

New Evidence Discovery for Maternal Lineage B of Goats (*Capra hircus*) in Chinese Tropical Zone

S. Guan, H.L. Zhou, G. Y. Hou, D.J. Wang, T.S. Xu,
D.F. Wang, L.G. Shi, G. Rong and M. Li

Institute of Tropical Grassland and Livestock Research,
Chinese Academy of Tropical Agricultural Sciences, Danzhou, 571737 Hainan, China

Abstract: Phylogenetic and genetic diversity of four local goat breeds were analyzed by mitochondrial Hypervariable Region (HVR) in 89 individuals from Chinese tropical zone. A total of 36 haplotypes which defined 64 polymorphic sites were found in the study. Comparing with the published mtDNA control region sequences, two mtDNA lineages (A and B) were identified by the phylogenetic analysis in which lineage A was 57.30%, lineage B was 42.70%, haplotype A was 47.22%, haplotype B was 36.11%. The interest in the study was that the proportion of lineage B was up to 80.95% in Hainan black goat, lineage A was only 19.05%. The genetic diversity showed that Hainan black goat had the lowest variability. The average diversity and nucleotide diversity were 0.957 ± 0.007 and 0.02887 ± 0.00256 , respectively. A mantel test and the Analysis of Molecular Variance (ANOVA) indicated that there was no significant geographical structuring in Chinese tropical zone goat breeds. Mismatch analysis showed that haplogroup A and B had not experienced population expansion events. According to the study and previous evidence, researchers speculated that lineage B of goat breed might originate from Hainan Island of China.

Key words: Hainan black goat, phylogenetic, lineage B, Chinese tropical zone, diversity

INTRODUCTION

Mitochondrial DNA (mtDNA) is an excellent tool to study the evolution and phylogenetic relationship of biological species, especially the control region of mtDNA was used for describing genetic diversity and origin of domesticated species due to its constant rate. It has been used widely to study goat domestication. In previous studies, mtDNA analysis on modern domestic goats identified four main maternal lineages, termed as lineage A-D. Lineage A is the most diverse and widely distributed across all continents. Lineage B was detected mainly in Eastern and Southern Asia including China, Mongolia, Laos, Pakistan, India and Malaysia. Lineage C is observed with a few samples from Mongolia, Switzerland and Slovenia. Lineage D is rare and was only observed in Pakistan, India and China (Luikart *et al.*, 2001; Mannen *et al.*, 2001; Chen *et al.*, 2005; Fan *et al.*, 2007; Wang *et al.*, 2008; Kang *et al.*, 2011). There is a new minority lineage E detected in India (Joshi *et al.*, 2004). Another new mtDNA lineage F was found in Sicilian goats (Sardina *et al.*, 2006). Lineage G has been defined and localized around the Fertile Crescent (Naderi *et al.*, 2007). However, the origin and evolution of Chinese domestic goats have not been defined entirely.

The history of goat feeding in China is very long and the native domestic goat breeds are abundant. In recent years, many studies on the origin, evolution and genetic diversity of the majority of local goat breeds in China have been carried out and those goat breeds are distributed mainly over Northern, Central, West and Southwest China. Most of the previous studies shows that the genetic diversity of Chinese goat breeds is rich and there are two main maternal origin (A and B). Maternal C and D are only detected in Tibet, Inner Mongolia, Shandong, Shanxi and Hebei breeds (Fan *et al.*, 2007; Wang *et al.*, 2008; Wu *et al.*, 2009a, b) with low frequency of haplotype. Hainan Province situates at the Southernmost of China. It belongs to tropical monsoon climate with very distinguishable rainy and dry seasons each year. There are about 9 million Hainan black goats raising in the island. Up to now, few reports about the mtDNA genetic diversity and evolutionary origins of the Hainan black goats. To find some differences from other Chinese goat breeds and to find meaningful and valuable data for the origin of the goats, the research of the mtDNA Hypervariable Region (HVR) of Chinese tropical zone goat breeds was carried out. The results of this study can provide some basic data in genetic resources for the sustainable development of husbandry and the preservation of biological diversities in China.

