MOLECULAR PHYLOGENY AND PHYLOGEOGRAPHY OF THE HIMALAYAN LANGUR SPECIES COMPLEX

Thesis Submitted to
Saurashtra University
Rajkot, Gujarat

For the award of the Degree of

DOCTOR OF PHILOSOHY
In
WILDLIFE SCIENCE

by
KUNAL AREKAR

Under the Supervision of

Supervisor	Co-supervisor
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December 2020

DECLARATION

I hereby declare that the thesis **"Molecular phylogeny and phylogeography of the Himalayan langur species complex"** is an original research conducted by me under the guidance of my supervisor **Dr. S. Sathyakumar** of the wildlife Institute of India, Dehradun and co-supervisor **Prof. Praveen Karanth** of the Centre for Ecological Sciences, Indian Institute of Science, Bengaluru. The thesis has been submitted to Saurashtra University, Rajkot (Gujarat) for the award of the degree of **Doctor of Philosophy in Wildlife Science** and has not formed the basis for the award of any other degree. It embodies my own work and observations, and in that respect, the investigation appears to advance knowledge on the subject.

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CERTIFICATE

This is to certify that the thesis by **Mr. Kunal Arekar** entitled **"Molecular phylogeny and phylogeography of the Himalayan langur species complex"** is an original and independent research work submitted to Saurashtra University, Rajkot (Gujarat) for the award of the degree of **Doctor of Philosophy in Wildlife Science**.

Mr. Kunal Arekar has put in more than six terms of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other university or institution.

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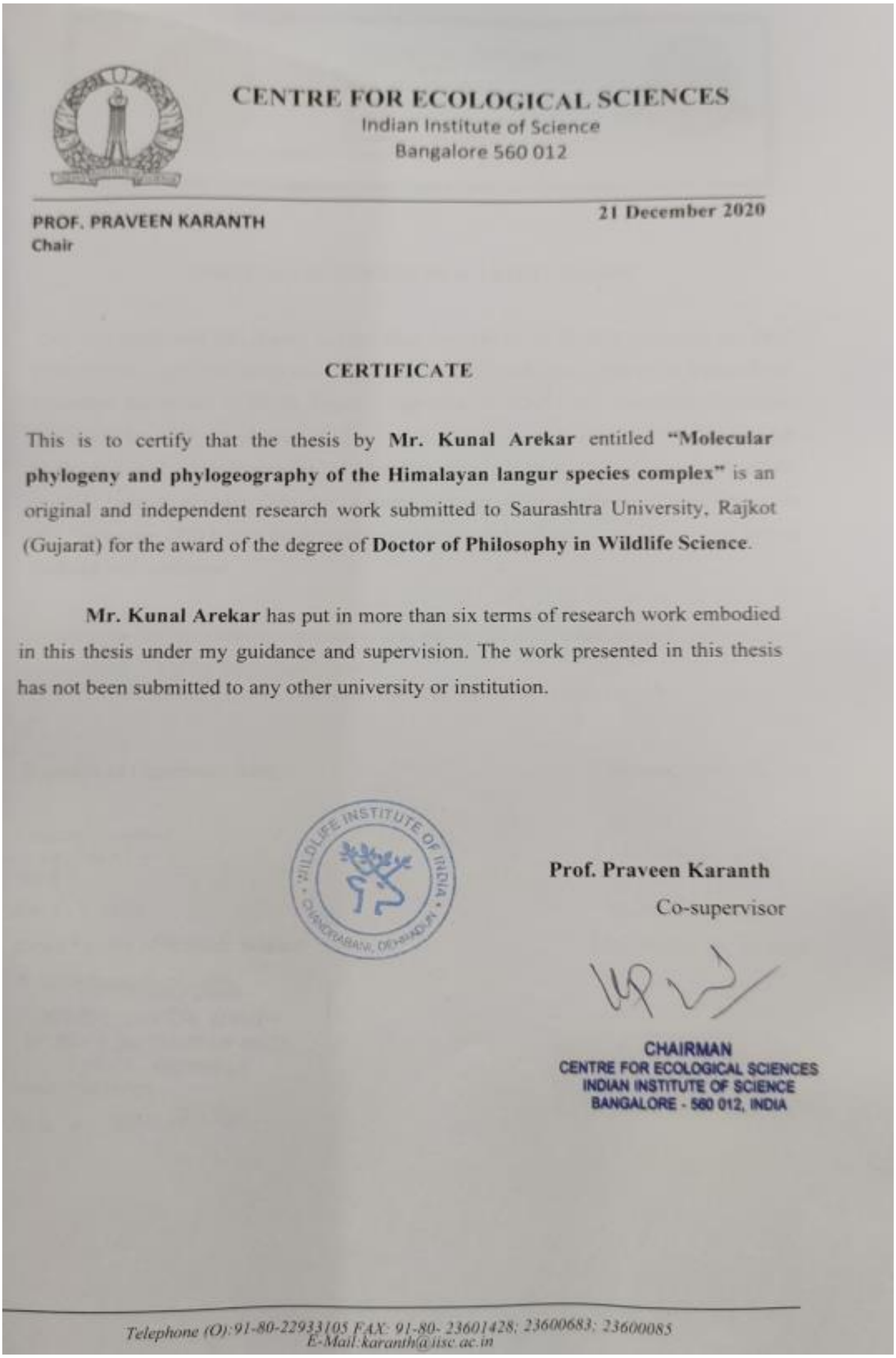

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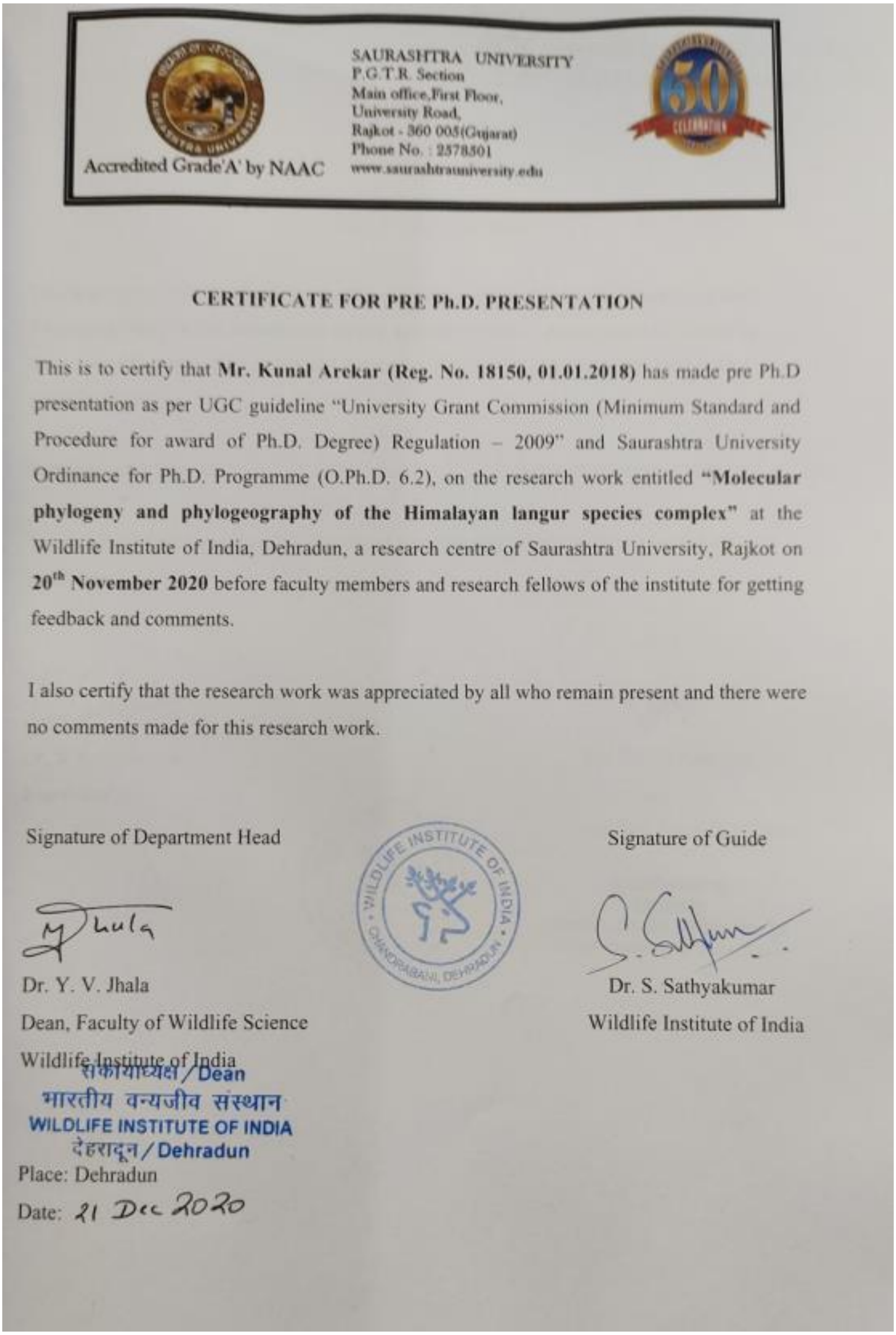

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I also certify that the research work was appreciated by all who remain present and there were no comments made for this research work.

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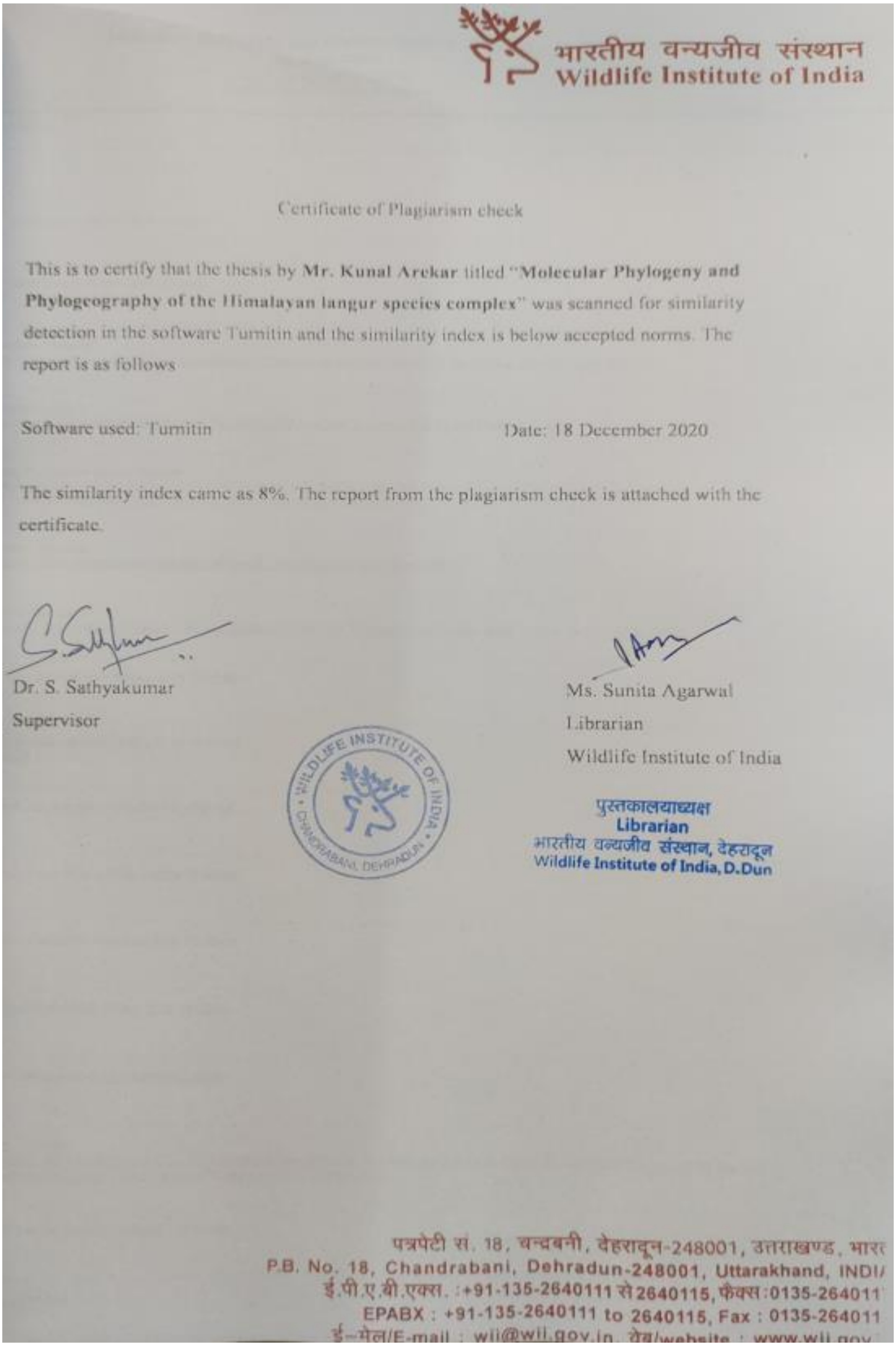

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Miss you Ajji

Dedicated to my parents and my grandparents

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मैं अकेला ही चला था जानिब-ए-मंज़िल मगर लोग साथ आते गये और कारवां बनता गया
- मजरूह सुलतानपुरी

میں اکیلا ہی چلا تھا جانب منزل مگر لوگ ساتھ آتے گئے اور کارواں بنتا گیا
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I set off alone towards my goal, but people came along and it began to turn into a caravan
- Majrooh Sultanpuri

* * * * *

This PhD has been a roller coaster ride with a lot of people to acknowledge and be thankful to. Many people joined in at different stages of this journey who supported and encouraged me in various ways. Below, I have mentioned all the people who have helped me get through this journey and if I miss someone, please know that your help is deeply appreciated.

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Contents

Chapter 1 Introduction

Chapter 2 Systematics of Himalayan langur using integrative taxonomy

Chapter 3 Understanding ecological requirements of the langurs of Himalayas through ecological niche modelling (ENM)

Contents

Chapter 4 Phylogeographic patterns and barriers to dispersal

List of Tables

List of Figures

List of Appendices

List of Plates

Executive Summary

Species is considered to be a fundamental unit in many fields from systematics to ecology, behaviour, evolution, developmental biology, genetics, molecular biology, physiology, anatomy and palaeontology. However, there is no consensus on how to define a species. It is one of the most puzzling problems known to biologists and for decades biologists are trying to find an answer to this eternal problem. There are multiple species concepts (about 24) proposed by different authors but none agree on a common definition of species. Much of early systematic works were based on morphology that used very few objective criteria and often were based on claims by experts regarding species relationships on particular taxonomic groups. Later, considerable advances in the field of molecular biology allowed evolutionary biologists to use molecular data for systematic work, an approach termed as 'molecular systematics'. However, the use of molecular data does not guarantee a robust description and identification as molecular data is also another singular data type, like morphological data. In order to achieve a robust delineation of species, we need to integrate methods from multiple and complimentary perspectives such as morphology, ecology, molecular biology, and behaviour referred to as integrative taxonomy. It not only helps in classifying species but also helps understand the processes that give rise to them.

Accumulation of genetic variation within different populations of a species is shaped by temporal changes in geography, climate and population demographic history via a combined effect of genetic drift, gene flow and selection. Physical barriers such as rivers and mountain ranges are known to prevent gene flow among populations, and over time, create population genetic structures. Given this background, in my thesis, I used an integrative taxonomy

approach to resolve the taxonomy of the Himalayan langurs and I further studied the role of different river valleys in the Himalayas as a potential barrier to gene flow.

In the first chapter, I give a brief overview of systematics and diversification followed by a detailed account of the history of taxonomic studies in Colobine primates, particularly langurs of the genus Trachypithecus and Semnopithecus. My model system, the Himalayan langurs, is the northernmost population of the Hanuman langur group (Semnopithecus spp.), its taxonomy is in flux given the presence of multiple classification schemes relying on morphological characters for species description. So, the first step is to resolve the taxonomy of the Himalayan langurs using an integrative taxonomy approach.

In the second chapter titled, “To investigate the Systematics of Himalayan langur using integrative taxonomy”, I use morphological data in conjunction with molecular data with an aim to identify if the Himalayan langurs are a distinct species from the northern plains langur (Semnopithecus entellus) and if the Himalayan langurs contain multiple species/subspecies. For generating the molecular data, I collected 176 fecal samples from 46 different locations across the distribution range of these langurs in the Himalayas. From these, I was able to successfully sequence a 746 bp of mitochondrial Cytochrome b (Cyt b) fragment from 76 fecal samples. However, for the molecular data analysis, I did not use all the 76 samples, but exemplar sequences from each location were used bringing the total to 26 sequences from India, this includes all the haplotypes. Further, I downloaded 30 sequences from GenBank and 1 S. hypoleucos sequence (also generated in this study) was used as outgroup. I reconstructed evolutionary relationship using Maximum likelihood (ML) and Bayesian approach and performed hypothesis testing analysis to compare the likelihood of the tree obtained in the phylogenetic analysis with the constraint tree based on the three currently recognised species

of Himalayan langurs. For morphological analyses, data was collected by direct observations during field survey as well as from photographs. I used six color-independent morphological characters to differentiate between S. entellus and the Himalayan langur. A total of 85 individuals were recorded from 82 different locations. I then built a phylogeny using the distance matrix based on the similarity in the morphological characters between each individual. The result of my analysis suggest that the Himalayan langurs form a monophyletic group which is separate from the northern plains langur (S. entellus) and our results do not support the splitting of the Himalayan langurs into multiple species/subspecies that are currently mentioned in any of the classification schemes.

