

Mitochondrial DNA diversity and origin of *Bos frontalis*

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***Bos frontalis* is an enigmatic bovine species found in selected regions of Myanmar, Bangladesh, China, Thailand, Bhutan and India. Though there are conflicting hypotheses regarding its origin, the most widely held view is that *Bos frontalis* originated from direct domestication of *Bos gaurus* (gaur). To study this, we analysed mtDNA control region and cytochrome *b* gene sequences of 41 *B. frontalis* from China and India, together with the available sequences in the public databases. Bayesian inference revealed clustering of *B. frontalis* and *B. gaurus* sequences into two separate clades. Application of relaxed molecular clock to these clades shows that *B. frontalis* and *B. gaurus* diverge between 0.134 and 0.207 million years ago. This time estimate lies much outside the ~10,000-year-old history of animal domestication. Based on this finding, we argue that *B. frontalis* should be treated as a separate species/sub-species and not merely a domestic form of *B. gaurus*. Our study also highlights the gene flow between domestic cattle and *B. frontalis* population.**

Keywords: *Bos frontalis*, domestication, gene flow, molecular clock.

MANY species belonging to the family Bovidae are closely associated with the human civilization. Apart from yak, buffalo and bison, Bovidae includes fully domesticated species like cattle and semi-domesticated species like *Bos frontalis*. The history of domestication of these bovid species is no more than ~10,000 years old. All of today's major bovid species like cattle, yak and buffalo were domesticated in the Near East, Indus valley and Southeast Asia ~10,000 years ago¹. In a time-period ranging from 7 to 10,000 yrs BP, both present-day domestic cattle, *Bos taurus* and the *Bos indicus* were domesticated independently in the Near East and in the Indus

Valley respectively^{2,3}. To meet the requirement during their long association with humans, the domestics were subjected to enormous selection and hybridization. Among bovines, the Eastern Himalayan region is considered as the hot spot of interspecies hybridization⁴. Earlier, many hybrids of cattle, yak and *Bos gaurus* (gaur) have been reported from this region⁵. The current work deals with *B. frontalis*, commonly referred to as Mithan or Gayal in India and Dulong in the Yunnan Province of China⁶. *B. frontalis* is a semi-domesticated species quite similar to *B. gaurus* in appearance and in distribution it ranges from northeast region of Myanmar through the Chit-tagong tracts of Bangladesh, Arunachal Pradesh in India to the Yunnan Province of China. Over the years the geographical distribution of *B. frontalis* has remained unchanged, but the population has dwindled rapidly in the respective regions⁷. Three separate hypotheses were presented to explain the origin of *B. frontalis*⁸. The first hypothesis presumes direct domestication from *B. gaurus*, whereas the second proposes hybrid origination resulting from cross-breeding between *B. gaurus* and *B. indicus*. The third hypothesis assumes domestication from extinct wild cattle. Previous studies based on blood types and protein markers support direct domestication from *B. gaur*⁹. As far as studies based on DNA markers are concerned, with few exceptions no study to measure the time to the most recent common ancestor (TMRCA) has been attempted on *B. frontalis* so far to measure the ancestry of this bovid¹⁰⁻¹³. These previous studies are far from comprehensive; they included very few samples from restricted geographical regions and employed simple phylogenetic approaches. Here, we have analysed novel sequences of *B. frontalis* and *B. gaurus* from China and India using Bayesian MCMC analyses under a relaxed molecular clock assumption to test the available hypotheses about its origin. We produced statistically significant calibrated estimates of time to the most common ancestor for *B. frontalis*.

We sampled 28 *B. frontalis* from the Yunnan Province of China and 13 from Arunachal Pradesh, India. In addition, one *B. gaurus* from the Yunnan Province was also sampled from the wild using non-invasive method after clearance from the Yunnan Province forest authorities.

