Population Genetic Structure and Phylogeny of Himalayan Gorals (*Naemorhedus goral*) in Central Nepal

Sapana Ulak

T.U. Registration No.: 5-2-37-1917-2017

T.U. Examination Roll No.: 7930041

Batch: 2079

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

A dissertation submitted

In partial fulfilment of the requirements for the award of the degree

of Master of Science in Zoology with special paper Ecology and Environment

---

March 2025

Population Genetic Structure and Phylogeny of Himalayan Gorals (*Naemorhedus goral*) in Central Nepal

Sapana Ulak

TU Registration No.: 5-2-37-1917-2017

M.Sc. Zoology (Ecology and Environment)

T.U. Examination Roll No.: 7930041

Supervisor
Laxman Khanal, PhD
Associate Professor

Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu

Dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in Zoology with special paper Ecology and Environment

---

March 2025

March 2025

E-mail: sapanaulak999@gmail.com

Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu, Nepal
Website: https://www.cdz.tu.edu.np/

Citation: Ulak, S. (2025). *Population genetic structure and phylogeny of Himalayan gorals (Naemorhedus goral) in Central Nepal (*MSc dissertation). Central Department of Zoology, Tribhuvan University.

Declaration

I hereby declare that the work presented in this dissertation "**Population genetic structure and phylogeny of Himalayan gorals (*Naemorhedus goral*) in Central Nepal**" has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

.....

Sapana Ulak

Exam Roll No.: 7930041

Email: sapanaulak999@gmail.com

Date: 28 March 2025

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.
☎ ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology T.U., Kirtipur

Recommendation

This is to recommend that the dissertation entitled "**Population genetic structure and phylogeny of Himalayan gorals (*Naemorhedus goral*) in Central Nepal**" has been carried out by Sapana Ulak for the partial fulfilment of Master's Degree of Science in Zoology with special paper Ecology and Environment. This is her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

.....

Supervisor
Laxman Khanal, PhD
Associate Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Date: 28 March 2025

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

☎ ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology T.U., Kirtipur

Letter of approval

On the recommendation of supervisor "Laxman Khanal, PhD" this dissertation submitted by Sapana Ulak entitled "**Population genetic structure and phylogeny of Himalayan gorals (*Naemorhedus goral*) in Central Nepal**" is approved for the examination in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Ecology and Environment.

.....

Head of Department
Kumar Sapkota, PhD
Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Date: 28 March 2025

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.
☎ ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

Certificate of acceptance

This dissertation work submitted by Sapana Ulak entitled "**Population genetic structure and phylogeny of Himalayan gorals (*Naemorhedus goral*) in Central Nepal**" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Ecology and Environment.

Evaluation committee

.....
Supervisor
Laxman Khanal, PhD
Associate Professor

.....
Head of Department
Kumar Sapkota, PhD
Professor

.....
External Examiner
Narayan Prasad Koju, PhD
Pokhara University

.....
Internal Examiner
Tej Bahadur Thapa, PhD
Professor

Date of examination: 04/09/2025

Acknowledgments

I would like to express my profound gratitude to my supervisor, Associate Professor Dr. Laxman Khanal for his invaluable guidance, encouragement, and immense support throughout my thesis journey. I am sincerely thankful to Professor Dr. Kumar Sapkota, Head of Central Department of Zoology, for his continuous motivation and administrative support.

I appreciate entire faculty members and my classmates for being very cooperative. I am grateful to the Department of National Parks and Wildlife conservation for granting me permission to collect samples from Langtang National Park and Gaurishankar Conservation Area.

I am immensely thankful to my friend, Mrs. Sabina Osthi, for her invaluable assistance during my field survey and lab work. I am indebted to Mr. Asmit Subba and Mr. Ankit Kumar Singh for helping me during my sample analysis. I would like to thank Mr. Tilak Paudyal and Anish Paudyal for helping me with the sample collection. I am truly thankful to Mr. Kanchha Ghale, Mr. Chhiring Tamang and Mr. Chhiring Chhembel Tamang for assisting me during my field work. I am forever grateful to my family for their unwavering support.

Special thanks to Chinese Academy of Science for providing me financial support for this study under KCRE Excellent Student Thesis Grant (10132024).

Sapana Ulak
Central Department of
Zoology Tribhuvan University

Abstract

Gorals (*Naemorhedus* spp.) are facing taxonomic incongruence due to morphological variations and phylogenetic disparities among the studies mainly due to incomplete lineage sorting. The Himalayan region is believed to have two species/subspecies of Himalayan gorals (*Naemorhedus goral*), however the population in Nepal remains excluded in phylogenetic studies. Limited studies have been conducted in gorals of Nepal which focus on distribution, threats, and conservation of Himalayan gorals, however, the genetic information is yet to be explored. This study aimed for understanding the genetic structure in Central Nepal and establishing the phylogenetic relationship of Himalayan gorals within the subfamily Caprinae. Fecal samples were collected from Langtang National Park and Gaurishankhar Conservation Area representing the Himalayan range and from Daunne Forest that lies in Nawalparasi district in the Mahabharat range. Mitochondrial cytochrome b (1002 bp) and control region (1069 bp) gene sequences were used as molecular markers. Population genetic analysis of the three subpopulations from Central Nepal along with phylogenetic analysis was performed to clarify the genetic characteristics, taxonomy, and phylogeny of Himalayan gorals. As a result, from twenty-nine sequences, five novel haplotypes of mitochondrial cytochrome b were identified, exhibiting low haplotype diversity (0.3960) and very low nucleotide diversity (0.0006). In contrast, 14 novel haplotypes of control region were identified with high haplotype diversity (0.8200) and moderate nucleotide diversity (0.0082). The median joining haplotype network was star-shaped indicating a recent population expansion with bottleneck or founder effect. The maximum likelihood (ML) tree of the obtained haplotypes, along with partial sequences from the Indian Himalayan region indicated that the haplotypes from Central Nepal are more closely related to those from Sikkim (Eastern Himalaya) than those from Uttarakhand (Western Himalaya). The maximum likelihood tree of the genus *Naemorhedus* suggested six valid species of gorals. The Bayesian time tree of mitochondrial cytochrome b within the subfamily Caprinae revealed that the genus *Naemorhedus* is most closely related to the genus *Capricornis* and next to the genus *Ovibos*. It is important to know the level of genetic diversity the goral populations in Nepal possess and identify some genetically unique subpopulations of conservation importance.

शोध सारांश

घोरल (*Naemorhedus* spp.) मा भएका अध्ययनहरू बीच मुख्यतया अपूर्ण वंश क्रमबद्धताको कारणले वर्गीकरण र फाइलोजेनेटिक असमानता पाइन्छ । हिमालय क्षेत्रमा हिमालयन घोरल (*Naemorhedus goral*) को दुई प्रजाति/उप-प्रजातिहरू छन् भन्ने विश्वास गरिन्छ, तर नेपालका घोरलहरू अझै पनि फाइलोजेनेटिक अध्ययनमा समावेश गरिएका छैनन । नेपालमा हिमालयन घोरलहरूको वितरण, खतरा र संरक्षणमा केन्द्रित सीमित अध्ययनहरू भएका छन्, तथापि, आनुवंशिक जानकारीहरू अन्वेषण गर्न बाँकी नै छ । यस अध्ययनको उद्देश्य हिमालयन घोरलहरूको आनुवंशिक विविधता र उपपरिवार Caprinae भित्र घोरलहरूको फाइलोजेनेटिक सम्बन्ध स्थापित गर्ने थियो । हिमाली क्षेत्रको प्रतिनिधित्व गर्ने लाङटाङ राष्ट्रिय निकुञ्ज र गौरीशंखर संरक्षण क्षेत्र तथा महाभारत क्षेत्रको नवलपरासी जिल्लामा पर्ने दाउने वनबाट घोरलहरूको दिसाको नमूना सङ्कलन गरिएको थियो । माइटोकोन्ड्रियल cytochrome b (१००२ bp) र control region (१०६९ bp) जीन डीएनए अनुक्रमहरू आणविक मार्करहरूको रूपमा प्रयोग गरियो । नतिजाको रूपमा, उनतीस नमूना अनुक्रमहरूबाट, माइटोकोन्ड्रियल cytochrome b को पाँच ह्याप्लोटाइपहरू पहिचान भयो, जसमा कम ह्याप्लोटाइप विविधता (०.३९६०) र धेरै कम न्यूक्लियोटाइड विविधता (०.००६) थियो । यसको विपरित, control region मा १४ ह्याप्लोटाइपहरू पहिचान भयो जसमा उच्च ह्याप्लोटाइप विविधता (०.८२००) र मध्यम न्यूक्लियोटाइड विविधता (०.००८२) थियो । Median joining haplotype network तारा-आकारको थियो जसले निकट बिगत समयमा भएको जनसंख्या विस्तारलाई संकेत गर्दछ । प्राप्त ह्याप्लोटाइपहरूको maximum likelihood (ML) फाइलोजेनेटिक सम्बन्धको नतिजाले मध्य नेपालका ह्याप्लोटाइपहरू उत्तराखण्ड (पश्चिमी हिमालय) को तुलनामा सिक्किम (पूर्वी हिमालय) सँग धेरैक नजिकको सम्बन्ध रहेको संकेत गर्दछ । *Naemorhedus* जीनसको ML फाइलोजेनेटिक सम्बन्धको नतिजाले गोरलहरूको छ वटा वैध प्रजातिहरू रहेको सुझाव दियो । उपपरिवार Caprinae भित्र माइटोकोन्ड्रियल साइटोक्रोम बी को Bayesian time tree ले जीनस *Neamorhedus*, *Capricornis* र *Ovibos* को बिचमा नजिकको आनुवंशिक सम्बन्ध रहेको देखायो । यस अध्ययनले नेपालमा हिमालयन गोरलहरूको विकासको इतिहासमा जानकारी प्रदान गर्दछ र आनुवंशिक रूपमा संरक्षणको महत्वको उप-जनसंख्या पहिचान गर्दछ ।

Table of Content

List of Tables

List of Figures

List of photographs

List of abbreviations

Abbreviated form	Details of abbreviations
BLAST	Basic Local Alignment Search Tool
GCA	Gaurishankar Conservation Area
HPD	Highest Posterior Distribution
IUCN	International Union for Conservation of Nature
LNP	Langtang National Park
ML	Maximum Likelihood
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction

1. Introduction

1.1 Background

Gorals (*Naemorhedus* spp.) belong to family Bovidae, often considered as small goat-antelope found in rugged, wooded mountainous terrain, associate in small groups of twelve or fewer individuals and presently occur in Asia only (Mammal Diversity Database, 2024; Mead, 1989). They are herbivore that occupy the mixed feeder niche and their restricted range is possibly led by the Holocene environmental changes and adverse anthropogenic causes (Isarankura Na Ayudhya et al., 2022). As of now, the IUCN has recognized four species of gorals: Himalayan goral (*Naemorhedus goral*), long-tailed goral (*N. caudatus*), Chinese goral (*N. griseus*) and red goral (*N. baileyi*). Among the gorals, *N. caudatus*, *N. griseus* and *N. baileyi* are categorized as 'Vulnerable' and *N. goral*, found in Himalayan region of central East Asia, including Nepal, Bhutan, China, northern India, and Pakistan is considered as 'Near Threatened' according to the IUCN Red List of Threatened Species (Duckworth & MacKinnon, 2008). In Nepal, Himalayan gorals are found at an elevation range of 300 m to 3000 m across the Churia, mid-hills and Himalayan foothills (Jnawali, 2011).

Gorals are experiencing population decline across their distribution range. The major causes of local extinction of goral include illegal hunting, habitat degradation, overgrazing and lack of public awareness (Zahidullah et al., 2016) which often lead to small and fragmented populations (Ashraf et al., 2016). Illegal hunting and trapping of gorals are done for meat and recreation, and few people do it for a living (Perveen & Khan, 2013). Shrinkage on geographical distribution of gorals due to global warming (Ahmad et al., 2020), cross contamination of parasites (Adhikari et al., 2021; Dar et al., 2022), bottleneck effect due to male biased mortality and female biased sex ratio (Abbas et al., 2015; i-Abbas et al., 2012) are additional threats to goral population. The gorals being a resident species along with small territories (Cho et al., 2014; Cho et al., 2016) makes it more vulnerable to threats. Population genetic studies, habitat, corridor and range management, international cooperation and mass awareness campaigns would be helpful in conservation of Himalayan goral (Abbas et al., 2015).

Despite of considerable efforts by several studies, the evolutionary relationship within family Bovidae is controversial (Yang et al., 2013). There is much debate regarding intra-genus species delimitation and phylogenetic status of the gorals within the subfamily Caprinae (Sun

et al., 2024). The taxonomy and phylogeny of gorals and serows was revised by using complete mitochondrial genome and found *N. baileyi*, *N. caudatus*, and *N. goral* are three genetically confirmed species while *N. griseus* and *N. goral* are synonyms (Mori et al., 2019). Serows and gorals are closely related taxa that form a monophyletic group (Liu & Jiang, 2017; Xiong et al., 2013). Mori et al. (2019) reported that they could not find sturdy basis for the presence of *N. griseus*, *N. bedfordi* and *N. evansi* as a distinct taxon which should rather be grouped together within *N. goral* (brown goral) thus ascertaining three goral and four serow species which were until recently also classified under *Naemorhedus*. Li et al. (2020) revisited the goral taxonomy and revealed *N. cranbrooki* and *N. evansi* are both distinct species and suggested for five valid species.

In the Himalayas, it was suggested that Sutlej River in Himachal Pradesh, India forms the boundary of distribution of *N. goral* (eastern) and *N. bedfordi* (western) (Groves & Grubb, 2011). In contrast, the border is defined east of Kathmandu where *N. goral* and *N. hodgsoni* represents western and eastern Himalayan species respectively (Hrabina, 2015). Two valid sub-species of Himalayan goral, recorded eastward from Nepal is *N. goral goral* and westward from Nepal is *N. goral bedfordi*; also distantly located subspecies might be considered as evolutionary significant units so that special attention should be provided for its long term conservation and management (Joshi et al., 2022). For this reason, sampling of the east and central Himalayan goral population has become priority to resolve this inconsistency (Hrabina et al., 2023) further recommending genetic monitoring of Himalayan goral population near Kathmandu and comparison with samples from Western and Eastern Himalayas.

Although, recent study recognizes six extant species of gorals further considering *N. canbrooki* and *N. evansi* (Hrabina et al., 2023). Gorals showed similarity with takin-muskox in mitochondrial genome-based phylogenetic tree rather than goat-antelopes (Sun et al., 2024). Therefore, taxonomy and phylogeny of gorals is debatable. Phylogenetics of gorals still remain unclear due to limited genetic data that do not represent the entire distribution areas of the genus (Li et al., 2020). The sample from Nepal has not been included throughout the study so taxonomic position and evolutionary relationship of Himalayan goral from Nepal is yet unknown.

Phylogeographic analyses, the geographical distribution of genealogical lineages and efforts had been led by using mitochondrial DNA that particularly considers population genetics (Avise, 2000). Population genetic studies are vital for conservation and management strategies

(Choi et al., 2015) as genetic diversity and connectivity helps in recognizing adaptation ability to different ecological conditions (Banks & Lindenmayer, 2014; Dharmarajan et al., 2014). Himalayan goral is found into different physiographic regions; Himalayas and Mahabharat range by which phylogeographic study will be meaningful. Phylogeography helps in identification of Evolutionary Significant Units (ESUs) that supports in development of conservation strategies (Craig, 1994).

1.2 Statement of the problem

Molecular phylogeny accompanies global assessment that provides strong evidence in evolutionary relationship among the taxa. Throughout the study for taxonomic and phylogenetic reassessment of gorals, the genetic information of Himalayan goral from Nepal was not included. Two valid sub-species of Himalayan goral, recorded eastward from Nepal is *N. goral goral* and westward from Nepal is *N. goral bedfordi* (Joshi et al., 2022) but Himalayan goral from Nepal is yet unrecognized in its sub-species level. Also, Central Nepal has been predicted to be the demarcation zone between the subspecies of Himalayan gorals- *N. goral goral* and *N. goral bedfordi* (Hrabina et al., 2023). Evolutionary relationship of Himalayan goral from Nepal with other bovids is still a mystery.