In the third chapter titled, “To understand ecological requirements of the langurs of Himalaya through ecological niche modelling (ENM)”, I attempt to understand if the monophyletic Himalayan langurs show divergence along the ecological axis too i.e. if the niche of the Himalayan population separate from S. entellus and does ecological data support the molecular and morphological results from chapter 2. I used presence only locality records for both the Himalayan langurs and S. entellus and built species distribution models (SDM) using Maxent. I first performed model selection to select for best features and regularisation multiplier (RM) values, I tested 48 models each for the Himalayan langurs and S. entellus dataset. I also calculated a measure of niche overlap between the two SDMs by estimating Schoener’s D value. Here I found that the ecological niches of the Himalayan langurs are separate from S. entellus as shown by the niche overlap analysis and further our analysis also showed that the Himalayan langurs prefer areas with high precipitation but with moderate temperatures whereas S. entellus is adapted to drier conditions.

My fourth chapter, titled “Phylogeographic patterns and barriers to dispersal”, deals with understanding the geographic distribution of genetic variation between different populations of Himalayan langurs and I further explored if the four major Himalayan river valleys – Sutlej, Bhagirathi, Gandaki and Kosi act as barriers to gene flow. Investigating patterns of genetic variation within a species helps us identify changes that have occurred due to recent geoclimatic events. Phylogeography enables us to study these patterns by linking microevolutionary field of population genetics with historical biogeography. Different glaciation events during the quaternary period has had significant effects on the current distribution of genetic variation among different species. There are a number of studies on the European and North American taxa to understand the effect of these glaciation events, however very few studies attempted to address this in the south Asian biota. Therefore, here I also explored how the Pleistocene glaciation event affected the distribution of the Himalayan langurs. I use various phylogenetic and population genetic tools along with statistical phylogeographic methods to understand these patterns. Results showed 22 haplotypes across the Himalayan langur distribution in two clusters, one consisting of the Western population i.e. all the sequences from the western Himalayan region (west from 28.07N, 83.26E) and the other consisting of the Eastern population i.e. all the sequences from the eastern Himalayan region (east from 28.19N, 83.65E). This suggests that most river valleys do not act as barriers to geneflow, except river Gandaki which split the Himalayan langurs into the western population (WP) distributed west of Gandaki and an eastern population (EP) distributed east of Gandaki. This result is also corroborated by the AMOVA analysis which partitioned 74.59% of variation among groups and only 7.04% of the variation among populations within groups. Further, the genetic diversity and demographic history analysis suggests recent population expansion, possibly after a bottleneck event, especially in the western Himalaya. The statistical phylogeography analysis supports a scenario where the Himalayan langurs colonised the

western Himalaya after they diverged from the populations in the east. My niche modelling analysis to understand the historical distribution showed that the Himalayan langurs were distributed in the low elevation central Nepal and adjoining parts of India, with a reduced distribution in the western Himalaya. And after LGM ended, the Himalayan langur distribution moved northwards from the low elevation areas and the post LGM expansion actually facilitated the movement of these langurs from central Nepal into western Himalaya where a high probability for the distribution of these langurs can be seen currently.

In the fifth chapter, I summarise the conclusions of each work chapter. My findings, based on multiple lines of evidence, indicate that the Himalayan langur is a distinct species than Semnopithecus entellus. Further, my analysis does not support splitting of the Himalayan langurs into multiple species/subspecies as per the current classification schemes. Therefore, I have subsumed all the currently recognised species/subspecies in the Himalayan langur complex into a single species, *Semnopithecus schistaceus* Hodgson, 1840. I also conclude that the climatic requirements of the Himalayan langurs and *S. entellus* are different. Further, my analyses also support recent population expansion of Himalayan langurs, in the western Himalaya, after a bottleneck event. And none of the Himalayan rivers studied here seem to act as barriers to gene flow, except for the river Gandaki in Nepal which seems to have caused the clustering of the WP and EP. Conservation implications of this study are also discussed briefly here.

Chapter 1

Introduction

1.1 Systematics

The “species problem” is one of the most puzzling problems known to biologists. For decades biologists are trying to find an answer to this eternal problem. There are multiple species concepts (about 24; Mayden, 1997) proposed by different authors but none agree on a common definition of species (Mayden, 1997; Wilkins, 2006). Ironically, species is considered as one of the fundamental units of biology and still, there is no consensus on how to define a species. And if there is no agreement on what species are, then it also hinders our ability to understand the process of speciation itself.

Different systematists have used different concepts to define a species (Mayden, 1997). This has led to ambiguity in species numbers and species boundaries. Recent studies however have attempted to find a solution to this problem. de Quiroz (1998, 2005, 2007,) and Pigliucci (2003) have defined species as metapopulation lineages or family resemblance concepts, where they try to unify alternative and incompatible concepts under one single species concept.

This “species problem” has been discussed time and again with respect to taxonomy (Coyne and Orr, 2004; Zachos, 2016; Minelli, 2020). However, traditionally, taxonomy have been relying almost completely on morphological characters and it does not take into account the evolutionary history of the organisms. It therefore, uses a single type of biological property to characterise different species. Taxonomy completely based on a specific character is sometimes not enough to distinguish between species or subspecies (Will et al., 2005). Such exemplars are quite often found in literature and are called as species complex (Karanth, 2017), which are described as a group of closely related species that are very similar in appearance with no clear boundaries between them.

Systematics on the other hand can be called as an extension of taxonomy which takes into account the evolutionary relationships (species phylogenies) between organisms while classifying and characterising them. Systematics and taxonomy are often used interchangeably, but taxonomy is a subset of systematics. Much of early systematic works were based on morphology that used very few objective criteria and often they were based on claims by experts regarding species relationships on particular taxonomic groups. Later, this scenario changed with more objective methods being developed to construct evolutionary history based on the shared attributes between organisms (Hillis, 1996).

About the same time, there were other advancements in the field of molecular biology, which allowed evolutionary biologists to use molecular data to construct phylogenies. This led to an exponential increase in the availability of data for phylogenetic reconstruction. Central to this advancement was the development of techniques such as DNA sequencing and Polymerase Chain Reaction (PCR), for large scale examinations of variation in the DNA. Driven by these advances in molecular biology, there have been improvements in the tools to analyse these molecular data. This approach, as a whole, is termed as 'Molecular Systematics' (Hillis, 1996).

The advent of molecular techniques such as PCR and DNA sequencing and the advancement of computer-based software for genetic analyses have accelerated the study of understanding the evolutionary processes that gives rise to a diversity of organisms. This has led to exploring more interesting and challenging evolutionary hypotheses in different biological systems. However, the use of molecular data does not guarantee a robust description and identification (Will et al., 2005). Also, the use of molecular data has given rise to several other questions such as, is molecular data better than morphological data? what genes to sequence? neutrality of molecular characters, and others. Molecular data is often considered as another singular data type, like morphological data, to characterise and describe species.

In order to achieve a robust delineation of species (Dayrat, 2005), we need to integrate methods from different disciplines. It is termed as “integrative taxonomy” and defined as the science that aims to delimit the species from multiple and complimentary perspectives such as morphology, ecology, molecular biology, and behaviour (Dayrat, 2005). It not only helps in classifying species but also helps understand the processes that give rise to them (Schlick-Steiner et al., 2010). Since past few decades, biologists have started to recognise the importance of integrative taxonomy for species description (Padial et al., 2010). However, there are many who are sceptical; nevertheless, a general view is to use integrative approach that would incorporate multiple approaches and methods. Many studies have already adopted this approach to describe and identify new species (Riedel et al., 2013) and to reclassify the old ones (Nag et al., 2011, 2014; Ashalakshmi et al., 2014).

1.2 Diversification

As mentioned in the previous section, species is considered to be a fundamental unit in many fields from systematics to ecology, behaviour, evolution, developmental biology, genetics, molecular biology, physiology, anatomy and palaeontology (de Queiroz, 2005). To study a species, it is important to understand how that species came into being – the evolution of that species; and to know how a species evolved, we need to identify the historical processes that may have guided the lineage splitting which may have led to the existence of contemporary taxa.

Speciation is an important process in the field of ecology and evolutionary biology as it generates and maintains biodiversity (Thorpe et al., 2010). Studying diversification and the processes that led to species formation is an active and challenging topic in the field of evolutionary biology (Lozier and Mills, 2009). Diversification is affected by processes that act at different scales across taxa and regions (Kisel et al., 2011; Vijayakumar, 2014). At macroevolutionary scale, diversification can be studied in a phylogenetic framework and visualised in the form of bifurcating trees. It is through these phylogenies that information about the microevolutionary processes (eg. mutation, genetic drift, natural selection) is transmitted. Understanding this gap between the macroevolutionary and microevolutionary processes can be investigated under the framework of phylogeography (Avise et al., 1987; Avise, 2000). Phylogeography explores the patterns of genetic variations within the context of geographical distribution of a species which is governed by the interaction between changes in the environment, and the ecology and dispersal ability of the species. Phylogeographic methods are generally more appropriate to investigate intraspecies patterns which result from more recent environmental changes. And with the recent advances, along with the molecular data, phylogeography now can utilise high resolution climatic data and meticulous statistical analyses (Hickerson et al., 2010).

Earth’s quaternary period has been dominated by repeated cycles of cooling and warming. During the cooling phase majority of earth’s surface was covered by ice and reduction in temperature and water availability caused great changes in the distribution of species. This in turn involved changes in the demography which provided opportunity for adaptations to occur. These changes had selective effects on genetic variation and configuration which can be studied using phylogeography. Thus, phylogeography can be used in the context of Pleistocene refugia theory to understand the contemporary species distribution caused by past climatic changes (Hewitt, 2004).

1.3 Model System

Colobinae is one of the two subfamilies under the family Cercopithecidae (Old World monkeys), the other being Cercopithecinae (Disotell, 2000; Finstermeier et al., 2013). Cercopithecines are referred to as cheek-pouch monkeys; they are monogastric in nature and have a diverse diet. It includes monkeys such as baboons, macaques, mangabeys, guenons and drills. Colobines on the other hand are predominantly leaf eating and exhibit foregut fermentation (Chivers and Hladik, 1980). Based on their geographic distribution the colobines are divided into two groups; the African and the Asian colobines. The African colobine monkeys are represented by the genera *Colobus, Piliocolobus* and *Procolobus* (Groves, 2001). The Asian colobines have been divided into five or six species groups, it depends on what classification scheme we follow (Napier and Napier, 1967; Davies and Oates, 1994; Groves, 2001; Brandon-jones, 2004). According to Brandon-Jones et al. (2004), the Asian colobines consists of seven genera. These includes *Semnopithecus* with 3 species, *Trachypithecus* with 10 species, *Simias* with 1 species, *Rhinopithecus* with 4 species, *Pygathrix* with 2 species, *Presbytis* with 10 species and *Nasalis* with 1 species (Brandon-Jones et al., 2004). Recently the number of species present within these genera have been revised given the new findings (Roos et al., 2014). However, the ambiguity in the taxonomy of many of these groups is not resolved (Sterner et al., 2006; Ting et al., 2008).

Here, I use an integrative taxonomy approach wherein I utilise data from multiple sources viz. morphology, DNA and ecology to resolve the ambiguity in the taxonomy of the Himalayan langur species complex (*Semnopithecus* spp.). Himalayan langur has been considered either as a separate species with multiple subspecies (Hill, 1939) or as multiples separate species (Groves, 2001) or a subspecies of the widely distributed northern plains Hanuman langur (*S. entellus*) (Pocock, 1939, 1928; Roonwal and Mohnot, 1977; Roonwal, 1984; Brandon-jones, 2004;). Within the Himalayan langur, the species and subspecies status of various populations is unclear with some authors describing all the populations as subspecies of *S. entellus* (Pocock, 1928, 1939; Roonwal and Mohnot, 1977; Roonwal 1984; Brandon-Jones, 2004), while others have described the Himalayan langur as a separate species from *S. entellus* with multiple subspecies (Hill, 1939), and finally some authors have split them in multiple species (Groves, 2001). Given this, the taxonomy of the Himalayan langur group is in flux and needs to be resolved using a methodical approach for a robust result.

Asian Colobines –

On the basis of molecular data (mitochondrial and nuclear) and fossil data available for the Old World monkeys; the date of dispersal of colobines into Asia, from Africa, is thought to be around 10 million years ago (mya) (Stewart and Disotell, 1998; Raaum et al., 2005). However, these dates are debated with some authors estimating the origin at 15 mya and dispersal into Asia by 11 mya (Delson, 1994). Perelman et al. (2011) estimates the date of origin of colobines to be 12 mya and another study by Pozzi et al. (2014) estimates the origin at 14 mya.

Presently, the colobines are distributed mainly in South and Southeast Asia (SEA). The colobines found in the Indian subcontinent include the Nilgiri langur (*Semnopithecus johnii*), purple face langur (*S. vetulus*) and the Hanuman langur group consisting of Northern plains sacred langur (*S.* entellus), Malabar sacred langur (*S. hypoleucos*), Tufted sacred langur (*S. priam*) and Himalayan langurs (*Semnopithecus* spp.) (Karanth et al., 2010, 2008; Roos et al., 2014). Langurs in the Northeastern states of India and in eastern Bhutan and eastern

Bangladesh comprises of Golden langur (*Trachypithecus geei*) and Capped langur (*T. pileatus*). Some exemplars of langurs that are primarily found in SEA include Phayre's langur (*T. phayrei*), Ebony langur (*T. auratus*), dusky langur (*T. obscurus*), Francois' langur (*T. francoisi*) and silvered langur (*T. cristatus*) (Karanth et al., 2008; Roos et al., 2014).

Classification of many groups of Asian colobines is a matter of debate. There have been many attempts to resolve the taxonomy of different groups of Asian colobines (Sterner et al., 2006; Ting et al., 2008; Perelman et al., 2011; Wang et al., 2012; Finstermeier et al., 2013). One such debate is seen regarding the phylogenetic placement of Capped and Golden langur (CG) lineage. Currently, the Golden langur and Capped langur are classified in the genus *Trachypithecus* (Groves, 2001). Previous studies conducted to resolve this issue have proposed three hypotheses for the phylogenetic placement of the CG lineage; first being ancient hybridisation between *Semnopithecus* and *Trachypithecus* (Karanth et al., 2008; Wang et al., 2015), second hypothesis is incomplete lineage sorting of ancestral mitochondrial haplotypes or Y-chromosomal haplotypes or alternately they also proposed ancient hybridisation between *Semnopithecus* and *Trachypithecus* (Osterholz et al., 2008) and the third being morphological convergence based on paleoclimatic changes (Wangchuk et al., 2008). A recent study tried to resolve this ambiguity using multiple nuclear and mitochondrial markers and a combination of concatenation and coalescent approach (Arekar et al., 2018). However, the ambiguity regarding the phylogenetic placement still remains and need more data to obtain robust results.

Similarly, there was ambiguity in the taxonomy of other colobine species found in the Indian subcontinent. Earlier, the Nilgiri langur of Southern Western ghats in India and the Purple-faced langur of Sri Lanka, were placed in the genus *Trachypithecus*, but later, studies based on molecular data, suggested them to be placed well within *Semnopithecus* clade (Messier and

Stewart, 1997; Zhang and Ryder, 1998; Brandon-Jones et al., 2004; Karanth et al., 2008). Previously, the taxonomy of Hanuman langurs in the Indian subcontinent was very ambiguous with a variety of classification schemes. Most authors considered it as a single species but with as many as 14-16 subspecies (Pocock, 1928, 1939; Ellerman and Morrison-Scott, 1966; Napier and Napier, 1967; Roonwal and Mohnot, 1977; Roonwal, 1984) whereas, few other authors elevated some of these subspecies to species level with 2 (Brandon-Jones, 2004), 4 (Hill, 1939) and 7 (Groves, 2001) species. In this regard, recent studies on Hanuman langur attempted to resolve the ambiguity in their taxonomy by using an integrative approach (Nag et al., 2011, 2014; Ashalakshmi et al., 2014). Their study mainly focused on the langur populations in South India. According to their result, there are 3 species of Hanuman langur in South India (South of the rivers Godavari and Tapti), *S. entellus*, *S. hypoleucos* and *S. priam* (Nag et al., 2011, 2014; Ashalakshmi et al., 2014). The ambiguity in the taxonomy of the langur populations in the Himalaya is yet to be resolved.