MATERIALS AND METHODS

Samples collection: The ear tissues of 89 individuals were sampled from four breeds in the study. The breeds were Shizong black goat, SZ (Yunnan Province), Longlin black goat, LL (Guangxi Province), Leizhou black goat, LZ (Guangdong Province) and Hainan black goat, HN (Hainan Province). There were no genetic correlation among the individuals which provided by the owners and local breeding records. Genomic DNA was extracted according to a modified phenol and chloroform method.

PCR amplification and sequencing: The Hypervariable Region (HVR) of the mtDNA control region sequences was amplified and sequenced. The primers F (5'-CAT TACA CCG CTC GCC TAC-3') and R (5'-GGG CTG ATT AGT CAT TAG TCC A-3') (Wu *et al.*, 2009b) were used to amplify a 606 bp DNA fragment. The PCR mixture was 25 μ L reaction volume, containing 0.4 μ M of each primer, 160 μ M of each dNTP, 1.5 mM MgCl₂, 1 unit Taq DNA polymerase enzyme and 1.5 μ L of 50 ng L⁻¹ DNA templates. The PCR amplifications were conducted using a PTC-100 Thermal Controller (MJ Research Inc. USA) by the following program, 2 min, 95°C; 35 cycles of 30 sec, 95°C, 30 sec, 50°C, 1 min, 72°C and a final extension of 10 min, 72°C. PCR fragments were recovered in low melting agarose gel and purified using Promega's Wizard PCR Preps DNA Purification kit (Promega, USA). The purified PCR samples were sequenced commercially.

Statistical analysis: All the 89 sequences (JQ717071-717159) of mtDNA HVI region were aligned with the goat mtDNA D-loop (AF533441) using the Clustal W program (Thompson *et al.*, 1994). Analysis of the mtDNA haplotypes diversity and nucleotide diversity were performed using MEGA software package version 3.1 (<http://www.megasoftware.net/mega.html>) (Kumar *et al.*, 2004). The neighbor-joining trees were constructed from Kimura 2-parameter distance, assuming $\alpha = 0.29$ for gamma distribution. To compare the phylogenetic analysis with other studies, 9 sequences randomly chosen from lineage A (AJ37563, AJ317633 and AJ317593), lineage B (AJ317832, AJ317833 and AJ317826),

lineage C (AJ317834, AJ317835 and AJ317836), lineage D (AB110587, AB110588 and AB110589) were combined (Luikart *et al.*, 2001; Sultana *et al.*, 2003). Bootstraps of 1000 replicates were accounted to test the robustness of phylogeny tree. Median-joining network (Bandelt *et al.*, 1999) and mismatch analysis were carried out using the program Network 4.1 with weights = 10 and $\epsilon = 0$ (<http://www.fluxus-engineering.com>) to examine the possible relationships among haplotypes the four goats breeds. Haplotype diversity and its Standard Error (SE), nucleotide diversity (Schneider and Excoffier, 1999) were calculated using the software DnaSP version 4.0 with mtDNA model (<http://www.ub.es/dnasp>). AMOVA procedure (Excoffier *et al.*, 1992) was performed using ARLEQUIN v3.11 to test the partition of the genetic variance within breeds, among regions and among breeds within regions.

RESULTS AND DISCUSSION

Sequence polymorphism of HVR in Chinese tropical zone goat breeds: About 64 polymorphic sites were found in the 89 sequences (JQ717071-717159) which defined as 36 haplotypes. The haplotype diversity and nucleotide diversity of total individuals were 0.957 ± 0.0070 and 0.02916 ± 0.00256 , respectively. The detailed information about haplotype and nucleotide diversity and nucleotide differences value of each population was summarized in Table 1. In addition, the distribution of mtDNA haplotypes was heterogeneous (Fig. 1). Hap_2 and Hap_30 were the most common haplotypes which was presented in seven samples respectively, showing a frequency of 0.0787, followed by Hap_3, Hap_13, Hap_22, Hap_32 and Hap_33 ($n = 5$, 0.0562). And there were 14 haplotypes were each represented in only one sample. Moreover, only Hap_2 and Hap_3 were shared by LZ, LL and HN goat breeds; Hap_6 and Hap_12 were shared by SZ and LL goat breeds; Hap_8 was shared by LL and HN goat breeds, other haplotypes were not shared and there was no one haplotype were shared by all four breeds. The number of haplotypes detected in each goat breed varies from 3-13. Haplotype A and B frequencies of each breed was listed in Table 1.

