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Letter to the editor

Ancient DNA traces a Chinese 5400-year-old cat specimen as leopard cat (*Prionailurus bengalensis*)

Now, widely distributed modern cats are the descendant of wildcat (*Felis silvestris lybica*), which are assumed to have originally taken advantage of resources in human settlements, before being tamed in the Near East via a commensal pathway (Driscoll et al., 2007; Larson and Fuller, 2014). Cats were previously thought to have been firstly reached in China almost 2000 years ago until archaeological evidence of felid remains at Quanhucun (QHC) site in Shaanxi, China was discovered, raising new acknowledgment of a plausible commensal process that happened in China around 5000 years ago (Driscoll et al., 2009; Hu et al., 2014). Based on the osteometric analysis, the cat specimens were recognized as *Felis* sp. due to the fact that the biometric measurements of the QHC samples were within the range of domestic cats, hereby two hypotheses were proposed that the Near Eastern cats may have been transported to China earlier than the previous knowledge (Driscoll et al., 2009), or the gene flow or local domestication once potentially occurred from Asian wild cats (*F. s. ornata* or *F. s. bieti*) (Hu et al., 2014). Meanwhile, isotopic evidence indicated the commensal relationship between the ancient QHC villagers and the symmetrically distributed cats (Hu et al., 2014).

However, another morphometric examination that considered mandible form rather than size cast doubt on the identification of cat bones. All the cat remains obtained from Chinese archaeological sites including one of the ancient QHC samples were identified as leopard cats (Vigne et al., 2016). Therefore, the hypothesis that the early domestication events originated from the local or introduced wildcats could be rejected. Furthermore, the new evidence revealed that these ancient cats were most likely the north-central leopard cat subspecies of China, which is currently widespread in Eastern and South-Eastern Asia (Vigne et al., 2016; Patel et al., 2017). However, the latter identification improved accuracy by restricting the range of species matches and the presence of *F. s. ornata* (Asian wildcat) could not be directly ruled out (Vigne et al., 2016) due to a lack of effective referential data, whereas, which could be potentially remedied using genetic approaches. In order to further elucidate the discordance of cat identification as well as commensal or early domestication hypotheses, here, we use ancient DNA technology and multiple up-to-date bioinformatical methods to determine the phylogeny and evolutionary history of this Chinese Neolithic cat and provide clearer evidence for the origin of the QHC sample as well as the cat domestication process in Eastern Asia.

Using the typical ancient DNA capture protocols (Fu et al., 2013), we first extracted the complete mitochondrial genome of a ~5400-year-old cat specimen (AMS-¹⁴C date of cal. 5590–5330 B.P. with $\pm 2\sigma$ at 95.4% probability; Hu et al., 2014). It was excavated from

the refuse pit (H172) of the QHC site in Hua County, Shaanxi Province, China (Hu et al., 2014) (Fig. 1A).

Three hypotheses have been proposed to clarify the cat domestication scenario: (1) Near Eastern cats may have been brought to China 5000 years ago, and sympatric Asian wild cats may have contributed to the domestic cat gene pool; (2) Independent domestication events may have occurred in East Asia (Hu et al., 2014); (3) Leopard cats may have had a brief association with locals or may have been domesticated separately in East Asia, but they were subsequently displaced by the introduced domestic cat from Southwest Asia (Vigne et al., 2016). These contradictory hypotheses highlighted the limitations of utilizing morphological approaches to identify species. The evolutionary positioning of this QHC cat sparked a heated debate about whether cats have experienced one or many domestication processes. To address the incongruence of morphological identification, we constructed the phylogeny of the Felidae family using a total of 135 sequences, including the mitochondrial genomes of 133 modern samples and the ancient sample from the QHC site, with one spotted linsang (*Priondon pardicolor*) as the outgroup (Table S1). Our results indicated the ancient QHC cat is genetically closer to the modern leopard cats (*Prionailurus bengalensis*) (Fig. 1B). Both maximum likelihood and Bayesian methods-based trees of complete mitochondrial genomes showed consistent results (Figs. 1B and S1A) and was supported by the attribution of the QHC cat in leopard cats rather than wild/domestic cats (*Felis silvestris*) (Fig. S1B). The pairwise genetic distance of the QHC sample is closer to the leopard cat than other Felidae species, which also supports the genealogy relatedness (Fig. S2).

