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# Genetic diversity and classification of Tibetan yak populations based on the mtDNA *COIII* gene

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**ABSTRACT.** To determine the level of genetic diversity and phylogenetic relationships among Tibetan yak populations, the mitochondrial DNA cytochrome c oxidase subunit 3 (*COIII*) genes of 378 yak individuals from 16 populations were analyzed in this study. The results showed that the length of cytochrome c oxidase subunit 3 gene sequences was 781 bp, with nucleotide frequencies of 29.2, 29.4, 26.1, and 15.2% for T, C, A, and G, respectively. A total of 26 haplotypes were identified, with 69 polymorphic sites, including 11 parsimony-informative sites and 58 single-nucleotide polymorphism sites. No deletions/insertions were found in sequence comparison, indicating that nucleotide mutation types were transitions and transversions. Haplotype and nucleotide

 

diversities were 0.562 and 0.00138, respectively, indicating a high level of genetic diversity in Tibetan yak populations. Phylogenetic relationship analysis indicated that Tibetan yak populations are divided into 2 groups.

**Key words:** Mitochondrial DNA cytochrome c oxidase subunit 3; Genetic diversity; Phylogenetic relationship; Tibetan yak

## INTRODUCTION

The yak (*Bos grunniens*) is currently regarded as an important renewable genetic resource, as it thrives in extremely harsh environments at high altitudes, hypoxia, severely cold winters, and cool moist summers; the yak survives short growing seasons with limited grazing resources (Wiener et al., 2003). As a unique species on the Qinghai-Tibetan Plateau and in adjacent mountains and subalpine regions, the yak is a multi-purpose domestic animal utilized by local people. More than 4.9 million domestic yaks live in Tibet of China, accounting for approximately 30% of all Chinese domestic yaks (Zhang, 2012).

Mitochondrial DNA (mtDNA) is a self-replicating, maternally inherited, circular DNA molecule that has been widely used to resolve taxonomic controversies (Delarbre et al., 1998; Boore et al., 1998; Lavrov and Lang, 2005; Gissi et al., 2008; Mipam et al., 2012). It is composed of 13 protein subunits, 22 mitochondrial tRNAs, 2 mitochondrial-specific ribosomal RNAs, and 12S and 16S rRNAs (Delarbre et al., 1998; Tiranti et al., 2000). The *COIII* gene is 1 of 13 protein-coding genes in the mitochondrial genome. Cloning and sequence analysis of the *COIII* gene are very valuable for studying molecular system evolution and species classification. Recent studies have examined the *COIII* gene in several species, including fish, frogs, mammals, birds (Griffiths et al., 1998), mosquitos (Pridgeon et al., 2009), and rats (Huang et al., 2004). In addition, previous studies have been performed on several mtDNA genetic markers in Tibetan yak populations (Zhao et al., 2011; Chai et al., 2011; Ji et al., 2012; Zhang et al., 2012). However, the *COIII* gene has not been investigated in Tibet yak populations. In this study, we sequenced the complete *COIII* gene in 378 individuals representing 16 Tibetan yak populations, including the Sibu, Riduo, Leiwuqi, Dingqing, Jiangda, Sangri, Cuona, Longzi, Pali, Sangsang, Kangbu, Zhongba, Jiali, Baqing, Nierong, and Gongbujiangda yak populations. The aim of this study was to evaluate the genetic diversity of Tibetan yak populations and explore their classification relationships.

## MATERIAL AND METHODS

### Sample collection and DNA extraction

A total of 378 ear tissue samples were collected from 16 healthy yak populations in Tibet. Details regarding sample number, name, and geographical distribution of the populations are summarized in Table 1. After collection, the samples dipped in 75% ethyl alcohol were transported to the lab and stored at -80°C. A DNA Extraction Kit (Tiangen, Beijing, China) was used to extract genomic DNA, which was detected on a 1% agarose gel and stored at -20°C until use.

