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The complete mitochondrial genome sequence and phylogenetic analysis of Niangya yak (Bos grunniens)

Chun Huang[a,b]*, Qiang Zhang[c]*, Xiaoyun Wu[a,b], Yangla Dawa[c], Donghai Fu[a,b], Hui Jiang[c], Min Chu[a,b], Renqing Dingkao[a,b], Xian Guo[a,b], Wangdui Basang[b], Yangji Cidan[c], Ping Yan[a,b] and Chunnian Liang[a,b]

[a]Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou, People's Republic of China; [b]Key Laboratory for Yak Breeding Engineering of Gansu Province, Chinese Academy of Agricultural Sciences, Lanzhou, China; [c]Institute of Husbandry and Pharmaceutical Sciences, Tebet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, China

ABSTRACT

In the present study, we report the complete mitochondrial genome of Niangya yak (Bos grunniens) and its phylogenetic inferences. The complete mitochondrial DNA is a circular molecule with 16,322 bp length consisting of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and a non-coding control region(D-loop). Both ND6 and 7 tRNAs (tRNA-Pro, tRNA-Glu, tRNA-Tyr, tRNA-Cys, tRNA-Asn, tRNA-Ala and tRNA-Gln) are encoded on the light strand, and the remaining genes are encoded on the heavy strand. The overall nucleotide composition is A(33.73%), T(27.28%), C(25.80%), G(13.19%) respectively. The content of C + G is 38.99%. Given that yak is indispensable for the Tibetan people, it is important to understand the genetic status of the population for further systematic genetics, evolutionary significance and protection of genetic resources. Therefore, to understand the evolutionary history of Niangya yak, the complete mitochondrial genome of Niangya yak was sequenced and compared with the mitochondrial genome of closely related Bos species.

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Niangya yak; (Bos grunniens); mitochondrial genome; phylogenetic analysis; protection of genetic resources

Niangya yak originated in Jiali County, Naqu District, Tibet Autonomous Region, mainly distributed in eastern Jiali County and northeastern townships. The yak has become strongly integrated into Tibetan socio-cultural life (Liang et al. 2016) and plays an important economic role in the mountainous regions of Asia (Medugorac et al. 2017). According to the archeological findings of the Neolithic cultural sites in Changdu and Linzhi, the Chinese and Tibetan classics before Zhou and Qin dynasties show that the local Qiang people domesticated wild yaks at least 4600 years ago (ChinaBreed 2014). Niangya yak is one of the most important breeds among the three excellent yak groups in Tibet. It is one of the most precious and pure characteristic livestock breed resources in Tibet (Ding 2016). Therefore, the study of complete and clear mitochondrial DNA sequences will greatly help us to identify the evolutionary significance units in its distribution range, and contribute to the management of yak breeds and the protection and utilization of yak genetic resources. We generated and characterized the complete mitochondrial genome sequence of Niangya yak and to investigate the molecular phylogenetics of the species to confirm its taxonomic status in yaks (Bos grunniens).

In this work, the 30 blood samples of Niangya yak were collected from Lhari County, Nagqu Prefecture,Tibet Autonomous Region (N30°38′, E93°13′). These specimens are preserved at −20 °C with an accession number: LZ2019-229 until DNA extraction in the Key Laboratory of Yak Breeding Engineering of Gansu Province, Lanzhou Institute of Husbandry and Pharmaceutical Sciences (Lanzhou, Gansu Province, China). Referring to manufacturer's instructions, we extracted the total genomic DNA from blood samples by using Easy Pure Blood Genomic DNA Kit (Transgen Biotch, Beijing, China). The extracted DNA was followed by 0.8% agarose gel electrophoresis to examine the quality of DNA for the PCR amplification.We amplified the whole mitochondrial genome with 6 pairs of primers by polymerase chain reaction method, PCR reactions were carried out in 50 μL reaction volumes using 5× PrimeSTAR GXL Buffer (10 μL), dNTP Mixture (2.5 mM of each, 4 μL), 1 μL of each primer, PrimeSTAR GXL DNA Polymerase (2 μL) and double distilled H_2O (30 μL). Following the library preparation, high-throughput sequencing was conducted with the Illumina HiSeq X™ Ten Sequencing System (Illumina, CA, USA) by Annoroad Gene Technology (Beijing, China), and assembled the sequencing results using DNASTAR5.0 software (Madison, WI, USA).

