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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 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 caudal 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.

Figure 1: The physical characteristics of Jianchang horse.

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.ccbb.utexas.edu/) was used for annotating Jianchang 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://paup.csit.fsu.edu/about.html), 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), and has been deposited in GenBank (KT998647). The nucleotide composition (32.2% A, 28.5% C, 13.4% G, 25.9% T) is biased towards A-T content (58.1%), which is consistent with other horse breeds mitochondrial genomes [5,10,11]. 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 ATA, while the other genes had ATG start codon. There are four types of termination codon. The ND1 and ND2 genes are terminated with AGA. The COX3, ND3 and ND4 genes end with an 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 the 13 protein-coding genes of the Jianchang horse.

Table 1: The primers of PCR to amplify mitochondrial genome of Jian chang horses.

Primer name	Primer sequence (5′–3′)	Tm (°C)
JCHM1F JCHM1R	GTTAATGTAGCTTAATAATAT CCTTGTTACGACTTGTCT	62
JCHM2F JCHM2R	GCTTAATTGAATCAGGCCATG GGAACAAGTGATTATGCTACC	58
JCHM3F JCHM3R	TCACCTCTAGCATTTCCAGT GATGATGTTAGTGGTTGTAGTG	61
JCHM4F JCHM4R	CTATGGCCTACTACAACCTA TGGTGGAGGCTTCTATGGTT	60
JCHM5F JCHM5R	CTACTAGCCATTATCCCCAT CAGTCCTTAGTATGCAGGAA	60
JCHM6F JCHM6R	GGTTAACATCCCAGACCAAGA GTCTGTGAGAAGCATGGTAAT	60
JCHM7F JCHM7R	GCAGTACTCCTTCTCCTAG GTCTTGGAATCCTAGTTGGA	61
JCHM8F JCHM8R	CCACTATGTCTTTCTCCATC GCGATTGTTGATTAGTCGGT	58
JCHM9F JCHM9R	CATGTTTCCCAGCATCCTATT TTGATCCGTATACTCCATCTG	60
JCHM10F JCHM10R	CGTATACTTCACCCTTCTCC GGATAGGCTGATAAGGCTGA	57
JCHM11F JCHM11R	CTGAATCAACACTACAACC ACGAATAGCTCTCCAATTAG	56
JCHM12F JCHM12R	GCAGCCTGATGACTATTAG CGAGTATGAAGATTTGTTG	54
JCHM13F JCHM13R	GGCCATAGCCTGATTCCTA GAGTCTAGTAGAAGTGATGC	58
JCHM14F JCHM14R	CCAACAATTATACACCGACT CATGTCTCTAGGAATGTGTA	60
JCHM15F JCHM15R	CCTCTTCATTCACGTAGGA CATGTCAGGTGGGTATAGT	55
JCHM16F JCHM16R	CCTCATGTGCTATGTCAGT GGATGCCTGTCTATGGAAA	60

Gene	Start position	Stop position	Start codon	Stop codon	Strand
tRNA-Phe	1	70			H
12S rRNA	71	1045			H
tRNA-Val	1047	1113			H
16S rRNA	1114	2691			H
tRNA-Leu	2692	2766			H
ND1	2769	3725	ATG	TAG	H
tRNA-Ile	3725	3793			H
tRNA-Gln	3791	3863			L
tRNA-Met	3866	3934			H
ND2	3935	4975	ATA	TAG	H
tRNA-Trp	4974	5043			H
tRNA-Ala	5049	5117			L
tRNA-Asn	5119	5191			L
tRNA-Cys	5224	5289			L
tRNA-Tyr	5290	5356			L
COX1	5358	6902	ATG	TAA	H
tRNA-Phe	6900	6968			L
tRNA-Asp	6977	7043			H
COX2	7044	7727	ATG	TAA	H
tRNA-Leu	7731	7798			H
ATP8	7800	8003	ATG	TAA	H
ATP6	7961	8641	ATG	TAA	H
COX3	8641	9423	ATG	T++	H
tRNA-Gly	9425	9493			H
ND3	9494	9838	ATA	T++	H
tRNA-Arg	9841	9909			H
ND4L	9911	10207	ATG	TAA	H
ND4	10201	11577	ATG	T++	H
tRNA-His	11579	11647			H
tRNA-Ser	11648	11707			H
tRNA-Leu	11709	11778			H
ND5	11785	13599	ATG	TAA	H
ND6	13586	14110	ATG	TAA	L
tRNA-Glu	14111	14179			L
CYTB	14184	15323	ATG	AGA	H
tRNA-Thr	15324	15395			H
tRNA-Pro	15397	15462			L
Control region	15463	16614			H

Table 2: List of genes encoded by the Jian chang horse mitochondrial genome.

