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# The genetic relationship of Vietnamese gaurs assessed by mtDNA sequences

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## Abstract

This study aimed to assess the genetic relationship of Vietnamese gaurs from Central Highland and other gaurs from the Southeast Asia and India. The results showed that 15 polymorphic sites were found in 16S sequences between these gaurs. Vietnamese gaurs exhibited 15 identical SNPs with Cambodian gaur, while exposed 7 identical SNPs to Malayan gaur. The genetic distance analysis demonstrated that Vietnamese gaur showed the lowest distance to *Bos frontalis* (0.0086±0.0022) and *Bos gaurus* (0.0182±0.0054). Phylogenetic tree analysis indicated 3 groups of gaurs. Vietnamese gaur located in clade III which concluded *Bos gaurus, Bos javanicus, Bos frontalis*.

**Keywords:** *Bos gaurus*, 16S gene, phylogeny

## Introduction

The Gaur is the biggest extant species of cow (Duckworth *et al*., 2016) [4]. Gaur was widespread throughout a large portion of mainland South and Southeast Asia. However, it only happens in a few Asian nations at the moment, including Bangladesh, Bhutan, Cambodia, China, Malaysia, Myanmar, Nepal, Thailand, and Vietnam (Ashokkumar *et al*., 2011) [2]. Currently, there are 3 subspecies of Gaur recognized, including *Bos gaurus readei* and *Bos gaurus hubbacki* were found in Malaysia, as well as *Bos gaurus gaurus* and *Bos gaurus readei* in India, Nepal, and Bhutan (Ahrestani, 2018) [1]. Based on the size of the skull and horns, *Bos gaurus* was proposed two sub species, concluding *B. gaurus laosiensis* is found in Cambodia, Lao PDR, west Malaysia, Myanmar, Thailand, and Vietnam. *B. gaurus gaurus* is found in India and Nepal (Groves, 2003; Groves and Grubb, 2011) [6, 5]. In Vietnam, gaurs have been found to distribute in provinces such as Lai Chau, Son La, Thanh Hoa, Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien - Hue, Kontum, Gia Lai, Dak Lak, Lam Dong, Dong Nai, Binh Phuoc. The population of Gaur has been greatly reduced and is threatened in many area of Vietnam. The previous study showed that there were about 350-500 animals in the Northwest, now there are about 30-50 in Son La (Xuan Nha, Sop Cop), Lai Chau (Muong Te, Muong Lay) and the Central Highlands have less than 300 animals (Polet and Ling, 2004) [10]. The conservation of gaur in Vietnam is very necessary and important. In addition, the genetic characteristics of these gaur populations need to be evaluated to facilitate conservation and to make more available information about these gaur populations. Therefore, this study attempted to assess the genetic variation and phylogeny of Vietnamese gaurs from Central Highland with other gaurs from South and Southeast Asia assessed by mtDNA.

## Materials and Methods

### Sample collection and DNA extraction

The samples were collected from the Museum Biology, Tay Nguyen Institute for Scientific Research (Figure 1), their location was characterized in Table 1. The skin tissue (4-5 mm) were collected from gaur samples. Total DNA were extracted from tissue samples using Gene JET Genomic DNA Purification Kit (K0721, Thermo scientific). Total DNA was resuspended with TE buffer and preserved at -20 °C.

**Fig 1:** The *Bos gaurus* samples were used for this study. A, B, C, D, E: samples GY5BS-GY9BS (Table 1). The other sequences were derived from Genbank (Table 2)

**Table 1:** Sample collection of *Bos gaurus* in Vietnam

| Sample | Day of collection | Location | Tissue | Source |
|---|---|---|---|---|
| GY5BS | 11/05/1994 | Bao Loc, Vietnam | Skin | mitochondrion |
| GY6BS | 28/03/1995 | Da Lat, Vietnam | Skin | mitochondrion |
| GY7BS | 28/03/1995 | Da Lat, Vietnam | Skin | mitochondrion |
| GY8BS | 16/09/1999 | Phan Rang, Vietnam | Skin | mitochondrion |
| GY9BS | 29/09/2000 | Lam Ha, Vietnam | Skin | mitochondrion |

