

Subspecific Status of the Korean Tiger Inferred by Ancient DNA Analysis

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ABSTRACT

The tiger population that once inhabited the Korean peninsula was initially considered a unique subspecies (*Panthera tigris coreensis*), distinct from the Amur tiger of the Russian Far East (*P. t. altaica*). However, in the following decades, the population of *P. t. coreensis* was classified as *P. t. altaica* and hence forth the two populations have been considered the same subspecies. From an ecological point of view, the classification of the Korean tiger population as *P. t. altaica* is a plausible conclusion. Historically, there were no major dispersal barriers between the Korean peninsula and the habitat of Amur tigers in Far Eastern Russia and northeastern China that might prevent gene flow, especially for a large carnivore with long-distance dispersal abilities. However, there has yet to be a genetic study to confirm the subspecific status of the Korean tiger. Bone samples from four tigers originally caught in the Korean peninsula were collected from two museums in Japan and the United States. Eight mitochondrial gene fragments were sequenced and compared to previously published tiger subspecies' mtDNA sequences to assess the phylogenetic relationship of the Korean tiger. Three individuals shared an identical haplotype with the Amur tigers. One specimen grouped with Malayan tigers, perhaps due to misidentification or mislabeling of the sample. Our results support the conclusion that the Korean tiger should be classified as *P. t. altaica*, which has important implications for the conservation and reintroduction of Korean tigers.

Keywords: *Panthera tigris coreensis*, phylogenetic relationship, mitochondrial DNA, Korean tiger, *Panthera tigris altaica*, Amur tiger

INTRODUCTION

The tiger, *Panthera tigris*, is one of the most endangered species in the world. In the 1900s, an estimated 100,000 tigers inhabited much of the Asian continent. However, today only 3,000 survive in limited areas (Driscoll et al., 2009; Tilson and Nyhus, 2010). Tigers have been traditionally classified into eight subspecies based upon geographic distribution and morphological differences. Recently a comprehensive molecular analysis of a tiger subspecies using mtDNA and microsatellite genotypes affirmed the distinctiveness of these sub-

species and identified a new subspecies, the Malayan tiger, *P. t. jacksoni* (Luo et al., 2004, 2008). Of these recognized subspecies, three are extinct, *P. t. sondica*, *P. t. balica*, and *P. t. virgata* (Driscoll et al., 2009) and one, *P. t. amoyensis*, only survives in zoos (Tilson et al., 2004; Morell, 2007; Luo et al., 2008).

From the Joseon Dynasty to the Japanese colonial period, tigers were seen as a threat to the Korean people and there was a lot of pressure to eliminate tigers from the wild (Endo, 2009; Kim and Lee, 2011). As a consequence, tigers that had once roamed the forests of the Korean peninsula became

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Table 1. Samples used in this study and haplotypes of the mitochondrial DNA

Museum ID	Provider	Type of sample	Collector (the year of date)	Mt DNA haplotypes	Haplotype ID
10146	National Museum of Nature and Science	Bone	Maeda Kenkiohi	<i>Panthera tigris altaica</i>	KOR1
A49797	National Museum of Natural History	Bone	Lord W Smith (1904)	<i>P. t. altaica</i>	KOR2
A49798	National Museum of Natural History	Bone	Lord W Smith (1904)	<i>P. t. altaica</i>	KOR3
A49799	National Museum of Natural History	Bone	Lord W Smith (1904)	<i>P. t. corbetti</i> II (<i>P. t. jacksoni</i>)	KOR4

KOR, Korean sample.

extinct and the only surviving population in the extreme northeastern Asia is the Amur or Siberian tigers in Russian Far East and northeast China.

The tiger is an animal of great significance in Korea. It is deeply embedded in the Korean history and culture with references found in numerous myths, folk tales, art work, and old sayings. Although extinct in the wild, the tiger lives on in the minds of the Korean people through the use of tiger symbols in logos and signs that represent Korea. Currently, the lack of tigers as a top predator has resulted in an uncontrolled population growth of large ungulates such as deer and wild boar causing both property damage and injuries around the country. Despite the continued attention to the tiger in Korea, there is a lack of scientific data to understand the evolutionary history of the species in this region.

