Complete Mitochondrial Genome Sequence of *Bos Frontalis* (Gayal) of Bangladesh

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Abstract

Background: Complete mitochondrial genome of *Bos frontalis* will aid in the investigation of evolutionary links between closely related species. *Bos frontalis* mitogenome contains 37 genes and a control region. We discover the first complete mitogenome of *Bos frontalis* found in Bangladesh which was obtained from whole-genome sequencing of *Bos frontalis*.

Results: *Bos frontalis* mitogenome is 16,347 bp long, with an AT-based nucleotide composition (60.21%). It contains 37 genes, including 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and a control region (D-loop). This circular genome starts with *ND6*(negative strand) and ends at *ND5*(positive strand). Protein coding genes lost 24 bases and tRNA genes gained 27 bases compared to closely related species. Phylogenetic analysis of the mitochondrial genome of 26 closely related species from 8 congeneric species was conducted by the maximum likelihood method with 1000 bootstrap iteration.

Conclusion: Our studied *Bos frontalis* mitochondrial genome has a similar gene arrangement compared with other *Bos* species. It has almost the same amount of AT and GC content, but notably the *ND6* gene lacks AT skew and GC skew than that of the other two comparing species. PCG's loss in size might be a continuous process of evolution. The mitochondrial genome is regarded as a critical tool for species identification and monitoring populations of conservation concern.

Introduction

Gayal is a semi-wild animal descended from a cross between gaurss and domestic cattle. The gayal originated in the hilly highlands of Bhutan, eastern India, eastern Bangladesh, northern Myanmar, and northwestern Yunnan, China, where elevations can reach 3000 meters. Gayal is a free-roaming mammal that does not have organized mating amid the damp forest of hills. The body and head are typically 2.5 to 3.3 meters long, with a tail length ranging from 0.7 to 1.05 meters. 1.65 to 2.2 meters is the shoulder zenith. Both sexes have a pair of horns, with horn lengths ranging from 0.6 to 1.15 meters. *B. frontalis* has dark reddish-brown to blackish-brown hair with white stockings. Adult males are around 25% larger and heavier than females. Over the shoulders, a characteristic hump of elevated muscle may be noticed; this can be caused by extended spinal processes on the vertebrae. It is occasionally referred to as a semi-domestic animal. It is only found in the Hill district of Bangladesh. Gayal (*Mithun*) is a highly productive grazer on steep hilly slopes that distinguishes itself from other animals. The Gayal is predominantly a meat animal and is popular among the tribal people of India's north-eastern area. However, the gayal is also utilized as a ceremonial animal and plays an important role in the economic, social, and folk life of the tribal people of north-eastern India. Gayal is in a conflict cluster with related cattle, Zebu, and wild Gaur, according to phylogenetic analyses based on mtDNA or Y-chromosomal DNA. For example, Chinese Gayal, or Dulong cattle, are known to exhibit Zebu or Taurine mtDNA footprints, implying a hybrid origin, and additional research has revealed a high mtDNA and Y-chromosomal DNA sequence similarity between Gayal and Guar. Experimentation has revealed a significant level of genomic branching in bovine species. As a result, mapping resequencing data from a single bovine species onto the entire genomes of

different species (for example, gayal vs cattle) creates a channel for biases and/or inaccuracies in the sequence calibration and SNP calling technique. To be more specific, this entire investigation yielded knowledge of the *Bos frontalis* mitogenome, which could be useful for future taxonomic classification, phylogenetic reconstruction, and conservation strategy implementation. We present the full mtDNA sequences of *Bos frontalis* from Chattogram's Hill district.

