

Dissecting the Matrilineal Components of Tongjiang Cattle from Southwest China

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Abstract Tongjiang cattle are a local cattle population of Sichuan Province, China, numbering approximately half a million in 2005. They have long been grouped into the Bashan breed, although they have a unique breeding history and phenotypic characteristics, as well as a restricted geographic distribution. Morphologically, they can be divided into two groups based on the basic coat color (black and russet). In order to dissect the matrilineal components of Tongjiang cattle and to compare the body size traits of the two morphological groups, we measured five body size traits among 59 Tongjiang cattle samples and further sequenced the mtDNA D-loop sequence of 54 individuals. Among the 54 mtDNAs, 37 (68.5%) were *Bos taurus* types and 17 (31.5%) were *Bos indicus* types. Four known *B. taurus* haplogroups (T1–T4) and one *B. indicus* haplogroup (I1) were detected in these samples. Two body size traits differed significantly ($P < 0.05$) between the black group and the russet group, although the two groups possessed similar matrilineal genetic structure. This is the first report to identify all four *B. taurus* haplogroups in one local Chinese cattle population. Our results suggest that the contribution of different matrilineal lineages to Chinese cattle might be more complex than we originally thought.

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Introduction

According to the livestock investigation in 2001, China has abundant cattle genetic resources, with 52 local breeds, 5 developed breeds, and 12 introduced breeds (Chen 2004). Based on the geographic distribution and phenotype characteristics, the local cattle breeds can be divided into three main groups such as a northern group (distributed in north China), a central group (in the middle and lower areas of the Yellow River and the Huaihe River), and a southern group (in South China). Their body size declines continuously along a north-to-south axis (Qiu et al. 1988). In recent years, many local cattle breeds (with unique characteristics) have been crossbred with the introduced breeds to improve production performance, but little attention has been paid to maintain these genetic resources. The population size of a few local cattle breeds has decreased dramatically, bringing the breeds close to extinction (Ma et al. 2002; Chen 2004). Fortunately, 12 local cattle breeds were recently added to the National Genetic Resources Protection list (http://www.agri.gov.cn/blgg/t20060609_626418.htm). The conservation and utilization of local breeds have become urgent for sustainable development of agriculture in China.

Previous studies of Chinese local cattle breeds using blood protein polymorphisms (Wang et al. 1991; Geng et al. 1995), mtDNA restriction fragment length polymorphisms (Lan et al. 1993; Wen and Wang 1995; Yu et al. 1999), and mtDNA D-loop and cytochrome *b* sequence variations (Lei et al. 2004, 2006; Lai et al. 2005, 2006) have cast many insights on the genetic diversity, origin, and taxonomy of these breeds. Most of the molecular data support the view that Chinese cattle originated from two matrilineal sources, *Bos taurus* and *Bos indicus* (Wang et al. 1991; Yu et al. 1999; Lei et al. 2004, 2006; Lai et al. 2005, 2006). Among the five known *B. taurus* mtDNA lineages (T and T1–T4; Troy et al. 2001; Mannen et al. 2004) and two *B. indicus* mtDNA lineages (I1 and I2; Lai et al. 2006) in cattle, lineages T2–T4, I1, and I2 have been detected in various Chinese local cattle breeds, and they present a marked pattern of geographic distribution (Lai et al. 2006; Lei et al. 2006). The *B. taurus* lineage T3 was predominant, compared with T2 and T4, in all studied Chinese local breeds (Lai et al. 2006). Cattle from South and Southwest China received more matrilineal contributions from *B. indicus* than from *B. taurus*. Moreover, the *B. indicus* lineage I1 was present with high frequency in most of the southern breeds, whereas I2 appeared in only three breeds from Southwest China with low frequency (Lai et al. 2006; Lei et al. 2006).