Furthermore, Himalayan gorals found in Nepal exist in two typical different physiographic regions; Himalayas and Mahabharat range. Although elevation range of Himalayan goral is wide, presence of barriers for genetic exchange and independent adaptation of subpopulations in isolated habitats may be significant in its evolution which remains unexplored till date. Also, being Near Threatened, it is important to know the level of genetic diversity they possess and identify some genetically unique subpopulations of conservation importance.

1.3 Research objectives

1.3.1 General objective

The general objective of this study was to assess the genetic makeup of the Himalayan goral populations in central Nepal and establish a phylogenetic relationship with congeners.

1.3.2 Specific objectives

i. To examine genetic diversity and population genetic structure of Himalayan gorals inhabiting in the Himalayas and Mahabharat range in central Nepal.

ii. To establish phylogenetic relationship of Himalayan gorals of Nepal within genus and among the species under subfamily Caprinae.

1.4 Research hypothesis

- Himalayan goral in Nepal, being isolated from other known species by the Himalayas, may present distinct phylogenetic lineages.
- Himalayan goral population in Himalayas and Mahabharat range may display notable genetic differences.
- Phylogeographic analysis may provide cue to the level of gene exchange between subpopulations of gorals.

1.5 Significance of the study

Phylogenetic species concept relies on evolutionary lineage and shared derived characters. The time and again monitoring of molecular phylogenetic study can reimpose the existing knowledge. The study examines population genetic structure and phylogeny of Himalayan goral from Central Nepal which will foster the inferences for its evolutionary history, ecological adaptation, migration pattern, population structure, level of gene flow, genetic divergence, and genetic diversity. More importantly, these findings will help to develop conservation strategies. Phylogeographic study of Himalayan goral in its native habitat will reflect the status of biodiversity, ecological interactions, and conservation threats in Nepal. In global scenario, the study will facilitate to recognize Himalayan goral from Central Nepal in its sub-species level and determine its actual position among Caprinae. The genetic data from this study allows for the identification of Management Units and will help to resolve ongoing taxonomic ambiguities.

1.6 Limitations of the study

The inclusion of only a single sample from Gaurishankar Conservation Area constrained the capability of examining genetic diversity and population structure in that region. Use of fecal sample introduced biases in DNA quality which affected amplification success and subsequent genetic analyses.

2. Literature review

2.1 Population genetics of Himalayan gorals

Population genetic studies describe current genetic structure and allocation of genetic variation within and among populations. Phylogeography concerns with the principles and historical evolutionary processes that govern the geographical distributions of genealogical lineages within and among closely related species. Historically isolated populations are major candidates for genetic adaptation because of likely exposure to divergent selection pressure without homogenizing effect of gene flow (Avise, 2000). Several studies of *Naemorhedus* species regarding population genetics have been conducted in various ways to investigate their genetic diversity, population structure and evolutionary history across various habitats. Population genetic structure is shaped by natural selection, genetic drift, mutation, isolation, and gene flow.

The study of population structure and genetic diversity of *N. caudatus* in South Korea, it was found that the goral from the lower northeast region differed significantly from the upper northeast population, likely due to intrinsic climatic and geographic characteristics (Choi et al., 2015). The assessment of phylogeographic relationships of Korean goral populations revealed that Seoraksan population exhibits a greater level of mitochondrial DNA diversity, highlighting the need of conservation of the observed endemic lineages (Jang et al., 2020). Through gut microbiome analysis, the study of geographical relationship and regional migration of *N. caudatus* population in South korea was made possible (Park et al., 2021). Only two haplotypes were obtained from twelve tissue samples of *N. caudatus* from South Korea with low sequence divergence between haplotypes indicating lower genetic diversity (Min et al., 2004).

To examine genetic connectivity of *N. griseus* populations from Beijing and Inner Mongolia in China by using partial mitochondrial control region, a total of eight haplotypes were obtained from samples of overall 76 individuals with no shared haplotypes (Yang et al., 2019). In Joshi et al. (2022), out of thirty-two partial mitochondrial cytochrome b sequences of Himalayan goral; twenty-six from Uttarakhand and six from Sikkim, seven and two haplotypes were obtained, respectively. Jang et al. (2020) studied phylogeography and population genetics of *N. caudatus* by using a partial gene fragment of mitochondrial control region from South Korea in which out of 38 fecal DNA samples, seven haplotypes were obtained of which five haplotypes were unique to Seoraksan population. Genetic diversity of *N. caudatus* was assessed

by using mitochondrial cytochrome b gene from different populations in South Korea from overall sixty-four fecal samples, only three haplotypes were obtained with two polymorphic sites with very low haplotype diversity (0.160) and nucleotide diversity (0.053%) which could be a warning signal (Kim, 2021). A greater number of unique haplotypes is an affirmative sign of a healthier and resilient population.

Captive breeding programs are necessary to prevent extinction but it is equally important to know that it also reduces genetic diversity due to high inbreeding (Ariyaraphong et al., 2021). The study of genetic structure of *N. caudatus* at 12 microsatellite loci of sixty eight goral individuals from South Korea revealed that genetic diversity was moderate in wild population but lower in captive group (Choi et al., 2015). Extensive knowledge on population genetic structure is required to ensure genetic diversity in both captive and wild population of gorals for effective translocation and reintroduction while required.

The sequence diversity of *N. caudatus* by using partial mitochondrial cytochrome b gene revealed that the genetic distance of haplotypes from different localities increased with respect to geographic distance due to habitat isolation (Koh et al., 2002). The genetic differentiation between population of Himalayan gorals should be studied in Nepal to unveil its taxonomic status and phylogeny and assessment of genetic diversity is essential to help securing long-term survival and adaptability.

The bovids are believed to have an ancient origin in the Miocene epoch, 18.5 million years ago (Ropiquet & Hassanin, 2005), thrived and diversified, evolved horn as a defense mechanism (Janis, 1982), formed social structure and some even domesticated while most of them adapted in wildlife just like in case of gorals (Stricklin, 2001). Future distribution ungulates are negatively affected by the altering climate, global warming limited species distributional zones and it is vital to encompass how future distributions might vary based on predicted climate change (Haq et al., 2023). It is a prime time to make some efforts until it is too late.

2.2 Phylogenetic relationships of gorals

With much efforts, phylogeny of the family Bovidae is still debatable (Yang et al., 2013). The phylogenetic relationship keeps altering when more sample species are taken into consideration (Zwickl & Hillis, 2002). Due to habitat degradation and human interference, Himalayan goral is facing threats (Sharma et al., 2023). Several efforts have been made to solve its taxonomy

through the study of phylogeny and population genetics that also aid in its conservation and management.

Since the very beginning, there has been many confusions regarding phylogeny of goral as it was kept together with serows earlier. Gorals and serows belong to different genera (Koh et al., 2002; Min et al., 2004) and their divergence occurred nearly two million years ago (An et al., 2010). The complete cytochrome b gene of red goral (*N. baileyi*) from the Shanghai Zoo was sequenced and compared with thirty sequences of *Naemorhedus* from the GenBank where maximum likelihood phylogeny showed polyphyletic relationships of *Naemorhedus* species (Xiong et al., 2013) while by using 13 protein-coding genes of mitochondrial DNA revealed monophyletic group of *Naemorhedus* species (Ren et al., 2018). By using mitochondrial cytochrome b gene, it was found that *N. goral* and *N. caudatus* may be conspecific but further analysis is needed with an additional specimens (Koh et al., 2002). The analysis of complete mitochondrial genome sequence of Himalayan goral showed that *N. goral* was closely related with *N. griseus* (Liu & Jiang, 2017) and *N. griseus* closest to *N. baileyi* (Liu & Zhang, 2018). The mitochondrial cytochrome b gene sequence analysis of Korean goral species *N. caudatus* by using fecal sample showed a single cluster of Korean and Russian gorals (Kim, 2021). The assessment of genetic differentiation has a great impact on its taxonomy and phylogenetic inference.

More rapid evolution of mitochondrial genome and maternal inheritance makes it very useful to infer evolutionary relationships (Wilson et al., 1985). The taxonomy and phylogeny of gorals has not been fixed as the series of recent research suggest different views; by using total mitochondrial genome, Mori et al. (2019) advised three species and li et al. (2020) put forward five valid species suggesting additional species *N. evansi* and *N. cranbrooki* and inferred that *N. griseus* is synonym of *N. goral* while Joshi et al. (2020) encouraged the existence of six goral species with few recognized subspecies and also recommended that all the distantly located subspecies might be considered as Evolutionary significant units so special attention should be provided for long term conservation and management. Genetic features and clarity in phylogeny is must for domestication, genetic improvisation and conservation of Himalayan goral (Yang et al., 2013).

3. Materials and methods

3.1 Study area

This study was conducted in the Himalayan and Mahabharat range of Central Nepal. The study area constitutes Langtang National Park and Gaurishankar Conservation Area from Himalayas and Daunne Forest in Nawalparasi East/Nawalpur District from Mahabharat range.

In Langtang National Park, the major sampling sites were Sherpa Gaon, Rimche and Dhimsha. Although Langtang National Park has an elevation range of 1500 m to 7234 m, sample sites of goral fall below 4000 m. The weather is generally dry except for January - February when snow falls and July - August when monsoon rain occurs. Yak and chauri herd ascend to higher altitude in warm weather. Sub-tropical Sal Forest, hill forest with Chirpine (2000–2600 m), temperate oak forest (2600–3000 m), silver fir, hemlock and Larch are found in the lower sub-alpine zone (3000–3600 m), rhododendron and Nepal alders are the main features of this area. Major fauna includes red panda (*Ailurus fulgens*), Himalayan black bear (*Ursus thibetanus laniger*), Assamese monkey (*Maccaca assamensis*), clouded leopard (*Neofelis nebulosa*), grey wolf (*Canis lupus*), leopard cat (*Prionailurus bengalensis*), Himalayan goral (*Naemorhedus goral*), musk deer (*Moschus Cyrysogaster*), Himalayan tahr (*Hemitragus jemlahicus*), serow (*Capricornis* sp.), snow leopard (*Panthera uncia*) and more than 250 species of birds (DNPWC, 2024).

Gaurishankhar Conservation Area has an elevation range of 968 m to 7181 m. *Pinus* Forest, lower temperate oak forest, temperate mixed broad leaves forest and alpine scrubs can be found here. Major fauna includes snow leopard, red panda (*Ailurus fulgens*), Himalayan goral, Himalayan tahr, mongoose (*Herpestes* sp.), weasel (*Mustela* sp.), yellow throated marten (*Martes flavigula*), wild boar (*Sus scrofa*), blue sheep (*Pesudois nayaur*), rhesus monkey (*Macaca mulatta*) and Assamese monkey. This region is quite rich in water resources. Higher altitude rangeland is used for grazing livestock.

The elevation range of Nawalparasi district is 91 m to 1936 m representing subtropical zone. It is inhabited by barking deer (*Muntiacus vaginalis*), Himalayan goral, langur (*Semnopithecus entellus*), golden jackal (*Canis aureus*), leopard (*Panthera pardus*), and small mammals. Highly variable precipitation occurs mostly from June to October. Major vegetation includes Sal tree, needle wood and Indian chestnut (Thapa et al., 2011). Chure hills are youngest hills with a fragile environment that has a crucial role in ecology and rich in biodiversity (Uprety et

al., 2023). It has a diverse topography with sub-tropical forest, riverine vegetation, and agricultural land with crops. The major sampling sites were Khalde, Buchohul, Gargare and Bihari bhir which lies in Dhobadi Rural Municipality, Nawalparasi, Nepal.

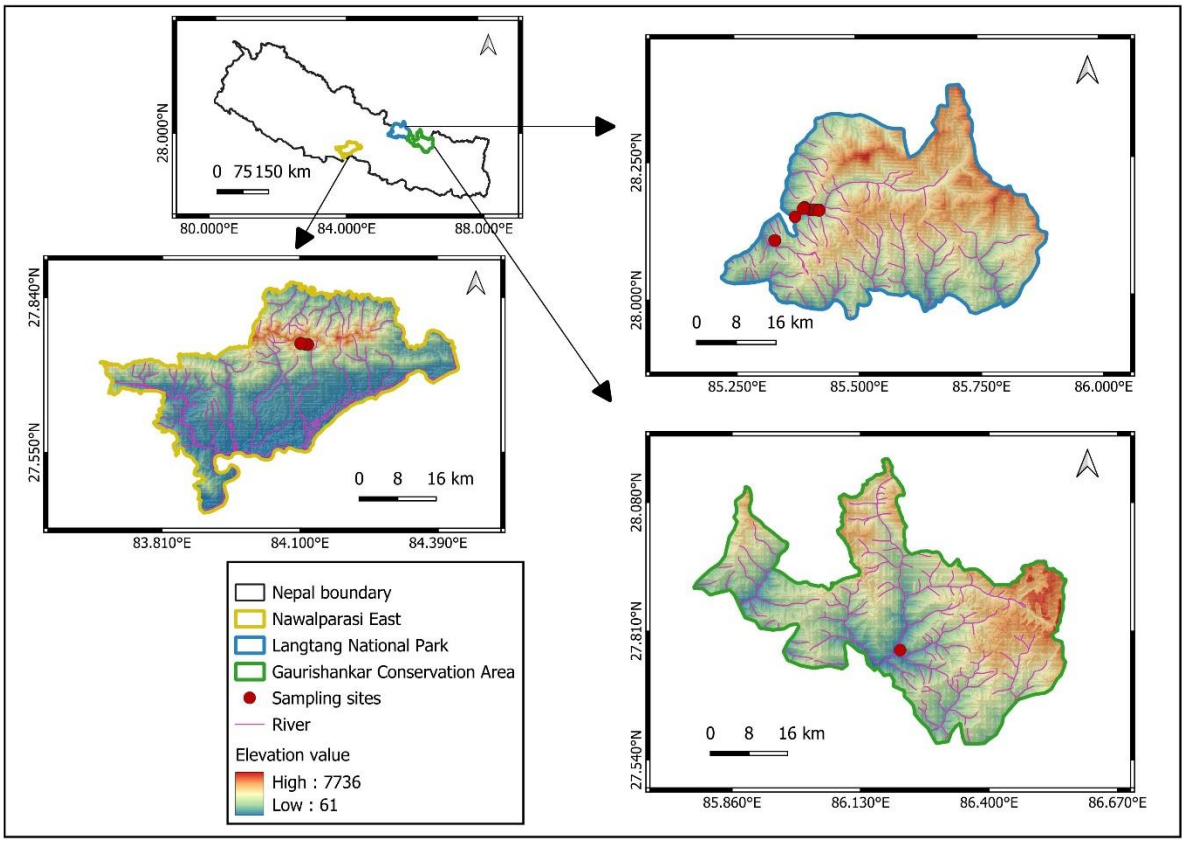


Figure 1. Map of Nepal showing the study areas and sampling sites at different elevations.

3.2 Field surveys and sample collection

The field survey was conducted in April–May 2024 during spring season/pre-monsoon when conditions were favorable for detecting and collecting samples. Fecal pellets were searched along the predetermined trails in the possible habitat of gorals, based on previous field observations. Interaction with local people aided significantly. The survey was specifically focused on the early morning and late evening, as gorals are crepuscular (Fakhar et al., 2008) which attributed to greater chances of goral sightings. Fecal samples of Himalayan goral were collected non-invasively ensuring no harm was caused to the animal and its habitat from Langtang National Park (LNP), Daunne Forest, and Gaurishankar Conservation area (GCA). When a sample was found, GPS coordinates and elevation were recorded initially. The entire sample collection was carried out following the method of (Khanal et al., 2021). Each sample

was collected using a separate plastic-wrapped sterile cotton buds and a plastic sample vial containing 2 ml of lysis buffer solution. A dry cotton swab was rolled over the surface of the fecal matter thoroughly and then immersed in lysis buffer, where it was stirred to dissolve the fecal matter and recover mucus cells present in the sample. This process was repeated several times for the same sample, with additional turning over of the fecal material. The pellets were preserved in a larger vial containing lysis buffer. While handling samples, strict precautions were taken to prevent contamination and the samples were secured in Ziplock bags. Only fresh and moist fecal matter was considered for analysis. Before fieldwork, I visited the Central Zoo to observe goral and its fecal matter which helped me to identify goral samples in field. The samples were recognized based on its distinct shape which is cylindrical with one end typically pointed and goral latrine sites were also identified. Fecal samples were collected at a minimum distance of 100 meters from each other to avoid sampling the same individual again. For each sample, habitat features such as vegetation type, slope orientation, and distance from the walking trail were recorded during the survey. Additionally, binoculars were used to scan and observe gorals, and photographs of the gorals were taken using a camera. To preserve the collected samples, ambient temperature was maintained until they were stored in a freezer at -20°C in the laboratory for further DNA extraction.

