Research Article

Genetic variability and phylogenetic analysis of the Insulin Like Growth Factor gene in ten goat breeds

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ABSTRACT

This study aims to evaluate genetic variability in the insulin-like growth factor 1 (*IGF1*) gene among ten goat breeds. Gene sequences for the *IGF1* gene from ten goat breeds *viz.*, Sirohi, Barbari, Black Bengal, Attappady Black, Malabari, Assam Hill, Zaraibi, San Clemente, Tibetan, and Shiba, were retrieved from the National Center for Biotechnology Information (NCBI). Sequences were aligned using ClustalW and analyzed with BioEdit software. Phylogenetic relationships were assessed using MEGA software, incorporating additional *IGF1* sequences from various Bovidae and Cervidae species. The analysis revealed eleven polymorphic sites within intron-3 of the *IGF1* gene, with three transitions and eight transversions. All identified single nucleotide polymorphisms (SNPs) were novel, except for a G>C mutation at bp position 5752, which was consistent with previous studies. Goat breeds displayed a genetic distance of 0.000, indicating identical or nearly identical *IGF1* sequences, while *Ovis aries* and *Bos taurus* also showed a close genetic relationship. *Ovis dalli* and other bovine species exhibited moderate genetic divergence, with more distant relationships observed in species such as *Pseudois nayaur* and *Budorcas taxicolor*. Phylogenetic analysis confirmed the close genetic similarity among the examined goat breeds and highlighted their evolutionary relationships within the broader group of ruminants. These findings noted close relationships between *Bos taurus* and *Capra hircus* and high homology of caprine *IGF1* with other ruminants. This study provides insights into the genetic variability of the *IGF1* gene and its implications for improving growth traits in goats.

Keywords: Goat, Genetic variability, IFG1, Phylogeny

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INTRODUCTION

Goat meat (chevon) is a high protein, low fat, and low cholesterol option suitable for human consumption (Banskalieva *et al.*, 2000), with strong market prospects. Goat production plays a crucial role in the economy of farmers in arid and semi-arid regions (Askari *et al.*, 2011). In many developing countries, domestic goats are a primary economic resource, with over 90% of the world's goat population. However, goats have not undergone strong artificial selection for production traits, resulting in slow growth rates and low meat yields. Therefore, improvements in `growth traits, such as weight and meat production, are needed for goats (Zeng *et al.*, 2021).

Growth traits determine an animal's economic value and are influenced by complex physiological processes. The Hypothalamic-pituitary somatic (HPS) axis regulates growth by secreting growth hormone (GH) from the pituitary and stimulating insulin-like growth factor 1 (*IGF1*) from the liver (Wang *et al.*, 2004). The *IGF1* gene, located on chromosome 5 in goats, has 6 exons and is crucial for cell differentiation, embryogenesis, reproduction, fetal development, and metabolism (Adam *et al.*, 2000; Shen *et al.*, 2003).

IGF1 also influences reproductive traits such as pregnancy duration, twin ovulations, and conception rates (Sirotkin et al., 2003; Echternkamp et al., 2004; Patton et al., 2007). High IGF1 levels promote muscle differentiation, skeletal growth, and increased growth rates (Oksbjerg et al., 2004; Beckman et al., 2004). Polymorphisms in the IGF1 gene are linked to growth, production, and reproduction traits in various species, including cattle, swine, sheep, goats, and chickens (Yilmaz et al., 2005; Li et al., 2006; Bian et al., 2008; Zhang et al., 2008; Li et al., 2010; Akis et al., 2010; Yue et al., 2014; Meira et al., 2019). The IGF1 gene's extensive physiological roles make it a key candidate for investigating growth and milk production traits in goats, and a promising target for marker-assisted selection.

Genetic variation is vital for the effective conservation and enhancement of livestock. To ensure the successful conservation of domestic goat breeds, molecular characterization is essential for assessing biodiversity and variation among indigenous breeds (Sharma *et al.*, 2013). In this view, this study aimed to evaluate genetic variability and perform a phylogenetic analysis of the *IGF1* gene sequences across various goat breeds.

