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Short communication

Molecular characteristics of Tibetan antelope (*Pantholops hodgsonii*) mitochondrial DNA control region and phylogenetic inferences with related species

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Abstract

Although Tibetan antelope (*Pantholops hodgsonii*) is a distinctive wild species inhabiting the Tibet-Qinghai Plateau, its taxonomic classification within the Bovidae is still unclear and little molecular information has been reported to date. In this study of Tibetan antelope, the complete control regions of mtDNA were sequenced and compared to those of Tibetan sheep (*Ovis aries*) and goat (*Capra hircus*). The length of the control region in Tibetan antelope, sheep and goat is 1067, 1181/1106 and 1121 bp, respectively. A 75-bp repeat sequence was found near the 5′ end of the control region of Tibetan antelope and sheep, the repeat numbers of which were two in Tibetan antelope and three or four in sheep. Three major domain regions, including HVI, HVII and central domain, in Tibetan antelope, sheep and goat were outlined, as well as other less conserved blocks, such as CSB-1, CSB-2, ETAS-1 and ETAS-2. NJ cluster analysis of the three species revealed that Tibetan antelope was more closely related to Tibetan sheep than Tibetan goat. These results were further confirmed by phylogenetic analysis using the partial control region sequences of these and 13 other antelope species. Tibetan antelope is better assigned to the Caprinae rather than the *Antilopinae* subfamily of the Bovidae.

Keywords: Tibetan antelope; mtDNA control region; Sequence characteristics; Phylogenetic relationships

1. Introduction

The Chiru animals inhabiting the Qinghai-Tibet Plateau of China and the adjacent mountainous regions of India, Nepal are known as Tibetan antelope (*Pantholops hodgsoni*). Tibetan antelope, a valuable wild species, regarded as 'the numen of plateau' by local inhabitants, is well adapted to high altitudes (∼3300–5200 m above sea level), harsh living conditions and difficult grazing environment, which contribute to its distinctive characteristics from other antelope species. In traditional Chinese medicine, the horn of Tibetan antelope is an important ingredient in the treatment of many diseases. In addition, the wool of Tibetan antelope is used for the well-known "Shahtoosh" shawl in the Cashmere region of Pakistan and India.

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Over the last two decades, due to habitat loss, environmental deterioration and illegal hunting driven by the enormous profits from "Shahtoosh" production, the Tibetan antelope population has declined dramatically and is now a seriously endangered wild animal species. It has been reported that Tibetan antelope is already extinct in India and Nepal. Tibetan antelope is currently listed on Appendix I of the Convention on International Trade of Endangered Species of Wild Fauna and Flora agreement (CITES) and considered as EN grade by IUCN (The World Conservation Union) (IUCN, 1996).

Previous reports on Tibetan antelope focused mainly on morphology, physiology, population distribution, seasonal reproduction and immigration. In comparison, information on molecular characteristics of nucleic and extra-nucleic DNA sequences is scarce (Gatesy et al., 1997; Hassanin and Douzery, 1999; Lei et al., 2003). Furthermore, the taxonomic classification of Tibetan antelope is still very ambiguous (Gatesy et al., 1997; Hassanin and Douzery, 1999; Lei et al., 2003).

In this study, we present the first extensive analysis of the completed mtDNA control region sequences of Tibetan antelope via DNA sequencing techniques. By comparing the total control region sequences of Tibetan antelope, sheep and goat, characterization of control regions and the phylogenetic relationship of the three species have been clarified. In addition, the classification status of Tibetan antelope was elucidated using mtDNA control region sequences of other antelope species deposited in the GenBank.

2. Materials and methods

2.1. Sample collection and DNA extraction

DNA samples were extracted from seven dried skins of unrelated Tibetan antelopes collected from Kekexile National Nature Reserve using method described by Rao et al. (2001). Additionally, 27 sheep samples from two different geographic localities (North and East Tibet) and 26 goat samples from four localities (North, East, Southeast and Central Tibet) were collected. DNA was extracted from whole blood according to a modified phenol and chloroform method.

2.2. mtDNA control region sequencing

mtDNA control regions of Tibetan antelopes ($n = 7$), sheep ($n = 27$) and goat ($n = 26$) were sequenced. PCR primers were designed according to an entire *Capra* mtDNA sequence deposited in GenBank (accession number: AF533441). Primers sequences were as follows: L-Thr: 5′-CTCCCTAAGACTCAAGGAAGAAGC-3′; H-Phe: 5′-GCATTTTCAGTGCCTTGCTTTA-3′, which binds nt 15352 and 40 of the above *Capra* sequence, respectively. PCR was performed as follows: 95 °C for 4 min, followed by 33 cycles consisting of 35 sc at 95 °C, 35 s at 60 °C and 1 min at 72 °C, with a final extension at 72 °C for 10 min. PCR fragments were purified using a Promega DNA purification kit (Promega, WI, USA) and then sequenced commercially (BGI, China) using the sequencing primers of L-16070 5′-CACGAGCTTGTTGACCATGCCG-3′ and H-16221 5′-GCGATTTTAGATGAGATGGCC-3′, which are internal to the above PCR primers.

2.3. Statistical analysis

The complete mtDNA control region sequences of 60 individuals were checked, contiged and aligned with the DNAStar (version 5.01) software package. The major conserved domains were identified by aligning with reference sequences. The basic statistical estimators, including nucleotide compositions, ratio of transitions and transversions and haplotype analysis, were computed with MEGA2 software (Kumar et al., 2001). The neighbor-joining trees were constructed from Tajima–Nei distances among haplotypes of Tibetan antelope, sheep and goat, assuming $\alpha = 0.29$ for gamma distribution. Bootstraps of 1000 replicates were considered to test the robustness of the phylogenetic tree (Saitou and Nei, 1987).

To trace the primary phylogenetic relationship among antelope species, control region sequences of 13 antelope species were obtained in GenBank. According to Macdonald's (1984) taxonomy criteria, these 13 antelope species represent five subfamilies within the Bovidae family. The detail information is as follows: *Cephalophinae leucogaster* (GenBank accession number: AJ235317), *Cephalophinae*; *Tragelaphus strepsiceros* (AF301712), *Tragelaphus*; *Antilocapra americana* (AY786169), *Antilocapra*; *Gazella gazella* (AJ235320), *Procapra przewalskii* (DQ017349), *Neotragus moschatus* (AJ235323), *Antidorcas marsupialis* (AJ235313), *Antilope cervicapra* (AJ235315), *Antilopinae*; *Alcelaphus buselaphus* (AF300923), *Hippotragus equines* (AJ235321), *Hippotragus niger* (AJ235322), *Oryx dammah* (AJ235325), *Addax nasomaculatus* (AJ235310), *Hippotraginae*. The downloaded sequences of 13 antelope species were firstly aligned with those of Tibetan antelope, sheep and goat. A phylogenetic tree was created using the distance method of observed divergence with the DNAMAN program and 1000 bootstrap trials were used to evaluate the reliability of every branch of the phylogenetic tree.

3. Results

3.1. mtDNA control region characteristics

The sequencing results showed that the complete control region of Tibetan antelope, sheep and goat were 1067, 1181/1106 and 1121 bp, respectively. The sequences for Tibetan antelope were deposited in GenBank (accession numbers: EF583925-EF583931). The length difference for sheep resulted from variable tan-