**Table 2:** 16S ribosomal sequences of gaurs and cattles from Genbank

| Sample | Accession number | Organism | Country | Source |
|---|---|---|---|---|
| AB074968-*Bos taurus* | AB074968 | *Bos taurus* | Japan | Mitochondrion |
| MG837552-*Bos taurus* | MG837552 | *Bos taurus* | China | Mitochondrion |
| MF663794-*Bos taurus* | MF663794 | *Bos taurus* | China | Mitochondrion |
| MN200938-*Bos taurus* | MN200938 | *Bos taurus* | China | Mitochondrion |
| KY766258-*Bos taurus* | KY766258 | *Bos taurus* | China | Mitochondrion |
| MF959941-*Bos frontalis* | MF959941 | *Bos frontalis* | China | Mitochondrion |
| GU985279-*Bos primigenius* | GU985279 | *Bos primigenius* | Ireland | Mitochondrion |
| JQ437479-*Bos primigenius* | JQ437479 | *Bos primigenius* | Poland | Mitochondrion |
| GU256940-*Bos indicus* | GU256940 | *Bos indicus* | China | Mitochondrion |
| MF667929-*Bos indicus* | MF667929 | *Bos indicus* | India | Mitochondrion |
| AY126697-*Bos indicus* | AY126697 | *Bos indicus* | Brazil | Mitochondrion |
| KX575711-*Bos indicus* | KX575711 | *Bos indicus* | Australia | Mitochondrion |
| AF492350-*Bos indicus* | AF492350 | *Bos indicus* | Germany | Mitochondrion |
| MK033130-*Bos mutus* | MK033130 | *Bos mutus* | China | Mitochondrion |
| KY829451-*Bos mutus* | KY829451 | *Bos mutus* | China | Mitochondrion |
| KR106993-*Bos mutus* | KR106993 | *Bos mutus* | China | Mitochondrion |
| KM233417-*Bos mutus* | KM233417 | *Bos mutus* | China | Mitochondrion |
| KX232521-*Bos grunniens* | KX232521 | *Bos grunniens* | China | Mitochondrion |
| AY684273-*Bos grunniens* | AY684273 | *Bos grunniens* | China | Mitochondrion |
| KR011113-*Bos grunniens* | KR011113 | *Bos grunniens* | China | Mitochondrion |
| MK279401-*Bos frontalis* | MK279401 | *Bos frontalis* | India | Mitochondrion |
| MF614103-*Bos frontalis* | MF614103 | *Bos frontalis* | China | Mitochondrion |
| MK279400-*Bos frontalis* | MK279400 | *Bos frontalis* | India | Mitochondrion |
| FJ997262-*Bos javanicus* | FJ997262 | *Bos javanicus* | Poland | Mitochondrion |
| AB915322-*Bos javanicus* | AB915322 | *Bos javanicus* | Malaysia | Mitochondrion |
| JN632606-*Bos javanicus* | JN632606 | *Bos javanicus* | France | Mitochondrion |
| JN632605-*Bos javanicus* | JN632605 | *Bos javanicus* | France | Mitochondrion |
| MK770201-*Bos gaurus* | MK770201 | *Bos gaurus* | Malaysia | Mitochondrion |
| MT345893-*Bos gaurus* | MT345893 | *Bos gaurus* | India | Mitochondrion |
| MT345892-*Bos gaurus* | MT345892 | *Bos gaurus* | India | Mitochondrion |
| MT360653-*Bos gaurus* | MT360653 | *Bos gaurus* | India | Mitochondrion |
| JN632604-*Bos gaurus* | JN632604 | *Bos gaurus* | France | Mitochondrion |
| MT360652-*Bos gaurus* | MT360652 | *Bos gaurus* | India | Mitochondrion |

## PCR

iProof HF Master Mix (1725310, Biorad) was applied for the target sequence amplification, PCRs are performed in a final volume of 25 µl containing 2.5µl Master Mix, 1 µl DNA template, 1 µl Forward and Reverse Primer, 20.5 µl distilled water. Primers of cytchome b amplification were as follows: F:5'-CCT CAR AAT GAT ATT TGK CCT CA-3', R:5'-CAG GMC TAT TCC TRG CHA TAC A-3' (Sarvani *et al*., 2018); and 16S included UH15155: GGA ATT CAT CTC TCC CGG TTT ACA AGA C; LCO1490: GGT CAA CAA ATC ATA AAG ATA TTG G. PCR was performed under the following conditions: one cycle of DNA denaturation at 98 °C in 3 min; 40 cycles at 98 °C in 10 s; annealing at 58 °C in 30 s; extension at 72 °C in 30 s; final extension at 72 °C in 10 min. After PCR running, the gel electrophoresis of PCR product is performed on 1% agarose gel.

