Short Communication

Whole Mitogenome of Golmud Yak *(Bos grunniens):* Sequencing, Characterization and Phylogenetic Analysis

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Abstract: Golmud yak *(Bos grunniens)* is mainly lived in the southern Qaidam Basin in Qinghai Province, China. Here, the complete mitogenome of the Golmud yak was firstly sequenced using Illumina high-throughput sequencing technique and the corresponding sequence characterization also has been identified. The mitogenome of Golmud yak was 16,324 bp length with an A + T-biased nucleotide composition (60.97%). It includes 22 tRNA genes, 13 protein-coding genes, 2 rRNA genes and a control region (D-loop region), which is consistent with that of other bovine species. The phylogenetic tree showed that Golmud yak was most closely related to Sibu, Huanhu, Zhongdian, Ashdan, Jiulong, Pali, Datong and Bazhou yak breeds, closer to Yushu, Niangya, Qinghai-Gaoyuan, Xueduo, Maiwa and wild yak, but far away from other yak breeds (i.e. Jinchuan, Gannan and Tianzhu white yak). The complete mitogenome sequence reported here would provide valuable information for breeding and improvement of Golmud yak.

Keywords: *Bos grunniens, Mitogenome, Annotation, Phylogeny*

Golmud Yak'ının *(Bos grunniens)* Tam Mitogenomu: Sekans, Karakterizasyon ve Filogenetik Analiz

Öz: Golmud yakı *(Bos grunniens),* çoğunlukla Çin'in Qinghai Eyaletindeki güney Qaidam Havzasında yaşar. Burada, Golmud yakının tam mitogenomu ilk olarak Illumina yüksek verimli sekans tekniği kullanılarak sekanslandı ve karşılık gelen sekans karakterizasyonu gerçekleştirildi. Golmud yakının mitogenomunun uzunluğu %60.97 A + T-taraflı nükleotit bileşimi ile 16.324 bp idi. 22 tRNA geni, 13 protein kodlayan gen, 2 rRNA geni ve diğer sığır türleriyle tutarlı olan bir kontrol bölgesi (D-loop bölgesi) içeriyordu. Filogenetik ağaç, Golmud yakının en yakın olarak Sibu, Huanhu, Zhongdian, Ashdan, Jiulong, Pali, Datong ve Bazhou yak ırkları ile ilişkili olduğunu gösterdi. Bu yaklar, Yushu, Niangya, Qinghai-Gaoyuan, Xueduo, Maiwa and yabani yaklara yakın iken, diğer yak ırklarından (örneğin, Jinchuan, Gannan ve Tianzhu beyaz yakı) oldukça uzaktı. Burada bildirilen tam mitogenom sekansı, Golmud yakının yetiştirilmesi ve geliştirilmesi için değerli bilgiler sağlayacaktır.

Anahtar sözcükler: *Bos grunniens, Mitogenom, Notlama, Filogeni*

INTRODUCTION

Yak, an ungulate, provides meat, milk and other necessities for local inhabitants at high altitude area on the Qinghai-Tibetan Plateau (QTP) and other adjacent alpine and subalpine areas [1]. China owns abundant yak genetic resources. At present, there are 22 officially recognized yak breeds (Qinghai-Gaoyuan, Huanhu, Xueduo, Yushu, Niangya, Sibu, Pali, Leiwuqi, Tibet-Gaoshan, Tianzhu, Gannan, Bazhou, Zhongdian, Jiulong, Maiwa, Changtai, Jinchuan, Muli, Pamier, Chawula, Datong and Ashdan) in China. In the paternal genetic study of Qinghai yak, the researcher explored the Y chromosome haplotype diversities and population genetic structure of nine Qinghai yak breeds/populations (Tanggulashan, Tianjun, Qumalai, Qilian, Guoleimude (Golmud), Ganglong, Xueduo,
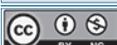
Huanhu and Datong) and showed that Qinghai Province might be one of the domestication or origin places of yak, in which Golmud yak *(Bos grunniens)* was first described [1]. Golmud yak has excellent properties of meat, milk and reproduction, which is mainly produced for meat in Haixi Mongol and Tibetan autonomous Prefecture of Qinghai province, China. Again, it also owns good adaptability to the cold ecological environment and arid mountainous areas. Recently, based on nucleotide variations of mtDNA, the maternal genetic diversities of Qinghai yak breeds (Qinghai-Gaoyuan, Huanhu, Xueduo and Yushu yak) were also systematically studied. It indicated that Qinghai indigenous yak breeds have rich maternal genetic diversity. And the genetic differentiation between Qinghai indigenous yak breeds was weak, but each Qinghai indigenous yak breed had unique maternal genetic information [2].

