergence and early dispersal of rice domestication. Based on the confirmation of the data that can be safely accepted, we attempt to depict a picture of the spatio-temporal distribution of the archaeological rice and propose a hypothetical framework of rice early dispersal routes that may require further information to enhance the articulation.



2 Refining the understanding of the “domestication” concept

Now, it is generally accepted that “domestication” can be described as a sustained multi-generational mutualistic relationship between humans and domesticates (e.g., Zeder, 2006; 2020; Purugganan, 2022). From this perspective, both sides of the interaction derive benefits from the establishment and long-term maintenance of this relationship. On the human side, through a certain level of manipulation, control, and care of a plant or animal, a human is able to increase the productivity of food and other resources of interest and guarantee a more stable and predictable supply. On the side of domestic plants or animals, specific phenotypic characteristics favored by people are selected, and thus, they gain a greater advantage in enhancing their reproductive success than other individuals not participating in this relationship. One of the current debates in documenting domestication relates to what kind of material evidence is essential in order to confirm the emergence of domestication. Some researchers take morphological and genetic changes in domesticated plants and animals as the core of the domestication process (e.g., Langlie et al., 2014; Martínez-Ainsworth and Tenaillon, 2016), while others consider them secondary consequences of domestication (Terrell et al., 2003; Smith, 2006; Zeder, 2006). Providing a constructive response to the disagreement, the explanation elaborated many times in the past 2 decades by Bruce Smith has already shed light on the issue. The creation of such a relationship involves three general categories of changes that are causally chained in the form of *behavioral change* → *genetic change* → *morphological change* (Smith, 2006).

Here, we intend to make a detailed statement of the mechanism suggested by Smith (2006) (Figure 1). For whatever reason, the domestication process is, in nature, initiated by a new pattern of human behavior. The new selective pressures sustainably brought by the behavior pattern functioning on target species for a period may cause changes in the genetic profile of the plant and animal population. This process may take place via two routes. One refers to the human behavior directly imposed on the plant or animal population, whose certain genotype positively responding to the behavior pattern is subsequently selected for. The other is that human environmental management activities indirectly have an

impact on the plant and animal population. The artificially modified physical factors of the environment inhabited by these species would select for the genotypes favored by this human-driven environment. Then, the new selected genotypes of plant and animal species may express in visible morphological changes. When the morphological changes and their causal relationship with specific human behaviors are recognized, humans make decisions on whether they would like to encourage or enhance the morphological changes by continuing to repeat their behavior patterns. In this way, the three categories of changes form a positive-feedback recycle promoting the domestication process. This model clarifies that genotypic and phenotypic changes in plant and animal domesticates are consequences of long-term human intervention in target species, rather than the very beginning of the creation of this relationship. It means that the concern on initial domestication should be moved to the phase of human behavioral changes that differ from the interactions between hunter-gatherers and wild plant and animal species.

Defining domestication in terms of causal human behavior stimulates a critical issue. The question is raised as to whether it is possible for researchers to identify a demarcating line as the exact front edge of the appearance of the new human behavior pattern that later results in the establishment of domesticates. The answer is no. Numerous experiences indicate that the markers of initial domestication have been mostly recognized in a *post hoc* way. Only the effects resulting from human engagement in the sustained interaction with target species for a period can be reliably confirmed as markers or syndromes of domestication. The second reason is that most perennial root crops do not produce or display obviously visible morphological changes responding to human-assisted propagation. The intractable problems in preservation brought about by high precipitation, high temperature, and soil acidity add difficulties in recovering signatures that are sensitive to a slight to mild degree of human intervention and cultivation. The third reason is intimately related to the mimicry of human behavioral patterns. Human actions in the initial stage of plant domestication almost simulate the natural elements and processes of the habitat to which the targeted plant species originally adapted in order to ensure a high probability of their successful survival and propagation in the new anthropogenic environment. Thus, it is extremely hard to distinguish anthropogenic evidence, which is sporadic and elusive, from natural factors in the context of initial domestication (Smith, 2011). Despite all the difficulties in capturing the signals of the subtle initial stage of domesticates being taken care of by humans, it is still meaningful for archaeologists to approach the empirically recognizable early stage of human behavioral intervention in the life cycle of target species to modify their reproductive patterns and make them increasingly distinct from their wild relatives.

Intentionality, as pointed out by many specialists in evolutionary biology, bioarchaeology, and genetics, is the pivotal feature in the domestication relationship with human involvement (e.g., Schultz et al., 2005; Zeder, 2006; 2020; Purugganan, 2022). The domestication relationship is not restricted to the mutualism between humans and other species. Non-human associated mutualism between insects, beetles, termites, and fungi is also defined as domestication (e.g., Harrington, 2005; Mueller et al., 2005; Schultz et al., 2021). In human-associated domestication, it is

human intentionality that initially triggers the mutualistic relationship, making the intervention between the two ends of the equation dominated by humans. In comparison, the two ends are more balanced in the other domestication relationships. Humans may deliberately or unconsciously take on actions without predicting genetic or phenotypic responses of plant species. They are unable to foresee the evolutionary consequence resulting from long-term changes successively taking place, together with social evolutions. Regardless of these facts, intentional purposes as the core appeal involved in human behaviors are concrete and cannot be excluded. It is where human agency comes into the picture that distinguishes between domestication and other similar mutualistic relationships in nature (Schultz et al., 2005; Zeder, 2006). Archaeological studies are normally required to reveal a long-term sustained domestication relationship by empirical evidence in advance and then trace the line further back to touch the earlier phase of forming or consolidation of the relationship.

3 Recognizing rice domestication

The improved understanding of domestication stimulated an increasing number of archaeologists to rethink and reexamine the appropriateness of a few widely employed approaches and the criteria for recognizing plant and animal domestication. Great attention has been paid to several of the most popular crops in the world, such as rice, wheat, and maize (e.g., Emshwiller, 2006; Crawford, 2012; Willcox, 2012; Faris, 2014). These annual seed plants are thought to respond to human management quickly compared to other kinds of plants. Three categories of changes can all be detected in archaeological records. Recent advances in analytical technologies allow researchers to integrate different lines of evidence and promote the ability to interpret genetic, physiological, behavioral, and cultural components during the process (Smith, 2006; Langlie et al., 2014). Here, we will concentrate our discussion on the appropriate methods of recognizing rice domestication and try to explain the intimately associated human behavioral implications. In the last 50 years, massive efforts have been invested in the study of rice domestication and have generated many methods of distinguishing wild and domesticated states of different forms of rice materials, laying a foundation for exploring the process on multiple layers. A series of macrofossil and microfossil analytical methods will be the major part of our reexamination. The underlying mechanisms of rice domestication are interpreted by articulating the causally chained successive changes in phenotype, genotype, and human behavior involved in each method.

3.1 Macrofossil evidence and its human behavioral implication

3.1.1 Morphology of spikelet base

Reduction of seed shattering is a key domestication trait that takes place in an early phase of human intervention in the rice life cycle by a set of specific harvesting and reproducing strategies. Genetic studies demonstrate that the gene mutation dominating this phenotypic feature, such as *sh4*, *SHA1*, and *qSH3*, existed prior to the

genetic differentiation of subspecies in *Oryza sativa*, a very early stage of rice domestication (Li et al., 2006; Lin et al., 2007; Sang and Ge, 2007; Ishikawa et al., 2022). In morphology, the scar at the spikelet base derived from separating the seed from the branch shows varied shapes due to differing development of the abscission layer controlled by these alleles. The clarification of this mechanism provides a reliable genetic foundation for the application that the morphology of spikelet base breakage can be used as a visible indicator of determining the strength of seed shattering in a rice population. The profile change of a non-shattering spikelet base in an archaeological sample distinct from that in a wild rice population signals a sustained period of selective pressure, which is commonly induced by year-by-year harvest through panicle cutting and seed sowing. It enables researchers to detect human behavioral changes approaching the beginning of their intentional taking care of rice.