## Sequencing

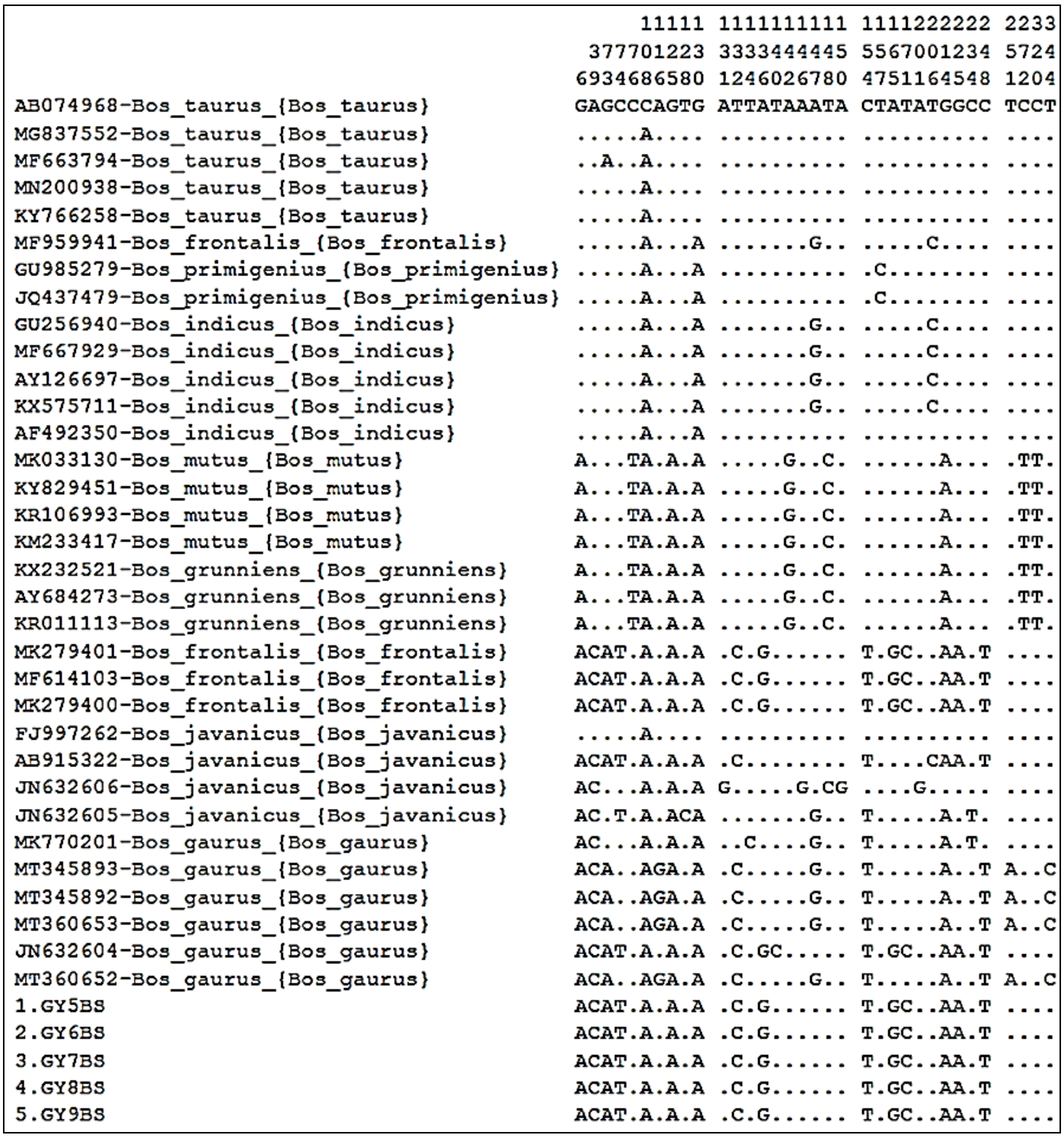
Amplified DNAs were purified using ExoSAP-IT PCR Clean up kit (Macrogen, Korea) and used as sequencing templates. The nucleotide sequences were determined using 3730XL DNA Analyzer (Macrogen, Korea). All PCR products are well-prepared, labeled and carefully packed for preventing the contamination among samples before sending out to nucleotide sequencing in Korea.

