Molecular Biology Reports
https://doi.org/10.1007/s11033-019-04675-0

SHORT COMMUNICATION

Complete mitochondrial genome of Indian mithun, *Bos frontalis* and its phylogenetic implications

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Received: 11 December 2018 / Accepted: 5 February 2019

Abstract

Mithun (*Bos frontalis*) is an endangered domestic bovine species native to the hilly areas of China, Bangladesh, Myanmar, Bhutan and India. It is believed to have been domesticated from gaur around 8000 years ago. However, a few studies suggest that mithun is either an independent species or a hybrid descendant of gaur and cattle. Therefore, to understand the evolutionary history of mithun, the complete mitochondrial genome of Indian mithun was sequenced and compared with the mitochondrial genome of closely related *Bos* species. The mitochondrial genome of mithun was 16,346 bp long and consisted of 22 tRNA genes, 13 protein-coding genes, 2 rRNA genes, and a control region. The phylogenetic assessments of Indian mithun along with other *Bos* species showed a very close genetic relationship of Indian mithun with gaur suggesting that Indian mithun might have evolved from gaur.

Keywords Mithun · Gayal · Gaur · Mitochondrial DNA · Domestication

Introduction

Bos frontalis, commonly known as "Mithun" in India, is a large domesticated ungulate which is native to the Asian continent. It has a narrow geographic range and occupies the hilly regions of China, Bangladesh, India, Myanmar and Bhutan [1, 2]. Their natural distribution in India is highly restricted to northeastern states [3] with individuals persisting as four distinct strains namely Arunachalee, Nagami, Mizorami and Manipuri [4]. Mithun remains as an economically important livestock species of Northeast India, where they are mainly reared for meat purpose and considered to be of greater socio-cultural value among the local tribal communities [3]. Nevertheless, presently, mithun is facing population decline primarily due to cross breeding with domestic cattle and over-exploitation for meat. Consequentially, the species has been catalogued as endangered by the IUCN [5, 6].

Mithun displays unique morphological traits such as a bony dorsal ridge and whitish stockings on both forelegs and hind legs and karyotype (2n = 58) which readily distinguish them from gaur (*Bos gaurus*; 2n = 56) and cattle (*Bos taurus* and *Bos indicus*; 2n = 60) [7–9]. However, karyotype analysis carried out on Indian mithun and Indian gaur has suggested that both the species possess same number of chromosomes (2n = 58) which is different from that of Malaysian and Chinese gaur [6]. Apart from morphological and karyotypic studies, a few genetic studies have also attempted to understand the evolutionary relationship of mithun, gaur and cattle. The widely held view on the origin of mithun is that it is evolved from wild gaur (*B. gaurus*) about 8000 years ago [3, 10, 11]. Contrary to this notion, Ma et al. [12] and Baig et al. [1] have reported mithun as an independent species rather than the direct descendant of gaur based on mtDNA cytochrome *b* gene and control region. While, a few studies based on restriction pattern of mtDNA, mtDNA control region and *SRY* gene have suggested that mithun is a hybrid descendant of gaur and cattle [13, 14]. However, these studies have utilized only small fragments of the mitochondrial and nuclear DNA to delineate the genetic relationship between mithun, gaur and cattle. Recently, Ren et al. [15] have reported that the Chinese mithun is genetically closer to cattle species than gaur based

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on whole mitochondrial genome. Therefore, it is necessary to understand the phylogenetic status of Indian mithun which might help to enhance our knowledge on the domestication history of mithun as India owns approximately 97.5% of total mithun population [3] and could be a likely hotspot of mithun genetic diversity [16]. In the present study, the whole mitochondrial genome of Indian mithun has been sequenced in order to understand the phylogenetic status and evolutionary history of mithun.

Materials and methods

Two muscle tissue samples of domestic mithun (BF16 and BF23) were collected opportunistically from slaughterhouses at Itangar and Bomdila, Arunachal Pradesh, India. Genomic DNA was extracted from the muscle tissue using phenol chloroform method [17]. The whole mitochondrial genome was amplified into 9.2 and 9.6 kb fragments using long range PCR with the following primer sets (BF1F 5′-AATATGCTCGCCATCATTCC-3′, BF1R 5′-ATTGCAGAGGGAAGTCATGG-3′; BF2F5′-TCACCAGCATAATTCCCACA-3′, BF2R 5′-GGCATGTCACCAAGGAGAGT-3′). These amplicons were used for library preparation using NEB Ultra DNA Library Prep Kit and subsequently sequenced on Illumina Hiseq2500.

