Genetic diversity and phylogenetic relationship of domestic pig breeds of northeast India based on Cytochrome b gene analysis

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Abstract
Northeast India contributes about 38.5% of the country's pork production. However, extensive crossbreeding between indigenous and exotic breeds in this region has caused decline in native pig populations. To conserve and restore the native pig breeds, characterising them at molecular level is a must. Therefore, current study determines genetic diversity and phylogenetic analysis of domestic pigs (Doom and Ghungroo pigs) of northeast India, using cytochrome b gene. Blood samples from 24 pigs were collected, followed by DNA extraction and gene was amplified using universal primers and sequenced. Sequence variation sites, genetic distance and phylogenetic analysis were performed at MEGA 11. A total of 18 polymorphic sites was found where 6 sites were common to Doom and Indian wild pig (15940, 16344, 16350, 16355, 16356 and 16357).

Doom pig showed least genetic distance with wild boars while Ghungroo pig showed the farthest distance. Phylogenetic analysis depicted that Doom and Ghungroo, clustering in one clade with Indian wild pig, indicates of having common maternal ancestor. This study highlights the evolutionary history and genetic diversity of domesticated pigs of northeast, India. Identifying the pig-specific mitochondrial DNA sequences will help in distinguishing domestic pigs from wild boars as well as in forensic and vegeto-legal cases.

Keywords: Indigenous pig breeds, cytochrome b gene, genetic variation, phylogenetic analysis, MEGA 11.

Introduction

Domestication of pigs was found to have occurred about 9000 – 10000 years before present (YBP) at various places across Asia and Europe. The present-day pigs are the development of local breeds that have occurred throughout the centuries, characterized by distinct phenotypes and productive capabilities[2]. Mitochondrial DNA (mt-DNA) is a powerful tool to study the genetical structure and to carry out matrilineal origin of domesticated pigs[1]. Compared to nuclear DNA, mitochondrial DNA has higher rate of mutation and does not undergo recombination, thereby making it ideal to study divergence between the animal species[4,20]. Cytochrome b gene is one of the regions of mitochondrial DNA, commonly used as a marker for identification of species and to establish phylogenetic relationships[5].

Doom (accession no. INDIA_PIG_0200_DOOM_09006) and Ghungroo (accession no. INDIA_PIG_2100_GHUNGROO_09001) are indigenous pig breeds from northeast India[7, 8]. They are protected and recognized by ICAR-NBAGR with the aim to utilize their genetic resources and creating a database on indigenous livestock species of India[11]. Indigenous pig breeds are more promising than commercial ones due to their high prolificacy, hardiness, disease resistance, adaptability and low maintenance[3]. However, due to high rate of crossbreeding for better pork productivity, these local breeds are declining at an alarming rate. Therefore, conserving and restoring local germplasm is essential for protecting the genetic makeup of indigenous pig breeds.

On the other hand, it also helps in preserving the cultural and economic benefits that these breeds provide to local farmers. Previous studies used 'cytochrome b' as a molecular marker to identify the genetic differences between Indian wild pigs and domestic pigs[10]. Another study determined the molecular characterization of cytochrome b gene by microsatellites in indigenous pig of India[15]. Despite the importance of cytochrome b gene in species identification and determining phylogenetic relationships, there are very few studies on domesticated pig breeds in India that utilize this marker.

Evaluating the phylogenetic relationships of domestic pigs (Doom and Ghungroo) will provide insights of their genetic diversity and the origin based on maternal lineages. The present study determines the genetic diversity and phylogenetic relationship of domestic pigs based on partial sequence of cytochrome b gene that will provide a base for future studies and conservation of these novel genotypes.

Material and Methods

Collection of samples: The study was conducted during October 2019 to July 2020 at Udalguri district of northeast, India. A total of 72 blood samples were collected from 24 (12 Doom and 12 Ghungroo) pigs by certified veterinarian of Animal Husbandry and Veterinary Department of Government of Assam, India. About 2 mL of blood was withdrawn from the auricular vein of the ear and collected in clean EDTA vials[13]. Each sample were labelled accordingly and stored at -20 ◦C till extraction of DNA.