**Table 1.** Distribution area, code, number, and sampling locations of samples.

| Distribution area | Population | Code | Number of samples | Sampling locations |
|---|---|---|---|---|
| Lasa | Sibu yak | SB | 10 | Lasa |
| | Riduo yak | RD | 51 | Riduo village |
| Changdu | Leiwuqi yak | LW | 25 | Leiwuqi county |
| | Dingqing yak | DQ | 50 | Dingqing county |
| | Jiangda yak | JD | 10 | Jiangda county |
| Shannan | Sangri yak | SR | 10 | Sangri county |
| | Cuona yak | CN | 26 | Cuona county |
| | Longzi yak | LZ | 49 | Longzi county |
| Rikaze | Pali yak | PL | 10 | Pali town |
| | Sangsang yak | SS | 10 | Sangsang town |
| | Kangbu yak | KB | 10 | Kangbu village |
| | Zhongba yak | ZB | 36 | Zhongba county |
| Naqu | Jiali yak | JL | 10 | Jiali county |
| | Baqing yak | BQ | 11 | Baqing county |
| | Nierong yak | NR | 50 | Nierong county |
| Linzhi | Gongbujiangda yak | GD | 10 | Gongbujiangda county |

## PCR amplification and sequencing

The *COIII* gene in each sample was amplified by polymerase chain reaction (PCR) using the primers PF 5'-TGTGAGCAGGAGCCGTAA-3' and PR 5'-CCATATTCGGTTCATTCC AGTC-3'. PCR amplifications were carried out in a 50-μL reaction tube containing 200 ng genomic DNA, 20 pmol of each primer, 25 μL 2X long Taq premixed DNA polymerase (TaKaRa, Dalian, China), and 19 μL sterilized dd$H_2O$. PCR amplifications were performed as follows: initial denaturation step at 95°C for 2 min, followed by 35 cycles at 95°C for 30 s, 57.3°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 7 min. PCR products were purified using the DNA Gel Extraction Kit (Tiangen) according to manufacturer instructions and sequenced on an ABI 3730xl automated sequencer (Invitrogen, Carlsbad, CA, USA).

## Data analyses

mtDNA *COIII* sequences were compiled using the SeqManII software (DNASTAR, Inc., Madison, WI, USA) and aligned using ClustalW 1.83 (Thompson et al., 1997) with default parameters. Nucleotide polymorphisms within the sequences were computed using MEGA 4.0 (Tamura et al., 2007). Genetic distances between populations were calculated using Kimura double parameters. A phylogenetic tree was constructed based on the Kimura's 2-parameter model using the MEGA 4.0 software with the neighbor-joining method. The confidence level of individual nodes of the phylogenetic tree was assessed using bootstrap resampling analysis (1000 replicates). Polymorphic sites, haplotype diversity, and nucleotide diversity were analyzed using DnaSP 4.0 (Rozas et al., 2003).

# RESULTS

## Genetic diversity among populations

Tibetan yak mtDNA *COIII* gene sequences were successfully obtained. The length of yak *COIII* sequences was 781 bp and the average ratios of T, C, A, and G were 29.2% (29.1-29.6%), 29.4% (29.1-29.6%), 26.1% (25.9-26.6%), and 15.2% (14.9-15.5%), respectively.

A total of 69 polymorphic sites were detected in the sequence comparisons of the Tibetan yak mtDNA *COIII* gene, including 11 parsimony-informative sites and 58 nucleotide polymorphism sites. No deletions or insertions were found in sequence comparison, indicating that nucleotide mutations included only transitions and transversions. The transition/transversion ratio was 12.89. Most base substitutions were T/C transitions, accounting for approximately 57% of all nucleotide mutations. The frequency of transformations was significantly higher than that of transversions.

Additionally, 26 haplotypes were identified in the study, with an average of 3.75 (Table 2). Haplotype diversity ranged from 0.315-0.844, with an average of 0.562 (Table 3), with the highest level of genetic diversity within the Sibu yak population and the lowest level of genetic diversity in the Riduo yak population. Nucleotide diversity ranged from 0.00051-0.00347, with an average of 0.00138, with the highest level of genetic diversity in Dingqing yak and the lowest level of genetic diversity in Jiali yaks. Haplotype and nucleotide diversities of Sibu, Gongbujiangda, Leiwuqi, Sangri, Longzi, and Jiangda yaks were relatively higher than in other populations. The haplotype and nucleotide diversities of Baqing, Jiali, Kangbu, Sangsang, Cuona, Nierong, Riduo, and Zhongba yaks were relatively lower than in other populations.