Table 1: Genetic diversity parameter and haplotype frequencies of four goat breeds

Abbreviation of breed	Genetic diversity parameter						Haplotype frequencies	
	No.	S	H	Hd \pm SE	Pi \pm SE	K	A	B
HN	21	31	12	0.938 \pm 0.0300	0.01612 \pm 0.00255	9.771	16.67% (2/12)	83.33% (10/12)
LZ	20	31	5	0.747 \pm 0.0570	0.0249 \pm 0.00260	15.068	60% (3/5)	40% (2/5)
LL	18	46	13	0.961 \pm 0.0300	0.02624 \pm 0.00325	15.902	61.54% (8/13)	38.46% (5/13)
SZ	30	47	9	0.880 \pm 0.0280	0.02511 \pm 0.00287	15.168	88.89% (8/9)	11.11% (1/9)
Total	89	64	36	0.957 \pm 0.0007	0.02916 \pm 0.00256	17.642	47.22% (17/36)	36.11% (13/36)

No. = Number of individuals; S = Number of polymorphic sites; H = Number of Haplotypes; Hd = Haplotype diversity; Pi = Nucleotide diversity; K = Average number of nucleotide differences

Haplotype ID	Haplotype sequences definition	Relative frequencies	Haplotype sharing by individual
Hap_1	TCCCGGACTTACAACTCAACAGAGTTTTFSGTTCAGCATTCCTTAGACCTTACTGCTCCCC	0.0112	[LL01]
Hap_2T.....G.....A.....	0.0787	[LL02 LL03 LL06 HN18 HN19 HN21 LZ01]
Hap_3T.....G.....A.....	0.0562	[LL04 HN11 LZ08 LZ12 LZ19]
Hap_4T.....G.....A.....	0.0112	[LL05]
Hap_5TAGG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[LL07 LL14]
Hap_6T.....G.....C.....C.....C.....G.....G.....G.....T.....C.....T.....T.....	0.0337	[LL09 SZ12 SZ32]
Hap_7T.....G.....G.....C.....G.....G.....G.....T.....C.....T.....T.....	0.0225	[LL10 LL11]
Hap_8T.....AG.....C.....T.....A.....ACCC.....C.....CTT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[LL12 HN12]
Hap_9T.....G.....G.....A.....AC.....C.....G.....C.....CG.....T.....C.....T.....T.....	0.0225	[LL13 LL17]
Hap_10T.....T.....G.....A.....T.....C.....G.....G.....T.....CG.....T.....T.....	0.0112	[LL15]
Hap_11T.....G.....G.....C.....C.....G.....G.....T.....C.....T.....T.....	0.0112	[LL16]
Hap_12T.....A.....C.....T.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0449	[LL18 SZ1 SZ18 SZ36]
Hap_13T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0562	[LL19 HN05 HN14 HN15 HN22]
Hap_14T.....AG.....C.....T.....A.....ACCC.....C.....CTT.....G.....TCC.....A.....T.....C.....A.....T.....T.....	0.0112	[HN01]
Hap_15T.....AG.....C.....T.....A.....ACCC.....C.....CTT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[HN02 HN13]
Hap_16T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....A.....T.....T.....	0.0225	[HN03 HN36]
Hap_17T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[HN06 HN24]
Hap_18T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[HN07 HN08]
Hap_19T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0112	[HN09]
Hap_20T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[HN16 HN23]
Hap_21T.....G.....G.....A.....T.....C.....C.....T.....T.....	0.0337	[LZ02 LZ18 LZ20]
Hap_22T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0562	[LZ03 LZ04 LZ21 LZ22 LZ23]
Hap_23T.....G.....G.....A.....T.....C.....C.....T.....T.....	0.0112	[LZ05]
Hap_24TC.....T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0112	[LZ07]
Hap_25T.....AG.....C.....T.....A.....ACCC.....C.....CTT.....G.....TC.....A.....T.....C.....A.....T.....T.....	0.0112	[LZ09]
Hap_26T.....AG.....C.....T.....A.....ACCC.....C.....TCCT.....G.....TCC.....A.....T.....C.....T.....T.....	0.0225	[LZ13 LZ15]
Hap_27G.....T.....G.....G.....A.....T.....C.....T.....T.....	0.0112	[LZ14]
