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The complete mitochondrial genome of the Tibetan fox (*Vulpes ferrilata*) and implications for the phylogeny of Canidae

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ABSTRACT

Canidae is a family of carnivores comprises about 36 extant species that have been defined as three distinct monophyletic groups based on multi-gene data sets. The Tibetan fox (*Vulpes ferrilata*) is a member of the family Canidae that is endemic to the Tibetan Plateau and has seldom been in the focus of phylogenetic analyses. To clarify the phylogenic relationship of *V. ferrilata* between other canids, we sequenced the mitochondrial genome and firstly attempted to clarify the relative phylogenetic position of *V. ferrilata* in canids using the complete mitochondrial genome data. The mitochondrial genome of the Tibetan fox was 16,667 bp, including 37 genes (13 protein-coding genes, 2 rRNA, and 22 tRNA) and a control region. A comparison analysis among the sequenced data of canids indicated that they shared a similar arrangement, codon usage, and other aspects. A phylogenetic analysis on the basis of the nearly complete mtDNA genomes of canids agreed with three monophyletic clades, and the Tibetan fox was highly supported as a sister group of the corsac fox within *Vulpes*. The estimation of the divergence time suggested a recent split between the Tibetan fox and the corsac fox and rapid evolution in canids. There was no genetic evidence for positive selection related to high-altitude adaption for the Tibetan fox in mtDNA and following studies should pay more attention to the detection of positive signals in nuclear genes involved in energy and oxygen metabolisms.

1. Introduction

The mitochondrial genome (mtDNA) has been used as an ideal marker of molecular evolutionary studies over the past three decades because of its convenience for the reconstruction of phylogeny, inference of population history, and estimation of divergence time [1–5]. mtDNA typically comprises a small closed circular DNA strand that generally encodes 37 genes: 13 protein-coding genes (PCGs), 2 rRNAs, and 22 tRNAs [6–8]. Compared with nuclear genes, mtDNA is characteristic of maternal inheritance, a high substitution rate, an absence of recombination, and a high copy number, which make it more effective in analyses [7,9,10]. In addition, the proteins involved in oxidative phosphorylation have provided insight into the molecular basis of adaptation to extreme environments [8,11–13].

Canidae is a family of carnivores that comprises about 36 extant species with a worldwide distribution [14]. Because of their close relationship with mankind, a great deal of attention has been devoted to studies on canids, including studies on their physiology, behavior, origins,

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and evolutionary relationships [15–17]. In recent decades, phylogenetic analyses based on morphologic and molecular data have been conducted for most extant canids and the most definitive analysis made use of a data set that combined three mitochondrial genes (*COI*, *COII*, and *CYTB*) [17–22]. Three distinct monophyletic groups within the family Canidae were defined as follows:

- the wolf-like canids, including the genera *Canis*, *Lycaon*, and *Cuon*;
- the fox-like canids, including the genera *Vulpes*, *Alopex*, and *Fennecus*;
- the South American canids, including the genera *Pseudolopex*, *Lycolopex*, *Atelocynus*, *Chrysocyon*, and *Speothos*.

As more morphological and molecular characteristics have been combined and analyzed, some phylogenetic issues (e.g., the phylogenetic positions of *Chrysocyon brachyurus* and *Speothos venaticus*) have become better resolved and the monophyly of these three clades are well supported [20,22]. Furthermore, some phylogeneticists have focused on the divergence time issues to comprehend the evolution history of the Canidae, although there are several points of conflict because of differences in molecular markers and fossil calibration points [22,23]. To date, only these three mitochondrial genes have been applied to illustrate the phylogenetic relationship of canids. The control region has been used to clarify intraspecies relationships among wolves from various areas because of its relatively homogeneous evolutionary rates [24]. Furthermore, the genome wide analysis indicated that the wolves inhabited in the Qinghai–Tibet Plateau were separated from the lowland ones, which was consistent with the geographic distribution of the wolf population in China [25].