The identification criteria for attributing a specific form of spikelet base to domestic or wild type remains controversial, although some researchers have attempted to propose their own strategies. Zheng et al. (2007) believe that wild, japonica, and indica types of rice can be distinguished through the shape of spikelet base breakage. Fuller et al. (2009) insist that the spikelet base of immature harvested rice is distinguishable from that of mature seeds, and mature specimens can be divided into wild and domestic types. However, neither of them has ever provided a baseline reference generated from systematic quantitative analysis of modern samples attributed to different domestication intensities. Later, Pan (2011); Pan (2017): 221–244) conducted an experimental archaeology project in order to test the criteria for correctly linking spikelet base morphology to the corresponding anthropogenic status, i.e., wild versus domestic, japonica versus indica, and mature versus immature. The results evidence that japonica and indica samples cannot be distinguished in terms of spikelet base morphology, and immature harvested spikelets do not show distinguishable diagnosis at the breakage scar. She, therefore, recommended a categorial duality principle for identifying the archaeological specimens as wild-type and domestic-type. The experiment further ascertains the quantitative criteria for tracing the initial phase of seed shattering reduction with a high confidence level. A rice sample with a domestic-type spikelet base percentage above 20% can be safely identified as a domestic population, and a sample with a domestic-type spikelet base percentage lower than 10% can be identified as a complete wild population. The sample with domestic-type specimens between 10% and 20% falls within a range overlapped by this parameter of wild and domestic samples, so its domestication intensity should be identified as indeterminant in terms of seed shattering. In reality, such an indeterminant sample has not been recognized in the archaeological records in China.

Archaeobotanical studies at a series of sites, including Huxi, Tianluoshan, Xuenan, Baligang, Jiahu, Majiabang, Caoxieshan, Sushui River Valley, etc. (e.g., Gao, 2012; Deng et al., 2015; Zheng et al., 2016; Zhang et al., 2018; Song et al., 2019; Qiu et al., 2021; Huan et al., 2022a), documented the spatial and temporal variations of the percentage of domestic-type individuals in a rice spikelet base assemblage during 9,000–4,000 BP. Despite the increase in data, the archaeological records involved in documenting the early phase of seed shattering reduction are still scarce. Analyses of rice spikelet bases have been reported from no more than six sites dated to 10,000–7,000 BP

TABLE 1 Percentage of domestic-type spikelet base of the archaeological context dated to before 7,000 BP [based on the data from [Zheng et al. \(2007\)](#); [Deng et al. \(2015\)](#); [Zheng et al. \(2016\)](#); [Zhang et al. \(2018\)](#); [Qiu et al. \(2021\)](#); [Luo, \(2022\)](#)]. *The original report did not include indeterminant spikelet base number in calculation of the percentage of domestic-type spikelet base, but it is included here.

Site name	Radiocarbon date	Percentage of domestic-type spikelet base (%)	Domestic-type spikelet base number	Wild-type spikelet base number	Indeterminant spikelet base number	Total number
Jiahu-Phase 1	9,000–8,700 BP	68.68*	261	69	50	380
Jiahu-Phase 2	8,600–8,200 BP	62.16*	46	13	15	74
Baligang	8,700–8,300 BP	66.40	251	34	93	378
Huxi	8,500 BP	38.89	49	77		126
Xuenan	8,400–8,000 BP	60	15	7	3	25
Jingtoushan	8,200–7,800 BP	58.02	228	20	145	393
Kuahuqiao	8,000–7,000 BP	41.67	50	70		120

(Table 1). According to the data published so far, all of them yielded rice populations with a domestic-type spikelet base percentage higher than 20%, the minimal level for being identified as domestic. In addition, very few sites have provided a chronologically continuous profile recording the change in seed shattering from wild to domestic, but the data from phase 1 and phase 2 at Jiahu are reported separately. This means that there is great potential to trace human manipulation of rice to a pioneering period much earlier than what we have previously discovered.

In large measure, human selection for non-brittle rice spikelets is also inevitably accompanied by several other phenotypic traits to be selected and altered. These include simultaneous maturation of seeds, seed compaction on highly visible terminal stalks, and loss of seed dormancy ([Smith, 2006](#)). From a behavioral perspective, the selective pressure resulting from new human-created environments gives rise to new rules of evolutionary success in the target rice population ([Smith, 2006](#): 18). This can also be explained by genetic mechanism. For example, a genetic study reveals that shattering QTLs and dormancy QTLs are linked to each other in several chromosomal regions of the rice gene ([Cai and Morishima, 2000](#)). Although these associated domestication syndromes are invisible in archaeological rice remains, changes in seed shattering reasonably imply that humans sustain the particular relationship with rice through activities including harvesting, storing, and broadcasting for clear purposes.

3.1.2 Seed size

Change in seed size of annual plants is also a visible marker that has been commonly analyzed to indicate the status of domestication ([Smith, 2006](#); [Zeder, 2006](#)), but whether this criterion is applicable to identifying the initial or early stage of rice domestication remains problematic ([Liu et al., 2007](#); [Crawford, 2012](#); [Pan et al., 2017](#)). First of all, the enlargement of seed size is not verified by the rice remains from an array of sites dated to earlier than 6,000 BP ([Liu et al., 2007](#)). Compared to seed shattering reduction, seed size increase seems to be a markedly lagged response adapted to human manipulations operated on the rice population. Secondly, changes in seed size variability distinct from the wild rice population have been repeatedly observed in archaeological samples from before

7,000 BP ([Liu et al., 2007](#); [Gao, 2012](#); [Deng et al., 2015](#)), but very little progress in statistical analysis and explanation of the phenomena has been made. Although it was 15 years ago that [Liu et al. \(2007\)](#) pointed out the issue for the first time, her observation and suggestion are still valuable today for digging up the human behavioral implications of seed size variability during initial rice domestication. Generally speaking, the phenotypic variation amplified due to plant and animal species populations exposed to altered selective contexts is widely seen in evolutionary processes (e.g., [Price et al., 2003](#); [Kelly et al., 2012](#); [Evin et al., 2015](#)). It is not difficult to understand that human care removed or restrained the natural selections operated on the wild rice population so that the abnormally grown seeds that were originally not able to survive until complete ripening would have the chance to grow and develop into a thin or sterile state (not immature). Their contribution to the seed stock for broadcasting in the next growing season will be conserved and even enhanced in a population. The validity of this trait indicating domestication requires sufficient verification derived from archaeological materials and comparison with modern reference samples. Thirdly, another shift in rice seed size, whereby the ratio of length to width of grain tends to decrease during 8,000–6,000 BP, was recognized by [Deng et al. \(2015\)](#) and [Gao \(2012\)](#). However, this tendency seems a little more subtle. The measurement of carbonized rice grain and statistical analysis may be influenced by a variety of factors, including rice variety, temperature and duration of firing during seed carbonization, sample size, etc., so the dynamics of change in the ratio of length to width should be carefully examined by multiple hypotheses. In addition, thickness has never been included in seed shape analysis ([Crawford, 2012](#): 616). If the seed becoming fatter in the early stage of rice domestication could be verified, it would be worthwhile to discern the genetic and behavioral mechanism of why the seed size changes in this manner. In sum, for many decades, numerous efforts to figure out a generalized pattern of seed size change indicating initial rice domestication through conventional measurement and statistical analysis have not arrived at a satisfying accomplishment. It also implies that a new method is necessary. The computer-assisted morphometric method of

processing data on seed morphological variation for distinguishing wild and domestic populations designed by [Rovner and Gyulai \(2007\)](#) may have great potential in this realm.

3.2 Microfossil evidence and its human behavioral implications

3.2.1 Morphometrics of double-peaked tubercle phytolith

In 1998, 25 years ago, [Pearsall et al. \(1995\)](#) and [Zhao et al. \(1998\)](#) developed a set of prediction formulas based on systematic discriminant analysis of double-peaked tubercle phytolith morphology of a series of modern rice samples for identifying wild and domestic specimens. The method was successfully employed in documenting the emergence of rice cultivation around 10,000 BP and the subsequent intensification of the domestication process until 7,000 BP at the Diaotonghuan site ([Zhao, 1998](#)). It was also used to investigate the double-peaked phytolith archaeologically unearthed from the Lower Yangzi River ([Wu et al., 2014](#)) and the Lower Huai River ([Luo et al., 2016](#); [Gu et al., 2022](#); [Qiu et al., 2022](#)). An increased intensity in rice domestication during 12,000–7,000 BP was documented in the two regions. The advantage of this morphometric method depends on the effective statistical method, but the relationship between human behavioral changes pertaining to cultivation and morphological changes in double-peaked phytolith has never been discussed.