Mitochondrial genome (mitogenome) is a kind of circular DNA molecule with fast evolution rate, matriarchal inheritance and rich polymorphism. Therefore, it is considered as an ideal tool in studies on animal matriarchal genetic diversity, population structure and classification [2-7]. At present, D-loop, *Cytb* and mitogenome are widely used in the molecular phylogenetic studies of bovine species [2-8]. In our previous study, based on the D-loop genetic variation, our results showed that the Golmud yak population had rich maternal genetic diversity and owned three maternal lineages [8]. For these reasons, we based on the whole mitogenome sequence genetic variation in-depth study of Golmud yak. Here, we obtained the complete mitogenome sequence of Golmud yak and analyzed its characterization and phylogeny with other yak breeds, which could provide valuable information for breeding and improvement of this population.

Material and Methods

Ethical Statement

This study was conducted according to the guidelines of the Council of China and animal welfare requirements. Based on the recommendations of the Regulations for the Administration of Affairs Concerning Experimental Animals of China and the Institutional Animal Care and Use Committee of Qinghai Province approved all animal experiments.

Sample Collection and Mitochondrial Genome Extraction

The blood sample of one Golmud yak *(Bos grunniens)* was collected in Guoleimude town of Golmud city, Qinghai, China (36°24'51"N, 94°53'42"E). The genomic DNA was extracted using DNA Extraction Kit (Aidlab Biotechnologies Co., Ltd, China) and stored at -20°C for later use. The complete genome of Golmud yak was sequenced using Illumina Nova 6000 platform (Beijing Nuohe Zhiyuan

Technology Co., Ltd, China) with sequencing depth of 22.9x. The reads were aligned to the wild yak mitochondrial reference genome (Accession number: NC_006380) using the Burrows-Wheeler Aligner v0.7.15, which were subsequently converted to BAM files. To improve alignment into the circularized genome, the 30 bp of sequence from the end of the mtDNA was attached to the beginning. InDel realignment was performed using the Genome Analysis ToolKit (GATK v3.8) [9], determining the circular mitogenome.

Mitogenome Map Construction, Phylogenetic Tree and Upload Sequence

Mitogenome map of Golmud yak was constructed using OGDRAW v1.3.1 software with default [10]. The corresponding mitogenome sequences of wild yak *(B. mutus)*, 16 other domestic yak breeds *(B. grunniens)* and American bison *(Bison bison)* (Acc. no.: NC_12346) in GenBank were used to compare the mitogenome sequence of Golmud yak with Bioedit 7.2.5 software and refined manually. Taking American bison *(Bison bison)* as an outgroup, a phylogenetic tree was constructed based on the Kimura's 2-parameter model using Mega 7.0 by neighbor-joining (NJ) method to explore the phylogenetic relationship between Golmud yak and other yak breeds [11].

Results

The annotated mitogenome sequence of Golmud yak was submitted to GenBank with the accession number OK271109.

The length of Golmud yak's mitogenome was 16,324 bp with an A+T-biased nucleotide composition (61.0%), consisting of 13 protein-coding genes, 22 tRNA genes, two rRNA genes and one non-coding region (D-loop region) *(Table 1; Fig. 1)*.

The similarities of mitogenome sequence between Golmud yak and Pali yak, Qinghai-Gaoyuan yak, Sibu yak, Ashdan yak, Tianzhu white yak, Bazhou yak, Huanhu yak, wild yak, Datong yak, Jinchuan yak, Xueduo yak, Maiwa yak, Yushu yak, Gannan yak, Niangya yak, Zhongdian yak and American bison *(Bison bison)* were 99.9%, 99.8%, 99.9%, 99.9%, 93.1%, 99.9%, 99.9%, 99.9%, 99.9%, 99.3%, 99.8%, 99.8%, 99.8%, 99.3%, 99.8%, 99.9% and 91.3%, respectively *(Table 2)*. The phylogenetic tree showed that Golmud yak was most closely related to Sibu, Huanhu, Zhongdian, Ashdan, Jiulong, Pali, Datong and Bazhou yak breeds, closer to Yushu, Niangya, Qinghai-Gaoyuan, Xueduo, Maiwa and wild yak, but far away from other yak breeds (i.e. Jinchuan, Gannan and Tianzhu white yak) *(Fig. 2)*.

