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# Oldest Directly Dated Remains of Sheep in China

SUBJECT AREAS:

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John Dodson<sup>1</sup>, Eoin Dodson<sup>1,5</sup>, Richard Banati<sup>1</sup>, Xiaoqiang Li<sup>2</sup>, Pia Atahan<sup>1</sup>, Songmei Hu<sup>3</sup>, Ryan J. Middleton<sup>1</sup>, Xinying Zhou<sup>2</sup> & Sun Nan<sup>4</sup>Received  
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requests for materials  
should be addressed to  
J.D. ([john.dodson@  
ansto.gov.au](mailto:john.dodson@ansto.gov.au))

<sup>1</sup>Australian Nuclear Science and Technology Organisation, Sydney, Australia, <sup>2</sup>Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, China, <sup>3</sup>Institute of Archaeology, Xi'an, Shaanxi, China, <sup>4</sup>Chang An University, Xi'an, China, <sup>5</sup>Garvan Institute, Sydney, Australia.

The origins of domesticated sheep (*Ovis* sp.) in China remain unknown. Previous workers have speculated that sheep may have been present in China up to 7000 years ago, however many claims are based on associations with archaeological material rather than independent dates on sheep material. Here we present 7 radiocarbon dates on sheep bone from Inner Mongolia, Ningxia and Shaanxi provinces. DNA analysis on one of the bones confirms it is *Ovis* sp. The oldest ages are about 4700 to 4400 BCE and are thus the oldest objectively dated *Ovis* material in eastern Asia. The graphitised bone collagen had  $\delta^{13}\text{C}$  values indicating some millet was represented in the diet. This probably indicates sheep were in a domestic setting where millet was grown. The younger samples had  $\delta^{13}\text{C}$  values indicating that even more millet was in the diet, and this was likely related to changes in foddering practices

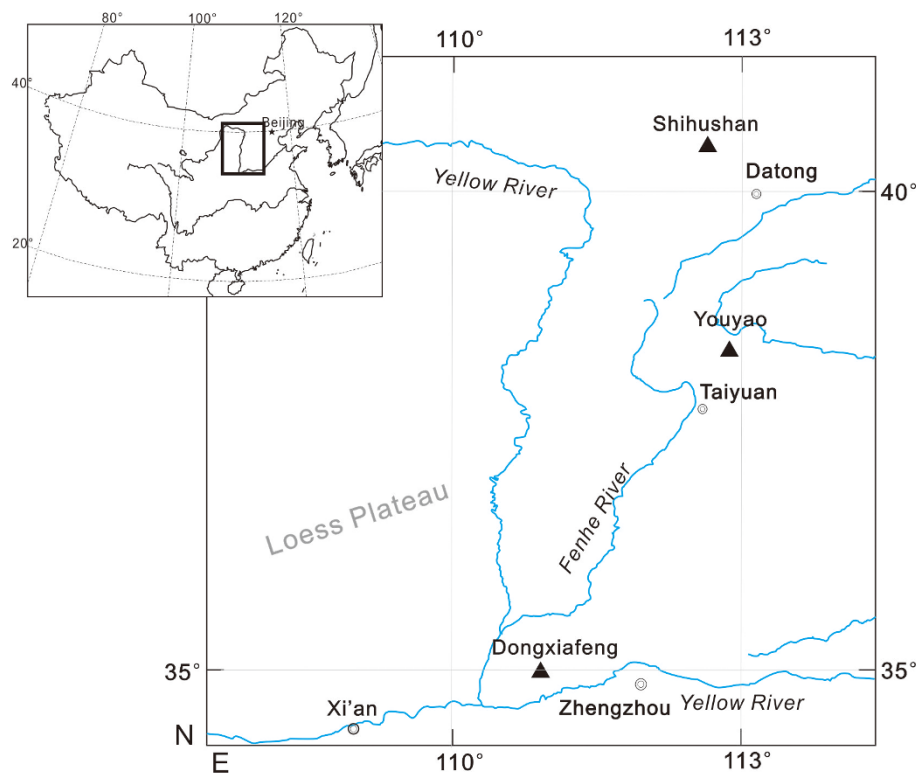
Fossil sheep of Pliocene age are known for East Asia<sup>1</sup>, but domesticated sheep are thought to have arrived several millennia after they were first domesticated in the Fertile Crescent region of Western Asia e.g. Refs. 2, 3. The pathway to first domestication remains unknown but is believed to have taken place about 10,500 to 11,000 years ago and relied on the fact that sheep were docile and herded naturally<sup>2-4</sup>. They were valuable sources of meat and probably milk and milk products, and the hides were useful for leather. It is likely that several thousand years later selective breeding added fibre for wool as a new product. Dated evidence from Turkey at about 8200 cal BC has revealed new insights into the domestication of sheep based on age and sex ratios and dung analysis<sup>5</sup>.