3.2.2 Morphometrics of bulliform phytolith

The investigation and application of bulliform or fan-shaped phytolith has a longer history in the study of rice remains. [Fujiwara's \(1993\)](#) pioneering work developed a discriminant formula based on his statistical studies of various rice varieties in Japan for distinguishing japonica and indica subspecies. However, his method is not ideally applicable to exploring archaeological evidence of rice agriculture in mainland East Asia and many adjacent areas because it did not include the precise identification of wild rice. It was noticed that the wild specimens might be incorrectly identified as japonica or indica ([Wang and Lv, 2012](#); [Wang et al., 2019a](#)). Since, efforts to distinguish bulliform phytolith from wild and domestic rice through a morphometric index have been made by several researchers ([Pearsall et al., 1995](#); [Zhang et al., 1998](#); [Gu, 2000](#); [Ma and Fang, 2007](#)), but it has proven to be highly difficult to generate a reliable quantitative standard for this aim. The morphometric parameters of bulliform phytolith are influenced by a number of factors, so the profiles of wild and domestic samples are usually overlapped with each other.

3.2.3 Fish-scale shaped decorations of bulliform phytolith

Another feature, the number of fish-scale-shaped decorations along the bottom of bulliform phytolith, was intensively investigated by Houyuan Lv's team to set up a new identification standard. They noticed that the bulliform phytolith of domesticated rice normally showed 8–14 fish-scale-shaped decorations, while that from wild rice commonly had less than 9 ([Lu et al., 2002](#)). A reference baseline for identification was provided by the systematic sampling of surface

soil in a wild, domesticated rice field and other vegetation, as well as the quantitative analysis of phytolith assemblages in these samples ([Huan et al., 2015](#); [Huan et al., 2020](#)). This indicates that the proportion of bulliform phytoliths with ≥ 9 fish-scale decorations (abbreviated to “PBFS” as follows) in domesticated rice soil samples was $57.6\% \pm 8.7\%$, while the PBFS in wild rice soil samples was $17.46\% \pm 8.29\%$. However, the PBFS in dry rice field soil samples was $11.5\% \pm 5.3\%$. This means that domesticated dry rice cannot be distinguished from wild rice by this method. Considering the limited number of available dry rice samples (only four), it requires more extensive data collection and dynamic explanation of the phenomenon to replenish the current understanding. Lv's team also proposed explanations of the linkage between the morphological change in fish-scale decorations and the biotic/abiotic factors modified during initial rice domestication ([Huan et al., 2020](#)). The bulliform cells in living grasses, like rice, play an important role in leaf rolling in response to environmental stresses such as water loss and high temperatures (e.g., [Moullia, 2000](#); [Li et al., 2010](#); [Zou et al., 2014](#); [Zhang et al., 2015](#); [Matschi et al., 2020](#)). The increase in the number of fish-scale decorations is caused by frequent folding, shrinkage, and distortions of bulliform cells squeezed by surrounding mesophyll, sclerenchyma, and parenchyma cells during leaf rolling. It is also supported by genetic studies indicating that a major part of rice leaf rolling genes cloned so far are relevant to the development of bulliform cells, and only a few are relevant to other cells ([Zhou et al., 2018](#)). Therefore, this morphological change may be regarded as a drought resistance response to the environmental fluctuations that may have been associated with water availability, salinity, and other hydrological conditions most probably altered by human behaviors. Human-sustained year-by-year perturbations and interventions in wild rice habitats can be reasonably considered as a dynamic leading to a new hydrological regime. Although people usually tend to mimic the physical conditions of or directly take advantage of the habitat where the wild rice population is grown during initial rice domestication, rice might be sensitive to such stresses, which would stimulate immediate physiological effects. Besides the above explanation, agronomic and genetic research has also indicated that moderate leaf rolling facilitates photosynthesis efficiency and raises grain yield (e.g., [Lang et al., 2004](#); [Zhao et al., 2016](#); [Cho et al., 2018](#)). Selection for rice plants with a high yield may be concurrently related to the selection for the leaf rolling phenotype. Therefore, the increase in the number of fish-scale decorations of bulliform cells may also be one of the consequences of purposeful yield enhancement.

Compared to the studies of macro-plant remains, interpretation of PBFS of archaeological data may require more careful verification of depositional context, chronological background, associated artifacts, and even material processing procedure. PBFS value is not an indicator directly related to the intensity of rice domestication. Its implication of rice cultivation is indirectly inferred based on the mechanism through which rice responds to environmental stresses by leaf rolling. Anthropogenic factors may or may not contribute to the formation of these external stresses. Therefore, to what extent and in what way human activity causes changes in PBFS should be cautiously differentiated. So far, the PBFS in most archaeological soil samples dated to 10,000–7,000 BP ranges between 20% and 60%, which falls within the range of between

average wild rice and domesticated rice. The interpretation that these rice populations were cultivated by people and the domestication process had already been initiated is reasonable. The complication exists in how to properly interpret the relative difference of PBFS values. To document a continuous domestication process, the PBFS values of the samples from one site or one region are generally expected to show an increasing tendency through time and transcend the PBFS of wild rice. However, the chronological changing pattern of PBFS is not consistent with the theoretical expectations and varies in each region, which adds challenges to clarifying the roles played respectively by the physical environment and human activity in the long-term evolutionary process. This also reminds us that the inter-regional comparison and interpretation of PBFS records needs to take more variables into consideration.

3.2.4 Phytolith in spikelet base

A new type of phytolith, FUSIFORM ECHINATE, observed in the rice spikelet base has been reported recently (Ge et al., 2022). The primary analysis of wild and domesticated rice specimens of the AA genome showed that fusiform echinate phytolith abundance was significantly different between shattering and non-shattering groups. The fusiform echinate phytolith abundance was reported to be 264.84 ± 162.8 for the *O. nivara* and *O. rufipogon* combined group and 771.75 ± 383.22 for the *O. sativa* group. Thus, the higher fusiform echinate phytolith abundance in one spikelet base may indicate a non-shattering phenotype of rice. This inspiring discovery provides a new line of evidence directly related to the loss of seed shattering, but its applicability to differentiating wild and domesticated specimens from archaeological contexts still needs to be improved. One problem regarding its reliability is the small sample size currently available. A large sample normally requires at least 30 specimens for statistical analysis, but each group reported in the study contained less than 7. The large standard deviations of the two groups mean that the data is spread far out, some of it being far away from the mean. Another issue, which may be minor and easier to resolve, relies on whether the fusiform echinate phytolith is exclusively found in rice. Therefore, a more systematic analysis of fusiform echinate phytolith in different rice varieties is expected.

3.3 Summary of methodology

To sum up, we suggest that the following criteria can be safely accepted as empirical evidence for recognizing rice domestication, especially in its early stages. The appearance of these visible or detectable phenotypic traits can all be unequivocally connected to and explained by certain genetic and behavioral mechanisms. Human intentionality is presented by a long-term sustained human-induced management or selective pressure imposed on rice, which results in highly indicative traits. The methods are listed by priority.

- (1) The percentage of domestic-type rice spikelet base can be used as evidence of the highest priority as it is directly associated with human harvest strategy, and seed shattering reduction is most probably the earliest domestication trait.
- (2) The proportion of bulliform phytoliths with ≥ 9 fish-scale decorations (PBFS) is sufficient to demonstrate rice domestication,

but it is less directly connected to explicit human behavior. Rather, the variation of PBFS indicates some complicated relationships among rice, human behaviors, and physical conditions. The anthropogenic factors must be carefully verified.

- (3) The morphometrics of double-peaked tubercle phytolith is also sufficient in indicating domestication, but its genetic and behavioral mechanisms are relatively unclear.
- (4) To some extent, seed size may reflect the intensity of rice domestication. However, this criterion is not properly applicable to distinguishing the initial stage as seed size change may take place long after humans begin to foster wild rice, and the genetic and anthropogenic mechanism causing the change is only partly known.

4 Geographic distribution of archaeological evidence during 10,000–7,000 BP

The archaeological sites yielding rice remains and the associated phenomena during 10,000–7,000 BP have served to outline a spatial and temporal framework of the threshold of rice domestication and its dispersal immediately following the scenario (Figure 2). Based on the above methodological clarifications, we will carefully examine the primary data and the demonstration of evidence by region.