There has been significant debate as to whether the tigers that once inhabited the Korean peninsula are indeed the same subspecies as the tigers that survives in the Russian Far East. In the early 20th century, Brass (1904) classified the Korean tiger as *P. t. coreensis* based on the morphological differences between Korean and the Amur tigers (fur color and pattern). Until 1965, CITES categorized the Korean tiger as an independent subspecies, *P. t. coreensis*. However, after 1965 the Korean tigers were reclassified as the same subspecies as the Amur tiger, *P. t. altaica*, without scientific confirmation. In the present study, the phylogenetic relationship of the Korean tiger was investigated by comparing data from ancient DNA samples with previously published sequences of other tiger subspecies. Accurate understanding of the subspecies status of the Korean tiger is essential to establish conservation units and to help inform future efforts to reintroduce tigers back in Korea.

MATERIALS AND METHODS

Korean tigers were extirpated from the wild and some of the remains such as bones and skins were scattered around the

world. To acquire specimens with reliable records on capture locality, collector, and year of capture, an intensive survey of known collections was conducted. Four specimens were found from two museums; one in the National Science Museum, Tokyo (NSMT), Japan and three in the National Museum of Natural History (NMNH) in Washington, DC, USA (Table 1). The information regarding their origin was crosschecked using several independent references. Chung (1978) reported that an American doctor, William Lord Smith, while visiting Korea caught wild boar, roe deer, and three tigers near Mokpo in 1902. In addition, there is a record that Dr. Smith hunted three tigers from the southwestern peninsula of Korea; two tigers by himself and one by his hunter (Hollister, 1912). According to the records of the NMNH, Smith collected samples from Korea in 1904 then donated the specimens to the NMNH in 1906. Even though there is a conflict between the times of specimen collection, all records indicate that the three tigers in the NMNH were captured by Smith in Korea. The single specimen in the NMNS was taken over from Tokyo museum in 1989, which was initially collected by Maeda Kenkichi who lived in Korea from 1878 to 1887 (Dajokan, 1880).

DNA extraction was carried out in a dedicated ancient DNA laboratory in the Laboratory of Genomic Diversity (LGD), Frederick, USA and replicated at the Conservation Genome Resource Bank for Korean Wildlife (CGRB), Seoul, South Korea. Whole genomic DNA was isolated in multiple extracts using QIAamp DNA Micro Kit (Cat. No. 56304; Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Multiple extracts from each individual were used to confirm the consistency of ancient DNA from contamination and to facilitate PCR of ancient material. In accordance with the procedure of Driscoll et al. (2009), five mitochondrial genes (*ND2*, *COI*, *ND5*, *ND6*, and *Cyt b*) were amplified in eight short fragments using eight sets of primers, including a negative control. Sequences were determined in forward and reverse directions from independent amplifications and multiple extracts.

Table 2. Polymorphic sites of tigers and mitochondrial gene information used in this study

Nucleotide position ^a	ND2 (394 bp)				COI (177 bp)				ND5 (84 bp)				ND6 (214 bp)				Cyt b (305 bp)				
	5332	5349	5515	5518	5533	5608	5674	5728	5737	7287	7304	13722	14591	14618	14711	15588	15595	15602	15691	15743	15765
ALT	C	T	A	G	G	C	T	G	C	C	G	T	T	C	A	T	G	G	C	A	C
KOR1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KOR2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KOR3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
COR3	T	-	-	-	-	-	-	-	T	-	-	C	-	-	G	-	A	-	-	-	-
COR2	T	-	-	-	-	-	-	-	T	-	-	C	-	-	G	-	A	-	-	-	-
AMO3_COR1	T	-	-	-	-	-	-	-	T	-	-	C	-	-	G	-	A	-	-	-	-
AMO2	T	-	-	-	-	-	-	-	T	-	-	C	-	-	G	-	A	-	-	-	-
TIG6	T	-	-	-	A	-	-	T	T	-	C	-	T	T	G	-	-	-	-	-	T
TIG4	T	-	-	-	A	-	-	-	T	-	-	C	-	T	G	-	-	-	-	-	T
TIG3	T	-	-	-	A	-	-	-	T	-	-	C	-	T	G	-	-	-	-	-	T
TIG2	T	-	-	-	A	-	-	-	T	-	-	C	-	T	G	-	-	-	-	-	T
TIG5	T	-	-	-	A	-	-	-	T	-	-	C	-	T	G	-	-	-	-	-	T
TIG1	T	-	-	-	A	-	-	T	T	-	-	C	-	T	G	-	-	-	-	-	T
SUM8	T	-	-	-	-	T	-	-	T	A	-	C	-	T	G	-	-	-	-	G	T
SUM7	T	-	-	-	-	T	-	-	T	A	-	C	-	T	G	-	-	-	-	G	T
SUM6	T	-	-	-	-	T	-	-	T	A	-	C	-	T	G	-	-	-	-	G	T
SUM5	T	-	-	-	-	T	-	-	T	-	-	C	-	T	G	-	-	-	-	G	T
SUM4	T	-	-	-	-	T	-	-	T	-	-	C	-	T	G	-	-	-	-	G	T
SUM2	T	-	-	-	-	T	-	-	T	-	-	C	-	T	G	-	-	-	-	G	T
SUM3	T	-	-	-	-	T	-	-	T	-	-	C	-	T	G	-	-	-	-	G	T
SUM1	T	-	-	-	-	T	-	-	T	-	-	C	-	T	G	-	-	-	-	G	T
AMO1	T	C	-	A	-	-	A	-	T	-	-	C	C	-	G	-	-	-	-	-	T
*COR5	T	-	-	-	-	-	-	-	T	-	-	C	-	-	G	-	-	-	T	-	T
*COR4	T	-	-	-	-	-	-	-	T	-	-	C	-	-	G	-	-	A	T	-	T
*COR8	T	-	G	-	-	-	-	-	T	-	-	C	-	-	G	-	-	-	-	-	T
*COR7	T	-	G	-	-	-	-	-	T	-	-	C	-	-	G	-	-	-	-	-	T
*COR6	T	-	G	-	-	-	-	-	T	-	-	C	-	-	G	-	-	-	-	-	T
KOR4	T	-	G	-	-	-	-	-	T	-	-	C	-	-	G	-	-	-	-	-	T