Methodology

Figure 1 depicts the computational framework used for this study. A fresh blood sample of an adult male *Bos frontalis* was collected from Rangunia, Chattogram, Bangladesh (Longitude / Latitude – 22.4701 N 92.0286 E) and delivered to the laboratory to be preserved at -80° C for subsequent examination in December 2019. The animal was not sedated, and blood was collected in accordance with the 'Regulations for Animal Experiments at Chattogram Veterinary and Animal Sciences University,' which were authorized by the Bangladesh Livestock Research Institute Ethics Committee, Bangladesh. Blood samples from the gayal were collected from the farm with the permission of the gayal owner by a certified veterinarian. The blood tissue was dissected and preserved in 95% ethanol. Following that, high molecular weight genomic DNA was extracted and purified using the AddPrep Genomic DNA extraction kit (AddBio, Korea) for future analysis of the DNA's quality and quantity. Purified DNA was sent to a commercial provider for library preparation and sequencing. The DNA was sequenced on the Illumina Novaseq 6000 platform in BGI, China. All techniques were carried out in compliance with the "Regulations for Animal Experiments at Chattogram Veterinary and Animal Sciences University's distinctive characteristic, the Indian and GOB ethical clearance." The mitochondrial genome reads were split from the whole genome sequence reads (SRR12782937) by mapping them against the reference Gayal mitochondrial dataset (NC_036020) with SAMTOOLS. Then the clean reads were assembled using the organelle assembler NOVOPlasty V.2.7.2. Database searches using the Basic Local Alignment Search Tool (BLAST) were carried out next to ensure that the right target genome was acquired. For the structural and functional annotation of the mitogenome, two web-based programs- MITOS and GeSeq are used. tRNAscan-SE which uses vertebrate mitochondrial genetic coding for tRNA structure prediction as its default search mode, was used to identify the transfer RNA genes. Furthermore, OGDRAW was used to construct a circular depiction of the whole mitogenome. Finally, mitogenomes were aligned and phylogenetic tree was constructed using the MEGAX.

Table 1

The mitochondrial genome of the Bangladeshi *Bos frontalis* has been summarized. The table represents the order of the genes, strand, position, Size (bp) and intergenic spacer (bp). In the intergenic spacer column, the (+) and (-) values denote intergenic nucleotides and overlapping sections between the genes, respectively.

Genes	Strand	Position	Size (bp)	Intergenic spacer (bp)
<i>ND6</i>	-	16039–16347, 1-219	528	0
<i>tRNA-Glu</i>	-	220–288	69	1
<i>CYTB</i>	+	293–1432	1140	5
<i>tRNA-Thr</i>	+	1437–1505	69	5
<i>tRNA-Pro</i>	-	1505–1570	66	0
<i>D-loop</i>	+	1571–2491	921	1
<i>tRNA-Phe</i>	+	2492–2558	67	1
<i>12S RNA</i>	+	2559–3514	956	1
<i>tRNA-Val</i>	+	3515–3581	67	1
<i>16S RNA</i>	+	3582–5150	1569	1
<i>tRNA-Leu2</i>	+	5151–5225	75	1
<i>ND1</i>	+	5228–6183	956	3
<i>tRNA-Ile</i>	+	6184–6252	69	1
<i>tRNA-Gln</i>	-	6250–6321	72	-2
<i>tRNA-Met</i>	+	6324–6392	69	3
<i>ND2</i>	+	6393–7434	1042	1
<i>tRNA-Trp</i>	+	7435–7501	67	1
<i>tRNA-Ala</i>	-	7503–7571	69	2
<i>tRNA-Asp</i>	-	7573–7645	73	2
<i>tRNA-Cys</i>	-	7678–7744	67	33
<i>tRNA-Tyr</i>	-	7745–7812	68	1
<i>COX1</i>	+	7814–9358	1545	2
<i>tRNA-Ser2</i>	-	9356–9426	71	-2
<i>tRNA-Asn</i>	+	9432–9499	68	6
<i>COX2</i>	+	9501–10184	684	2