MATERIALS AND METHODS

The goat breeds considered for this study were Sirohi, Barbari, Black Bengal, Attappady Black, Malabari, Assam Hill Goat, Zaraibi, San Clemente, Tibetan Goat, and Shiba Goat. Attappady Black, Assam Hill, and Black Bengal are prominent Indian goat breeds raised for chevon production. Notably, the Black Bengal breed is recognized for its high prolificacy. The Sirohi and Barbari breeds in India are dual-purpose goats, raised for both milk and meat production (Aggarwal et al., 2022). The Zaraibi, also known as the Egyptian Nubian goat, is a dual-purpose breed from Egypt and is renowned for its prolificacy (Aboul-Naga et al., 2012). San Clemente goats, native to San Clemente Island in California, USA, are known for their dual-purpose utility and high butterfat content in milk (Cooper, 2022). The Tibetan goat breed, found in the mountain valleys and plateaus of Tibet, is valued for its fiber production (Li and Zhao, 2004). Lastly, the Shiba goat breed, originating from the Kyushu district of Japan, is primarily raised for meat production (Sakurai et al., 2004).

Retrieval and Comparative Analysis of *IGF1* Gene Sequences

The gene sequences of the insulin-like growth factor 1 (*IGF1*) gene for above mentioned ten distinct goat breeds were retrieved from the National Center for Biotechnology Information (NCBI) database (Table 1). These sequences were aligned using the ClustalW algorithm to account for gaps and substitutions, ensuring maximum alignment accuracy. This alignment was performed with BioEdit software (version 7.2). Following the multiple sequence alignment, the sequences were analyzed for the presence of missing nucleotides, and both transition and transversion mutations.

Phylogenetic analysis

For the phylogenetic analysis, the study expanded beyond the goat breeds to include available *IGF1* gene

sequences from a variety of species within the Bovidae family, including Ovis aries (GenBank Accession No. KX432965.1), Ovis dalli (GenBank Accession No. HQ285865.1), Bos taurus (GenBank Accession No. HG797647.1), Bubalus bubalis (GenBank Accession No. XM_006058814.4), Bos frontalis (GenBank Accession No. EF686015.1), Bos grunniens (GenBank Accession No. KF303538.1), Bos mutus (GenBank Accession No. XM_005898608.2), Bos javanicus (GenBank Accession No. XM_061417108.1), Bison bison (GenBank Accession No. XM_010853427.1), Bubalus kerabau (GenBank Accession No. XM_055575265.1), Budorcas taxicolor (GenBank Accession No. XM_052640485.1), Oryx dammah (GenBank Accession No. XM_040234858.1), and Pseudois nayaur (GenBank Accession No. JQ993346.1). Additionally, species from the Cervidae family, including Cervus elaphus (GenBank Accession No. OU343099.1) and Rangifer tarandus platyrhynchus (OX596097.1), were included in the analysis to explore potential evolutionary relationships beyond the Bovidae family. Phylogenetic analysis was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method. The Kimura 2-parameter model was applied, which is particularly appropriate for estimating evolutionary distances by considering both transition and transversion nucleotide substitutions. To ensure the reliability of the phylogenetic tree, a bootstrap analysis was performed with 1,000 replicates, providing a measure of confidence for each branch in the tree. In addition to the phylogenetic tree, a pairwise distance matrix was computed to quantify the genetic distances among the various goat breeds, as well as the species from the Bovidae and Cervidae families. This matrix was also generated using MEGA version 11.0, offering detailed insights into the genetic divergence and relatedness among the examined species based on IGF1 gene sequences.

