The Complete Mitochondrial Genome and Phylogenetic Analysis of Chinese Jianchang Horse (*Equus caballus*)

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**Abstract**

The Jianchang horse (*Equus caballus*), a famous breed in the southwest regions of China, has excellent mountainous adaptation. The domestic Jianchang horse is popular in China, but information on its origin and evolution is still limited. In this study, we generate complete mitochondrial genome sequences of Jianchang horse. It is 16,614 bp in length, containing 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes and a non-coding control region (D-loop region). And it was found to be similar to other horse mitochondrial genomes. However, there were 105 nucleotide substitutions in the 13 protein-coding genes. The phylogenetic tree analysis was shown that Jianchang horse was significantly clustered as the clade with Debao and Yunnan horse. This genomic data provides useful information for further studies on the genetic diversity and origin of the Jianchang horse population.

**Keywords:** *Equus caballus*; Mitochondrial genome; Jianchang horse

**Introduction**

The Jianchang horse is a small horse breed that is popular in China, particularly in the southwest regions of China. The Jianchang horse breed is from the mountainous regions of Liangshan in the Sichuan Province, and has excellent mountainous adaptation when compared to other horses. It was domesticated to fulfill local people's needs for riding and transport across mountainous regions over 1000 years ago.

Jianchang horses are well known to be quiet, hardy, powerful, and not vulnerable to environmental stress, these attribute to work effortlessly in the mountainous regions of China. As shown in Figure 1, it is typically small with an average height of 110 cm to 120 cm and a weight of 180 kg to 250 kg. It has small ears and head, long and dense bristle, and caulal seta and mane. It has a sloped croup, slender but well-developed legs, and hard hooves. Its hair is mainly in the bay color, but other colors like black appear occasionally [1].

The sequence diversity of the mtDNA control region has been used for analyzing the origin and diversification of domestic horses [2-4]. It is widely believed that horses were domesticated from one or several ancestral horse populations [5-7]. However, information on its origin and evolution is still limited. In this study, we generate complete mitochondrial genome sequence of Jianchang horse and the complete mtDNA mitochondrial genome was used for phylogenetical analysis. The diversity and origin of the domestic horses in China by mtDNA D-loop have been reported [8]. However, the mtDNA D-loop sequence was not fully show the diversity and origin of the Jianchang horse breeds in the southwest regions of China. It is the first time that molecular evidences were provided for the origin of the Jianchang horse population.

**Materials and Methods**

**Blood sample and DNA isolation**

Blood samples were collected from Jianchang horses, and stored at -70°C. Genomic DNA was extracted from 0.2 mL of whole blood with a DNA extraction kit (Tiangen, Beijing, China).

**PCR amplification and sequencing**

Two microliters DNA was amplified in 30 or 35 cycles with specific primer pairs (Table 1) using the long and accurate DNA polymerase (PrimeSTAR Max DNA Polymerase, TaKaRa, China). The primers were designed based on the Equus caballus (Accession No. X79547). PCR cycling conditions were as follows: 95°C initial denaturation for 4 min, 35 cycles of 95°C denaturation for 40 s, 60°C annealing for 40 s, and 72°C extension for 90 s. A final extension was performed at 72°C for 7 min. The PCR products were separated by electrophoresis in 2.0% agarose gel, and purified using a Gel Extraction Kit (Sangon, Shanghai, China). The purified products were subcloned into the pMD-18T vector (Takara, Japan) and sequenced by Beijing AuGCT Biotechnology Company. SeqMan software (DNASTAR Inc., USA) were employed to assemble a continuous sequence. DOGMA (http://dogma.cbb.utexas.edu/) was used for annotating ihanchang horse mitochondrial genome. tRNA genes were defined with tRNAscan-SE 1.2 (http://lowelab.ucsc.edu/tRNAscan-SE/).