Hap_28C.....T.....G.....G.....A.....T.....C.....C.....T.....T.....	0.0112	[LZ16]
Hap_29C.....T.....G.....G.....A.....T.....C.....C.....T.....T.....	0.0112	[LZ17]
Hap_30T.....T.....G.....G.....A.....C.....C.....TG.....C.....CG.....T.....C.....T.....T.....	0.0787	[SZ23 SZ24 SZ39 SZ15 SZ17 SZ20 SZ27]
Hap_31TT.....C.....G.....A.....C.....G.....C.....G.....G.....T.....T.....C.....TTT.....	0.0225	[SZ23 SZ28]
Hap_32T.....G.....G.....C.....C.....G.....C.....G.....G.....T.....T.....T.....	0.0562	[SZ25 SZ26 SZ11 SZ16 SZ30]
Hap_33T.....G.....G.....C.....C.....G.....C.....G.....G.....T.....T.....T.....	0.0562	[SZ10 SZ40 SZ21 SZ24 SZ31]
Hap_34T.....A.....C.....T.....T.....T.....A.....ACCC.....C.....TCCT.....A.....TCC.....A.....T.....C.....C.....T.....T.....	0.0449	[SZ19 SZ23 SZ29 SZ33]
Hap_35T.....G.....G.....A.....T.....C.....G.....G.....T.....C.....T.....T.....	0.0112	[SZ22]
Hap_36T.....G.....G.....C.....C.....CG.....G.....GA.....T.....T.....T.....	0.0112	[SZ25]

Fig. 1: Haplotype definition, relative frequencies and haplotype sharing sequences

Phylogenetic analysis: A neighbour-joining phylogeny was constructed for the four goat breeds with 10 sequences from GenBank (Fig. 2). In the phylogeny tree, all sequences of the samples divided into two distinct lineage (A and B), lineage A was predominant including 51 individuals and accounting for 57.30% of all samples and lineage B including 38 individuals, accounted for 42.7% of all samples. Lineage A and B were presented in all breeds, the frequencies of individual in lineage were shown in Table 2. The result showed that the proportion of lineage B was up to 80.95% in Hainan black goat and lineage A was only 19.05%. However, the proportion of lineage A accounted for 76.67% and lineage B accounted for 23.33% in YN. The proportion of lineage A and lineage B in LL and LZ were equilibrium relatively. Furthermore, the median-joining network shown that 36 haplotypes divided two clusters named haplogroup A and B which was shown in Fig. 3.

Population genetic structure and expansion: The hierarchical analysis of molecular variance revealed that there was a large percentage (95.75%, $p < 0.0001$) of total mtDNA variation existed within populations and a smaller percentage (4.25%, $p = 0.02346$) was among populations as shown in Table 3. This result showed that there was no significant geographical structuring in Chinese tropical zone goat breeds. In addition, the Fu's F_s neural test were used to detect the population expansion for all goat breeds in Chinese tropical zone at lineage level because of the small sample size in our sampling (<30 individuals). The mismatch distribution analysis of complete dataset, haplogroups A and B of mtDNA D-loop were as shown in Fig. 4. The complete dataset of all goat breeds in Chinese tropical zone had a positive Fu's

F_s and p -value (1.820, $p = 0.85800$) and Fu's F_s and p -value of haplogroups A and B (1.08352, $p = 0.71500$ and -0.57600 , $p = 0.45800$, respectively). These results suggested that might one population expansion event happened in Chinese tropical zone goat breeds with small departure from neutrality but not significant, haplogroup A and B showed no population expansion and relatively stable population sizes because of no significant difference from neutrality and these populations were relatively stable.

