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Short Communication

Mitogenome Characterization and Diversity of the Nangqian Grey Yak *(Bos grunniens)*

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ABSTRACT

Nangqian grey yak *(Bos grunniens)* is a unique yak population in Qinghai Province, China. In this study, the whole mitogenome sequences of 18 Nangqian grey yaks were sequenced based on the next-generation sequencing (NGS) technology and annotated. The total length of whole mitogenome sequence is between 16.323 bp and 16.325 bp, including a non-coding control region (D-loop region), 22 tRNA genes, 13 protein-coding genes and two rRNA genes (12*S rRNA* and 16*S rRNA*). Maternal genetic diversity based on the mitogenome variations **was** analyzed. A total of 12 haplotypes were identified among 18 complete mitogenome sequences, the haplotype diversity and nucleotide diversity of Nangqian grey yak were 0.948±0.033 and 0.001±0.001, respectively. Compared with the wild yak population and six other domestic yak breeds/populations in China, the haplotype diversity of Nangqian grey yak population was higher, indicating abundant maternal genetic diversity in Nangqian grey yak. The phylogenetic tree showed that Nangqian grey yak was most closely related to Tibet alpine, Xueduo, Changtai, Sibu, Zhongdian, Tianzhu white, Ashdan, Jinchuan, Jiulong, Pamir, Pali, Qinghai plateau, Huanhu, Datong, Bazhou and wild yak breeds/populations, closer to Chawula, Muli, Gannan, Niangya and Yushu yak breeds, but far away from other yak breeds (i.e. Leiwuqi and Maiwa yak).

Keywords: Grey yak, Mitogenome, Annotation, Genetic diversity

Introduction

Yak *(Bos grunniens)* is mostly found on the Qinghai-Tibetan Plateau (QTP) and nearby alpine and subalpine regions at heights ranging from 3.000 to 6.000 meters above sea level [1]. There are now 23 domestic yak breeds that are officially recognized, comprising 21 indigenous breeds and two improved breeds that are kept in China [2]. Qinghai Province of China is home to four indigenous breeds (Yushu, Qinghai-Plateau, Huanhu, and Xueduo) as well as two developed breeds (Datong and Ashdan) [2]. With the exception of the Tianzhu white yak breed, which is white, the majority of these yak breeds are black or black blown [3]. Additionally, a small number of yaks in various breeds or populations have golden, grey, or mixed coat colors [4]. It should be noted that certain individuals with the same coat color have been bred artificially over an extended period of time to form herds, such as the Nangqian grey yak [5]. Nangqian County is situated in the southern part of the Yushu Tibetan Autonomous Prefecture, Qinghai Province, China, at an average elevation of more than 4.000 meters. One of the key businesses in this County is the yak industry, and there are more than 200.000 yaks in total. The Nangqian grey yak *(Bos grunniens)* has a high degree of adaptation to resist extreme conditions such as high altitude, acute cold, and strong UV radiation. Mitochondrial DNA (mtDNA) is a circular DNA molecule that is self-replicating, non-recombinogenic, and maternal inheritance, which is characterized by rapid evolution, simple structure and straightforward sequencing [6]. It is frequently employed as an excellent molecular marker in research on the

Fig 1. Nangqian grey yak

taxonomy, phylogeny, and genetic diversity of mammals [7]. The maternal genetic diversities of wild yak and some Chinese domestic yak breeds/populations (e.g. Qilian, Pamir, Qinghai-Plateau, Huanhu, Xueduo, and Yushu yak) were recently also comprehensively analyzed based on nucleotide variations of the complete mitogenome [8-11]. According to these previously reported findings, both domestic yak breeds/populations and wild yak exhibited higher maternal genetic variations, which were clustered into three lineages with three possible maternal origins [8-11]. In this study, we sequenced and annotated the whole mitogenome sequences of 18 Nangqian grey yaks and investigated its characterization, maternal genetic diversity and phylogeny, which would be helpful for the future conservation and molecular breeding of this rare yak genetic resource.

Materials and Methods

Ethical Approval

All experiments in this study were performed following requirements of animal welfare and were based on the recommendations of the Regulations for the Administration of Affairs Concerning Experimental Animals of China. Animal experiments were approved by the Institutional Animal Care and Use Committee of the Academy of Animal Science and Veterinary Medicine, Qinghai University.

Sample Collection

Here, the blood samples of 18 Nangqian grey yaks *(Bos grunniens)* were collected in Nangqian County of Yushu Tibetan Autonomous Prefecture, Qinghai Province, China (96°28′44”N, 32°12′26”E) *(Fig. 1)*. To ensure that the representative of yak samples, all experimental animal individuals are collected through the way of asking herdsmen, consulting the pedigree records and random sampling. The voucher specimen (Sample No.: NQ202201-202218; Approval date:20220822) was kept in the Plateau Livestock Genetic Resources Protection and Innovative Utilization Key Laboratory of Qinghai Province (Xining, Qinghai, China).