The Tongjiang cattle population is reared in Sichuan Province of Southwest China, particularly in Tongjiang County. According to our field investigation in 2005, Tongjiang cattle number about half a million, and more than 40% are distributed in Tongjiang County. Morphologically, Tongjiang cattle show some features of a mixture of *B. taurus* and *B. indicus* and can be divided into two groups,

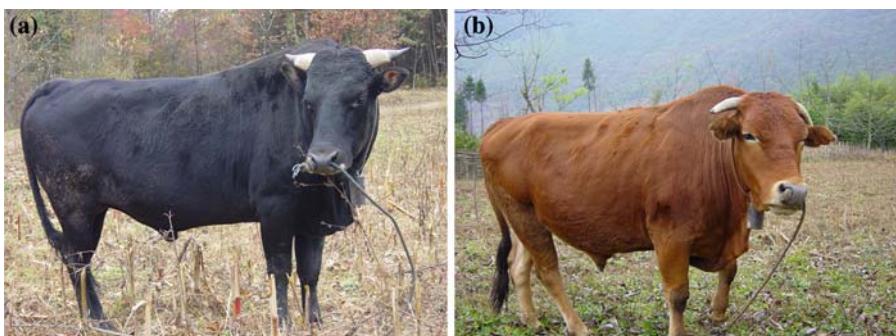


Fig. 1 Tongjiang cattle with distinct black (**a**) and russet (**b**) body coat color

a black group and a russet group, based on the basic coat color (Fig. 1). The body-weight range is 450–700 kg in adult males and 300–450 kg in adult females. Tongjiang cattle have long been bred and isolated as a single local cattle population. At the same time, another cattle population, called Xuanhan cattle, was reared in the adjacent Xuanhan County (Fig. 2). Owing to a few morphological differences and the adjacent geographic distribution of the two populations, the Tongjiang population has been considered a subgroup of Xuanhan cattle (Qiu 1987). Based on geographic distribution and phenotypic characteristics, Qiu and colleagues (1988) proposed that Xuanhan cattle (including Tongjiang cattle) and two other

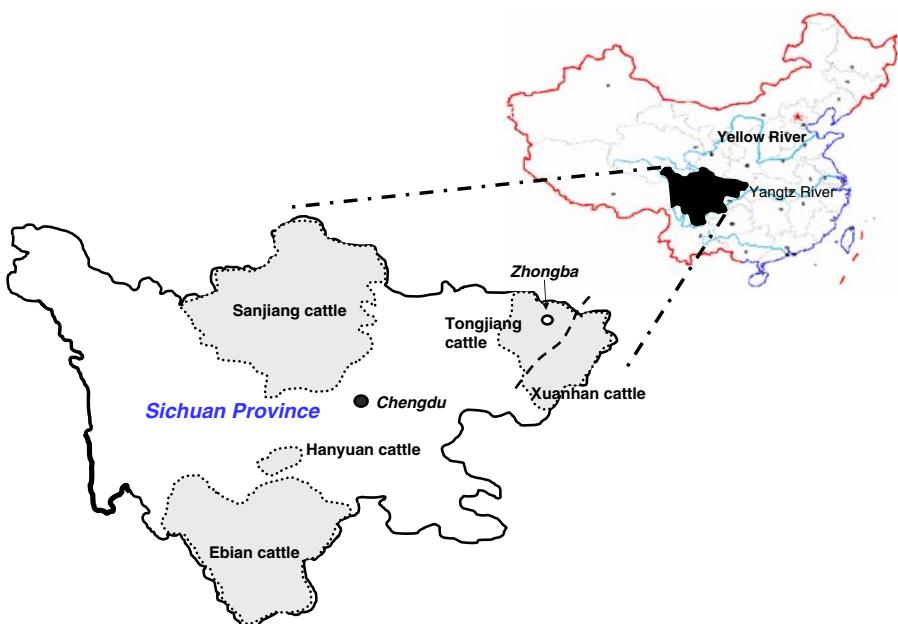


Fig. 2 The geographic distribution of Tongjiang cattle and four reported local cattle breeds or populations from Sichuan Province, China

local cattle populations (one from the boundary habitat between Sichuan and Hubei and the other between Sichuan and Shaanxi) should be grouped together and given the name “Bashan breed.” In contrast, based on breeding history and phenotypic characteristics, we are inclined to consider Tongjiang cattle as a unique local breed rather than a subpopulation of the Bashan breed. In order to scrutinize the traditional breed classification of Tongjiang cattle, we studied here the matrilineal origin and genetic diversity of this local population based on mtDNA D-loop sequence variation. We also compared the body size traits of the two morphological groups in Tongjiang cattle.