1.4 Objectives and Research questions

This study is conducted with the main idea of resolving the taxonomy of the Himalayan langur and establishing the phylogenetic position of this population with respect to the rest of the *Semnopithecus* species in the Indian subcontinent. This study will also help us to solve the final piece of puzzle in the Hanuman langur species complex.

1. **To investigate the Systematics of Himalayan langur using integrative taxonomy**
   - Is the Himalayan population distinct from the langurs of the northern plains?
   - What is the number of morphologically distinct species of langurs in the Himalaya?
   - Does the molecular data support the morphological clustering?

2. **To understand ecological requirements of the langurs of Himalaya through ecological niche modelling (ENM).**
    - Does the distribution of the Himalayan population separate from the langurs of the northern plains?
    - Do the putative species, based on the morphological and molecular axis, show divergence along the ecological axis too?
    - What is the habitat preferred by different species of the Himalayan langur species complex?

3. **Phylogeographic patterns and barriers to dispersal**
    - Are the populations of Himalayan langurs from one slope of the river valley sister to the populations found on the opposite slope of the valley at the same altitude? Or alternatively, are they sister to the populations of langurs found at lower altitude on the same slope of the river valley?
    - Are the populations in adjacent valleys sister to each other?

1.5 Thesis layout

This thesis is organised into 5 Chapters. Chapter 1 details the general introduction and background to this study, the taxonomic complexity of the Himalayan langur population. Chapters 2 – 4 are the work chapters which are presented here as papers. In chapter 2, I have used morphological and molecular data to first understand if the Himalayan langur is a distinct species from the northern plains sacred langur (*S. entellus*); and then to investigate if the Himalayan langur populations corresponds to the three currently recognised species i.e., *S. schistaceus*, *S. ajax* and *S. hector* (Groves, 2001). Chapter 3 deals with the second objective where I examine if the Himalayan langur distribution separate from the distribution of *S. entellus*. Here I have used ecological niche modelling to predict the distribution of these two lineages as well as the distribution of the potential species/subspecies within the Himalayan langur group. In chapter 4, I talk about the genetic variation between different populations within the Himalayan langur group. I also explore whether the deep river valleys and the permafrost snow-capped peaks act as a barrier to gene flow between adjacent populations. Lastly, chapter 5 concludes and summarises the synthesis of my work chapters. I also discuss the conservation implications of the findings of my work.

1.6 Study Area

My study area comprises of a large portion of the Himalaya which includes, Nepal and the Indian states of Jammu and Kashmir (J&K), Himachal Pradesh (H.P.), Uttarakhand and Sikkim (Fig. 1.1). The Himalaya is the highest mountain range in the world which runs from west-northwest to east-southeast in a 2400 km long arc (Wadia, 1931). The Himalaya constitutes three parallel ranges; the Shivalik or lesser Himalaya in the south with an average elevation of 1500 – 2000 m (Kohli, 2002), then comes the lesser Himalaya with elevation ranging from 3700 – 4500 m and the greater Himalaya in the north with elevations above 4500 m. To the north, the Himalaya are bound by the Tibetan plateau and to the south lies the Indo-Gangetic plains (Mani, 1974; Le Fort, 1975).

Geology and Climate

The formation of the Himalayan range was a result of orogeny along the convergent boundary of the Indian plate and the Eurasian plate (Mani, 1974; Valdiya, 1998). The date of this collision is highly debated with proposed ages ranging from ca. 65 million years ago (mya) to 37 mya (Searle and Treloar, 2019). The Himalaya consists mainly of the sedimentary and metamorphic rocks uplifted from an ancient ocean (Searle and Treloar, 2019). The width of the Himalayan range varies from around 350 km in the west to 150 km at its eastern limits (Apollo, 2017; Le Fort, 1975). It is geologically an active range with 5 mm/year increase in the altitude (Searle and Treloar, 2019). The Himalaya contain the third largest deposits of snow and ice after

Antarctica and the Arctic. About 15000 Himalayan glaciers store ~12000 km^3 of freshwater (Cruz et al., 2007). Such a massive reservoir of water gives rise to some of the major perennial rivers of the Himalaya such as Indus, Ganges, Brahmaputra, Sutlej, etc. which are the lifeline of millions of people in south Asia (Cruz et al., 2007).

The Himalaya with its vast size and a complex topography experiences a wide range of climate. Two major influencers of the Himalayan climate are the monsoon and the westerlies (Bohner, 2006; Bookhagen and Burbank, 2010). The westerlies are a major source of moisture for the northwest part of the Himalaya, whereas the monsoon is responsible for more than 80% of the moisture in the south and southeast (Bookhagen and Burbank, 2010; Bolch et al., 2012;). Due to their high altitude, the Himalaya prevent the crossing over of the cold continental air from the north into India and also compels the southwest monsoon to give up most of their moisture before crossing the mountains northward. This results in high precipitation, both in the form of snow and rain, on the south of the mountain range but arid conditions to the north (Bolch et al., 2012). The mean temperatures in the Himalaya fall at the rate of 6 °C for every 1000 m rise in the altitude (Mani, 1981; Romshoo et al., 2018), however, there is a lot of local variation in temperatures within the Himalaya. This results in a variety of climates from nearly tropical climate at the foothills to tundra climate and permanent snow cover at high elevations. The distribution of precipitation is also unequal in the Himalaya, with the east receiving precipitation as high as 3000 mm annually than the west which gets 600 – 1000 mm annual precipitation (Mani, 1981).

Flora and Fauna

Himalaya is one of the 34 globally recognised biodiversity hotspots (Mittermeier et al., 2004). With varied landscapes and soil formations and a variety of climatic conditions, the Himalaya support an array of distinct floral and faunal communities. As mentioned earlier, the elevation zones in the Himalaya extend from tropical (<500m) to alpine (>6000m). The main vegetation regime from low to high elevation comprise of tropical and subtropical rainforests, temperate broadleaf deciduous or mixed forest, temperate coniferous forest and high-altitude steppe and cold desert (Chettri et al., 2008; Sharma et al., 2008). Local differences in the relief and climate as well as the exposure to sunlight and wind, cause substantial variation in the species present in each regime. There are almost 10,000 species of plants with ~3000 being endemic and 1827 animal species with almost 150 – 200 being endemic (Xu et al., 2019). The eastern Himalayan region is richer in biodiversity than the western region.

Fig. 1.1: Digital elevation model (DEM) of the study area. My study area encompasses the following regions; 1 – Jammu and Kashmir, 2 – Himachal Pradesh, 3 – Uttarakhand, 4 – Nepal, 5 – Sikkim.

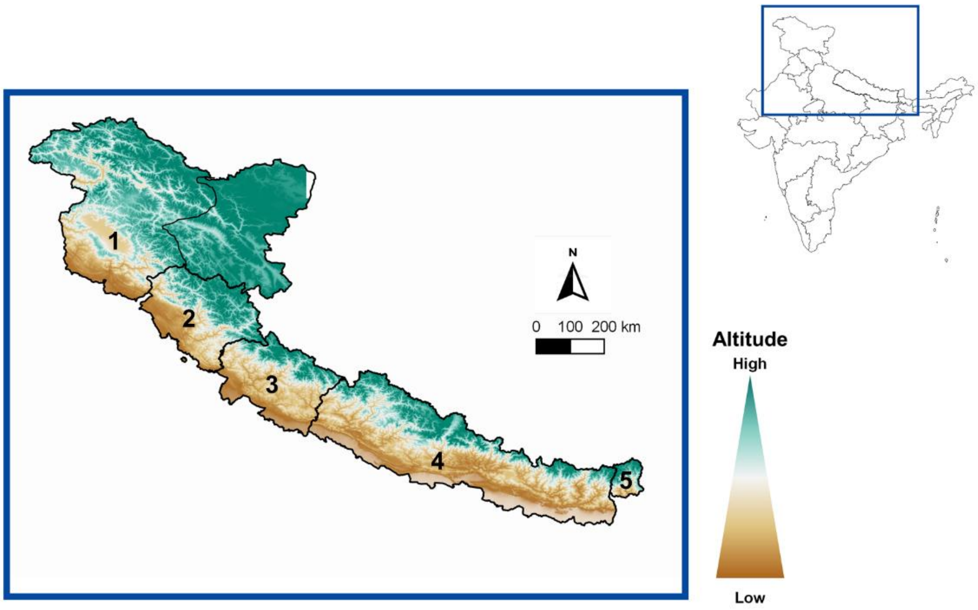

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Plate 1: Himalayan langurs from different regions of the Himalaya from west to east.
a) Dachigam, Jammu and Kashmir; b) Dalhousie, Himachal Pradesh; c) Shimla, Himachal Pradesh; d) Rishikesh, Uttarakhand; e) Munsiyari, Uttarakhand; f) Dadeldhura, Nepal; g) Ramnagar, Nepal; h) Thimphu, Bhutan

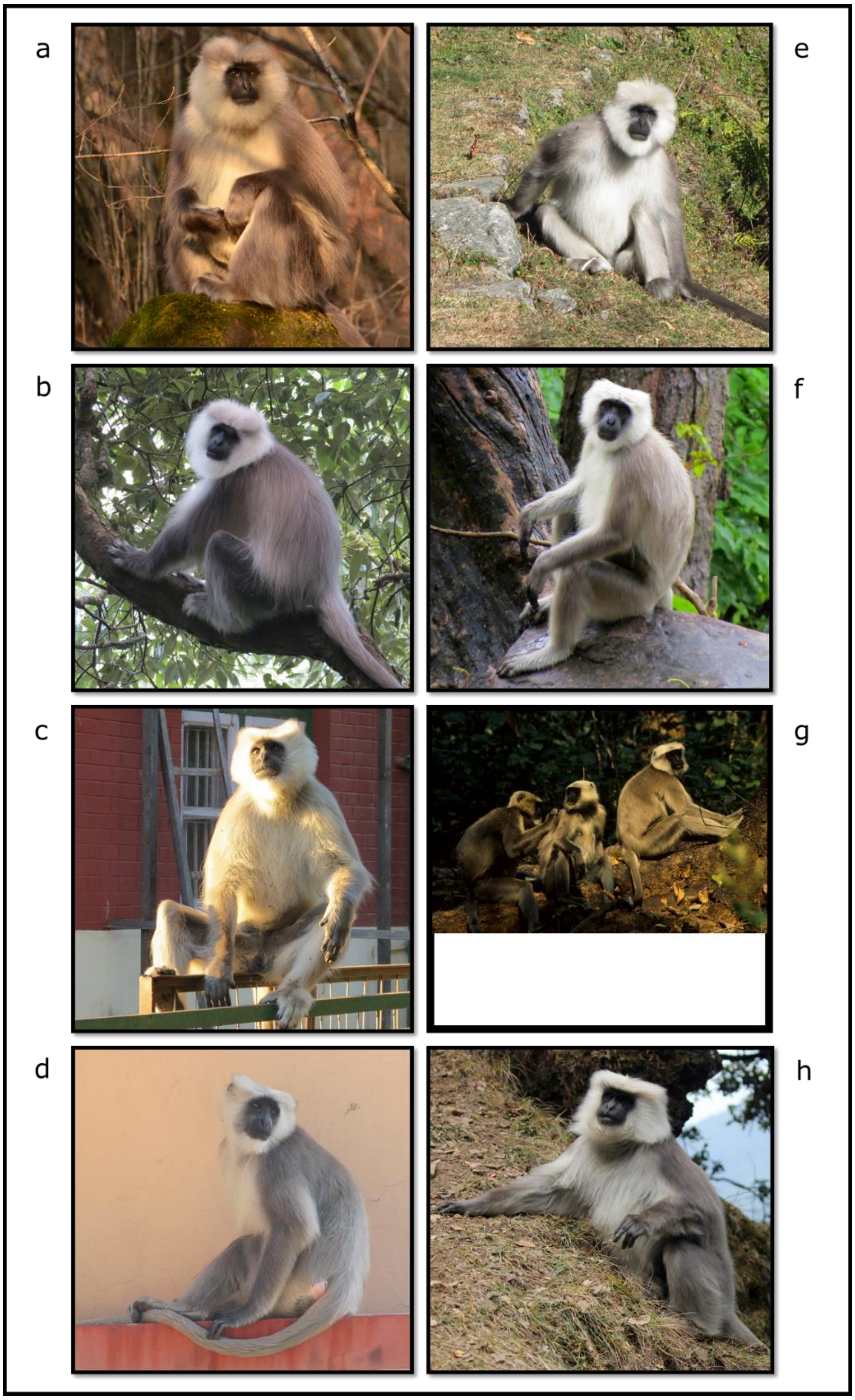

*Photo credits: a) Mehreen Khaleel, f) Amar Kunwar, g) Andreas Koenig, h) Himani Nautiyal

Chapter 2

Systematics of Himalayan langur using integrative taxonomy

2.1 Introduction

Species is the fundamental unit of study in many fields of biology such as systematics, ecology, evolution, behaviour and many more (de Queiroz, 2007). Thus, the first step in these studies is accurate and unambiguous identification of species for which it is imperative to have a well resolved taxonomy for the group of interest. Nevertheless, taxonomy is replete with "problematic groups" wherein species identity and classification remain unresolved. One such group are the colobine monkeys broadly referred to as Hanuman langur or Gray langur or Sacred langur (Groves, 2001) (Genus: *Semnopithecus*, Subfamily: Colobinae). Hanuman langur is a widely distributed primate in the Indian subcontinent (Newton, 1988) which exhibits extensive morphological variation across its range. A multitude of classification schemes have been proposed mostly during early-mid 20th century to resolve the taxonomic status of Hanuman langurs (Pocock, 1928, 1939; Hill, 1939; Ellerman, & Morrison-Scott, 1966; Napier & Napier, 1967; Roonwal & Mohnot, 1977; Roonwal, 1984; Groves, 2001; Brandon-jones, 2004).

Hanuman langurs are broadly divided into two categories; the northern type (NT) which is characterised by a forward looping tail towards the head and the southern type (ST) with the tail looping backwards away from the head (Roonwal, 1979, 1984). The Tapti-Godavari rivers in central India form the borderline with NT distributed to the north and ST distributed to the south of these rivers (Roonwal, 1979, 1984). Recent studies support the splitting of ST Hanuman langur into two species, namely *S. priam* and *S. hypoleucos*, based on an integrative approach wherein multiple lines of evidence from molecular, morphological and ecological data were used (Nag et al., 2011, 2014; Ashalakshmi et al., 2014). Similarly, genetic and morphological data suggest that the plains population of NT Hanuman langur is a separate species, *S. entellus* (Karanth et al., 2010). However, taxonomy of the Himalayan population (hereafter Himalayan langur) is still unresolved. In order to understand the exact number of species of Hanuman langur in the Indian subcontinent, it is important to resolve the taxonomy of all the populations of Hanuman langur.

Himalayan langur is the northernmost population of Hanuman langur (Sugiyama, 1976) distributed in the Himalayan region of India, Nepal and parts of Pakistan and Bhutan (Blanford, 1888; Pocock, 1939). Altitudinal range extends from the Himalayan foothills up to 4270m asl (above sea level) (Bishop, 1977). These are one of the few colobine monkeys living in temperate climate (Nijman, 2013) while the rest are distributed predominantly in tropical regions (Bishop, 1979). Himalayan langurs are classified in the NT category based on the tail loop character (Roonwal, 1979, 1984). Morphologically, the Himalayan langur can be distinguished from the plains population (*S. entellus*) by the tail carriage pattern (discussed in methods section; Fig. 2.1) and by their pelage – the langurs from the Himalaya have a bushy white head which is very distinct from the darker grey-brown body (Fig. 2.2) (Bishop, 1979). Many authors have also talked about differences in the behavioural characters between Himalayan langurs and their conspecifics in the plains, for example reduction in vocalisation profile such as whoop vocalisation and canine grinding (Bishop, 1979; Dolhinow, 1978; Sugiyama, 1976) (Sugiyama 1976; Bishop, 1979); and reduction in use of certain aspects of sexual behaviours like reduction in use of female headshake to initiate sex (Bishop, 1979). These behavioural differences could possibly indicate that Himalayan langurs and *S. entellus* are distinct lineages.

A variety of classification schemes have been proposed to resolve the taxonomy of the Himalayan langurs (Table 2.1). One of the earliest comprehensive classifications of Indian colobines was by Pocock (1928). He assigned the Himalayan langurs to five subspecies *schistaceus*, *ajax*, *achilles*, *lanius* and *hector* under the species *Pithecus entellus*. Later Pocock (1939) renamed *Pithecus* as *Semnopithecus* with three subspecies under it; *schistaceus, ajax* and *achilles*. The subspecies *lanius* and *hector* from Pocock's (1928) earlier classification were not included here. Hill (1939) considered the Himalayan langur to be a single species *S. schistaceus* with five subspecies *hector*, *lanius, achilles*, *schistaceus* and *ajax*.