The recent development of sequencing technologies makes it practical to sequence the complete mitochondrial genome at a reasonable cost. Unlike partial mtDNA markers, the complete mtDNA contains an increased phylogenetic signal with reduced effects of homoplasy [9], and the order of the mitochondrial genes provides more accurate information that allows it to be widely used as a highly heterogeneous marker in evolutionary studies [4,6,26–28]. With the recent rapid growth in the taxonomic coverage of complete mtDNA sequences, the analysis of mtDNA sequences has proven useful in the clarification of the colonization of two lineages of wolves in Japan [10], the origin and domestication history of dogs [1], and phylogenetic issues among various vertebrate taxa [29].

A member of the family Canidae (Carnivora), the Tibetan fox (*V. ferrilata*) is an endemic species that is widely distributed in the steppes and semi-deserts of the Tibetan Plateau north across central China, Nepal, and northern India [30]. According to the International Union for Conservation of Nature and Natural Resources, the Tibetan fox is listed as of "least concern"; however, hunting and habitat destruction by humans are the main threats to its population [30]. Little biological and ecologic information, especially phylogeny based on molecular data, is available because this species is rarely involved in phylogenetic analyses of the canids [17,31]. All aspects of the fox's natural history need study [32], and the complete mtDNA sequence of this species would be conducive to studies on molecular phylogeny, population conservation, and even further elucidation of its high-altitude adaptations. In this study, we present the complete mtDNA genome of the Tibetan fox and compare its structure and composition with other complete canine mtDNAs that have already been published. We also performed a phylogenetic analysis based on the complete mtDNA genome and estimated the divergence times, with the intention of resolving the phylogenetic relationship between this species and other canids.

2. Materials and methods

2.1. Specimen collection and DNA extraction

The sample that was used to sequence the complete mtDNA was taken from a specimen of Tibetan fox preserved in the Wildlife Park of Xining. The Tibetan fox died a natural death and then was made specimen in 2013. Permission to collect and use the sample was issued by Xining Wildlife Park. We snipped the pelage close to the anus to avoid affecting the appearance of this specimen. The total genomic DNA of the Tibetan fox was extracted using a DNeasy blood and tissue kit (Qiagen) and was examined on a 1.0% agarose/TBE gel.

2.2. Polymerase chain reaction amplification and sequencing

We designed 12 primer pairs with Primer 5.0 on the basis of the alignment of the complete mtDNA of *V. vulpes* (GQ374180) and *V. corsac* (KJ140137), and the primers were modified by sequencing (Table 1). The fragments were amplified using Taq DNA polymerase (TaKaRa) under

Table 1
Primer sequences used in this study.

Primer No	Primer ID	Forward and reverse primer sequence (5′–3′)
1	1-F	TCCCTCTAGAGGAGCCTGTTC
	1-R	TCCGAGGTCACCCCAACC
2	2-F	GACGAGAAGACCCTATGGAGC
	2-R	GGGTATGGGCCCGATAGCTT
3	3-F	GTCTGACAAAAGAGTTACTTTGATAGAG
	3-R	ACCTACTATACCGGCCCATGC
4	4-F	GCTCAGCCATTTTACCTATGTTC
	4-R	GCGAATTTAACTTTGACAAAGTCATGT
5	5-F	GAAGAAAGGAAGGAATCGAACC
	5-R	GCGAAGAGTTGTAGTGAAATCATAT
6	6-F	GCTACCTAATGACCCACCAAAC
	6-R	GCGTAGGGATGATAATTTTTAGCATT
7	7-F	GTATTTGCTGCCTGCGAAGC
	7-R	CGCTTATCTGGAGTTGCACC
8	8-F	CCGCAAGAACTGCTAATTCATG
	8-R	TAGTGGTGGGATTGGTTGTGC
9	9-F	TTCATGTGCTCCGGGTCAATTATC
	9-R	ATTCCATGTGGGAATAATGATAAC
10	10-F	TCGTAACAAATCCCACAAAGCTC
	10-R	CAAGACCAATGTAATTAGTATACT
11	11-F	TAATGCCAACCATTAGCATTATC
	11-R	ACGACTCATCTTGGCATTTTCAG
12	12-F	CGCGATGAAGAGTCTTTGTAGTAT
	12-R	GGTTTGCTGAAGATGGCGGTATAT