Table 1: GenBank accession numbers of Insulin-like growth factor 1 gene sequences of goat breeds along with their natural habitats

| Breed | GenBank Accession No. | Region | | | | | | |
|---------------------------|-----------------------|--------------------------------------|--|--|--|--|--|--|
| Sirohi | MT954973.1 | Rajasthan, India | | | | | | |
| Barbari | MT954972.1 | Uttar Pradesh and Rajasthan, India | | | | | | |
| Black Bengal | MT954974.1 | West Bengal, India | | | | | | |
| Attappady Black | KP256001.1 | Kerala, India | | | | | | |
| Malabari | KP256000.1 | Kerala, India | | | | | | |
| Assam Hill | MG948341.1 | Assam and Meghalaya, India | | | | | | |
| Zaraibi (Egyptian Nubian) | OM418860.1 | Northeast of the Nile Delta, Egypt | | | | | | |
| San Clemente | XM_005680535.3 | San Clemente Island, California, USA | | | | | | |
| Tibetan | JF896276.1 | Tibet | | | | | | |
| Shiba | D11378.1 | Kyushu, Japan | | | | | | |

RESULTS AND DISCUSSION

Comparative sequence analysis

A comparative sequence analysis of the *IGF1* gene across various goat breeds identified a total of eleven polymorphic sites within intron-3, using the GenBank sequence (accession no. D26119.2) as a reference. Among these, three were transition mutations and eight were transversions. To the best of the authors' knowledge, all identified SNPs in this study are novel, with the exception of the G>C mutation at bp position 5752. In the Shiba and Tibetan goats, two novel mutations, T>A and A>G, were identified at bp positions 5722 and 5724, respectively. The G>C mutation at bp position 5752 was also observed in the Sirohi, Barbari, and Black Bengal breeds. Additionally, an A>C mutation

at bp position 5844 was found in both the Barbari and Black Bengal breeds, with the Black Bengal breed also exhibiting a T>C mutation at bp position 5845. Several other novel mutations, including G>T, T>A, C>G, A>T, and A>C, were detected in the Sirohi, Barbari, and Black Bengal breeds at bp positions 5847, 5848, 5851, 5852, and 5853, respectively. Furthermore, a novel A>G mutation was identified at bp position 5855 in both the Barbari and Black Bengal breeds. In the Assam Hill goat, adenine was missing at bp position 5637, while the Sirohi breed exhibited an absence of the AT motif at bp positions 5844 to 5845. Additionally, the thymine residue at bp position 5845 and adenine at bp position 5855 were absent in the Barbari and Sirohi breeds, respectively (Table 2 and Fig. 1).

Table 2: Single nucleotide polymorphisms (SNPs) identified in intron-3 of the caprine *IGF1* gene across ten goat breeds using GenBank sequence (accession no. D26119.2) as reference

| Nucleotide position (bp) | Reference nucleotide | Polymorphic nucleotide | Type of mutation | Breed | | | | | | |
|-----------------------------|-------------------------|------------------------|------------------|----------------------------------|--|--|--|--|--|--|
| 5722 | Thymine | Adenine | Transversion | Shiba and Tibetan | | | | | | |
| 5724 | Adenine | Guanine | Transition | Shiba and Tibetan | | | | | | |
| 5752 | Guanine | Cytosine | Transversion | Sirohi, Barbari and Black Bengal | | | | | | |
| 5844 | Adenine | Cytosine | Transversion | Barbari, Black Bengal | | | | | | |
| 5845 | Thymine | Cytosine | Transition | Black Bengal | | | | | | |
| 5847 | Guanine | Thymine | Transversion | Sirohi, Barbari and Black Bengal | | | | | | |
| 5848 | Thymine | Adenine | Transversion | Sirohi, Barbari and Black Bengal | | | | | | |
| 5851 | Cytosine | Guanine | Transversion | Sirohi, Barbari and Black Bengal | | | | | | |
| 5852 | Adenine | Thymine | Transversion | Sirohi, Barbari and Black Bengal | | | | | | |
| 5853 | Adenine | Cytosine | Transversion | Sirohi, Barbari and Black Bengal | | | | | | |
| 5855 | Adenine | Guanine | Transition | Barbari and Black Bengal | | | | | | |