Total genomic DNA was extracted by standard phenol-chloroform extraction method¹⁴. Highly informative 310 bp fragment of D-loop region of mtDNA was amplified using cattle primers² AN2 (L15960 5'-GGTAATG-TAC-ATAACATTAATG-3') and AN3 (H16334; 5'-CGAGAT-GTCTTATTTAAGAGG-3'). We found these primers to be genus-specific and provide amplification for both *B. frontalis* and *B. gaurus*. PCR amplifications were performed in a 50 µl of volume containing 2.5 mM MgCl₂, 200 µM dNTP, 1 µM of each primer and 0.25 U *Taq* polymerase. For amplification 4 min of denaturation was followed by 35 cycles of 40 sec at 95°C. Annealing was performed for 45 sec at 55°C followed by extension

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of 40 sec at 72°C. The products were then finally extended at 72°C for 4 min. Amplicons were purified using spin columns (Watson BioTechnologies, Inc., Shanghai). Purified products were sequenced directly for both the DNA strands using the ABI PRISM BigDye™ Terminator Cyler Sequencing Ready Reaction Kit (Applied Biosystems, CA, USA). In parallel to the D-loop, ~300 bp of cytochrome *b* (*cyt b*) gene was also amplified and sequenced in 28 *B. frontalis* from the Yunnan Province and Arunachal Pradesh with the inclusion of a single *B. gaurus* sample from the Yunnan Province using universal primers¹⁵. The resulting raw sequences were checked using DNASTAR 6.0 (DNASTAR Inc., Madison, WI) and deposited in GenBank (accession no. HM215205-HM215246 and HM215175-HM215204).

The newly resulted D-loop sequence information of 41 *B. frontalis* was combined with 27 sequences of *B. frontalis* available in the GenBank. Control region sequence information from other related species, viz. *Bos gaurus*, *Bos grunniens*, *Bos javanicus*, *Bos indicus* and *Bos taurus* was also included and *Bubalus bubalis* sequence was used as an outgroup (Table 1). The resulting dataset of 136 D-loop sequences was aligned using ClustalX 1.81 program¹⁶ with default parameters and subjected to diversity and demographic indices estimation. The nucleotide diversity¹⁷ was calculated using the program¹⁸ DnaSP version 5.10. Using the same program, Fu's *F_s* was estimated to decipher the genetic signals of past population expansion¹⁹. Based on BIC criterion of model selection, the appropriate model of evolution was selected by using the program²⁰ jModelTest2.2. For phylogenetic tree construction two methods were utilized: first we used maximum likelihood method available in the online program PhyML 3.0 (www.phylogeny.fr). Heuristic searches were performed using 1000 bootstrap replicates. Secondly, we used Bayesian MCMC method to construct the phylogenetic tree using the program²¹ BEAST version 1.5.8. The same program was used to measure TMRCA and substitution rate under the relaxed molecular clock model assumption²². For divergence estimation, we used 28 samples from the Yunnan Province and Arunachal Pradesh and a sole gaur sample from the Yunnan Province for which sequence information for both D-loop and *cyt b* was generated. Demographic model of exponential growth was applied in the form of coalescence prior as it provides better fit for the divergence evaluation²³. Calibration of *B. frontalis* node was carried out from fossil and molecular estimates derived from the *Bison–Bos* split. A time estimate of 2 million years and 1.4 million years for *Bison–Bos* split derived from fossil and molecular information was employed respectively^{24,25}. During the process of divergence estimation, the D-loop and *cyt b* gene sequence information was combined together. In the Markov chain Monte Carlo analysis, samples were drawn from the posterior at every 1000 steps over a total of 10,000,000 steps, followed by a burn-in of 1,000,000

cycles. Convergence of the chains to the stationary distribution was checked visually by plotting posterior estimates using the program Tracer²⁶. Effective sample size (ESS) for each parameter sampled from the MCMC analysis was almost always found to exceed 300, by an order of magnitude. Three independent MCMC chains were run to obtain good congruence between runs and high ESS.