**Table 2.** Distribution of haplotypes of Tibetan yak populations.

| Haplotype | Number of haplotypes | Distribution of haplotypes |
|---|---|---|
| Hap-1 | 45 | DQ (6), LW (6), LZ (9), NR (8), RD (3), ZB (5), BQ (2), GD (2), SR (4) |
| Hap-2 | 1 | JD (1) |
| Hap-3 | 1 | SS (1) |
| Hap-4 | 60 | CN (7), DQ (10), LW (3), LZ (6), NR (7), RD (5), ZB (1), BQ (2), GD (4), JD (3), KB (3), PL (5), SB (2), SR (2) |
| Hap-5 | 1 | DQ (1) |
| Hap-6 | 1 | JL (1) |
| Hap-7 | 1 | LW (1) |
| Hap-8 | 2 | LW (2) |
| Hap-9 | 4 | LZ (4) |
| Hap-10 | 1 | GD (1) |
| Hap-11 | 1 | LZ (1) |
| Hap-12 | 2 | ZB (2) |
| Hap-13 | 242 | NR (33), SS (8), SR (4), SB (4), PL (4), KB (7), JL (8), JD (6), GD (3), BQ (7), ZB (28), RD (42), CN (19), LW (11), DQ (32), LZ (26) |
| Hap-14 | 1 | RD (1) |
| Hap-15 | 1 | NR (1) |
| Hap-16 | 1 | SB (1) |
| Hap-17 | 1 | SB (1) |
| Hap-18 | 1 | JL (1) |
| Hap-19 | 1 | PL (1) |
| Hap-20 | 1 | SB (1) |
| Hap-21 | 1 | SS (1) |
| Hap-22 | 2 | LZ (2) |
| Hap-23 | 1 | LZ (1) |
| Hap-24 | 1 | SB (1) |
| Hap-25 | 3 | LW (2), NR (1) |
| Hap-26 | 1 | DQ (1) |

## Population classification

The average genetic distance between the populations of Tibetan yaks was 0.001452 (Table 4). The genetic distance between Kangbu and Cuona yaks was the smallest (0.000523), while the genetic distance between Sibu and Dingqing yaks was the largest (0.002912).

**Table 3.** Haplotype diversity and nucleotide diversity of different Tibetan yak populations.

| Populations | Number of haplotypes | Haplotype diversity | Nucleotide diversity |
|---|---|---|---|
| Sibu yak | 6 | 0.844 | 0.00219 |
| Riduo yak | 4 | 0.315 | 0.00057 |
| Leiwuqi yak | 6 | 0.750 | 0.00194 |
| Dingqing yak | 5 | 0.546 | 0.00347 |
| Jiangda yak | 3 | 0.600 | 0.00162 |
| Sangri yak | 3 | 0.711 | 0.00182 |
| Cuona yak | 2 | 0.409 | 0.00052 |
| Longzi yak | 7 | 0.674 | 0.00150 |
| Pali yak | 3 | 0.644 | 0.00097 |
| Sangsang yak | 3 | 0.378 | 0.00128 |
| Kangbu yak | 2 | 0.467 | 0.00060 |
| Zhongba yak | 4 | 0.383 | 0.00084 |
| Jiali yak | 3 | 0.378 | 0.00051 |
| Baqing yak | 3 | 0.582 | 0.00126 |
| Nierong yak | 5 | 0.529 | 0.00119 |
| Gongbujiangda yak | 4 | 0.778 | 0.00185 |
| Mean | 3.75 | 0.562 | 0.00138 |