In the present study, no mutations were observed in intron-3 of the IGF1 gene in the Attappady Black, Malabari, Assam Hill, Zaraibi, and San Clemente goat breeds. A possible explanation for this could be that intron-3 of the IGF1 gene is highly conserved across these breeds, suggesting that the sequence has remained unchanged over time due to strong evolutionary pressures to preserve its function. Zeng et al. (2021) in their study on Dazu Black goats, identified five mutations in the IGF1 gene, specifically A/G, C/T, T/C, C/T, and G/C variations at bp positions 1617, 1620, 4700, 5524, and 5752, respectively. Notably, the G/C mutation at bp position 5752 is consistent with the findings of the present study. In contrast, Sharma et al. (2013) reported 18 polymorphic sites across various regions of the *IGF1* gene, including the 5'UTR, exon-1,

exon-2, exon-3, exon-4, intron-1, and intron-2, in nine Indian goat breeds. However, in the present study, no variations were detected within these regions.

Phylogenetic analysis

The matrix of pairwise genetic distances between different goat breeds and animal species, based on *IGF1* gene sequences, was calculated using MEGA11 software and is presented in Table 2. This matrix provides insights into the evolutionary relationships among these species, highlighting the genetic similarity or divergence in their *IGF1* gene sequences. Among the goat breeds analyzed, the genetic distance was 0.000, indicating that these breeds have identical or nearly identical *IGF1* gene sequences. The species *Ovis aries* and *Bos taurus* also showed a genetic

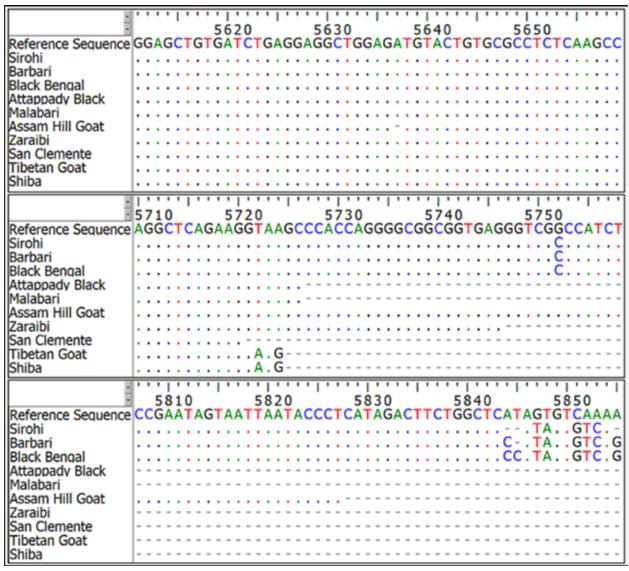


Fig. 1: Multiple sequence alignment of the IGF1 gene across various goat breeds, performed using the ClustalW method in BioEdit software, with GenBank accession no. D26119.2 as the reference sequence.

distance of 0.000 from these goat breeds, reflecting a close genetic relationship in terms of the *IGF1* gene. In contrast, *Ovis dalli* exhibited a genetic distance of 0.021 from the goat breeds and *Ovis aries*, suggesting a slight genetic divergence. Other bovine species, including *Bos taurus*, *Bubalus bubalis*, *Bos grunniens*, *Bos mutus*, *Bos frontalis*, *Bison bison*, *Bos javanicus* and *Bubalus kerabau*, displayed a genetic distance of 0.021, indicating a moderate divergence from the goat breeds and *Ovis aries*. Species such as *Pseudois nayaur*, *Budorcas taxicolor*, *Oryx dammah*, *Cervus elaphus* and *Rangifer tarandus platyrhynchus* showed greater genetic distances (e.g., 0.036, 0.029), reflecting more significant genetic differences from the goat breeds.