The diversity and demographic indices are depicted in Table 2. The results from Fu's *F_s* show little departure from neutrality and were not found to be significant. Both maximum likelihood (ML) and Bayesian inference (BI) tree based on D-loop segment show similar topology (ML tree not shown). Bayesian analysis of D-loop dataset produced a tree with posterior support ranging from 0.99 to 1.0 for the monophyly of each species (Figure 1). Splitting of *B. frontalis* and *B. gaurus* sequences into two separate clades was apparently visible in the tree inferred from D-loop sequence information. Moreover, the *B. frontalis* clade exhibits further divergence into two discrete clades designated as F1 and F2. Similar dichotomy between *B. frontalis* and *B. gaurus*, and within *B. frontalis* has been found when the tree was built combining D-loop and *cyt b* gene segments (Figure 2). TMRCA estimates using palaeontological and molecular information for node calibration fall outside the time scale of animal domestication (Table 3). Of the 41 samples sequenced by us, two sequences of *B. frontalis* cluster tightly with the *B. indicus* and one with the *B. taurus*.

Previous studies based on small sample size and single locus laid support in favour of domestication of *B. frontalis* from its wild counterpart, *B. gaurus*^{10–12}. A study based on Y-chromosome-specific *SRY* gene also underestimated the distinction between *B. gaurus* and *B. frontalis* haplotypes¹³. We include samples from a large geographical region, typed fast (D-loop) and slow evolving (*cyt b*) loci, and tested different models along with calibration information drawn from fossil and molecule to provide better insight into the origin and diversity of *B. frontalis*. Low departure of Fu's *F_s* may be either due to highly restricted distribution of the species or due to the constant population size which the species has maintained over the years. We argue that noticeable separation of *B. frontalis* and *B. gaurus* in the Bayesian MCMC analysis cannot be explained simply by direct domestication of *B. gaurus* (Figures 1 and 2). Reconstruction of phylogenetic tree by means of D-loop and concatenated sequence illustrates two separate clades designated as F1 and F2 in the *B. frontalis* cluster, which within 95% HPD level yield a time depth of 1.301 to 0.090 million years for palaeontological and molecular dating respectively. Clustering of sequences in the F1 and F2 clades was not found according to their geographical origin. On the contrary, there was admixing of sequences from China, India and Bhutan. Notably, similar duality has been identified in the D-loop analysis of *B. indicus* cattle^{27,28}. Both gayal and

