Research Journal of Animal Sciences 1 (4): 114-119, 2007
ISSN: 1993-5269

The Origin and Taxonomic Status of the Gayal Based on Cytochrome B Gene Partial Sequences

[1,2]S. Li, [2]H. Chang, [2]G. Ma, [2]H. Chen, [2]D. Ji and [2]R. Geng
[1]College of Bioscience and Biotechnology,
[2]College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China

Abstract: The gayal (*Bos frontalis*) is a rare semi-wild and semi-domestic bovine species. There still have remarkable divergences on the gayal's origin and taxonomic status. In the present study, the cytochrome b (*Cyt b*) gene partial sequences (447 bp) of 13 gayals were sequenced and analyzed. Combined with the homologous fragments of other bovine *Cyt b* sequences cited in GenBank, the phylogenetic trees of genus Bos were reconstructed by Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods with *Bubalus bubalis* as outgroup. Sequence analysis showed that, among he 447 sites compared for 13 gayals, 33 variable sites (7.38% of all sites) and 4 different haplotypes were observed, showing abundant mitochondrial genetic diversity in gayals. Both NJ and MP trees demonstrated that gayals in this study were markedly divided into 3 embranchments: One embranchment clustering with *Bos gaurus*, another clustering with *Bos taurus* and the third clustering with *Bos indicus*. The results of phylogenetic analysis suggested that the gayal might be the domesticated form of the gaur and a great proportion of the gayal bloodline was invaded by other bovine species.

Key words: Gayal (*Bos frontalis*), cytochrome b gene, origin, taxonomic status

INTRODUCTION

The gayal (*Bos frontalis*), also called mithan or mithun, in China is only found in the Dulong River and Nujiang River Basin in Yunnan Province and Menyu and Luoyu regions in Tibet Autonomous Region where the altitude ranges between 1500 M-4100 M. It is also found in Assam in India, East Bengal and Kachin state in the northern part of Burma (Simoons, 1984; Nyunt and Win, 2004; Namikawa, 2005; Mao *et al*., 2005). The gayal is a rare semi-wild and semi-domestic livestock. The gayal in Yunnan Province was named as "Dulong Cattle" because it was firstly domesticated by Dulong Tribe.

The gayal was classified as a separate subgenus together with Bali cattle (*Bos banteng*), the kouprey (*Bos sauveli*) and the gaur (*Bos gaurus*), distinct from European cattle (*Bos taurus*) and zebu cattle (*Bos indicus*) (Williamson and Payne, 1977). There were three major hypotheses about the origin of the gayal (Walker *et al*., 1968; San *et al*., 1980; Winter *et al*., 1984; Payne, 1991; Lan *et al*., 1993; Ritz *et al*., 2000; Tanaka *et al*., 2004; Verkaar *et al*., 2004): That it was a domesticated gaur; that it was a hybrid descendant, from crossing of gaur (*Bos gaurus*) and ordinary domestic cattle (*Bos indicus* or *Bos taurus*) and that it was an independent species descended from a wild Indian bovine which is now extinct. Of these: The first is most favored.

As one of important protein-coding genes in Mitochondrial DNA (mtDNA), Cytochrome b (*Cyt b*) gene contains abundant phylogenetic information among intra- and interspecies and it is considered to be a good marker to study on genetic differentiation and phylogenetic relationships among species within the same genus or the same family (Browers *et al*., 1994; Zardoya and Meyer, 1996). *Cyt b* gene is widely used in the studies on origin, taxonomy and phylogeny of the subfamily Bovinae (Kikkawa *et al*., 1997; Birungi and Arctander, 2001; Hassanin and Ropiquet, 2004). In the present study, *Cyt b* gene partial sequences of 13 gayals in China were sequenced and analyzed. These data, combined with *Cyt b* sequences of other bovine species in GenBank, were used to perform phylogenetic analysis, in order to explore the origin and taxonomic status of the gayal in molecular level and to provide some molecular biological gists for evaluating and protecting this rare genetic resource.