To determine the genetic link between this ancient QHC cat and present leopard cats, we then zoomed down the scale into *Prionailurus* species and reconstructed the phylogeny of leopard cats, taking the flat-headed cat (*Prionailurus planiceps*) as the outgroup (Table S1). The divergence time of flat-headed cat and leopard cat (2.04–4.31 million years ago, Mya) estimated previously (Johnson et al., 2006) were used for node calibration on the root, as well as the carbon date of the ancient sample in this study for tip calibration, respectively. We estimated the mean substitution rate to be 1.58×10^{-8} substitutions/site/year (95% HPD $1.06\text{--}2.28 \times 10^{-8}$) and the early divergence between the mainland and sundaic leopard cats around 1.60 Mya (95% HPD, 1.02 Mya–2.18 Mya), while the age of the tree root was inferred as 3.03 Mya (95% HPD, 1.97 Mya–4.12 Mya; Fig. 1C). The split time between mainland and sundaic leopard cats approaches the previous estimations (0.98/2.67 Mya; Luo et al., 2014; Patel et al., 2017). The slight differences are likely due to the use of different time priors of interspecific divergences (Luo

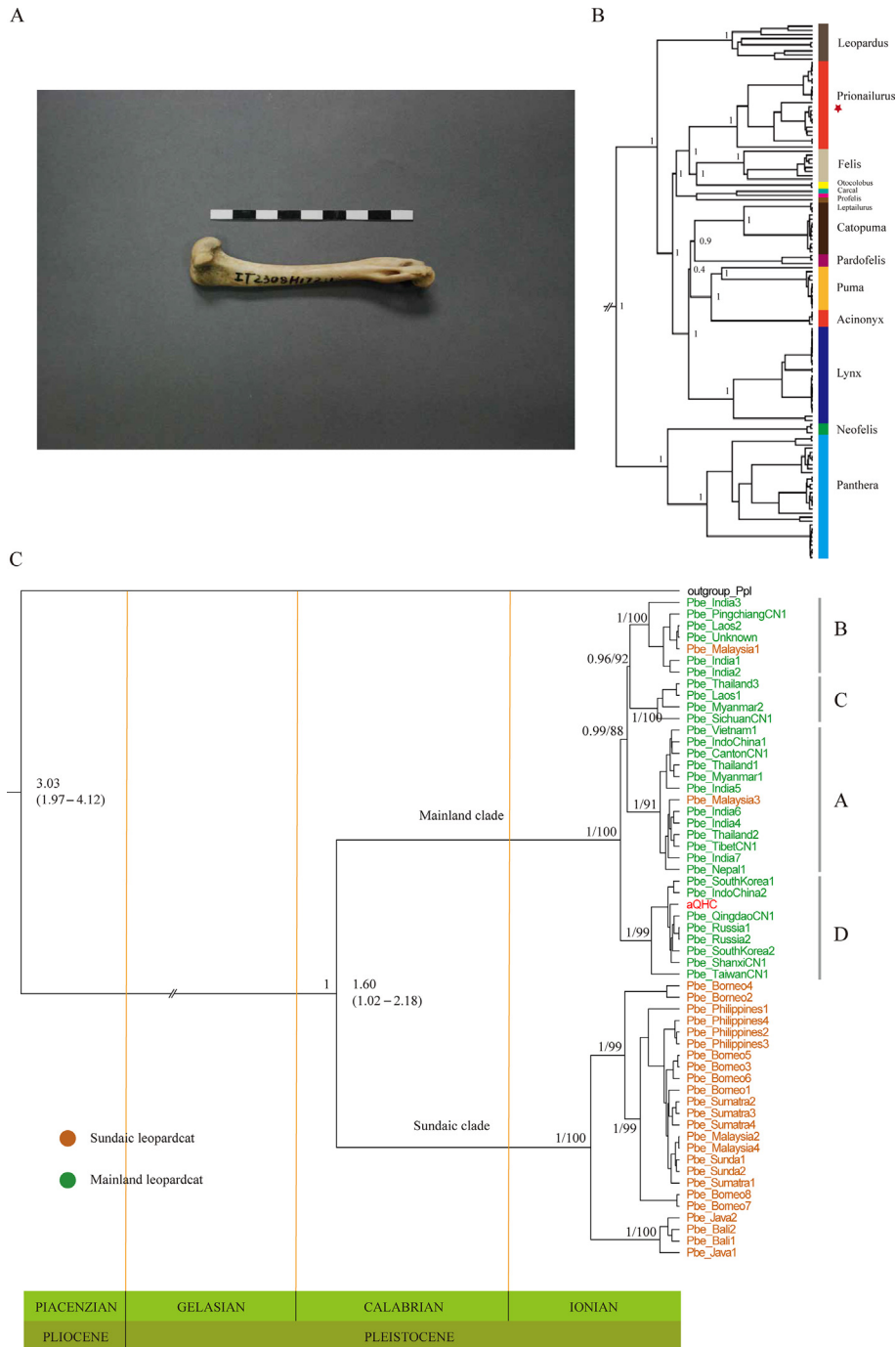


Fig. 1. Phylogenetic analysis of ancient QHC sample. **A:** Right humerus of the QHC cat. **B:** Phylogeny was reconstructed for the Felidae family (Table S1) based on the Bayesian method. Different color bars represent various genera (marked genus name aside) in the Felidae family. The ancient QHC sample was marked with a red star. Posterior values are listed on the nodes. **C:** Phylogeny reconstructed for the leopard cats (Table S1) based on the Bayesian method. The inferred TMRCA for the root and leopard cats were shown with 95% HPD in the parentheses. Posterior values (the first value) were shown for the main clades and verified with the bootstraps (the second value) estimated based on the maximum likelihood method. The samples were marked with consistent colors with their geographical region and haplogroups A–D were marked in the mainland lineage.