Polymorphism of HVR in Chinese tropical zone goat breeds: Haplotype diversity and nucleotide diversity of mtDNA are two important indices for assessing population polymorphism and genetic differentiation (Pereira *et al.*, 2005). The average haplotype diversity and nucleotide diversity of the study were 0.957 ± 0.0070 and 0.02916 ± 0.00256 , respectively. Its worth to note that the haplotype diversity, nucleotide diversity and average number of nucleotide differences in LL was higher than other groups, the nucleotide diversity and average number of nucleotide differences in HN was very low, this result was not consistent with Wu *et al.* (2009a). The results showed that HN was the lowest variability breed and its genetic diversity was lower than other goat breeds (Wang *et al.*, 2008; Wu *et al.*, 2009b; Zhao *et al.*, 2011). The results of present study indicated that the Hainan black goat breed was a purebred and it should be protected suitably. In addition, all the 89 sequences defined as 36 haplotypes but there was no 1 haplotype was shared by all of the four breeds. The most common haplotypes (Hap_2 and Hap_3) were shared by LZ, LL and HN goat breeds. The number of detected haplotypes in each goat breed was varied from 3-13. The

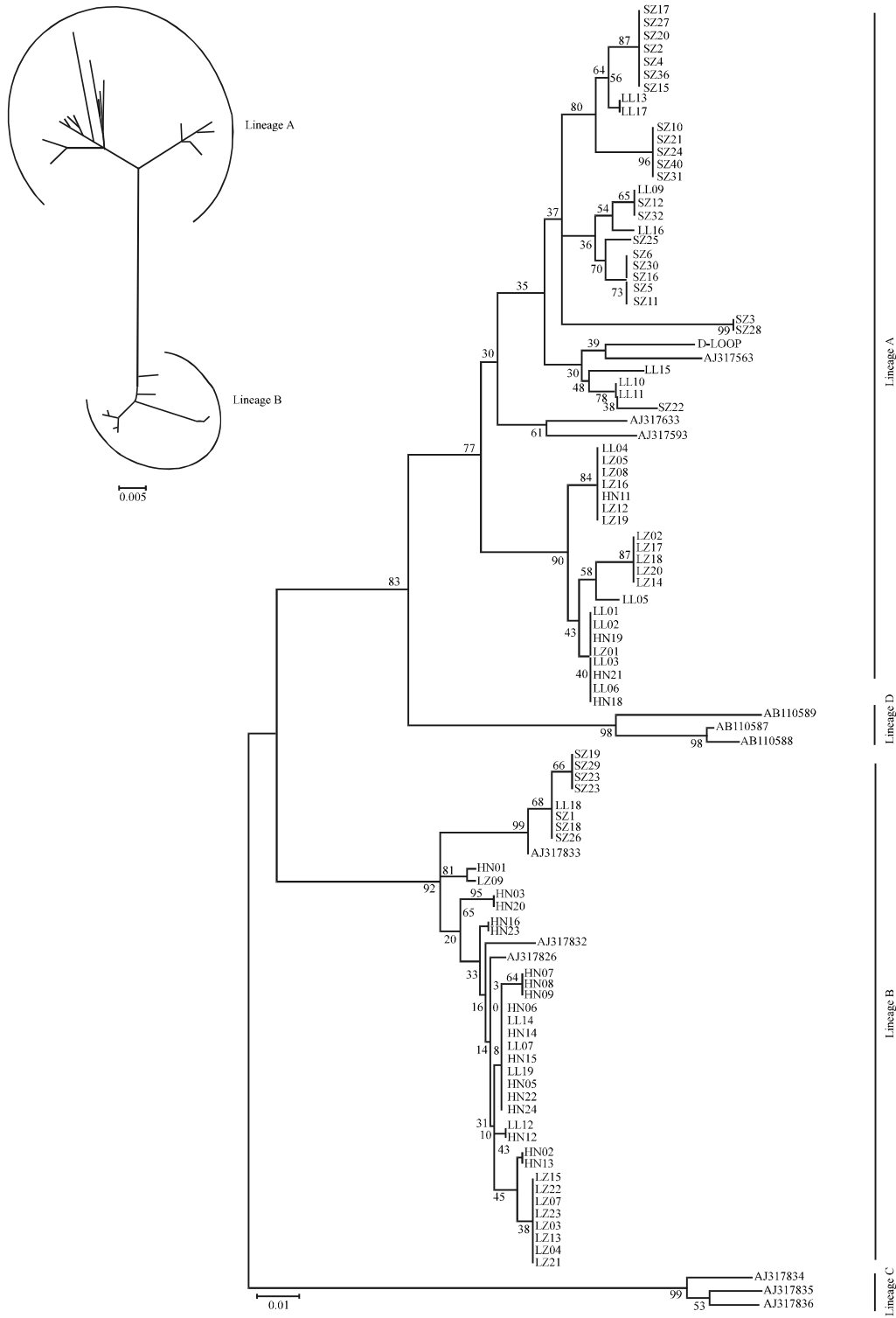


Fig. 2: The phylogenetic tree of all four goat breeds

haplotype frequency of LZ was very low (25.00%), in LL was very high (72.22%). The frequency of haplotype A

and B of each breed was differences. Its special to note that haplotype A and B in HN group was 16.67 and