Extraction of DNA: DNA was extracted from the samples using DNeasy Blood and Tissue kit (QIAGEN, Germany) in a final elution volume of 50-100 µL and the DNA samples were stored at -20°C.

PCR amplification and DNA sequencing: Cytochrome b sequences were amplified using universal primers: Forward primer: 5'TACCATGAGGACAAATATCATTCTG3' and Reverse primer: 5'CCTCCTAGTTTGTTAGGGATTG ATCG3'[20]. PCR amplification was performed in a MiniAmp Plus thermal cycler (Applied BioSystems). PCR amplification was conducted in a final volume of 25 µL with 100 ng of extracted DNA, using 2.5 µL of 10X PCR buffer, 0.5 µL of 10 mM dNTPs, 5 pmol/µL of each forward and reverse primer and 3 units/µL of Taq DNA polymerase (Applied Biosystems). The PCR was performed using the following conditions: initial denaturation at 95° C for 5 minutes, 35 cycles of denaturation at 94° C for 45 s, annealing at 52°C for 50 s, extension at 72°C for 1 minute with a final extension of 72°C for 7 minutes.

The PCR products were loaded on to 2% agarose gel containing ethidium bromide. The amplified DNA was run on a horizontal electrophoresis at 100 V for 40-45 minutes. The gel was visualized at gel documentation system (Life Technologies). The PCR products were sequenced using Automated DNA Sequencer (Model 3730XL, from Applied BioSystems, USA).

Ethical approval: The Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC), Bodoland University vide letter no.-IAEC/BIOTECH/2019/3. All procedures were conducted according to the guidelines mentioned by the IAEC.

Submission of nucleotide sequences to NCBI: The partial fragment sequences of cytochrome b sequences of Doom and Ghungroo pig breeds obtained in FASTA format were submitted to NCBI (National Centre for Biotechnology Information) for generating accession number.

Analysis of nucleotide sequences: BLASTN search was performed on the Doom and Ghungroo pig cytochrome b gene sequences to generate similarity scores (i.e. 90 to 100 %) with other suidae sequences. The cytochrome b gene sequences of the present study along with sequences retrieved from gene bank were run for multiple sequence alignment and pairwise sequence alignment using Clustal W[17]. Among all the models tested using MEGA 11, the general time reversible (GTR+G) using discrete gamma distribution model has the lowest Bayesian Information Criterion (BIC) score. Therefore, it is regarded as the most suitable model for nucleotide substitution to determine the variation sites.

Kimura 2-parameter model was used to generate the genetic distance between the samples of the current study and all the suidae sequences retrieved from gene bank[12,16]. The aligned sequences were used to establish phylogenetic relationship by neighbour-joining tree method bootstrapping at 1000 replications using Kimura-2 parameter using MEGA 11[16].

Results

Accession numbers of cytochrome gene of Doom and Ghungroo pig breeds: The 437 bp of cytochrome b gene sequences of Doom and Ghungroo pig breeds were submitted to NCBI for generation of accession number. The accession numbers of Doom and Ghungroo pig as published in NCBI are tabulated in table 1.

BLAST similarity score of cytochrome b gene of Doom and Ghungroo pig: The partial fragment of cytochrome b sequences of Doom and Ghungroo pig generated a homology of 99 % with 25 domestic pig breeds around the world and 5 wild boars retrieved from gene-bank. After generating the BLAST similarity scores, indigenous pig breeds and wild boars were selected according to their closest similarity score to generate variation position/sites by Clustal W at MEGA 11, outlined in table 2. The indigenous pigs and wild boars selected based on closest similarity included 11 indigenous pig breeds from India, China, Japan and European countries and 5 wild boars of Asian-European origin. The same indigenous pigs and wild boars were also used for generating genetic-distances and phylogenetic tree to ensure a comprehensive understanding of their genetic relationships with the samples of the current study.

