

## Characterization of the complete mitochondrial genome of Tibet Gaoshan Yak (*Bos grunniens*)

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### ABSTRACT

Tibet Gaoshan Yak (*Bos grunniens*) is a local Yak breed that mainly produces meat in Tibet Autonomous Region, China. In this study, the complete mitochondrial genome of Tibet Gaoshan Yak was sequenced. The total length of the mitochondrial genome is 16,323 bp, and the base composition is 33.71% for A, 13.21% for G, 27.27% for T, and 25.81% for C. The genome includes 13 protein-coding genes (*ND1-ND6*, *ND4L*, *COX1-COX3*, *ATP6*, *ATP8*, and *CYT6*), two rRNA genes (12S rRNA and 16SrRNA), 22 tRNA genes, and a noncoding control region (D-loop). Phylogenetic analysis showed that Tibet Gaoshan Yak has the closest relationship with Polled Yak. The sequence analysis provided in this study will be helpful to the management of Yak breeds, the origin, and evolution of Yak, and the protection and utilization of genetic resources.

### ARTICLE HISTORY

Received 28 May 2021  
Accepted 28 October 2021

### KEYWORDS

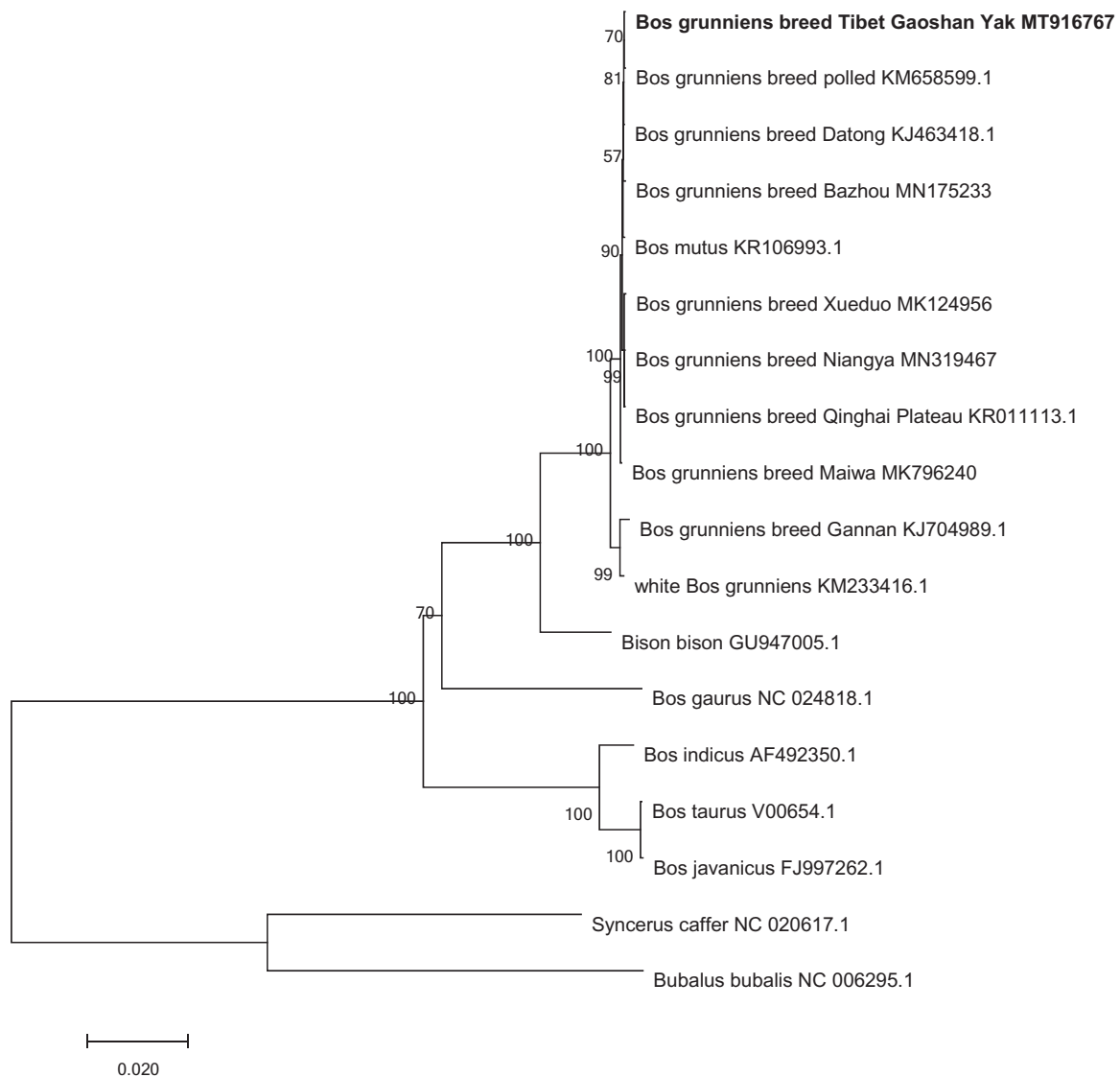
Tibet Gaoshan Yak;  
mitochondrial genome;  
phylogenetic analysis