et al., 2014) or intraspecific divergence estimates (Patel et al., 2017). The time of the most recent common ancestor (TMRCA) was estimated to be 0.27 Mya for mainland leopard cats and 0.41 Mya for sundaic leopard cats, respectively (Fig. 1C), which were younger than the coalescence time for these two leopard cat lineages. Henceforth, a large divergence was also revealed within the leopard cat populations (Fig. 1C). Mainland leopard

cats could be divided into four haplogroups (Patel et al., 2017), and the ancient QHC cat was mainly clustered with eight samples from Northeast Asian (Korean Peninsula, Russian Far East), China (Shandong, Shanxi, Taiwan), and Indochina. The estimated TMRCA for the ancient QHC cat and other samples excluding the sample from Taiwan in haplogroup D was 43.13 thousand years ago (Kya, 95% HPD, 25.78 Kya–65.82 Kya).

To further verify the evolutionary position of this QHC leopard cat among all its modern counterparts, haplotype network and genetic distance were performed. The ancient QHC cat showed consistent clustering with the phylogenetic tree (Fig. S3). The present-day mainland Asian samples were split into four haplogroups based on the tree topology, indicating a high genetic diversity of the present leopard cats' populations. Haplogroups A–C were shared by the leopard cats of the southern samples from South Asia, Southern China, and Mainland Southeast Asia, except for two samples from Malaysia, while the Northeast Asian and Northern Chinese samples, including the QHC cat, were only in haplogroup D (Fig. 1C). Furthermore, the ancient QHC cat held the minimal genetic distance with leopard cats from Northeast Asia, Northern China (Shandong and Shanxi), and Indochina, followed by other mainland and sundaic samples (Table S2). The consistency of geographic and genetic patterns for mainland Asian leopard cats suggested the large divergence possibly caused by long-term geographical isolation. In addition, the ancient QHC cat is likely to be local originated and genetically closer to the Northeastern Asian and Northern Chinese leopard cats.