Genes	Strand	Position	Size (bp)	Intergenic spacer (bp)
<i>tRNA-Lys</i>	+	10188–10254	67	4
<i>ATP8</i>	+	10256–10456	201	2
<i>ATP6</i>	+	10417–11097	681	-39
<i>COX3</i>	+	11097–11880	784	0
<i>tRNA-Gly</i>	+	11881–11949	69	1
<i>ND3</i>	+	11950–12295	346	1
<i>tRNA-Arg</i>	+	12297–12365	69	2
<i>ND4L</i>	+	12366–12662	297	1
<i>ND4</i>	+	12656–14033	1378	-6
<i>tRNA-His</i>	+	14034–14103	70	1
<i>tRNA-Ser1</i>	+	14104–14163	60	1
<i>tRNA-Leu1</i>	+	14165–14234	70	2
<i>ND5</i>	+	14235–16055	1821	1

Results And Discussion

Genome organization and base composition

The complete mitochondrial genome of *Bos frontalis* is a 16,347 bp closed circular and double-stranded molecule containing 13 PCGs (*COX 1–3*, *ND1–6*, *ND4L*, *CYTb*, *ATP6* and *ATP8*), 22 tRNA genes (one for each amino acid and two each for leucine and serine), 2 rRNA genes (*rrnS* or *12S* and *rrnL* or *16S*), and a major (CR). The heavy (H) strand encodes 28 of these 37 genes, whereas the light (L) strand encodes the remaining nad6 and 8 tRNAs (Table 1; Fig. 2). As a result, there is no significant difference in the lengths of PCGs, rRNAs and tRNAs within the mitogenomes of different *Bos* species (Fig. 3). This finding supports the consistency of the genome size and gene architecture of Bangladeshi *Bos frontalis* in comparison to the mitochondrial genomes of Indian Mithun and Gaur, which have previously been reported. The mitochondrial genome's AT and GC content are 60.92% and 39.08% respectively, indicating that the nucleotide composition is overall biased towards adenine and thymine (Fig. 4). Previously described *Bos* species have shown a similar patterns. Furthermore, the mitochondrial genome on average has positive AT (0.085) and CG (0.40) skews (Fig. 4), indicating that adenine and cytosine are more abundant than their corresponding nucleotides, guanine and thymine. Moreover, it contains a total of 90bp of intergenic spacer (IGS) sequences, which are interspersed at 33 regions across the mitochondrial genome with varying ranges from 1 to 33 bp. Among these, four different overlapping regions have been speculated, ranging from 2 to 39 bp; while *ATPase subunit 8* (*ATP8*) and *ATPase subunit 6* (*ATP6*) having

the most overlapped area (39 bp). The putative control region (D-loop) of the mitochondrial genome is located between the *tRNA-Pro* and *tRNA-Phe*, consisting of 921 bp. Other closely related bovine species, such as Indian Mithun and *Bos gaurus*, have shown similar result.

Table 2

Amino acid's codon frequency and relative synonymous codon usage (RSCU) are taken into 4 decimal points. Cat represents color legends used for codons.