Subsequent classification schemes (Ellerman, & Morrison-Scott, 1966; Napier, & Napier, 1967; Roonwal, & Mohnot, 1977; Roonwal, 1984) synonymised *Semnopithecus* with *Presbytis* and subsumed all the Himalayan species into a single species *P. entellus* along with the subspecies *entellus* from the northern plains. The subspecies *lanius* (Hill, 1939) was changed to *lania* and the subspecies *hector* (Hill, 1939) was removed.

Later, Groves (2001) reverted back to using *Semnopithecus* for Hanuman langurs and recognized three species of Himalayan langur *S. schistaceus*, *S. ajax* and *S. hector*. He elevated the three subspecies from previous classification schemes to species level. Lastly, Brandon-Jones (2004) included all the Himalayan species as subspecies of *S. entellus*, except for *achilles* and *lanius* (Hill, 1939) which he did not include in the classification.

Thus, the Himalayan langur has a convoluted taxonomic history falling into three broad groups of taxonomic schemes (TS). TS1 – Various populations of Himalayan langurs are placed in multiple subspecies under either *Pi. entellus*, *S. entellus* or *P. entellus* (Pocock, 1928, 1939; Ellerman, and Morrison-Scott, 1966; Napier, and Napier, 1967; Roonwal, and Mohnot, 1977; Roonwal, 1984; Brandon-Jones et al., 2004). TS2 – Himalayan langur are considered a distinct species itself with multiple subspecies (Hill, 1939). TS3 – Himalayan langurs are split into multiple species (Groves, 2001).

With the advent of molecular techniques many recent studies have used genetic data to resolve taxonomic ambiguities in primates of the Indian subcontinent (Chakraborty et al., 2007; Karanth et al., 2010, 2008; Osterholz et al., 2008; Wangchuk et al., 2008; Ashalakshmi et al., 2014; Arekar et al., 2019). However, the use of molecular data does not guarantee a robust description and identification (Will et al., 2005). Molecular data is often considered as another singular data type, such as morphological data, which can be used as a line of evidence to characterise and describe species. In order to achieve a robust delineation of species, we need to integrate methods from different discipline (Dayrat, 2005).

Given this background, I have used morphological and molecular data to understand if the Himalayan langurs are a distinct species from *S. entellus* and does the Himalayan langurs comprise of multiple species/subspecies.

2.2 Methods

2.2.1 Molecular data

Data collection

I conducted field work in four Himalayan states of India – Jammu and Kashmir (J&K), Himachal Pradesh (HP), Uttarakhand and Sikkim. These states were chosen for field work based on distribution records from past studies (Hill, 1939; Pocock, 1939; Sugiyama, 1976; Bishop, 1979; Choudhury, 2001) as well as from IUCN website (www.iucnredlist.org). Here I included the field data as well as published sequences from older studies (Karanth et al., 2010; Ashalakshmi et al., 2014; Khanal et al., 2018).

I collected 176 faecal samples from 46 locations (Fig. 2.3 A, Table 2.4) across the distribution range of Himalayan langur in India with multiple samples collected from each location. Additionally, five faecal samples of *S. entellus* were collected from one location in the northern plains (22.88220N, 88.39970E). Out of these, I used 26 samples for the mitochondrial dataset and six samples for the nuclear dataset, for the molecular phylogenetic analysis. Fresh faecal samples were collected by following the troops in the morning and the evening hours. Samples were collected by two different methods – First, as described in Kawamoto et al. (2013), a sterile cotton swab was rolled multiple times over the surface of the faeces and thoroughly rinsed in the lysis buffer (White & Densmore, 1992). The second method involved collection of the whole faeces which was then stored in absolute alcohol. These samples were stored at -20 °C in the laboratory until DNA extraction. Samples stored in lysis buffer were first treated with starch to remove potential PCR inhibitors like bilirubin and bile salts (Zhang et al., 2006; Kawamoto et al., 2013) and then DNA was extracted by using *Wizard® SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA* and stored in pure nuclease free water at -20 °C until further use. DNA from whole faecal samples was extracted using the commercially available QIAamp DNA stool mini kit (QIAGEN Inc.), following the manufacturer's protocol with slight modifications as mentioned in Mondol et al. (2009), however, I did not add the carrier RNA (Poly A) (Kishore et al., 2006). Each extraction had a negative control to monitor contamination. The quantity of extracted DNA was measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc.).

PCR amplification and sequencing

A 775 bp (i.e. amplicon length) region of mitochondrial cytochrome *b* (Cyt-*b*) gene was PCR amplified using the primer pair Cytb_278F (5' – GCCTATTTCTACACGTAGGCCG – 3') and Cytb_1052R (5'– CCAATTGCAATGAAGGGTTGGT – 3'). A 25 µl of reaction was set with standard 1X reaction buffer premixed with 1.5 mM $MgCl_2$ (New England BioLabs® Inc.), 0.25mM of dNTPs (Bangalore Genei, Bangalore), 0.3 µM of each primer (Sigma-Aldrich Inc.), 1.5U Taq polymerase (New England BioLabs® Inc.) and 2 µl of template DNA (DNA concentration between samples varied from 20 ng/µl to 80 ng/µl). I also added 2 µl of BSA (Bovine Serum Albumin, Fisher Scientific) to augment the PCR reaction. Further, the template DNA was diluted to 1:5 (DNA extract : water) ratio and used for the reaction in order to reduce the amount of PCR inhibitors. The PCR cycling conditions were carried out with initial denaturation at 94 °C for 5 mins followed by 50 cycles with denaturation at 94 °C for 40 sec, annealing at 57.5 °C for 30 sec and elongation at 72 °C for 30 sec and final extension at 72 °C for 10 mins. Additionally, I also PCR amplified 633 bp of a partial nuclear X-chromosomal region (X2) from five Himalayan langur samples and one *S. entellus* using the following primer pair X61_F (5' – TCTTGTGACCTGATGCCAAATCTA – 3') and X61_R (5' – GCTTGGAACAAATGAACATTCCTG – 3') (Peng et al., 2009). A 25 µl of PCR reaction was set with 0.5 µM of each primer (Sigma-Aldrich Inc.), concentrations of all the other reagents were as described above. A touch-down PCR reaction was set with initial denaturation at 94 °C for 3 mins followed by 10 touch-down cycles starting at 60 °C with decrease of 1 °C in annealing temperature per cycle and extension at 72 °C for 60 sec; this was followed by 50 repetitions of regular PCR cycle with denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec and elongation at 72 °C for 60 sec; the final extension was set to 72 °C for 4 mins. All the PCR products were outsourced for purification and sequencing to Medauxin, Bangalore.

Phylogenetic analysis

The sequence files obtained were viewed and edited manually in ChromasLite v2.01 (Technelysium Pty. Ltd.). Sequences of Himalayan langur as well as *S. entellus* were also downloaded from previous molecular studies (Table 2.5) (Ashalakshmi et al., 2014; Khanal et al., 2018). The sequences were aligned using MUSCLE algorithm (Edgar, 2004) incorporated in MEGA v7 (Kumar et al., 2016).

I used jModelTest 2.1.3 (Darriba et al., 2012) to pick the best model of sequence evolution. Phylogenetic reconstruction was performed using Maximum Likelihood (ML) and Bayesian methods. ML analysis was performed in RAxML7.4.2 incorporated in raxmlGUI v1.3 (Stamatakis, 2006). I used GTR+G model in RAxML as there is no provision to use other models. 1000 replicates were performed to assess support for different nodes. I used MrBayes 3.2.2 (Ronquist et al., 2012) to perform the Bayesian analysis with HKY+G nucleotide substitution model. Two parallel runs with four chains each were run for 10 million generations with sampling frequency every 1000 generations. Convergence between the two runs was determined based on standard deviation of split frequencies. The program Tracer v1.6 (Rambaut et al., 2013) was used to determine stationarity, an effective sample size (ESS) value of >200 for each parameter was used as a cut-off for run length. The first 25% of trees were discarded as burn-in.

Hypothesis testing

I compared the Bayesian tree (Fig. 2.4), with an *a priori* hypothesis where the phylogeny was constrained to be consistent with Groves' (2001) three species of Himalayan langurs; *S. ajax*, *S. hector* and *S. schistaceus*. RAxML7.4.2 incorporated in raxmlGUI v1.3 (Stamatakis, 2006) was used to generate a constraint tree. The likelihood of the constraint tree was then compared with the best tree using the SH test (Shimodaira and Hasegawa, 1999) and AU test (Shimodaira, 2002) in PAUP* Version 4.0a (build 164) (Swofford, 2001). For both the SH and AU test, I performed 10000 bootstrap replicates and the nonparametric bootstrap with reestimated log likelihoods (RELL) approximation (Kishino et al., 1990) was used for resampling the loglikelihoods.

2.2.2 Morphological data

Data collection

Morphological data was collected by direct observations during field survey as well as from photographs. I used a total of 85 samples for the morphological analysis – 35 samples for Himalayan langur, 28 samples for *S. entellus* and 22 samples of *S. hypoleucos*. For the Himalayan langur, out of the 35 samples, 7 were from Nepal (photographs provided by Prof. Naomi Bishop, Prof. John Bishop, Prof. Andreas Koenig, Prof. Carola Borries, Mr. Ganga Ram Regmi, Mr. Sagar Dahal, Ms. Mehreen Khaleel), 1 was from Bhutan (Photograph provided by Ms. Himani Nautiyal) and the remaining 27 were from the Indian Himalayan region (collected for this study). Samples for *S. entellus* and *S. hypoleucos* were obtained from a previous study (Nag et al., 2011). Based on past studies (Nag et al., 2011) and my field observations, six color-independent morphological characters were used to differentiate between *S. entellus* and the Himalayan langur. These included the four characters described by Nag et al. (2011) and two characters unique to Himalayan langurs. Characters specific to Himalayan langur included tail carriage (Roonwal, 1984, 1979) and demarcation between the head and the body (HBC) (Hill, 1939; Pocock, 1939; Bishop, 1979; Groves, 2001). Within the NT langurs, two forms of tail carriage are observed, in *S. entellus* from the northern plains the tail loops over the back and the tip of the tail hangs perpendicular to the ground, here onwards TC3 (Fig. 2.1b), whereas, in the Himalayan populations the tail loops well behind the back and the tip ends above the base of the tail, here onwards TC4 (Fig. 2.1a). I coded them TC3 and TC4 to be consistent with Nag et al.’s (2011) coding system. The tail carriage pattern was recorded when the individual was walking on a flat surface and not while climbing up or down the hill and nor while it was running or standing (as per Roonwal, 1984). The Himalayan langur has a distinct demarcation between the head and the body; the head is bushy and white coloured distinct from the grey-brown body (Fig. 2.2a) (Hill, 1939; Pocock, 1939; Bishop, 1979; Groves, 2001). Langurs from the plains (*S. entellus*) have a uniform colour without much of a distinction/contrast between the head and the body (Fig. 2.2b). I call this character Head-Body contrast (HBC) character. Apart from these two characters I also used the four characters described in Nag et al. (2011) i.e. presence or absence of crest, presence or absence of streak between the eye and the ear, Northern or Southern type tail loop (TL) and the extent of blackness (EOB) on the hand; these characters are not seen in Himalayan langurs and therefore were coded '0'(absent). I coded TL as 1 to indicate that Himalayan langurs belong to NT langur group with forward looping tail along with *S. entellus*. I recorded these characters by direct observations using 10 X 50 binoculars (Olympus) and through photographs taken from a digital camera (Canon PowerShot SX20 IS). These characters were scored for multiple adult individuals per location. Furthermore, additional data points were obtained from literature records (Roonwal, 1984, 1981). The point coordinates for these data points were extracted from google maps using the names of the locations provided by Roonwal (1981, 1984) (Fig. 2.3B, Table 2.6).

Analysis

I typed 85 adult individuals from 82 locations (Table 2.6). I also included one of the southern species from this complex, *S. hypoleucos* (St1 and St2 morphotypes in Nag et al., 2011) in the analysis. The characters were coded as described in Table 2.2. All the characters were coded in a way that they are consistent with Nag et al.'s (2011) coding system. The codes for *S. hypoleucos* were similar to ones used in Nag et al. (2011).

I prepared a character matrix with the terminal taxa in the rows and the six morphological characters in the columns. Using this matrix, first the mean character difference was calculated between the individuals in PAUP* Version 4.0a (build 164) (Swofford, 2001). Then a Neighbour Joining (NJ) tree was built using these distances, with the default settings. Mid-point rooting was used to root the tree.

2.3 Results

Phylogenetic analysis

I generated 76 Cyt-*b* sequences, many of these sequences were identical falling in 11 unique haplotypes. To reduce the size of the data set and to keep the numbers of identical sequences low, I used exemplar sequences from each location for the phylogenetic analysis, bringing the total to 26 sequences from India (it includes all the Haplotypes). The final alignment contained 746 bp of Cyt-*b* sequence from 57 samples (Table 2.5). It includes 26 samples sequenced in this study, 30 sequences downloaded from previous studies and 1 *S. hypoleucos* sequence used as outgroup (Table 2.5). For nuclear dataset, partial X61 sequence was successfully amplified from five Himalayan langur and one *S. entellus* sample (Table 2.5). Sequences generated in this study have been deposited in GenBank, Accession numbers MT919045 – MT919070.

The mitochondrial (Fig. 2.4) as well as nuclear (Fig. 2.5) phylogenies both showed that the Himalayan langurs and *S. entellus* form reciprocally monophyletic group. In the mitochondrial dataset, both the Bayesian and ML analysis recovered two major clades, the *S. entellus* clade – containing the sequences from the northern plains; and the Himalayan clade – containing sequences from the Himalayan region (Fig. 2.4). Within the Himalayan clade was a well-supported subclade consisting of haplotypes from the western Himalaya (west from 28.07N – 83.26E, which was nested within samples from eastern Himalaya (east from 28.19N – 83.65E). Both, the Bayesian and ML trees, showed similar topology where in all the major clades were retrieved. Furthermore, in the Bayesian tree (Fig. 2.4), two samples i.e.128_Nepal and 129_Nepal were placed within the clade containing samples from the Indian Himalayan Region (IHR), whereas in the ML tree (Appendix 1), these two samples were sister to the above-mentioned clade.

Hypothesis testing

The likelihood score of the best tree was significantly higher than that of the constraint tree for both SH test and AU tests. Therefore, these trees based on the molecular data did not support the splitting of Himalayan langurs into three species (Table 2.3).

Morphological data

Our final NJ tree (Fig. 2.6) was based on morphological data collected from 64 adult individuals from 60 localities. The NJ tree retrieved a distinct cluster consisting of Himalayan samples that was sister to *S. entellus*. The UPGMA method also generated a similar topology.

2.4 Discussion

The so-called Hanuman langur has been known to be a species complex since a long time. Nevertheless, recent studies have brought some clarity to their confused taxonomy. These studies suggest that the so-called Hanuman langur consists of at least three species: *S. entellus*, distributed in the plains of North India (Karanth et al., 2010), *S. hypoleucos* distributed in peninsular India and *S. priam* distributed in peninsular India and Sri Lanka (Nag et al., 2011, 2014; Ashalakshmi et al., 2014). However, the taxonomic status of the Himalayan population of this complex remained unresolved. Here I address this issue by applying multiple lines of evidence to resolve the taxonomic status of the Himalayan langur.

Phylogenetic species concept (PSC) II (Donoghue, 1985; Mishler, 1985; de Queiroz, 1998) identifies species as monophyletic groups on the basis of shared derived characters. My analysis using the genetic data shows that Himalayan langurs and *S. entellus* are reciprocally monophyletic. Results from the morphological analysis suggests that the Himalayan langur is a separate species as per the Phenetic Species Concept (Sokal and Crovello, 1970).

Among the plethora of classification schemes only Hill (1939) placed the Himalayan langurs in a separate species, *S. schistaceus*, with multiple subspecies – *ajax*, *achilles*, *lanius* and *hector* (Table 2.1). My study supports this classification, however there is no support for further splitting this species into multiple subspecies/species (see result under hypotheses testing). Later, Groves (2001), elevated *ajax* and *hector* to species level and subsumed *achilles* and *lanius* into *schistaceus* (Table 2.1). However, the molecular and morphological analysis in this study shows the Himalayan langurs to be monophyletic. In the molecular phylogeny, within the Himalayan clade, a well-supported western clade can be seen nested within the samples from eastern Himalaya (Fig. 2.4). The western clade containing sequences from J & K to western Nepal whereas, sequences from Central Nepal to Sikkim constitutes the eastern Himalayan samples. This suggest that there could be ta distinct monophyletic lineage within the Himalayan clade. However, this lineage does not match the distribution of any subspecies or species described by Hill (1939) and Groves (2001).