The earliest claimed domesticated sheep in China are based on archaeological associations with ages between 5–7000 years from Banpo village near Xi'an and Baoji, also in Shaanxi Province<sup>6,7</sup>. Other early ages are associated with sites at Shizhaocun, in Gansu<sup>8</sup> and Hetaozhuang in Minhe, Qinghai Province<sup>9</sup>. None of these ages have been determined directly on remains of sheep, and they need revisiting to check their veracity. Presumably, sheep arrived before these early dates and it is believed sheep were first widespread across Gansu-Qinghai sometime between 5600 and 4000 BP, and on the central Plains of China by 4500-4000 BP<sup>10</sup>.

While early remains of sheep have been reported for several sites in China, the usually fragmentary and incomplete nature of those remains has tended to limit whether domesticated animals can be distinguished from wild varieties<sup>11</sup>. Ancient DNA analysis is able to resolve this issue and distinguish domestication with a high degree of certainty, as well as provide specific information about relationships between ancient and modern sheep populations. Previous work using ancient DNA analysis on sheep remains from the middle reaches of the Yellow River valley dating to ca. 2100-1800 BCE found strong genetic links to modern domestic varieties in eastern Asia<sup>12,11</sup>. Thus far those studies have been unable to propose specific geographic origins for ancient sheep, due in part to a scarcity of genetic information from both eastern Asia and surrounding regions.

Stable carbon isotopes on fossil bone collagen are useful for palaeodiet studies. Collagen  $\delta^{13}\text{C}$  values indicate long-term diet averages. In northern China  $\delta^{13}\text{C}$  values  $\leq 18\text{‰}$  are typically regarded as mainly C3 food consumers: those with values  $\delta^{13}\text{C}$  between  $-18\text{‰}$  and  $-12\text{‰}$  are interpreted to have mixed C3 and C4 food consumption; and those with  $\delta^{13}\text{C}$  values  $\geq -12\text{‰}$  are interpreted to be mainly C4 consumers<sup>13-15</sup>.

In summary, these data suggest domesticated sheep came to China in the Neolithic and became widespread during the Bronze Age when societies became sedentary, but the ages of these events remain tentative in the absence of reliable dates. Here we identified sheep bone palaeontologically from sites in northern Shaanxi Province, and confirmed the identification of *Ovis* with DNA analysis on one sample. The AMS ages determined



**Figure 1** | Map showing the location of key sites where sheep bone was analysed for this paper. Figure 1 was designed, composed and prepared by XZ using Correl Draw software.

on the bone samples is independent of any archaeological association. In addition the stable isotopic composition was measured to provide insight into the diet of the sheep.

**Study Specimens.** The present study is part of a broader analysis on the impact of Prehistoric to Bronze Age cultures on environments of northern and western China<sup>16–22</sup>. In the course of this, several bone samples were collected from sediment at Shihushan, Dongxiafeng and Youyao in northern Shaanxi, and Haba Lake in Ningxia (Figure 1). The bones were removed from clayey sediment and transported to Xi'an for identification. In each case the bones were in a close archaeological context with pottery sherds, charcoal and sometimes associations of slag associated with metal working. The cultural setting is equivalent to the Yangshao. Six of the bone samples were identified as sheep (*Ovis*), and an additional sample from Huoshiliang (Shaanxi Province) was provided by the Shaanxi Provincial Museum. There exists a full list of taxa identified at Huoshiliang site<sup>23</sup>. About 10 gm of each *Ovis* bone was transported to the laboratories of the Australian Nuclear Science and Technology Organisation (ANSTO) in Sydney for further analysis.

