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Mitochondrial diversity and phylogeographic structure of native cattle breeds from Yunnan, Southwestern China

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ABSTRACT

Little is so far known about genetic diversity and phylogeographic structure of native cattle breeds from Yunnan in Southwestern China, a frontier zone that connects China with South and Southeast Asia. Herein we analyzed a 910-bp fragment of mitochondrial DNA (mtDNA) D-loop region sequences of 280 individuals from 6 Yunnan native cattle breeds, of which 251 sequences were newly determined. There were 93 variable sites that defined 117 haplotypes among all sequences. Phylogenetic analysis of all haplotypes revealed that Yunnan native cattle had two distinct mtDNA lineages - taurine and zebu. The taurine sequences fell into four haplogroups T1a, T2, T3 and T4, whereas the zebu sequences grouped into two haplogroups I1 and I2. In addition, our results revealed patterns of gradient changes in frequencies of taurine and zebu mtDNA lineages across different geographic regions of Yunnan. Furthermore, three kinds of methods (haplotype network, analysis of molecular variance, and Mantel tests) consistently showed no significant phylogeographic structuring among Yunnan native cattle breeds. This might be attributed to historical strong gene flow and genetic admixture among native cattle breeds in different geographic regions.

1. Introduction

Cattle have a prominent status among all livestock species and play a significant role in human history since the early Neolithic age (Mahgoub et al., 2013). Archaeological and genetic evidence suggested that modern domestic cattle were mainly domesticated from two different *Bos primigenius* populations in Near East and Indus Valley (Loftus et al., 1994a, 1999; Troy et al., 2001), which resulted in humpless/taurine (*Bos taurus*) and humped/zebu cattle (*Bos indicus*) (Bruford et al., 2003). The divergence time between taurine and zebu cattle was estimated to be 0.2–1.0 million years ago, far predated cattle domestication time approximately 8000–10,000 years before present (Loftus et al., 1994a,b).

Despite longstanding interests in studying genetic diversity, origins and evolution of domestic cattle worldwide, there are still many native cattle breeds in some regions that have never been extensively assessed using molecular markers. For instance, Yunnan in Southwestern China is one among those regions where native cattle were never systematically genetically assessed. Like Arabian Peninsula (Mahgoub et al., 2013), Yunnan also represents an admixture zone for taurine and zebu cattle (Yu et al., 1999).

Mitochondrial DNA (mtDNA) has been widely used to characterize genetic diversity and population genetic structure of domestic animals. Previous studies revealed five major mtDNA haplogroups (T, T1, T2, T3 and T4) in taurine cattle (Troy et al., 2001; Mannen et al., 2004). The T1 haplogroup predominated in African cattle, while the T, T2 and T3 haplogroups were mainly found in Near Eastern and European cattle (Troy et al., 2001), and the T4 haplogroup was relatively rare and only found in Northeast Asian cattle (Mannen et al., 2004). In addition, the I1 and I2 haplogroups were identified in zebu cattle across Asian countries (Chen et al., 2010). Intriguingly, both taurine and zebu cattle have spread out from their domestication centers – Near East and Indian subcontinent, respectively. Historically, these two types of cattle met with each other and subsequently admixed in contact zones. For instance, taurine cattle have spread out from Near East to Central Asia and then to Northern China, and finally to Southwestern China (Flad et al., 2007). Zebu cattle have spread out from Indian subcontinent to Southeast Asia and then to Southwestern China and further upward to Central China (Higham, 1996). The distribution of both taurine and zebu cattle in China shaped a decreasing genetic component pattern for taurine cattle from Northern to Southern China and for zebu cattle from Southern to Northern China, respectively (Lai et al., 2006; Lei et al., 2006). However, the exact admixture time of taurine and zebu cattle in Yunnan of Southwestern China remains uncertain.

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Unfortunately, little is so far known about genetic diversity and phylogeographic structure among Yunnan native cattle breeds.

There are 6 officially recognized native cattle breeds in Yunnan, including Wenshan cattle, Dengchuan cattle, Dianzhong cattle, Dehong humped cattle, Zhaotong cattle, and Diqing cattle. These native breeds represent important livestock genetic resources and exhibit some features of merit, such as good adaptability, tolerance to rough feeding, and strong disease resistance (Yu et al., 1999; Gou et al., 2010). However, in recent years, artificial insemination using frozen bovine semen of exotic commercial breeds has become common practice, making Yunnan native cattle breeds being at risk of loss. Thus, it is urgent to understand their genetic background before losses of these invaluable cattle genetic resources.

Yunnan is a frontier zone that connects China with South and Southeast Asia. For domestic cattle, it is an admixture zone for taurine and zebu cattle. Apparently, some individuals of Yunnan native cattle breeds are offspring of crosses between taurine and zebu cattle. It was speculated that Indian zebu cattle might have entered into China via Yunnan (Jia et al., 2007). Molecular genetic evidence would provide new insight into migration and admixture pattern of taurine and zebu cattle in this geographic area. Therefore, here we determined mtDNA D-loop region sequences to investigate genetic diversity and phylogeographic structure of 6 Yunnan native cattle breeds.

The main purposes of this study were to: (1) dissect genetic components of each Yunnan native cattle breed, (2) test whether there existed a gradient geographic distribution pattern for taurine and zebu cattle, and (3) test whether there had phylogeographic structure among breeds.