The complete mitochondrial genome sequences were aligned and edited using the software Lasergene [18]. Mitochondrial genome sequences were annotated using MITOS web server [19]. OGDRAW was used to draw the mitochondrial genome map [20]. The tRNA secondary structures were predicted using tRNAscan-SE webserver [21]. MEGA software was used to calculate the nucleotide composition and construct phylogenetic tree [22]. The neighbor joining tree was constructed using 16,364 bp sequences with 5000 bootstrap values.

Results and discussion

The total sequencing reads generated by Illumina Hiseq2500 were 490,245 for BF16 and 608,732 for BF23 in which 90% of the bases had a base calling accuracy of 99.9%. The assembled mitochondrial genomes of mithun were 16,346 bp in size (MK279400 & MK279401) and consisted of 22 tRNA genes, 13 protein-coding genes, 2 rRNA genes, and

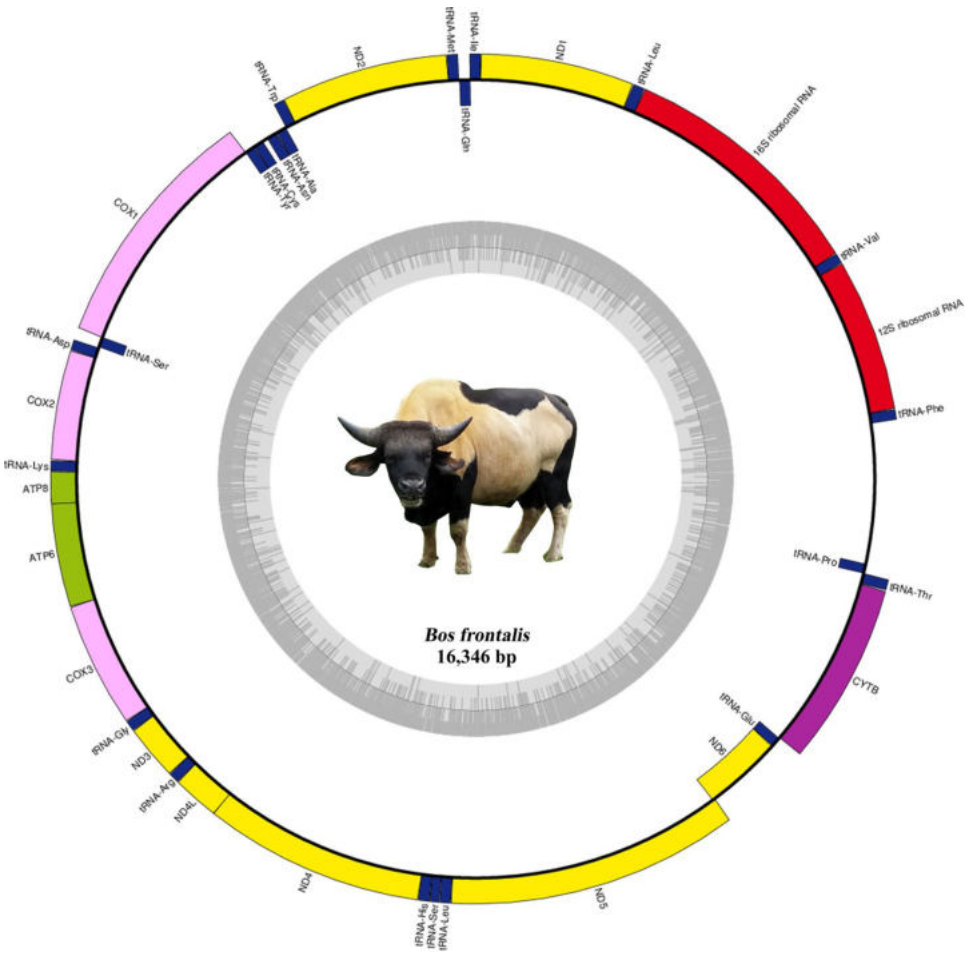

Fig. 1 The mitochondrial genome map of Indian mithun. The colored blocks outside the circle denote 28 genes encoded on the '+' strand and the colored blocks inside the circle denote the remaining 9 genes encoded on the '−'strand. The total GC content of the mitochondrial genome is represented by an inner ring

a control region which are typical characteristics of a vertebrate mitochondrial genome (Fig. 1; Table 1). As observed in other vertebrates, most of the mtDNA genes were encoded on the '+' strand except 9 genes (eight tRNA genes and ND6 gene). The gene arrangement of the mitochondrial genome was consistent with those in related bovid species. The average base nucleotide composition was 33.6% for A, 26% for C, 13.3% for G and 27.1% for T with A + T bias of 60.7%. The tRNA gene size ranged from 66 to 75 bp. The non-coding control region was 903 bp long, which is located between $tRNA^{Phe}$ and $tRNA^{Pro}$. The putative structures of tRNAs were determined, all of which exhibited the typical clover-leaf structure except tRNA Serine-GCT (Fig. 2).