Table 2: Individual frequencies of lineage in each goat breed

Abbreviation of breed	Individual frequencies of lineage	
	A	B
HN	19.05% (4/21)	80.95% (17/21)
LZ	55% (11/20)	45% (9/20)
LL	72.22% (13/18)	27.78% (5/18)
SZ	76.67% (23/30)	23.33% (7/30)
All	57.30% (51/89)	42.70% (38/89)

Table 3: Analysis of Molecular Variance (AMOVA) of the four goat breeds based on mtDNA genetic variance

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	p-value
Among populations	3	29.765	0.21819	4.25	0.02346
Within populations	89	437.439	4.91504	95.75	<0.0001
Total	92	467.204	5.13324	-	-

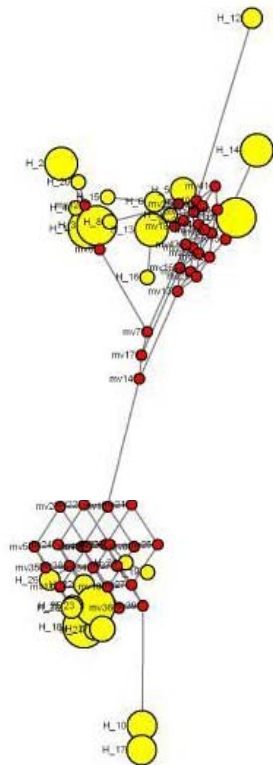


Fig. 3: Median-joining network of haplotype for all four goat breeds

83.33%, respectively as haplotype A was very low, haplotype B was very high. This finding was different from the previous studies on other Chinese goat breeds (Wang *et al.*, 2008; Zhao *et al.*, 2011). In contrast, the frequency of haplotype A and B in SZ was 88.89 and 11.11%, respectively. This result was similar with the previous studies (Zhao *et al.*, 2011).

Lineage: The neighbour-joining phylogeny tree and the median-joining network (Fig. 2 and 3) of these goat breeds shown that there were two lineages (A and B) in Chinese tropical zone, in which lineage A and B were accounting for 57.30 and 42.7%, respectively not found lineages (C-G) ((Naderi *et al.*, 2007). This finding was consistent with the previous studies on other goats breeds in China (Zhao *et al.*, 2011; Liu *et al.*, 2009).