Other classification schemes (Pocock, 1928; Pocock, 1939; Roonwal and Mohnot, 1977; Roonwal, 1984; Brandon-Jones, 2004) have placed the Himalayan langurs as a subspecies of *S. entellus* (TS1, Table 2.1). Pocock (1928) proposed five subspecies within the Himalayan langur lineage – *schistaceus*, *ajax*, *achilles*, *lanius* and *hector*. Later (Pocock, 1939) he described only three subspecies – *schistaceus*, *ajax* and *achilles* and removed *lanius* and *hector*.

Roonwal and Mohnot (1977) and Roonwal (1984) described four subspecies – *schistaceus*, *ajax*, *achilles* and *lania* (they renamed *lanius* as *lania*). And finally, Brandon-Jones (2004) described three subspecies – *schistaceus*, *ajax* and *hector*. My analyses do not confirm to the above-mentioned classifications.

In the morphological (Fig. 2.6) as well as the molecular (Fig. 2.4) tree, the *entellus* clade is monophyletic and corresponds to Hill's (1939) and Groves' (2001) *Semnopithecus entellus*. Hill and Groves elevated the subspecies *Semnopithecus entellus entellus* to *S. entellus*. Recent molecular studies too suggest assigning species status to *S. entellus* (Karanth et al., 2010; Ashalakshmi et al., 2014).

Brandon-Jones (2004) had split the langurs from the plains into two subspecies – *Semnopithecus entellus achates* distributed in Gujarat, Madhya Pradesh, southern Rajasthan, western Maharashtra, western Andhra Pradesh, southern Karnataka, northern Chhattisgarh and probably Uttar Pradesh; and *Semnopithecus entellus entellus* distributed in southern West Bengal, southern Bihar, Jharkhand, north-eastern Maharashtra, southern Chhatisgarh, Orrisa and probably an introduced population in western Bangladesh. In this study, the sequences from Kolkata form a separate clade which is sister to the rest of the *S. entellus* sequences (Fig. 2.4). These sequences are geographically distributed within the range of *S. e. entellus* described by Brandon-Jones. However, the support for this node is very poor, suggesting that this relationship might change with addition of more markers and samples. Also, the morphological analysis does not show any difference between these two lineages. Additionally, see Nag et al. (2011) for discussion on Brandon-Jones' classification of the southern type Hanuman langur.

Two of the characters used in this study – HBC and forms of tail carriage (TC3 and TC4), can be used as field identification characters for differentiating the Himalayan langur and *S. entellus*. Ido not recommend the use any of the external morphological characters listed in the earlier classification schemes for distinguishing between different subspecies of Himalayan langurs. The morphological characters used in earlier classification schemes to describe these subspecies, are highly plastic, variable, and subjective in nature (Nag et al. 2011). For e.g., Pocock (1939) in the identification key, describes *schistaceus* as follows "General colour paler, salty or greyish-buff; coat shorter and less woolly" and for *achilles* he writes, "General colour dark earthy brown; coat thick and woolly". Hill (1939) describes *schistaceus* as "A slatey-grey race, with shorter, less woolly coat than those found at higher altitudes". These characters tend to differ based on what month of the year the langur is being observed and what is the altitude at that location. Roonwal (1981) describes four types of tail carriage in the NT langurs, however I recorded only two tail carriage types that are used in this study, the other two tail carriage types have not been observed.

This study, using morphological and molecular data, comprehensively illustrate that the Himalayan langur and *S. entellus* are distinct lineages. However, it does not show any support for presence of multiple species/subspecies within the Himalayan langur complex. In the next chapter, I have used a third line of evidence i.e. ecological niche modelling, to substantiate the findings from this chapter.

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2.6 Figures

Fig. 2.1: Tail carriage pattern in the Himalayan langur (a) and *Semnopithecus entellus* (b).

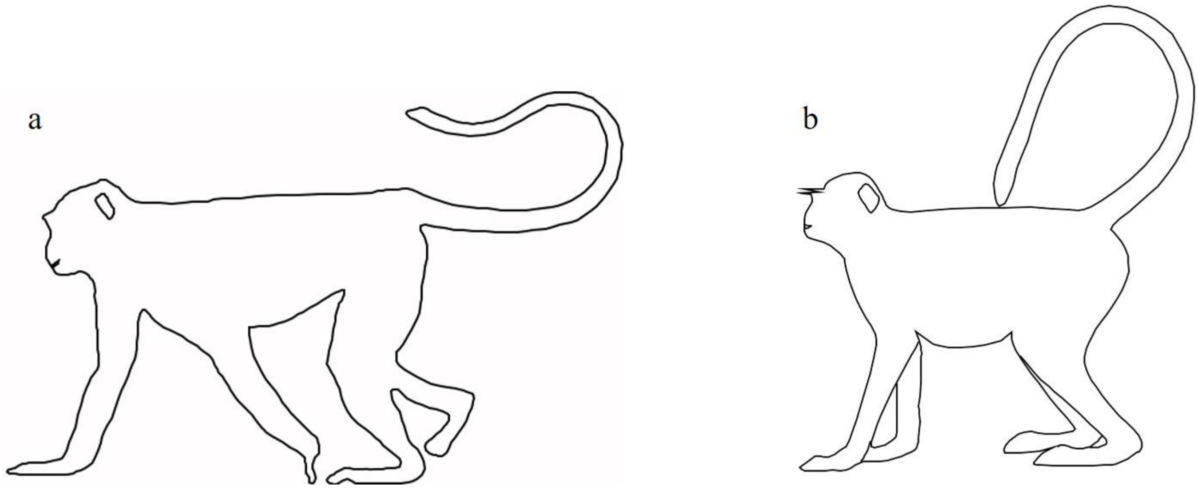


Fig. 2.2: Contrast between the head and the dorsal region of the body in the Himalayan langur (a) and *Semnopithecus entellus* (b).

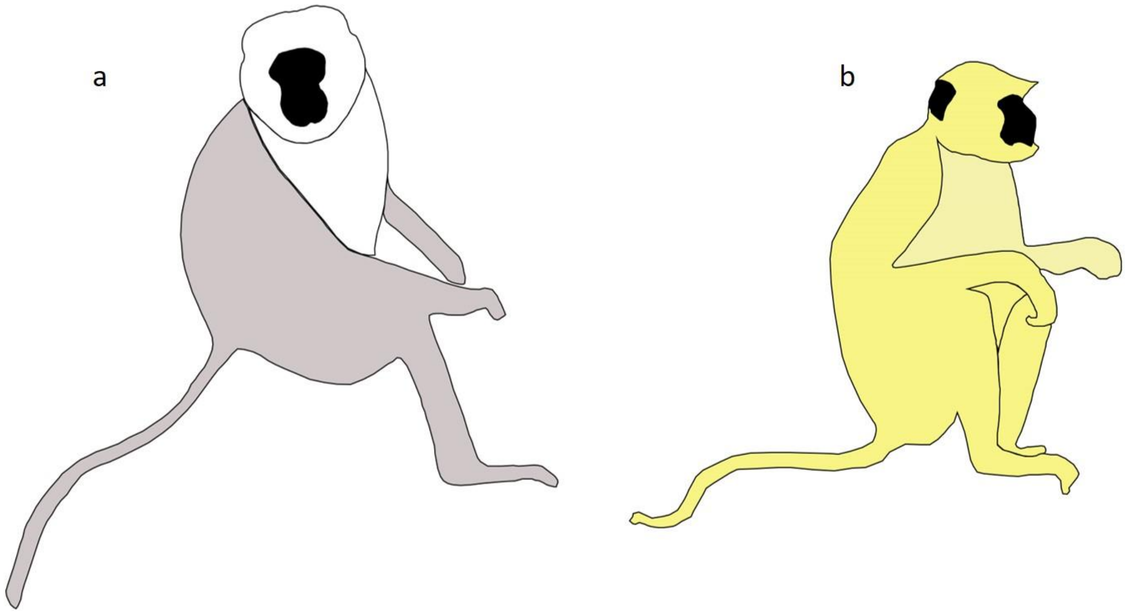


Fig. 2.3: Locations of samples used in molecular (A) and morphological (B) analyses.

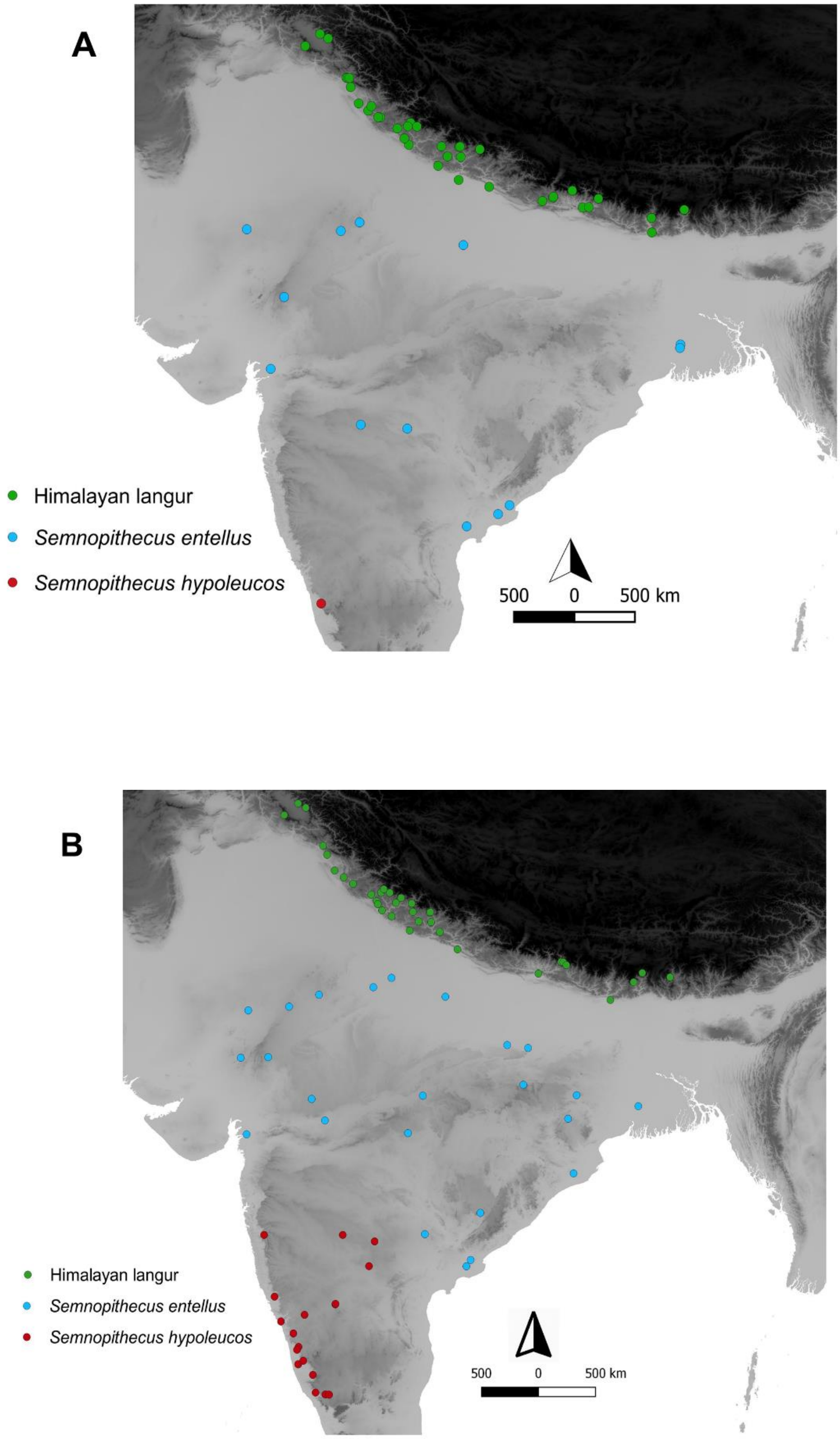


Fig. 2.4: Bayesian tree of Himalayan langurs based on the mitochondrial cytochrome *b* (Cyt-b) gene. The numbers at the nodes above the branches indicate Bayesian posterior probability (BPP) values whereas numbers below the branches indicate Maximum likelihood bootstrap support (MLBS) support. Support values are shown only for main nodes. * indicates BPP <0.85 and MLBS <85; - indicates MLBS values not available for that node. HP = Himachal Pradesh; JnK = Jammu & Kashmir; Uk = Uttarakhand.

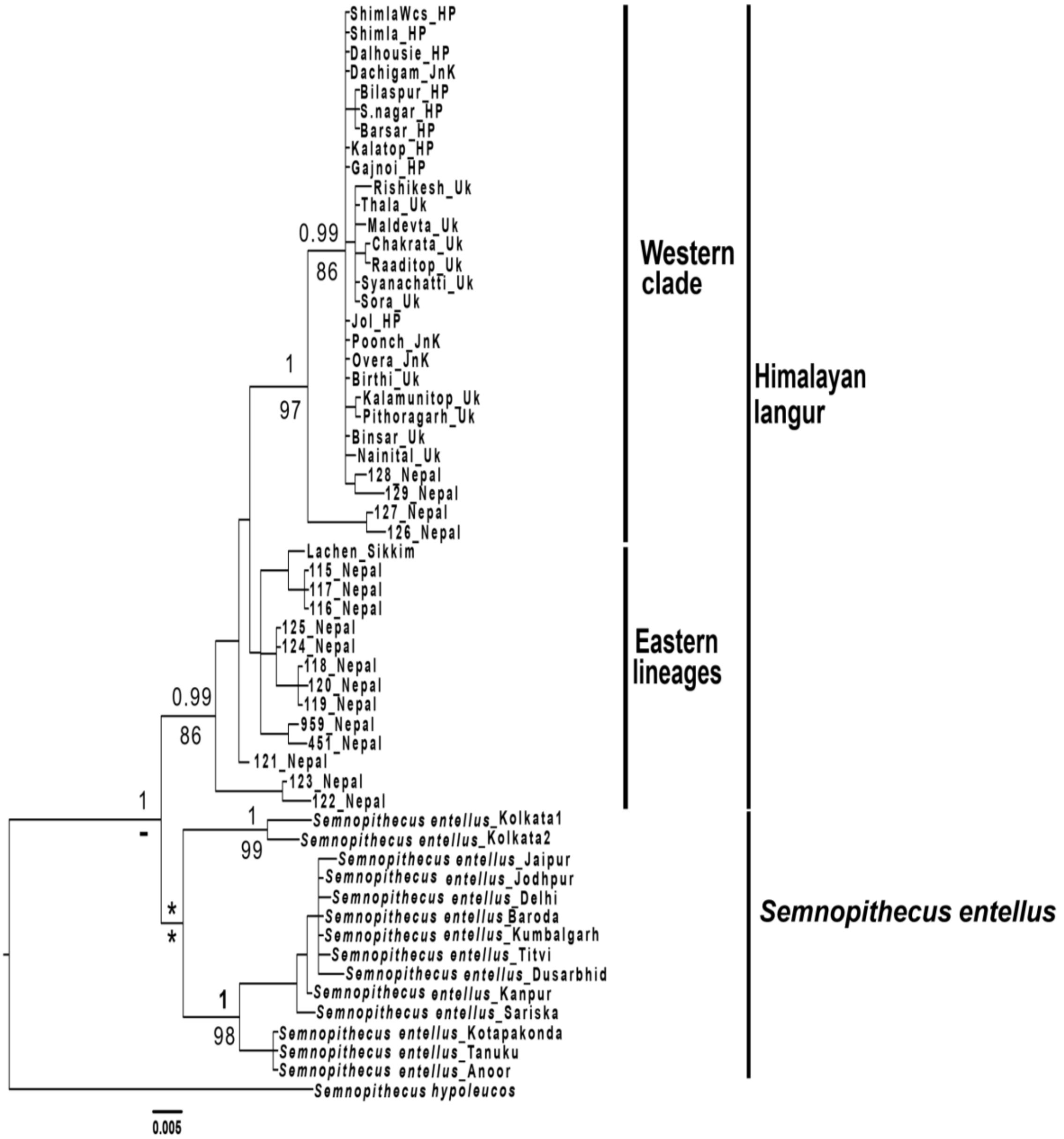


Fig. 2.5: Maximum likelihood (ML) cladogram of the Himalayan langurs for nuclear X2 gene. Numbers at the node indicated ML bootstrap support. Only the node support values >75 are shown, Bayesian phylogeny showed posterior probability values of < 0.75 for all the nodes. SE – *Semnopithecus entellus*; HyL – Himalayan langur