There were no differences in size, nucleotide composition and gene organization between the two Indian mithun mitochondrial genomes. However, they differed from each other by 45 mutations. Followed by, Indian mithun mitochondrial genomes were compared with that of two Chinese mithun mitochondrial genomes (MF614103 & MF959941). Indian mithun BF16 and BF23 had one and 44 mutations respectively with reference to the Chinese mithun-MF614103. However, Chinese mithun-MF959941 differed vastly from the other Chinese mithun-MF614103 and Indian mithun by 1033 and 1034 mutations respectively. Mithun mitochondrial genomes were further compared with gaur and found to be different with 10–49 mutations. But the Chinese mithun-MF959941 differed from the gaur with 1032 mutations and showed more relatedness to cattle species (241–245 mutations). Therefore, in order to understand the phylogenetic status of Indian mithun, a neighbor joining tree was constructed along with two Chinese mithun and seven congeneric species such as *B. gaurus, B. taurus, B. indicus, B. javanicus, B. mutus, B. grunniens* and *B. primigenius*. The African buffalo, *Syncerus caffer* was used as an outgroup. In the phylogenetic tree, Indian mithun and Chinese mithun-MF614103 clustered together with gaur suggesting a very close genetic relationship between mithun and gaur (Fig. 3). It supports the hypothesis that mithun is a direct descendent of gaur which has been previously reported by several studies using Cytochrome *b* gene [23, 24], 16S rRNA gene [11], Y chromosomal DNA [25], SNP genotyping [3] and whole mtDNA [6]. On the other hand, Chinese mithun-MF959941 clustered along with *B. primigenius, B. taurus*, and *B. indicus*, which demonstrated close genetic relationship between these species. Previous studies have also indicated the close proximity of mithun with cattle using different markers [13, 15].

Our phylogenetic analyses suggested the presence of two maternal lineages for mithun. The occurrence of two types of mithun, one which is a descendant of gaur, while the other which is a hybrid of mithun bull and cattle, has also been indicated by Dorji et al. [11]. Similarly, Baig et al. [1] have reported three different haplotypes for mithun. The recent studies [3, 5, 6, 26] based on whole genome sequencing and SNP genotyping have supported all the three proposed hypotheses for the origin of mithun. Therefore, the phylogenetic status of mithun remains contentious. Perhaps, the evolutionary history of mithun is more complex as reported in the case of many other livestock species. A comprehensive phylogenetic analysis with more number of mithun, gaur and cattle samples particularly from India and China would improve the

Table 1 Gene order and features of mitochondrial genome of Indian mithun

Name	Start	End	Strand	Size (bp)	Anticodon
Control region	1	363		363	
tRNA-Phe	364	430	+	67	GAA
12S rRNA	431	1386	+	956	
tRNA-Val	1387	1453	+	67	TAC
16S rRNA	1452	3021	+	1570	
tRNA-Leu	3023	3097	+	75	TAA
ND1	3106	4050	+	945	
tRNA-Ile	4056	4124	+	69	GAT
tRNA-Gln	4122	4193	−	72	TTG
tRNA-Met	4196	4264	+	69	CAT
ND2	4265	5302	+	1038	
tRNA-Trp	5307	5373	+	67	TCA
tRNA-Ala	5375	5443	−	69	TGC
tRNA-Asn	5445	5517	−	73	GTT
tRNA-Cys	5550	5616	−	67	GCA
tRNA-Tyr	5617	5684	−	68	GTA
COI	5686	7224	+	1539	
tRNA-Ser	7228	7296	−	69	TGA
tRNA-Asp	7304	7371	+	68	GTC
COII	7373	8053	+	681	
tRNA-Lys	8060	8126	+	67	TTT
ATP8	8128	8322	+	195	
ATP6	8289	8963	+	675	
COIII	8969	9751	+	783	
tRNA-Gly	9753	9821	+	69	TCC
ND3	9822	10,166	+	345	
tRNA-Arg	10,169	10,237	+	69	TCG
ND4L	10,238	10,531	+	294	
ND4	10,528	11,895	+	1368	
tRNA-His	11,906	11,975	+	70	GTG
tRNA-Ser	11,976	12,035	+	60	GCT
tRNA-Leu	12,037	12,106	+	70	TAG
ND5	12,113	13,909	+	1797	
ND6	13,917	14,435	−	519	
tRNA-Glu	14,439	14,507	−	69	TTC
Cyt b	14,512	15,645	+	1134	
tRNA-Thr	15,655	15,724	+	70	TGT
tRNA-Pro	15,724	15,789	−	66	TGG
Control region	15,807	16,346		540	