4.1 The Middle Yangzi region

The Middle Yangzi region is one of the areas yielding the earliest dated archaeological records of rice cultivation. The sites bearing rice remains of 10,000–7,000 BP are scattered across a large area extending from the main stream to tributaries. Since the end of the last century, the Diaotonghuan site has been widely accepted as a representative of early rice cultivation in East Asia, and the Xianrendong site, despite its scarcity of rice remains, has been attributed to the same culture due to its very close location and similar pattern of artifact assemblage composed of pottery sherds, lithics, and animal bones. The thousands of years-long rice cultivation at Diaotonghuan was demonstrated by a continuous increase in proportions of domestic double-peaked phytolith from layer G to B (Zhao et al., 1998). A dramatic shift throughout the profile is shown in layers D and E, so their radiocarbon dates were crucial. The youngest radiocarbon date of the two layers is $15,531 \pm 214$ cal. BP, which is derived from the bone of layer D (School of Archaeology and Museology, Peking University and Jiangxi Provincial Institute of Cultural Heritage and Archaeology, 2014: 266–267). However, it seems to have been rejected by some researchers (e.g., Kuzmin, 2006). Eventually, Zhao (1998) accepted the chronology in terms of the seriation of artifact assemblages and ceramic typology proposed for the Neolithic culture sequence in southern China. A transition from wild rice utilization to rice cultivation starting by 11,000 BP and lasting until 7,000 BP is documented in this area. No macro-plant rice remains dated to around 10,000 BP have been uncovered so far.

Pengtoushan and Bashidang of 9,000–8,000 BP, located 530 km away from Diaotonghuan in the west, are the subsequent important

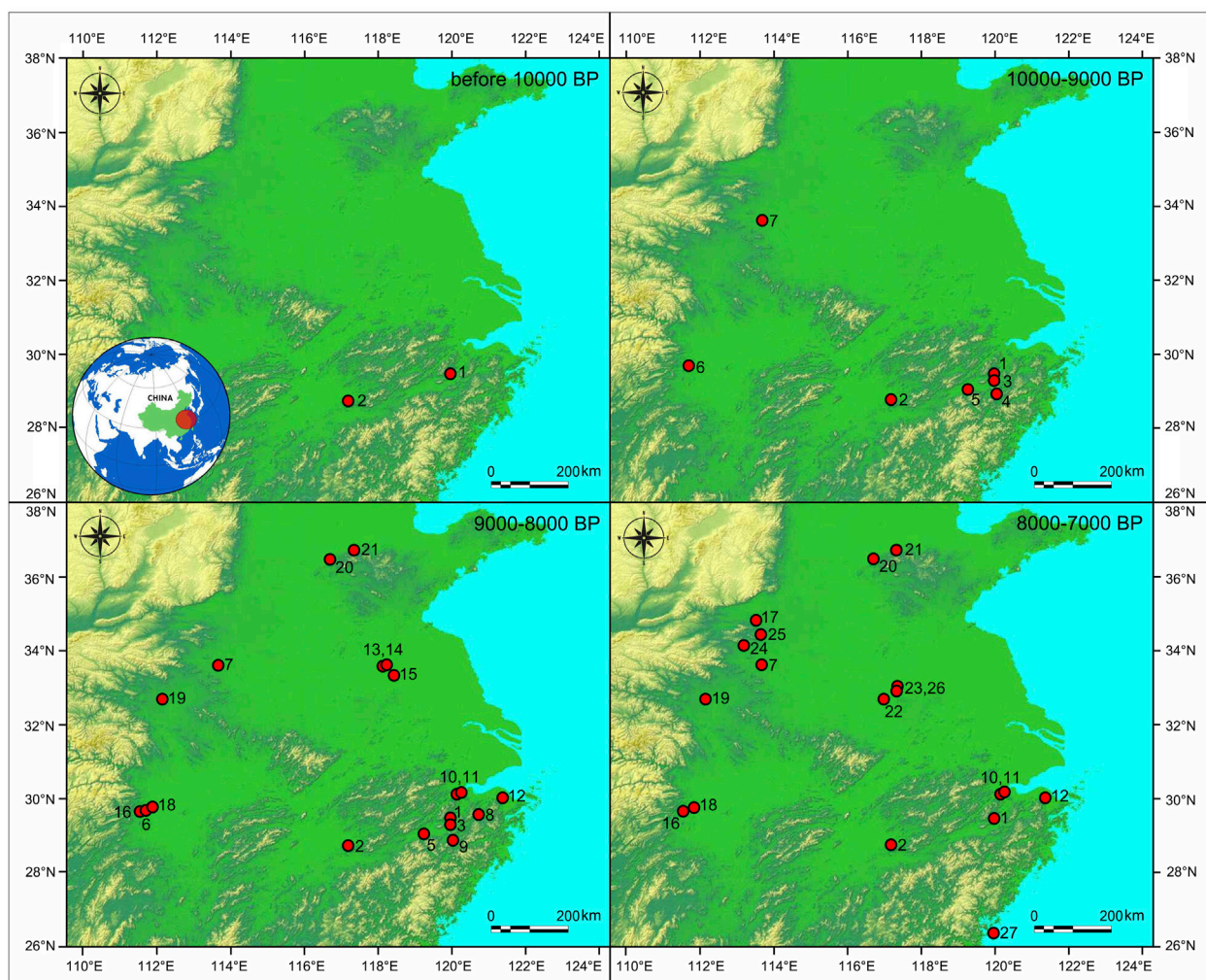


FIGURE 2

Maps of the archaeological sites yielding rice remains of 10,000–7,000 BP in China. 1. Shangshan; 2. Xianrendong and Diaotonghuan; 3. Qiaotou; 4. Miaoshan; 5. Hehuashan; 6. Pengtoushan; 7. Jiahu; 8. Xiaohuangshan; 9. Huxi; 10. Kuahuqiao; 11. Xiasun; 12. Jingtoushan; 13. Shunshanji; 14. Hanjing; 15. Xuenan; 16. Shanlonggang; 17. Zhuzhai; 18. Bashidang; 19. Baligang; 20. Yuezhuan; 21. Xihe; 22. Xiaosungang; 23. Shuangdun; 24. Tanghu; 25. Peiligang; 26. Yuhucun; 27. Dapingding.

sites. The rice remains from Pengtoushan are the husks and chaff tempered in pottery sherds, so the observation is constrained to the micro-scale impressions of the double-peaked tubercle structure of the husk. The researchers suggested that morphometric analysis of double-peaked structure revealed an ancient “japonica-prone” rice population with mixed characteristics of wild, japonica, and indica varieties (Zhang et al., 2003). A morphometric analysis of carbonized rice grains from Bashidang, conducted by the same researcher, classified them as “a primitive ancient cultivated population that was evolving towards indica type” (Zhang and Pei, 2002). However, our understanding of these descriptions should not be restricted to the literal meaning. In fact, Zhang and his colleagues intended to emphasize that the rice remains from the two sites can hardly be categorized as japonica, indica, or wild, although they might have been cultivated then. These were published 20 years ago. No updated research has been reported. However, rice cultivation associated with groundwater table fluctuations in 8,600–8,000 BP at Bashidang is evidenced by

double-peaked phytolith and pollen data (Liu et al., 2017). In addition, Shanlonggang, a nearby site attributed to the late phase of Pengtoushan culture and dated to around 8,000 BP, yielded carbonized seeds including rice. The morphometric analysis of rice grains based on the discriminant formula developed by Zhao and Gu (2009) categorized 44% of specimens as domestic type and 56% as wild type (Gu et al., 2016). Although the evidence still seems ambiguous and needs strengthening, the rice remains from Pengtoushan culture have been generally accepted as being domesticated.

