SAARC J. Agric., 17(1): 23-35 (2019) *DOI: https://doi.org/10.3329/sja.v17i1.42759*

PHYLOGENETIC ANALYSIS OF BLACK BENGAL AND JAMUNAPARI GOATS IN BANGLADESH BASED ON PARTIAL SEQUENCE OF *CYTOCHROME B* GENE

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ABSTRACT

Goats, among the livestock species, are considered the most prolific ruminant especially under callous climatic conditions. The aim of the present study was to depict the current phylogenetic status and genetic diversities of Black Bengal (BBG) and *Jamunapari* goat of Bangladesh and the world. *Cytochrome b (cytb)* gene (1140 bp) of mitochondrial DNA of Black Bengal goats (*Capra hircus*) was amplified by Polymerase Chain Reaction (PCR) for the first time in Bangladesh. The sequence from BBG had no nucleotide (nt) difference and 100% homology with the BBG (*C. hircus*) of India and also the goats (*C. hircus*) from China (Yangtze River Delta White Goat), Thailand (Wild Cervidae), Japan (Bezoar goat) and South Africa (Domestic goat). The sequence had 1-5 nt differences and 99% homology with the goats (*C. hircus*) from China, Thailand and Japan (other goats), and also with the goats (*C. hircus*) from Malaysia, South Korea, France, Italy, Pakistan, Slovenia, Switzerland and USA. Phylogenetic tree constructed with Black Bengal Goat (BBG-K-2) and *Jamunapari* goats (SG-1) of Bangladesh with cytochrome b nucleotide sequences were closely related to China-HM7. China-YP xj46, Pakistan-Lineage C1, Pakistan-Lineage C2, Slovenia-ChSo1, Switzerland-ChTo2992 and shared 98.8% to 99% and 98.3% to 98.6% similarity, respectively and 1-1.2% and 1.4 to 1.7% genetic distance, respectively. Based on Ctb gene Sequence collected from Bangladeshi Black Bengal Goats (BBG-K-2) and Jamunapari goats (SG-1) that were closely related and shared with the same genetic lineage of China HM18 and India-BBG-DQ073048, respectively, suggesting a common origin.

Keywords: Domestic Goats, Genetic Diversity, mtDNA, cytb Gene, Phylogenetic Analysis

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Received: 16.01.2019 *Accepted: 20.06.2019*

INTRODUCTION

Archaeological evidences indicated that goats were the first ruminant animal to be domesticated 10,500 years ago around the Fertile Crescent (Fernández et al., 2006; Zeder, 2008). There were two suggested wild species of the genus *Capra* (*C. aegagrus* and *C. falconari*), with the closest candidate *C. aegagrus*, which domestic goat gene pool was derived from (Mannen et al., 2001). Analyses of the control region (the displacement-loop) of mitochondrial DNA (mtDNA) and nuclear DNA are most useful examinations and are informative genomic elements for explicating the origin, diversity, genetic relationship and diversification of livestock including goat (Dorji et al., 2010).

In Bangladesh, the rearing of goats is a profitable household enterprise for rural populations due to the animal's prolific breeding potential, survivability and consumption of locally collected feed (Islam et al., 2011). Economy of Bangladesh is mainly driven by agricultural product and livestock is the most viable sector. Livestock is contributing about 2.73% of overall Gross Domestic Products (GDP) and 4.31% export earnings from leather and leather goods of total export, 20% of the population is directly and 50% is partly dependent on this sector (Draft Sixth Five Year Plan, 2010). Goat (66.6 million) and Sheep (29.1 million) in Bangladesh have valuable contributions to the economy of the country (DLS, 2018).