2. Materials and methods

2.1. Sampling and genomic DNA extraction

Ear skin tissues from 251 individuals of 6 indigenous cattle breeds were collected from remote villages of Yunnan Province, Southwestern China (Fig. 1; Table 1). Effort was made to collect samples from unrelated individuals, based on the information provided by local farmers. The detailed information about breeds, sampling locality, sample sizes are given in Table 1. All samples were preserved in 100% ethanol and later stored at −80 °C in the laboratory. Genomic DNA was extracted from ear skin tissues using TIANamp genomic DNA kit (TIANGEN Biotech, Beijing).

Table 1
Breed, sampling locality, sample sizes, number of haplotypes (*h*), haplotype diversity (Hd) and nucleotide diversity (Pi) for each Yunnan native cattle breed used in the study.

Breed	Locality	Samples[a]	*h*	Hd	Pi
Wenshan (WS)	Guangnan county, Yunnan	53	27	0.927	0.02358
Dengchuan (DC)	Eryuan county, Yunnan	30	20	0.977	0.02661
Dianzhong (DZ)	Shuangbai county, Yunnan	53	24	0.935	0.02341
Dehong humped (DHH)	Mangshi county, Yunnan	34	14	0.626	0.00193
Zhaotong (ZT)	Ludian county, Yunnan	32 + 14 (gb)	33	0.974	0.02547
Diqing (DQ)	Diqing prefecture, Yunnan	49 + 15 (gb)	40	0.981	0.01961

[a] The suffix of sample size (gb) indicates that these sequences were extracted from GenBank database.

2.2. PCR amplification and sequencing

The mtDNA D-loop region was amplified by using the primers L15737 (5′-CTGCAGTCTCACCATCAACC-3′) and H992 (5′-GATTATAG AACAGGC TCCTC-3′) (Loftus et al., 1994a; Lai et al., 2006; Salim et al., 2015). Polymerase chain reaction (PCR) amplifications were conducted in a 50 μl volume containing 5 μl of 10 × reaction buffer, 3.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM each primer, 1.25 U *Taq* DNA polymerase (TaKaRa Biosystems), and approximately 30 ng–50 ng genomic DNA. The PCR cycling consisted of initial denaturation at 95 °C for 3 min and 35 cycles at 95 °C for 1 min, 60 °C for 30 s, and 72 °C for 90 s, with a final extension at 72 °C for 4 min. To avoid potential contamination during PCR amplification, negative controls were always set. The PCR products were checked by electrophoresis in a 1% agarose gel and successful amplifications were sent to TSINGKE Biological Technology (Kunming) for Sanger sequencing, using two amplification

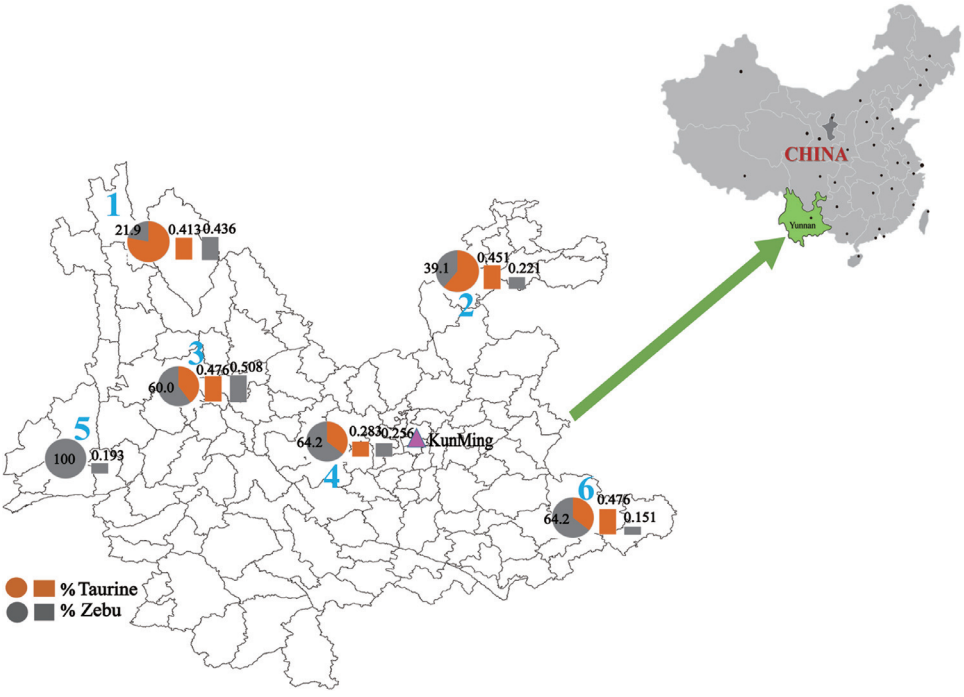

Fig. 1. Localities of Yunnan native cattle breeds analyzed in the study and mapping of nucleotide diversity and frequencies of taurine and zebu lineage. Yellow color represents taurine lineage and gray color represents zebu lineage. Number notes: 1, Diqing cattle; 2, Zhaotong cattle; 3, Dengchuan cattle; 4, Dianzhong cattle; 5, Dehong humped cattle; 6, Wenshan cattle.