4.2 The Lower Yangzi region

The Lower Yangzi region has yielded the richest materials for documenting early rice domestication spanning a wide chronological range from 10,000 to 6,000 BP. The earliest phase of Shangshan culture can be traced back to more than 10,000 years

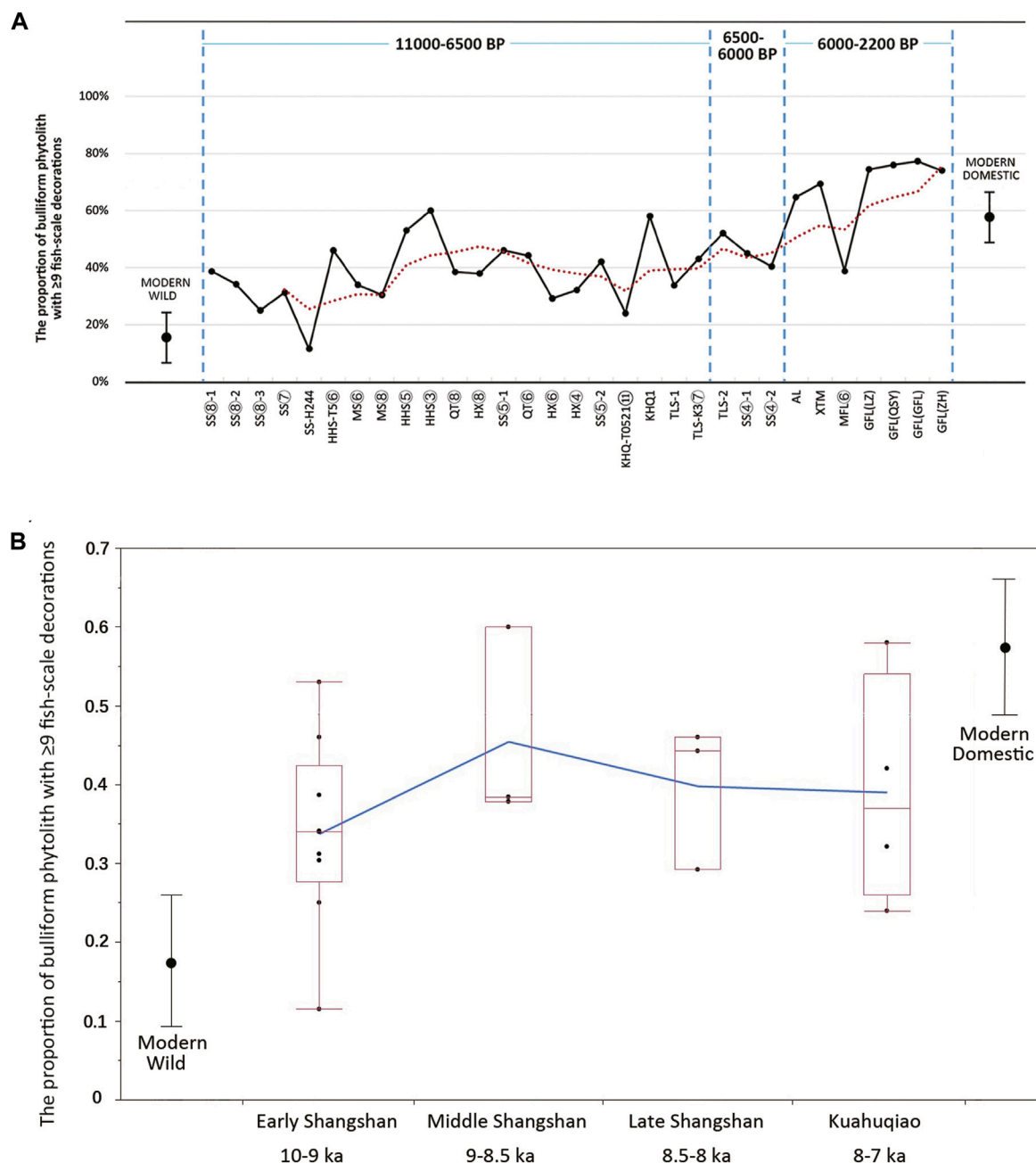


FIGURE 3

Charts of the changes in rice phytoliths from the Neolithic sites in the Lower Yangzi region. (A) Chronological change in the proportion of bulliform phytolith with ≥9 fish-scale decorations throughout the Neolithic period [based on the data adopted from Wu et al. (2014); Huan et al. (2014); Ma et al. (2016); Huan et al. (2021); Huan et al. (2022a)]. The red dotted trendline calculated by moving average shows an increasing tendency. (B) The proportion of bulliform phytolith with ≥9 fish-scale decorations in the Shangshan Culture and Kuahuqiao Culture sites [based on the data adopted from Wu et al. (2014); Huan et al. (2014); Ma et al. (2016); Huan et al. (2021)].

ago (Zhejiang Provincial Institute of Cultural Heritage and Archaeology and Pujiang Museum, 2016). A major part of pottery sherds are densely tempered with rice husks and chaff. However, convincing macro-plant evidence of domestication prior to 8,500 BP is not yet available. The earliest dated evidence comes from the Huxi site (Zheng et al., 2016), but the most intriguing point of it has been largely neglected. The rice spikelet base morphology and the change in rice phytolith assemblages are complementary to

each other in verifying loss of seed shattering no later than 8,600 BP. The percentage of non-brittle spikelet bases being 38.89% is sufficient to indicate a sustained selective pressure favoring seed retention. The phytolith analysis of the profile in a probable ditch structure showed a relatively high density of double-peaked tubercle phytoliths with a few bulliform phytoliths during 9,000–8,400 BP compared to a lower density of double-peaked tubercle phytoliths, with huge increases in bulliform phytoliths after 8,400 BP. This

means that, in the later phase, more spikelets were removed from the wetland environment and more rice leaves remained, indicating a panicle-cutting harvest strategy likely being practiced at Huxi. It might have accelerated a continuous increase in anthropogenesis involving rice cultivation and management of several species of grass sharing the niche. A long-term process of loss of seed shattering has been documented in the subsequent cultures of Kuahuqiao, Hemudu, Majiabang, Songze, and Liangzhu (Zheng et al., 2007; Allaby et al., 2017), but most of the existing evidence is from later than 7,000 BP. Exploration focusing on seed shattering in the early-middle Holocene still needs work.

An abundance of phytolith analyses also evidenced rice domestication, in particular, clarifying rice cultivation during 10,000–8,500 BP (equivalent to the early and middle phases of Shangshan culture). Both double-peaked tubercle phytoliths and bulliform phytoliths have been carefully investigated at many sites. A gradual process of rice domestication throughout the Neolithic period was demonstrated by phytolith data (Huan et al., 2014; Wu et al., 2014; Ma et al., 2016; Huan et al., 2021; Huan et al., 2022b) (Figure 3A). It indicated that rice domestication was initiated in Shangshan culture and its intensity approaching the level of modern rice agriculture took place after the Late Majiabang culture, approximately dated to 6,500–6,000 BP. More detailed research focusing on Shangshan culture, including five sites, revealed a complicated spatial and temporal pattern of the early stage (Huan et al., 2014; Wu et al., 2014; Ma et al., 2016; Huan et al., 2021). The rice domestication intensity indicated by PBFS slowly improved during 10,000–9,000 BP; it reached a relatively high level in 9,000–8,500 BP and slightly declined with fluctuations after 8,500 BP (Figure 3B). The PBFS value of the Hehuashan site is markedly higher than those of the other sites, which was explained by its location closer to the main channel of the Upper Qiantang River (Huan et al., 2021). However, phytolith analysis at the same site conducted by Qiu et al. (2019) did not show similar results, probably because of the different sampling strategy and location (Qiu, 2021). Although the tendency of rice domestication is generally consistent with an expected pattern, it is necessary to conduct detailed site-by-site investigations to figure out the complex interrelationship of human behavior, water environment, and rice responses to various changes and comprehensively understand the homogeneity and diversity of human behaviors of Shangshan culture.

Only two sites, Kuahuqiao and Jingtoushan, as well as some strata in Shangshan culture sites are attributed to 8,000–7,000 BP. Rice domestication at Kuahuqiao was well verified by multiple lines of evidence (Zheng et al., 2007; Pan et al., 2017). The newly excavated Jingtoushan site yielded carbonized rice grains (Sun et al., 2021). The analysis of rice spikelet base morphology showed at least 60% of specimens identified as domestic type, clearly indicating that rice domestication was underway (Luo, 2022). However, the pollen and phytolith data of a geological core at the edge of the Jingtoushan site did not provide robust evidence for local rice cultivation (Liu et al., 2020; Deng et al., 2021). Considering that the subsistence pattern of Jingtoushan is characterized by marine resource exploitation, the relationship between Jingtoushan people and the source of domestic rice should be examined with caution.