The complete mitochondrial genome annotation was done using Genbank and deposited with an accession

CONTACT Ping Yan pingyanlz@163.com; Chunnian Liang chunnian2006@163.com Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou, People's Republic of China; Key Laboratory for Yak Breeding Engineering of Gansu Province, Chinese Academy of Agricultural Sciences, Lanzhou, China
*These authors contributed equally to this work.

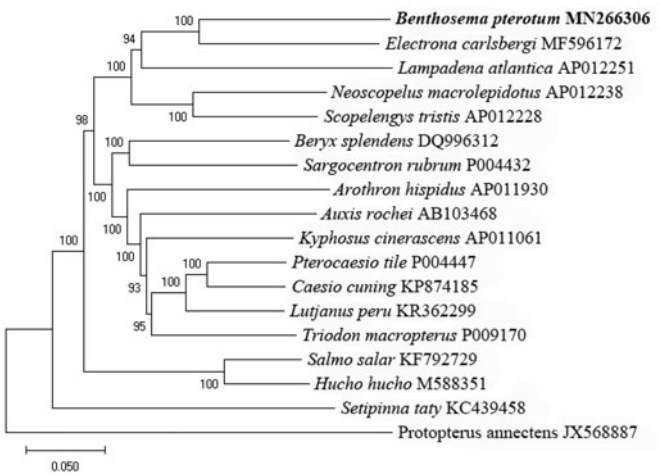

Figure 1. Phylogenetic relationships of mitochondrial genomes of 19 species based on the neighbor-joining (NJ) methods. The result was validated by 1000 bootstraps and the bootstrap values are shown next to the branches.

number: MN319467. The entire mitochondrial DNA is a circular DNA molecule with a total length of 16,322 bp, which contains the base composition of 33.73% for A, 27.28% for T, 25.80% for C and 13.19% for G, and the content of C + G is 38.99%. The alignment of these genes is conservative compared to other yak subspecies and Bovidae. Furthermore, this mitochondrial DNA contains the typical structure including a non-coding control region (D-loop), 22 transfer RNA genes, 2 ribosomal RNA subunit genes (12 s and 16 s rRNA) and 13 protein-coding genes. The total encoding length of 13 protein coding genes (PCGs) in Niangya yak was 11,400bp, accounting for 69.84% of the complete mitochondrial DNA. The total AT content of 13 PCGs in Niangya yak was 60.75%. These PCGs regions are composed of seven NADH dehydrogenases (ND1-6 and ND4L), three cytochrome c oxidases (COX1-3), two ATPases (ATP6 and ATP8) and one cytochrome b (CYTb) genome, and only ND6 encoded on the L-strand. The two rRNAs are 957 bp (12S rRNA) and 1571 bp (16S rRNA) in length, respectively, and are separated by tRNA-Val whose length is 67 bp. The length of the two rRNA genes accounts for 15.49% of the whole length of mitochondrial genes.

In order to understand the phylogenetic status of Niangya yak in Tibet, we constructed a phylogenetic tree using MEGA7.0 software combined with the neighbor-joining (NJ) method of 1000 bootstrap replicates (Cummings 2004). The molecular phylogenetic analysis revealed that 19 species are divided into two branches as a whole. As can be seen from the Figure 1, Niangya yak and Bison Bison are grouped together, which is consistent with the views of Songchang Guo (Guo et al. 2008) that Tibet yaks are closely related to Bison Bison. Furthermore, Niangya yak has a closer genetic relationship with Qinghai Plateau yak.

Only by proper classification and understanding,can yak resources be used reasonably and creat more wealth. The yak is a source of necessary materials for the Tibetan people to survive bravely on the roof of the world. The molecular data presented in this study provides new mitochondrial DNA resources for the better consideration of phylogeny.This research also provides a further help in the study of the origin, evolution and classification of yaks and help spur investigation of phylogeography, evolutionary significance unit (ESU) and to address genetic linkages among this species.

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.

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