## Sequence analysis

The sequences will be compared with the other groups derived from Genbank using MEGA12 program. The 16S sequences were aligned using CLUSTAL W. Tamura & Nei model which used as genetic distance model. Neighbor-joining method was used for phylogenetic tree construction. Bootstrap analyses (1000 replications) are applied to estimate the confidence in branching order.

## Results

In this study, we used 2 genes for genetic analysis of gaur, including cytochrome b and 16S genes. However, sequencing on 16S gene showed better results. Therefore, the 16S gene sequence is used for genetic variation and genetic relationship analysis on the gaurs of Vietnam and some other groups derived from Genbank. The PCR product length of 16S rRNA gene was nearly 460 bp, after eliminating noisy sequences at two ends, a verified 448 bp sequence was finally obtained. The result of gaur's 16S sequence alignment showed that there are 15 polymorphic sites, representing 3.34% of the total analyzed DNA sequence (Figure 2). The mutation types being observed in these sequences is substitution, there is none of deletion or insertion. The polymorphic variation was determined at positions of 6, 39, 73, 74, 108, 125, 130, 132, 136, 154, 165, 171, 214, 225, 248.



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                                                11111 1111111111 1111222222 2233
                                            377701223 3333444445 5567001234 5724
                                           6934686580 1246026780 4751164548 1204
AB074968-Bos_taurus_{Bos_taurus}           GAGCCCAGTG ATTATAAATA CTATATGGCC TCCT
MG837552-Bos_taurus_{Bos_taurus}           .....A.... .......... .......... ....
MF663794-Bos_taurus_{Bos_taurus}           ..A..A.... .......... .......... ....
MN200938-Bos_taurus_{Bos_taurus}           .....A.... .......... .......... ....
KY766258-Bos_taurus_{Bos_taurus}           .....A.... .......... .......... ....
MF959941-Bos_frontalis_{Bos_frontalis}     .....A...A .......G.. .....C.... ....
GU985279-Bos_primigenius_{Bos_primigenius} .....A...A .......... .C........ ....
JQ437479-Bos_primigenius_{Bos_primigenius} .....A...A .......... .C........ ....
GU256940-Bos_indicus_{Bos_indicus}         .....A...A .......G.. .....C.... ....
MF667929-Bos_indicus_{Bos_indicus}         .....A...A .......G.. .....C.... ....
AY126697-Bos_indicus_{Bos_indicus}         .....A...A .......G.. .....C.... ....
KX575711-Bos_indicus_{Bos_indicus}         .....A...A .......G.. .....C.... ....
AF492350-Bos_indicus_{Bos_indicus}         .....A...A .......... .......... ....
MK033130-Bos_mutus_{Bos_mutus}             A...TA.A.A .....G..C. ......A... .TT.
KY829451-Bos_mutus_{Bos_mutus}             A...TA.A.A .....G..C. ......A... .TT.
KR106993-Bos_mutus_{Bos_mutus}             A...TA.A.A .....G..C. ......A... .TT.
KM233417-Bos_mutus_{Bos_mutus}             A...TA.A.A .....G..C. ......A... .TT.
KX232521-Bos_grunniens_{Bos_grunniens}     A...TA.A.A .....G..C. ......A... .TT.
AY684273-Bos_grunniens_{Bos_grunniens}     A...TA.A.A .....G..C. ......A... .TT.
KR011113-Bos_grunniens_{Bos_grunniens}     A...TA.A.A .....G..C. ......A... .TT.
MK279401-Bos_frontalis_{Bos_frontalis}     ACAT.A.A.A .C.G...... T.GC..AA.T ....
MF614103-Bos_frontalis_{Bos_frontalis}     ACAT.A.A.A .C.G...... T.GC..AA.T ....
MK279400-Bos_frontalis_{Bos_frontalis}     ACAT.A.A.A .C.G...... T.GC..AA.T ....
FJ997262-Bos_javanicus_{Bos_javanicus}     .....A.... .......... .......... ....
AB915322-Bos_javanicus_{Bos_javanicus}     ACAT.A.A.A .C........ T....CAA.T ....
JN632606-Bos_javanicus_{Bos_javanicus}     AC...A.A.A G.....G.CG ....G..... ....
JN632605-Bos_javanicus_{Bos_javanicus}     AC.T.A.ACA .......G.. T.....A.T. ....
MK770201-Bos_gaurus_{Bos_gaurus}           AC...A.A.A ..C....G.. T.....A.T. ....
MT345893-Bos_gaurus_{Bos_gaurus}           ACA..AGA.A .C.....G.. T.....A..T A..C
MT345892-Bos_gaurus_{Bos_gaurus}           ACA..AGA.A .C.....G.. T.....A..T A..C
MT360653-Bos_gaurus_{Bos_gaurus}           ACA..AGA.A .C.....G.. T.....A..T A..C
JN632604-Bos_gaurus_{Bos_gaurus}           ACAT.A.A.A .C.GC..... T.GC..AA.T ....
MT360652-Bos_gaurus_{Bos_gaurus}           ACA..AGA.A .C.....G.. T.....A..T A..C
1.GY5BS                                    ACAT.A.A.A .C.G...... T.GC..AA.T ....
2.GY6BS                                    ACAT.A.A.A .C.G...... T.GC..AA.T ....
3.GY7BS                                    ACAT.A.A.A .C.G...... T.GC..AA.T ....
4.GY8BS                                    ACAT.A.A.A .C.G...... T.GC..AA.T ....
5.GY9BS                                    ACAT.A.A.A .C.G...... T.GC..AA.T ....
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**Fig 2:** Polymorphic nucleotide positions in 16S gene sequences from gaurs' samples. The dot (".") represents the similar nucleotide positions