Recently, molecular studies of goats based on mitochondrial DNA (mtDNA) sequences have been carried out to investigate the origin and phylogeny of goats (Mannen et al., 2001). Mitochondrial DNA is very useful for its multiple presences in cells. The most of animal mtDNA is coding 37 genes (Avise, 1994). One of them, *Cytochrome b* (*Cyt b*) is one of the genes encoded by mitochondrial deoxyribonucleic acid (mtDNA). The mtDNA sequence has been used extensively in the study of genetic evolution because it is easy to obtain, has a high value in evolution, and generally follows a pattern of inheritance compatible with phylogenetic reconstruction (Jain et al., 2007). The uniqueness of *Cyt b* is one of the protein-coding genes involved in the transport of electrons in the respiratory chain; it can be determined as a target for evolutionary analysis and species identification, particularly useful for comparing species within the same genus or the same family and also can be used to study genetic diversity through mtDNA sequences (Manceau et al., 1999; Castresana, 2001; Mohammadi et al., 2018). Amino acid sequence on *Cyt b* gene can be used to clarify Kejobong goat phylogenetic status among several Asian local goats (Lestari et al., 2018).

Characterize and molecular sequencing of *Cytochrome b* gene of Mitochondrial DNA in Bangladeshi goat genome was evident in our previous endeavor (Chowdhury et al., 2011). The present study was investigated to current phylogenetic status and genetic diversities of *Black Bengal* and *Jamnapari* goat of Bangladesh and the world in order to understand the genetic basis of this breed.

MATREIALS AND METHODS

Breed selection

There were two different local breeds *Black Bengal* and *Jamnapari* goats of Bangladesh selected for this research study.

Study area

This study was conducted in three different goat farms located in semi-urban area of Savar region of Dhaka and Khulna for Black Bengal goats, Gazipur area for *Jamnapari* goats in Bangladesh during the period 2010 to 2011. Three goat farms are designated as farm code A, B, and C. The samples were collected from the three goats of selected farms and brought to the Department of Microbiology and Hygiene, Bangladesh Agricultural University, for laboratory analysis.

Sample collection

A total of 3 blood samples from individual goats of 2.5 years of age were collected from jugular vein each from three selected goat farms. Blood samples of *Black Bengal* goat were taken from Khulna (BBG-K-2), Dhaka and Jamunapari goats from Sardagonj of Gazipur (SG-1) area of Bangladesh. All blood samples (5ml in EDTA Containing tubes) were aseptically collected and stored at -20°C until used at Microbiology laboratory. The goat samples were unrelated genetically based on the information of the owners and local breeding data. Processing of blood samples was followed by Chowdhury et al. (2011).

DNA extraction, amplification, and sequencing of *cyt b* gene

DNA was extracted from whole blood using the method as described by Chowdhury et al (2011) for sequence analysis *Cyt b* gene in mtDNA. All DNA samples were brought to the final concentration of 50 ng/µL and stored at -80°C. The forward primer (5′-ATG ACC AAC ATC CGA AAG ACC C-3′ (nt 1-22)) and reverse primer (5′-TCT TCA TTT TAG AAG GTT GTT TCC-3′ (nt 1140-1117) that generated 1140 bp polymerase chain reaction (PCR) product were used to amplify 1140 bp of *Cytb* gene as described by Takada et al (1997) and Chowdhury et al (2011).

Sequence Alignment and identification

Partial sequences, obtained using forward and reverse primers of mtDNA *Cyt b* sequences were combined to full length sequences (420 bp for Black Bengal) via the SeqMan Genome Assembler (DNAstar, USA) and were compared to the Gene Bank database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/GenBank) by means of the basic local alignment search tool (BLAST) to identify close phylogenetic relatives. The nucleotide sequences then were translated into amino acids form by mitochondrial vertebrate genetic code. All mtDNA *Cyt b* sequences were analyzed using Molecular Evolutionary Genetics Analysis 6 program (Tamura et al., 2013) and aligned by ClustalW (Thompson et al., 1994).

Partial *cyt b* gene sequence (N-terminal part) of mtDNA of *Jamnapari* goat from Gazipur was published (Chowdhury et al., 2011). Based on the resulted partial *cyt b* gene sequence (N-terminal part) of mitochondrial DNA of *Black Bengal* goat (Bangladesh-BBG-K-2) of Khulna district, similar sized sequence was taken from that of *Jamnapari* goat (Bangladesh-*Jamnapari*-SG) and that of 42 other goats of different countries from Gene Bank Databases. Multiple alignment was carried out using Lasergene MgAlign program of DNASTAR Software (http://www.dnastar.com, Product Key: NXRAY-GQ8NJ-EKJW7). Sequence distances were obtained using MgAlign Distance ClustalW.