4.3 The Upper Huai River region

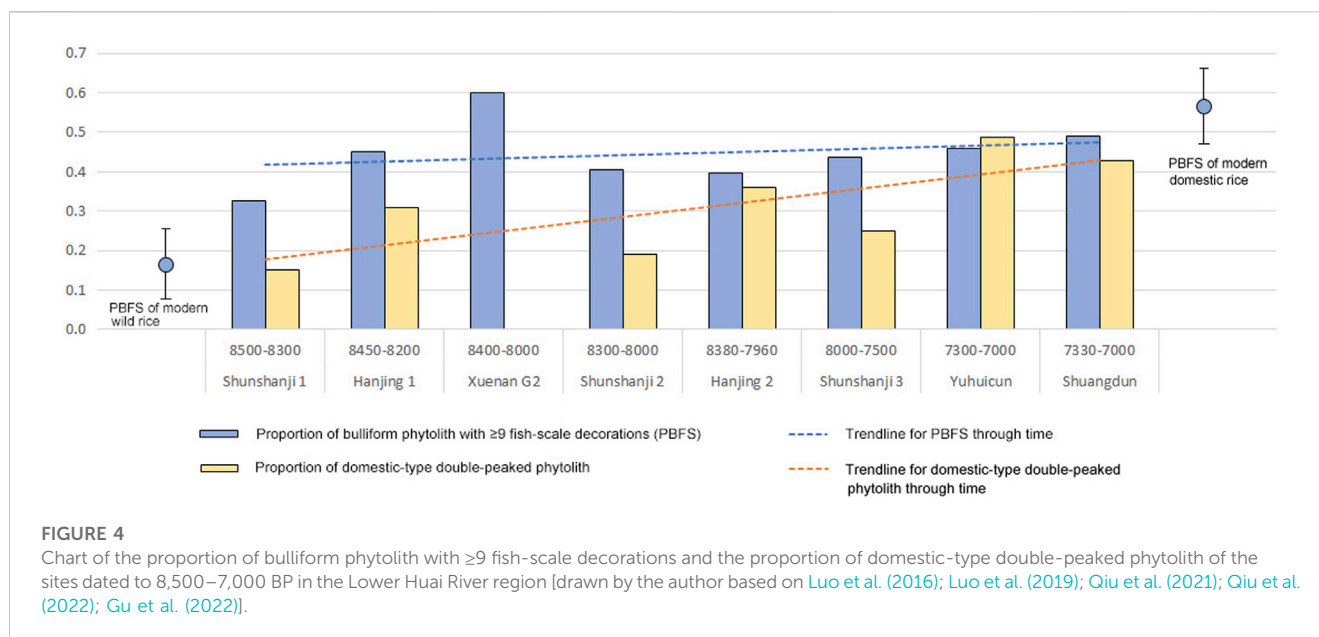
This region is characterized by its transitional topography and terrain. Stretching north-south along the west part of the Huang-Huai-Hai Plain, the sites are scattered on the interface where the Huai River and the Qinling Mountain meet. Many streams that originate here run southeast and converge with the Huai River, forming a densely connected river network that may provide abundant water resources for local agricultural practice. Rice cultivation in the region has a deep history rooted in the subsistence of Peiligang and Yangshao cultures (e.g., Yang et al., 2016; Wang et al., 2017; Wang et al., 2019b; Wang et al., 2019c; Cheng et al., 2022a; Huan et al., 2022a; Cheng et al., 2022b; Sun et al., 2022).

The Jiahu site dated to 9,000–7,500 BP yielded the oldest rice remains in the Upper Huai River. A recent study of rice spikelet base evidenced that the rice population was domesticated during 9,000–8,200 BP (Zhang et al., 2018). Rice domestication was also supported by the morphometric analysis of carbonized rice grains, revealing not only their larger size compared to the counterparts of other later Neolithic sites (Liu et al., 2007) but also a wider distribution of measurements uncommonly seen in the wild rice population (Zhang et al., 2009).

The Baligang, Zhuzhai, and Tanghu sites all have long chronological sequences from the Peiligang period to the Zhou period, providing well-documented sequences of agricultural development. According to archaeobotanical research, the agricultural pattern in the earliest phase of Baligang was only represented by rice cultivation, while the other two started with an agricultural system involving both rice and millet cultivations. The analysis of rice spikelet base morphology at Baligang reflected that an established domesticated rice population had already existed in the pre-Yangshao period and was sustained throughout the entire Neolithic and Bronze Age (Deng et al., 2015). Its earliest phase can be traced back to 8,600–8,400 BP. Phytolith investigation suggested that wetland rice cultivation was sustainedly practiced and anthropogenic water conditions changed over time (Weisskopf et al., 2015). Rice cultivation at Zhuzhai and Tanghu as early as ca. 7,800 BP was documented by the morphometric analysis of phytoliths (Zhang et al., 2012; Wang et al., 2018), while rice only played a minor role in daily cuisine and local farming systems (Bestel et al., 2018; Wang et al., 2018). Peiligang, the well-known site dated to 8,200–7,700 BP, yielded carbonized plant remains including rice, common millet, and other fruits (Li et al., 2020), but no detailed archaeobotanical report has been published. Despite the archaeobotanical data directly relating to the early phase of rice domestication in this region not being as abundant as those from the Lower Yangzi, systematic investigations in recent years have provided very high-quality records. Furthermore, because of this, the Upper Huai River region was suspected to be another potential rice domestication center (Huan et al., 2022b), but it is not empirically supportive so far.

4.4 The Lower Huai River region

In the recent decade, early rice domestication in the Lower Huai River has been traced back to 8,500–8,400 BP at the Hanjing, Xuenan, and Shunshanji sites. Phytolith analysis played a



significant role in verifying rice agricultural practices, while most sites yielded only a few carbonized rice remains. It is almost impossible to learn about the traits, such as seed shattering and seed size, and infer the corresponding human behaviors.

A set of paddy-like features was revealed at the Hanjing site, which was composed of three pieces of depressed ground and 18 ditches connected to a contemporaneous paleochannel. As evidenced by the phytolith data, it might have been the earliest rice paddy, dated to ca. 8,400 BP (Qiu et al., 2022). This means that rice cultivation in this region was relatively mature when it had just appeared. Following this, a protracted process of rice domestication spanning 1,500 years is demonstrated by the phytolith data from a series of sites of Shunshanji and Shuangdun cultures (Luo et al., 2016; Luo et al., 2019; Qiu et al., 2021; Gu et al., 2022; Qiu et al., 2022). The PBFS values of all sites in this region are all higher than that of wild rice and slowly increase over time, with an exceptional peak at the Xuenan site. In contrast, the domestic type of double-peaked phytolith shows a more remarkable rising tendency but with more fluctuations (Figure 4). The rice arable system at Yuhuicun might be rain-fed, unlike the paddy at Hanjing (Gu et al., 2022). At the Xiaosungang site, the low percentage and ubiquity of rice in the macro-plant remain assemblage implies that rice might not have been a major starch source in diets around 7,000 BP (Cheng et al., 2016).

4.5 The Lower Yellow River region

The earliest appearance of rice in the Lower Yellow River dates back to approximately 8,000 BP. Charred rice grains and fragments were uncovered from Xihe (Jin et al., 2014) and Yuezhuang (Crawford et al., 2007; Crawford et al., 2016) sites. Only two features of each site yielded rice in very small numbers, so the occurrence of rice in Houli culture was quite incidental. This does not allow morphological observations that are essential for determining whether the rice is wild or domestic. Furthermore,

rice did not reappear in archaeological records until the beginning of the seventh millennium BP; thus, we lack hints to explain the source of Houli rice. Two hypotheses have been proposed: 1) it was imported from its original domestication place; 2) its distribution reached the Lower Yellow River and was locally grown in the Houli period (Crawford et al., 2007). The second seems less plausible, but some researchers suggested that, based on the thermal niche simulation, rice at Houli period sites could have been locally grown or exploited by local hunter-gatherers during the climatic optimum (Guedes et al., 2015). However, although climatic factors may influence human decision-making, they cannot explain everything because the subsistence pattern in nature is a major cultural selection in human society largely involving perception, subjectivity, preference, and so on. A better understanding of Houli rice is seriously restricted by the shortage of useful archaeobotanical information. Before the sophisticated hypotheses can be tested, we must expect some research progress on both charred seeds and phytoliths from the period prior to 7,000 BP to strengthen the database for the study of Houli rice.