Mitochondrial Genome Extraction and Statistical Analysis

DNA Extraction Kit (Aidlab Biotechnologies Co., Ltd, China) was used to extract genomic DNA. All the data of Nangqian grey yak were obtained on the Illumina NOVA 6.000 platform with 2×150 bp paired-end reads. Then the read pairs were aligned to the yak reference mitogenome (Accession number: NC_006380) using the Burrows-Wheeler Aligner (BWA) v0.7.15, which were subsequently converted to BAM files using samtools v0.1.19 software. Indel realignment and recalibration were performed with the Genome Analysis Toolkit (GATK v3.8) [12]. Mitogenome characterization of Nangqian grey yak was identified using comparative genome approach. Furthermore, Dnasp5.10 [13] and Arlequin3.11 [14] were used to detect the polymorphic sites, the number or type, type of haplotype/haplogroups, haplotype diversity and nucleotide diversity. Taking American bison (*Bison bison*) as an outgroup, a phylogenetic tree was constructed using neighbor-joining (NJ) method in Mega 5.0 software [15] (Kimura 2-parameter model, 1000 replicates) to explore the phylogenetic relationship between Nangqian grey yak and other 23 yak breeds/populations.

Results

The annotated mitogenome haplotype sequences of Nangqian grey yak were submitted to GenBank with the accession number OP598192, OR085996~OR086006. The length of the mitogenomes was 16.323 bp~16.325 bp and it composed of noncoding control region *(D-loop)*, two rRNA subunit genes (12*S rRNA* and 16*S rRNA*), 22 tRNA genes and 13 protein coding genes *(Fig. 2, Table 1)*. Noncoding control region *(D-loop)* was located between the *tRNA*^{Pro} and *tRNA*^{Phe}, which was the control region for the mitochondrial genome transcription and replication. The two rRNA genes were between 957 bp (12*S rRNA)*

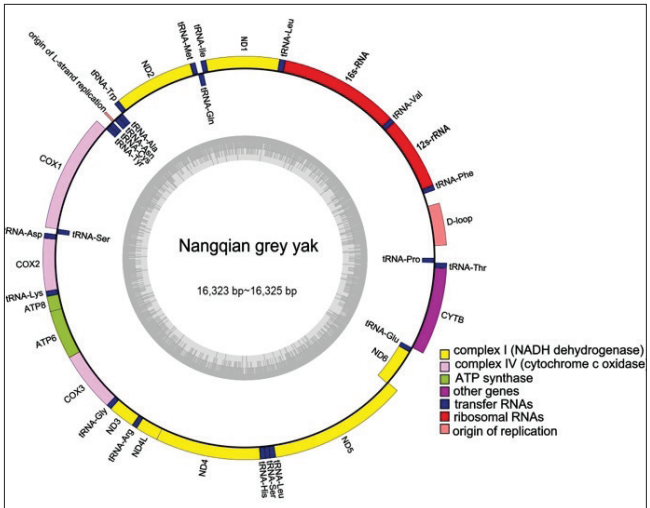

Fig 2. Pattern map of mitogenome of Nangqian grey yak

Table 1. *Structural characteristics of mitogenomes of Nanngqian grey yak (Bos grunniens)*

Gene	Position		Size(bp)	Start Codon	Stop Codon	Strand
	From	To				
D-loop	166~168	717~718	551~552			H
tRNA^{phe}	894~896	960~962	67			H
12S rRNA	961~963	1917~1919	957			H
tRNA^{Val}	1918~1920	1984~1986	67			H
16S rRNA	1985~1987	3554~3556	1570			H
tRNA^{Leu}	3556~3558	3630~3632	75			H
ND1	3633~3635	4589~4591	957	ATG	TAA	H
tRNA^{Ile}	4589~4591	4657~4659	69			H
tRNA^{Gln}	4655~4657	4726~4728	72			L
tRNA^{Met}	4729~4731	4797~4799	69			H
ND2	4798~4800	5841~5843	1044	ATA	TAG	H
tRNA^{Trp}	5840~5842	5906~5908	67			H
tRNA^{Ala}	5908~5910	5976~5978	69			L
tRNA^{Asn}	5978~5980	6051~6053	74			L
OL	6054~6056	6084~6086	31			H
tRNA^{Cys}	6084~6084	6150~6152	67			L
tRNA^{Tyr}	6151~6153	6218~6220	68			L
COX1	6220~62222	7764~7766	1545	ATG	TAA	H
tRNA^{Ser}	7762~7764	7830~7832	69			L
tRNA^{Asp}	7838~7840	7905~7907	68			H
COX2	7907~7909	8590~8592	684	ATG	TAA	H
tRNA^{Lys}	8594~8590	8660~8662	67			H
ATP8	8662~8664	8862~8864	201	ATG	TAA	H
ATP6	8823~8825	9503~9505	681	ATG	TAA	H
COX3	9503~9505	10287~10289	785	ATG	TAA	H
tRNA^{Gly}	10287~10289	10355~10357	69			H
ND3	10365~10367	10712~10714	348	ATA	TAG	H
tRNA^{Arg}	10703~10705	10771~10773	69			H
ND4L	10772~10774	11068~11070	297	ATG	TAA	H
ND4	11062~11064	12439~12441	1378	ATG	TAA	H
tRNA^{His}	12440~12442	12509~12511	70			H
tRNA^{Ser}	12510~12512	12569~12571	60			H
tRNA^{Leu}	12571~12573	12640~12642	70			H
ND5	12632~12634	14461~14463	1830	ATA	TAA	H
ND6	14445~14447	14972~14974	528	ATG	TAA	L
tRNA^{Glu}	14973~14975	15041~15043	69			L
Cytb	15046~15048	16185~16187	1140	ATG	AGA	H
tRNA^{Thr}	16189~16191	16258~16260	70			H
tRNA^{Pro}	16258~16260	16323~16325	66			L