Table 3 shows the genetic distances between testing groups and other *Bos* species which is based on the 16S sequence. The lowest results of these comparisons are with *Bos frontalis* (0.0086±0.0022) and *Bos gaurus* (0.0182±0.0054), the highest is with *Bos mutus* and *Bos grunniens* (both are 0.0346±0.0065). Table 4 shows the genetic distances within groups, the result is showing a high homogeneity of Vietnamese gaur individuals in genetic aspect.

**Table 3:** Matrix of Tamura & Nei genetic distance between *Bos* species. Lower triangular matrix values were mean genetic distance, Upper triangular matrix values were standard errors

| Group | BT | BF | BP | BI | BM | BGr | BJ | Bga | VN |
|---|---|---|---|---|---|---|---|---|---|
| *Bos taurus* (BT) | | 0.0065 | 0.0031 | 0.0034 | 0.0066 | 0.0066 | 0.0043 | 0.0073 | 0.0084 |
| *Bos frontalis* (BF) | 0.0261 | | 0.0064 | 0.0064 | 0.0073 | 0.0073 | 0.0052 | 0.0050 | 0.0022 |
| *Bos primigenius* (BP) | 0.0054 | 0.0258 | | 0.0032 | 0.0065 | 0.0065 | 0.0045 | 0.0072 | 0.0083 |
| *Bos indicus* (BI) | 0.0067 | 0.0255 | 0.0058 | | 0.0069 | 0.0069 | 0.0044 | 0.0070 | 0.0085 |
| *Bos mutus* (BM) | 0.0214 | 0.0316 | 0.0205 | 0.0218 | | 0.0000 | 0.0057 | 0.0075 | 0.0086 |
| *Bos grunniens* (BGr) | 0.0214 | 0.0316 | 0.0205 | 0.0218 | 0.0000 | | 0.0057 | 0.0075 | 0.0086 |
| *Bos javanicus* (BJ) | 0.0184 | 0.0232 | 0.0188 | 0.0183 | 0.0246 | 0.0246 | | 0.0051 | 0.0059 |
| *Bos gaurus* (BGa) | 0.0293 | 0.0206 | 0.0291 | 0.0274 | 0.0315 | 0.0315 | 0.0238 | | 0.0054 |
| *Bos gaurus* (VN) | 0.0322 | 0.0086 | 0.0322 | 0.0336 | 0.0346 | 0.0346 | 0.0246 | 0.0182 | |

**Table 4:** The matrix of genetic distance within testing groups

| | Distance (d) | Standard errors (s.e) |
|---|---|---|
| *Bos taurus* | 0.0017926 | 0.0012166 |
| *Bos frontalis* | 0.0172936 | 0.0044864 |
| *Bos primigenius* | 0.0000000 | 0.0000000 |
| *Bos indicus* | 0.0017928 | 0.0012557 |
| *Bos mutus* | 0.0000000 | 0.0000000 |
| *Bos grunniens* | 0.0000000 | 0.0000000 |
| *Bos javanicus* | 0.0239757 | 0.0052858 |
| *Bos gaurus* | 0.0127626 | 0.0034554 |
| *Bos gaurus (VN)* | 0.0000000 | 0.0000000 |