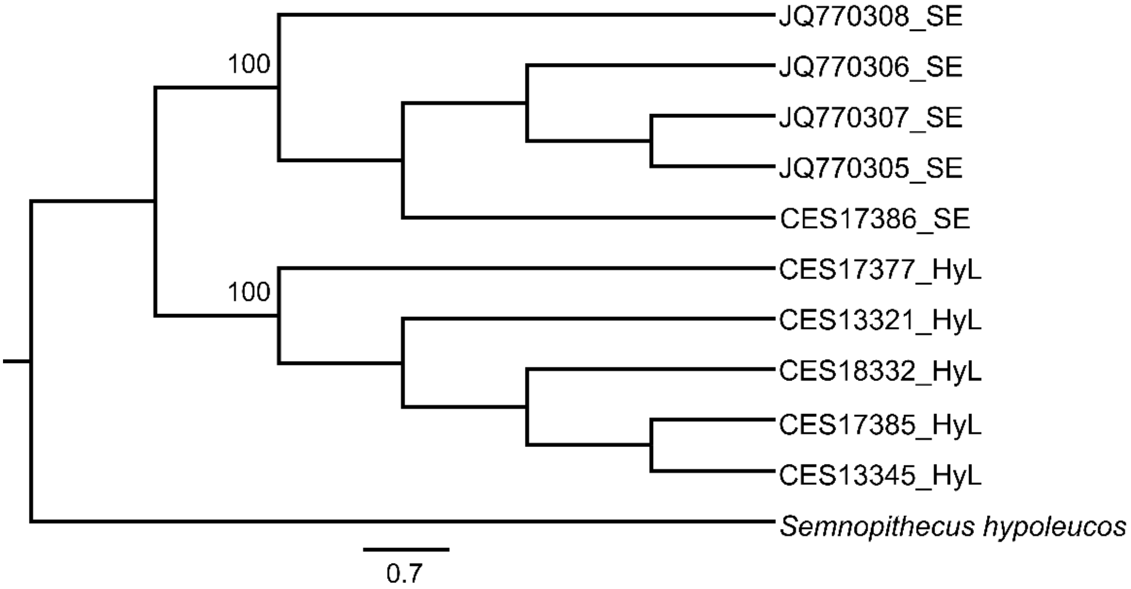
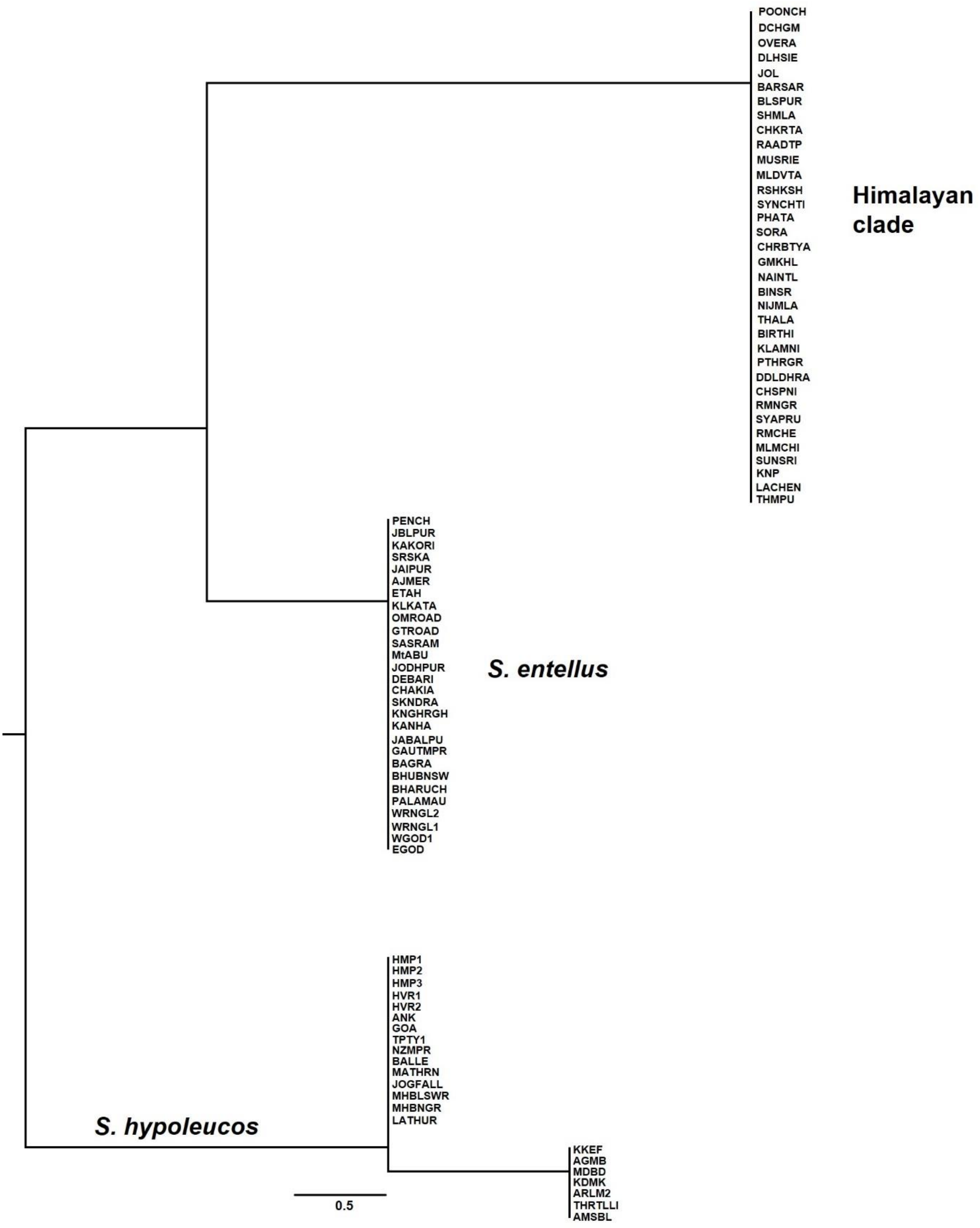


Fig. 2.6: Neighbour-Joining tree based on 6 colour independent characters. *S. hypoleucos* is one of the two species of Hanuman langur from peninsular India



2.7 Tables

Table 2.1: Different Taxonomic Schemes (TS) for Himalayan langur proposed by various Authors

	TS 1					**TS 2**	**TS 3**
Scientific names	***a***	***b***	***c***	†***d***	***e***	***f***	***g***
Pi. e. schistaceus	X						
Pi. e. ajax	X						
Pi. e. achilles	X						
Pi. e. lanius	X						
Pi. e. hector	X						
S. e. schistaceus		X			X		
S. e. ajax		X			X		
S. e. achilles		X			X		
S. e. hector					X		
P. e. schistaceus			X	X			
P. e. ajax			X	X			
P. e. achilles			X	X			
P. e. lania			X	X			
S. schistaceus						X	
S. s. ajax						X	
S. s. achilles						X	
S. s. lanius						X	
S. s. hector						X	
S. schistaceus							X
S. ajax							X
S. hector							X

*Also includes Ellerman and Morison-Scott (1966) and Napier and Napier (1967)
Pi = *Pithecus*; *S* = *Semnopithecus*; *P* = *Presbytis*; *e.* = *entellus*

Table 2.2: Coding system used for the 6 morphological characters used in this study. The coding system is designed to be consistent with Nag et al. (2011), unless mentioned otherwise.

	Characters					
	Crest	Streak	EOB	Tail loop (TL)	Tail carriage (TC)	HBC
Himalayan langur	0	0	0	1	3	1
*Semnopithecus entellus**	0	0	3	1	0	0
Semnopithecus hypoleucos	0	1	3/4†	2	1	0
*Semnopithecus priam***	1	0	1	2	2	0

**S. entellus* = Nt from Nag et al. (2011).

†*S. hypoleucos* contains two morphotypes (St1/St2; Nag et al., 2011).

** I did not use this species in the analysis.

EOB – Extent of blackness; HBC – Head-Body contrast

Crest, Streak and HBC, 0 = absent, 1 = present; TL - 1 = Northern type, 2 = Southern type; EOB - not visible = 0, till finger tips = 1, till knuckles = 2, till wrist = 3, till elbow = 4; TC = tail carriage (Fig. 1), TC3 = 0, TC4 = 3, TC1 (Fig. 3c in Nag et al., 2011) = 1

Table 2.3: Topology test results

Tree	-ln L	Diff -ln L	SH	AU	p-value
Best tree	2107.65046	(best)			
Constrained tree	2371.94530	264.29484	0.0000*	~0*	*P < 0.05

Table 2.4: Locations of all the fecal samples collected for the study.

Latitude	Longitude	Elevation	No. of samples collected
33.68274	74.44421	3068	3
34.07608	74.56408	1796	6
34.07115	74.59235	2010	5
34.12110	74.99444	2087	7
33.95963	75.29607	2200	4
32.53807	75.95441	1817	1
32.53807	75.96306	1960	3
32.53407	75.97314	2031	4
32.53180	76.01758	2489	1
32.53511	76.04634	2269	6
32.52777	76.08361	1622	9
32.19456	76.13210	697	3
31.60419	76.43049	749	4
31.34576	76.78393	1025	3
31.34579	76.78499	993	1
31.50311	76.90276	1120	5
31.10310	77.15263	2091	3
31.10350	77.15423	2088	2
31.10409	77.15710	2106	5
31.10799	77.16646	2161	2
31.10026	77.23568	2321	1

30.68908	77.87001	2094	1
30.70000	77.87174	2041	5
30.46354	78.06320	2000	3
30.45103	78.08196	1831	2
30.33809	78.12877	764	5
30.77358	78.25728	2217	4
30.10681	78.29535	352	4
30.12418	78.31133	354	2
30.90474	78.36508	2012	5
30.05882	78.51107	593	1
30.76847	78.59856	1521	6
30.02100	78.63456	550	2
29.88449	78.67303	1550	3
30.38834	78.83901	2100	4
30.57578	79.04742	1587	6
29.31781	79.34721	619	1
29.34665	79.38558	1164	6
30.36582	79.46301	1476	4
30.05083	79.50986	1698	7
29.68749	79.73640	2004	6
30.03160	80.16652	1873	3
30.04284	80.19897	2609	5
29.66763	80.23032	1205	7
27.41350	88.19775	2129	1
27.75934	88.53851	2808	4

Table 2.5: List of samples with their accession numbers, Sample IDs and location coordinates. We used these samples to construct the phylogeny shown in Fig. 2.4. Names in the first column matches the tip names in Fig. 2.4.

Samples	**Sample-ID**	**Latitude**	**Longitude**	**Accession Number**	**Sample type**
Himalayan langur (samples from India)					
ShimlaWcs_HP[#]	CES15308	31.10026	77.23568	MT919045	Fecal
Shimla_HP[#]	CES13325	31.10409	77.1571	MT919046	Fecal
Dalhousie_HP[#]	CES15324	32.53407	75.97314	MT919047	Fecal
Dachigam_JnK[#]	CES13345	34.1211	74.9944	MT919048	Fecal
Bilaspur_HP[#]	CES15311	31.34576	76.78393	MT919049	Fecal
S.nagar_HP[#]	CES15313	31.50311	76.90276	MT919050	Fecal
Barsar_HP[#]	CES15320	31.60419	76.43049	MT919051	Fecal
Kalatop_HP[#]	CES15327	32.5318	76.01758	MT919052	Fecal
Gajnoi_HP[#]	CES12317d	32.5277	76.0836	MT919053	Fecal
Rishikesh_Uk[#]	CES13324	30.10681	78.29535	MT919054	Fecal
Jol_HP[#]	CES13335	32.19456	76.1321	MT919055	Fecal
Poonch_JnK[#]	CES18302	33.68274	74.44421	MT919056	Fecal
Overa_JnK[#]	CES18304	33.95963	75.29607	MT919057	Fecal
Thala_Uk[#]	CES17340	30.05083	79.50986	MT919058	Fecal
Birthi_Uk[#]	CES17346	30.0316	80.16652	MT919059	Fecal
Kalamunitop_Uk[#]	CES17351	30.04284	80.19897	MT919060	Fecal
Pithoragarh_Uk[#]	CES17358	29.66763	80.23032	MT919061	Fecal
Binsar_Uk[#]	CES17362	29.68749	79.7364	MT919062	Fecal
Maldevta_Uk[#]	CES17384	30.33809	78.12877	MT919063	Fecal
Nainital_Uk[#]	CES17366	29.34665	79.38558	MT919064	Fecal
Chakrata_Uk[#]	CES17305	30.699997	77.87174	MT919065	Fecal
Syanachatti_Uk[#]	CES17312	30.90474	78.36508	MT919066	Fecal
Raaditop_Uk[#]	CES17316	30.77358	78.25728	MT919067	Fecal
Sora_Uk[#]	CES17317	30.76847	78.59856	MT919068	Fecal
Lachen_Sikkim[#]	CES18329	27.75934	88.53851	MT919069	Fecal
Himalayan langur (samples from Nepal)					
115_Nepal[†]		27.45944	87.31611	MH271115	
117_Nepal[†]		26.93527	87.33222	MH271117	
116_Nepal[†]		27.45944	87.31611	MH271116	

125_Nepal[†]		28.19166	83.65194	MH271125	
121_Nepal[†]		27.8411	84.7663	MH271121	
124_Nepal[†]		28.22694	83.67388	MH271124	
118_Nepal[†]		28.17250	85.35027	MH271118	
120_Nepal[†]		27.8508	84.9913	MH271120	
119_Nepal[†]		28.1597	85.3480	MH271119	
123_Nepal[†]		28.4513	84.3758	MH271123	
122_Nepal[†]		28.4513	84.3758	MH271122	
127_Nepal[†]		28.5858	81.28500	MH271127	
128_Nepal[†]		28.8358	80.1505	MH271128	
126_Nepal[†]		28.07333	83.26166	MH271126	
129_Nepal[†]		29.9447	80.9408	MH271129	
959_Nepal*		26.9625	85.826	AF293959	
451_Nepal		-	-	AY519451	
***Semnopithecus entellus* individuals**					
*S. entellus*_Kolkata1[#]	CES15329	22.8822	88.3997	MT919070	Fecal
*S. entellus*_Kolkata2[††]		22.755	88.38	AF293958	
*S. entellus*_Jaipur[††]		26.991	75.77	AF293957	
*S. entellus*_kanpur[††]		26.4792	80.325	JQ734760	
*S. entellus*_Jodhpur[††]		27.05	72.27	JQ734761	
*S. entellus*_Sariska[††]		27.3	76.466	JQ734691	
*S. entellus*_Delhi[††]		-	-	JQ734690	
*S. entellus*_Baroda[††]		22	73.16	JQ734726	
*S. entellus*_Kumbalgarh[††]		24.5969	73.6618	JQ734727	
*S. entellus*_Titvi[††]		19.838	78.2375	JQ734724	
*S. entellus*_Dusarbhid[††]		19.9766	76.5083	JQ734725	
*S. entellus*_Kotapakonda[††]		16.3	80.45	JQ734733	
*S. entellus*_Tanuku[††]		16.7451	81.616	JQ734702	
*S. entellus*_Anoor[††]		17.0629	82.0436	JQ734701	
***S. hypoleucos*[#]	CES09401	13.5074	75.0368	MT758700	Tissue

[#] Sequenced in this study; *S* = *Semnopithecus*.

HP = Himachal Pradesh; JnK = Jammu & Kashmir; Uk = Uttarakhand.

[†] Khanal et al., 2018; *Karanth et al., 2010; [††] Ashalakshmi et al., 2014

***S. hypoleucos* – used as outgroup

Table 2.6: Details of the locations and characters used for the morphological tree.