Phylogeny 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), Xinihe horse (DQ297638) and Przewalskii horse (AF011409) (Figure 2).

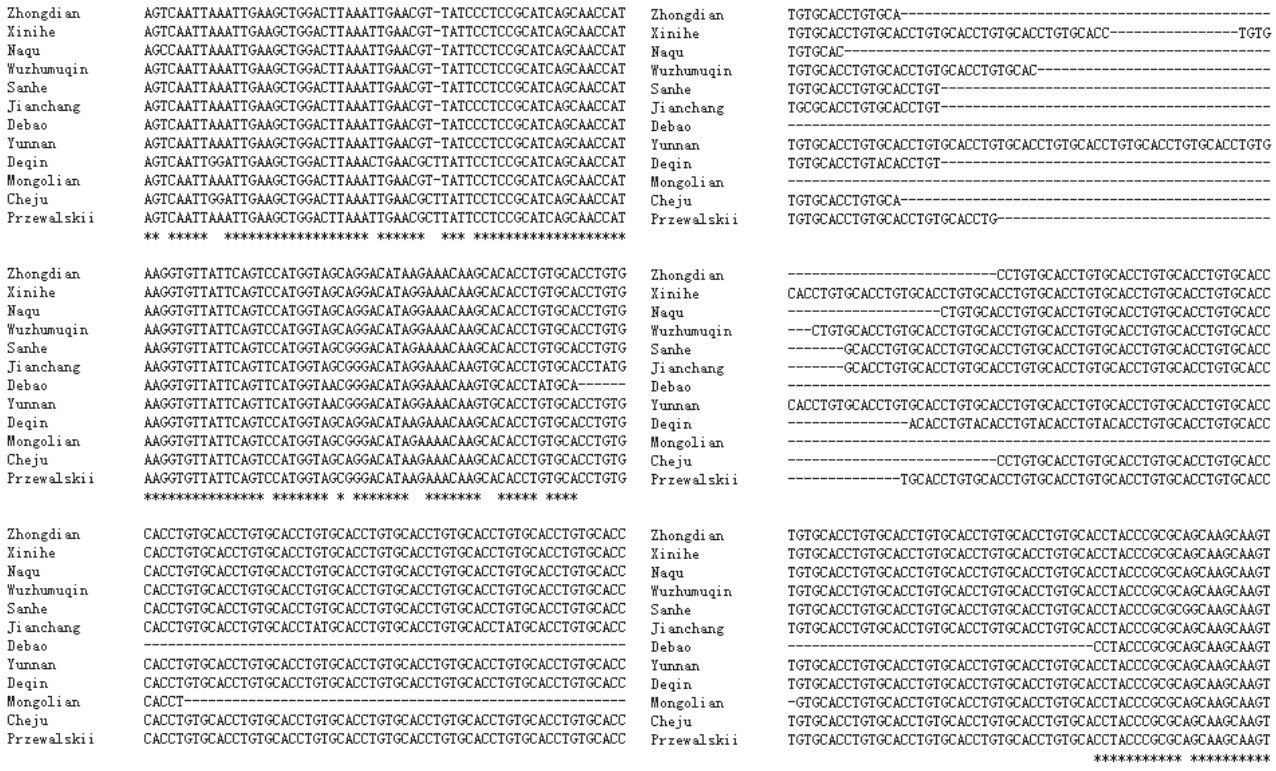

Figure 2: Multiple sequence alignments of mtDNA D-loop of twelve Chinese horse breeds. There are variable numbers of 8 bp repeat fragments (ACCTGTGC) in control region among the breeds.

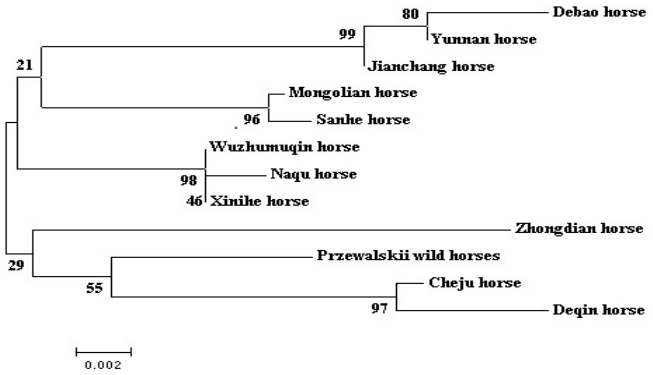

Figure 3: The phylogenetic tree of the Jianchang horses and other 11 horses.

The phylogenetic tree of 11 horses includes that from Jianchang, Mongolian, Cheju, Yunnan, Zhongdian horse, Naqu horse, Deqin horse, Sanhe horse, Wuzhumuqin horse, Xinihe horse and Przewalskii horse based on D-loop sequence.

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 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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