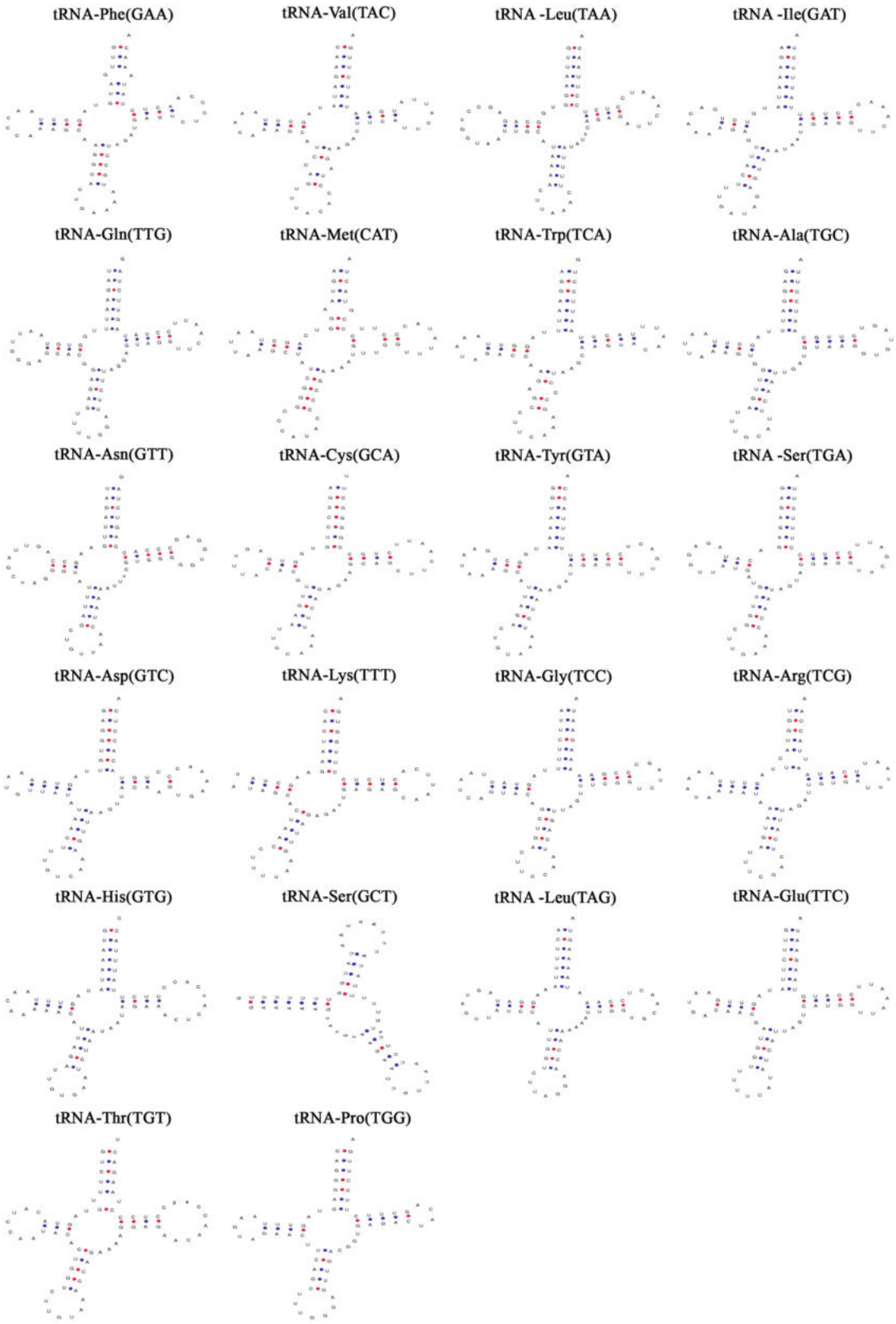

Fig. 2 Predicted structures of 22 tRNA genes of Indian mithun. Base pairing between G and C are represented by red dots. Base pairing between A and U is represented by blue dots. (Color figure online)