**Phylogenetic analysis**

The alignment of the nucleotide sequences of 11 mtDNA control regions of horses was performed with ClustalW (http://www.ebi.ac.uk/)

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clustalw/) using default settings. The phylogenetic tree was constructed using two methods: Maximum likelihood (ML) and Maximum parsimony (MP). ML analysis was performed with MEGA5 (http://www.megasoftware.net/), based on mtDNA control regions of horses [9]. MP analysis was conducted using PAUP 4.0 Beta 10 program (http://www.megasoftware.net/), with indels treated as missing character states. For the MP analysis, we performed using a heuristic search with the tree bisection and reconnection (TBR), and branch swapping algorithm. ML analysis was based on General Time Reversible model (GTR). The reliability of the resulting MP and ML tree topologies was tested using bootstrap analyses through 1 000 replicates for MP and 100 for ML.

**Results and Discussion**

**Characters of Jianchang horse’s mitochondrial genome**

The complete mtDNA sequence of the Jianchang horse is composed of 16,614 bp, with 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes and one D-loop region (Table 2). Table 1: The primers of PCR to amplify mitochondrial genome of Jian chang horses. The length of mitochondrial genome of Jianchang horse were shorter than that from Swedish horse (16,660 bp), and longer than that from Naqu Tibetan horse (16592 bp) [12]. There are variable numbers of 8 bp repeat fragments (ACCTGTGC) in control region among the breeds.

All protein-coding genes were found to be H-strand encoded, whereas ND6 was L-strand encoded. The initiation codons for ND2 and ND3 started with TAA, while the other genes had ATG start codon. There are four types of termination codon. The ND1 and ND2 genes are incomplete termination codon of T, Cyt b ends with AGA, and the rest have a termination codon of TAA. In addition, compared with the Swedish horse mitochondrial genome (X79547), there are 105 nucleotide substitutions in positions of Jianchang horses and other horses based on 420 bp D-loop sequences, which were retrieved from GenBank databases, including Jianchang (KT998647), Cheju (AF014406), Yunnan horse (AF014416), Mongolian horse (AF014413), Zhongdian horse (EF597512), Naqu horse (EF597513), Deqin horse (EF597514), Sanhe horse (DQ297635), Wuzhumuqin horse (DQ297637), Debao horse (FJ392562.1), Xinjie horse (DQ297638) and Przewalskii horse (AF014409) (Figure 2).

**Phylogenetic analysis**

Phylogenetic tree analysis was employed to find the phylogenetic positions of Jianchang horses and other horses based on 420 bp D-loop sequences, which were retrieved from GenBank databases, including Jianchang (KT998647), Cheju (AF014406), Yunnan horse (AF014416), Mongolian horse (AF014413), Zhongdian horse (EF597512), Naqu horse (EF597513), Deqin horse (EF597514), Sanhe horse (DQ297635), Wuzhumuqin horse (DQ297637), Debao horse (FJ392562.1), Xinjie horse (DQ297638) and Przewalskii horse (AF014409) (Figure 2).
The different horse breeds were classified into three main clusters according to the phylogenetic clades (Figure 3). Obviously, Jianchang horse was significantly clustered as the clade with Debao and Yunnan horse. In addition, the Cheju and Deqin horse was clustered as another clade with Przewalskii and Zhongdian horse. Mongolian and Sanhe horse belongs to a cluster. Wuzhumuqin horse was clustered together with Xinihe and Naqu horse. Based on the mtDNA control region, cytochrome b diversity and microsatellite markers, previous studies have shown that Chinese horses have multiple maternal origins and high genetic diversity [13-15]. The Chinese breeds could be divided into five major groups by genotyping these animals for 27 microsatellite loci [13]. Previous study had also shown that there were two haplotypes of Y chromosome discovered in the domestic horse breeds in China [16], which develop into an important tool for horse population genetics[17].

Conclusions

In summary, we have sequenced the complete mtDNA sequence of the Jianchang horse. It has a typical mitogenome structure, containing 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes and a non-coding control region (D-loop region). Data presented in our study provide a structural basis for future studies on mitogenome function in Jianchang horse. The phylogenetic tree analysis, therefore, will also contribute to the understanding the genetic diversity and origin of the Jianchang horse population in the future.

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