Table 1. D-loop and cytochrome *b* sequences used in the present study

Species	Accession number	Origin	Species	Accession number	Origin
<i>Bos gaurus</i>	DQ377060	India	<i>Bos frontalis</i>	HM215233	Arunachal Pradesh (India)*
<i>Bos gaurus</i>	DQ377058	India	<i>Bos frontalis</i>	HM215234	Yunnan (China)*
<i>Bos gaurus</i>	DQ377056	India	<i>Bos frontalis</i>	HM215235	Yunnan (China)*
<i>Bos gaurus</i>	DQ377061	India	<i>Bos frontalis</i>	HM215236	Arunachal Pradesh (India)*
<i>Bos gaurus</i>	DQ377059	India	<i>Bos frontalis</i>	HM215237	Yunnan (China)*
<i>Bos gaurus</i>	DQ377057	India	<i>Bos frontalis</i>	HM215238	Yunnan (China)*
<i>Bos frontalis</i>	EF693814	Cambodia	<i>Bos frontalis</i>	HM215239	Yunnan (China)
<i>Bos frontalis</i>	EF693813	Cambodia	<i>Bos frontalis</i>	HM215240	Arunachal Pradesh (India)*
<i>Bos frontalis</i>	DQ995680	Bhutan	<i>Bos frontalis</i>	HM215241	Arunachal Pradesh (India)*
<i>Bos frontalis</i>	DQ995678	Bhutan	<i>Bos frontalis</i>	HM215242	Arunachal Pradesh (India)*
<i>Bos frontalis</i>	DQ995676	Bhutan	<i>Bos frontalis</i>	HM215243	Yunnan (China)*
<i>Bos frontalis</i>	DQ995674	Bhutan	<i>Bos frontalis</i>	HM215244	Arunachal Pradesh (India)*
<i>Bos frontalis</i>	DQ995672	Bhutan	<i>Bos frontalis</i>	HM215245	Arunachal Pradesh (India)*
<i>Bos frontalis</i>	DQ995670		<i>Bos gaurus</i>	HM215246	Yunnan (China)*
<i>Bos frontalis</i>	DQ995659	Bhutan	<i>Bos frontalis</i>	HM215175	Arunachal Pradesh (India) [#]
<i>Bos frontalis</i>	DQ995661	Bhutan	<i>Bos frontalis</i>	HM215176	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995663	Bhutan	<i>Bos frontalis</i>	HM215177	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995665	Bhutan	<i>Bos frontalis</i>	HM215178	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995667	Bhutan	<i>Bos frontalis</i>	HM215179	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995668	Bhutan	<i>Bos frontalis</i>	HM215180	Arunachal Pradesh (India) [#]
<i>Bos frontalis</i>	DQ995669	Bhutan	<i>Bos frontalis</i>	HM215181	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995666	Bhutan	<i>Bos frontalis</i>	HM215182	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995664	Bhutan	<i>Bos frontalis</i>	HM215183	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995662	Bhutan	<i>Bos frontalis</i>	HM215184	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995660	Bhutan	<i>Bos frontalis</i>	HM215185	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995658	Bhutan	<i>Bos frontalis</i>	HM215186	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995681	Bhutan	<i>Bos frontalis</i>	HM215187	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995679	Bhutan	<i>Bos frontalis</i>	HM215188	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995677	Bhutan	<i>Bos frontalis</i>	HM215189	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995681	Bhutan	<i>Bos frontalis</i>	HM215190	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995675	Bhutan	<i>Bos frontalis</i>	HM215191	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995673	Bhutan	<i>Bos frontalis</i>	HM215192	Yunnan (China) [#]
<i>Bos frontalis</i>	DQ995671	Bhutan	<i>Bos frontalis</i>	HM215193	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215205	Yunnan (China)*	<i>Bos frontalis</i>	HM215194	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215206	Yunnan (China)*	<i>Bos frontalis</i>	HM215195	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215207	Arunachal Pradesh (India)*	<i>Bos frontalis</i>	HM215196	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215208	Arunachal Pradesh (India)*	<i>Bos frontalis</i>	HM215197	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215209	Yunnan (China)*	<i>Bos frontalis</i>	HM215198	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215210	Yunnan (China)*	<i>Bos frontalis</i>	HM215199	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215211	Arunachal Pradesh (India)*	<i>Bos frontalis</i>	HM215200	Arunachal Pradesh (India) [#]
<i>Bos frontalis</i>	HM215212	Yunnan (China)*	<i>Bos frontalis</i>	HM215201	Arunachal Pradesh (India) [#]
<i>Bos frontalis</i>	HM215213	Yunnan (China)*	<i>Bos frontalis</i>	HM215202	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215214	Yunnan (China)*	<i>Bos gaurus</i>	HM215203	Yunnan (China) [#]
<i>Bos frontalis</i>	HM215215	Arunachal Pradesh (India)*	<i>Bos frontalis</i>	HM215204	Arunachal Pradesh (India) [#]
<i>Bos frontalis</i>	HM215216	Yunnan (China)*	<i>Bos javanicus</i>	EF693808	Cambodia
<i>Bos frontalis</i>	HM215217	Arunachal Pradesh (India)*	<i>Bos javanicus</i>	EF693804	Cambodia
<i>Bos frontalis</i>	HM215218	Arunachal Pradesh (India)*	<i>Bos javanicus</i>	EF693807	Cambodia
<i>Bos frontalis</i>	HM215219	Yunnan (China)*	<i>Bos javanicus</i>	EF693805	Cambodia
<i>Bos frontalis</i>	HM215220	Yunnan (China)*	<i>Bos javanicus</i>	AF162489	Cambodia
<i>Bos frontalis</i>	HM215221	Yunnan (China)*	<i>Bos javanicus</i>	AF162486	Cambodia
<i>Bos frontalis</i>	HM215222	Yunnan (China)*	<i>Bos grunniens</i>	GQ464146	China
<i>Bos frontalis</i>	HM215223	Yunnan (China)*	<i>Bos grunniens</i>	DQ856603	China
<i>Bos frontalis</i>	HM215224	Yunnan (China)*	<i>Bos grunniens</i>	DQ139215	China
<i>Bos frontalis</i>	HM215225	Yunnan (China)*	<i>Bos grunniens</i>	DQ139187	China
<i>Bos frontalis</i>	HM215226	Yunnan (China)*	<i>Bos grunniens</i>	AY521156	China
<i>Bos frontalis</i>	HM215227	Yunnan (China)*	<i>Bos grunniens</i>	AY521150	China
<i>Bos frontalis</i>	HM215228	Yunnan (China)*	<i>Bos grunniens</i>	GQ464261	China
<i>Bos frontalis</i>	HM215229	Yunnan (China)*	<i>Bos grunniens</i>	EF494177	China
<i>Bos frontalis</i>	HM215230	Yunnan (China)*	<i>Bos grunniens</i>	AY374125	China
<i>Bos frontalis</i>	HM215231	Yunnan (China)*	<i>Bos grunniens</i>	DQ007219	China
<i>Bos frontalis</i>	HM215232	Yunnan (China)*	<i>Bos grunniens</i>	DQ995719	Nepal