Variation position/sites of cytochrome b gene of Doom and Ghungroo pig: For generating variation position or sites of sequences of cytochrome b gene of Doom and Ghungroo pigs, the nucleotide sequences of the current samples including sequences retrieved from NCBI were run for multiple sequence alignment and pairwise sequence alignment using Clustal W at MEGA 11. The variable positions of 437 bp of cytochrome b gene of Doom and Ghungroo pig are shown in table 2. The complete genome mitochondrial sequence of *Sus scrofa domesticus* (acc. no. ON715893) was taken as a reference to generate the number of nucleotide positions. In the table, "." (dots) represents identical nucleotides.

The NCBI gene bank accession number of DNA sequence is given in brackets ( ) consisting of alpha-numeric letters. A highly variable region was found between 15924 and 16357 sites. There was a total of 18 polymorphic sites that were identified (Table 2) with no observed insertions and deletions in cytochrome b gene.

The sample of the current study i.e. Doom pig (DB-S1; accession no. PP951122) showed identical nucleotides with Indian wild pig (IWP; accession no. PP951121) at 6 positions. These positions have substitutions of A in place of C at 15940, C in place of T at three positions 16344, 16350 and 16355, T in place of A at 16356 and A in place of G at 16357. Doom pig sample of the present study also showed identical nucleotide with Ryukyu wild boar (RWB;

accession no. AB015073) at one position (16350) where T is substituted by C. The Doom pig (MZ846190) retrieved from NCBI showed similar nucleotides with the Asian (Indian wild pig, Lanyu wild boar, Ryukyu wild boar, Yunan wild boar) and European wild boars at 4 positions 16110, 16185, 16218 and 16332.

At position 16110, C is substituted by T, at position 16185, G is substituted by A, at position 16218, T is substituted by C and at 16332, C is substituted by T. The sample of the present study Ghungroo pig (GB-S2) only showed identical nucleotides at two positions with Indian wild pig and Ryukyu wild boar. At position 16344, T is substituted C and at position 16350, again T is substituted by C. The sequences of Ghungroo pig (OM634652) retrieved from NCBI did not show any similar nucleotides with sequences of indigenous and wild pigs.

Genetic distance analysis of Cytochrome b of Doom and Ghungroo pig: The genetic distance between the samples of the current study and sequences of wild boar and indigenous pig breeds was generated based on Kimura 2-parameter model[12,16] presented in table 3. In the table, lower triangular matrix values depict the mean genetic distances and upper triangular matrix depicts the standard errors. The Doom pig (0.0140±0.0059) of the current study was found to have closest distance with Indian wild pig with a mean of 0.0238±0.0079, Lanyu wild boar (mean, 0.0301±0.0014) and European wild boar (mean, 0.0119±0.0009). It also showed close distance with indigenous pig breeds of European origin i.e. Pietran (mean, 0.0123±0.0009) and Mangalica (mean, 0.0123±0.0009). The Doom pig (mean, 0.0104±0.0008) sequence retrieved from NCBI too showed close genetic distance with the same wild boars and indigenous pigs.

The indigenous pig breeds: Indian breeds (Ghungroo, Tenyi Vo, Niang Megha, Zovawk), Chinese breeds (Ya Chen, Ma Shen) and Japanese breeds (Ohmini miniature pig, Satsuma), all showed the close genetic distance among the groups. Ghungroo breed sample of the present study was found to have the nearest genetic distance with Satsuma (S) indigenous pig breed of Japan with a mean of 0.0044 ± 0.0020 (Table 3) and with Ohmini miniature pig (OMP) of Japan (0.0026 ± 0.0015). The Ghungroo pig sample retrieved from NCBI of mean 0.0002±0.0008 had closest genetic distance with that of indigenous pig breeds of India that included Tenyi Vo (0.0014±0.0003), Niang Megha (0.0014±0.0003) and Zovawk (0.0016±0.0003).