MATERIALS AND METHODS

Applying simple random sampling in typical colony methods, in the central area of habitat (in Nu River City of

Corresponding Author: Shiping Li, College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China

Table 1: *Cyt b* sequences cited in GenBank and accession number

Species	Accession No.
Bos taurus	V00654
Bos indicus	NC_005971
Bos gaurus	AF348593
Bos sauveli	AY689189
Bos javanicus	D82889
Bos grunniens	AY955225
Bubalus bubalis	D88635

DNA extraction and sequencing

Yunnan Province), 13 gayals were selected. Blood samples were collected and taken back to the lab in an icebox, then kept at -20°C until use. The *Cyt b* gene sequences of other cattle cited in GenBank for phylogenetic analysis were showed in Table 1.

Total genomic DNA was extracted from blood using standard procedures, involving treatment with SDS and proteinase K and subsequent phenol/chloroform extraction (Chen and Leibenguth, 1995). The *Cyt b* gene partial sequence was amplified from total genomic DNA using Polymerase Chain Reaction (PCR) with the two designed primers (forward: 5'-CCCTCCTGGGA ATCTGCCTAA-3'; reverse: 5'-ATTGTTGGAGCCT GTTTCGTG -3', which were situated at positions 104-124 and 601-621 in cattle *Cyt b* gene, respectively). The standard PCR conditions were as follows: 4 min at 94°C; 30 cycles of denaturation/annealing/extension with 40 sec at 94°C for denaturation, 40 sec at 52°C for annealing and 50 sec at 72°C for extension and 7 min at 72°C. Each PCR was performed in 25 μL reaction volume with 2.0 units *Taq* DNA polymerase (TaKaRa biotechnology (Dalian) Co. Ltd in China) and about 100 ng DNA as template. The PCR products (each about 518 bp) were analyzed in 10% PAGE gel with a vacant comparison. Purification and sequencing procedure were carried out by Shanghai Sangon Biological Engineering Technology and Service Co. Ltd in China.

Statistics analysis: *Cyt b* partial sequences of 13 gayals were edited and aligned with reference to the *Cyt b* sequence of the domestic cow (*Bos taurus*) (Accession No. V00654) using DNASTAR package and were checked manually. Pairwise comparisons of observed sequence differences, number of transitions and transversions and nucleotide composition by codon position were analyzed using the computer program MEGA 3.1 (Kumar *et al*., 2004). The Haplotype Diversity (Hd) and nucleotide diversity (Pi) were calculated by the software DNAsp 4.1 (Rozas *et al*., 2003). Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods were used to reconstruct the phylogenetic trees. The NJ phylogenetic tree was reconstructed on the basis of Kimura two-parameter model using the computer program MEGA 3.1 (Kumar *et al*., 2004). The MP phylogenetic tree was generated by heuristic search routines with 1000 random-addition sequences and TBR branch swapping using the software PAUP* 4.0 (Swofford, 2000). Levels of resolution at internal nodes of two phylogenetic trees were evaluated by bootstrap resampling with 1000 iterations (Felsenstein, 1985).

RESULTS AND DISCUSSION

Nucleotide composition of *Cyt b* partial sequences of gayals: After sequenced and aligned, a 447 bp fragment of *Cyt b* gene (positions 163-609 in cattle *Cyt b* gene) was obtained in all 13 gayals (Fig. 1). No insertions/deletions were observed. The average nucleotide frequencies of T,C, A and G were 28.1, 26.5, 30.1 and 15.3%, respectively (Table 2). A remarkable imbalance in base usage was observed at third positions, with infrequent use of G (3.7%) and a bias towards A+C (80.2%). The low number of Gs and high number of As at third positions indicate that the likelihood of an A to G transition is much lower than a G to A transition (Birungi and Arctander, 2001).