Materials and Methods

Measurement of Body Size and Sampling

Five body size traits, including hip height (HH), body length (BL), chest girth (CG), circumference of cannon bone (CCB), and body live weight (BLW), were measured among 59 Tongjiang cattle from the Zhongba village in north Tongjiang County (Fig. 2). The mean age of these cattle was 3.8 ± 1.3 years, ranging from 1.5 to 7.0 years (Table 1). Ear tissue samples were collected in 54 samples for mtDNA analysis.

Amplification and Sequencing of mtDNA

Genomic DNA was extracted by the standard phenol/chloroform method. The entire mtDNA D-loop sequence was amplified and sequenced using the same method described in our recent studies (Lai et al. 2005, 2006). In brief, PCR amplification

Table 1 Body size traits in Tongjiang cattle

Color group	Sex (N)	Age (years)	Body size trait				
			HH (cm)	BL (cm)	CG (cm)	CCB (cm)	BLW (kg)
Black	♀ (7)	2–6	93–114	114–150	143–165	13–16	250–370
	♂ (18)	1.5–6	105–135	107–150	140–180	13–20	238–463
Subtotal	–	3.8 ± 1.4	116.6 ± 9.2	130 ± 12.4	161.6 ± 12.6	16.3 ± 1.8	353.3 ± 72.4
Russet	♀ (13)	1.5–7	98–116	111–179	142–174	14–17	246–425
	♂ (21)	1.5–6	100–140	112–158	152–201	15–21	292–630
Subtotal	–	3.8 ± 1.3	118.1 ± 11.0	135 ± 15.2	171.0 ± 15.3	17.0 ± 1.8	412.9 ± 100.0
Total	–	3.8 ± 1.3	117.4 ± 10.2	133.3 ± 14.2	167.0 ± 14.9	16.7 ± 1.8	388.3 ± 93.7

HH, hip height; BL, body length; CG, chest girth; CCB, circumference of cannon bone; BLW, body live weight; Values (mean and standard deviation) for CG and BLW are significantly different between the black group and the russet group ($P < 0.05$)

was performed in a 50 μ l reaction mixture containing 100 ng DNA, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 50 mM KCl, 10 pM each primer (forward, 5'-CTG CAG TCT CAC CAT CAA CC-3' (Loftus et al. 1994); reverse, 5'-GAT TAT AGA ACA GGC TCC TC-3'), and 1 U *Taq* polymerase (S_{ABC}) following 35 cycles of 50 s at 94°C, 30 s at 58°C, and 90 s at 72°C. PCR products were purified on spin columns and were directly sequenced for both strands using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI Prism 3100 DNA sequencer according to the manufacturer's manual.

Data Analysis

We first constructed a rooted neighbor-joining (NJ) phylogenetic tree using *Bos grunniens* (GenBank acc. no. AY521137) as the outgroup to discern the *B. taurus* and *B. indicus* status of the 54 mtDNAs sequenced in this study. The *B. taurus* and *B. indicus* types were further aligned to the respective reference sequences of *B. taurus* (acc. no. V00654; Anderson et al. 1982) and *B. indicus* (acc. no. L27733; Loftus et al. 1994). Sequence variation was exposed using MEGA 2.1 (Kumar et al. 2001), and gaps in the aligned sequences were excluded in the following analyses.

We classified the *B. taurus* and *B. indicus* types according to the respective nomination systems defined by Troy et al. (2001), Mannen et al. (2004), and our recent study (Lai et al. 2006). The distribution pattern of each matrilineal component in Tongjiang cattle was then estimated and compared with the other local breeds or populations from Sichuan Province. To discern whether Tongjiang cattle with distinct black and russet color have different genetic diversities, we estimated the haplotype diversity and nucleotide diversity (Nei 1987) using DnaSP 4.10 (Rozas et al. 2003). We also compared the means and standard deviations of the five measurement traits based on the classification of the body coat color. The 54 mtDNA D-loop complete sequences of Tongjiang cattle have been deposited in GenBank under acc. nos. EF417933–EF417986.

Results

Distribution Pattern of mtDNA Lineages

The rooted NJ tree revealed two major clades corresponding to *B. taurus* and *B. indicus*, with strong bootstrap support (Fig. 3). Among the 54 mtDNAs, 37 (68.5%) were sorted into *B. taurus* clade and 17 (31.5%) were *B. indicus* types. In total, 18 haplotypes defined by 30 nucleotide substitutions were recognized in Tongjiang *B. taurus* samples. Among them, haplotype C2 was predominant, appearing in 10 individuals, whereas 10 haplotypes were found in only one sample. Four haplotypes were identified in 17 *B. indicus* individuals (Fig. 4).