## Methods

In the course of this, several bone samples were collected from sediment at Shihushan (probably belonging to the Yangyao Culture), and Youyao (Zhukaigou Culture: 2200–

1500 BC) in northern Shaanxi, the Dongxiafeng site (belonging to the Erlitou Culture: 1900–1500 BC) in northern Shaanxi, Haba Lake in Ningxia (Xia Dynasty: 2199–1600 BC), and Huoshiliang (Longshan to Xia Culture: 2150–1900 BC) (Figure 1). The bones were removed from clayey sediment and transported to Xi'an for identification. In each case the bones were in a close archaeological context with pottery sherds, charcoal and sometimes associations of slag associated with metal working.

Collagen was extracted from each sample and tested to determine whether preservation was sufficient and contaminants had been effectively removed to achieve reliable radiocarbon results. For this the total collagen yield (% by weight), nitrogen and carbon percentages and the C/N atomic ratio were measured, following established protocols<sup>23–26</sup>. Once the collagen had been extracted and determined to meet standards for reliable dating, they were processed to graphite<sup>27</sup>. The <sup>14</sup>C content was measured on the STAR Accelerator at ANSTO and AMS ages were calculated after estimating fractionation effects from the  $\delta^{13}\text{C}$  values determined on the samples.

DNA analysis was carried out on five of the bone samples to provide independent corroboration that the bones were indeed from sheep. DNA extraction was conducted on subsamples of the same bone fragments that were prepared for radiocarbon analysis.

In preparation, the metal utensils used during sample processing were wiped with 70% ethanol and UV irradiated, mortars and pestles were soaked in 11–14% sodium hypochlorite solution (w/v) for 30 min, rinsed with MilliQ water, and then dried in an oven set at 60°C before being used to grind bone samples. Separate mortars and pestles were used for each sample (Table 1). Approximately 500 mg of cleaned bone was used for DNA analysis.

Bone fragments were carefully crushed, as opposed to grinding, in order to prevent localised high temperatures and further DNA damage. A negative control was used from this point onwards, in order to detect introduced contaminating DNA, from the other samples or elsewhere (Table 1).

DNA extraction was performed according to a published protocol that relies on DNA absorption to silica particles<sup>28</sup>.

**Table 1** | Samples and bone fragments used for DNA analysis, including original samples sizes and DNA Nanodrop results

Site	Sample ID	Bone type	Bone Fragment mass (mg)	Bone Powder mass (mg)	DNA conc. (ng/μL)
Shihushan	OZP087/SI3001	Compact	554	429	52.2
Shihushan	OZP086/SI2998	Spongiose	556	385.4	9.6
Shihushan	OZP085/SI2997	Spongiose	496	502.8	42.2
Youyao	OZO960/SI2950	Compact	440	464.4	65.4
Dongxiafeng	OZO949/SI2921	Compact	491	500	51.2
Negative Control	-	-	-	0	13.5



**Table 2 | Radiocarbon and stable carbon isotope analyses on sheep bone. Calibrated ages were calculated in Calib (v. 7.0.1) using the IntCal13 calibration curve<sup>29,30</sup>**