Nucleotide variations of *Cyt b* partial sequences of gayals: Among 447 sites compared for 13 gayals, a total of 33 variable sites (7.38% of all sites) were observed, all of which were phylogenetically informative sites and only one site (the 407th site) was amino acid substitution site. Of the 33 variable sites. The transition sites and transversion sites were 31 and 2, respectively. The transition/transversion Ratio (R) was 15.5, showing a high transition bias (Irwin *et al*., 1991). Interestingly, the transitional rate between pyrimidines (T-C) was higher than that between purines (A-G) with a ratio 4.33, similar to the report of Tamura and Nei (1993). 13 *Cyt b* sequences generated 4 different haplotypes (Hap01~Hap04): Hap01 including 2 sequences (d01 and d09), Hap02 including 6 sequences (d02, d06, d10, d12, d13 and d14), Hap03 including 2 sequences (d03 and d08) and Hap04 including 3 sequences (d04, d05 and d11). The Haplotype diversity (Hd) and nucleotide diversity (Pi) were 0.744±0.091 and 0.0336±0.0069, respectively, showing abundant mitochondrial genetic diversity in gayals.

Phylogenetic analysis: In this study, phylogenetic analysis was based on 11 *Cyt b* partial sequences (447bp), including 4 haplotype sequences of 13 gayals and 7 homologous fragments of *Cyt b* sequences cited in GenBank (Table 1). The NJ and MP phylogenetic trees of genus Bos (Fig. 2) were reconstructed with *Bubalus bubalis* (Accession No. D88635) as outgroup. Support for individual branches of 2 phylogenetic trees was

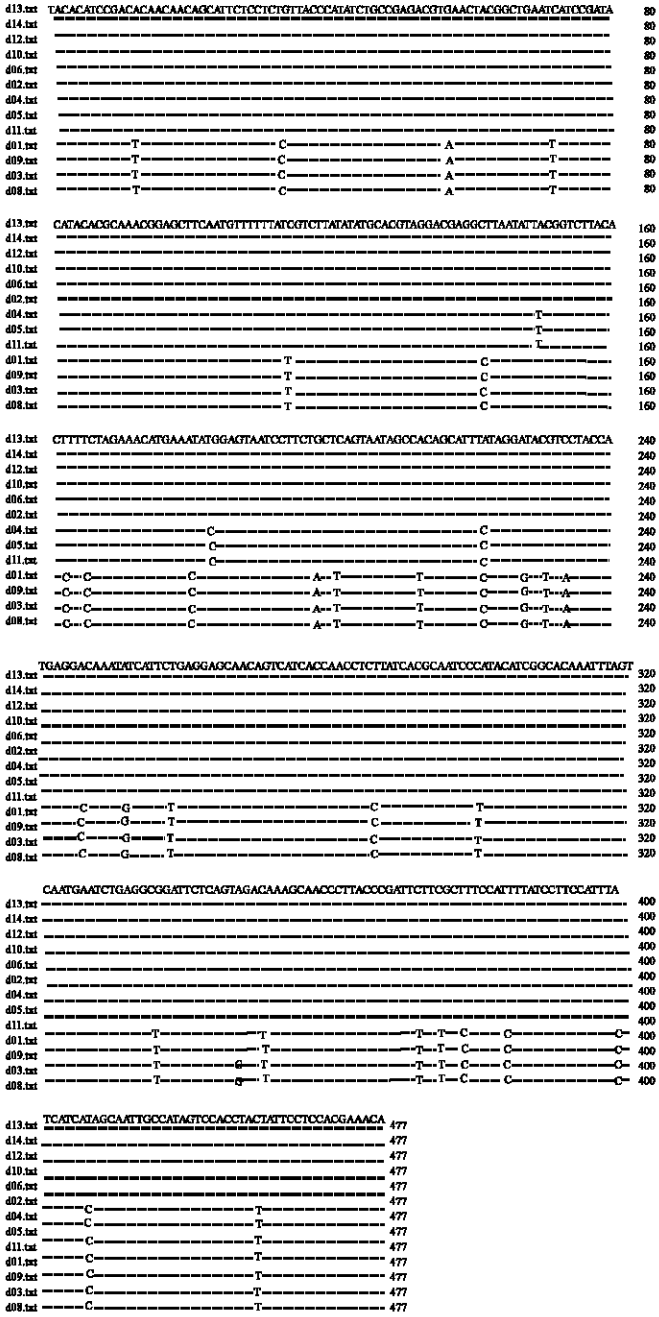

Fig. 1: The aligned result of *Cyt b* gene partial sequences of 13 gayals d01, d02, d03, d04, d05, d06, d08, d09, d10, d11, d12, d13 and d14 are original serial numbers of 13 gayals, respectively

assessed by Bootstrap Percentages (BP) computed after 1000 replicates of the closest stepwise addition option.