The haplogroup classification of the Tongjiang sample revealed that it contained all four known *B. taurus* haplogroups (T1–T4) and one *B. indicus* haplogroup (I1) (Fig. 4). Among the 37 *B. taurus* sequences, 28 samples (75.7%) could be assigned

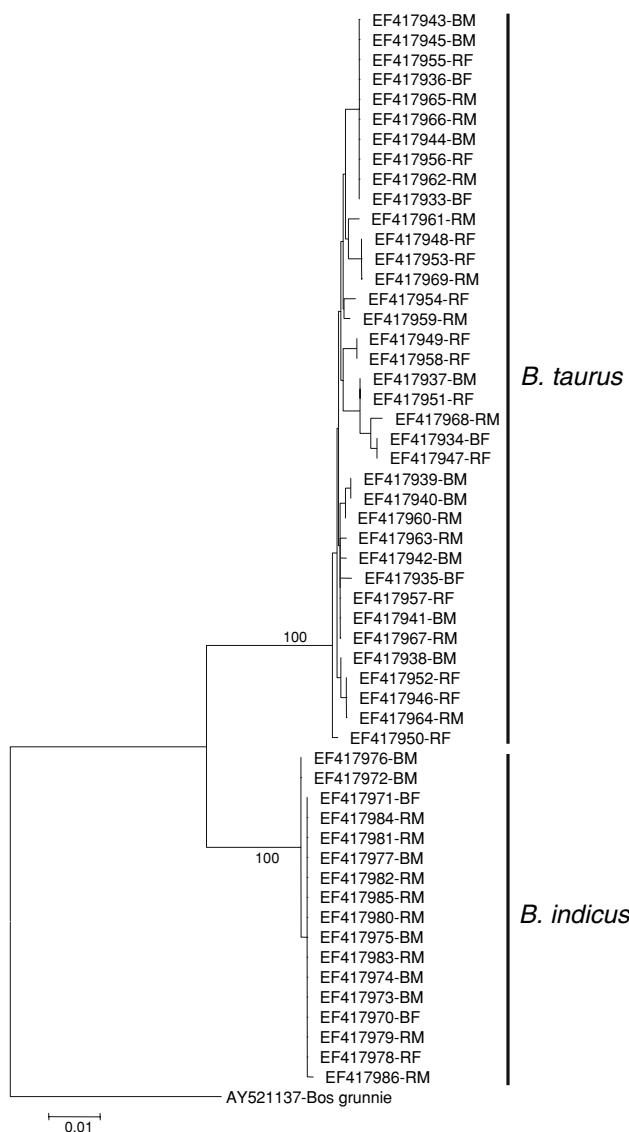


Fig. 3 Neighbor-joining tree of complete mtDNA D-loop sequences of 54 Tongjiang cattle. The tree was rooted by *Bos grunniens* (GenBank acc. no. AY521137). The values on the branches are bootstrap support based on 1,000 replications. Animals with black (B) and russet (R) coat color are designated by a letter combination with gender (M, male; F, female) after their GenBank accession number (e.g., BM indicates a male animal with black coat color)

to haplogroup T3, and this distribution pattern was consistent with our previous observation (Lai et al. 2006). Haplogroups T1, T2, and T4 were found in 1, 3, and 5 samples, respectively (Table 2; Fig. 4). The absence of *B. indicus* haplogroup I2 in the Tongjiang sample was not surprising, as this matrilineal component is prevalent