Amino Acid	Codon	Frequency	RSCU	Cat	Amino Acid	Codon	Frequency	RSCU	Cat
Ala	GCG	0.0017	0.0458	a	Leu1	CUU	0.0181	0.8289	c
	GCA	0.0121	0.0321	b		CUC	0.0241	1.1053	d
	GCU	0.0088	0.9466	c	Lys	AAG	0.0089	0.4592	a
	GCC	0.0149	1.5878	d		AAA	0.0298	1.5407	b
Arg	CGG	0.0032	0.6667	a	Met	AUG	0.0115	0.4969	a
	CGA	0.0063	1.3333	b		AUA	0.0347	1.5031	b
	CGU	0.0046	0.9697	c	Phe	UUU	0.0192	0.8428	a
	CGC	0.0049	1.0303	d		UUC	0.0264	1.1572	b
Asn	AAU	0.0313	1.0531	a	Pro	CCG	0.0109	0.5188	a
	AAC	0.0281	0.9469	b		CCA	0.0247	1.1097	b
Asp	GAU	0.0077	0.7941	a		CCU	0.0292	1.3925	c
	GAC	0.0118	1.2059	b		CCC	0.0192	0.9147	d
Cys	UGU	0.0077	0.9643	a	Ser1	AGU	0.0089	0.4581	a
	UGC	0.0083	1.0357	b		AGC	0.0192	0.9901	b
Gln	CAG	0.0158	0.6790	a	Ser2	UCG	0.0103	0.5320	a
	CAA	0.0307	1.3210	b		UCA	0.0270	1.3892	b
Glu	GAG	0.0131	0.9200	c		UCU	0.0230	1.1823	c
	GAA	0.0155	1.0800	d		UCC	0.0281	1.4483	d
Gly	GGG	0.0026	0.5000	a	Thr	ACG	0.0080	0.3544	a
	GGA	0.0077	1.5000	b		ACA	0.0304	1.3418	b
	GGU	0.0040	0.7778	c		ACU	0.0255	1.1266	c
	GGC	0.0063	1.2222	d		ACC	0.0267	1.1772	d
His	CAU	0.0261	1.1166	a	Trp	UGG	0.0055	0.5352	a
	CAC	0.0207	0.8834	b		UGA	0.0149	1.4648	b
Ile	AUU	0.0298	1.0400	a	Tyr	UAU	0.0319	1.1385	a

Amino Acid	Codon	Frequency	RSCU	Cat	Amino Acid	Codon	Frequency	RSCU	Cat
	AUC	0.0275	0.9600	b		UAC	0.0241	0.8615	b
Leu2	UUG	0.0132	0.6052	a	Val	GUG	0.0017	0.3529	a
	UUA	0.0270	1.2368	b		GUA	0.0100	2.0588	b
Leu1	CUG	0.0092	0.4211	a		GUU	0.0034	0.7059	c
	CUA	0.0393	1.8026	b		GUC	0.0043	0.8824	d

Protein-coding genes (PCGs) and codon usage

The mitochondrial genome encodes 13 PCGs totaling 11379 bp in size, accounting for 69.61% of the mitochondrial genome. The AT and GC concentrations are almost 62% and 38%, respectively (Figure 4), demonstrating that the PCGs have nucleotide compositional inclination towards adenine and thymine. Furthermore, the positive AT (0.085) and CG (0.400) skews of PCGs show that adenine is more abundant than thymine, while cytosine is more abundant than guanine. *The Cox1* and *Cox3* genes have a slightly negative AT skew, whereas the other PCGs have a positive AT skew. Being repeatedly observed in other bovine and mammalian species (11,15–17), the PCGs are organized into seven *NADH dehydrogenase subunits* (*ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5* and *ND6*), three *cytochrome c oxidase subunits* (*COX1*, *COX2* and *COX3*), two *ATPase subunits* (*ATP8* and *ATP6*) and one *cytochrome b* (*CYTb*). The PCGs vary greatly in size, with *ATP8* (201bp) being the smallest while *ND5* (1821bp) being the largest. Furthermore, four neighboring pairs of PCGs (*ATP8-ATP6*, *ATP6-COX3*, *ND4L-ND4* and *ND5-ND6*), as well as vertebrates (11,16,17), share a common gene location. Following the exclusion of stop codons, the relative synonymous codon usage (RSCU) is determined and summarized in Figure 5. The RSCU analysis reveals that GCU (A), GUA (V), and AAA (K) codons are the most often used. Furthermore, proline, threonine, leucine 1, asparagine and serine are the most frequently used amino acids in mitochondrial proteins, in that order.