Construction of Phylogenetic tree

The multiple sequence alignment of the retrieved reference sequences from NCBI, EMBL or DDBJ and representative isolates' sequences were performed with the ClustalW (Larkin, Blackshields et al., 2007) software. Aligned sequences were exported to the GeneDoc software for sequence trimming and conserved region identification. Refined sequences were further exported to the Molecular Evolutionary Genetic Analysis (MEGA) (Tamura and Dudley, 2007) software for phylogenetic tree construction using the Neighbor joining algorithm and selecting 1000 bootstrap replication. Further analysis of the genes was carried out using the Distance and Pattern analysis tool in the MEGA software. The phylogenetic tree was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). A bootstrap consensus tree was inferred from 1000 replicates (Felsenstein, 1985).

Nucleotide sequence accession numbers

The partial mtDNA Cytochrome B gene sequences obtained in this study have been deposited in the GenBank database under the accession numbers MN066604 for Black Bengal goat (BBG-K-2) and MN066605 for Jamunapari goat (SG-1). The mtDNA *Cytb gene* sequences were displayed within the phylogenetic tree and the accession numbers are listed in Table 1. Isolates name have been abbreviated using the following format: country/organization/location/isolate no.

Table 1. List of *Cyt b gene* sequences of goats used in phylogenetic analysis

Serial no.	Goat Species	Country	Accession No.
1.	Capra hircus (Isolate: BBG-K-2)	Bangladesh	MN066604
2.	Capra hircus (Isolate: SG-1)	Bangladesh	MN066605
3.	Capra hircus (Isolate:D-LK18)	China	GU229281
4.	Capra hircus (Isolate: G32, breed: Yangtze River Delta White Goat)	China	EU130773
5.	Capra hircus (Isolate: G45, breed: Yangtze River Delta White Goat)	China	EU130774
6.	Capra hircus (Isolate: HM7, breed:	China	EU130780

Serial no.	Goat Species	Country	Accession No.
	Yangtze River Delta White Goat)		
7.	Capra hircus (Isolate: HM13, breed: Yangtze River Delta White Goat)	China	EU130775
8.	C. hircus (Isolate: HM18, breed: Yangtze River Delta White Goat)	China	EU130776
9.	C. hircus (Specimen_voucher:YP xj46)	China	DQ089480
10.	C. cylindricornis (East caucasian tur)	France	AF034737
11.	C. caucasica (West caucasian tur)	France	AF034738
12.	C. ibex (Ibex)	France	AF034735
13.	C. falconeri (Markhor)	France	AF034736
14.	C. nubiana (Nubian ibex)	France	AF034740
15.	Capra hircus (Isolate: V07-146)	France	GU295658
16.	C. aegagrus (wild goat)	France	AF034739
17.	Capra hircus (breed: black Bengal)	India	DQ073048
18.	C. hircus	India	EF056502
19.	C. aegagrus cretica (subspecies-cretica)	Israel	AF217255
20.	Capra hircus (Strain- breed Baladi)	Israel	AF217254
21.	C. hircus	Italy	AF533441
22.	C. hircus	Japan	AB004073
23.	C. hircus	Japan	AB004074
24.	C. hircus	Japan	D84201
25.	C. hircus (Strain: Laos native, Isolate: Laos-1)	Japan	AB044307
26.	C. hircus (Strain : Laos native, Isolate: Laos-5)	Japan	AB044308
27.	C. falconeri (Markhor)	Japan	AB044309
28.	C. hircus (haplotype: ChMy50)	Malaysia	DQ514545
29.	C. hircus (Strain: Lineage C1)	Pakistan	AB110594
30.	C. hircus (Strain: Lineage C2)	Pakistan	AB110595
31.	C. hircus (Strain: Lineage D1)	Pakistan	AB110596
32.	C. hircus (Strain: Lineage D2)	Pakistan	AB110597
33.	C. aegagrus blythi (Strain: Sindh Ibex 1)	Pakistan	AB110592
34.	C. aegagrus blythi (Strain: Sindh Ibex 2)	Pakistan	AB110593