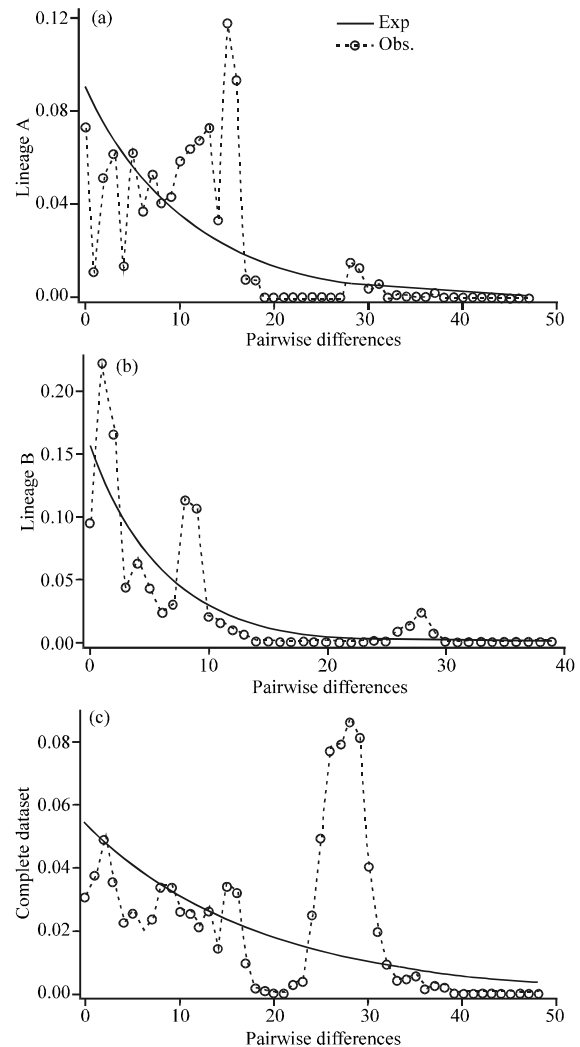


Fig. 4: Mismatch distributions of mtDNA haplogroup (A and B) and complete dataset

However, Fan *et al.* (2007) found lineage C and D in Tibet breeds. Wang *et al.* (2008) found lineage C in Hebei and Inner Mongolia breeds, lineage D was found in Hebei, Shandong and Inner Mongolia breeds. Wu *et al.* (2009a) found lineage C in Shanxi, Shandong and Inner Mongolia breeds, lineage D was found in Qinghai and Tibet breeds. In addition, lineage C and D were not found in other

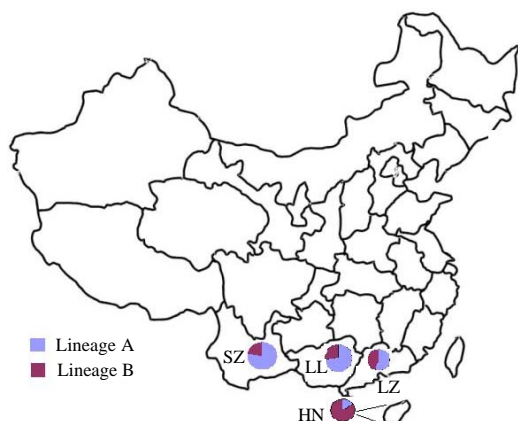


Fig. 5: Geographic distribution of lineage (A and B) of goat breeds in subtropical and tropical of China, detail number showed in Table 4. The area of each circle is proportional to sample size