China has the largest number of Yaks in the world (Wiener et al. 2011). Tibet Gaoshan Yak is a local Yak breed in China, mainly produced in the alpine grassland of the eastern Tibet Autonomous Region. Tibet Gaoshan Yak has strong adaptability to the extreme environment of high altitude, low oxygen content, large temperature difference and short growing period of forage in its distribution area. It is an important animal species indispensable for local people's production and life (Huang et al. 2012). However, up to now, there is no report on the complete sequence of mitochondrial genome in Tibet Gaoshan Yak. Mitochondrial DNA is a circular molecule and it is a powerful molecular tool in evolutionary and phylogenetic studies (Wang et al. 2011); this study reported the complete mitochondrial DNA sequence of Gaoshan Yak also in comparison with other breeds.

In this study, blood samples of Tibet Gaoshan Yak were collected from Baqing County, Naqu City, Tibet Autonomous Region (N31. 924638, E94. 059203), preserved at  $-20^{\circ}\text{C}$  in the Key Laboratory of Yak Breeding Engineering of Gansu Province, Lanzhou Institute of Husbandry and Pharmaceutical Sciences (Lanzhou, Gansu Province, China) with a storage number: LZ2020-008. Referring to the manufacturer's instructions, total genomic DNA was extracted from 20  $\mu\text{l}$  of blood by using Easy Pure Blood Genomic DNA Kit (Transgen Biotch, Beijing, China). Six pairs of primers (Table 1) (Wang et al. 2011) were used to amplify mitochondrial DNA by

polymerase chain reaction. The thermocycler settings were as follows:  $95^{\circ}\text{C}$  for 3 min; 35 cycles of  $95^{\circ}\text{C}$  for 20 s,  $52^{\circ}\text{C}$  for 40 s, and  $72^{\circ}\text{C}$  for 4 min, followed by  $72^{\circ}\text{C}$  for 10 min. PCR products were detected by 1% agarose gel electrophoresis. PCR products with appropriate band size were then sent to Xi'an Qincuo Biotechnology Co., Ltd (Xi'an, China) for sequencing. The sequencing results were assembled by using DNASTAR 7.1 (Burland 2000) software.

The complete mitochondrial genome of Tibet Gaoshan Yak was determined and deposited in GenBank with an accession number: MT916767. The mitochondrial genome of the Tibet Gaoshan Yak was a circular molecule with 16,323 bp in length, and the composition of the overall nucleotide is 33.71% for A, 13.21% for G, 27.27% for T and 25.81% for C, including 13 protein-coding genes (*ND1-ND6*, *ND4L*, *COX1-COX3*, *ATP6*, *ATP8* and *CYT6*), two rRNA genes (12S rRNA and 16S rRNA), 22 tRNA genes and a noncoding control region (D-loop). The gene sequence and composition of the mitochondrial genome of the Tibet Gaoshan Yak is similar to other Yak species (Huang et al. 2019). The overlap of ATPase genes seemed to be a common phenomenon in most vertebrate mitochondrial genomes (Li et al. 2014). We found three overlaps in the protein-coding genes of this sequence, including ATP6 overlaps with ATP8 for 40 bp, ND4 overlaps with ND4L for 7 bp and ND5 overlaps with ND6 for



**Figure 1.** Molecular phylogenetic analysis by maximum likelihood method.

17bp. The alignment of these genes is conservative compared to other Yak subspecies and Bovidae.

Based on 18 complete bovine mitochondrial genomes, we constructed a phylogenetic tree using MEGA6.0 (Tamura et al. 2013) software combined with the Maximum Likelihood (ML) method of 1000 bootstrap replicates. The tree with the highest log likelihood ( $-43,542.1851$ ) is shown in Figure 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8747)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 55.9107% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences. Codon positions included were 1st + 2nd +

3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 16,275 positions in the final dataset. The molecular phylogenetic analysis revealed that 18 species are divided into two branches as a whole and Tibet Gaoshan Yak has a closer genetic relationship with Polled Yak (Figure 1). In conclusion, the complete mitochondrial genome sequence of Tibet Gaoshan Yak can be used to further study of its origin evolution and breeding improvement.

### Disclosure statement

The authors declare that there are no financial and personal relationships with other people or organizations that can inappropriately influence our work, and no professional or other personal interest of any nature or kind in any product, service and company that could be construed as influencing the position presented in, or the review of the manuscript.

### Funding

This study was supported by grants from the Agricultural Science and Technology Innovation Program [CAAS-ASTIP-2014-LIHP5-01], the Major

Science and Technology Projects in Tibet Autonomous Region [XZ201901NA02], the National Beef Cattle Industry Technology & System [CARS-37], the science and technology program of Gansu Province [20JR5RA580].

### Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, reference number MT916767.

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