Discussion

Through the prediction of gene structure, we can get detailed information of gene distribution and structure of mitogenome. In this study, the gene composition,

Table 1. *Characteristics of the mitogenome of Golmud yak*

Gene/ Region	Position		Size (bp)	Base Composition (%)				Start Codon	Stop Codon	Strand
	From	To		A	C	G	T			
D-loop	1	893	893	32.36	25.42	13.77	28.44			H
tRNA^{Phe}	895	961	67	34.33	22.39	19.4	23.88			H
12S rRNA	962	1918	957	36.47	18.18	22.57	22.78			H
tRNA^{Val}	1919	1985	67	38.81	11.94	19.4	29.85			H
16S rRNA	1984	3555	1572	38.1	20.74	17.05	24.11			H
tRNA^{Leu}	3557	3631	75	32.0	17.33	29.08	26.36			H
ND1	3640	4584	945	32.28	29.21	12.28	26.24	ATA	TAA	H
tRNA^{Ile}	4590	4658	69	40.58	15.94	10.14	33.33			H
tRNA^{Gln}	4656	4727	72	36.11	9.72	27.78	26.39			L
tRNA^{Met}	4730	4798	69	27.54	18.84	27.54	26.09			H
ND2	4799	5836	1038	37.19	27.36	8.0	27.46	ATA	TAG	H
tRNA^{Trp}	5841	5907	67	37.31	16.42	20.9	25.37			H
tRNA^{Ala}	5909	5977	69	39.13	10.14	23.19	27.54			L
tRNA^{Asn}	5979	6052	74	31.08	14.86	28.38	25.56			L
OL	6053	6082	30	35.48	25.81	29.03	9.68			L
tRNA^{Cys}	6085	6151	67	29.85	19.4	26.87	23.88			L
tRNA^{Tyr}	6152	6219	68	29.41	16.18	20.59	33.82			L
COX1	6221	7759	1539	28.53	25.54	16.37	29.56	ATG	TAA	H
tRNA^{Ser}	7763	7831	69	24.64	14.49	28.99	31.88			L
tRNA^{Asp}	7839	7906	68	36.76	16.18	17.65	29.41			H
COX2	7908	8588	681	34.21	22.76	14.54	28.49	ATG	TAA	H
tRNA^{Lys}	8595	8661	67	31.34	20.9	17.91	29.85			H
ATP8	8663	8857	195	41.54	23.08	6.15	29.23	ATG	TAA	H
ATP6	8824	9498	675	33.04	26.81	11.41	28.74	ATG	TAA	H
COX3	9504	10286	783	27.64	29.63	15.2	29.12	ATG	TA-	H
tRNA^{Gly}	10288	10356	69	31.88	15.94	20.29	31.88			H
ND3	10357	10701	345	30.92	12.14	28.9	28.03	ATA	TA-	H
tRNA^{Arg}	10704	10772	69	39.13	11.59	10.14	39.13			H
ND4L	10773	11066	294	31.63	23.47	11.9	32.99	ATG	TAA	H
ND4	11063	12430	1368	33.48	26.97	10.09	29.46	ATG	T--	H
tRNA^{His}	12441	12510	70	41.43	8.57	15.71	34.29			H
tRNA^{Ser}	12511	12570	60	31.67	16.67	18.33	33.33			H
tRNA^{Leu}	12572	12641	70	37.14	15.71	20.0	27.14			H
ND5	12648	14444	1797	32.83	10.63	10.63	27.38	ATA	TAA	H
ND6	14452	14970	519	20.23	7.7	29.67	42.39	ATA	TAA	H
tRNA^{Glu}	14974	15042	69	27.54	11.59	21.74	39.13			L
Cytb	15047	16180	1134	31.66	29.01	12.96	26.37	ATG	AGA	L
tRNA^{Thr}	16190	16259	70	35.71	24.59	15.71	24.59			H
tRNA^{Pro}	16259	16324	66	24.24	28.79	13.64	33.33			L

structure and arrangement of mitogenome for Golmud yak are similar to that of most other mammals *(Fig. 1).* Control region (D-loop) is 893 bp length with an A + T content (60.8%) higher than G + C (39.19%). The two rRNAs *(12S rRNA and 16S rRNA)* are 957 bp and 1572 bp length, respectively. The 22 tRNAs range in size from 60 bp *(tRNA^{Ser})* to 75 bp *(tRNA^{Leu})* with a total length of 1511 bp. The start codon and stop codon of 13 protein-coding genes of Golmud yak mitogenome are same to that of other bovine species [6,12] *(Table 1).*