3.3 DNA extraction, PCR amplification and sequencing

The genomic DNA extraction from non-invasive fecal samples was carried out by using a QIAamp DNA Stool Mini Kit (QIAGEN, Germany) following the manufacturer's protocol. Gel electrophoresis was performed to assess the DNA quality. The extracted DNA samples were stored with an elution buffer at -20°C until further processing. The PCR amplification of mitochondrial cytochrome b gene was done by using the set of primers; forward primer L14724: 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' and reverse primer H15315: 5'-GGAATTCATCTCTCCGGTTTACAAGAC-3' (Xiong et al., 2013) while mitochondrial control region was amplified by using the set of primers; forward, FL-1: 5'-AGTATACTGGTCTTGTAAACC-3' and reverse primer; HS-3: 5'-AGGCATTTTCAGTGCCTTGC-3' (Okumura, 2004).

PCR amplification was carried out in 20 µl reaction volumes that contained 10 µl 2× master mix (consisting of dNTPs, Taq polymerase and $MgCl_2$), 0.5 µl each of forward and reverse primers, 1.0 µl template DNA and 8.0 µl deionized distilled water. The PCR conditions were an initial denaturation for 5 minutes at 94°C, followed by 35 cycles of 30 seconds denaturation

at 94°C, 30 seconds annealing at 54°C for mitochondrial cytochrome b while 56°C for mitochondrial control region, 1 minute extension at 72°C and a final extension at 72°C for 5 minutes. PCR products were analyzed by conducting electrophoresis on 1.5% agarose gel and successful amplicons were preceded for Sanger sequencing using the same primer pairs as in PCR amplification employing Big Dye Terminator Cycle Kit v.3.1 (Invitrogen) on an ABI 3730XL sequencer (Applied Biosystems, Waltham, MA, USA). The sequences from two primers were assembled into a single FASTA file by using SeqMan tool in the DNASTAR Lasergene package (DNASTAR laser gene v.7.1).

3.4 Population genetic structure analysis

The obtained mitochondrial cytochrome b and control region sequences were aligned by means of Clustal W (Thompson et al., 1994) algorithm in MEGA11 software (Tamura et al., 2021). Sequences were proofread manually and trimmed off the edges of the alignment as required. The alignment files were created separately for each gene and imported into DnaSP v5.0 (Librado & Rozas, 2009) to identify polymorphic sites (s), no. of haplotypes (h), haplotype diversity (hd), nucleotide diversity (π), average number of nucleotide differences (k), Tajima's D, and Fu's Fs statistic. The Nexus haplotype data files were generated for mitochondrial cytochrome b and control region on which geographical traits were added. Inter-haplotypic relationship was portrayed that relied on median-joining haplotype network which was analyzed and demonstrated by using PopART v1.7 (Population Analysis with Reticulate Trees) (Leigh et al., 2015) that visualized genealogical relatedness among haplotypes from different geographical areas in central Nepal.

Phylogenetic relationship among the obtained haplotypes of mitochondrial cytochrome b and among control region genes from this study were constructed. Furthermore, sequences from the previous study (Joshi et al., 2022); partial mitochondrial cytochrome b (GenBank Accession numbers: MT845345–MT845351 from Uttarkashi, Uttarakhand, India and MT845352–MT845353 from East Sikkim, Sikkim, India) and control region haplotypes (GenBank accession numbers: MZ448632–MZ448636 from Uttarkashi, Uttarakhand, India) retrieved from NCBI (http://www.ncbi.nlm.nih.gov) were also incorporated in the next instance. These Phylogenetic analyses of haplotypes were relied on Maximum likelihood (ML) method in RAxML GUI 2.0.10 (Edler et al., 2021) on which best fitting model was searched and selected with the least Bayesian information criterion (BIC) and Akaike information criterion (AIC) values. GTR+G+I model was selected for ML tree of mitochondrial cytochrome b haplotypes.

HKY+G model was selected for ML tree of mitochondrial cytochrome b haplotypes along with partial sequences of *N. goral*. GTR+G+I model was selected for ML tree of mitochondrial control region haplotypes. Rapid bootstrap with 1000 replications was selected to test the confidence coefficient of the tree topology. *Capra ibex* was used as an outgroup to root the tree. Pairwise sequence divergence was calculated using the Kimura 2 parameters (K2P) distance matrix in MEGA11(Tamura et al., 2021).

3.5 Phylogenetic analyses

The resulting haplotypes of the complete mitochondrial cytochrome b and control region of this study were used to retrieve similar sequences and to compare with those of other bovids by using BLAST. The mitochondrial DNA sequences of genus *Naemorhedus* and other species of subfamily Caprinae were downloaded from the NCBI GenBank to explore the phylogenetic relationship within its genus and subfamily Caprinae. If a complete mitochondrial cytochrome b or control region sequence was not available as such it was extracted from the complete mitochondrial genomes via alignment. Some partial sequences were also downloaded. The downloaded sequences were aligned with the mitochondrial cytochrome b haplotypes from this study by means of Clustal W (Thompson et al., 1994) algorithm in MEGA11 (Tamura et al., 2021). Phylogenetic tree of genus *Naemorhedus* with an outgroup *Capricornis sumatrensis* and within subfamily Caprinae with an outgroup *Hippotragus leucophaeus* was constructed through the Maximum Likelihood (ML) algorithm in RAxML GUI 2.0.10 (Edler et al., 2021) program with 1000 rapid bootstraps and best fit models were determined using least BIC value. Bayesian Inference (BI) trees were constructed by using the software Bayesian Evolutionary Analysis by Sampling Trees (BEAST) version 1.10.4 (Drummond et al., 2012). Input file was prepared in BEAUti v1.10.4 version (Drummond et al., 2012) with Relaxed clock (lognormal), site models and a Yule model were applied. Markov's Chain Monte Carlo (MCMC) was run for 50,000,000 generations with a tree sampling frequency 2000 and later set for 10% burn in. For time calibration, three priors were used: 12.90±1.19 mya for divergence of an outgroup *Hippotragus leucophaeus*, 12.71±1.21 for the most recent common ancestor of the subfamily Caprinae, and 6.46±0.99 mya for the most recent common ancestor of genus *Naemorhedus* (Ropiquet & Hassanin, 2005). The XML file was created in BEAUti and was run in BEAST v.10.5.0 (beta 5). The log file generated by beast was examined and evaluated in Tracer v1.7.2 (Rambaut et al., 2018) to verify that the effective sample sizes exceeded 200 for all parameters.

The consensus tree was generated in Tree Annotator v.10.5.0 discarding initial 10% burn-in. The final tree was visualized in Figtree v1.4.4 with Bayesian posterior probabilities as nodal support values and 95% HPD of time of divergence as nodal bars.

4. Results

4.1. Population genetic structure of Himalayan gorals

4.1.1 Genetic diversity of mitochondrial cytochrome b and control region

A total of forty fecal samples of Himalayan goral were collected: twenty-seven from Langtang National Park (LNP), twelve from Daunne Forest, and one from Gaurishankar Conservation area (GCA). The elevation range for the collected samples was from 1833 m to 2913 m in LNP and 898 m to 966 m in Daunne Forest. Out of the forty samples collected, only 29 samples were successfully sequenced for both the mitochondrial cytochrome b and control region: twenty sequences from LNP, eight sequences from Daunne Forest and one sequence from GCA.

In the mitochondrial cytochrome b sequences from all populations, 7 polymorphic sites were detected across 1002 bp by sequence alignment, corresponding to polymorphism rate of 0.70%. Five mitochondrial cytochrome b sequence haplotypes were recognized including CYD1 from Daunne Forest, CYL1, CYL18, CYL21 from Langtang National Park (LNP) and CYG1 from Gaurishankar Conservation Area (GCA). The haplotype diversity (hd) was 0.369±0.110, and the nucleotide diversity (π) was 0.0006±0.0002. Genetic parameters of mitochondrial cytochrome b indicated low haplotype diversity of and very low nucleotide diversity signifying high level of inbreeding which reduces the fitness and limits the adaptive ability.

In the mitochondrial control region sequences from all populations, 42 polymorphic sites were detected across 1069 bp with a polymorphism rate of 3.93%. Fourteen mitochondrial control region sequence haplotypes were recognized: CRD2, CRD5, CRD6, CRD10 from Daunne Forest; CRL1, CRL2, CRL4, CRL7, CRL14, CRL20, CRL21, CRL22, CRL24 from LNP and CRG1 from GCA. The haplotype diversity (hd) was 0.820±0.067 and the nucleotide diversity (π) was 0.0082±0.0015. Genetic parameters of control region indicated high haplotype diversity and moderate nucleotide diversity reflecting recent alterations in the population. Shared haplotype sequences were found between LNP and Daunne Forest in case of both mitochondrial DNA cytochrome b and control region haplotypes that confirming the existence of some gene flow between these two populations. The average number of nucleotide differences (k) for mitochondrial cytochrome b was low (0.606) revealing minimal differences between haplotypes. For mitochondrial control region, (k) was higher (8.798) indicating

apparent differentiation between haplotypes. Neutrality test for overall sub-populations by Tajima's D and Fu's Fs test statistic for mitochondrial cytochrome b showing negative values was statistically significant at ($p < 0.05$) indicating population expansion after bottleneck effect or positive selection and presence of excess allele numbers due to recent population expansion. Neutrality test for mitochondrial control region showed weak negative values of Tajima's D and Fu's Fs which was statistically insignificant ($p > 0.10$) (Table 1).

Table 1. Genetic diversity parameters and demographic analyses of mitochondrial cytochrome b and control region from three different goral populations in Central Nepal.

Genes	**Features**	**Population**			
		LNP	DF	GCA	Total
Cyt b 1002bp	Sequences	20	8	1	29
	s	4	1	-	7
	h	3	2	-	5
	hd±Sd	0.353±0.123	0.250±0.180	-	0.369±0.110
	π±Sd	0.0006±0.0003	0.0003±0.0002	-	0.0006±0.0002
	k	0.568	0.25	-	0.606
	Tajima's D	-1.4354	-1.0548	-	-1.9663
	Fu's Fs	0.031	-0.182	-	-2.000
CR 1069 bp	Sequences	20	8	1	29
	s	19	23	-	42
	h	9	5	-	14
	hd±Sd	0.705±0.111	0.786±0.151	-	0.820±0.067
	π±Sd	0.0029±0.0009	0.0082±0.0024	-	0.0082±0.0015
	k	3.189	8.786	-	8.798
	Tajima's D	-1.5296	-0.0501	-	-0.6590
	Fu's Fs	-1.538	2.072	-	-0.200

Note: LNP: Langtang National Park, DF: Daunne Forest, GCA: Gaurishankar Conservation Area, s: Number of polymorphic sites, h: Number of haplotypes, hd±Sd: Haplotype diversity with standard deviation, π±Sd: Nucleotide diversity with standard deviation, k: Average number of nucleotide differences.

4.1.2 Genetic differentiation of haplotypes for populations in central Nepal

The Maximum likelihood phylogenetic tree (Figure 2) displayed that the mitochondrial cytochrome b haplotypes clustered into a major clade excluding the outgroup. The major clade included four closely related haplotypes CYD1, CYL1, CYL18 and CYL21. CYD1 from Daunne Forest and CYL1 from LNP were grouped together with a high bootstrap value with a least genetic divergence and more interestingly they belong to different geographical areas showing gene flow between subpopulations and recent divergence. CYG1 from GCA formed a distinct lineage. It expressed higher genetic differentiation in comparison with the rest of the haplotypes from LNP and Daunne Forest.

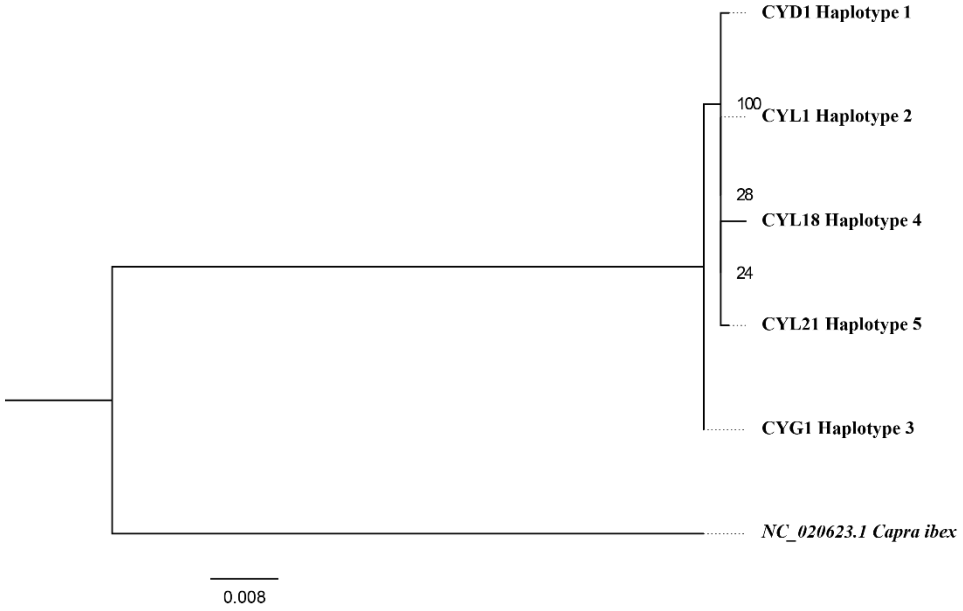


Figure 2. Maximum likelihood phylogenetic tree of five novel haplotypes of mitochondrial cytochrome b (1002 bp) sequences of *Naemorhedus goral* from Central Nepal with an outgroup *Capra ibex*. The tip label refers to haplotype number and node labels indicate percentage bootstrap values (1000 replications). The scale bar represents number of substitutions per site.

Note: In given phylogenetic trees (Figure 2, Figure 4, Figure 5), the name of the sequences was coded. For example, in the CYD1 sequence; "CY" refers to mitochondrial cytochrome b, "D" refers to first letter of geographical area and the number after it is the fecal sample collection number. If instead of "D", there is "G" then it represents to GCA and if there is "L" which represents LNP. If there is "CR" instead of "CY" that refers to mitochondrial control region.

The genetic distances between mitochondrial cytochrome b haplotypes calculated by using Kimura-2-model showed lowest genetic distance of (0.001) between CYL1 hap_2 and CYD1 Hap_1, and between CYL21 Hap_5 and CYL1 Hap_2. The maximum genetic distance among these haplotype sequences was 0.005 between CYG1 Hap_3 and CYL18 Hap_4. The genetic distance between mitochondrial cytochrome b haplotype sequences in the given matrix indicated quite low differentiation (Table 2).

Table 2. Estimates of pairwise genetic distance between mitochondrial cytochrome b haplotype sequences from Central Nepal using K2P model.

Sequences	**1**	**2**	**3**	**4**	**5**
1. CYD1 Haplotype 1		0.002	0.001	0.002	0.001
2. CYG1 Haplotype 3	0.003		0.001	0.002	0.002
3. CYL1 Haplotype 2	0.001	0.002		0.002	0.001
4. CYL18 Haplotype 4	0.004	0.005	0.003		0.002
5. CYL21 Haplotype 5	0.002	0.003	0.001	0.004	

Note: Genetic distances below diagonal and standard errors above diagonal.

The median-joining haplotype network (Figure 3) also displayed the genetic relationship between different haplotypes in a similar topology, with one to several mutational steps existing between different sub-populations from respective contrasting geographical areas. Among five novel mitochondrial cytochrome b haplotypes, the frequencies of haplotypes 1, 3 and 4 were 3.45% each, haplotype 5 had frequency 10.34% whereas haplotype 2 in central position had the highest frequency of 79.31% (23/29) on which shared haplotypes between LNP and Daunne Forest occurred suggesting an ancestral haplotype. Out of 23 sequences that constitutes haplotype 2; seven sequences (30.43%) sequences are from Daunne Forest, and 16 sequences (69.57%) are from LNP. This star shaped network reflected recent population extension with rapid lineage diversification either from founder effect or population bottleneck and a chance of positive selection can be perceived favoring the central (haplotype 2) which was the most frequent haplotype surrounded by closely related haplotypes with only few mutations revealing reduced genetic diversity. Also, the central haplotype was shared between the sub-populations from LNP and Daunne Forest which marked the occurrence of gene flow. It can be speculated that Himalayan goral from LNP moved to Dauune forest or vice versa. Even though, LNP and GCA are geographically very close, but Haplotype showed distinct

genetic integrity and exclusive to GCA. Beside this, haplotype 4 and haplotype 5 were unique to LNP and haplotype 1 was restricted to Daunne Forest.