Phylogenetic tree of Cytochrome b of Doom and Ghungroo pig: Neighbour-Joining (NJ) tree method was applied for phylogenetic construction, bootstrapping at 1000 replications using Kimura-2 parameter using MEGA 11 (Fig. 1). The *Babyrousa babyrussa* (Bb) was taken as an outgroup for the construction of phylogenetic tree. The NJ phylogenetic tree revealed that the samples of the current study including the Indian wild pig (IWP) are confined to one cluster, placed next to Asian indigenous pig breeds.

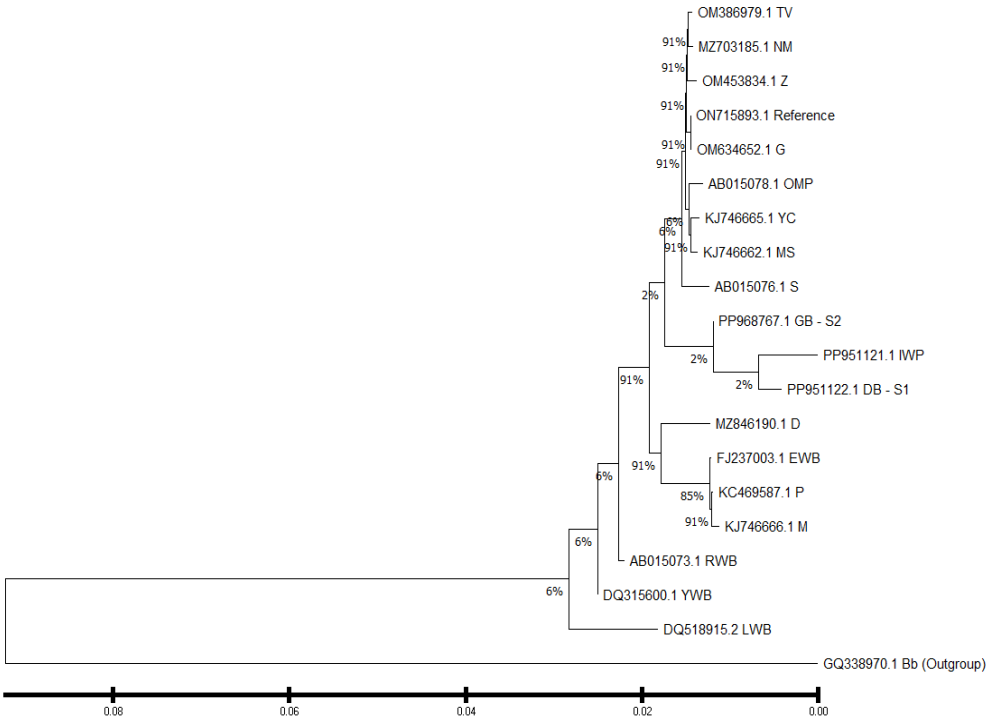

Figure 1: Phylogenetic tree of partial fragment of Cytochrome b gene. DB-S1= Doom, GB–S2= Ghungroo, IWP = Indian wild Pig, LWB = Lanyu Wild Boar, RWB= Ryukyu Wild Boar, YWB= Yunnan Wild Boar, EWB= European Wild Boar, G= Ghungroo, D= Doom, TV= Tenyi Vo, NM= Niang Megha, Z= Zovawk, YC= Ya Cha, MS= Ma Shen, OMP= Ohmini Miniature Pig, S= Satsuma, P= Pietran, M= Mangalica, Bb (Outgroup)= *Babyrousa babyrussa.*

Table 1
Accession number of Doom and Ghungroo pig breeds

S.N.	Pigs	NCBI Accession numbers
1.	Doom pig	PP951122
2.	Ghungroo pig	PP968767

Table 2
Variable positions of 437 bp of cytochrome b gene of Doom and Ghungroo pig.