Serial no.	Goat Species	Country	Accession No.
35.	C. hircus (haplotype ChGr642)	South Africa	DQ514544
36.	Capra hircus (Isolate: a-105)	South Korea	EU259119
37.	Capra hircus (Isolate: b-109)	South Korea	EU259120
38.	Capra hircus (Isolate: e-139)	South Korea	EU259132
39.	C. hircus (haplotype: ChSo1)	Slovenia	DQ514547
40.	C. hircus (haplotype: ChTo2992)	Switzerland	DQ514548
41.	Capra hircus (Isolate:Goat01)	Thailand	FJ556564
42.	Capra hircus (Isolate:Goat03)	Thailand	FJ556557
43.	C. hircus	USA	X56289
44.	C. aegagrus (wild goat)	Japan	AB004069

RESULTS AND DISCUSSION

We analyzed cytochrome b sequences to identify Bangladeshi goat phylogeny as well as to discern the genetic diversity of goat breeds/populations. Two different goat breeds, *Black Bengal* (MN066604) and *Jamunapari* (MN066605) goats, were detected in Bangladesh. Two sequencing reactions (forward and reverse) for each sample gave 606 bp sequence for a *Jamunapari* goat (Chowdhury et al., 2011) and 420 bp sequence (Fig. 1) for a BBG from Khulna those after analysis were confirmed as partial cyt b gene sequence (N-terminal part) of goat mtDNA. Nuclcotide (nt) differences and percentage homologies of the genome fragment sequence (420 bp) of a BBG with the concerned sequences of goats of other countries are given in Table 1.

1 ATTGTAAACA ACGCATTTAT TGACCTCCCA ACCCCATCAA ACATCTCATC
ATGATGAAAC TTTGGATCCC TCCTAGGAAT TTGCCTAATC TTACAAATCC
TGACAGGCCT ATTCCTAGCA ATACACTATA CATCCGACAC AATAACAGCA
TTTTCCTCTG TAACTCACAT TTGTCGAGAT GTAAATTATG GCTGAATCAT
CCGATACATA CACGCAAACG GAGCATCAAT ATTCTTTATC TGCCTATTCA **420**
TACATATCGG ACGAGGTCTA TATTATGGAT CATATACCTT TCTAGAAACA
TGAAACATTG GAGTAATCCT CCTGCTCGCG ACAATGGCCA CAGCATTCAT
AGGCTATGTT TTACCATGAG GACAAATATC ATTTTGAGGG
GCAACAGTCA TCACTAATCT TCTTTCAGCA

Figure 1. Partial Cytochrome b (CYTB) gene sequence (420 bp, N-terminal part) of itochondrial DNA from a *Black Bengal* goat (*Capra hircus*) (Bangladesh-BBG-K-2) of Khulna district.

Phylogenic analysis of the *Cyt b* region of mtDNA has been used extensively to identify and characterize goat species, and investigate the molecular distribution of the goat worldwide (Lestari et al., 2018). mtDNA is an important tool for phylogenetic studies and has been widely used to study genetic differentiation, genetic complexity, evolutionary relationships and origins of many domestic animals

(Wilson et al., 1985; Carmela et al., 2000) including cattle (Loftus et al., 1994), buffalo (Babar et al., 2011a), sheep (Loehr et al., 2006) and goats (Joshi et al., 2004; Babar et al., 2011b). Sequence data from the BLRI isolates of different goat breeds in this study indicated that the two goat breeds existing during the period of 2010–2011 were closely related to native breeds of Bangladesh and surrounding countries.