The neighbor-joining tree was applied to assess the phylogenetic relationship of Vietnamese gaurs and other genus of *Bos* in the world (Figure 3). There groups were determined in the phylogenic tree based on 16S sequence analysis. The Clade I included *Bos taurus*, *Bos javanicus*, *Bos primigenius*, and *Bos indicus* with the bootstrap value is 83% (Nijman *et al*., 2002). The clade II concluded *Bos mutus* and *Bos grunniers* with the bootstrap value is 99%. The clade III consisted the *Bos gaurus*, *Bos javanicus*, *Bos frontalis* with the bootstrap value is 98%. The Vietnamese *Bos gaurus* was located in the clade III. *Bos gaurus* and *Bos javanicus* was distributed in clade I and clade III.

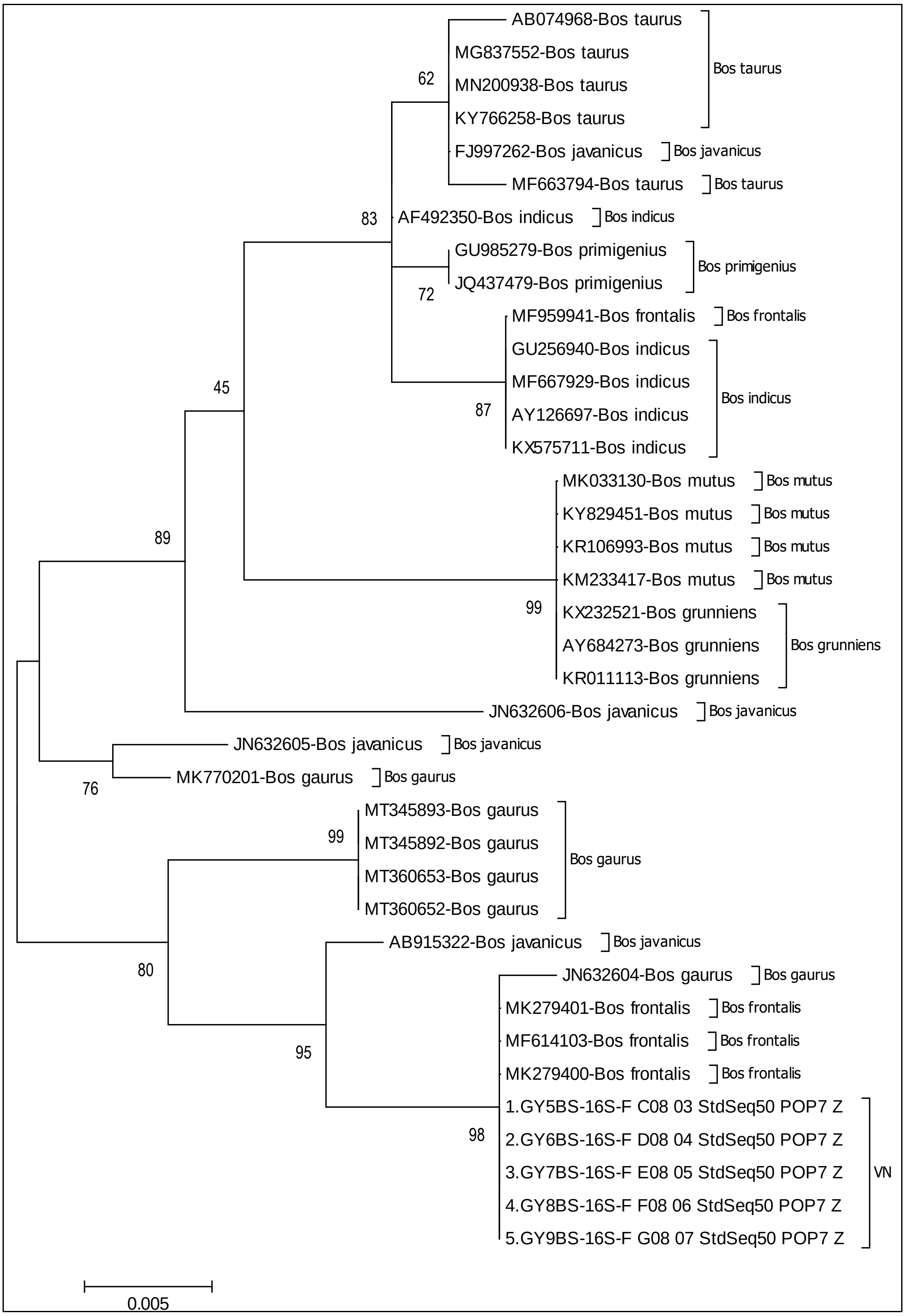

**Fig 3:** Phylogenetic tree constructed from 16S ribosomal sequences of gaurs and cattles by the neighbor-joining analysis method. Bootstrap resampling was done 1000 times, and resulting bootstrap values are shown on the corresponding branches

## Discussion

Gaur has found to be distributed in Southeast Asia and India (Atkulwar *et al.*, 2020) [3]. In this study, the Vietnamese *Bos gaurus* was located in the same clade with Cambodian *Bos gaurus* (JN632604) which was indicated by 15 similar SNPs. The Malayan gaur (MK770201) showed 7 indentical SNPs to Vietnamese gaur (Rosli *et al.*, 2019) [11]. These results suggested that the Vietnamese gaur showed the closer relationship to Cambodian gaur than Malayan gaur. This result was consist to the previous study, in which Cambodian gaur and Malayan gaur formed distinct clades (Kamalakkannan *et al.*, 2020) [7].

In this study, divergence times for all branching points in the topology were also calculated using the Maximum Likelihood method based on the Tamura-Nei model. The highest divergence time was founded in *Bos grunniens* (55000 YBP-years before present). *Bos mutus* and *Bos indicus* showed a lower divergence time (30300 YPB) than *Bos grunniens*. The divergence time of Malayan gaur and *Bos javanicus* (JN632605) is about 28800 YBP, while the Vietnamese gaur and Cambodian gaur shared the divergence time of 18800 YBP. The earlier divergence of Malayan gaur is resulted in the higher genetic distance and SNP numbers compared to Vietnamese gaur than Cambodian gaur.