	Nucleotide positions																	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	9	9	9	0	0	1	1	1	2	2	2	3	3	3	3	3	3	3
	2	3	4	0	3	1	8	9	1	2	7	2	3	4	5	5	5	5
	4	6	0	0	5	0	5	9	8	2	7	3	2	4	0	5	6	7
Animals																		
Reference (ON715893)	G	T	C	G	T	C	G	T	T	C	T	C	C	T	T	T	A	G
DB – S1 (PP951122)	.	.	A	.	.	.	.	.	.	.	.	.	.	C	C	C	T	A
GB – S2 (PP968767)	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.
Wild boars																		
IWP (PP951121)	.	.	A	.	.	T	A		.	.	.	T	T	C	C	C	T	A
LWB (DQ518915)	.	.	.	.	.	T	A	.	.	.	.	.	T	.	.	.	.	.
RWB (AB015073)	.	.	.	.	.	T	A	.	C	.	.	.	T	.	C	.	.	.
YWB (DQ315600)	.	C	.	.	.	T	A	.	.	.	.	.	T	.	.	.	.	.
EWB (FJ237003)	.	.	.	.	C	.	A	.	.	.	.	.	T	.	.	.	.	.
Indigenous pig breeds																		
G (OM634652)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D (MZ846190)	.	.	.	.	.	T	A		C	A	.	.	T	.	.	.	.	.
TV (OM386979)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NM (MZ703185)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Z (OM453834)	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
YC (KJ746665)	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
MS (KJ746662)	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OMP (AB015078)	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
S (AB015076)	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P (KC469587)	.	.	.	.	C	.	A	.	.	.	.	.	T	.	.	.	.	.
M (KJ746666)	.	.	.	.	C	.	A	.	.	.	C	.	T	.	.	.	.	.

Note: DB-S1= Doom, GB–S2= Ghungroo, IWP = Indian wild Pig, LWB = Lanyu Wild Boar, RWB= Ryukyu Wild Boar, YWB= Yunnan Wild Boar, EWB= European Wild Boar, G = Ghungroo, D= Doom, TV= Tenyi Vo, NM= Niang Megha, Z= Zovawk, YC= Ya Cha, MS= Ma Shen, OMP= Ohmini Miniature Pig, S= Satsuma, P= Pietran, M= Mangalica.

Within this cluster, Doom pig sample of the current study was in close relationship with Indian wild pig. The Doom breed (retrieved from gene bank) forms a clade along with European indigenous pigs. Within the Asian indigenous clade, Satsuma pig (S) of Japan with low bootstrap value showed separate cluster. At the level of the European clade, the Ryukyu wild boar (RWB) with 91% bootstrap probability and Yunan and Lanyu wild boar, both with low bootstrap value are designated as an isolated clade.

The northeast Indian pigs, included Tenyi Vo and Niang Megha with 91% bootstrap probability (Fig. 1). Within the northeast Indian pigs, Zovawk indigenous pig breed is inclined in a separate cluster, with bootstrap value of 91%. The minor clade included Ghungroo and complete genome of *Sus scrofa domesticus* (reference) that generated bootstrap probability of 91%.

Discussion

The results of the current study provide an outline of the partial cytochrome b gene of domestic pigs (Doom and Ghungroo pig) comparing them with the other pig sequences obtained from gene bank NCBI. Cytochrome b gene which is a mitochondrial DNA (mt-DNA), is utilized as a molecular marker for exploring the evolutionary lineage of various animal species[6, 21]. The cytochrome b gene when compared with other phylogenetic markers was found to show greater compatibility than standard mammalian phylogeny marker, showing nucleotide variation at greater level in short sequences[10,18]. Earlier studies have confirmed that the cytochrome b gene can be applied to distinguish between the two subspecies i.e. wild pig and domestic pig[10].

Assessing the variable positions showed that Indian wild pig had identical nucleotides with Asian and European wild

boars at three sites and these nucleotide substitutions can be used for differentiating wild boars and indigenous pigs of Asian and European origin as suggested by previous reports[10]. The Doom pig sample of the current study is showing six similar nucleotides with the wild boars and that of Ghungroo pig showing only two similar nucleotides with the wild boars. Also, the Ghungroo pig gene sequences retrieved from NCBI did not show any nucleotides similarity.

