Genetic Background of Yunnan Gayal Revealed by MtDNA D-loop and *SRY* Gene Sequence Variation

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Introduction

Yunnan gayal are mainly distributed in the narrow valley of the Dulong river in western Yunnan, China, and the adjacent region, and also named “Dulong” cattle. Many zoologists regarded the gayal as the domestic gaur for morphological similarity between the gayal (*Bos frontalis*) and the gaur (*Bos gaurus*), but Walker (1968) classified the gayal as a species in the genus Bos. The evidences from both karyotype and mtDNA supported that gayal originated from gaur. A single Robertsonian translocation (involving cattle chromosomes 2 and 28) differentiates the karyotypes of the domestic cattle (2n = 60) and gayal (2n = 58), while the same 2; 28 translocation has been reported for *Bos gaurus* (2n = 58) (Chi *et al*. 2005). The proposed close relationship between the wild gaur and the domestic gayal or mithan (*Bos frontalis*) was further verified by analyzing the sequences of three mitochondrial and two Y-chromosomal DNA segments (Verkaar *et al*. 2004). The genetic ground of Yunnan gayal is still not clear. It had been taken as the offspring of gayal or the hybrid of gayal and taurine or zebu cattle (Lan *et al*. 1993). It is essential to clarify genetic contributions of these Bos species to Yunnan gaygal.

Material and methods

The sources of animal. Blood or tissue of 23 individuals was collected from 22 Yunnan gayal and 1 gaur. Yunnan native catlle was used for comparison: Wenshan cattle (n = 27), Nujiang cattle (n = 11) and Diqing cattle (n = 10). Of 71 individuals, 39 bulls were used for *SRY* gene analysis: gayal (n = 16), gaur (n = 1), Wenshan cattle (n = 15), Nujiang cattle (n = 3) and Diqing cattle (n = 4).

PCR amplification and Data analysis. Based on the cattle mtDNA complete sequence

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(Anderson *et al*. 1982) and *SRY* complete gene sequence (Verkaar. 2004), primer sets (Table 1) were designed to amplify 455 bp of the mtDNA D-loop and 1305 bp of *SRY* gene segments. A neighbor-joining (NJ) tree was constructed using PAUP packages (V4) (Swofford 2000). The median network plots of the mtDNA D-loop and *SRY* gene sequences (Bandelt *et al*. 1995) were constructed using the Network4.1 program.

Table 1. Oligonucleotide primers used in this study

DNA segment	Sequence (5`-3`)	Position
Control region	ACACGCCCATACACAGACCACAG	15917-15939
	TCCAAGCATCCCCCAAAATAAAA	59-81
SRY	TTGGGGGCGGAGAAATAAAT	916-935
	CTGTGCCTCCTCAAAGAATGG	1370-1390
	CAGCAAGCAGCTGGGATATGT	1318-1340
	GTACTTACTTATTGGGCTTCTTAT	1828-1851
	GCTAACAAAGGCGCTCTTTATCTC	1770-1793
	ACAAAGAGGTGGAAAGTAG	2254-2272

Results and discussion

Among 22 Yunnan gayal mtDNA D-loop sequences analyzed, 5 could be assigned to *Bos taurus* while the remaining samples belonged to *Bos indicus* type (Figure 1). The mtDNA type of gaur (collected from Yunnan) belonged to gaur-gayal clade (Figure 1). A median network (Figure 2) of *SRY* gene sequences was constructed based on 1305 bp fragments from one gaur, 16 gayal bulls and previously sequenced *SRY* fragments from yak, gayal, and gaur bulls. Taurine cattle, zebu, gayal-gaur, and yak occupied separate branches in the network. Seen from paternal lineage, all Yunnan gayal and gaur were *Bos frontalis-Bos gaurus* type.