Location	Code	Latitude	Longitude	Morphotype	Morphological characters					
					Crest	**Streak**	**EOB**	**TL**	**TC**	**HBC**
East Godavari	EGOD$^{\#\#}$	16.983	81.783	SE	0	0	3	1	0	0
West Godavari	WGOD1$^{\#\#}$	16.745	81.616	SE	0	0	3	1	0	0
Warangal	WRNGL1$^{\#\#}$	17.95	79.981	SE	0	0	3	1	0	0
Warangal	WRNGL2$^{\#\#}$	-	-	SE	0	0	3	1	0	0
Palamau	PALAMAU$^{\#\#}$	23.416	84.6	SE	0	0	3	1	0	0
Bharuch	BHARUCH$^{\#\#}$	21.7	72.966	SE	0	0	3	1	0	0
Bhubaneshwar	BHUBNSW$^{\#\#}$	20.233	85.833	SE	0	0	3	1	0	0
Bagra	BAGRA$^{\#\#}$	8.833	85.65 1	SE	0	0	3	1	0	0
Gautampur	GAUTMPR$^{\#\#}$	23.55874	83.85467	SE	0	0	3	1	0	0
Jabalpur	JABALPU$^{\#\#}$	23.166	85.952	SE	0	0	3	1	0	0
Kanha	KANHA$^{\#\#}$	22.283	85.616	SE	0	0	3	1	0	0
Kangerghati	KNGHRGH$^{\#\#}$	18.75	82.166	SE	0	0	3	1	0	0
Sikandra,	SKNDRA*	27.220585	77.95396	SE	0	0	3	1	0	0
Chakia, Varanasi	CHAKIA*	25.051296	83.22068	SE	0	0	3	1	0	0
Debari, Udaipur	DEBARI**	24.595273	73.8173	SE	0	0	3	1	0	0
Jodhpur	JODHPR**	26.351535	73.03284	SE	0	0	3	1	0	0
Mt. Abu	MtABU**	24.572363	72.73386	SE	0	0	3	1	0	0
Sasaram, Bihar	SASRAM**	24.939757	84.04294	SE	0	0	3	1	0	0
Gautampura Road	GTROAD**	23.025352	75.52593	SE	0	0	3	1	0	0
Omkareshwar Road	OMROAD**	22.218455	76.04802	SE	0	0	3	1	0	0
Kolkata	KLKATA**	22.755	88.38	SE	0	0	3	1	0	0
Etah, UP	ETAH**	27.572389	78.66916	SE	0	0	3	1	0	0
Ajmer	AJMER**	26.490855	74.6384	SE	0	0	3	1	0	0
Jaipur	JAIPUR**	26.938642	75.81552	SE	0	0	3	1	0	0
Sariska	SRSKA**	76.378741	76.37874	SE	0	0	3	1	0	0
Kakori, UP	KAKORI**	26.864305	80.78896	SE	0	0	3	1	0	0
Jabalpur, MP	JBLPUR**	23.148986	79.90204	SE	0	0	3	1	0	0
Pench National park	PENCH**	21.743269	79.31647	SE	0	0	3	1	0	0
Hampi	HMP1$^{\#\#}$	15.318	76.457	SH1	0	1	3	2	1	0
Hampi	HMP2$^{\#\#}$	15.3335	76.466	SH1	0	1	3	2	1	0
Hampi	HMP3$^{\#\#}$	-	-	SH1	0	1	3	2	1	0

Haveri	HVR1##	14.922	75.248	SH1	0	1	3	2	1	0
Haveri	HVR2##	-	-	SH1	0	1	3	2	1	0
Ankola	ANK##	14.674	74.317	SH1	0	1	3	2	1	0
Bondla	GOA##	15.605	74.066	SH1	0	1	3	2	1	0
Tolpetty	TPTY1##	11.933	76.067	SH1	0	1	3	2	1	0
Nizampur	NZMPR##	17.678	78.005	SH1	0	1	3	2	1	0
Balle	BALLE##	11.925	76.212	SH1	0	1	3	2	1	0
Matheran	MATHRN##	18 .999	73 .280	SH1	0	1	3	2	1	0
Jog, Gersoppa	JOGFALL##	14.224	74.808	SH1	0	1	3	2	1	0
Mahabhaleshwar	MHBLSWR##	17.921	73.655	SH1	0	1	3	2	1	0
Mahbubnagar	MHBNGR##	16.75	77.783	SH1	0	1	3	2	1	0
Lathur	LATHUR##	17.922	76.749	SH1	0	1	3	2	1	0
Kukke Subrahmanya	KKEF##	12.667	75.574	SH2	0	1	4	2	1	0
Agumbe	AGMB##	13.064	74.998	SH2	0	1	4	2	1	0
Moodbidre	MDBD##	13.069	74.993	SH2	0	1	4	2	1	0
Kudremukha	KDMK##	13.199	75.195	SH2	0	1	4	2	1	0
Aralam	ARLM2##	12.003	75.681	SH2	0	1	4	2	1	0
Theerthahalli	THRTLLI##	13.718	75.011	SH2	0	1	4	2	1	0
Amasebail	AMSBL##	13.612	74.949	SH2	0	1	4	2	1	0
Poonch	POONCH	33.68274	74.44421	HyL	0	0	0	1	3	1
Dachigam	DCHGM	34.1211	74.9944	HyL	0	0	0	1	3	1
Overa	OVERA	33.95963	75.29607	HyL	0	0	0	1	3	1
Dalhousie	DLHSIE	32.53407	75.97314	HyL	0	0	0	1	3	1
Jol, Shahpur	JOL	32.19456	76.1321	HyL	0	0	0	1	3	1
Barsar	BARSAR	31.60419	76.43049	HyL	0	0	0	1	3	1
Bilaspur	BLSPUR	31.34576	76.78393	HyL	0	0	0	1	3	1
Shimla	SHMLA	31.10409	77.1571	HyL	0	0	0	1	3	1
Chakrata	CHKRTA	30.7	77.87174	HyL	0	0	0	1	3	1
Raaditop	RAADTP	30.77358	78.25728	HyL	0	0	0	1	3	1
Mussoorie	MUSRIE	30.45103	78.08196	HyL	0	0	0	1	3	1
Maldevta	MLDVTA	30.33809	78.12877	HyL	0	0	0	1	3	1
Rishikesh	RSHKSH	30.10681	78.29535	HyL	0	0	0	1	3	1
Syanachatti	SYNCHTI	30.90474	78.36508	HyL	0	0	0	1	3	1
Phata	PHATA	30.57578	79.04742	HyL	0	0	0	1	3	1
Sora	SORA	30.76847	78.59856	HyL	0	0	0	1	3	1
Chirbatiya	CHRBTYA	30.38834	78.83901	HyL	0	0	0	1	3	1

Gumkhal	GMKHL	29.88449	78.67303	HyL	0	0	0	1	3	1
Nainital	NAINTL	29.34665	79.38558	HyL	0	0	0	1	3	1
Binsar	BINSR	29.68749	79.7364	HyL	0	0	0	1	3	1
Nijmula	NIJMLA	30.36582	79.46301	HyL	0	0	0	1	3	1
Thala	THALA	30.05083	79.50986	HyL	0	0	0	1	3	1
Birthi	BIRTHI	30.0316	80.16652	HyL	0	0	0	1	3	1
Kalamunitop	KLAMNI	30.04284	80.19897	HyL	0	0	0	1	3	1
Pithoragarh	PTHRGR	29.66763	80.23032	HyL	0	0	0	1	3	1
Dadeldhura, Nepal	DDLDHRA	29.2982	80.5694	HyL	0	0	0	1	3	1
Chisapani, Nepal	CHSPNI	28.6422	81.2657	HyL	0	0	0	1	3	1
Ramnagara, Nepal	RMNGR	27.7333	84.45	HyL	0	0	0	1	3	1
Syapru Besi, Nepal	SYAPRU	28.1766	85.3461	HyL	0	0	0	1	3	1
Ramche, Nepal	RMCHE	28.1614	85.429832	HyL	0	0	0	1	3	1
Melemchi, Nepal	MLMCHI	28.05	85.55	HyL	0	0	0	1	3	1
Sunsari, Nepal	SUNSRI	26.74548	87.279883	HyL	0	0	0	1	3	1
Sachen	KNP	27.4135	88.19775	HyL	0	0	0	1	3	1
Lachen	LACHEN	27.75934	88.53851	HyL	0	0	0	1	3	1
Bhutan	THMPU	27.5986	89.6280	HyL	0	0	0	1	3	1

##Nag et al., 2011; *Roonwal 1981; **Roonwal, 1984

Morphotypes: SE = *S. entellus*; SH1 & SH2 = *S. hypoleucos*; HyL = Himalayan langur.

EOB = Extent of Blackness; HBC = Head Body contrast; TL = Tail loop;

Crest, Streak and HBC, 0 = absent, 1 = present; TL - 1 = Northern type, 2 = Southern type; EOB - not visible = 0, till finger tips = 1, till knuckles = 2, till wrist = 3, till elbow = 4; TC = tail carriage (Fig. 1), TC3 = 0, TC4 = 3, TC1 = 1 (Fig. 3(c) in Nag et al., 2011).

Chapter 3

Understanding ecological requirements of the langurs of Himalaya through ecological niche modelling (ENM)

3.1 Introduction

Delimiting species boundaries is an important aspect of a species' biology with a vital role in various applications in the fields of ecology, evolutionary biology, behaviour, conservation biology, reserve planning, epidemiology, invasive species management and others (Yom-tov and Kadmon, 1998; Corsi et al., 1999; Peterson and Shaw, 2003; Thuiller et al., 2005; Karanth et al., 2009; Milanovich et al., 2010; Urbina-Cardona and Flores-Villela, 2010; Welk et al., 2002; Yackulic et al., 2013). With the advancement in technology, there is an increase in the number of available tools that can be used to predict species' distributions (Elith et al., 2006). Species distribution models relate the presence or abundance of the species with the environmental predictors and predict the distribution of the species (Elith et al., 2006). These models can give excellent results when we have a sound knowledge about a species' ecological requirements (Austin and Meyers, 1996; Diekotter et al., 2006). But this comes with a note of caution; the reliability of the predictions from the models should be carefully evaluated (Elith et al., 2006; Yackulick et al. 2013; Nag et al., 2014). Presence only data might be found from secondary sources like herbariums, museums, species lists, incidental observation records and so on with no information on sampling extent. Such type of data may not tell us the available habitat for a species (Pearce and Boyce, 2006). Another issue is, knowing how many sampling points are needed for accurate predictions; with a smaller number of sampling points or if the sampling points are collected from close by areas not covering the entire range of the species, it will result in incorrect predictions. Also, the selection of variables to model species distribution should be based on the species' ecology and behaviour. Wrongly chosen variable will also give incorrect predictions; and also, to obtain a robust result, we might need to use multiple variables (Nag et al., 2014). Considering all the doubts, one should be wary of the predictions made especially with measures having direct applications such as conservation planning.

Species distribution models have been used in a number of studies to understand species distributions (Bauer and Peterson, 2005; Peterson, 2001), to estimate species invasion (Thuiller et al., 2005), identify effects of climate change (Thuiller, 2005) and habitat alterations (Manel et al., 2000), to delimit species boundaries (Graham et al., 2004; Wiens and Graham, 2005; Raxworthy et al., 2007; Nag et al., 2014; Wang et al., 2019), to characterise species ecological requirements (Austin and Meyers, 1996; Diekotter et al., 2006; Luoto et al., 2006; Zhang et al., 2014). A handful of similar studies have been conducted using primates as model system, these studies mostly concentrated on setting up conservation priorities by assessing species status (Ortiz-Martínez et al., 2008; Boubli and De Lima, 2009; Kumara et al., 2009; Thorn et al., 2009; Torres et al., 2010; Vidal-Garciaa and Serio-Silva, 2011). A few studies have been conducted on distribution of Asian colobines with an aim to delimit species (Nag et al., 2014).

The most widely distributed non-human primate in the Indian subcontinent is Hanuman or common langur. Most of the studies conducted on langurs deals with their foraging ecology (Sugiyama, 1976; Schulke, 2001; Sharma, 2001; Punekar, 2002; Vasudev et al., 2008) or behaviour (Chhangani and Mohnot, 2006; Lal and Rajpurohit, 2010) and to some extent on their taxonomy (Karanth et al., 2010; Nag et al., 2011; Ashalakshmi et al., 2014). A recent study by Nag et al. (2014) used ENM approach to predict the distribution and delimit the species boundaries of various populations of Hanuman langur in peninsular India (Nag et al., 2014). But there is a lack of reliable data on precise distribution of different populations of Himalayan langurs. It is imperative to understand the ecological requirements of these langurs given the fact that they are used as model systems in many ecological and behavioural studies, also in biomedical studies (Karanth, 2010).

In chapter 2, it is observed that the morphological and molecular data shows Himalayan langur as a distinct lineage from *S. entellus*. However, within Himalayan langur lineage, it does not support presence of multiple species/subspecies. Ecological data is another data type which can be used to identify and delimit species boundaries (Graham et al., 2004; Wiens and Graham, 2005; Raxworthy et al., 2007; Nag et al., 2014; Zhang et al., 2014; Wang et al., 2019). Species distribution and geographic range limits are constrained by various ecological processes (Darwin, 1859; Orr and Smith, 1998) (for e.g. temperature, precipitation). In such cases, closely related species gets adapted to the local conditions in their respective habitats. Eventually, with time, these species become genetically divergent and more adapted to the local environmental conditions (Schluter, 2001; Holt and Keitt, 2005; Khimoun et al., 2013). With respect to recently evolved taxa, investigating their relationship with their respective niches can provide insights into their ecological distinctiveness and mechanism responsible for their diversification (Wiens and Graham, 2005; Knouft et al., 2006; Kozak and Wiens, 2006; Raxworthy et al., 2007; Mccormack et al., 2009).

In this chapter, I address the following questions: 1] Does the distribution of the Himalayan population separate from *S. entellus*? 2] What are the ecological requirements of the Himalayan langur and *S. entellus*? 3] Does ecological data support results from the molecular and morphological data from chapter 2?

3.2 Methods

Data collection

For ecological data, I obtained 192 occurrence records of the Himalayan langurs (Fig. 3.1). Out of these, 79 records were from the field surveys conducted for this study, 58 occurrence records were obtained from previous studies (Minhas et al., 2012; Khanal et al., 2018; Minhas et al.,

2018) and 55 occurrence records were downloaded from GBIF (Global Biodiversity Information Facility) database (www.gbif.org). For *S. entellus*, 69 occurrence records were used (Fig. 3.1), out of these, 21 records were obtained from Nag et al. (2014) and 48 records were downloaded from GBIF database (www.gbif.org). For the occurrence records downloaded from GBIF database, I plotted these occurrence records on the map and included only those records which fell within the known distribution zones of the respective taxa (iucnredlist.org). Further, I used 22 environmental layers, 19 were bioclimatic layers downloaded from www.worldclim.org, one altitude layer (USGS website), two more layers – slope and aspect were derived from the altitude layer in ArcGIS 10.2.1 (Table 3.1). All the layers were of 30 arcsec resolution, projected in WGS84 projection. These bioclimatic variables were clipped to the region from 68 °E to 97.4 °E and from 6.7 °N to 37 °N using ArcGIS 10.2.1. These clipped layers were then exported to ASCII format using QGIS 2.18.12. The 22 environmental layers were tested for multicollinearity by calculating Pearson's correlation coefficient (r). The layers with $r \leq 0.75$ were selected for further analysis (Table 3.2).

Model selection

I first performed model selection using the maximum entropy algorithm available in Maxent v3.4.1 (Phillips et al., 2006). Advantage of using Maxent is that it uses presence-only data as well as both categorical and continuous variables to make predictions. (Baldwin, 2009). Maxent has been widely used in ecological studies for predicting species distribution (Raxworthy et al., 2007; Rissler & Apodaca, 2007; Lozier & Mills, 2009; Vidal-Garciaa & Serio-Silva, 2011; Joshi & Karanth, 2012; Zhou et al., 2012; Yang et al., 2013; Kumar et al., 2014; Matyukhina et al., 2014; Nag et al., 2014; Bose et al, 2015; Sen et al., 2016; Qin et al., 2017; Khanal et al., 2018; Younger et al., 2018;)

Performance of Maxent depends on the choice of features and regularisation multiplier (RM) (Phillips et al., 2006). I tested 48 models, each for Himalayan langur and *S. entellus*, by employing different combinations of features and RM (Table 3.4) in MaxEnt v3.4.1 (Phillips et al., 2006). Maxent was run with some modifications as described in next section. Here, the output format was chosen as 'Raw'. The model (combination of features and RM) with the highest AUC value was selected as the best model.

MaxEnt algorithm

The best models were run using MaxEnt v3.4.1. Two separate Maxent analysis were conducted, one for Himalayan langur and one for *S. entellus*. Maxent was run with the following modifications. Random test percentage was set to 30%, maximum number of background points was set to 10000 and the replicates were set to 10 with replicated run type changed to Subsample. We performed 5000 iterations with the convergence threshold set to 1 x 10^{-5}. Jackknife test was used to estimate the contribution of each environmental variable. The feature type and RM value were based on the best model selected (Table 3.4). To overcome the sampling bias, a bias file was created in ArcGIS 10.2.1 by applying Gaussian kernel density function to 10000 background points (Elith et al., 2010). The output format was chosen as Cloglog (Phillips et al., 2017). AUC values were examined to check for the predictive ability of the model. AUC value of >0.75 is considered satisfactory, >0.8 is considered good and >0.9 is considered to have very good predictive ability.

I used ENMTools software (Warren et al., 2010) to measure the niche overlap between the two distribution models. Schoener's D (Schoener, 1968) was estimated as a measure of niche overlap by calculating the difference between the suitability score at each grid cell, after suitabilities were standardised (Warren and Seifert, 2011).

3.3 Results

Model prediction

The best model selected for the Himalayan langurs had features ‘LQPTH’ and a RM value of 2.5; whereas for *S. entellus*, the best model had features ‘Auto’ with a RM value of 3 (Table 3.4). Based on these models and the environmental variables selected, we obtained different distribution maps for *S. entellus* and the Himalayan langurs (Fig. 3.2). In the distribution maps, the warmer colour indicates suitable area whereas the cooler colours indicate unsuitable areas. The AUC values for the training and test data for the Himalayan langur dataset were 0.9663 and 0.9621, respectively. And the AUC values for the training and test data for *S. entellus* were 0.889 and 0.87, respectively. These AUC values indicate that the potential distribution of these species fits well with our data.