Jamunapari (MN066605) and *Black Bengal* (MN066604) goats are compared with 22 different goat breeds from different countries by drawing phylogenetic (Table 2). The phylogeny tree shows two main clusters which in each cluster there were several subclusters (Fig. 3). Almost all of *Jamunapari* goats in this study were in the first cluster together with India, Pakistan, China, Japan, Thailand, Malaysia, Japan, France, S. Africa and BBG-K-2 goats, but some of them (S. Korea-a-105, S. Korea-b-109, S. Korea-e-139) formed separate subcluster with most of the other *Jamunapari* (MN066605) and *Black Bengal* (MN066604) goats. On the other hand, the second cluster was filled by goats from Israel, Italy, USA Switzerland, Slovenia, France, Japan, China and Pakistan. Two sequencing reactions (forward and reverse) for each sample gave 606 bp sequence for a *Jamunapari* goat (Chowdhury et al. 2011) and 420 bp sequence for a BBG from Khulna those after analysis were confirmed as partial cytb gene sequence (N-terminal part) of goat mitochondrial DNA. The sequence from BBG had no nucleotide (nt) difference and 100% homology with the BBG (*C. hircus*) of India and also the goats from China, Thailand, Japan and South Africa. The sequence had 1-5 nt differences and 99% homology with the goats from China, Thailand and Japan (other goats), and also with the goats from Malaysia, South Korea, France, Italy, Pakistan, Slovenia, Switzerland and USA. Same similarity (99%) with only 1 nt difference was found with a Japanese wild goat (Bezoar *C. aegagrus*), with 2 nt difference was found with a Bangladeshi *Jamunapari* (MN066605) goat , and with 3 nt difference was found with Israeli goats (*Capra hircus* and *C. aegagrus cretica*) (Table 2). The sequence had 12-13 nt difference (97% homology) with Sindh Ibex goats (*C. aegagrus blythi*) from Pakistan, and with other Indian goat (*C. hircus*). Sixteen nt difference (96% homology) was found with Markhor goats (*C. falconeri*) of Japan and France. Eighteen to 28 nt difference (96%-93% homology) was found (Table 2) with other goats of France (*C. caucasica*, *C. cylindricornis*, *C. aegagrus*, *C. nubiana* and *C. ibex*). This is possible because of geographical distance and distribution. Chowdhury *et al*., 2011 reported sequencing of Bangladeshi *Jamunapari* (MN066605) goat has unique at positions 17 and 21 containing G and G, respectively. For this reason, *Jamunapari* goat phenotypically has been showed more height, weight and milk production in relation to BBG of Bangladesh. In our present study, BBG of Bangladesh showed unique at positions 17 and 21 containing T and T, respectively, which may be considered as nucleotide markers of *Black Bengal* goats. Since these differences BBG gives at least triplet numbers of kid at six months interval of reproduction period.

Table 2. Nuclcotide (nt) differences and percentage homologies of the sequence (mitochondrial cytb gene partial sequence, 420 bp) of Black Bengal (Accession No.: MN066604) goat (BBG-K-2) with that of other goats

Serial no.	Accession No. /Identity	Country	No. of nt differences	Percentage homology (%)
1.	MN066605	Bangladesh	2	99
2.	GU229281	China	3	99
3.	EU130773	China	2	99
4.	EU130774	China	1	99
5.	EU130780	China	4	99
6.	EU130775	China	0	100
7.	EU130776	China	0	100
8.	DQ089480	China	5	99
9.	AF034737	France	21	95
10.	AF034738	France	18	96
11.	AF034735	France	28	93
12.	AF034736	France	16	96
13.	AF034740	France	27	94
14.	GU295658	France	1	99
15.	AF034739	France	21	95
16.	DQ073048	India	0	100
17.	EF056502	India	13	97
18.	AF217255	Israel	3	99
19.	AF217254	Israel	3	99
20.	AF533441	Italy	1	99
21.	AB004073	Japan	0	100
22.	AB004074	Japan	1	99
23.	D84201	Japan	5	99
24.	AB044307	Japan	0	100
25.	AB044308	Japan	1	99
26.	AB044309	Japan	16	96
27.	DQ514545	Malaysia	1	99
28.	AB110594	Pakistan	4	99
29.	AB110595	Pakistan	4	99
30.	AB110596	Pakistan	3	99