ND, NADH dehydrogenase subunit; COI, cytochrome c oxidase subunit I; Cyt b, cytochrome b gene.
Panthera tigris subspecies: ALT, *P. t. altaica*; COR, *P. t. corbetti*; AMO, *P. t. amoyensis*; TIG, *P. t. tigris*; SUM, *P. t. sumatra*; *COR, *P. t. corbetti* II (*P. t. jacksoni*); KOR, Korean sample.
^aNucleotide positions correspond to the complete *Felis catus* mitochondrial sequence (accession no.: U20753.1).

Mitochondrial gene sequences of tiger subspecies from a previous study were retrieved from GenBank for comparative phylogenetic analysis (Luo et al., 2004). Sequences were aligned using CLUSTAL_X (Thompson et al., 1997), and further modifications were made by eye using Geneious 5.3 (Drummond et al., 2011). Polymorphic sites were extracted using DNAsp v4.10 (Rozas et al., 2003). The most likely evolutionary model parameters were identified using the Akaike Information Criterion (AIC) in Modeltest v3.06 (Posada, 2008). The most suitable model of DNA substitution was HKY+G. An optimal phylogenetic tree was constructed using distance neighbor-joining method in PAUP version 4.0b10 (Swofford, 2002). Eight fragments were combined for a phylogenetic analysis. The robustness of the tree was evaluated by bootstrap resampling (1,000 random replications) (Felsenstein, 1985).

RESULTS

DNA was successfully recovered from bone powders of tigers that were collected in Korea around 100 year ago. Five segments (*ND2*, *COI*, *ND5*, *ND6*, and *Cyt b*) of mitochondrial

DNA were amplified and sequenced from all samples using eight primer sets to yield a total of 1,174 base pairs including 22 subspecies diagnostic variable sites (Table 2) (Luo et al., 2004).

Analysis of the sequences revealed two haplotypes from the four individuals in this study. When compared to previously published sequences, one haplotype shared by three individuals (KOR1 from NMNS; KOR2 and KOR3 from NMNH) was identical to the Amur subspecies, *P. t. altaica* (Table 2, Fig. 1). In contrast, the other haplotype (KOR4) matched a haplotype found in the Malayan tiger, *P. t. jacksoni* (Table 2, Fig. 1), which corresponded to COR6, COR7, and COR8 in Luo et al. (2004). The two haplotypes had five nucleotide differences.

DISCUSSION

Tigers are currently listed as an endangered species class I by the Ministry of Environment in South Korea. Although many people will not be convinced that tigers still survive on the peninsula without any scientific evidence, it is broadly believed by the public that they are not extinct. As the Korean tiger disappeared before the genetic techniques were widely employed, it was an unresolved question as to whether to classify the Korean tiger as an independent subspecies, *P. t. coreensis*, or as the Amur tiger, *P. t. altaica*. Our results help resolve the subspecies status of the Korean tiger relative to the Amur tiger.

