



Once lost, twice found: Combined analysis of ancient giant panda sequences characterises extinct clade

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This year, two studies have reported ancient DNA sequences from giant pandas (*Ailuropoda melanoleuca*). We present the combined analysis of these ancient panda sequences to reveal a lost clade which survived through the Last Glacial Maximum and became extinct sometime subsequent to the Middle Holocene. This result indicates a major loss of mitochondrial diversity occurring during the recent evolutionary history of pandas.

The giant panda is an iconic species that currently occurs in fragmented populations across six mountain ranges on the eastern margin of the Tibetan Plateau (Figure 1). However, this represents a tiny fraction of their distribution during the Pleistocene, when they occurred across most of southern China as well as adjacent parts of Myanmar, Thailand, and Vietnam (Jablonski et al., 2012). Although pandas have frequently been the subject of genetic studies (Li et al., 2010; Zhao et al., 2012), until very recently, ancient DNA sequences have proved elusive (Basler et al., 2017), potentially reflecting advanced DNA degradation typical of ancient bones deposited in tropical and subtropical environments (Kehlmaier et al., 2017; Min-Shan Ko et al., 2018). This year, a study by Sheng et al. (2018) reported the successful recovery of around 5,000 bp of mitochondrial sequence from one 8,470 ± 45-year-old individual and another 5,025 ± 35-year-old individual from a natural sinkhole in Jiangdongshan, Tengchong County, Yunnan Province, southwestern China, which is outside the current distribution of pandas. This study found the c. 5,000-year-old haplotype to be nested within the mitochondrial diversity of extant pandas, whereas the c. 8,500-year-old haplotype formed a divergent sister lineage to this clade. A second study by Min-Shan Ko et al. (2018) reported the successful recovery of the mitochondrial genome of a 21,703 ± 208-year-old individual from Cizhutuo cave in Guangxi Province, southern

China, also outside the current distribution of pandas. This study also found the ancient haplotype to form a divergent lineage that is sister to all sampled extant panda haplotypes. We investigated the interrelationships of these three ancient haplotypes.

We cropped the c. 22,000 year mitochondrial genome to c. 5,000 bp overlapping the two Jiangdongshan sequences and replicated the analysis described in Sheng et al. (2018) using BEAST 1.8.2 (Drummond, Suchard, Xie, & Rambaut, 2012) (Supporting Information). We constrained the coalescence time of extant panda haplotypes using a normal prior with a mean of 72,000 years and SD 10,000 years based on the results of Min-Shan Ko et al. (2018). The substitution rate of the relaxed clock model was estimated within an open, uniform prior of 0%–20% per million years based on this node calibration and the radiocarbon dates of the ancient samples.

Consistent with previous studies (Min-Shan Ko et al., 2018; Sheng et al., 2018), we did not find evidence of strong mitochondrial phylogeographic structuring in modern pandas. Nuclear genomic studies do, however, support a primary structuring of living pandas into three populations according to mountain range, comprising a Qinling Mountains population; a Minshan Mountains population; and a third population encompassing the Qionglai, Daxiangling, Xiaoxiangling, and Liangshan Mountains (Zhao et al., 2012). Our results do suggest some congruent patterns of mitochondrial diversity, since the northeastern Qinling population and the two western populations combined each possesses some exclusive mitochondrial lineages established prior to 10,000 years ago (Figure 1). Given the overall lack of reciprocal monophyly among populations, and several examples of more recent coalescence of haplotypes between populations, the mitochondrial phylogeography is consistent with incomplete post-divergence lineage sorting and/or recent maternal gene flow.