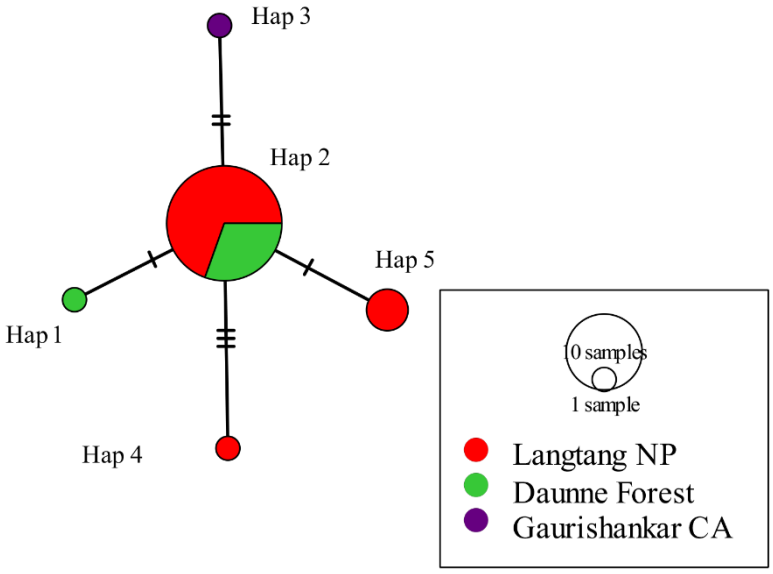


Figure 3. Median-joining haplotype network constructed among five haplotypes of mitochondrial cytochrome b sequences of *Naemorhedus goral* from Central Nepal. Different color represents different geographical areas. The haplotypes are linked through edges on which each hatch line depicts a single mutation step between haplotypes regardless of its length. The size of each circle representing a haplotype is proportional to number of sequences associated with that respective haplotype.

The given Maximum Likelihood phylogenetic tree (Figure 4) includes five mitochondrial cytochrome b haplotypes (1002 bp by sequence alignment) obtained from this study representing three sub-populations from central Nepal. The haplotypes from LNP and GCA represents central Himalayas. The haplotype 1 from Daunne Forest represents Mahabharat range of Central Nepal. There were two partial sequences of mitochondrial cytochrome b of *Naemorhedus goral* from East Sikkim, Sikkim, India (Central Himalayas) and seven partial sequences (404 bp) of mitochondrial cytochrome b from Uttarkashi, Uttarakhand, India (western Himalayas) (Joshi et al., 2022) which were included here with a keen interest. Sikkim, India is towards east and Uttarakhand, India is towards west from the central Nepal, all of them lying within a similar range. Moreover, Joshi et al. (2022) claimed the presence of two valid sub-species of *Naemorhedus goral*; *N. goral. bedfordi* in the western and *N. goral. goral* in the central Himalayas. In this phylogenetic tree, two major clades were visualized. The first major clade had Nepal haplotypes and Sikkim haplotypes as subclades. The second clade included haplotypes from Uttarakhand, India with two subclades containing (haplotypes 4, haplotype 3,

haplotype 5 and haplotype 6) in one and (haplotypes 1, haplotype 2 and haplotype 7) in another. All five haplotypes of Central Nepal clustered together, in combined representing Central Himalayas which was closely related with the *Naemorhedus goral* haplotypes from Sikkim, India (MT845352.1–MT845353.1) in comparison with *Naemorhedus goral* haplotypes (MT845345.1–MT845351.1) from Uttarakhand, India (Western Himalayas) (Figure 4). Localized genetic differentiation was also observed within haplotypes from Central Nepal. Thus, these unique subpopulations from different regions must be considered as evolutionary significant units.

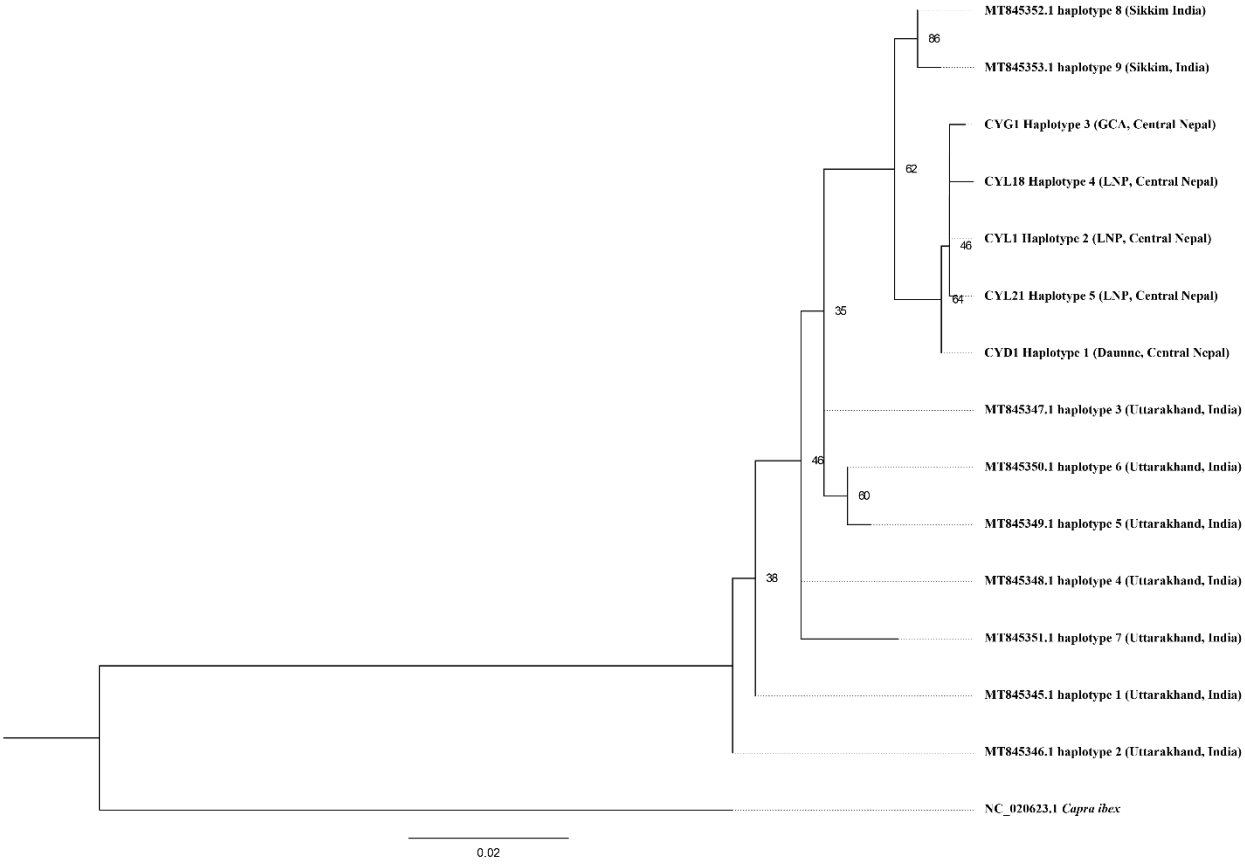


Figure 4. Maximum likelihood phylogenetic tree of mitochondrial cytochrome b haplotype sequences of *Naemorhedus goral* from central Nepal, along with partial mitochondrial cytochrome b sequences from Uttarakhand, India (Western Himalayas) and Sikkim, India (Eastern Himalayas) is shown. Tip labels refer to haplotype number and node labels indicate percentage bootstrap values (1000 replications). Scale bar represents sequence divergence.

Comparatively, the mean genetic distances between the group of mitochondrial cytochrome b sequences of *N. goral* from Uttarakhand and geographically different populations of Central

Nepal were found to be greater than the mean genetic distance of goral populations from Uttarakhand and Sikkim. The maximum mean genetic distance was 0.023 between goral populations from Uttarakhand and Langtang National Park showing significant genetic differentiation (Table 3).

Table 3. Estimates of pairwise genetic distance matrix between group averages of mitochondrial cytochrome b sequences from Central Nepal and Indian Himalayan Region using K2P model.

Sequences	**1**	**2**	**3**	**4**	**5**
1. *Naemorhedus goral* (Daunne Forest)		0.002	0.001	0.007	0.006
2. *Naemorhedus goral* (GCA)	0.003		0.002	0.007	0.007
3. *Naemorhedus goral* (LNP)	0.002	0.003		0.007	0.006
4. *Naemorhedus goral* (Uttarakhand)	0.021	0.021	0.023		0.007
5. *Naemorhedus goral* (Sikkim)	0.010	0.017	0.012	0.019	

Note: Genetic distances below diagonal and standard errors above diagonal.

The Maximum Likelihood phylogenetic tree of mitochondrial control region haplotypes from LNP, GCA and Daunne Forest (Figure 5) clustered into two major clades indicating an apparent genetic divergence between these groups of haplotypes. The first major Clade was further divided into two branches; first branch formed a tiny clade that included three haplotypes 1, haplotype 5 and haplotype 4 from Daunne Forest while the second branch was distinct with haplotype 6 from GCA showing that the haplotypes from GCA and Daunne Forest are closely related. The second major clade almost entirely includes haplotypes from LNP except for one haplotype i.e., haplotype 2 from Daunne Forest which was closely related to haplotype 11 from LNP. The independent branches in second major clade showed genetic differentiation among haplotypes.

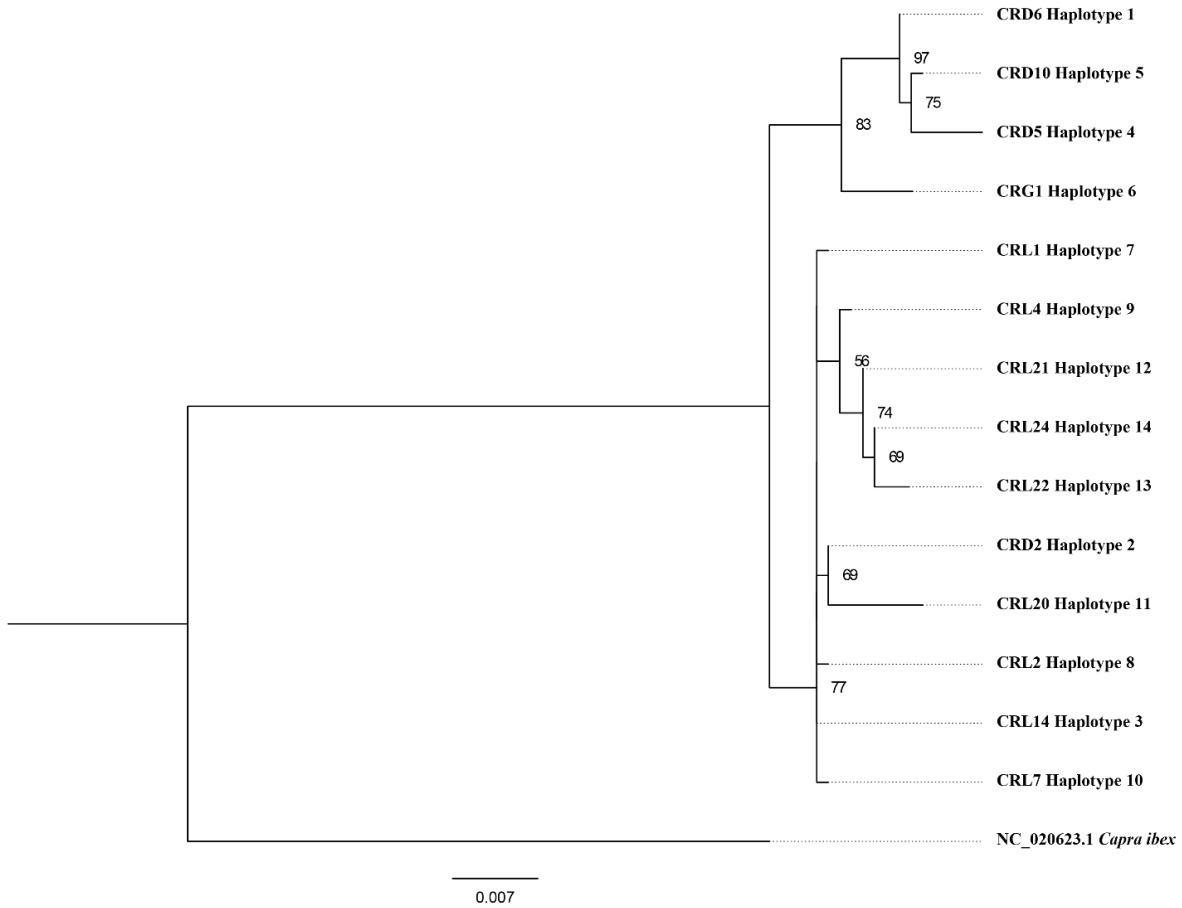


Figure 5. Maximum likelihood phylogenetic tree of mitochondrial control region haplotypes sequences of *Naemorhedus goral* from Central Nepal with an outgroup *Capra ibex*. The tip label refers to haplotype number and node labels indicate percentage bootstrap values (1000 replications). Scale bar indicates genetic divergence.

Among fourteen mitochondrial control region haplotypes, the frequencies of haplotype 2 and haplotype (4–14) were 3.45% each while haplotype 1, haplotype 3 and haplotype 12 had the frequencies 13.79%, 41.38% (12/29) and 6.89% respectively. Haplotype 3 had the highest frequency which additionally included shared sequences between the populations from Daunne Forest and LNP; only one sequence (8.33%) from Daunne Forest and 11 sequences (91.67%) from LNP. The median joining network showed similar topology as depicted by ML phylogenetic tree of mitochondrial control region haplotypes. A large cluster of haplotypes from LNP shown in green circles, connected through edges with one to few hatch lines as mutational steps occurred between respective haplotypes. Haplotype 3 is in central position dominating the LNP region but also present in a minor fraction in Daunne Forest. Haplotype 1, haplotype 2, haplotype 4 and haplotype 5 are unique to Daunne Forest forming a small cluster

in pink circles. Haplotype 6 was isolated in GCA. The green cluster topology suggests the recent localized population expansion with low genetic differentiation (Figure 6).

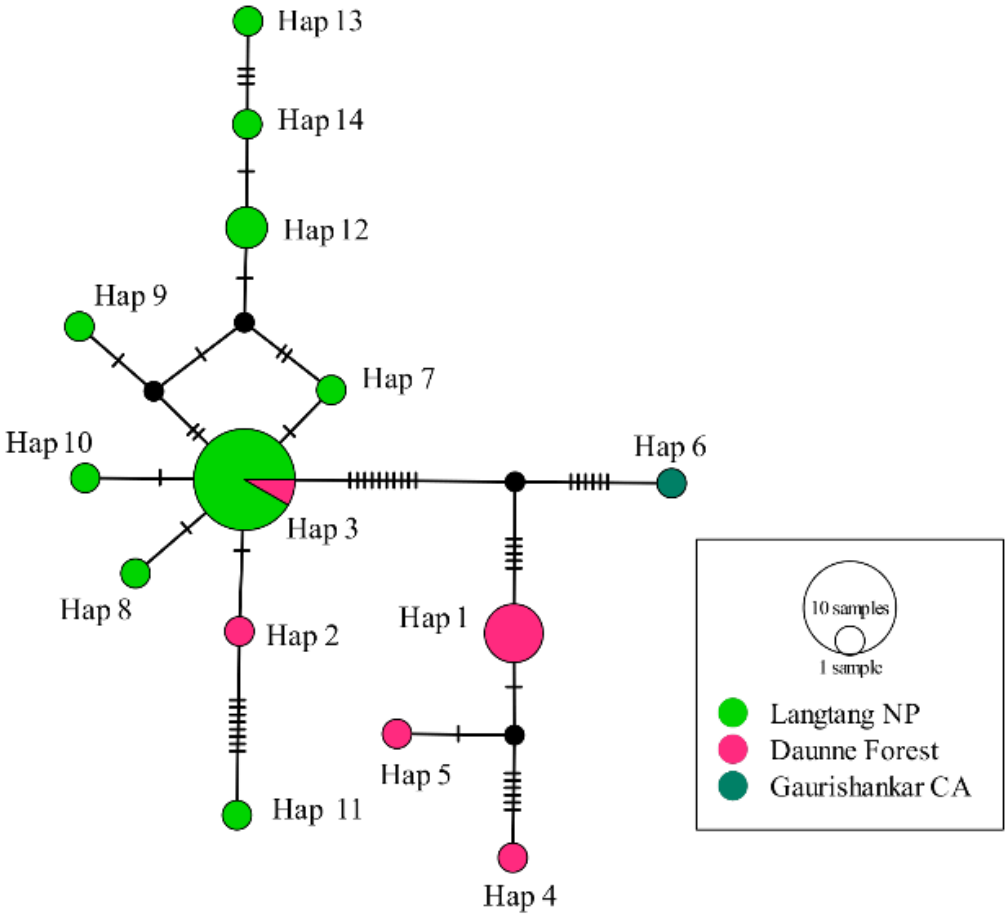


Figure 6. Median-joining haplotype network constructed among fourteen haplotypes of mitochondrial control region of *Naemorhedus goral* from Central Nepal. Different color represents different geographical areas. The haplotypes are linked through edges on which each hatch line depicts a single mutation step between haplotypes regardless of its length. The size of circle representing a haplotype corresponds to the number of sequences that are assigned to the respective haplotype. The small black dots are intermediate haplotypes that are absent in our samples but are essential to connect the observed haplotypes to the network.