Mitochondrial DNA and *SRY* gene data showed that the extant gayal in Yunnan of China was a crossing population between male gayal (15/16) and female taurine (5/22) or zebu (17/22). In gayal samples, a single sample (D39) is identical to wild gaur in *SRY* sequence, which suggested that gaur immigrated in Yunnan and also contributed partly to the present crossing population. The wild gaur (2n = 58) and the described gayal were clustered in the same clade in

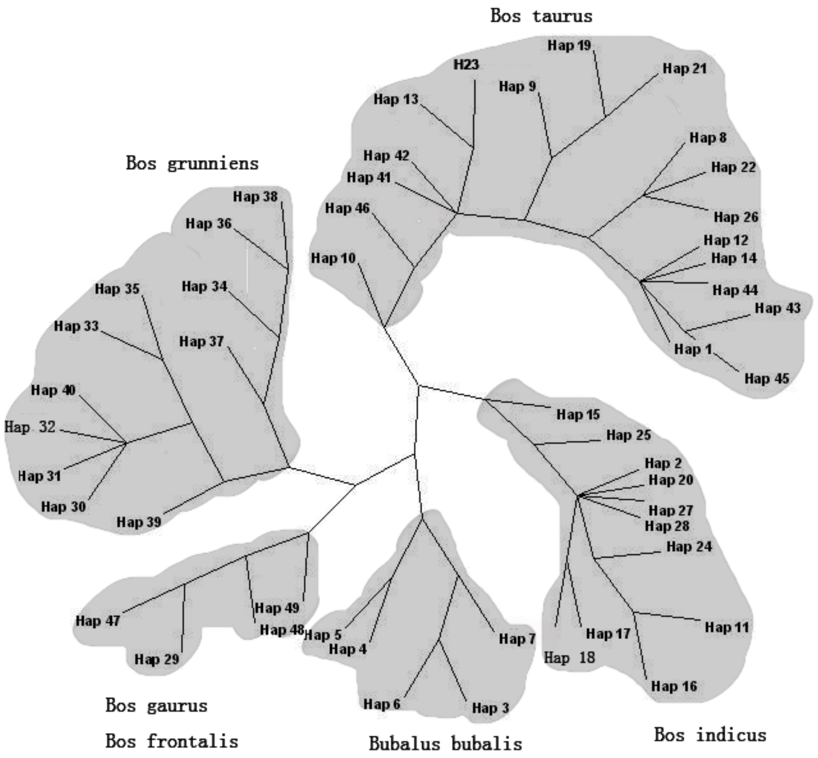

Figure 1. Neighbour-joining phylogeny of Yunnan gayal mtDNA control-region haplotypes.

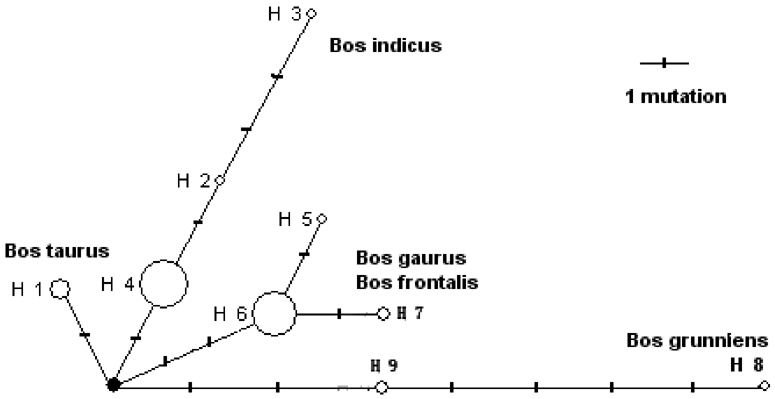

Figure 2. Median network of *SRY* gene sequences of *Bos species*. Black dots represent hypothetical sequences, which have not been found in the sequencing exercise.

phylogenetic trees of maternal and paternal lineage (Figure 1 and Figure2), suggesting a close relation of the wild gaur and the domestic gayal.

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