**Table 4.** Kimura 2-parameter genetic distance for Tibetan yak populations.

| Groups | LZ | JD | DQ | LW | NR | RD | ZB | BQ | GD | SR | SS | CN | KB | PL | SB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JD | 1.570 | | | | | | | | | | | | | | |
| DQ | 2.570 | 2.577 | | | | | | | | | | | | | |
| LW | 1.741 | 1.876 | 2.851 | | | | | | | | | | | | |
| NR | 1.334 | 1.393 | 2.397 | 1.583 | | | | | | | | | | | |
| RD | 1.077 | 1.096 | 2.109 | 1.408 | 0.906 | | | | | | | | | | |
| ZB | 1.181 | 1.270 | 2.254 | 1.459 | 1.018 | 0.716 | | | | | | | | | |
| BQ | 1.326 | 1.366 | 2.383 | 1.578 | 1.160 | 0.901 | 1.021 | | | | | | | | |
| GD | 1.695 | 1.644 | 2.715 | 1.937 | 1.520 | 1.296 | 1.447 | 1.482 | | | | | | | |
| SR | 1.687 | 1.824 | 2.812 | 1.798 | 1.536 | 1.414 | 1.448 | 1.517 | 1.824 | | | | | | |
| SS | 1.407 | 1.491 | 2.468 | 1.711 | 1.243 | 0.914 | 1.035 | 1.249 | 1.695 | 1.721 | | | | | |
| CN | 1.125 | 1.038 | 2.105 | 1.495 | 0.942 | 0.579 | 0.790 | 0.920 | 1.224 | 1.491 | 0.988 | | | | |
| KB | 1.155 | 1.054 | 2.129 | 1.525 | 0.970 | 0.611 | 0.827 | 0.945 | 1.232 | 1.515 | 1.027 | 0.523 | | | |
| PL | 1.477 | 1.285 | 2.406 | 1.849 | 1.284 | 0.946 | 1.198 | 1.237 | 1.412 | 1.798 | 1.413 | 0.769 | 0.769 | | |
| SB | 1.910 | 1.875 | 2.912 | 2.245 | 1.730 | 1.391 | 1.569 | 1.715 | 2.054 | 2.234 | 1.772 | 1.362 | 1.386 | 1.655 | |
| JL | 1.121 | 1.156 | 2.151 | 1.489 | 0.950 | 0.558 | 0.720 | 0.957 | 1.411 | 1.540 | 0.889 | 0.602 | 0.641 | 1.026 | 1.411 |

Data in the table must be multiplied by $10^{-3}$.

A neighbor-joining phylogenetic tree was constructed using Kimura 2-parameter genetic distances (Figure 1 and Table 4). The results showed that Tibetan yak populations were divided into 2 major groups; the Dingqing yak was in 1 group, while the other yaks clustered in another group. Two subgroups were created between the 15 groups (Figure 1). The Leiwuqi, Sangri Gongbujiangda, Baqing, Longzi, Nierong, Zhongba, Riduo, Sangsang, and Jiali populations clustered into 1 subgroup. The Sibu, Jiangda, Cuona, Kangbu, and Pali populations clustered into another subgroup.

## DISCUSSION

### Genetic diversity of mtDNA *COIII* of Tibetan yak populations

Studies examining genetic diversity can reveal the origin, genetic variation, and evo-