In case of mitochondrial control region haplotypes, the maximum mean genetic distance was 0.018 between goral populations from Langtang National Park and Gaurishankar Conservation Area (Table 4).

Table 4. Estimates of pairwise genetic distance matrix between group averages of mitochondrial control region haplotypes from Central Nepal using K2P model.

Sequences	1	2	3
1. *Naemorhedus goral* (Daunne Forest)		0.003	0.003
2. *Naemorhedus goral* (GCA)	0.014		0.004
3. *Naemorhedus goral* (LNP)	0.016	0.018	

Note: Genetic distances below diagonal and standard errors above diagonal.

4.2. Phylogenetic analyses

4.2.1 Phylogenetic relationship within genus *Naemorhedus*

The Maximum likelihood tree (Figure 7) included five mitochondrial cytochrome b haplotypes from Nepal, along with other twenty available mitochondrial cytochrome b sequences of multiple *Naemorhedus* species from different localities, retrieved from GenBank, for phylogenetic analysis among genus *Naemorhedus*, using an outgroup *Hippotragus leucophaeus*. The ML tree showed genetic differentiation among six species of gorals, which formed distinct clades. Three major clades were found. The first major clade included *Naemorhedus goral* sequences from Nepal and India, representing the Himalayas, with unique genetic integrity compared to all other *Naemorhedus* sequences. The second major clade included *Naemorhedus caudatus* from South Korea and *Naemorhedus griseus* from China. The third clade included *Naemorhedus cranbrooki*, *Naemorhedus baileyi* and *Naemorhedus evansi*. The major clades further branched into distinct sub-clades and this ML tree topology strongly supported six valid species of goral i.e *N. goral*, *N griseus*, *N. baileyi*, *N. caudatus*, *N. cranbrooki* and *N. evansi*, *N. goral* from Nepal and India showed the closest evolutionary relationship. The mitochondrial cytochrome b haplotypes from Central Nepal showed similarity with sequence from Sikkim in comparison to Uttarakhand. The species from China, Korea and Southeast Asia formed a separate group, indicating the evolution of genus *Naemorhedus* in their corresponding geographic regions. Each species made a separate cluster showing genetic diversity among *Naemorhedus* species.

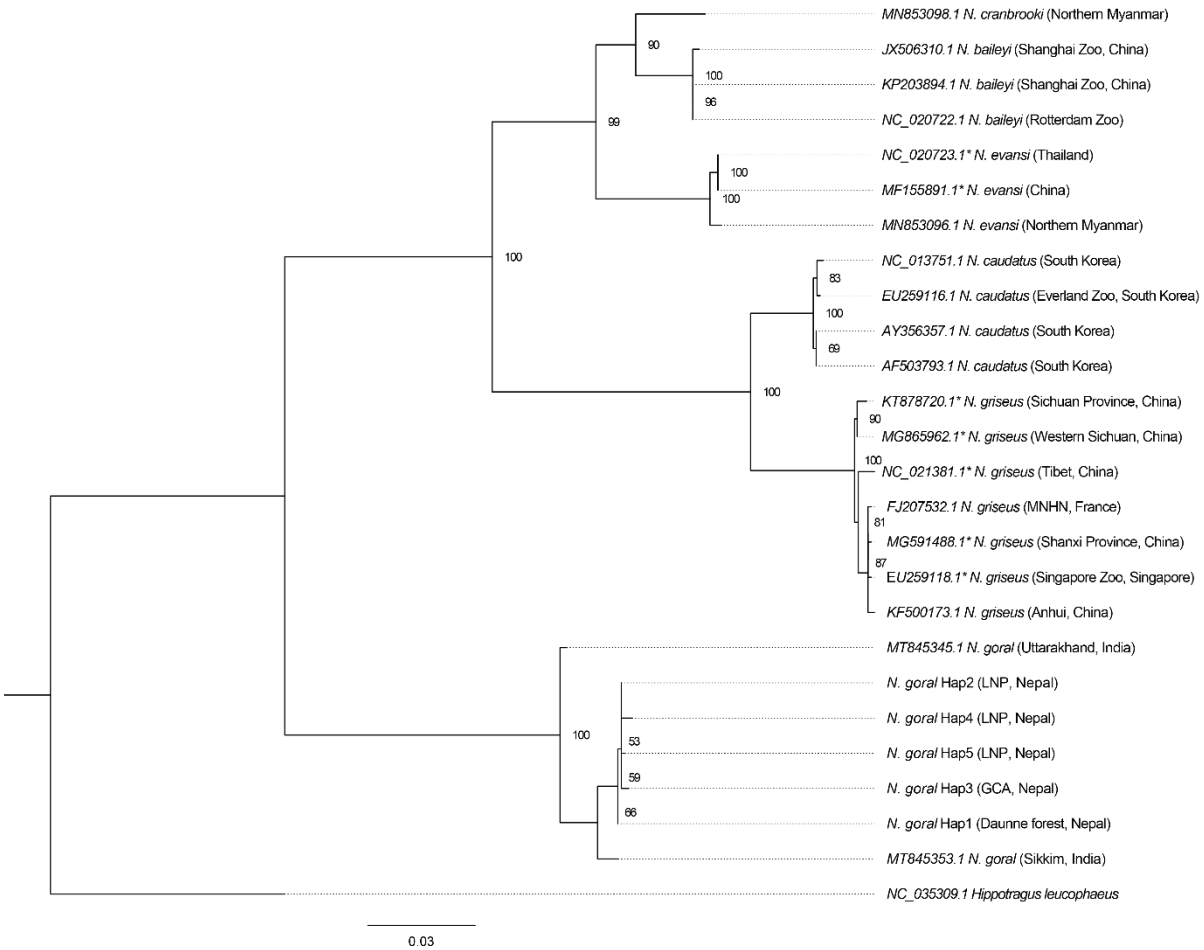


Figure 7. Maximum likelihood phylogenetic tree of mitochondrial cytochrome b sequences of *Naemorhedus goral* showing the relationships among genus *Naemorhedus* from given localities using an outgroup *Hippotragus leucophaeus* with bootstrap values included.

*Revised sequences from GenBank as mentioned in Appendix 2.

In the given matrix, the lowest mean genetic distance was 0.033 between the group of mitochondrial cytochrome b sequences of *N. cranbrooki* and *N. baileyi* while the highest mean genetic distance was 0.161 between the group of mitochondrial cytochrome b sequences of *N. goral* and *N. griseus* (Table 5).

Table 5. Estimates of pairwise genetic distance matrix between group averages of mitochondrial cytochrome b sequences of genus *Naemorhedus* using K2P model.

Sequences	**1**	**2**	**3**	**4**	**5**	**6**
1. *Naemorhedus baileyi*		0.006	0.008	0.013	0.012	0.017
2. *Naemorhedus cranbrooki*	0.033		0.008	0.014	0.013	0.018
3. *Naemorhedus evansi*	0.056	0.057		0.013	0.013	0.017
4. *Naemorhedus caudatus*	0.107	0.119	0.118		0.007	0.019
5. *Naemorhedus griseus*	0.112	0.124	0.123	0.047		0.018
6. *Naemorhedus goral*	0.139	0.153	0.140	0.160	0.161	

Note: Genetic distances below diagonal and standard errors above diagonal.

4.2.2 Phylogenetic relationship within subfamily Caprinae

The Bayesian tree of mitochondrial cytochrome b sequences (Figure 8) represented twelve genus of subfamily Caprinae. The placement of mitochondrial cytochrome b sequences from this study among Caprinae was demonstrated on this tree, showing their closeness to *Naemorhedus goral* sequence from Sikkim, India within the genus *Naemorhedus*. Other *Naemorhedus* species were slightly more diverse than *Naemorhedus goral* from the Himalayas but still within the goral lineage. The genus Naemorhedus was found to be closest to the genus *Capricornis* (serows), sharing a recent common ancestor. The *Capricornis* and *Obivos* (muskox) together formed a sister group with goral.

In the given Bayesian time tree (Figure 8), the basal split represented divergence time of subfamily Caprinae, which occurred 12.71 million years ago. The divergence time of *Naemorhedus, Capricornis* and *Ovibos* clade was around 6.4 million years ago. The compact clustering of regional *Naemorhedus* sequences suggested recent speciation phenomenon likely due to geographical barriers. Overall topology of time tree indicated adaptive radiation of subfamily Caprinae evolved in diverse ecological niche.

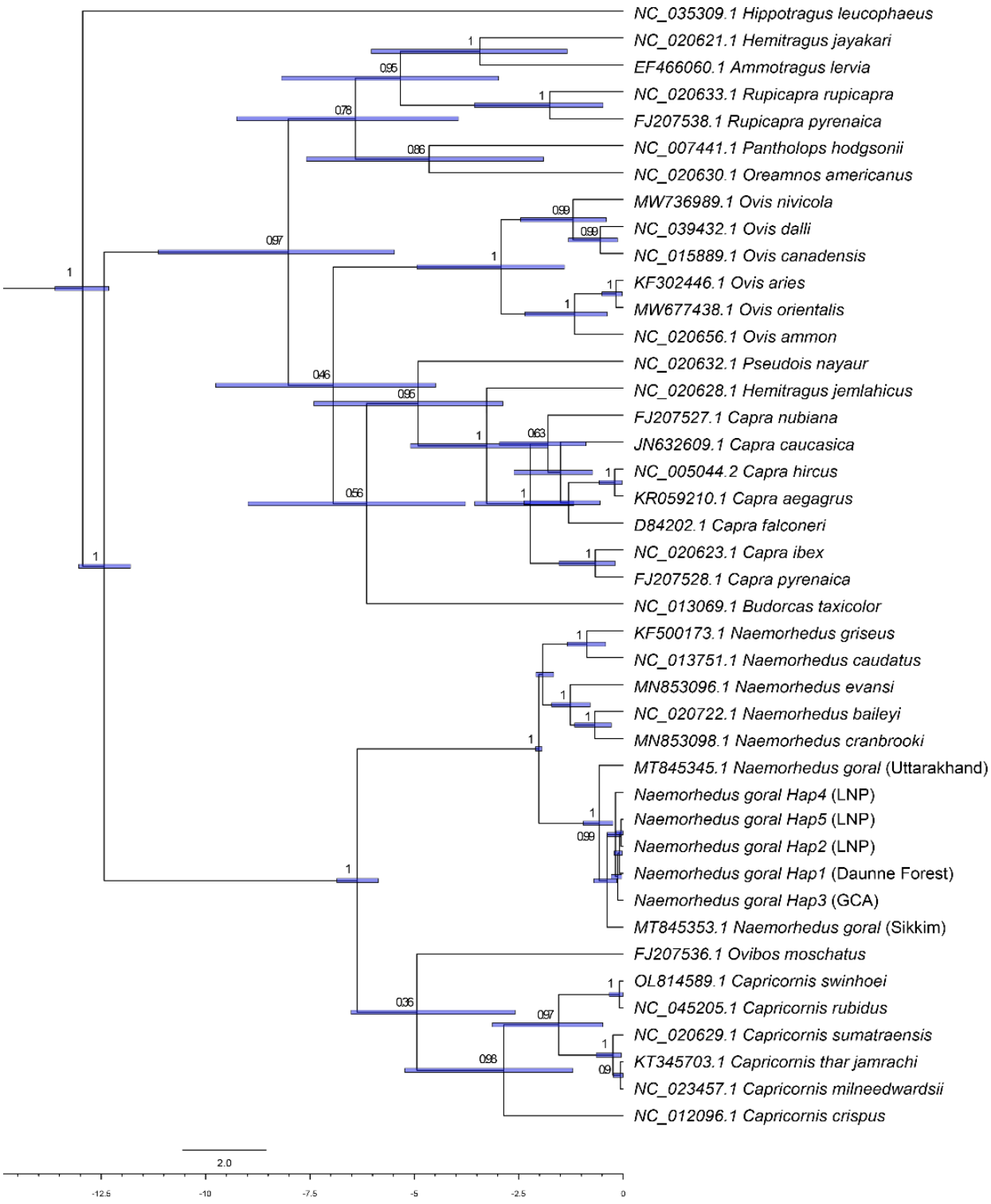


Figure 8. Bayesian time tree showing divergence time estimate for subfamily Caprinae based on mitochondrial cytochrome b. The blue bars indicate 95% highest posterior densities of divergence times. Time scale in X-axis represents million years ago before present. Nodal values showing Bayesian posterior probabilities.

5. Discussion

This study assessed the level of genetic diversity and population genetic structure of Himalayan gorals in Himalayan and Mahabharat region of central Nepal using mitochondrial DNA sequences retrieved from the non-invasively collected fecal samples. The goral populations in central Nepal displayed low level of genetic diversity and gene flow between the Himalayan and Mahabharat ranges. It identified unique haplotypes for both the genes and observed a star-shaped median joining haplotype networks suggesting recent population expansion. Phylogenetic analyses among the gorals revealed that the gorals from central Nepal are genetically closer to the populations from eastern Himalaya (Sikkim) than those from the western Himalaya (Uttarakhand).

5.1. Population genetic structure of Himalayan gorals

The mitochondrial DNA is widely used molecular marker in genetic studies because of its maternal inheritance (Wilson et al., 1985), no recombination and its faster mutation rate (Brown et al., 1979). The only non-coding area and the most rapidly evolving region of mitochondrial DNA control region which is useful for population genetic studies and phylogenetics (An et al., 2010).

The phylogeographic analysis of Himalayan goral in Central Nepal revealed low haplotype diversity and very low nucleotide diversity of mitochondrial cytochrome b gene. However, in the case of mitochondrial control region, haplotype diversity was high and nucleotide diversity was moderate which was like the findings of a study on vulnerable Chinese gorals *N. griseus* populations from two geographically separated populations of Beijing and northeast Inner Mongolia in China indicating rapid population growth from small effective population size (Yang et al., 2019). The neutrality test of mitochondrial cytochrome b also suggested recent population alteration. This contrasting result obtained from these two molecular markers was likely because control region is highly variable non-coding region useful for studying genetic diversity within populations whereas mitochondrial cytochrome b is protein coding gene with a relatively slow mutation rate. Since mitochondrial control region evolves rapidly driven by genetic drift and local adaptations capturing recent evolutionary events reflecting greater differentiation than cytochrome b sequences.

In mammals, nucleotide diversity can range generally from 0.003 to 0.04 with maximum value 0.1 and greater the value, greater will be the genetic diversity (Neigel & Avise, 1993) and if

mean genetic distance between haplotypes is greater than 0.01 then it is considered genetically diverse (Nei, 1987). In this study, nucleotide diversity of overall mitochondrial cytochrome b haplotypes was extremely low (0.0006). The genetic distances between Haplotypes from Himalayan populations (LNP and GCA) and Chure population (Daunne Forest) with range of (0.001-0.004) which is small genetic differentiation. Shared haplotype between these two different elevation ranges indicated subsequent colonization or migration and the reason for some level of genetic similarity.