## Acknowledgements

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## References

1. Ahrestani FS. *Bos frontalis* and *Bos gaurus* (Artiodactyla: Bovidae). Mamm. Species. 2018;50(959):34-50.
2. Ashokkumar M, Swaminathan S, Nagarajan R, Desai AA. Animal diversity, natural history and conversation, Distribution, ecology and conservation of the gaur. Daya Publishing House; c2011. p. 77-94.
3. Atkulwar A, Farah S, Gadhikar Y, Baig M. Mitochondrial DNA diversity in wild gaur (*Bos gaurus gaurus*): evidence from extant and historical samples. Mitochondrial DNA B: Resour. 2020;5(2):1556-1560.
4. Duckworth JW, Sankar K, Williams AC, Samba Kumar N, Timmins RJ. *Bos gaurus*. The IUCN Red List of Threatened Species; c2016. p. e.T2891A-46363646.
5. Groves C, Grubb P. Ungulate taxonomy. John Hopkins University Press, Baltimore, Maryland, USA; c2011. p. 317.
6. Groves CP. Taxonomy of ungulates of the Indian subcontinent. J Bombay. Nat. Hist. Soc. 2003;100(2/3):341-362.
7. Kamalakkannan R, Bhavana K, Prabhu VR, Sureshgopi D, Singha HS, Nagarajan M. The complete mitochondrial genome of Indian gaur, *Bos gaurus* and its phylogenetic implications. Sci. Rep. 2020;10(1):119-36.
8. The Himalayan Times: Bison population increasing in CNP; c2016. https://thehimalayantimes.com/nepal/bison-population-increasing-cnp (accessed: 12/04/2022)
9. Nijman IJ, Otsen M, Verkaar EL, De Ruijter C, Hanekamp E, Ochieng JW, *et al*., Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity. 2003;90(1):10-16.
10. Polet G, Ling S. Protecting mammal diversity: opportunities and constraints for pragmatic conservation management in Cat Tien National Park, Vietnam. Oryx. 2004;38(2):186-196.
11. Rosli N, Sitam FT, Rovie-Ryan JJ, Gan HM, Lee YP, Hartini Ithnin, *et al*., The complete mitochondrial genome of Malayan Gaur (*Bos gaurus hubbacki*) from Peninsular Malaysia. Mitochondrial DNA B Resour. 2019;4(2):2535-2536.
12. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution. 1993;10:512-526.