(Contd)

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Table 1. (Contd)

Species	Accession number	Origin	Species	Accession number	Origin
<i>Bos grunniens</i>	DQ995721	Nepal	<i>Bos taurus</i>	EF417950	China
<i>Bos grunniens</i>	DQ995728	Nepal	<i>Bos taurus</i>	EF417942	China
<i>Bos indicus</i>	GQ890140	India	<i>Bos taurus</i>	EU233366	China
<i>Bos indicus</i>	GQ890092	India	<i>Bos taurus</i>	AB177777	China
<i>Bos indicus</i>	GQ890126	India	<i>Bos taurus</i>	DQ166074	China
<i>Bos indicus</i>	GQ890066	India	<i>Bos taurus</i>	AB177795	China
<i>Bos indicus</i>	GQ890026	India	<i>Bos taurus</i>	AF516713	China
<i>Bos indicus</i>	GQ890117	India	<i>Bos taurus</i>	EU281522	China
<i>Bos indicus</i>	BIU51811	India	<i>Bos taurus</i>	EU281536	China
<i>Bos indicus</i>	AY972130	India	<i>Bos taurus</i>	DQ520591	China
<i>Bos indicus</i>	GU256940	China	<i>Bos taurus</i>	AB085926	Japan
<i>Bos indicus</i>	EF524184	China	<i>Bos taurus</i>	AB085919	Nepal
<i>Bos indicus</i>	EF524178	China	<i>Bos taurus</i>	AB085920	Nepal
<i>Bos indicus</i>	EF417976	China	<i>Bos taurus</i>	AB065130	Nepal
<i>Bos indicus</i>	EU233340	China	<i>Bos taurus</i>	DQ166053	China
<i>Bos indicus</i>	EU233324	China	<i>Bos taurus</i>	DQ166088	China
<i>Bos indicus</i>	DQ887760	China	<i>Bos taurus</i>	EF417960	China
<i>Bos indicus</i>	GU256940	China	<i>Bos taurus</i>	FJ971088	Mongolia
<i>Bos indicus</i>	EF524120	China	<i>Bos taurus</i>	AB117066	Mongolia
<i>Bos indicus</i>	DQ887761	China	<i>Bos taurus</i>	AB268583	Bhutan
<i>Bos indicus</i>	AB570174	Nepal	<i>Bos taurus</i>	AB268582	Bhutan
<i>Bos indicus</i>	AB570162	Bhutan	<i>Bubalus bubalis</i>	NC006295	China

*D-loop sequences generated in the present study.