Serial no.	Accession No. /Identity	Country	No. of nt differences	Percentage homology (%)
31.	AB110597	Pakistan	2	99
32.	AB110592	Pakistan	12	97
33.	AB110593	Pakistan	12	97
34.	DQ514544	South Africa	0	100
35.	EU259119	South Korea	2	99
36.	EU259120	South Korea	1	99
37.	EU259132	South Korea	1	99
38.	DQ514547	Slovenia	4	99
39.	DQ514548	Switzerland	5	99
40.	FJ556564	Thailand	0	100
41.	FJ556557	Thailand	1	99
42.	X56289	USA	5	99
43.	AB004069	Japan	1	99

Our results were in general agreement with the pattern described in previous studies (Liu et al., 2007; Wang et al., 2008). From NCBI BLAST search of the GenBank, EMBL, DDBJ and PDB databases, no sequence of Jamunapari goat was available. However, unpublished Gene Bank sequences of cytochrome b gene of mitochondrial DNA of Black Bengal goats (BBG) in India (Accession nos. DQ093614 and DQ 073048) had only 3-4 nt differences (Table 2) with high homology (99%) with the present Jamunapari goat sequence (Chowdhury et al., 2011). Results of sequence analysis of cytochrome b gene of mitochondrial DNA revealed that China-HM13 were closely related to Black Bengal goat (BBG-K-2) of Bangladesh, China-HM18, Japan-AB004073, S. Korea-a-105, S. Korea-b-109, S. Korea-e-139, Thailand-Goat01, Japan-AB004069 and also shared 99.5% to 100% similarity at the nucleotide level. Significant divergence rates (0 to 0.5%) observed among the goats of above countries. Black Bengal Goat (MN066604) of Bangladesh showed that S. Korea-a-105, S. Korea-b-109, S. Korea-e-139, Japan-AB004069 with 99.5% to 99.8% identity were most closely related to BBG-K-2 collected from the Khulna area of Bangladesh. Moreover, it revealed that there was slight divergence (0.2% to 0.5%) of those countries (Fig. 2). Whereas analyses of cytochrome b gene sequences suggested that Jamunapari goats (SG-1) have maximum identity of 99.5% to 99.8%, and were most closely related to BBG-K-2, China-D-LK18, China-G32, China-HM13, China-HM18, India-BBG-DQ073048, Japan-AB004073, Japan-AB004074, Pakistan-Lineage D1, Pakistan-Lineage D2, S. Africa-ChGr642, S. Korea-a-105, S. Korea-b-109, S. Korea-e-139, Thailand-Goat01, Japan-AB004069 (Fig. 2). The slight divergence rate (0.5% to 1.2%) was also found (Fig. 3).

Genetic relationships of *Black Bengal* (MN066604) Goat (BBG-K-2) and *Jamunapari* (MN066605) goats (SG-1) of Bangladesh with cytochrome b nucleotide sequences were closely related to China-HM7, China-YP xj46, Pakistan-Lineage C1, Pakistan-Lineage C2, Slovenia- ChSo1, Switzerland-ChTo2992 and shared 98.8% to 99% and 98.3% to 98.6% similarity, respectively and 1-1.2% and 1.4 to 1.7% genetic distance (Fig. 2), respectively. China-HM7 showed 98.8% to 100% similarities with China-YP xj46, Pakistan-Lineage C1, Pakistan-Lineage C2, Slovenia- ChSo1, Switzerland-ChTo2992 and slight divergence rate (0% to 0.2%).