Transfer RNA (tRNAs) and Ribosomal RNA (rRNAs)

There are a total of 2443bp long 22 tRNA genes in the *Bos frontalis* mitogenome, ranging in size from 60 bp (*tRNA-Ser1*) to 75 bp (*tRNA-Leu2*). Most of the tRNA genes (14) are encoded by the H-strand (*tRNA-Thr*, *tRNA-Phe*, *tRNA-Val*, *tRNA-Leu2*, *tRNA-Ile*, *tRNA-Met*, *tRNA-Trp*, *tRNA-Asn*, *tRNA-Lys*, *tRNA-Gly*, *tRNA-Arg*, *tRNA-His*, *tRNA-Ser1*, *tRNA-Leu1*), while the remaining 8 tRNAs (*tRNA-Glu*, *tRNA-Pro*, *tRNA-Gln*, *tRNA-Ala*, *tRNA-Asp*, *tRNA-Cys*, *tRNA-Tyr*, *tRNA-Ser2*) are encoded by the L-strand (Table 1). MITOS (5) and tRNA scan-SE (7) are used to determine all tRNA sequences and structures. Except for *tRNA-Ser1* and *tRNA-Lys* which lack a stable dihydrouridine arm loop, rest of the tRNA genes have the characteristic cloverleaf secondary structure (Figure 6). Such aberrant tRNA structures have been observed in mammals

in general, including the previously reported Indian Gaur and Mithun (11,12,18). In addition to the conventional Watson-Crick base pairs, these tRNAs contain a total of 9 mismatched base pairs (Figure. 6). G-A (two) and C-A (three) pairs are among them, and they are known as non-canonical pairs generating weak connections in tRNA secondary structures. One C-U and three U-U combinations are among the remaining four mismatches.

There are two *rRNA* genes, one 1569bp *16S ribosomal RNA* gene and one 956bp *12S ribosomal RNA* gene, for a total size of 2525 bp. The H (+) strand contains both rRNA genes. The *16S ribosomal RNA* is found between *tRNA-Val* and *tRNA-Leu2*, and the *12S ribosomal RNA* gene is found between *tRNA-Phe* and *tRNA-Val*. (Table. 1; Figure 2).

Phylogenetic relationship

The evolutionary analysis uses 26 bovine species from 8 congeneric species, including *Bos frontalis*, *Bos gaurus*, *Bos javanicus*, *Bos indicus*, *Bos primigenius*, *Bos taurus*, *Bos grunniens*, and *Bos mutus*. In this study, our sequenced species, *Bos frontalis* (MW763078.1), clustered with Mithun, Gaur, mutus, javanicus, and grunniens. The presence of Bangladeshi *Bos frontalis* with Indian mithun (MK279401.1) and Cambodian gaur (NC024818, JN632604) species indicates a tight genetic link between mithun and gaur (Figure 7). This finding clearly supports the unequivocal concept of Mithun being a direct descendant of Gaur, which is supported by the findings of prior research using *cytochrome b* (CYTB) gene (19,20), *16S ribosomal RNA* gene (21), SNP genotyping (22), entire mtDNA (3), and Y chromosomal DNA markers (23). One of the Mithuns, on the other hand, has been paired with *Bos javanicus* (AB915322.1), a Southeast Asian cattle species known as Banteng or Tembadau. The dispersed grouping of Banteng with *Bos frontalis*, *Bos gaurus* and *Bos taurus* respectively indicates its hybrid nature, likewise reported earlier (24).

In cluster 2, there is a strong phylogenetic relationship between *Bos frontalis*, *Bos Gaurus*, *Bos Jananicus*, *Bos Mutus*, and *Bos Grunniens*, and in cluster 1, there is a relationship between *Bos Taurus*, *Bos Javanicus* (706), *primigenius*, and *Indicus*.

Similar findings have also been obtained in previous studies using different markers (3,25). These findings indicate that hybridization between domestic mithun and cattle occurs (21,25,26) and that it is common in China.

Conclusion

To summarize, this study provides comprehensive information about the Bangladeshi *Bos frontalis* mitogenome, which was gathered from the Rangunia Hill track in Bangladesh. The outcome of this study will pave the road for future animal geneticists and evolutionary biologists to conduct research. This research will be used as a multifunctional genetic resource for future taxonomic, evolutionary, and phylogenetic studies of closely related species. Furthermore, because it is closely related to Chinese

gayal, Indian gayal, and Mithun, this unique Bangladeshi resource necessitates appropriate conservation methods and effective execution.