Haplotype and nucleotide diversity of mitochondrial control region were comparatively higher in Daunne Forest than in LNP showing recent population expansion following bottleneck or founder effect. The unique haplotypes restricted to respective geographical populations in Central Nepal indicated population differentiation due to their isolation and possible adaptive evolution with respect to its ecological essence. The population differentiation between different geographic populations of *N. caudatus* in South Korea was possibly due to human made barriers and recent environmental changes or persistent natural environmental condition of respective geography over a very long period of time (Choi et al., 2015).

In this study, out of 29 sequences from Central Nepal, only 5 haplotypes were obtained while in previous study in Indian Himalayan region, 9 haplotypes (7 haplotypes from Uttarakhand and 2 haplotypes from Sikkim) were identified out of 32 sequences (Joshi et al., 2022). The haplotypes from Central Nepal showed similarity with haplotypes from Sikkim representing eastern Himalayas which suggested that the Himalayan goral from Central Nepal corresponds to the *N. goral goral* subspecies. In case of mean genetic distance between groups, sequences from Central Nepal varied significantly from sequences from Uttarakhand with range (0.021–0.023), greater than that between Uttarakhand and Sikkim (0.019). There was significant genetic differentiation between gorals of Central Nepal and Indian Himalayan region. Also, geographically, Sikkim is in proximity from Central Nepal than Uttarakhand. Nepal haplotypes were no wonder distinct from haplotypes from Sikkim and Uttarakhand due to historical geographic isolation. Limited gene flow within these regions led to greater genetic divergence.

5.2. Phylogenetic relationship among gorals

The mitochondrial DNA, particularly protein-coding genes serves as a powerful tool in molecular evolution (Boore & Brown, 1998). Several efforts have been made to solve

taxonomic ambiguities of gorals especially with the help of mitochondrial DNA which is efficient and more reliable too. There has been possible misidentification of samples from the distribution range of *N. griseus* causing inconsistency in goral taxonomy. Mori et al. (2019) and Li et al. (2020) had suggested that *N. goral* and *N. griseus* are synonyms but Joshi et al. (2020) disagreed and raised concerns about the annotation of *N. goral* sequences that clustered in the *N. griseus* clade. The GenBank sequences KT878720.1, MG591488.1, MG865962.1 which were initially annotated as *N. goral* were later revised to *N. griseus* (Hrabina et al., 2023; Joshi et al., 2022) and even questioned the existence of *N. goral* in China. Naemorhedus goral (NC021381) in GenBank was also revised to *N. griseus* (Hrabina et al., 2023). Furthermore, additional sampling is required to clarify the variation between *N. griseus* and *N. goral*.

After considering these several revisions, the Maximum Likelihood tree of genus *Naemorhedus* in this study supported six species which accords with Joshi et al. (2022). The major clade 1 that included *N. baileyi*, *N. cranbrooki* and *N. evansi*; representing South eastern region as in a previous study (Hrabina et al., 2023), major clade 2 that included *N.caudatus* and *N. griseus,* major clade 3 which contained *N. goral* from Himalayas. Joshi et al. (2022) considered *N. bedfordi* and *N. goral goral* as subspecies of goral due to relatively low sequence divergence below 0.030. In Hrabina et al. (2023), the phylogenetic trees based on cytochrome c oxidase I (COI) gene also depicted six species of gorals i.e., *N. goral, N. caudatus, N. griseus, N. baileyi, N. evansi, N.cranbrooki*. The Bayesian tree showed that the genus *Naemorhedus* is closest to genus *Capricornis* which accords with the previous results (Liu & Zhang, 2018; Yang et al., 2013). Gorals and serows portrayed the sister group to musk-oxen (Hassanin et al., 2009).

In this study, *Naemorhedus, Capricornis* and *Ovibos* formed a clade and *Hemitragus* showed polyphyly as in Yang et al. (2013) because of its two different affiliations as *Hemitragus jemlahicus* was closely related to genus *Capra* while *Hemitragus jayakari* was closely related with *Ammotragus lervia*. *N. goral* from Himalayas was more diverse than all other goral species but still resided within the goral lineage. *Obivos moschatus* was also included in clade of gorals and serows. According to Sun et al. (2024), goral was closely related to *Ovibos* (muskox) on mitochondrial genome level and clustered in takin-muskox clade on the genome tree. The divergence time between genus Naemorhedus and *Capricornis* may be around 2 million years ago (An et al., 2010). The existing extant diversity of the subfamily Caprinae is due to adaptive radiation during the late Miocene around 10.96 million years ago with a key innovation of short metacarpels (Ropiquet & Hassanin, 2005).

6. Conclusions and recommendations

6.1. Conclusions

The population genetic analyses of Himalayan gorals from Central Nepal, based on mitochondrial cytochrome b and control region identified five haplotypes of mitochondrial cytochrome b and fourteen haplotypes of the control region. Low haplotype diversity and very low nucleotide diversity were observed in mitochondrial cytochrome b haplotypes while high haplotype diversity and moderate nucleotide diversity were found in control region haplotypes. The star shaped median-joining haplotype network and neutrality tests indicated a recent population expansion with a bottleneck or founder effect. Shared haplotypes between subpopulations of Daunne Forest and LNP suggested some level of gene flow. The ML phylogeny of haplotypes from the Himalayas showed that haplotypes from Central Nepal were more closely related to those from Sikkim (Eastern Himalaya) than from Uttarakhand (Western Himalaya). The maximum likelihood tree of genus *Naemorhedus* suggested six valid species of gorals. The Bayesian tree of the subfamily Caprinae showed that genus *Naemorhedus* is closely related to *Capricornis*, followed by *Ovibos*.

6.2. Recommendations

Comprehensive goral sampling and analysis across their entire geographical distribution is crucial to resolve the taxonomic and phylogenetic vagueness. As a significant portion of the Himalayas lies within Nepal, population genetic studies of all Himalayan goral subpopulations throughout Nepal should be conducted which also facilitates clarification of geographical boundaries of the two subspecies of *N. goral*: *N. goral bedfordi* (Western Himalayas) and *N. goral goral* (Eastern Himalayas). The habitat connectivity of goral subpopulations should be assessed, and their genetic makeup should be monitored time and again. Only protected areas are not enough for conservation, protection outside Protected Areas should be emphasized with an active involvement of local people enhancing awareness and education. Himalayan goral from Central Nepal aligns with sub-species *N. goral goral*. Unique subpopulations of gorals must be considered as Evolutionary significant units and formulate conservation actions. Identification and management of ecological corridors between isolated goral subpopulations is imperative to mitigate habitat fragmentation.

7. References

Abbas, F.-i., Mian, A., Akhtar, T., & Rooney, T. P. (2015). Status and future management of grey goral (*Naemorhedus goral bedfordi*) in Pakistan. *Journal of Bioresource Management*, *2*(4), 2. https://doi.org/10.35691/JBM.5102.0036

Adhikari, J. N., Adhikari, R. B., Bhattarai, B. P., Thapa, T. B., & Ghimire, T. R. (2021). A small-scale coprological survey of the endoparasites in the Himalayan goral *Naemorhedus goral* (Hardwick, 1825) in Nepal. *Biodiversitas Journal of Biological Diversity*, *22*(3), 1285-1290. https://doi.org/10.13057/biodiv/d220326

Ahmad, S., Yang, L., Khan, T. U., Wanghe, K., Li, M., & Luan, X. (2020). Using an ensemble modelling approach to predict the potential distribution of Himalayan gray goral (*Naemorhedus goral bedfordi*) in Pakistan. *Global Ecology and Conservation*, *21*, e00845. https://doi.org/10.1016/j.gecco.2019.e00845

An, J., Okumura, H., Lee, Y.-S., Kim, K.-S., Min, M.-S., & Lee, H. (2010). Organization and variation of the mitochondrial DNA control region in five Caprinae species. *Genes and Genomics*, *32*, 335–344. https://doi.org/10.1007/s13258-010-0023-8

Ariyaraphong, N., Pansrikaew, T., Jangtarwan, K., Thintip, J., Singchat, W., Laopichienpong, N., . . . Srikulnath, K. (2021). Introduction of wild Chinese gorals into a captive population requires careful genetic breeding plan monitoring for successful long-term conservation. *Global Ecology and Conservation*, *28*, e01675. https://doi.org/10.1016/j.gecco.2021.e01675

Ashraf, N., Anwar, M., Hussain, I., Mirza, S. N., Latham, M. C., & Latham, A. D. M. (2016). Habitat use of Himalayan grey goral in relation to livestock grazing in Machiara National Park, Pakistan. *Mammalia*, *80*(1), 0099. https://doi.org/10.1515/mammalia-2014-0099

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Harvard University Press.

Banks, S. C., & Lindenmayer, D. B. (2014). Inbreeding avoidance, patch isolation and matrix permeability influence dispersal and settlement choices by male *Agile antechinus* in a fragmented landscape. *Journal of Animal Ecology*, *83*(2), 515–524. https://doi.org/10.1111/1365-2656.12128

Boore, J. L., & Brown, W. M. (1998). Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. *Current Opinion in Genetics & Development*, *8*(6), 668–674. https://doi.org/10.1016/S0959-437X(98)80035-X

Brown, W. M., George Jr, M., & Wilson, A. C. (1979). Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences*, *76*(4), 1967–1971.

Cho, C.-U., Gyun, G.-H., Yang, J.-J., Lim, S.-J., Lee, A.-N., Park, H.-B., & Lee, B.-K. (2014). Home range and behavioral characteristics of the endangered Korea Gorals (*Naemorhedus caudatus*) with GPS collar. *Korean Journal of Environment and Ecology*, *28*(1), 1–9. https://doi.org/10.13047/KJEE.2014.28.1.1

Cho, C., Kim, K., & Kwon, G. (2016). Habitat altitude and home range of the endangered long-tailed goral (*Naemorhedus caudatus*): seasonal and monthly home range and altitude change. *Mammalia*, *80*(5), 481–489. https://doi.org/10.1515/mammalia-2015-0024

Choi, S. K., Chun, S., An, J., Lee, M.-Y., Kim, H. J., Min, M.-S., . . . Kim, K. S. (2015). Genetic diversity and population structure of the long-tailed goral, *Naemorhedus caudatus*, in South Korea. *Genes and Genomics Systems*, *90*(1), 31–41. https://doi.org/10.1266/ggs.90.31

Craig, M. (1994). Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, *9*(10), 373–375. https://doi.org/10.1016/0169-5347(94)90057-4

Dar, J. A., Fazili, M. F., Bhat, B. A., Ahmad, R., & Wani, I. N. (2022). Prevalence of gastrointestinal helminth parasites of Himalayan goral (*Naemorhedus goral*) in Kazinag National Park, Jammu and Kashmir, India. *Indian Journal of Animal Research*, *56*(5), 587–593. https://doi.org/10.35691/JBM.5102.0036

Dharmarajan, G., Beasley, J., Fike, J., & Rhodes Jr, O. (2014). Effects of landscape, demographic and behavioral factors on kin structure: testing ecological predictions in a mesopredator with high dispersal capability. *Animal Conservation*, *17*(3), 225–234. https://doi.org/10.1111/acv.12086

DNPWC. (2024). *Protected area*. Retrieved 2024 from https://dnpwc.gov.np/en/conservation-area-detail/76/

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*(8), 1969–1973. https://doi.org/10.1093/molbev/mss075

Duckworth, J. W., & MacKinnon, J. (2008). *Naemorhedus goral. The IUCN Red List of Threatened Species 2008: e.T14296A4430073*. Retrieved 01 December 2024 from https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T14296A4430073.en

Edler, D., Klein, J., Antonelli, A., & Silvestro, D. (2021). raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution*, *12*(2), 373–377. https://doi.org/10.1111/2041-210X.13512Citations

Fakhar, I. A. F., Akhtar, T., & Mian, A. (2008). Food and feeding preferences of Himalayan gray goral (*Naemorhedus goral bedfordi*) in Pakistan and Azad Jammu and Kashmir. *Zoo Biology*, *27*(5), 371–380. https://doi.org/10.1002/zoo.20202

Groves, C., & Grubb, P. (2011). Ungulate taxonomy. John Hopkins University Press, Baltimore, Maryland. *Gazella*, 317 pp. https://doi.org/10.56021/9781421400938

Haq, S. M., Waheed, M., Ahmad, R., Bussmann, R. W., Arshad, F., Khan, A. M., . . . Elansary, H. O. (2023). Climate change and human activities, the significant dynamic drivers of Himalayan goral distribution (*Naemorhedus goral*). *Biology (Basel)*, *12*(4), 610. https://doi.org/10.3390/biology12040610

Hassanin, A., Ropiquet, A., Couloux, A., & Cruaud, C. (2009). Evolution of the mitochondrial genome in mammals living at high altitude: new insights from a study of the tribe Caprini (Bovidae, Antilopinae). *Journal of Molecular Evolution*, *68*, 293–310.

Hrabina, P. (2015). A new insight into the taxonomy and zoogeography of recent species of goral (*Nemorhaedus,* Bovidae, Ruminantia). *Gazella*, *42*(1), 33–91. https://doi.org/www.zoopraha.cz/gazella/vyadani-casopisu/13892-gazella-42-2015

Hrabina, P., Pernerova, L., Suchomel, J., & Robovsky, J. (2023). Utility of cytochrome c oxidase I for the deciphering of unstable phylogeny and taxonomy of gorals, genus *Nemorhaedus* Hamilton Smith, 1827 (Bovidae, Ovibovina). *ZooKeys*, *1181*, 81–110. https://doi.org/10.3897/zookeys.1181.108019

i-Abbas, F., Ruckstuhl, K. E., Mian, A., Akhtar, T., & Rooney, T. P. (2012). Distribution, population size, and structure of Himalayan grey goral *Naemorhedus goral bedfordi* (Cetartiodactyla: Bovidae) in Pakistan. *Mammalia*, *76*(2). https://doi.org/10.1515/mammalia-2011-0038

Isarankura Na Ayudhya, J., Merceron, G., Wannaprasert, T., Jaeger, J.-J., Chaimanee, Y., Shoocongdej, R., & Suraprasit, K. (2022). Dental mesowear and microwear for the dietary reconstruction of Quaternary Southeast Asian serows and gorals. *Frontiers in Ecology and Evolution*, *10*, 1000168. https://doi.org/10.3389/fevo.2022.1000168

Jang, J. E., Kim, N. H., Lim, S., Kim, K. Y., Lee, H. J., & Park, Y. C. (2020). Genetic integrity and individual identification-based population size estimate of the endangered long-tailed goral, from Seoraksan National Park in South Korea, based on a non-invasive genetic approach. *Animal Cells and Systems*, *24*(3), 171–179. https://doi.org/10.1080/19768354.2020.1784273

Janis, C. J. B. R. (1982). Evolution of horns in ungulates: ecology and paleoecology. *Biological Reviews*, *57*(2), 261–318. https://doi.org/10.1111/j.1469-185X.1982.tb00370.x

Jnawali, S. R. J. (2011). The status of Nepal mammals: The national red list series. 47.

Joshi, B. D., Singh, V. K., Singh, H., Bhattacharjee, S., Singh, A., Singh, S. K., . . . Thakur, M. (2022). Revisiting taxonomic disparities in the genus *Naemorhedus*: new insights from Indian Himalayan Region. *Mammalia*, *86*(4), 373–379. https://doi.org/doi:10.1515/mammalia-2021-0152

Khanal, L., Chalise, M. K., Fan, P. F., Kyes, R. C., & Jiang, X. L. (2021). Multilocus phylogeny suggests a distinct species status for the Nepal population of Assam macaques (*Macaca assamensis*): implications for evolution and conservation. *Zoological Research*, *42*(1), 3–13. https://doi.org/10.24272/j.issn.2095-8137.2020.279

Kim, B.-J. (2021). Simple assessment of taxonomic status and genetic diversity of Korean long-tailed goral (*Naemorhedus caudatus*) based on partial mitochondrial cytochrome b gene using non-invasive fecal samples. *Proceedings of the National Institute of Ecology of the Republic of Korea*, *2*(1), 32–41. https://doi.org/10.22920/PNIE.2021.2.1.32

Koh, H.-S., Yang, B.-G., Lee, B.-K., & Lee, J.-H. (2002). Sequence diversity of mitochondrial cytochrome b gene in Grey goral *Naemorhedus caudatus* (Artiodactyla, Bovidae) from Korea. *Animal Systematics, Evolution and Diversity*, *18*(1), 13–21.