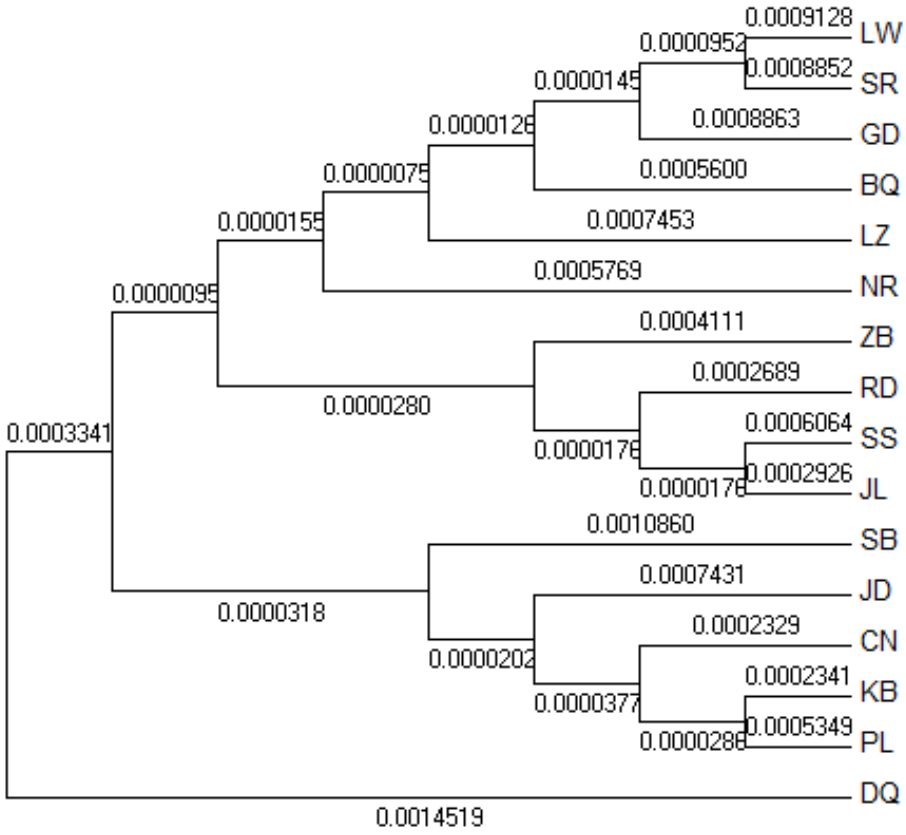


**Figure 1.** Neighbor-joining phylogenetic tree of Tibetan yak populations based on CO*III* gene sequences.

lution of a species (Zheng et al., 2012). Nucleotide and haplotype diversities are 2 important indicators used for measuring genetic variation of mtDNA within a population (Zhou et al., 2006). In our study, the average haplotype diversity among the 16 yak populations was 0.562, while the average nucleotide diversity was 0.00138. Although the genetic diversity of mtDNA *COIII* observed in our study was high, it was lower than that reported by Yang et al. (2009), Chai et al. (2011), Zhang et al. (2012), and Ji et al. (2012). However, our results were similar to those obtained using sequence-related amplified polymorphism marker analysis of 11 Tibetan yak populations (Zhao et al., 2012). The differences observed in genetic diversity may be associated with the conservation of different genes used in various studies. The abundant genetic diversity observed in the Sibu yak population was similar to that observed by Guo et al. (2008). However, the genetic diversity of the Riduo yak population was lower. The abundant genetic diversity of Tibetan yaks plays an important role in the sustainable development of the Tibetan yak industry. It may also serve as a valuable genetic resource for yak breeding (Zhong et al., 2011). In summary, the development, effective utilization, and protection of genetic diversity of Tibetan yaks have become increasingly important for the development of the Tibetan yak industry.

## Population classification

Over the past few years, previous studies have examined the phylogenetic relationships between Tibetan yak populations (Zhao et al., 2011; Zhang et al., 2012; Ji et al., 2012). However, we observed some differences from previous reports, which is primarily because of the different research methods and populations used. Ji et al. (2012) studied the relationships between Tibetan yak populations and then divided the 11 yak populations into 5 categories: Pali, Jiangda, Baqing, Sangri, and Leiwuqi yak types. However, Zhao et al. (2011) indicated that Tibetan yak populations could be divided into 3 categories, Pali, Baqing, and Sibu yak types. Moreover, Zhang et al. (2012) suggested that Tibetan yak populations could be divided into 2 categories, with the Kangbu and Jiali yaks as 1 cluster, and the remaining populations as another cluster. In our study, cluster analysis showed that 16 Tibetan yak populations could be divided into 2 categories. Dingqing yak comprised 1 category, while the remaining populations formed another category. This is consistent with the results of microsatellite DNA marker analysis for Tibetan yak populations (Li et al., 2013). The genetic distance between Kangbu and Cuona yaks was the lowest in this study (0.000523), indicating a relatively close genetic relationship between the 2 populations. The genetic distance between Sibu and Dingqing yaks was the largest (0.002912), indicating a relatively remote genetic relationship. The genetic diversity, genetic distance, and cluster analyses of Tibetan yak populations were not directly correlated to geographical distribution. Corroborating the views of Zhang et al. (2012), Zhao et al. (2012), and Guo et al. (2008), the data indicate that the effect of the geographical environment on genetic relationships between populations has gradually weakened.

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