Table 3
Matrix output of genetic distance of the indigenous pig breeds and wild boars.

	Reference	**DB-S1**	**GB-S2**	**IWP**	**LWB**	**RWB**	**YWB**	**EWB**	**G**	**D**	**TV**	**NM**	**Z**	**YC**	**MS**	**OMP**	**S**	**P**	**M**	**Bb (Outgroup)**
Reference		0.0059	0.0032	0.0079	0.0014	0.0031	0.0031	0.0009	0.0001	0.0008	0.0003	0.0003	0.0003	0.0003	0.0003	0.0015	0.0020	0.0009	0.0009	0.0168
DB-S1	0.0140		0.0093	0.0119	0.0075	0.0076	0.0080	0.0076	0.0059	0.0083	0.0059	0.0059	0.0063	0.0064	0.0064	0.0064	0.0063	0.0076	0.0080	0.0301
GB-S2	0.0045	0.0023		0.0055	0.0052	0.0054	0.0059	0.0053	0.0032	0.0062	0.0032	0.0032	0.0039	0.0041	0.0039	0.0040	0.0039	0.0053	0.0057	0.0277
IWP	0.0238	0.0024	0.0049		0.0062	0.0063	0.0067	0.0072	0.0079	0.0071	0.0079	0.0079	0.0075	0.0083	0.0083	0.0082	0.0082	0.0072	0.0075	0.0303
LWB	0.0301	0.0213	0.0114	0.0164		0.0023	0.0024	0.0016	0.0014	0.0015	0.0014	0.0014	0.0014	0.0014	0.0014	0.0033	0.0033	0.0015	0.0015	0.0168
RWB	0.0098	0.0213	0.0114	0.0164	0.0062		0.0022	0.0033	0.0031	0.0030	0.0031	0.0031	0.0030	0.0030	0.0030	0.0031	0.0031	0.0033	0.0034	0.0167
YWB	0.0098	0.0238	0.0138	0.0189	0.0062	0.0053		0.0033	0.0031	0.0034	0.0031	0.0031	0.0030	0.0031	0.0031	0.0032	0.0032	0.0033	0.0034	0.0165
EWB	0.0119	0.0213	0.0114	0.0213	0.0365	0.0117	0.0117		0.0009	0.0008	0.0009	0.0009	0.0009	0.0010	0.0010	0.0037	0.0038	0.0002	0.0003	0.0168
G	0.0002	0.0140	0.0045	0.0238	0.0302	0.0098	0.0098	0.0121		0.0009	0.0003	0.0003	0.0003	0.0004	0.0004	0.0015	0.0020	0.0009	0.0009	0.0168