The study on the origin and phylogeny of yak is helpful to reveal its genetic background, evolutionary history and phylogenetic relationship. In previous research reports, there were many studies on the phylogeny of domestic and wild yak maternal lines based on nucleotide variations of mtDNA D-loop region, *Cyt-b* and *COIII* gene [3,6], while there were relatively few studies on the composition and origin of yak maternal lines by using complete mitogenome sequence variation [5]. The wild yak and Qinghai domestic yak breeds were composed of three maternal lineages
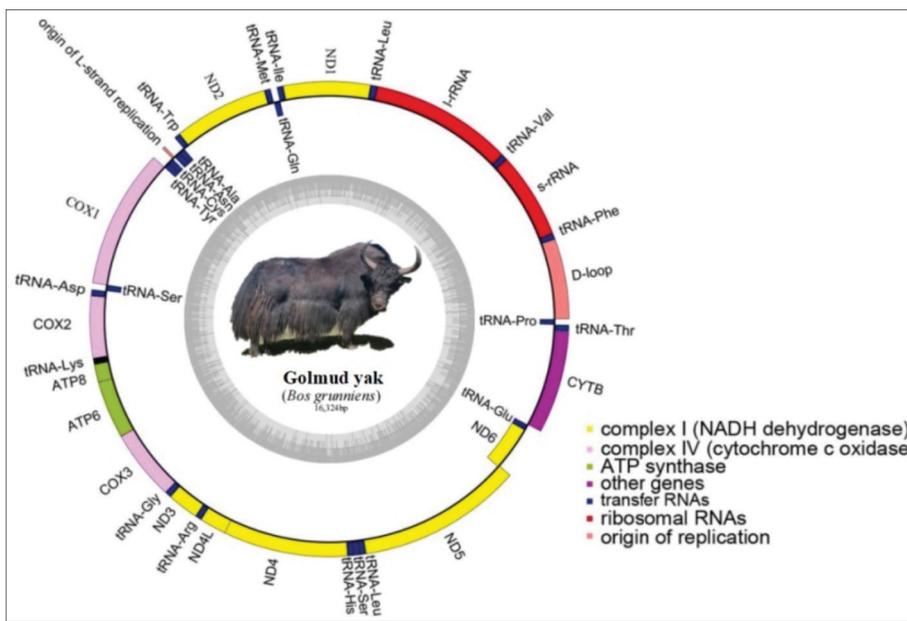
**Fig 1.** Mitogenome map of Golmud yak *(Bos grunniens)*

Table 2. *Identity of mitogenome sequence alignment among Golmud yak, other 16 yak breeds/populations and American bison (Bison bison)*

Breed	GLM	PL	QG	SB	ASD	TZ	BZ	HH	WY	DT	JC	XD	MW	YS	GN	NY	ZD	AB
GLM	1																	
PL	**0.999**	1																
QG	**0.998**	0.998	1															
SB	**0.999**	0.999	0.998	1														
ASD	**0.999**	0.999	0.998	0.999	1													
TZ	**0.931**	0.931	0.931	0.931	0.931	1												
BZ	**0.999**	0.999	0.998	0.999	0.999	0.931	1											
HH	**0.999**	0.999	0.998	0.999	0.999	0.931	0.999	1										
WY	**0.999**	0.999	0.999	0.999	0.999	0.931	0.999	0.999	1									
DT	**0.999**	0.999	0.998	0.999	0.999	0.931	0.999	0.999	0.999	1								
JC	**0.993**	0.993	0.993	0.993	0.993	0.935	0.993	0.993	0.993	0.993	1							
XD	**0.998**	0.998	0.999	0.998	0.998	0.931	0.998	0.998	0.998	0.998	0.993	1						
MW	**0.998**	0.998	0.999	0.998	0.998	0.931	0.998	0.998	0.998	0.998	0.993	1	1					
YS	**0.998**	0.998	0.999	0.999	0.998	0.931	0.998	0.998	0.999	0.998	0.993	0.999	0.999	1				
GN	**0.993**	0.993	0.993	0.993	0.993	0.935	0.993	0.993	0.993	0.993	0.999	0.993	0.993	0.993	1			
NY	**0.998**	0.998	0.999	0.999	0.998	0.931	0.998	0.998	0.999	0.998	0.993	0.999	0.999	0.999	0.993	1		
ZD	**0.999**	0.999	0.999	0.999	0.999	0.931	0.999	0.999	0.999	0.999	0.993	0.998	0.998	0.998	0.993	0.999	1	
AB	**0.913**	0.913	0.912	0.913	0.912	0.971	0.912	0.912	0.913	0.913	0.913	0.912	0.912	0.912	0.913	0.913	0.913	1