It can be seen from Fig. 2 that both the NJ and MP phylogenetic trees support almost the same topology. There were three embranchments for gayals in both phylogentic trees. The first embranchment, consisting of Hap01 and Hap03, clustered together with *Bos gaurus* at the BP value 100% in NJ tree and 85% in MP tree. The second embranchment, Hap02, clustered together with *Bos taurus* at the BP value 95% in NJ tree and 84% in MP tree. The third embranchment, Hap04, clustered together with *Bos indicus* at the BP value 98% in NJ tree and 87% in MP

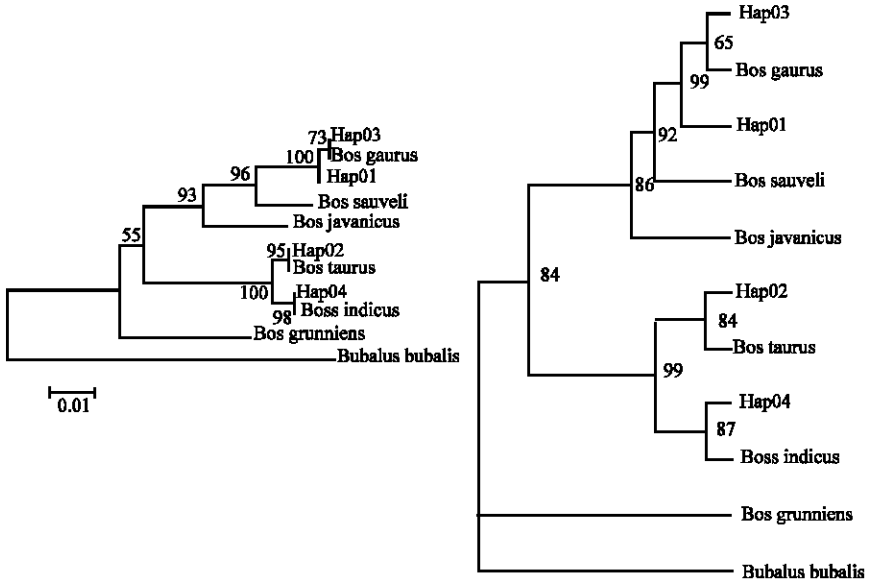

Fig. 2: Molecular phylogenetic trees of genus Bos based on *Cyt b* gene partial sequences using NJ (left) and MP (right) methods Numbers at nodes represent Bootstrap Percentages (BP) (%) with 1, 000 replicates

Table 2: Nucleotide composition of *Cyt b* partial sequences of 13 gayals

Nucleotide	Average %	First codon position %	Second codon position %	Third codon position %
T	28.1	28.1	40.1	16.1
C	26.5	16.8	25.0	37.6
A	30.1	28.9	18.8	42.6
G	15.3	26.2	16.1	3.7

tree. The results suggest that the gayal might have close relationships with *Bos taurus*, *Bos indicus* and *Bos gaurus*.

Origin and taxonomic status of the gayal: The result of phylogenetic analysis showed that the gayals in our study were divided into three embranchments (Fig. 2). The second and third embranchments close clustered with *Bos taurus* and *Bos indicus*, respectively, which suggested that the gayal might contain a maternal origin of *Bos taurus* or *Bos indicus*. However, the researches on descriptive characteristics, karyotype, blood protein polymorphism and microsatellite analysis (Walker *et al*., 1968; San *et al*., 1980; Simoons, 1984; Nie *et al*., 1999; Tu *et al*., 2000; Ritz *et al*., 2000; Tanaka *et al*., 2004) have shown that *Bos frontalis* is distinctly different from *Bos taurus* and *Bos indicus*. Therefore, the gayal couldn't originated from *Bos taurus* or *Bos indicus* and its maternal bloodline of *Bos taurus* or *Bos indicus* might be the mtDNA introgression of *Bos taurus* or *Bos indicus* into the gayal's ancestor through interbreeding during historic times. This scenario is apparently reasonable, as the gayal can interbreed with domestic cattle (*Bos taurus* and *Bos indicus*) and the female offspring may be fertile, but the male offspring may not always be fertile (Simoons, 1984; Huque *et al*., 2001; Nyunt and Win, 2004; Tanaka *et al*., 2004). Furthermore, according to the investigation in the field, there are crossbreed descendants of gayal and domestic cattle from Dulong River Basin to Nu River Basin and the half-domestic and half-wild breeding pattern increased the chance of the gayal contacting with domestic cattle. In our study, 9 *Cyt b* sequences of gayals (69.23% of 13 sequences) belonged to the second and third embranchments, which indicated that a great proportion of the gayal bloodline was invaded by other bovine species and the protection of the gayal was facing a formidable situation.