	1111111111 1111111111 111111		
	55566666666 66666666666 666666		
	9990000000 0000111112 2222331112		
	1384555556 8889111282 3455000672		
	0972035787 3453029758 2805126935		
ST	TGATCTTGCA ACTGCTTCGA CCATCGTAAA	N	HG
c1C.....G..	1	T3
c2C.....CGG.	10	T3
c3C..... T..G.G	1	T3
c4C..... G..	3	T3
c5C..... GG..	1	T3
c6C..T....G..	1	T3
c7C..G.....CG..	1	T3
c8	.A.....C.....G..	1	T3
c9G.....C.....GG..	2	T3
c10G.....C..A.G..	2	T3
c11T.....C.....G..	1	T3
c12T..A.CT.....G..	3	T3
c13	C.....C.....G..	1	T3
c14T.....C..CG..	1	T1
c15C.....A..C..G..	3	T2
c16	...C.....A.....A.G..	2	T4
c17	...CT...T..CA.....T..A.G..	1	T4
c18	..GCT.....A.....T..A.G..	2	T4
		249	
		620	
		617	
		ST TTG	N HG
		i1 ...	5 I1
		i2 ..C	9 I1
		i3 .CC	1 I1
		i4 C.C	2 I1

(a)

(b)

Fig. 4 Mitochondrial DNA sequence variations of (a) 18 *Bos taurus* haplotypes in 37 Tongjiang cattle samples and (b) 4 *Bos indicus* haplotypes in 17 Tongjiang cattle samples. Variable sites were scored relative to the standard sequences of *B. taurus* (acc. no. V00654) and *B. indicus* (L27733). The number of individuals sharing a haplotype is given in the column N, followed by the haplogroup status (column HG) of each haplotype. Gaps are excluded, and a filled dot denotes identity with the reference sequence

in South Asia and the Philippines and presents at a low frequency in cattle samples from South and Southwest China (Lai et al. 2006).

Genetic Diversity and Statistical Analysis of Body Size Traits

In all 54 Tongjiang cattle samples, haplotype diversity was 0.895 ± 0.028 and nucleotide diversity was 0.02306 ± 0.00183 . When we sorted these samples into the black and russet groups, the black group had lower haplotype diversity but slightly higher nucleotide diversity compared with the russet group (Table 3).

The measurements of the five body size traits among 59 Tongjiang cattle samples are shown in Table 1. The means and standard deviations of the traits were 117.4 ± 10.2 cm for hip height, 133.3 ± 14.2 cm for body length, 167.0 ± 14.9 cm for chest girth, 16.7 ± 1.8 cm for cannon bone circumference, and 383.3 ± 93.7 kg for body live weight. Among those traits, hip height, body length, and cannon bone circumference in the black group were not significantly different from the russet group. The two remaining traits, chest girth and body live weight, did differ significantly ($P < 0.05$) between the color groups, with higher values in the russet group.

Table 2 Matrilineal components of five local cattle breeds or populations distributed in Sichuan Province

Breed/ population	GenBank nos.	N	Matrilineal component (%)				
			T1	T2	T3	T4	I1
Tongjiang	EF417933–86	54	1 (1.9)	3 (5.6)	28 (51.9)	5 (9.3)	17 (31.5)
Bashan	AY521083–87; AY902385–86	7	0 (0.0)	1 (14.3)	1 (14.3)	2 (28.6)	3 (42.9)
Hanyuan	AY521090–93	4	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)
Ebian	AY521088–89; AY902387–89	5	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	0 (0.0)
Sanjiang	AY521112–16; AY902396–97	7	0 (0.0)	0 (0.0)	4 (57.1)	1 (14.3)	2 (28.6)
Subtotal ^a	–	23	0 (0.0)	1 (4.3)	14 (60.9)	3 (13.0)	5 (21.7)
Total	–	77	1 (1.3)	4 (5.2)	42 (54.5)	8 (10.4)	22 (28.6)

^a Pooled samples from Bashan, Hanyuan, Ebian, and Sanjiang cattle, which were reported in our recent study (Lai et al. 2006)

Table 3 Genetic diversity of Tongjiang cattle samples

Color group (N)	Species N		Haplotype diversity ($h \pm SD$)	Nucleotide diversity ($\pi \pm SD$)
	<i>Bos taurus</i>	<i>Bos indicus</i>		
Black group (21)	13	8	0.871 ± 0.050	0.02446 ± 0.00232
Russet group (33)	24	9	0.913 ± 0.031	0.02234 ± 0.00284
Total (54)	37	17	0.895 ± 0.028	0.02306 ± 0.00183