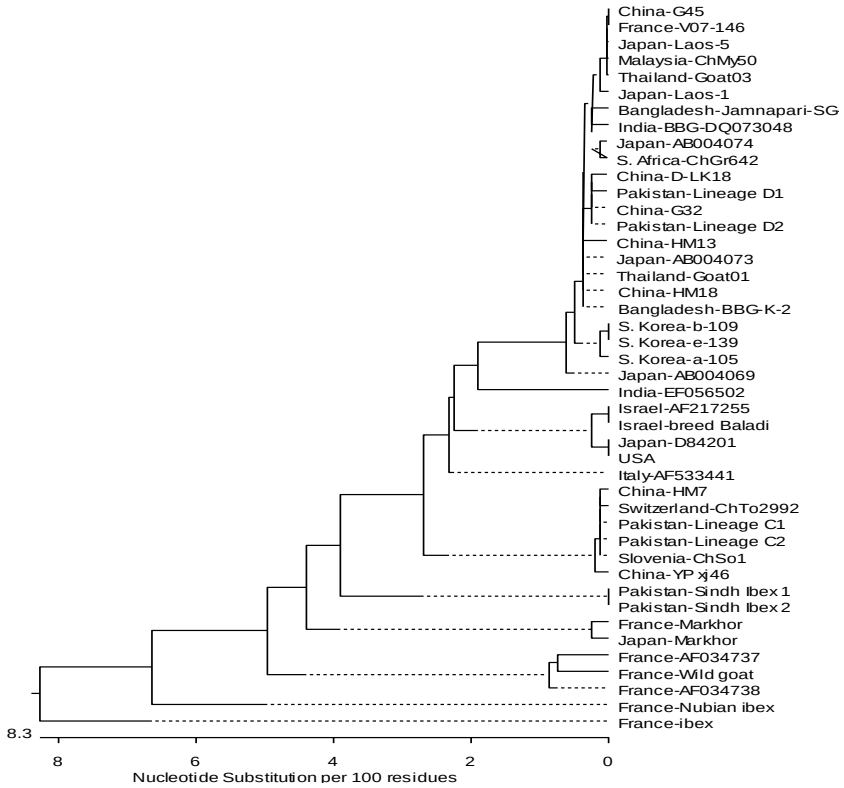

Figure 3. Phylogenetic tree showing the relationships of Black Bengal (BBG-K-2) and Jamunapari goats (SG-1) of Bangladesh with other goats based on partial cytochrome b gene (420 bp seq) sequence

There was 99% to 100% similarity observed for France-V07-146 and Japan-Laos-5 goat in relation to goats of Bangladesh-BBG-K-2, Bangladesh-Jamnapari-SG-1, China-D-LK18, China-G32, China-HM13, China-HM18, India-BBG-DQ073048, Japan-AB004073, Japan-AB004074, Japan-Laos-1, Malaysia-ChMy50, Pakistan-Lineage D1, Pakistan-Lineage D2, S. Africa-ChGr642, S. Korea-a-105, S. Korea-b-109, S. Korea-e-139, Thailand-Goat01, Thailand-Goat03, Japan-AB004069 and significant divergence rates (0% to 1%) observed. There was high divergence showed

(7.1% to 7.6% and 6.8% to 7.2%) from the sequencing of cytochrome b gene of France-ibex goat and France-Nubian ibex with Black Bengal Goat (BBG-K-2) and Jamnapari goats (SG-1) of Bangladesh (Fig. 2). Sequenced Cytb gene collected from Bangladeshi goat Black Bengal (BBG-K-2) were closely related and shared with the same genetic lineage of India (DQ073048), China HM18, respectively, suggesting a common origin whereas Bangladeshi Jamnapari goat (SG-1) showed 99.5% similarity with India (DQ073048) and BBG-K-2 (MN066604) of Bangladesh (Fig. 3).

CONCLUSIONS

Black Bengal and *Jamunapari* goats of Bangladesh have a close genetic relationship to several local goats in Southeast Asia. We speculated that gene flow among goat populations facilitated by the traditional seasonal pastoralism and annual long-distance migrations in history as well as trade would account for the pattern discerned in regional goat pools.

ACKNOWLEDGEMENTS

We acknowledge Bangladesh Livestock Research Institute, Savar, Dhaka for funding of this research work. The cooperation for the deposition of DNA sequence into GenBank provided by Dr. Jayedul Hassan of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh is accordingly acknowledged.

Conflicts of interest

Authors have no financial and other conflicts of interest to declare.

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