Leigh, J. W., Bryant, D., & Nakagawa, S. (2015). popart: Full‐feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210x.12410

Li, G., Sun, N., Swa, K., Zhang, M., Lwin, Y. H., & Quan, R. C. J. M. R. (2020). Phylogenetic reassessment of gorals with new evidence from northern Myanmar reveals five distinct species. *Mammal Review*, *50*(4), 325–330. https://doi.org/10.1111/mam.12200

Librado, P., & Rozas, J. J. B. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Liu, Y., & Jiang, L. J. M. D. P. A. (2017). The complete mitochondrial genome sequence of the Himalayan goral, *Naemorhedus goral* (Cetartiodactyla: Caprinae). *Mitochondrial DNA Part A*, *28*(2), 233–235. https://doi.org/10.3109/19401736.2015.1115856

Liu, Y., & Zhang, M. J. C. G. R. (2018). The complete mitochondrial genome of *Naemorhedus griseus* (Artiodactyla: Bovidae) and its phylogenetic implications. *Conservation Genetics Resources*, *10*, 179–183. https://doi.org/10.2307/3504167

Mammal Diversity Database. (2024). Mammal Diversity Database (Version 1.13) [Data set]. *Zenodo*. https://doi.org/10.528/zenodo.10595931

Mead, J. I. (1989). *Nemorhaedus goral Mammalian Species*(335), 1–5. https://doi.org/10.2307/3504167

Min, M.-S., Okumura, H., Jo, D.-J., An, J.-H., Kim, K.-S., Kim, C.-B., . . . Voloshina, I. V. (2004). Molecular phylogenetic status of the Korean goral and Japanese serow based on partial sequences of the mitochondrial cytochrome b gene. *Molecules and Cells*, *17*(2), 365–372. https://doi.org/10.1016/S1016-8478(23)13052-4

Mori, E., Nerva, L., & Lovari, S. (2019). Reclassification of the serows and gorals: the end of a neverending story? *Mammal Review*, *49*(3), 256–262. https://doi.org/10.1111/mam.12154

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia university press. https://doi.org/10.7312/nei-92038-016

Neigel, J. E., & Avise, J. C. (1993). Application of a random walk model to geographic distributions of animal mitochondrial DNA variation. *Genetics*, *135*(4), 1209–1220. https://doi.org/10.1093/genetics/135.4.1209

Okumura, H. (2004). Complete sequence of mitochondrial DNA control region of the Japanese serow *Capricornis crispus* (Bovidae: Caprinae). *Mammal Study*, *29*(2), 137–145. https://doi.org/10.3106/mammalstudy.29.137

Park, C. E., Cho, B.-J., Kim, M.-J., Park, H. C., & Shin, J.-H. J. M. (2021). Geographical relationships between long-tailed goral (*Naemorhedus caudatus*) populations based on gut microbiome analysis. *Microorganisms*, *9*(9), 2002. https://doi.org/10.3390/microorganisms9092002

Perveen, F., & Khan, A. (2013). Migratory status of the goral, *Naemorhedus goral* (Hardwicke)(Artiodactyla: Bovidae) in Kohistan, Pakistan. *International Journal of Biodiversity and Conservation*, *5*(10), 671–677. https://doi.org/10.5897/IJBC2013.0595

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. J. S. b. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Ren, Z., Wang, Y., & Zhu, J. (2018). Complete mitochondrial genome of *Naemorhedus goral* (Caprinae), a threatened species from the Himalayan and Hindu Kush regions. *Conservation Genetics Resources*, *10*, 855–858.

Ropiquet, A., & Hassanin, A. (2005). Molecular phylogeny of caprines (Bovidae, Antilopinae): the question of their origin and diversification during the Miocene. *Journal of Zoological Systematics and Evolutionary Research*, *43*(1), 49–60. https://doi.org/10.1111/j.1439-0469.2004.00290.x

Sharma, H. P., Regmi, S., Pant, B., & Belant, J. L. (2023). Effects of anthropogenic and ecological factors on Himalayan goral in Dhorpatan Hunting Reserve, Nepal. *Global Ecology and Conservation*, *46*. https://doi.org/10.1016/j.gecco.2023.e02562

Stricklin, W. R. (2001). The evolution and domestication of social behaviour. In *Social behaviour in farm animals* (pp. 83–110). CABI publishing Wallingford UK. https://doi.org/10.1079/9780851993973.0083

Sun, N., Ma, X.-Y., Shi, G.-H., Yang, X.-H., Li, W., Feng, C.-G., . . . Lu, J.-Q. J. B. g. (2024). Chromosome-level genome provides insight into the evolution and conservation of the threatened goral (*Naemorhedus goral*). *BMC Genomics*, *25*(1), 92. https://doi.org/10.1186/s12864-024-09987-5

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thapa, A., Thapa, S., & Poudel, B. S. (2011). Habitat and distribution of Goral in Mahabharat Goral Conservation Area in Nawalparasi district of the western Nepal. *Nepal Journal of Forestry*, *XIV*.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Uprety, Y., Tiwari, A., Karki, S., Chaudhary, A., Yadav, R. K. P., Giri, S., . . . Dhakal, M. J. F. (2023). Characterization of forest ecosystems in the Chure (Siwalik Hills) landscape of Nepal Himalaya and their conservation need. *Forests*, *14*(1), 100. https://doi.org/10.3390/f14010100

Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., . . . Sage, R. D. J. B. J. o. t. L. S. (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, *26*(4), 375–400. https://doi.org/10.1111/j.1095-8312.1985.tb02048.x

Xiong, Z., Chen, M., Zhang, E., & Huang, M. (2013). Molecular phylogeny and taxonomic status of the red goral by cytbgene analyses. *Folia Zoologica*, *62*(2), 125–129. https://doi.org/10.25225/fozo.v62.i2.a7.2013

Yang, C., Xiang, C., Qi, W., Xia, S., Tu, F., Zhang, X., . . . Yue, B. J. B. S. (2013). Phylogenetic analyses and improved resolution of the family Bovidae based on complete mitochondrial genomes. *Biochemical Systematics and Ecology*, *48*, 136–143. https://doi.org/10.1016/j.bse.2012.12.005

Yang, J., Zhu, G. F., Jiang, J., Xiang, C. L., Gao, F. L., & Bao, W. D. (2019). Non-invasive genetic analysis indicates low population connectivity in vulnerable Chinese gorals: concerns for segregated population management. *Zoological Research*, *40*(5), 439–448. https://doi.org/10.24272/j.issn.2095-8137.2019.058

Zahidullah, W. A., Khan, S., Akhtar, N., & Saeed, K. (2016). Analysis of Goral population and its extinction causes in District Buner, Khyber Pukhtunkhwa. *International Journal of Pure and Applied Zoology*, *4*(4), 294–301.

Zwickl, D. J., & Hillis, D. M. (2002). Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology*, *51*(4), 588–598. https://doi.org/10.1080/10635150290102339

8. Appendices

Appendix 1. The GenBank accession numbers of sequences from other species of the Caprinae subfamily used in phylogenetic analysis.

SN	Species name	Accession number	Gene
1	*Pseudois nayaur*	NC020632.1	Complete mitochondrial genome
2	*Ovis nivicola*	MW736989.1	Mitochondrial cytochrome b
3	*Ovis aries*	KF302446.1	Complete mitochondrial genome
4	*Ovis ammon*	NC020656.1	Complete mitochondrial genome
5	*Ovis orientalis*	MW677438.1	Mitochondrial cytochrome b
6	*Ovis canadensis*	NC015889.1	Complete mitochondrial genome
7	*Ovis dalli*	NC039432.1	Complete mitochondrial genome
8	*Capricornis swinhoei*	OL814589.1	Mitochondrial cytochrome b
9	*Capricornis milneedwardsii*	NC023457.1	Complete mitochondrial genome
10	*Capricornis sumatraensis*	NC020629.1	Complete mitochondrial genome
11	*Capricornis rubidus*	NC045205.1	Complete mitochondrial genome
12	*Capricornis thar jamrachi*	KT345703.1	Complete mitochondrial genome
13	*Capricornis crispus*	NC012096.1	Complete mitochondrial genome
14	*Capra ibex*	NC020623.1	Complete mitochondrial genome
15	*Capra hircus*	NC005044.2	Complete mitochondrial genome
16	*Capra nubiana*	FJ207527.1	Complete mitochondrial genome
17	*Capra caucasica*	JN632609.1	Complete mitochondrial genome
18	*Capra pyrenaica*	FJ207528.1	Complete mitochondrial genome
19	*Capra aegagrus*	KR059210.1	Complete mitochondrial genome
20	*Capra falconeri*	D84202.1	Mitochondrial cytochrome b
21	*Pantholops hodgsonii*	NC007441.1	Complete mitochondrial genome
22	*Hemitragus jemlahicus*	NC020628.1	Complete mitochondrial genome
23	*Hemitragus jayakari*	NC020621.1	Complete mitochondrial genome
24	*Budorcas taxicolor*	NC013069.1	Complete mitochondrial genome
25	*Ammotragus lervia*	EF466060.1	Complete mitochondrial genome
26	*Rupicapra rupicapra*	NC020633.1	Complete mitochondrial genome
27	*Rupicapra pyrenaica*	FJ207538.1	Complete mitochondrial genome
28	*Ovibos moschatus*	FJ207536.1	Complete mitochondrial genome
29	*Oreamnos americanus*	NC020630.1	Complete mitochondrial genome
30	*Hippotragus leucophaeus*	MF043256.1	Complete mitochondrial genome

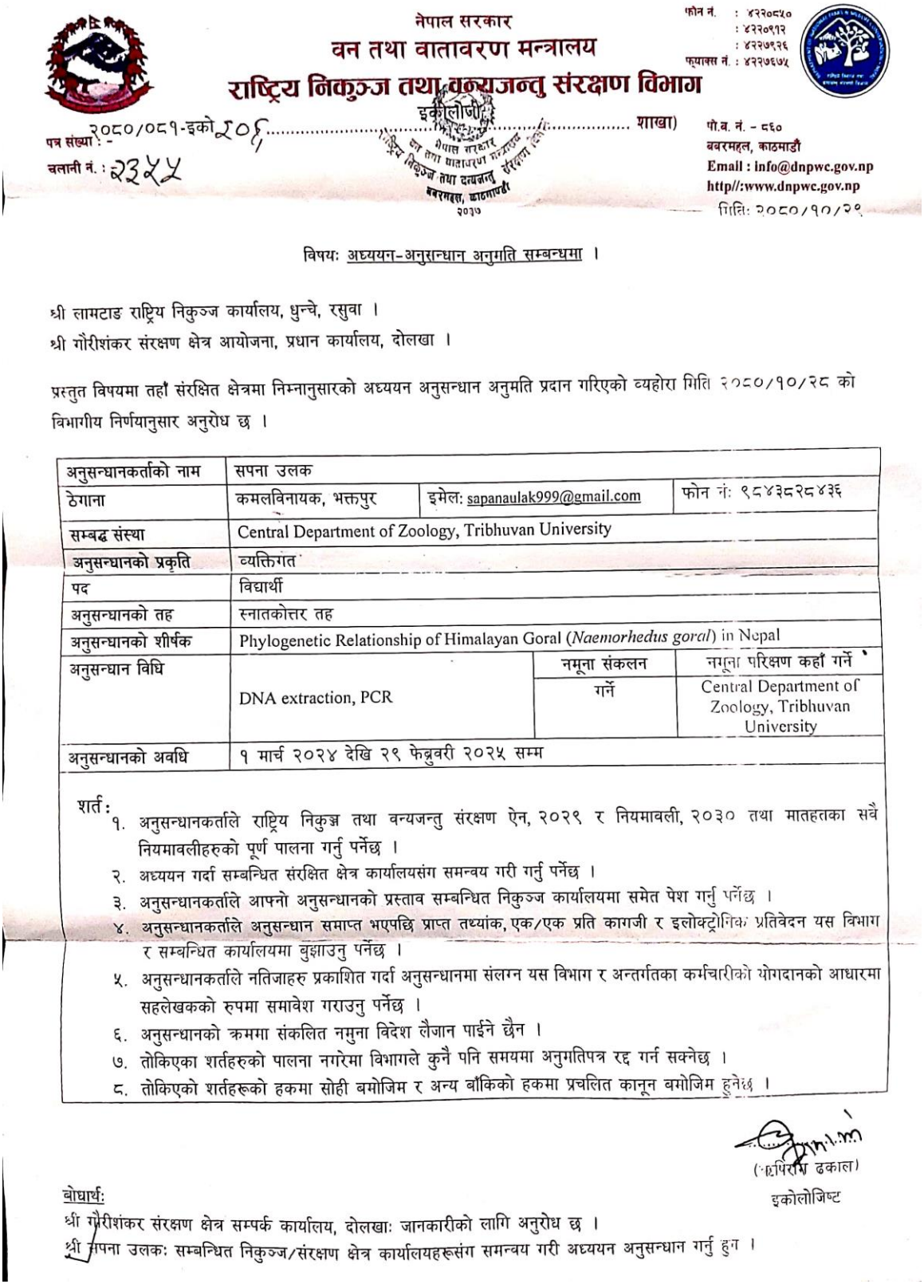
Appendix 2. The GenBank accession numbers of *Naemorhedus* species sequences used for phylogenetic analysis.

SN	Species name	Accession no.	Locality	Gene
1	*Naemorhedus goral*	MT845353.1	East Sikkim, India	Partial mt cyt b
2	*Naemorhedus goral*	MT845352.1	East Sikkim, India	Partial mt cyt b
3	*Naemorhedus goral*	MT845351.1	Uttarakhand, India	Partial mt cyt b
4	*Naemorhedus goral*	MT845350.1	Uttarakhand, India	partial mt cyt b
5	*Naemorhedus goral*	MT845349.1	Uttarakhand, India	partial mt cyt b
6	*Naemorhedus goral*	MT845348.1	Uttarakhand, India	partial mt cyt b
7	*Naemorhedus goral*	MT845347.1	Uttarakhand, India	Partial mt cyt b
8	*Naemorhedus goral*	MT845346.1	Uttarakhand, India	Partial mt cyt b
9	*Naemorhedus goral*	MT845345.1	Uttarakhand, India	Partial mt cyt b
10	*Naemorhedus caudatus*	EU259116.1	Everland Zoo, South Korea	mt genome
11	*Naemorhedus caudatus*	NC013751.1	South Korea	mt genome
12	*N. caudatus raddeanus*	EU259117.1	South Korea	mt cyt b
13	*Naemorhedus caudatus*	AY356357.1	Seoul, South Korea	mt cyt b
14	*Naemorhedus caudatus*	AF503793.1	South Korea	Partial mt cyt b
15	*Naemorhedus griseus*	NC020723.1	France	mt genome
16	*Naemorhedus griseus**	KT878720.1	Tangjiahe Natural Reserve, Sichuan Province, China	mt genome
17	*Naemorhedus griseus**	MG865962.1	Western Sichuan, China	mt genome
18	*Naemorhedus griseus**	NC021381.1	Mount Qomolangma Nature Reserve, Tibet, China	mt genome
19	*Naemorhedus griseus*	FJ207532.1	MNHN, France	mt genome
20	*Naemorhedus griseus**	MG591488.1	Shanxi Province, North China	mt genome
21	*Naemorhedus griseus**	EU259118.1	Singapore Zoo, Singapore	mt cyt b
22	*Naemorhedus griseus*	KF500173.1	Anhui, China	mt genome
23	*Naemorhedus cranbrooki*	MN853099.1	Putao county, Northern Myanmar	mt genome
24	*Naemorhedus cranbrooki*	MN853098.1	Putao county, Northern Myanmar	mt genome
25	*Naemorhedus evansi*	MN853096.1	Northern Myanmar	Partial mt genome
26	*Naemorhedus evansi**	MF155891.1	Guizhou Fanjingshan National Nature Reserve, China	mt genome
27	*Naemorhedus evansi**	NC020723.1	Thailand	mt genome
28	*Naemorhedus baileyi*	NC020722.1	France	mt genome
29	*Naemorhedus baileyi*	JN632663.1	France	mt genome

30	*Naemorhedus baileyi*	KP203894.1	Shanghai Zoo, China	mt genome
31	*Naemorhedus baileyi*	JX506310.1	Shanghai Zoo, China	mt genome
32	CYD1 Haplotype 1	This study	Daunne Forest, Nepal	mt cyt b
33	CYL1 Haplotype 2	This study	Langtang NP, Nepal	mt cyt b
34	CYG1 Haplotype 3	This study	Gaurishankhar CA, Nepal	mt cyt b
35	CYL18 Haplotype 4	This study	Langtang NP, Nepal	mt cyt b
36	CYL21 Haplotype 5	This study	Langtang NP, Nepal	mt cyt b
37	CRD2 Haplotype 2	This study	Daunne Forest, Nepal	Control region
38	CRD6 Haplotype 1	This study	Daunne Forest, Nepal	Control region
39	CRD10 Haplotype 5	This study	Daunne Forest, Nepal	Control region
40	CRD5 Haplotype 4	This study	Daunne Forest, Nepal	Control region
41	CRL1 Haplotype 7	This study	Langtang NP, Nepal	Control region
42	CRL2 Haplotype 8	This study	Langtang NP, Nepal	Control region
43	CRL4 Haplotype 9	This study	Langtang NP, Nepal	Control region
44	CRL7 Haplotype 10	This study	Langtang NP, Nepal	Control region
45	CRL14 Haplotype 3	This study	Langtang NP, Nepal	Control region
46	CRL20 Haplotype 11	This study	Langtang NP, Nepal	Control region
47	CRL21 Haplotype 12	This study	Langtang NP, Nepal	Control region
48	CRL22 Haplotype 13	This study	Langtang NP, Nepal	Control region
49	CRL24 Haplotype 14	This study	Langtang NP, Nepal	Control region
50	CRG1 Haplotype 6	This study	Gaurishankar CA, Nepal	Control region

*Revised sequences: In GenBank, NC020723.1 and MF155891.1 were annotated as *Naemorhedus griseus* which were later revised to *Naemorhedus evansi* (Hrabina et al., 2023; Joshi et al., 2022; Li et al., 2020). In GenBank, KT878720.1, MG591488.1, MG865962.1 were annotated as *Naemorhedus goral* which were later revised to *Naemorhedus griseus* (Hrabina et al., 2023; Joshi et al., 2022). *Naemorhedus goral* (NC021381) in GenBank was revised to N griseus (Hrabina et al., 2023).