Overall, the analysis reveals that goat breeds, *Ovis aries*, and *Bos taurus* are genetically very similar based on *IGF1* sequences. *Ovis dalli* is slightly more divergent but still closely related, whereas the other bovine species

exhibit moderate genetic divergence. Species such as *Pseudois nayaur* and *Budorcas taxicolor* show greater genetic divergence from the goat breeds.

This phylogenetic tree depicted the evolutionary relationships among various animal species based on the sequences of the Insulin-like Growth Factor 1 (*IGF1*) gene (Figure 2). The tree structure showed the genetic distances and the degree of relatedness among the species. The tree indicated a high level of similarity between different breeds of *Capra hircus* (e.g., Assam Hill Goat, Attappady Black, Sirohi, Barbari, etc.), as they are closely grouped together with very low branch lengths, indicating minimal genetic divergence. These goat breeds have low bootstrap values (close to 0), suggesting high confidence in their close relationship. *Ovis aries* is shown as more distantly related to *Capra hircus* breeds, suggesting a greater evolutionary distance, yet still closely related within the same clade

of ruminants. Bos taurus Cattle is placed in a separate branch, suggesting it shares a common ancestor with sheep and goats but diverged earlier. The tree included various outgroup species like Cervus elaphus, Rangifer tarandus, Bison bison, Bubalus bubalis and others. These species were placed on more distant branches, reflecting their more distant evolutionary relationship to the Capra hircus breeds and other ruminants. Other ruminants such as Bos javanicus, Bos mutus, and Ovis dalli are also included, showing their genetic distances within the broader group of ruminants.

The tree clearly demonstrated that different breeds of *Capra hircus* are genetically very similar to each other, as expected, given their classification within the same species. The more distant relationship between *Capra hircus* and other ruminants like *Ovis aries* and *Bos*

taurus reflects their evolutionary divergence within the family Bovidae. The placement of outgroup species like deer, buffalo, and bison highlights the broader evolutionary context within which these ruminants evolved. Overall, this tree is a visualization of the genetic relationships among a range of species based on the IGF1 gene, providing insights into their evolutionary history. Consistent with the findings of the present study, Sahoo et al. (2019) reported that phylogenetic analysis of the insulin-like growth factor receptor (IGF1R) based on amino acid sequences revealed a close relationship between Bos taurus and Capra hircus. Similarly, Naicy et al. (2017) found that the caprine IGF1 protein exhibited the highest homology with ruminants compared to other mammals, aligning with the results of the current study.

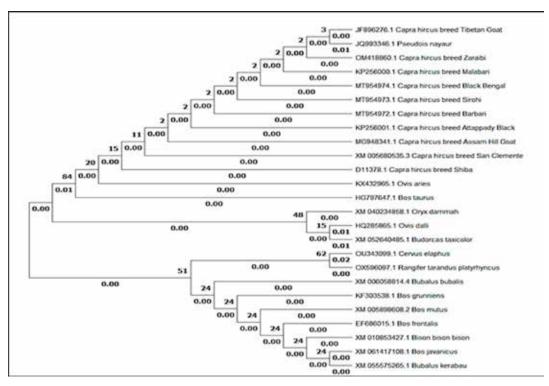


Fig. 2: Phylogenetic tree based on IGF1 gene nucleotide sequences from goat breeds and 15 other species, illustrating the highly conserved nature of IGF1 among ruminants.

CONCLUSION

This study provides a comprehensive analysis of genetic variability in the *IGF1* gene among ten distinct goat breeds from India and other regions, shedding light on their evolutionary relationships and potential for genetic improvement. The identification of eleven novel polymorphic sites within intron-3 of the *IGF1* gene, including both transition and transversion mutations, enhances our understanding of genetic diversity in these breeds. Notably, the genetic distance matrix and phylogenetic analysis revealed that the goat breeds studied exhibit very close genetic relationships, with identical or nearly identical *IGF1* gene sequences.