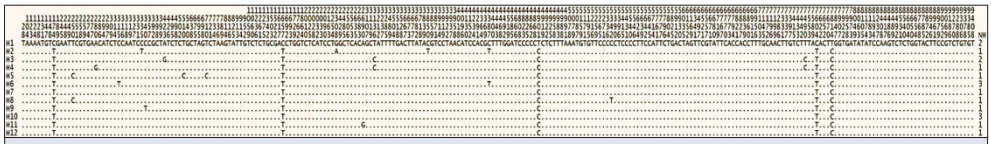

Fig 3. Frequencies of 12 haplotypes based on mitogenome sequence variations of Nangqian grey yak. NH on the right represents the number of individuals (sequences) that share each haplotype

Table 2. Comparison of genetic diversity indexes among Nangqian grey yak and other six domestic or wild yak breeds/populations in China

Breed/Population	Number of Sequences	Haplotype Diversity	Nucleotide Diversity	Reference
Huanhu yak	21~39	0.905~0.939	0.002	Wang XD et al.[8] Li GZ et al. [9]
Xueduo yak	23~30	0.992~0.989	0.002	Wang XD et al. [8] Li GZ et al. [9]
Yushu yak	20~32	0.963~0.976	0.003	Wang XD et al. [8] Li GZ et al. [9]
Qinghai Plateau yak	39	0.973	0.003	Li GZ et al. [9]
Qilian yak	22	0.948	0.003	Wang XD et al. [8]
Pamir yak	25	0.990	0.003	Wang XD et al. [8]
Wild yak	21~24	0.991~0.993	0.003~0.004	Li GZ et al. [9] Wang ZF et al. [10] Ma ZJ et al. [11]
Nangqian grey yak	18	0.948	0.001	This study

and 1.570 bp (16*S rRNA)* in length and were separated by *tRNA*^{Val}. 13 protein-coding genes ranged from 201 bp *(ATP8)* to 1.830 bp *(ND5),* and 22 tRNA genes ranged from 60 bp *(tRNA*^{ser}*)* to 75 bp *(tRNA*^{Leu}*)*. The nucleotide composition of mitogenomes was. A: 33.71%, T: 27.27%, C: 25.81%, G: 13.21%. The A+T content was 60.98% and the G+C content was 39.02%, showing a clear bias in nucleotide composition. All of the mitochondrial genes in Nangqian grey yak are encoded in the heavy chain except for eight tRNAs *(Gln*, *Ala*, *Asn, Cys*, *Tyr*, *Ser*, *Glu* and *Pro)* and *ND6* genes in the light chain. Duplication of ATPase genes appears to be common in the mitochondrial genomes of most vertebrates [16,17]. Here, there are 4 overlaps in the 13 protein-coding genes, respectively. For instance, *ATP6* and *ATP8* overlap by 40bp, *COX3* and *ATP6* overlap by 1bp, *ND4* and *ND4L* overlap by 7bp, *ND6* and *ND5* overlap by 17bp in the Nangqian grey yak mitogenome. Among the protein-coding genes, ATA is the starting codon of *ND2*, *ND3* and *ND5*, and ATG is the starting codon of *ND1*, *COX1*, *COX2*, *COX3*, *ATP6*, *ATP8*, *ND4*, *ND4L*, *ND6* and *Cytb*. Three complete stop codons are labeled, i.e. TAG *(ND2 and ND3),* AGA (*Cytb*) and TAA (*ND1*, *COX1*, *COX2*, *COX3*, *ATP8*, *ATP6*, *ND4*, *ND4L*, *ND5* and *ND6*) *(Fig. 2, Table 1).*