Appendix 3. Permission Letter from the Department of National Park and Wildlife Conservation, Government of Nepal



नेपाल सरकार
वन तथा वातावरण मन्त्रालय
राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभाग
इकोलोजी शाखा)

फोन नं. : ४२२०८५०
: ४२२०९१२
: ४२२७९२६
फ्याक्स नं. : ४२२७६७५

पो.ब. नं. - ८६०
बबरमहल, काठमाडौं
Email : info@dnpwc.gov.np
http//:www.dnpwc.gov.np

पत्र संख्या : - २०८०/०८१-इको/२०६
चलानी नं. : २३४५

मिति: २०८०/१०/२९

विषय: अध्ययन-अनुसन्धान अनुमति सम्बन्धमा ।

श्री लामटाङ राष्ट्रिय निकुञ्ज कार्यालय, धुन्चे, रसुवा ।
श्री गौरीशंकर संरक्षण क्षेत्र आयोजना, प्रधान कार्यालय, दोलखा ।

प्रस्तुत विषयमा तहाँ संरक्षित क्षेत्रमा निम्नानुसारको अध्ययन अनुसन्धान अनुमति प्रदान गरिएको व्यहोरा मिति २०८०/१०/२८ को विभागीय निर्णयानुसार अनुरोध छ ।

अनुसन्धानकर्ताको नाम	सपना उलक		
ठेगाना	कमलविनायक, भक्तपुर	इमेल: sapanaulak999@gmail.com	फोन नं: ९८४३८२८४३६
सम्बद्ध संस्था	Central Department of Zoology, Tribhuvan University		
अनुसन्धानको प्रकृति	व्यक्तिगत		
पद	विद्यार्थी		
अनुसन्धानको तह	स्नातकोत्तर तह		
अनुसन्धानको शीर्षक	Phylogenetic Relationship of Himalayan Goral (*Naemorhedus goral*) in Nepal		
अनुसन्धान विधि	DNA extraction, PCR	नमूना संकलन गर्ने	नमूना परिक्षण कहाँ गर्ने Central Department of Zoology, Tribhuvan University
अनुसन्धानको अवधि	१ मार्च २०२४ देखि २९ फेब्रुवरी २०२५ सम्म		

शर्त:

१. अनुसन्धानकर्ताले राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली, २०३० तथा मातहतका सबै नियमावलीहरुको पूर्ण पालना गर्नु पर्नेछ ।
२. अध्ययन गर्दा सम्बन्धित संरक्षित क्षेत्र कार्यालयसंग समन्वय गरी गर्नु पर्नेछ ।
३. अनुसन्धानकर्ताले आफ्नो अनुसन्धानको प्रस्ताव सम्बन्धित निकुञ्ज कार्यालयमा समेत पेश गर्नु पर्नेछ ।
४. अनुसन्धानकर्ताले अनुसन्धान समाप्त भएपछि प्राप्त तथ्यांक, एक/एक प्रति कागजी र इलोक्ट्रोनिक प्रतिवेदन यस विभाग र सम्बन्धित कार्यालयमा बुझाउनु पर्नेछ ।
५. अनुसन्धानकर्ताले नतिजाहरु प्रकाशित गर्दा अनुसन्धानमा संलग्न यस विभाग र अन्तर्गतका कर्मचारीको योगदानको आधारमा सहलेखकको रुपमा समावेश गराउनु पर्नेछ ।
६. अनुसन्धानको क्रममा संकलित नमुना विदेश लैजान पाईने छैन ।
७. तोकिएका शर्तहरुको पालना नगरेमा विभागले कुनै पनि समयमा अनुमतिपत्र रद्द गर्न सक्नेछ ।
८. तोकिएको शर्तहरूको हकमा सोही बमोजिम र अन्य बाँकिको हकमा प्रचलित कानून बमोजिम हुनेछ ।

(हरिराम ढकाल)
इकोलोजिष्ट

बोधार्थ:
श्री गौरीशंकर संरक्षण क्षेत्र सम्पर्क कार्यालय, दोलखा: जानकारीको लागि अनुरोध छ ।
श्री सपना उलक: सम्बन्धित निकुञ्ज/संरक्षण क्षेत्र कार्यालयहरूसंग समन्वय गरी अध्ययन अनुसन्धान गर्नु हुन ।

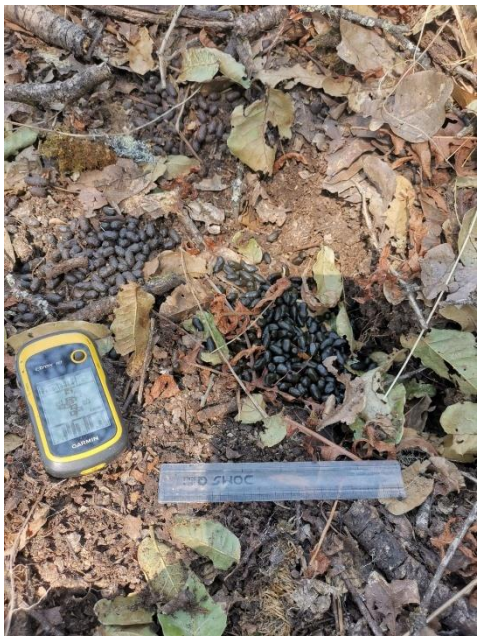
Appendix 4. Estimates of Pairwise genetic distance between mitochondrial cytochrome b haplotype sequences from Central Nepal and partial sequences from Indian Himalayan region using K2P model.

Sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. CYD1 Hap_1 (Daunne Forest)		0.002	0.001	0.002	0.001	0.008	0.008	0.006	0.007	0.008	0.008	0.008	0.011	0.005
2. CYG1 Hap_3 (GCA)	0.003		0.001	0.002	0.002	0.008	0.008	0.008	0.007	0.007	0.008	0.008	0.011	0.007
3. CYL1 Hap_2 (LNP)	0.001	0.002		0.002	0.001	0.008	0.008	0.006	0.007	0.008	0.008	0.008	0.011	0.005
4. CYL18 Hap_4 (LNP)	0.004	0.005	0.003		0.002	0.009	0.009	0.007	0.007	0.008	0.009	0.008	0.011	0.006
5. CYL21 Hap_5 (LNP)	0.002	0.003	0.001	0.004		0.009	0.009	0.007	0.008	0.008	0.009	0.008	0.011	0.006
6. MT845345.1 *N. goral*	0.019	0.019	0.019	0.022	0.022		0.003	0.008	0.005	0.004	0.007	0.006	0.008	0.007
7. MT845346.1 *N. goral*	0.021	0.021	0.021	0.025	0.025	0.003		0.008	0.006	0.005	0.008	0.007	0.008	0.008
8. MT845353.1 *N. goral* (Sikkim)	0.012	0.018	0.012	0.015	0.015	0.019	0.021		0.007	0.008	0.008	0.008	0.011	0.003
9. MT845347.1 *N. goral* (Uttarakhand)	0.015	0.015	0.015	0.018	0.018	0.009	0.012	0.015		0.003	0.004	0.003	0.007	0.006
10. MT845348.1 *N. goral* (Uttarakhand)	0.018	0.018	0.018	0.021	0.021	0.006	0.009	0.018	0.003		0.005	0.004	0.006	0.007
11. MT845349.1 *N. goral* (Uttarakhand)	0.021	0.021	0.021	0.025	0.025	0.016	0.018	0.021	0.006	0.009		0.003	0.007	0.008
12. MT845350.1 *N. goral* (Uttarakhand)	0.018	0.018	0.018	0.021	0.021	0.013	0.015	0.018	0.003	0.006	0.003		0.006	0.007
13. MT845351.1 *N. goral* (Uttarakhand)	0.031	0.031	0.031	0.034	0.034	0.019	0.021	0.031	0.015	0.012	0.015	0.012		0.010
14. MT845352.1 *N. goral* (Sikkim)	0.009	0.015	0.009	0.012	0.012	0.016	0.018	0.003	0.012	0.015	0.018	0.015	0.028	

Appendix 5. Estimates of Pairwise genetic distance between mitochondrial control region haplotype sequences from Central Nepal using K2P model.

Sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. CRD2 Hap_2 (Daunne Forest)		0.005	0.004	0.004	0.004	0.001	0.001	0.002	0.001	0.001	0.003	0.002	0.003	0.002
2. CRD5 Hap_4 (Daunne Forest)	0.020		0.002	0.002	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
3. CRD6 Hap_1 (Daunne Forest)	0.015	0.007		0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005
4. CRD10 Hap_5 (Daunne Forest)	0.017	0.007	0.002		0.004	0.004	0.004	0.005	0.004	0.004	0.005	0.005	0.005	0.005
5. CRG1 Hap_6 (GCA)	0.016	0.017	0.011	0.012		0.004	0.004	0.004	0.004	0.004	0.005	0.004	0.005	0.004
6. CRL1 Hap_7 (LNP)	0.002	0.022	0.015	0.017	0.016		0.001	0.002	0.001	0.001	0.003	0.002	0.002	0.002
7. CRL2 Hap_8 (LNP)	0.002	0.022	0.015	0.017	0.016	0.002		0.002	0.001	0.001	0.003	0.002	0.003	0.002
8. CRL4 Hap_9 (LNP)	0.004	0.024	0.017	0.019	0.016	0.004	0.004		0.002	0.002	0.003	0.002	0.002	0.002
9. CRL7 Hap_10 (LNP)	0.002	0.020	0.015	0.015	0.016	0.002	0.002	0.004		0.001	0.003	0.002	0.002	0.002
10. CRL14 Hap_3 (LNP)	0.001	0.021	0.014	0.016	0.015	0.001	0.001	0.003	0.001		0.003	0.002	0.003	0.002
11. CRL20 Hap_11 (LNP)	0.008	0.022	0.023	0.025	0.024	0.009	0.009	0.011	0.009	0.009		0.003	0.004	0.004
12. CRL21 Hap_12 (LNP)	0.005	0.025	0.018	0.020	0.017	0.003	0.005	0.003	0.005	0.004	0.012		0.002	0.001
13. CRL22 Hap_13 (LNP)	0.009	0.027	0.022	0.022	0.021	0.007	0.009	0.007	0.007	0.008	0.016	0.004		0.002
14. CRL24 Hap_14 (LNP)	0.006	0.024	0.019	0.019	0.018	0.004	0.006	0.004	0.004	0.005	0.013	0.001	0.003	

Appendix 6. Photographs of fecal samples of Himalayan gorals.



Photograph 1. Fecal samples of gorals found in Dhimsa, LNP.

Appendix 7. Photographs of Himalayan gorals from Langtang National Park

Photograph 2. Himalayan goral in Rimche, LNP

Photograph 3. Himalayan goral in Dhimsha, LNP

Appendix 8. Sampling records from Langtang National Park including GPS coordinates, location and slope facing direction.

Sample Name	Lattitude (N)	Longitude (E)	Elevation (m)	Slope facing	Location	Date
GL1 (Goral Langtang 1)	28˚09'41.9"	085˚24'08.4"	2649	southeast	Sherpa village to Rimche	12/27/2080
GL2	28˚09'39.3"	085˚24'38.6"	2612	southeast		
GL3	28˚09'39.8"	085˚24'44.2"	2665	south		
GL4	28˚09'39.2"	085˚24'47.8"	2658	southwest		
GL5	28˚09'56.0"	085˚23'30.9"	2639	Southwest	Khangjim to sherpa village	
GL6	28˚09'57.4"	085˚23'29.4"	2670	south		12/28/2080
GL7	28˚09'57.1"	085˚23'28.9"	2669	south		
GL8	28˚09'52.3"	085˚23'23.6"	2640	southeast		
GL9	28˚09'50.4"	085˚23'20.3"	2631	southeast		
GL10	28˚09'50.4"	085˚23'20.3"	2631	southeast		
GL11	28˚09'38.1"	085˚25'16.6"	2599	south	Rimche	
GL12	28˚09'39.5"	085˚25'16.0"	2641	southwest		
GL13	28˚09'39.4"	085˚25'16.3"	2638	southwest		
GL14	28˚09'39.5"	085˚25'16.5"	2625	southwest		
GL15	28˚09'39.8"	085˚25'17.3"	2641	south		
GL16	28˚09'39.9"	085˚25'17.8"	2643	south		
GL17	28˚09'39.6"	085˚25'17.5"	2634	south		
GL18	28˚09'38.1"	085˚25'17.5"	2597	south		
GL19	28˚09'35.2"	085˚25'18.0"	2561	south		
GL20	28˚08'56.0"	085˚22'19.4"	1833	north	Way to thulo syafru	12/29/2080
GL21	28˚06'22.5"	085˚19'49.1"	2889	southeast	Dhimsa	1/1/2081
GL22	28˚06'22.3"	085˚19'48.5"	2892	southeast		
GL23	28˚06'22.2"	085˚19'48.3"	2885	southeast		
GL24	28˚06'22.7"	085˚19'47.5"	2897	south		
GL25	28˚06'23.1"	085˚19'47.1"	2908	south		
GL26	28˚06'23.2"	085˚19'46.7"	2910	south		
GL27	28˚06'23.5"	085˚19'46.6"	2913	southwest		

Appendix 9. Sampling records from Daunne Forest including GPS coordinates and location.

Sample name	Sampling vial size	Lattitude (N)	Longitude (E)	Elevation (m)	Location	Date
1	big (with pellets)	27.75352˚	84.11558˚	966	Khalde	1/19/2081
1	small					
2	big (with pellets)	27.75279˚	84.11683˚	926		
2	small					
3	big (with pellets)	27.75259˚	84.11693˚	914		
3	small					
4	big (with pellets)	27.75273˚	84.11695˚	938	Buchohul	1/20/2081
4	small					
4B	small					
5	big (with pellets)	27.75336˚	84.11156˚	909.3	Gargare	
5	small					
6	big (with pellets)	27.75352˚	84.11160˚	898		
6	small					
7	big (with pellets)	27.75414˚	84.10191˚	913.8	Bihari Bhir	
7	small					
8	big (with pellets)	27.75406˚	84.10231˚	935		
8	small					
8B	small					
9	small	27.75430˚	84.10176˚	923		1/21/2081
9B	small					
10	small	27.75418˚	84.10211˚	928		
10B	small					
11	small	27.75420˚	84.10194˚	928		
11B	small					
12	big (with pellets)	27.75544˚	84.10159˚	937		
12	small					
13	big (with pellets)	27.75482˚	84.10201˚	944		1/22/2081