Bb (Outgroup)	M	P	S	OMP	MS	YC	Z	NM	TV	D
0.1696	0.0123	0.0123	0.0044	0.0026	0.0020	0.0021	0.0016	0.0014	0.0014	0.0104
0.1936	0.0238	0.0213	0.0164	0.0164	0.0164	0.0164	0.0164	0.0140	0.0140	0.0262
0.1735	0.0138	0.0114	0.0068	0.0068	0.0068	0.0068	0.0068	0.0045	0.0045	0.0161
0.1936	0.0238	0.0213	0.0263	0.0263	0.0263	0.0263	0.0213	0.0238	0.0238	0.0213
0.1663	0.0358	0.0358	0.0117	0.0117	0.0309	0.0309	0.0307	0.0310	0.0308	0.0343
0.1666	0.0126	0.0117	0.0108	0.0108	0.0098	0.0098	0.0089	0.0098	0.0098	0.0098
0.1651	0.0126	0.0117	0.0108	0.0108	0.0098	0.0098	0.0089	0.0098	0.0098	0.0117
0.1696	0.0012	0.0007	0.0154	0.0135	0.0128	0.0131	0.0118	0.0121	0.0121	0.0112
0.1696	0.0125	0.0125	0.0044	0.0026	0.0021	0.0023	0.0017	0.0016	0.0016	0.0106
0.1633	0.0122	0.0121	0.0172	0.0172	0.0112	0.0115	0.0109	0.0113	0.0111	
0.1696	0.0128	0.0126	0.0044	0.0026	0.0023	0.0026	0.0016	0.0011		0.0009
0.1696	0.0128	0.0127	0.0044	0.0026	0.0025	0.0028	0.0018		0.0003	0.0009
0.1681	0.0125	0.0124	0.0053	0.0035	0.0025	0.0027		0.0003	0.0003	0.0009
0.1726	0.0131	0.0131	0.0044	0.0026	0.0019		0.0004	0.0004	0.0004	0.0009
0.1726	0.0130	0.0128	0.0044	0.0026		0.0003	0.0004	0.0004	0.0004	0.0009
0.1711	0.0144	0.0135	0.0053		0.0015	0.0016	0.0018	0.0015	0.0015	0.0040
0.1726	0.0163	0.0154		0.0022	0.0020	0.0020	0.0022	0.0020	0.0020	0.0040
0.1696	0.0010		0.0038	0.0037	0.0009	0.0009	0.0009	0.0009	0.0009	0.0008
0.1711		0.0002	0.0040	0.0038	0.0009	0.0010	0.0009	0.0009	0.0009	0.0008
	0.0169	0.0168	0.0173	0.0170	0.0172	0.0173	0.0167	0.0168	0.0168	0.0163

Note: DB-S1= Doom, GB–S2= Ghungroo, IWP = Indian wild Pig, LWB = Lanyu Wild Boar, RWB= Ryukyu Wild Boar, YWB= Yunnan Wild Boar, EWB= European Wild Boar, G= Ghungroo, D= Doom, TV= Tenyi Vo, NM= Niang Megha, Z= Zovawk, YC= Ya Cha, MS= Ma Shen, OMP= Ohmini Miniature Pig, S= Satsuma, P= Pietran, M= Mangalica, Bb (Outgroup)= *Babyrousa babyrussa*.

Furthermore, Ghungroo pig generating the farthest distance from Doom pig and other wild boars. This shows the low genetic diversity of Ghungroo pig indicating the existence of inbreeding within the population. Recent study showing low haplotype genetic diversity of Ghungroo pig population supports the current findings in regard to Ghungroo pig[14].

The Doom pig having the nearest genetic distance from the wild boars' states that it is the most recent domesticated pig breed as also revealed by Das et al[9] in their study analysing the mitochondrial genome of Indian wild pigs.

The phylogenetic tree constructed depicted that the samples of the present study are grouped under one clade originating from Asian indigenous pig breeds. The results of the phylogenetic tree corroborate with the genetic distance i.e. the close distance between Indian wild pig and Doom breed pig suggests that they are closely related to each other and Doom pig is the most primitive among the indigenous pig breeds of India. Recent study too revealed through D-loop sequences of Indian pigs that Doom pig has the least genetic distance with Indian wild pig[9]. The inclusion of Doom pig of northeast India in the European clade could suggest of common maternal haplotypes due to practice of crossbreeding carried out in India for better production[14,19].

Conclusion

It can be concluded that the study investigated the partial fragment sequence of cytochrome b gene of mitochondrial DNA of two domestic pigs (Doom and Ghungroo), comparing them with the other sequences of wild boars and indigenous pigs generated from NCBI gene bank. The sequence variation sites revealed that Doom pig had more identical nucleotides with Indian wild pig than Ghungroo pig. Therefore, these sites common only to Doom and Indian wild pig can be used to distinguish between indigenous pigs and wild boars.

Furthermore, the least genetic distance between Doom pig and Indian wild pig also shows their close resemblance maternally. Even though the analysed cytochrome b sequences are short, they are unambiguous and will provide a foundation in future studies in determining the forensic cases as well as vegeto-legal cases including conservation of these novel genotypes.

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