4.6 The Min River valley

The newly reported discovery of rice remains earlier than 7,000 BP in the Min River valley refreshed our understanding of early rice dispersal along the east coast. Dapingding, located along the river valley 50 km away from Fuzhou city, has a chronological sequence including the Dapingding, Tanshishan, and Huangguashan cultures from 7,600 to 3,500 BP (Wu, 2018; Zuo et al., 2022). Carbonized rice grains were uncovered from the filled soil of a burial and charred rice husks were also found densely tempered in pottery sherds (Wu, 2018), but they did not yield any morphometric information. Typical rice phytoliths were uncovered from pottery sherds, cultural midden, and burials. The PBFS value of bulliform phytolith rose from 44% in the Dapingding period to 72% in the Tanshishan and Huangguashan periods, implying a seemingly

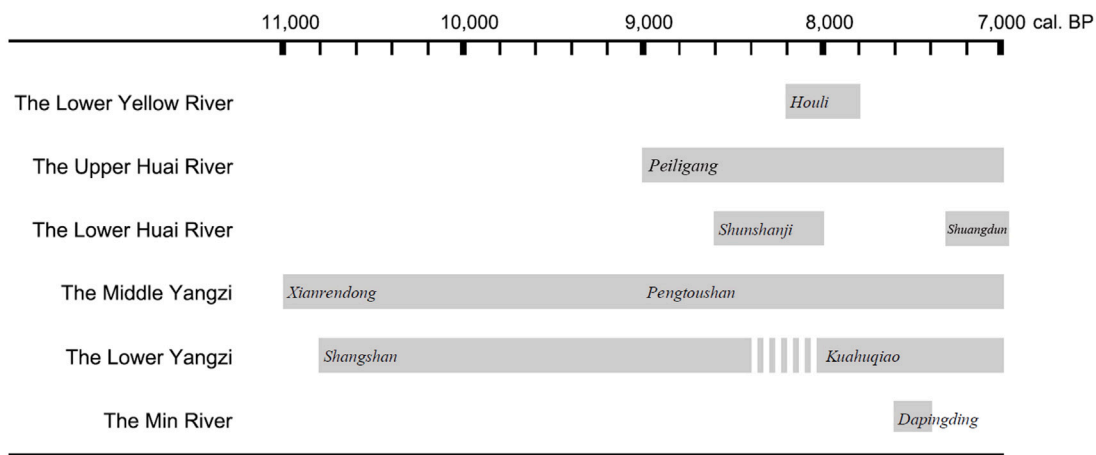


FIGURE 5
Chronology of the earliest occurrence of rice during 10,000–7,000 BP in the six regions.

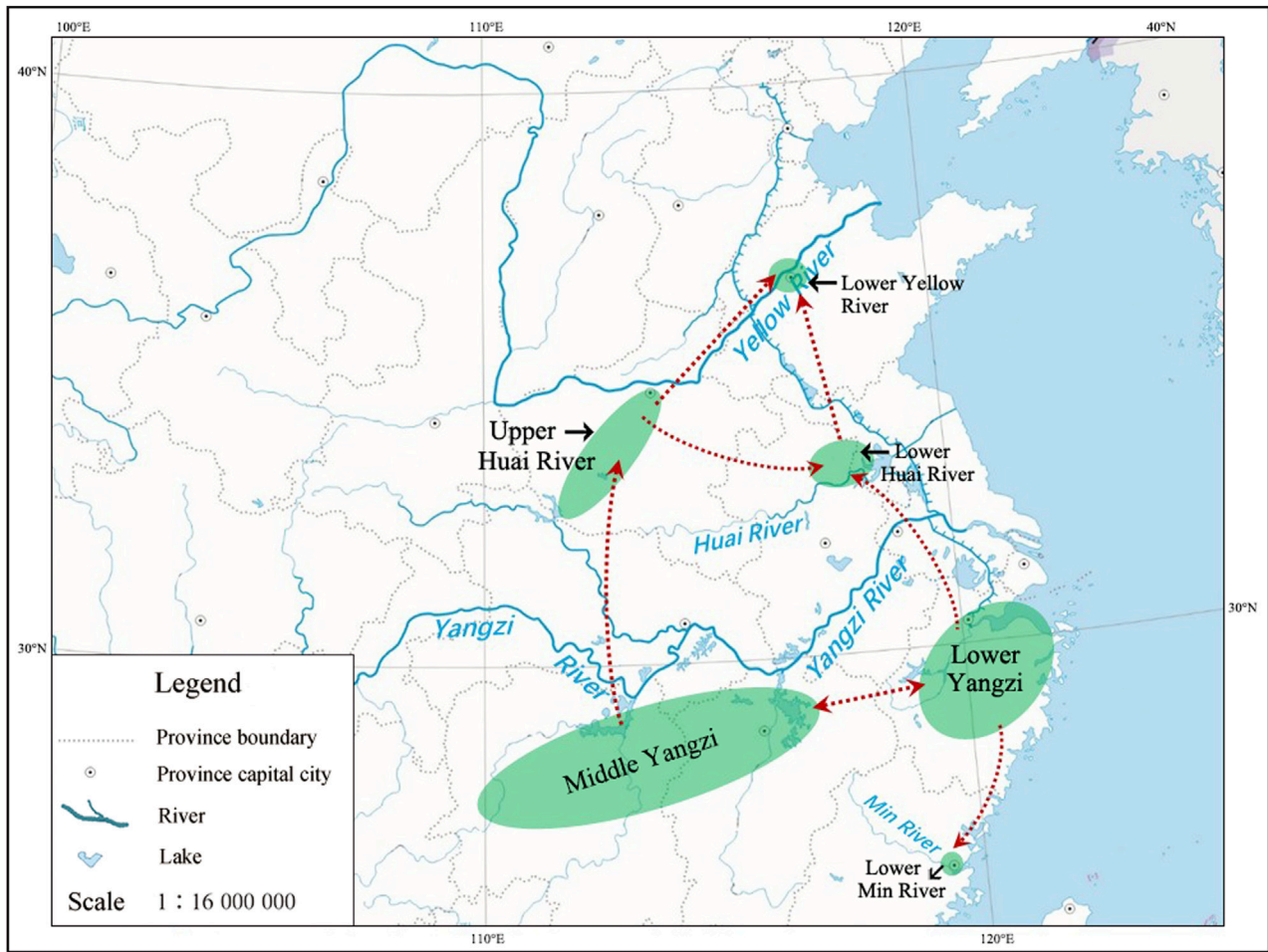


FIGURE 6
Probable routes of rice early dispersals across the large area covering the six sub-regions discussed in this article.

intensified process of rice cultivation (Zuo et al., 2022). The researcher suggested that the first appearance of rice in the Min River valley should be cautiously regarded as incidental because no archaeological rice record is known for the period of 7,000–6,000 BP. Moreover, it is inferred to be introduced from the Lower Yangzi through a coastal route (Zuo et al., 2022).

4.7 A proposed framework of initial rice domestication and early dispersals

By outlining the temporal and spatial pattern of archaeological rice records of 10,000–7,000 BP, a framework of initial rice domestication and early dispersals can be proposed. Surprisingly, we discovered that rice rapidly dispersed across a vast area closely following the threshold of its being cultivated by humans, while the rate of rice domestication in the early stage was rather slow (Allaby et al., 2017).

The Middle and Lower Yangzi regions parallelly regarded as the original places of rice domestication are supported by not only empirical evidence (Zhao, 2011) but also regression modeling (Silva et al., 2015; Long et al., 2022). None of the rice remains from the other areas date back to as early as those from these two regions. The rice dated to later than 10 millennia BP in other regions could have been introduced from either of them (Figures 5, 6). Before 9,000 BP, rice utilization or cultivation near the east end of the Middle Yangzi, Xianrendong, and Diaotonghuan, expanded westward a great distance and was well established in the region near the west end, in Pengtoushan culture. Subsequently, the Peiligang communities in the Upper Huai River acquired rice cultivation, most likely from Pengtoushan culture, the nearest neighbor to the south of them. It is reasonable to infer that the interactions between the Peiligang and Pengtoushan cultures, indicated by the double-eared vessels incorporated in the pottery assemblage of Pengtoushan, enabled the northward dispersal of the rice and the adoption of rice cultivation (Chen, 2018).