In summary, our study resolved the long-term controversial morphological identification of Chinese ancient cats and proved the ancient QHC sample as a local leopard cat through the genetic perspective. Deep divergency within the present leopard cat population possibly due to geographical isolation, while the QHC leopard cat was found to be genetically closer to the Northeast Asian and Northern Chinese leopard cats (Figs. 1C and S4). The ancient leopard cats were attracted by the house rodents along with the development of millet agriculture, which fitted the need of QHC villagers in the Neolithic period, facilitating a commensal relationship with the local people (Weissbrod, 2010; Zeder, 2012; Hu et al., 2014; Vigne, 2020). Today, the extant leopard cats are still widely distributed in East Asia, including Shaanxi and Henan province, and can adapt to the cultivated environment to hunt rodents, especially murids (Rajaratnam et al., 2007), but entirely been feral. Maybe due to a lack of strong human-driven cultural factors, local people have a weaker binding with the leopard cat, and the free management strategy of cats also made it easier for leopard cats escaped into the wild. Besides, a genetic replacement like European pigs (Frantz et al., 2019), hereby an introduced Near Eastern cat could be a later direct factor to extinguish the local domestication process. However, when and how the process occurred in ancient China still needs more samples and further analysis to answer. As for the status of the leopard cat during the Neolithic time in Northern China, there are two clues: 1) one cat specimen from the QHC site was detected with an extremely high $\delta^{13}\text{C}$ and low $\delta^{15}\text{N}$ values (-12.3‰ , 5.8‰) based on isotopic analysis, suggesting the dependence of eating habits on agricultural products (Hu et al., 2014); 2) all the five specimens from northern China showed domestication-related phenotype that the size range to be smaller than the average modern wild leopard cat (Vigne et al., 2016). The evidence of these two layers firmly confirmed that the Neolithic leopard cats remained in a commensal relationship with humans. Furthermore, multiple leopard cat remains in pits were discovered at different archaeological sites (Quanhucun, Wuzhuangguoliang, and Xiawanggang) over a long time period (6000 B. P.–4000 B. P.), also indicating a conscious and purposeful act of domesticating the leopard cat, not occasionally happened, hinting at a domesticating attempt by the local people in ancient China (Vigne et al., 2016).

Though the selection analysis on the 13 coding genes in the mitochondrial genome got non-significant signals (see Supplementary data), the evidence of the close relationship between the Neolithic leopard cats and the local people (Hu et al., 2014) as well as the morphological change of the Neolithic cats including the QHC sample (Vigne et al., 2016) both indicated that they have already been in

the domestication track. Nevertheless, after the late Neolithic, extant leopard cats in this adjacent area became feral and were replaced by the domestic cats (*F. catus*) originated from wild cats (*F. s. lybica*) (Driscoll et al., 2007). Taken together, whether they once were domesticated and for some reason then escaped into the wild or were just short-lived with the local villager remains unclear. Only further archaeological discoveries and increasing nuclear genomes and analysis of Chinese cat remains can potentially answer these questions. Even so, our study suggested a new angle that the haplogroup D in the mainland observed in present-day sub-groups of leopard cat likely had already emerged at least the Neolithic in Northern China. The funerary form, scale, and domestication traits hint a potential leopard cat domestication episodes or early wild introgression in North China.

Data availability

The mitochondrial genome sequence data reported in this article have been deposited in the Genome Warehouse in National Genomics Data Center (National Genomics Data Center Members and Partners, 2020), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, with accession number GWHBJB101000000 that is publicly accessible at <https://bigd.big.ac.cn/gwh>. All data needed to evaluate the conclusions in the article are present in the article and/or the Supplementary Data. Additional data related to this article may be requested from the authors.

Conflicts of interest

The authors declare no conflicts of interest.

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Supplementary data

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