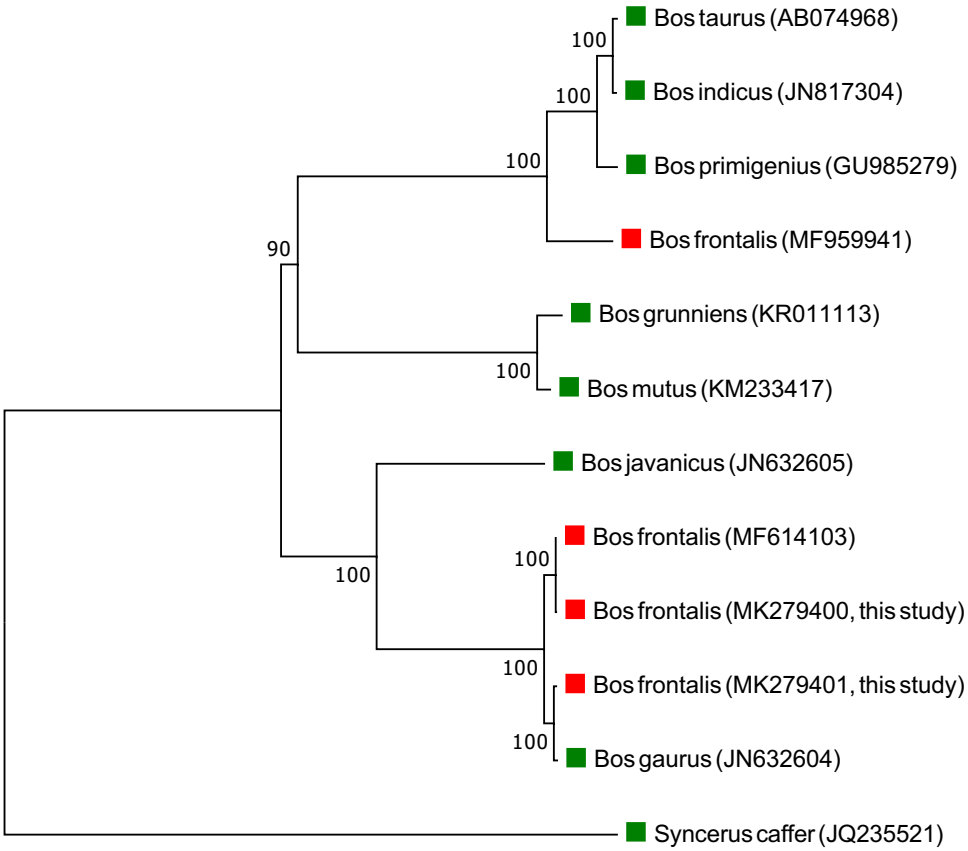

Fig. 3 Molecular phylogeny of the genus *Bos* inferred from the whole mitochondrial genome. Neighbor joining tree was constructed using 16,364 bp sequences of eight *Bos* species including mithun. The *Syncerus caffer* was used as an outgroup. Numbers adjacent to the nodes represent bootstrap values. GenBank accession numbers of each sequence is given in parentheses

current understanding of the evolutionary history of mithun. One of the major limitations to such type of study is that, as mithun falls under the IUCN endangered category, collection of tissue or blood samples is of serious ethical concern. Non-invasive samples such as dung, mucus, hair etc., can also be used as alternative to tissue or blood samples. But the low quality and quantity of DNA obtained from such sources often causes increased risk of failure in DNA sequencing particularly when targeting the complete mitochondrial genome or whole genome.

Conclusions

We sequenced for the first time the complete mitochondrial genome of Indian mithun using next generation sequencing. Our results showed that mithun of Northeast India are genetically closer to gaur. But in the case of China, two highly diverse mitochondrial genomes have been reported. Our results thus, indicate the possibility of having multiple origins for domestic mithun.

Acknowledgements We are grateful to the Science and Engineering Research Board (SERB), Department of Science and technology, Government of India, New Delhi for financial Assistance (EMR/2015/000937). The first author is grateful to the DST-INSPIRE (IF160266) for the support in the form of research fellowship.

Compliance with ethical standards

Conflict of interest There is no conflict of interest.

Ethical approval The study was performed in compliance with ethical standards of international and national guidelines for the care and use of animals. This study does not require Ethical approval as the tissue samples used were collected from the dead specimens at slaughterhouses.

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