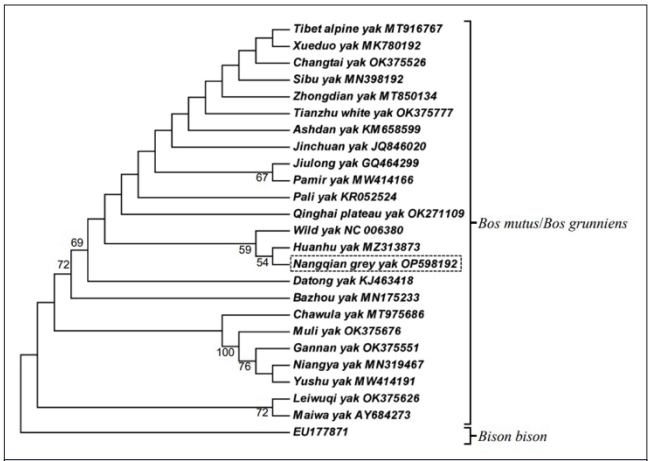

Fig 4. Phylogenetic relationship between Nangqian grey yak and 23 other yak breeds/populations in China based on mitogenome sequence variations. The support values (>50) next to the nodes are based on 1000 bootstrap replicates

After excluding four InDel sites, a total of 29 polymorphic sites were detected in the 18 mitogenomes alignment analysis, including 12 single polymorphic sites and 17 parsimony information sites. Totally, 12 haplotypes were identified in this study *(Fig. 3),* with the haplotype diversity and nucleotide diversity of Nangqian grey yak were 0.948±0.033 and 0.001±0.001, respectively *(Table 2).* The phylogenetic tree showed that Nangqian grey yak was most closely related to Tibet alpine, Xueduo, Changtai, Sibu, Zhongdian, Tianzhu white, Ashdan, Jinchuan, Jiulong, Pamir, Pali, Qinghai plateau, Huanhu, Datong, Bazhou and wild yak breeds/populations, closer to Chawula, Muli, Gannan, Niangya and Yushu. However, distant genetic relationships were found between Nangqian grey yak and the rest of domestic yak breeds (i.e. Leiwuqi and Maiwa yak) *(Fig. 4).*

Discussion

In this study, the mitogenome characterization and

maternal genetic diversity of Nangqian grey yak were analyzed for the first time. Our preliminary analysis showed that the mitogenome of Nangqian grey yak composed of noncoding control region (D-loop), two rRNA subunit genes, 22 tRNA genes and 13 protein coding genes, which indicated that the gene composition, structure and arrangement of Nangqian grey yak mitogenome are similar to that of most of other mammals [18-23].

Haplotype diversity was served as one of important indicators of maternal genetic diversity in animal populations. Compared with the genetic diversity index of the reported wild yak and six Chinese domestic yak breeds/populations [8-11], the Nangqian grey yak had a higher haplotype diversity, indicating rich maternal genetic diversity *(Table 2)*. In this study, the phylogenetic tree showed that Nangqian grey yak exhibited a mostly close genetic relationship with a majority of the yak breeds/populations (i.e. Tibet alpine, Xueduo, Changtai, Sibu, Zhongdian, Tianzhu white, Ashdan, Jinchuan, Jiulong, Pamir, Pali, Qinghai plateau, Huanhu, Datong, Bazhou and wild yak), closer relationship with the Chawula, Muli, Gannan, Niangya and Yushu breeds, but far away from other a few of yak breeds (i.e. Leiwuqi and Maiwa yak). To certain extent, the above result basically showed the clustering relationship and differentiation degree among them. However, to further thoroughly elucidate the genetic differences between Nangqian grey yak and other yak breeds/populations, a further extensive study of yak at whole-genome level is warranted in the future.

To sum up, the mitogenome of Nangqian grey yak was composed of noncoding control region (D-loop), two rRNA subunit genes, 22 tRNA genes and 13 protein coding genes. It owned rich maternal genetic diversity. Nangqian grey yak was most closely related to Tibet alpine, Xueduo, Changtai, Sibu, Zhongdian, Tianzhu white, Ashdan, Jinchuan, Jiulong, Pamir, Pali, Qinghai plateau, Huanhu, Datong, Bazhou and wild yak breeds/populations, closer to Chawula, Muli, Gannan, Niangya and Yushu, but far away from other yak breeds (i.e. Leiwuqi and Maiwa yak).

Availability of Data and Materials

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, accession number OP598192, OR085996-OR086006.

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Competing Interests

All authors reported no potential conflicts of interest.

Author Contribution Statement

ZJ participated in the conception and design; CP, MY performed the experiment and involved in data analysis; CP drafted the original manuscript; ZJ helped to revise the manuscript; CP, YH, LJ and ZJ carried out sampling; all authors critically reviewed and approved the final manuscript.

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