provinces breeds in China and these results revealed that the proportion for lineage C and D were very low. Overall up to now, lineages (E-G) were not found in China and lineages (C and D) were not found in Southwest China and Chinese tropic zone. All the previous research results showed that the proportion for lineage A was very high in all other Chinese goat breeds (Wang *et al.*, 2008; Wu *et al.*, 2009b; Zhao *et al.*, 2011). Nevertheless, the interesting thing of the study was that the proportion of lineage B was up to 80.95% in Hainan black goat and lineage A was only 19.05%. This result was very different from the previous studies of other goat breeds in China. Its not present in the previous research about other Chinese goats breeds (Liu *et al.*, 2009; Wang *et al.*, 2008; Zhao *et al.*, 2011). Researchers also found another interesting phenomenon, the climate gradually changed (from temperate to subtropical to tropical) with the geographical distribution (from Yunnan to Guangxi to Guangdong to Hainan Island). While the lineage A of goat breed gradually weakened, the lineage B gradually increased with the change and lineage B was up to a highly percentage in Hainan Island breed (Fig. 5). Whether the origin of species evolution was related to climate changes except the geographical distribution, researchers can not give any conclusions, perhaps the result was just a coincidence. Luikart *et al.* (2001) revealed that lineage A existed in the all breeds but lineage B occurred only in Eastern and Southern Asia breeds (India, Pakistan, Mongolia and Malaysia). Wu *et al.* (2009a) and Zhao *et al.* (2011) speculated that lineage B might originated from Southwestern Region of China. However, researchers think that their evidence was insufficient to prove the origin of lineage B. According to all the previous evidence and the study, researchers speculated that lineage B might originated from Hainan Island of

China or other counties near Hainan Island or the Southeast Asian and then they gradually pervade to the Southwest and Northeast China, the number of which gradually reduced or even disappeared as the geographical distribution farther and farther. It was not found in the Inner Mongolia and Liaoning Cashmere breeds (Wang *et al.*, 2008). Maybe the genetic information gradually lost in spreading and evolution with the geographical and breeding environment changes. In order to fully prove the origin of lineage B of goat breed, researchers will collect the samples of goat breeds from near countries (Vietnam, Laos, Cambodia, Thailand and Myanmar) to further study.

Population genetic structure and expansion: Goat is a portable food resource accompanying human migratory movements from time immemorial. The commercial trade and extensive transport of goats would account for the observed pattern by having favored genetic exchange (Azor *et al.*, 2005; Liu *et al.*, 2009; Amills *et al.*, 2009; Zhao *et al.*, 2011). In previous studies, Naderi *et al.* (2007) reported that 77% of mitochondrial DNA variation distributed within breeds. In Indian goats, Joshi *et al.* (2004) found that 83% of the total molecular variance was included in the within-breed component. In the study, the mtDNA genetic variance component within breed was up to 95.75 and only 4.25% of genetic variation was observed among the four populations. This result showed that there was no significant geographical structuring in Chinese tropical zone goat breeds which was similar with Zhao *et al.* (2011) but which was different from other previous studies (Luikart *et al.*, 2001; Chen *et al.*, 2005; Wang *et al.*, 2008; Liu *et al.*, 2009; Amills *et al.*, 2009; Wu *et al.*, 2009a). In this study, the mismatch analysis results suggested that might one population expansion event happened in Chinese tropical zone goat breeds with small departure from neutrality but not significant. But, haplogroup A had not experienced population expansion events because of no significant difference from neutrality. It was different from the previously reports (Liu *et al.*, 2006; Hou *et al.*, 2008; Zhao *et al.*, 2011). There was no population expansion event happened in haplogroup B too. And these populations also were relatively stable which was same to the previously reports (Liu *et al.*, 2009; Zhao *et al.*, 2011) but it was different from Chen *et al.* (2005) and Wu *et al.* (2009b).

CONCLUSION

There were two lineages (A and B) in Chinese tropical zone goat breeds. The proportion of lineage B was up to 80.95% in Hainan black goat, lineage A was only 19.05%. Researchers speculated that lineage B of goat breed might originate from Hainan Island of China or other counties

near Hainan Island. Hainan black goat had the lowest genetic variability. It was a purebred and it should be protected suitably. There were no significant geographical structure and population expansion events in these populations.

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