Winter *et al*. (1984) put forward that the gaur was the wild ancestor of the gayal according to karyotype, red blood cells and haemoglobin type. This view was subsequently supported by Ritz *et al*. (2000), Tanaka *et al*. (2004) and Verkaar *et al*. (2004). Our data of phylogenetic analysis indicated that the first embranchment of gayals was close allied with the gaur (*Bos gaurus*) (Fig. 2). And the nucleotide divergence between *Bos frontalis* (Hap01 and Hap03) and *Bos gaurus* was only 0.11%, farther less than the divergences between *Bos frontalis* and *Bos sauveli*/*Bos javanicus* (3.24%/4.36%). These results suggested that the wild ancestor of the gayal might be the gaur. And the hypothesis that the gayal was an independent bovine species (Walker *et al*., 1968; San *et al*., 1980) was not supported by the results presented in this study.

CONCLUSION

The gayal might be the domesticated form of the gaur and a great proportion of the gayal bloodline was invaded by other bovine species.

ACKNOWLEDGEMENT

We thank the anonymous reviewers for the comments on the manuscript. This research was supported by the Project of the National Natural Science Foundation of P. R. China (Grant No. 30571323).

We also appreciate the following people for their hard work: Kaidian Zhao, vice stationmaster of Yunnan Livestock Melioration Station, Apu Zhao, vise stationmaster of Husbandry and Veterinary station in Nu River City in Yunnan Province and the staff of Gayal Protection and Reproduction Base.

REFERENCES

Birungi, J. and P. Arctander, 2001. Molecular systematics and phylogeny of the Reduncini (Artiodactyla: Bovidae) inferred from the analysis of mitochondrial cytochrome b gene sequences. J. Mamm. Evol., 8: 125-147.

Browers, N., J.R. Stauffer and T.D. Kocher, 1994. Intra- and Interspecific Mitochondrial DNA Sequence Variation within Two Species of *Rock-Dwelling Cichlids (Teleostei: Cichlidae*) from Lake Malawi, Africa. Mol. Phylogenet. Evol., 3: 393-400.

Chen, H. and F. Leibenguth, 1995. Studies on multilocus fingerprints, RAPD markers and mitochondrial DNA of a gynogenetic fish (*Carassius auratus gibelio*). Biochem. Genet., 33: 297-306.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783-791.

Hassanin, A. and A. Ropiquet, 2004. Molecular phylogeny of the tribe Bovini (Bovidae, Bovinae) and the taxonomic status of the Kouprey, *Bos sauveli* Urbain 1937. Mol. Phylogenet. Evol., 33: 896-907.

Huque, K.S., M.M. Rahman and M.A. Jalil, 2001. Study on the growth pattern of Gayals (*Bos frontalis*) and their crossbred calves. Asian-Aust. J. Anim. Sci., 14: 1245-1249.

Irwin, D.M., T.D. Kocher and A.C. Wilson, 1991. Evolution of the cytochrome b gene of mammals. J. Mol. Evol., 32: 128-144.

Kikkawa, Y., H. Yonekawa and H. Suzuki, 1997. Analysis of genetic diversity of domestic water buffaloes and anoas based on variations in the mitochondrial gene for cytochrome b. Anim. Genet., 28: 195-201.

Kumar, S., K. Tamura and M. Nei, 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics, 5: 150-163.