The north-south routes along the east coast were constantly available throughout the Neolithic, which allowed active exchanges among the communities in the lower reaches of the Yellow River, the Huai River, the Yangzi River, and the Min River (Figure 6). The rice cultivation in the Lower Huai River seems to have been introduced most likely from the Upper Huai River. However, the interactions between Shunshanji and contemporaneous cultures were complicated. In terms of the cultural connections shown by pottery typology, the first and second phases of Shunshanji dated to 8,500–8,000 BP more or less shared features with Houli, Peiligang, and Pengtoushan, while the third phase dated to 8,000–7,500 BP was more closely related to Kuahuqiao (Nanjing Museum and Sihong Museum, 2016). The data in hand is insufficient for evaluating whether the culture in the Lower Yangzi might have impacted its neighbor to the north, so the alternative possibility that it came from the Lower Yangzi cannot be completely excluded. The sporadic occurrences of rice in the Lower Yellow River might have been imported from the Lower Huai River or the Upper Huai River via the Yellow River watercourse. The Houli communities seemed to be quite hesitant about adopting rice. In south China, the Min River valley witnessed a relatively late arrival of rice cultivation, most probably coming from the Lower Yangzi.

Many details are yet to be discovered, and the proposed diffusion routes need to be tested. Given that this map of the long journey of rice in its early phase of domestication is drawn based on data that were strictly assessed in the sense of human behavior, rather than on the description of phenotypic traits, further discussions on ecological inheritance, social learning and cultural transmission, subsistence tradition, and so on, can be coherently incorporated into the explanatory framework.

5 A prospect of the study of initial rice domestication and early dispersals from a human behavioral perspective

It is predictable that, in future archaeological studies of rice domestication, how to design a clearly-aimed research strategy, by what standard to assess and accept evidence, and making appropriate interpretations of abundant data will become increasingly significant. Rice domestication is not merely a biological problem; the process is inevitably woven into the daily life and cultural practices of human society. Rice cultivation, on the one hand, reshaped the morphological and genetic profile of rice, and on the other hand, is also constrained by its intrinsic life cycle and physiological characteristics. Research difficulty, in the face of the enrichment of datasets derived from archaeological materials, lies in the ability to distinguish natural factors and anthropogenesis that operate on the rice population and the environment inhabited by it. Only by clarifying the multiple-layered components tangled in the process can we understand how active or passive humans might be and what elements have promoted or prohibited the interactions between humans and rice. According to the paradigm proposed by Zeder (2006) and Smith (2006), there is a wide range of issues regarding multiple realms of rice domestication that can be elaborately discussed, but here, we intend to concentrate the following discussion on a human-behavior-centered view to detect the evidence and evolutionary process of initial rice domestication.

First of all, conceptually, the scientific perception of “domestication” does not advocate a terminal point or stage that has been pursued during the domestication process but rather emphasizes the differences in domestication intensity. Depending on which one trait or combination of traits, or what time is chosen to be observed, the consequences of domestication should be understood as relative. For rice, domestication has been constantly underway throughout the past 10 millennia and is still proceeding today. As long as the interactions between humans and rice are sustained, rice domestication will never be completed. Therefore, it is inappropriate to ask questions regarding when rice was fully domesticated or when rice domestication was completed. Instead, a meaningful scientific question requires a precise definition of the state of rice domestication, such as how long it took to domesticate a wild population into one with a 50% reduction of seed shattering.

Secondly, we should not neglect that the significant progress in biochemistry and molecular genetics has also provided some techniques to acquire information closely related to human behaviors that cannot be revealed by conventional methods. Strontium isotope analysis has the potential to contribute to the study. It can exclude, not confirm, the possible geographic source of the analyzed biological sample. By integrating the information

derived from other materials, it may ascertain whether the rice was planted at the place of its being unearthed. If an archaeological rice population can be proven to be imported from elsewhere, it implies that human management should have been indispensable. It may also generate the inference that the human communities engaged in different subsistence patterns might be exchanging their cultigens and other resources. Our understanding of how the early diffusion of rice resulted in rice evolutionary differentiation associated with domestication will be enriched by these studies. Although researchers are often worried about the contamination problem caused by post-depositional conditions, some efforts have shown that the contaminating strontium from the depositional environment can be successfully removed from organic samples uncovered archaeologically (e.g., [Carnap-Bornheim et al., 2007](#)).

DNA technology has been considered another exciting tool for detecting genetic data of rice domestication. Here, we refer to the application of DNA techniques in particular in investigating archaeological remains. Unlike animal and human bones, plant remains do not yield DNA fragments that are good enough for polymerase chain reaction and sequencing. The reasons for this may include preservation conditions, a fragile state of material, and difficulties in the extraction of very tiny amounts of ancient DNA fragments. Desiccated and waterlogged rice remains commonly contain more ancient DNA available for analysis than carbonized spikelets and grains, which usually lead to failure in such studies. Contamination is also a notoriously inevitable concern, particularly because the tissue structure of rice is typically fragile and less dense than that of bone, making the processing of tested material even more challenging. With the high-throughput, next-generation sequencing technology, ancient DNA research is able to increase the efficiency of DNA extraction and the amount of DNA that could be targeted in a single experiment ([Shapiro and Hofreiter, 2014](#); [Brown et al., 2015](#)). If these problems can be properly resolved in investigating archaeological rice, the materials may produce direct evidence of domestication genes or molecular signatures of selection under domestication. It will further provide invaluable information to assess the extent of the domestication bottleneck experienced by a certain rice population, to trace the genetic kinship of a modern population back to some ancient candidates, or to testify rice dispersals on different scales.

Although we are now able to capture the signatures of rice domestication at an early stage, an array of human actions that were necessary for ensuring successful year-to-year harvest have yet to be taken into account. Previously, researchers presumed that rice cultivation might have been very primitive in its early development and a series of field management strategies such as weeding, irrigation, deinfestation, and fertilization might have been carried out as late as the artificial paddy was created. However, the recovery of Hanjing rice paddy reminds us that the elaborate facilities and manipulating methods must have appeared early beyond expectation. These phenomena draw our attention to the intentionality of early rice cultivators. Some lines of evidence may suggest that a certain form of resource allocation or adjustment might be deliberately conducted to achieve some purposes even though a few natural processes and ecological laws were taken advantage of. For example, crop-weed competition and anthropogenic control of weeds can be reflected by the fine-resolution temporal and spatial analysis of macro- and micro-fossils. Plant diseases and crop failures resulting from fungal

infection or insect pests may be more challenging to detect, but advanced environmental genomic technology may make breakthrough contributions. Systematic geoearchaeological methods, including geophysical and geochemical techniques, still have great potential in revealing human manipulation and regulation of water conditions.

Finally, we would like to expand on the implications of archaeological rice records. Rice is significant in archaeological research, not only because it became a world-popular starchy staple food thousands of years later but also because it should be regarded as a diagnostic indicator of a whole set of human behaviors managing a variety of plant species in the niche. This means that, as just one of the consequences of holistic human ecosystem engineering, rice was not the only plant species engaged in the agroecological system created by humans. Identification of rice domestication provides the best clue of further detecting a wider human behavioral background for understanding how and why a long-term sustained agricultural pattern could achieve success. For example, water caltrop, foxnut, and acorns might have been managed by people at the Kuahuqiao site ([Pan et al., 2017](#)). More definitely, broomcorn and foxtail millets were planted at the Baligang, Zhuzhai, Tanghu, and Peiligang sites, along with rice cultivation. These all imply that the early rice cultivators were multiple-plant agriculturalists who were skilled in maintaining a long-lasting productive ecosystem through diverse forms of anthropogenic interventions in many plant species' life cycles. The habitat managed by the early rice cultivators, therefore, might have allowed more subsistence resilience and trial and error in rice domestication.

Author contributions

YP conceived the project, collected and analyzed the literature and datasets, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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