We identified two mtDNA haplotypes from four 100-year-old specimens of Korean tigers. One haplotype was identical to that of the Amur tiger (*P. t. altaica*) (Fig. 2) while the other matched that of Malayan tigers (*P. t. jacksoni*) (Table 2, Fig. 1). It is surprising that the haplotype of Malayan tigers

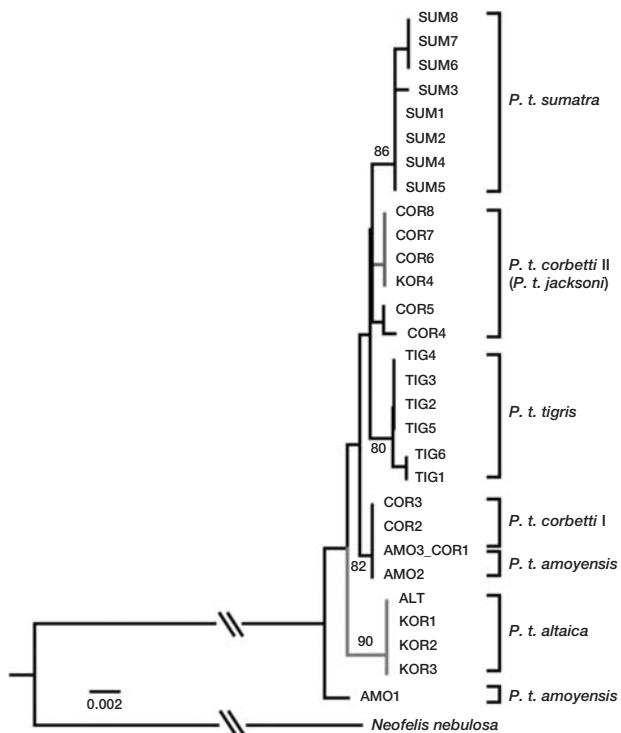


Fig. 1. Phylogenetic relationships of the Korean tigers using combined mitochondrial DNA. A distance neighbor-joining tree was constructed with 1,174 bp of mitochondrial DNA in PAUP version 4.0b10. Node bootstrap values greater than 80% are shown.



Fig. 2. Amur tiger (*Panthera tigris altaica*) in its wild habitat of Russian Far East (photo taken by Sooyong Park).

was found in this study because it is highly unlikely that the Malayan tiger inhabited the Korean peninsula a century ago.

The surprising result can be explained by either 1) a mishandling of the specimens between 1902 or early 1903 when Dr. Smith and his hunter collected the samples and 1906 when they were donated by C. Hart Merriam to the NMNH on behalf of Dr. Smith (database of the NMNH) (Hollister, 1912; Chung, 1978), 2) mislabeling during 100 years of storage at the museum, or 3) cross-contamination during the experiment due to the nature of ancient DNA. The last source has the lowest possibility since all lab work was carried out in a dedicated laboratory for ancient DNA where the DNA of the Malayan tiger was not handled. However, there are reliable records showing that Dr. Smith, the collector of Korean tigers, caught the three tigers in Mokpo, Korea in 1902 or early 1903 and that these tigers were deposited at the National Museum of National History, USA (Hollister, 1912; Chung, 1978). Without further information on the specimens and their storage, it is not possible to verify the precise basis of the questionable results.

In conclusion, except for the individual identified as Malayan tigers, the results presented here suggest that the Korean tiger is not an independent subspecies, *P. t. coreensis*. Instead of considering the Korean tiger as a different subspecies, we recommend that it should be regarded as *P. t. altaica*. This conclusion is also supported by an ecological view as tigers can disperse approximately 400 km and their home range size of female is around 400 km², 1,300 km² for male (Miquelle et al., 2005; Goodrich et al., 2010). In addition, it is unlikely that there has been a physical barrier to prevent dispersal of tigers in and out of the Korean peninsula.

The current population size of Amur tigers (*P. t. altaica*) is less than 600 due to several factors including loss of prey, habitat destruction/fragmentation, and over hunting for fur and Traditional Chinese Medicine (TCM) (Carroll and Miquelle, 2006). As our results show that the Korean tiger belongs to the Amur tiger, *P. t. altaica*, either captive or wild populations of the Amur tiger could serve as a source population for the restoration of the Korean tiger in the future. It may also be relevant that the former Caspian tiger has been shown recently to be near indistinguishable in genetic terms from *P. t. altaica* raising the prospect of tiger restoration of Caspian tiger habitat with re-located Amur Tiger founders (Driscoll et al., 2009, 2011). As the Amur tigers have the potential to be source populations for both Korean and Caspian tigers, further emphasis needs to be placed on the conservation of the Amur tigers in Russian Far East. Therefore, in order to preserve or restore the tigers in the Korean peninsula continuous effort is necessary for the conservation of the Amur tiger.

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