GLM: Golmud yak; PL: Pali yak; QG: Qinghai-Gaoyuan yak; SB: Sibu yak; ASD: Ashdan yak; TZ: Tianzhu white yak; BZ: Bazhou yak; HH: Huanhu yak; WY: Wild yak; DT: Datong yak; JC: Jinchuan yak; XD: Xueduo yak; MW: Maiwa yak; YS: Yushu yak; GN: Gannan yak; NY: Niangya yak; ZD: Zhongdian yak; AB: American bison (Bison bison); The numbers in bold represent the results of identity between Golmud yak and other yak breeds (populations) as well as American bison (Bison bison) in this study

(lineage I, II and III), and it was speculated that there were three maternal origins in yak. In lineage III, there were only a few wild yak, Xueduo yak and Golmud yak individuals [6,8]. This indicated that Golmud yak and Xueduo yak have significantly unique maternal genetic information, but the phylogenetic analysis and genetic relationship of Golmud yak with other yak breeds were not clarified. In this study, the phylogenetic tree showed that Golmud yak was most closely related to Sibu, Huanhu, Zhongdian, Ashdan, Jiulong, Pali, Datong and Bazhou yak breeds, closer to Yushu, Niangya, Qinghai-Gaoyuan, Xueduo, Maiwa and wild yak, but far away from other yak breeds (i.e. Jinchuan, Gannan and Tianzhu white yak). To some extent, the clustering result among these yak breeds/populations are basically consistent with their differentiation degree, which showing the genetic relationship among them.
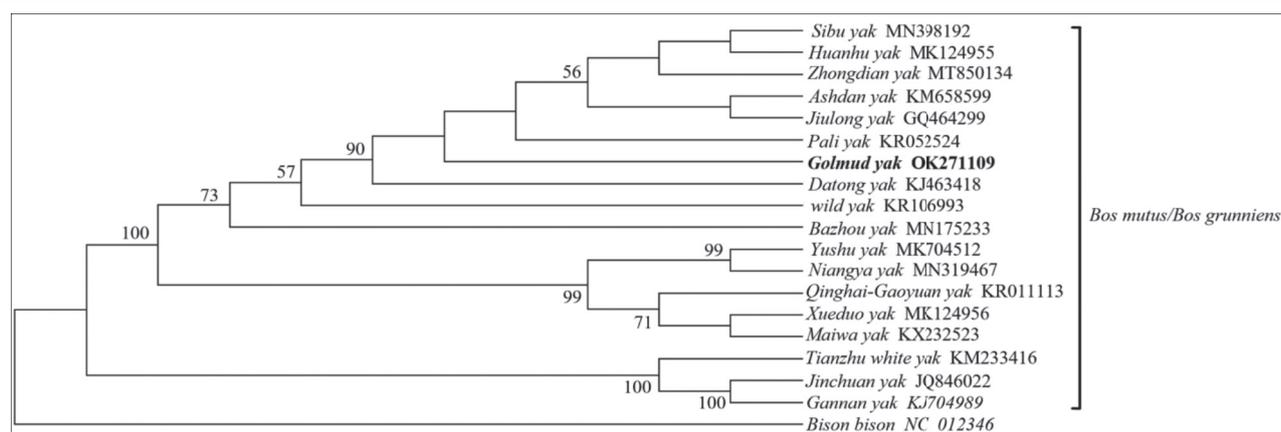
Fig 2. Phylogenetic relationship between Golmud yak and 17 yak breeds/populations in China based on mitogenome sequence variations. The support values (>50) next to the nodes are based on 1000 bootstrap replicates

In this study, the mitogenome characterization and phylogeny of Golmud yak were analyzed for the first time, which laid a foundation for the protection and utilization of this genetic resources and provided a theoretical basis for the breeding and improvement of the yak population.

Availability of Data and Materials

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at *(https://www.ncbi.nlm.nih.gov/)* under the accession no. OK271109. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA792074, SAMN24377418, and SRS11397677, respectively.

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Ethical Statement

This study was conducted according to the guidelines of the Council of China and animal welfare requirements.

Competing Interests

No potential conflict of interest was reported by the author(s).

Author Contribution Statement

ZJ was involved in the conception and design; GZ performed the experiment and data analyses; GZ wrote the original manuscript; ZJ revised the manuscript and approved the final version to be published; GZ, SM, YL, WX and ZJ carried out sampling; all authors reviewed and approved the final manuscript.

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