Discussion

All domesticated cattle are believed to be derived from *Bos primigenius*, containing two separate species, *B. taurus* (humpless) and *B. indicus* (humped), with independent domestication (Loftus et al. 1994). Among the four *B. taurus* mtDNA lineages (T and T1–T3) characterized by Troy et al. (2001), haplogroups T, T2, and T3 appeared in Anatolia and the Middle East, and T3 was the predominant haplogroup; haplogroup T1 was dominant in Africa. Based on the distribution pattern of these lineages, Troy et al. (2001) concluded that European cattle were of Near East origin and African cattle were independently domesticated. Mannen et al. (2004) found a new *B. taurus* mtDNA lineage (T4), which was specific to northeast Asian cattle, and claimed an independent matrilineal domestication. Chen et al. (1990) suggested that Chinese *B. indicus* cattle had two main origins, one from Southeast Asia and another from Africa and West Asia. Based on an extensive distribution of *B. indicus* mtDNA lineage I1 in cattle breeds from South and Southwest China, we thought that Chinese *B. indicus* cattle were probably introduced from South Asia or India after the initial domestication (Lai et al. 2006). Another *B. indicus* lineage, I2, was found only in a few breeds located around the Yunnan–Guizhou plateau, which is geographically closer to South Asia, and is probably descended from Indian zebu via Burma (Lei et al. 2006).

In the present study, we characterized the matrilineal components of the Tongjiang cattle population from Southwest China. We identified all four *B. taurus* mtDNA lineages (T1–T4) in this breed. There was no introgression of yak lineage in this sample (Yu et al. 1999; Lai et al. 2006). Of interest, haplogroup T1 was also found in two samples from two breeds sampled in North China, in a recent study by Lei et al. (2006), and shared the same sequence with the T1 type in our sample. Haplogroup T3 was the predominant haplogroup in Tongjiang cattle, and this distribution pattern of *B. taurus* mtDNA lineages was consistent with previous reports (Lai et al. 2006; Lei et al. 2006). Compared with the other four local breeds or populations from the same province (reported in our previous study, Lai et al. 2006), Tongjiang cattle presented some differences in their haplogroup distribution frequency (Table 2). However, the sample size of each of the four reported cattle populations (from 4 to 7 samples per population) was small, and we do not know whether the observed difference would be robust when more samples are analyzed. We speculate that the small sample size of the four local breeds or populations might not be enough to sample the T1 type. An alternative explanation is that the T1 lineage was recently introduced into the Tongjiang matrilineal pool from other parts of China. The presence of the T1 lineage in Tongjiang cattle from Southwest China (this study) and other cattle breeds from North China (Lei et al. 2006), although at very low frequency, suggests that the contribution of different matrilineal lineages to Chinese cattle might be more complex than we had thought. In the two *B. indicus* mtDNA lineages that were defined in our recent study (Lai et al. 2006), only I1 was detected in Tongjiang cattle, with a high proportion (31.5%). That distribution is consistent with the genetic pattern described in Lai et al. (2006) and Lei et al. (2006).

The Tongjiang cattle in the current study were sampled in a single village, and the chances are that they may not be representative of the whole diversity within this local population. We chose this sampling strategy for two reasons. First, during the past decades, Tongjiang cattle received extensive gene flow from introduced cattle breeds via hybridization, and the population size of purebred Tongjiang cattle was gradually reduced. Samples from Zhongba village, which was relatively isolated by geography and poor transportation, offered a high chance of maintaining authentic genetic components. Second, most of the families in this mountainous village bred both female and male cattle to reproduce their own herd. This unique family-based breeding history helped to reduce genetic exchange with the outside gene pool. Indeed, the relatively high haplotype diversity observed in our sample, compared with other reported Chinese cattle breeds or populations (Lai et al. 2006; Lei et al. 2006), suggests that genetic diversity was well maintained in the cattle from Zhongba.

Of the five body size traits, two (chest girth and body live weight) were significantly higher in the russet group than in the black group ($P < 0.05$) in Tongjiang cattle. This morphological difference, however, had no correlation with matrilineal genetic diversity and the distribution of *B. taurus* and *B. indicus* components within each group. Thus, mtDNA sequence variation did not reflect morphological differentiation between these two groups. This observation is consistent with our recent studies of chickens (Liu et al. 2006a, b), yaks (Lai et al. 2007), and cattle (Lai et al. 2006), finding no breed-specific matrilineal lineage in domestic animals.

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