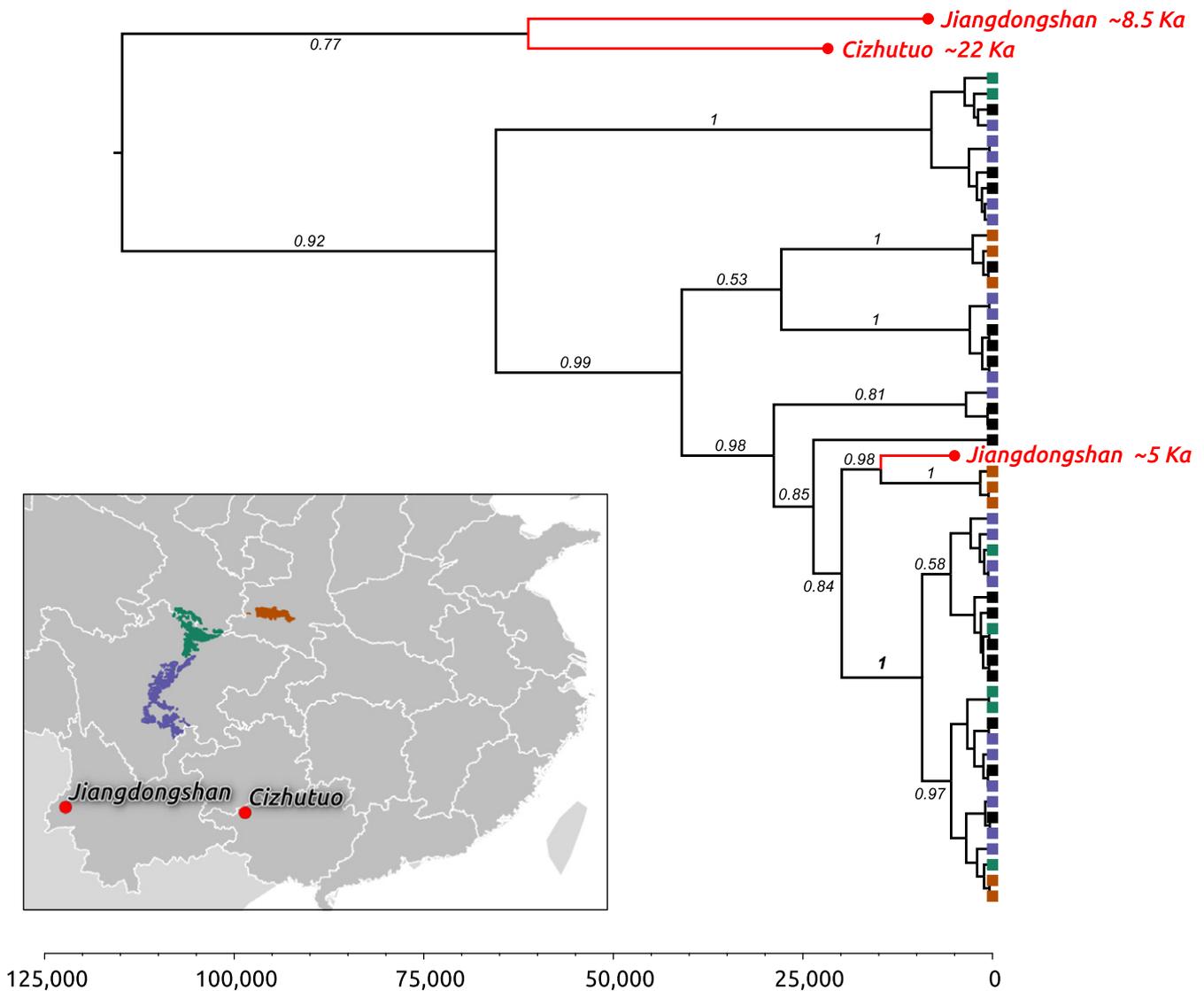


FIGURE 1 Mitochondrial phylogeny of pandas. Ancient lineages are shown in red, with sampling localities and ages indicated. Modern haplotypes are coloured according to their assignment to three population clusters identified previously by the analysis of nuclear genomes (Zhao et al., 2012): the northeastern Qinling Mountains population (orange); the northwestern Minshan Mountains population (green); and the southwestern Qionglai, Daxiangling, Xiaoxiangling, and Liangshan Mountains population (blue). Black haplotypes indicate captive individuals which either lack locality information or are admixtures of wild populations, and are ignored for phylogeographic inference. Branch labels are Bayesian posterior probabilities. Branch lengths are proportional to time (x axis in years) with node heights centred on the median of the posterior sample. Inset map of China shows the current distribution (Swaigood, Wang, & Wei, 2016) of panda populations in orange, green, and blue, respectively, and the approximate sampling localities of ancient haplotypes in red

Our analysis provides new information on the relationships of the ancient panda haplotypes. It supports the two divergent ancient mitochondrial haplotypes identified as sister to all modern panda haplotypes by separate studies (Min-Shan Ko et al., 2018; Sheng et al., 2018) as monophyletic with 77% posterior confidence (Figure 1). They form the sister lineage of a clade comprising all extant panda haplotypes plus that of the third ancient sample (92% posterior confidence). The estimated coalescence time of the lost clade is c. 61,000 years (95% credibility interval 24,000–130,000 years), which is similar to that estimated for extant panda haplotypes, c. 65,000 years (95% credibility interval 46,000–86,000 years).

Uniting the currently available ancient mitochondrial sequences sheds new light on the lost panda clade. Current sampling indicates that this clade occurred in both Yunnan and Guangxi Provinces, south of the current distribution of pandas (Figure 1). Sympatry of the lost and extant panda clades during the Middle Holocene is suggested as a possibility by their co-occurrence in Jiangdongshan, albeit separated in time by c. 3,500 years. Alternatively, the lost clade may have become extinct and this region subsequently colonized for a relatively brief time by the extant clade before pandas finally became extinct in this region. In either case, the lost clade survived through the Last Glacial Maximum and went extinct sometime subsequent to c. 8,500 years ago. Most strikingly, genetic divergence within the lost panda clade is

broadly similar to that occurring within the extant clade. Thus, the Holocene extinction of the lost clade appears to have resulted in a reduction in mitochondrial diversity equivalent to the total mitochondrial diversity present in living pandas. Moreover, since our estimated divergence within the lost clade is based on only two haplotypes, it is possible that sequencing other ancient pandas would uncover additional basal lineages, thus revealing an even greater diversity loss than suggested by the current dataset.

Nuclear genomic studies suggest that modern panda populations harbour reasonable genome-wide genetic diversity, being similar to that occurring in modern humans (Zhao et al., 2012). However, interpreting the mitochondrial data as a proxy suggests that this diversity may represent a mere fraction of that occurring in the recent evolutionary history of pandas. Whole genome data from ancient pandas would allow this hypothesis to be robustly tested, and shed light on the genetic affinities of these ancient populations with those existing today.

AUTHOR CONTRIBUTIONS

A.B. and J.L.A.P. conceived and designed the study; A.B. analysed data, J.L.A.P. prepared figures; A.B., J.L.A.P., G.S., X.L., and M.H. interpreted results and wrote the manuscript.

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