#Cytochrome *b* sequences generated in the present study.

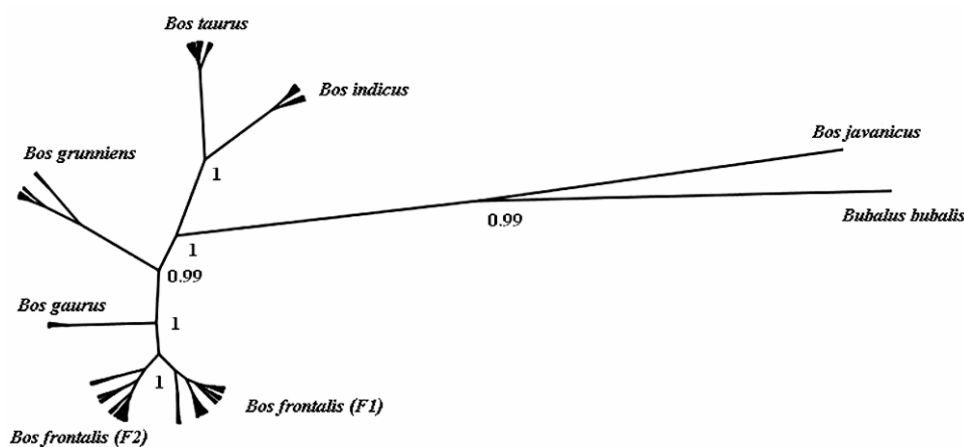


Figure 1. Phylogenetic tree constructed from D-loop sequences using Bayesian MCMC method. Note the separation of sequences into *Bos frontalis* and *Bos gaurus* clades and further splitting of *B. frontalis* sequences into two sub-clades, F1 and F2. Support for branches is depicted in the form of posterior probability ranging from 0.99 to 1.0.

gaur exhibit chromosome incompatibility; the chromosome number in gayal is commonly reported^{6,29,30} as $2n = 58$. On the other hand, two chromosome variations such as $2n = 56$ (refs 31 and 32) and $2n = 58$ (refs 29 and 33) have been reported for *B. gaurus* kept in captivity. The history of animal domestication is complex. Earlier conclusive genetic evidence in favour of the occurrence of two wild progenitor, viz. *Bos primigenius primigenius* and *Bos primigenius namadicus* to explain the separate domestication of *B. taurus* and *B. indicus*

respectively, was offered^{34,35}. Our statistically supported phylogenetic and TMRCA analyses show that *B. frontalis* and *B. gaurus* are independent sub-species/species and share a common ancestor in the range 0.134–0.207 million years ago (Table 3). Also, this time estimate is in congruence with the time depth of 0.127 million years estimated earlier for the divergence within Bison and Bos from their most recent common ancestor³⁶. Inference of divergence time under relaxed molecular clock assumption by us confers a relatively large time-frame for the