Abbreviations

AT: adenine-thymine

ATP: ATPase subunit

BP: base pair

CYTB: cytochrome b

COX1: cytochrome oxidase subunit1

GC: guanine-cytosine

mRNA: messenger ribonucleic acid

mtDNA: mitochondrial DNA

NADH: NADH dehydrogenase subunit

pcg: protein-coding gene

rRNA : ribosomal ribonucleic acid

SNP: single nucleotide polymorphism

tRNA : transfer ribonucleic acid

Declarations

Authors contribution

Gautam Kumar Deb: Project Planning ,Conceptualization, Data Curation; Razia Khatun: Investigation,Project Planning; Sheikh Mohammed Jahangir Hossain: Project Planning,Valiadation Checking; Shamsur Rahaman: Raw reads assembly, Annotation, Data Analysis and Visualizations; Md. Anamul Bahar Bhuiyan: Raw reads Submission to NCBI and Mapping Data; Sabrina Mobassirin: Raw reads Assembly, Annotation, Assembled Data Submission to NCBI, Data Analysis, Visualizations and Writing-original draft; Sajia Afrin: Assembled Data Submission to NCBI, Data Annotation, Visualizations and Writing-original draft; Md. Sirazul Islam: Sample Collection, Preparation, Evaluation and Writing-draft, review & Editing; Nathu Ram Sarker: Supervision on Sample Collection, Data Valiadation; Mohammed

Shamsul Alam Bhuiyan: Data Validation and Result Reviewer; AMAM Zonaed Siddiki: Funding Acquisition, Investigation, Project administration, Supervision, Validation, Writing-review & Editing.

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Availability of Data

Accession numbers of mitogenomes are available at NCBI under the study Accession Number: MW763078.1.

Ethics approval and consent to participate

All animal experiments and sample collecting procedures were carried out by the Institute's qualified veterinarians. The Ethical Committee (EC) of Chattogram Veterinary and Animal Sciences University (CVASU) granted ethics approval for animal experiments as required by the Bangladesh Livestock Research Institute (BLRI), Ministry of Fisheries and Livestock (MOFL), Government of Bangladesh.

Consent of Publications

Not Applicable

Declaration of Interest

The authors reported no potential conflicts of interest.

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Figures

Figure 1

The computational framework for the *Bos frontalis* mitogenomic study is a five-step procedure.

Figure 2

The whole mitochondrial genome of *Bos frontalis* has been mapped in a circular pattern. The colored blocks outside the circle represent the 28 H-strand genes, while the colored blocks inside the circle represent the remaining 9 L-strand genes. The inner ring represents the mitochondrial genome's entire GC content (grey color). The mitogenome map is generated via the OGDRAW webserver

Figure 3

The sizes of PCGs, rRNAs, and tRNAs with six closely related Bos species. The figure illustrates that our species has the lowest size for PCGs, the highest size for tRNA genes and rRNA genes have an equal size to the 4 other species.

Figure 4

The AT and GC content, as well as the skewness, of the Bangladeshi *Bos frontalis* and two other *Bos* species.

Figure 5

Codon usage frequency and RSCU (Relative synonymous codon usage) of Bangladeshi *Bos frontalis* mitochondrial protein-coding genes. The stop codon is not present. Different colors on the X-axis of both images represent the triplet codon families corresponding to the amino acids, whereas the codon usage frequency and RSCU are plotted on the Y-axis of the figure.

Figure 6

Predicted secondary structures of the 22 tRNA genes in Bangladeshi *Bos frontalis*. MITOS and tRNAscan-SE are used to determine all tRNA sequences and structures.

Figure 7

The mitochondrial genome sequences were used to determine the phylogenetic relationship between the eight *Bos* species. Except for the Bangladeshi *Bos frontalis* (MW763078.1), mitochondrial genome sequences were obtained from the NCBI database. MEGAX as used to construct phylogenetic tree and to simplify phylogenetic analysis.