This close genetic similarity among the goat breeds, as well as with *Ovis aries* and *Bos taurus*, underscores the potential for utilizing *IGF1* gene variations in breeding programs aimed at improving growth traits. The absence of mutations in certain goat breeds, such as Attappady Black, Malabari, Assam Hill, Zaraibi, and San Clemente, may indicate that the *IGF1* gene region analyzed is highly conserved across these breeds, reflecting evolutionary pressures to maintain its functional integrity. The observed genetic divergence in *Ovis dalli* and other bovine species suggests that while these species are related, they have evolved distinct genetic profiles compared to the goat breeds.

Table 3: Pairwise genetic distances between different goat breeds and animal species based on the IGF1 gene sequences

| | | | | | _ | _ | _ | _ | $\overline{}$ | _ | _ | _ | _ | | | | | _ | | | _ | _ | _ | _ | |
|-----|-------|-------|-------|-------|-------|-------|--------|-------|---------------|-------|---------|-------|-------|---------|---------|-------|-------|-------|-------|-------|-------|---------|-------|-------|---|
| CE | | | | | | | | | | | | | | | | | | | | | | | | 0.021 | E |
| ООМ | | | | | | | | | | | | | | | | | | | | | | | 0.036 | 0.014 | 1 .11 . |
| BT | | | | | | | | | | | | | | | | | | | | | | 0.007 | 0.044 | 0.022 | ע שעיוון יי ע עט יי יויש טשיי וט יט יי יויש טשיי ועי יט יט יויש עשיין יי עווא וויאן עאין וען וען יען יען יען יי |
| PN | | | | | | | | | | | | | | | | | | | | | 0.029 | 0.021 | 0.044 | 0.036 | |
| BK | | | | | | | | | | | | | | | | | | | | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | |
| BJ | | | | | | | | | | | | | | | | | | | 0.000 | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | , |
| BBS | | | | | | | | | | | | | | | | | | 0.000 | 0.000 | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | 1 |
| BF | | | | | | | | | | | | | | | | | 0.000 | 0.000 | 0.000 | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | E |
| BM | | | | | | | | | | | | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | ō |
| BG | | | | | | | | | | | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | 000 |
| BBL | | | | | | | | | | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.028 | 0.014 | 0.007 | 0.029 | 0.007 | : |
| BT | | | | | | | | | | | | | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | 1 |
| QD | | | | | | | | | | | | 0.021 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.029 | 0.014 | 0.007 | 0.044 | 0.022 | |
| 0A | | | | | | | | | | | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | |
| SB | | | | | | | | | | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | |
| TG | | | | | | | | | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | |
| SC | | | | | | | | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | |
| ZB | | | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | - |
| AHG | | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | |
| MB | | | | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | - |
| AB | | | | 0.000 | 0.000 | 0.000 | 00000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | - |
| BB | | | 0.000 | 0.000 | 0.000 | 0.000 | 0000 0 | 0.000 | 0.000 | 00000 | 1 0.021 | 0.000 | 0.021 | 1 0.021 | 1 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | l 0.014 | 0.036 | 0.029 | |
| BR | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 1 0.021 | 1 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | l 0.014 | 0.036 | 0.029 | |
| SR | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.007 | 0.021 | 0.014 | 0.036 | 0.029 | |
| | BR | BB | AB | MB | AHG | ZB | SC | TG | SB | OA | OD | BT | BBL | BG | BM | BF | BBS | BJ | BK | PN | BT | ODM | CE | SR | 6 |

SR = Sirohi, BR = Barbari. BB = Black Bengal, AB = Attappady Black, MB = Malabari, AHG = Assam Hill Goat, ZB = Zaraibi, SC = San Clement, TG = Tibetan Goar, OA = Ovis aries, OD = Ovis dalli, BT = Bos taurus, BBL = Bubalus bubalis, BG = Bos grunniens, BM = Bos mutus, BF = Bos frontalis, BBS = Bison bison, BJ = Bos javanicus, BK = Bos kerabau, PN = Pseudois nayaur, BT = Budorcas taxicolor, ODM = Oryx dammah, CE = Cervus elaphus

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