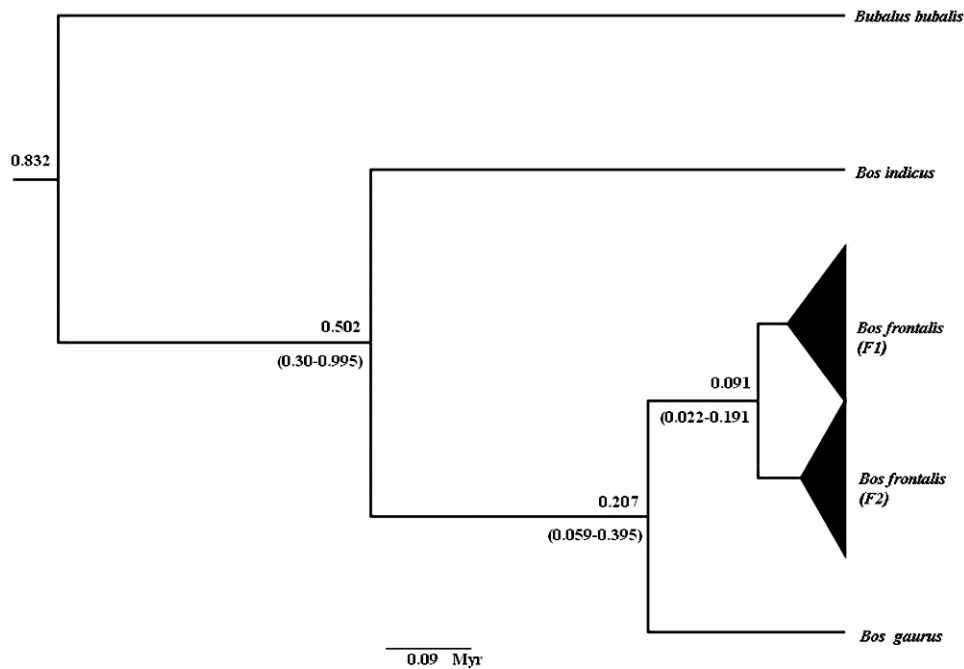


Figure 2. Bayesian inference tree produced from combined D-loop and *cyt b* gene segment under molecular clock assumption. *B. frontalis* sequences collapsed into two clades F1 and F2. Numbers above nodes denote mean estimates of divergence times (million years (myr)), with numbers below nodes showing the 95% Highest Posterior Density (HPD). The posterior probability of all labelled nodes was 1.0 (Table 3).

Table 2. Diversity and demographic indices estimated from 63 *Bos frontalis* D-loop sequences

	F1	F2	Both
(<i>n</i>)	29	34	63
Nucleotide diversity	0.024	0.018	0.037
Fu's <i>F_s</i>	-1.15	-1.07	-2.06

Table 3. Divergence time estimates (in million years) for *B. frontalis* under relaxed molecular clock assumption

Data type	Based on palaeontological data	Based on molecular data	Posterior
Combined (D-loop + <i>cyt b</i>)	0.207 (0.059–0.392)	0.134 (0.087–0.604)	1
D-loop only	0.221 (0.083–0.601)	0.152 (0.113–0.892)	1

separation of *B. frontalis* from its most recent common ancestor. Furthermore, this separation time is so large that it cannot be explained by the ~10,000 yrs BP history of animal domestication. Similar to our finding, based on *cyt b* gene analysis of *B. frontalis* and *B. gaurus*, a single previous study also confirms *B. frontalis* as a species distinct from *B. gaur*¹². In spite of supporting direct domestication of *B. frontalis* from gaur, presence of two diverse forms of gaur to explain the possible domestication of *B. frontalis* from *B. gaur* was also speculated¹¹. Some work-

ers also reported two different 'breeds' of *B. frontalis* population in the northeastern region of India³⁷. However, previous studies involving mtDNA markers failed to recognize the concept of breed in the domestics^{38,39}. Thus, taking into consideration the geographic, cytogenetic and phylogenetic evidences, maternal origin of *B. frontalis* from wild progenitor similar but not identical to *B. gaurus* cannot be ruled out. Thus, against the previously held view that *B. frontalis* is simply a domesticated form of gaur, our divergence time estimates for *B. gaurus* and *B. frontalis* provided support in favour of the hypothesis that assumes its origin from an extinct wild progenitor. Another reason that leads to discrepancies in the origins of *B. frontalis* study was due to incomplete information on samples and lack of inclusion of *B. gaurus* samples from the same geographical region. In addition, occurrence of two haplotypes of *B. indicus* and one of *B. taurus* in 41 *B. frontalis* sampled by us reinforces the gene flow between domestic cattle and *B. frontalis* population. This introgression of domestic cattle poses a serious threat to the diversity of *B. frontalis* in their respective regions^{10,12}. In the future typing of more samples of *B. frontalis* and *B. gaurus* for Y-chromosome and autosomal markers would shed further light on the origin and diversity of this interesting bovid.

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