Lan, H., X.K. Xiong, S.Y. Lin, A.H. Liu and L.M. Shi, 1993. Mitochondrial DNA polymorphism of cattle (*Bos taurus*) and mitham (*Bos frontalis*) in Yunnan Province. Acta Genetica Sinica, (In Chinese) 20: 419-425.

Mao, H.M., W.D. Deng and J.K. Wen, 2005. The biology characteristics of Gayal (*Bos frontalis*) and potential exploitation and utilization. J. Yunnan Agric. Uni. (In Chinese), 20: 258-261.

Namikawa, T., 2005. Cattle: Why the cattle genetic diversity is widely in eastern Asia. Rep. Soc. Res. Native Livestock, 22: 97-100.

Nie, L., Y. Yu, X.Q. Zhang, G.F. Yang, J.K. Wen and Y.P. Zhang, 1999. Genetic diversity of cattle in South China as revealed by blood protein electrophoresis. Biochem. Genet., 37: 257-265.

Nyunt, M.M. and N. Win, 2004. Mithan (*Bos frontalis*) in Myanmar. Rep. Soc. Res. Native Livestock, 21: 19-22.

Payne, W.J.A., 1991. Domestication: A forward step in Civilization. In: Hickman C.G. (Ed.). Cattle Genetic Resources. New York: Elsevier Science Publishers, pp: 51-72.

Ritz, L.R., M.L. Glowatzki-Mullis, D.E. MacHugh and C. Gaillard, 2000. Phylogenetic analysis of the tribe Bovini using microsatellites. Anim. Genet., 31: 178-185.

Rozas, J., J.C. Sánchez-DelBarrio, X. Messeguer and R. Rozas, 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics, 19: 2496-2497.

San, X.N., Y.F. Chen, L.H. Luo, X.M. Cao and J.Z. Song, 1980. The karyotype analysis of Gayal. *Hereditas* (Beijing), (In Chinese), 2: 25-27.

Simoons, F.J., 1984. Gayal or Mithan. In: Mason I.L. (Ed.). Evolution of Domesticated Animals. London: Longman, pp: 34-38.

Swofford, D.L., 2000. PAUP*: Phylogenetic analysis using parsimony (*and other Methods), version 4.0. Sinauer Associates, Sunderland, Mass.

Tamura, K. and M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol., 10: 512-526.

Tanaka, K., H. Mannen, Y. Kurosawa, K. Nozawa, M. Nishibori, Y. Yamamoto, H. Okabayashi, K. Tsunoda, T. Yamagata, Y. Suzuki, K. Kinoshita, Y. Meada, M.M. Nyunt, T. Daing, T. Hla, N. Win, T. Tur, P. Aung and A. Cho, 2004. Cytogenetic analysis of Mithan in Myanmar. Rep. Soc. Res. Native Livestock, 21: 123-127.

Tu, Z.C., L. Nie, Y. Yu, J.K. Wen and Y.P. Zhang, 2000. Blood Protein Polymorphism in *B. frontalis*, *B. grunniens*, *B. taurus* and *B. indicus*. Biochem. Genet., 38: 413-416.

Verkaar, E.L.C., I.J. Nijman, M. Beeke, E. Hanekamp and J.A. Lenstra, 2004. Maternal and Paternal Lineages in Cross-Breeding Bovine Species. Has Wisent a Hybrid Origin? Mol. Biol. Evol., 21: 1165-1170.

Walker, E.P., F. Warnick and S.T. Hamlet, 1968. Mammals of the World. Balttmore: The Johns Hopkins Press. pp: 1431-1442.

Williamson, G. and W.J.A. Payne, 1977. An Introduction to Animal Husbandry in the Tropics. London: Longman, pp: 199-204.

Winter, H., B. Mayer and W. Schleger, 1984. Karyotyping, red blood cells and haemoglobin typing of the mithan (*Bos frontalis*), its wild ancestor and its hybrids. Res. Vet. Sci., 36: 276-283.

Zardoya, R. and A. Meyer, 1996. Phylogenetic performance mitochondrial protein coding genes in resolving relationship among vertebrate. Mol. Biol. Evol., 13: 933-942.