Site	ANSTO Code	C%	N%	C/N (atomic)	$\delta^{13}\text{C}$ (‰) (graphite)	Conventional $^{14}\text{C}$ age (BP $\pm 1\sigma$ )	Calibrated age (2 $\sigma$ ) BCE
Shihushan	OZP087/SI3001	43.3	16.0	3.1	$-19.2 \pm 0.1$	$5740 \pm 40$	4695–4490
Shihushan	OZP086/SI2998	45.2	15.6	3.4	$-18.9 \pm 0.1$	$5695 \pm 35$	4667–4454
Shihushan	OZP085/SI2997	44.5	15.4	3.4	$-19.0 \pm 0.1$	$5665 \pm 40$	4601–4372
Youyao	OZO960/SI2950	45.3	15.0	3.4	$-14.3 \pm 0.1$	$3750 \pm 45$	2292–2029
Dongxiafeng	OZO949/SI2921	45.0	15.4	3.4	$-17.0 \pm 0.1$	$3360 \pm 45$	1713–1570
Huoshiliang	OZO951/SI2891	45.0	15.6	3.4	$-15.5 \pm 0.1$	$3750 \pm 50$	2334–1983
Haba Lake	OZN204/SI1592	44.0	15.0	3.3	$-17.0 \pm 0.1$	$4090 \pm 30$	2859–2499

## Results

Seven radiocarbon ages on sheep collagen have been obtained and have a calibrated age range (with errors) from 4700 to 1570 BCE (Table 2). Three radiocarbon ages of about 5700 BP (4700 to 4400 BCE) are statistically identical, and they overlap the Neolithic (Yangshao) and Bronze Age boundary. These are the oldest directly dated sheep material from eastern Asia. The  $\delta^{13}\text{C}$  values of the older bones indicate the sheep had a diet based largely on C3 plants but the  $\delta^{13}\text{C}$  values are close to the boundary of diets based on C3 and C4 plants (Table 1).

Out of the five bone samples only the Youyao bone produced a specific amplification product. The 78 nucleotide sequence shares 100% identity with *Ovis aries* mitochondrial DNA (mtDNA) and in addition the sequence also shares 100% identity with mtDNA from *O. orientalis anatolica*, *O. vignei* and *O. ammon* which are Turkish and Central Asian species, that are possible ancestors of domestic sheep.

## Discussion

The identification of the bones on physical characteristics and DNA fragment confirms the bone samples are from the genus *Ovis* (sheep). The radiocarbon age of 5700 BP (4700 to 4400 BCE) for the three bones from Shihushan is the oldest independent age for sheep in eastern Asia. Since the bones were found in association with other domestic species and in an archaeological setting of the Yangshao Culture it is a reasonable conclusion that the sheep were domesticated. This is further supported by the stable isotope analyses on graphite prepared from bone collagen that had values ranging from  $-19.2$  to  $-14.3\%$ . In all likelihood some millet was consumed by the sheep (the Asian millets are the C4 plants *Panicum miliaceum* and *Setaria italica*), but the bulk of the diet was comprised of C3 plants. The younger samples from Youyao and Huoshiliang had less negative  $\delta^{13}\text{C}$  values, suggesting that millet was a larger proportion of the diet in these cases, and this possibly related to changes in foddering practices occurring over time. This is quite unlike the diet of pigs, rats and dogs from similar settings which had even less negative values and suggest they had diets strongly influenced by millet, including meat from possibly domesticated animals that were fed on millet<sup>19</sup>. Deer, cattle and sheep  $\delta^{13}\text{C}$  values indicate that in general wild animals and herds grazed beyond the agricultural zone but sheep had some C4 plants, possibly millet, in their diet<sup>19</sup>. The younger *Ovis* samples suggest that the amount of millet in the diet increased and possibly indicates a change in foddering practice. It is unfortunate that a longer DNA sequence could not be recovered from the collagen, particularly from the older samples, as this could shed light on the early ancestors of the domesticated sheep.

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## Author contribution

J.D., X.L., P.A., X.Z. and S.N. participated in the fieldwork, and collected the samples. S.H. identified the bones and provided the museum specimen for analysis, E.D., R.B. and R.M. carried out the DNA analysis and interpretation, J.D. and P.A. assisted in the radiocarbon analysis and all contributed to the writing of the manuscript.

## Additional information

**Competing financial interests:** The authors declare no competing financial interests.

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