Variable selection and their importance

After testing all the variables for multicollinearity, the final set of variables for the Himalayan langur dataset consisted of 5 bioclimatic variables and 2 topographic variables (Table 3.2). Whereas, the final set of variables for *S. entellus* dataset consisted of 11 bioclimatic variables and one topographic variable (Table 3.2). All the variables were treated as continuous.

For the Himalayan langur, precipitation of driest quarter (Bio17) was the highest ranked variable (Table 3.3). Jackknife test also illustrates the importance of Bio17 (Fig. 3.5). The response curve (Fig. 3.3) shows that the habitat suitability increases with the precipitation of the driest quarter (Bio17) but eventually attains stationarity. Annual mean temperature (Bio1) and mean diurnal range (Bio2) were the next two contributing variables (Table 3.3).

Precipitation seasonality (Bio15) was the most contributing variable towards the distribution of *S. entellus* (Table 3.3). The response curve for Bio15 (Fig. 3.4) shows that the values for habitat suitability were high towards higher values of precipitation seasonality (Bio15), suggesting that *S. entellus* prefers areas with high variation in rainfall. Mean temperature of warmest quarter (Bio10) and annual mean temperature (Bio1) were the next two highly contributing variables (Table 3.3).

Niche overlap between *S. entellus* and Himalayan langur was 17 percent (Schoener's D value = 0.17). However, when we compare the areas with high probability of distribution (> 0.75), there was no overlap.

3.4 Discussion

I used ecological niche modelling based on maximum entropy (MaxEnt) algorithm to determine the distribution range of the Himalayan langur and *S. entellus*. It predicted the distribution range of the Himalayan langurs (Fig. 3.1a) with high accuracy (AUC = 0.96). The distribution was mainly governed by precipitation of the driest quarter (Bio17). Response curves (Fig. 3.3) for the top three contributing variables (Table 3.3) suggests that the Himalayan langurs prefers areas with high precipitation but with moderate temperatures. Interestingly, slope and aspect did not contribute to the model prediction. A recent study (Khanal et al., 2018) also showed that precipitation plays a major role in the distribution of the Himalayan langurs in Nepal. Langurs in the Himalaya inhabit broadleaf subtropical forest at lower altitudes and temperate broadleaf forest at higher altitudes (Sugiyama, 1976; Bishop, 1979; Curtin, 1982; Sayers & Norconk, 2008; Minhas et al., 2013). These forests receive high rainfall during the monsoon as well as precipitation in the form of snowfall in winter (Singh & Singh, 1987; Bhattarai & Vetaas, 2003; Singh et al., 1995). In contrast, precipitation seasonality was the highest contributing factor in the distribution of *S. entellus* (Table 3.3). This suggests that *S. entellus* can tolerate high temperatures, and it doesn't require high levels of precipitation. This indicates that *S. entellus* is adapted to drier conditions than the Himalayan langur.

I also used ENM to determine if the ecological niches of the Himalayan langur and *S. entellus* are separate. The ENM distinctly demarcated ecological niches of these two species. The AUC value for both the distribution models was significant, implying that the results greatly differ from the random predictions. Precipitation was the most important variable in demarcating these two species, with *S. entellus* requiring less precipitation and the Himalayan langurs need higher precipitation. Bishop (1979) also pointed out that climate is the factor that governs the distribution of the Himalayan langurs and *S. entellus*.

Niche models are regularly being used for conservation planning and species inventory (Anciaes and Peterson, 2006; Milanovich et al., 2010; Sen et al., 2016b; Calixto-pérez et al., 2018; Adhikari et al., 2019; Vieira et al., 2019; Da Re et al., 2020; Liu et al., 2020). Increasingly more studies have been comparing niche models between species or populations in order to help in either species delimitation or test various hypotheses about niche conservation (Rissler and Apodaca, 2007; Warren et al., 2008; Nag et al., 2014; Zhang et al., 2014; Younger et al., 2018). I used ENMtools (Warren et al., 2010) to checked for niche overlap between these two species to determine if they are divergent in their ecological axis. The niche overlap between these two taxa was not significant suggesting that their ecological niches are separate.

However, it must be noted that the presence of langurs in hot and drier areas like Jodhpur (23.2389N, 73.0243E) could be due to provisioning of food by the local inhabitants (Mohnot, 1971). This type of food provisioning can positively affect different life history traits and reproductive success in these populations (Borries et al., 2001).

We have used a range of ecological variables in this analysis. Identifying the specific ecological variables that contributes the most for the ecological differentiation between lineages can help us understand the specific traits responsible for adaptive differences between closely related species/subspecies. However, it is restricted by the variety of variables used. In this analysis, I included a total of 22 environmental variables: 19 bioclimatic and 2 hydrological variables (Table 3.1). The maxent based analysis of these variables showed that the bioclimatic variables (temperature and precipitation based variables) are more important than the hydrological variables in explaining the ecological differences between the Himalayan langur and *S. entellus*. This is in agreement with the idea proposed earlier that the distribution of the Himalayan langur and *S. entellus* is governed by climatic factors (Bishop, 1979).

Delimiting species and understanding their distribution is one of the important aspects in ecology and evolutionary biology (Sites and Marshall, 2003; Camargo and Sites, 2013). Ecological species concept (Van Valen, 1976) is one of the many species concepts used to define a species. Van Valen (1976) defines it as “A species is a lineage (or a closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range”. In this study, the niche modelling analysis too shows that the Himalayan langur and *S. entellus* occupy distinct ecological regions and thus shows separation on the ecological axis.

As described in chapter 2, the Himalayan langur complex is split into multiple species and/or subspecies by various authors (Pocock, 1928, 1939; Hill, 1939; Ellerman, & Morrison-Scott, 1966; Napier & Napier, 1967; Roonwal & Mohnot, 1977; Roonwal, 1984; Groves, 2001; Brandon-jones, 2004). IUCN currently recognises Groves (2001) classification where he split the Himalayan langurs into three species – *S. schistaceus, S. ajax* and *S. hector*. This niche modelling analysis does not predict habitat suitability corresponding to the distribution of any of the species/subspecies described in the above classification schemes. Using additional environmental variables of different kinds might be useful in predicting distribution of various species/subspecies within the Himalayan langur complex. As of now, it appears to be a single species.

Based on the analyses conducted in chapters 2 and 3, I found that the Himalayan langur is a lineage distinct from *S. entellus* supported by divergence on three lines of evidence corresponding to three different species concepts viz. The phylogenetic species concept (PSC) II (Donoghue, 1985; Mishler, 1985; de Queiroz, 1998), the Phenetic Species Concept (Sokal & Crovello, 1970) and the ecological species concept (Van Valen, 1976). Therefore, I subsumed the currently recognised subspecies of Himalayan langur within a single species *Semnopithecus schistaceus* Hodgson, 1840

3.5 References

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3.6 Figures

Fig. 3.1: Locations of samples used in the ecological niche modelling analysis.

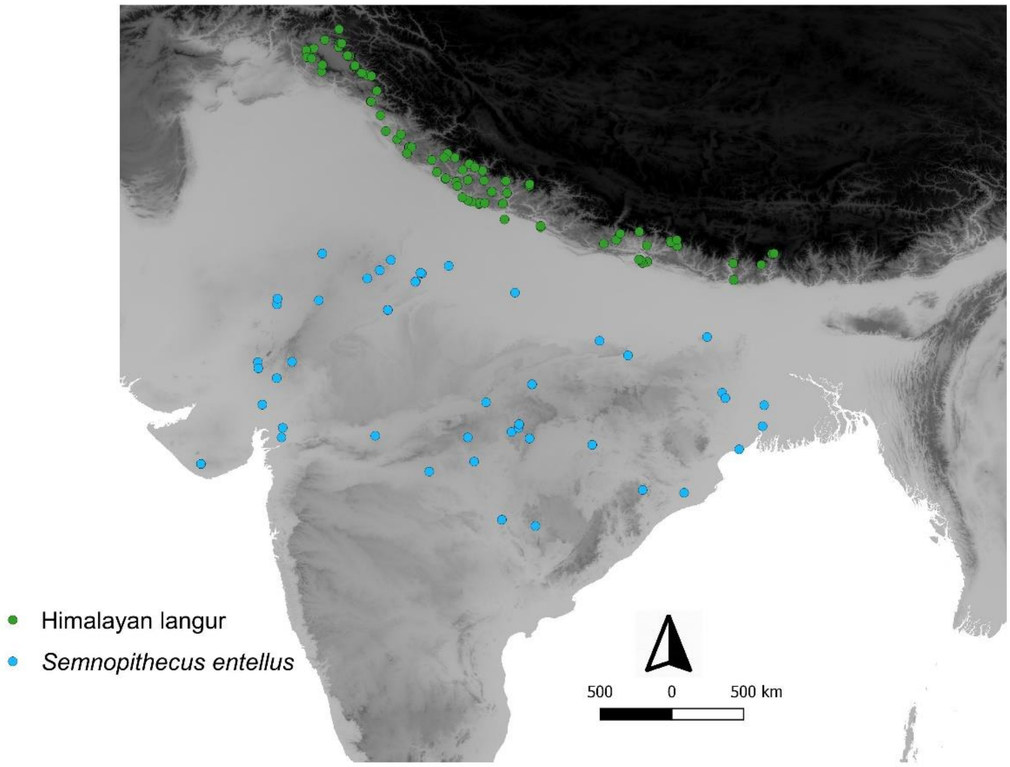


Fig. 3.2: Species distribution maps of Himalayan langur (a) and *S. entellus* (b).

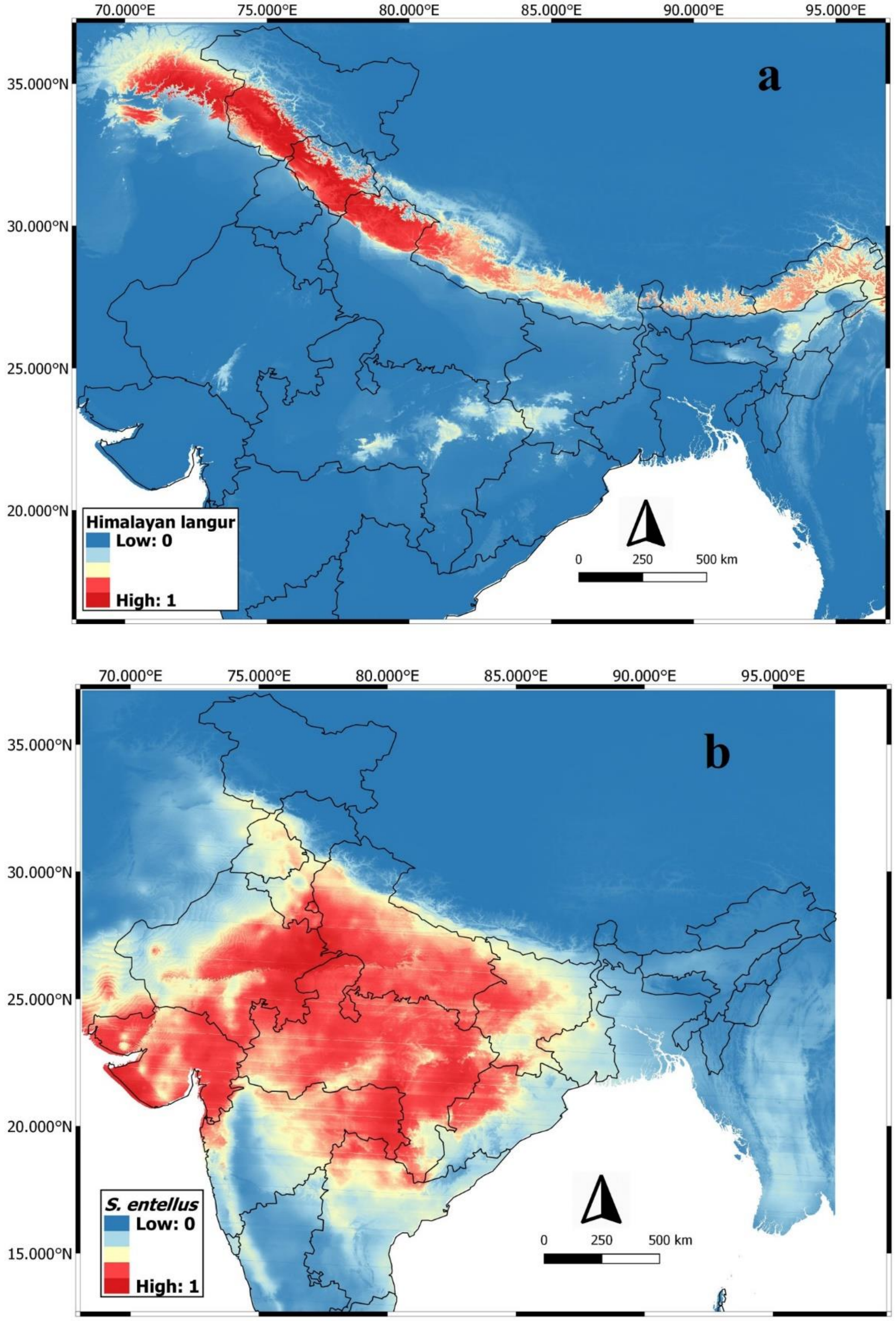


Fig. 3.3: Response curves for top three variables of importance in the MaxEnt based niche modelling analysis of the Himalayan langur

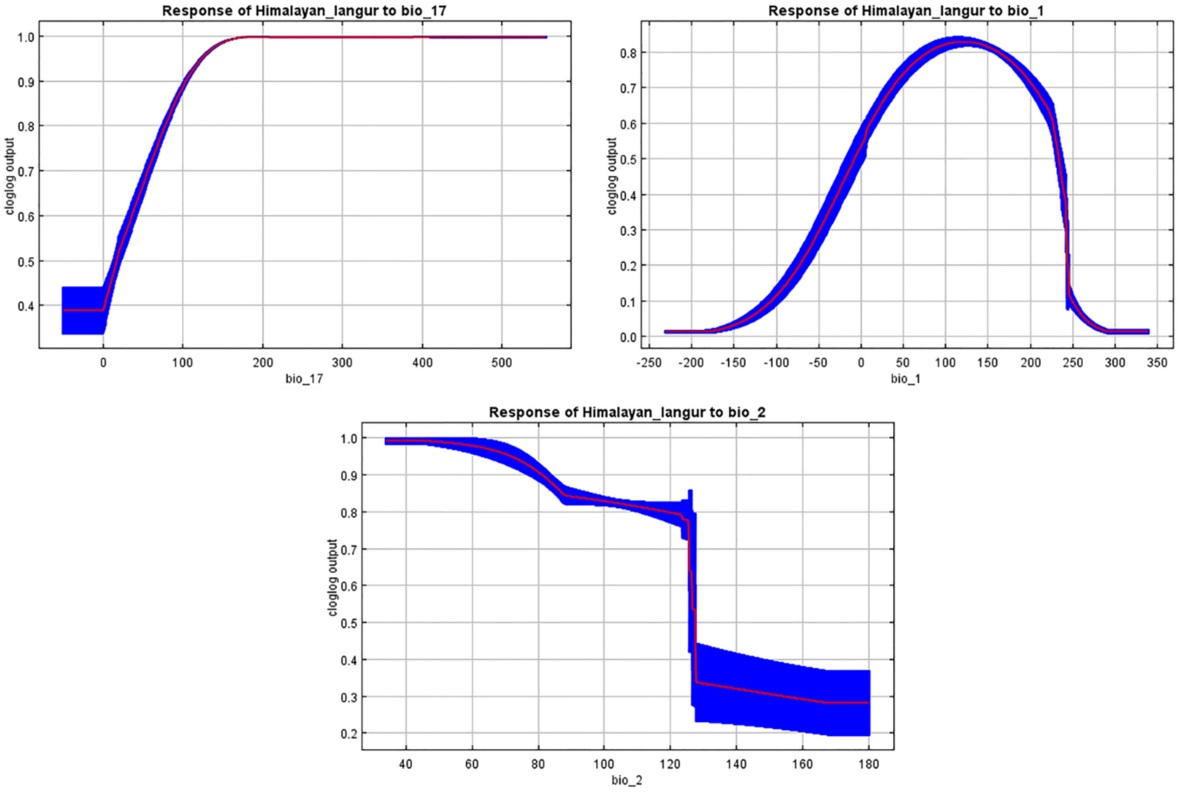


Fig. 3.4: Response curves for top three variables of importance in the MaxEnt based niche modelling analysis of *Semnopithecus entellus*

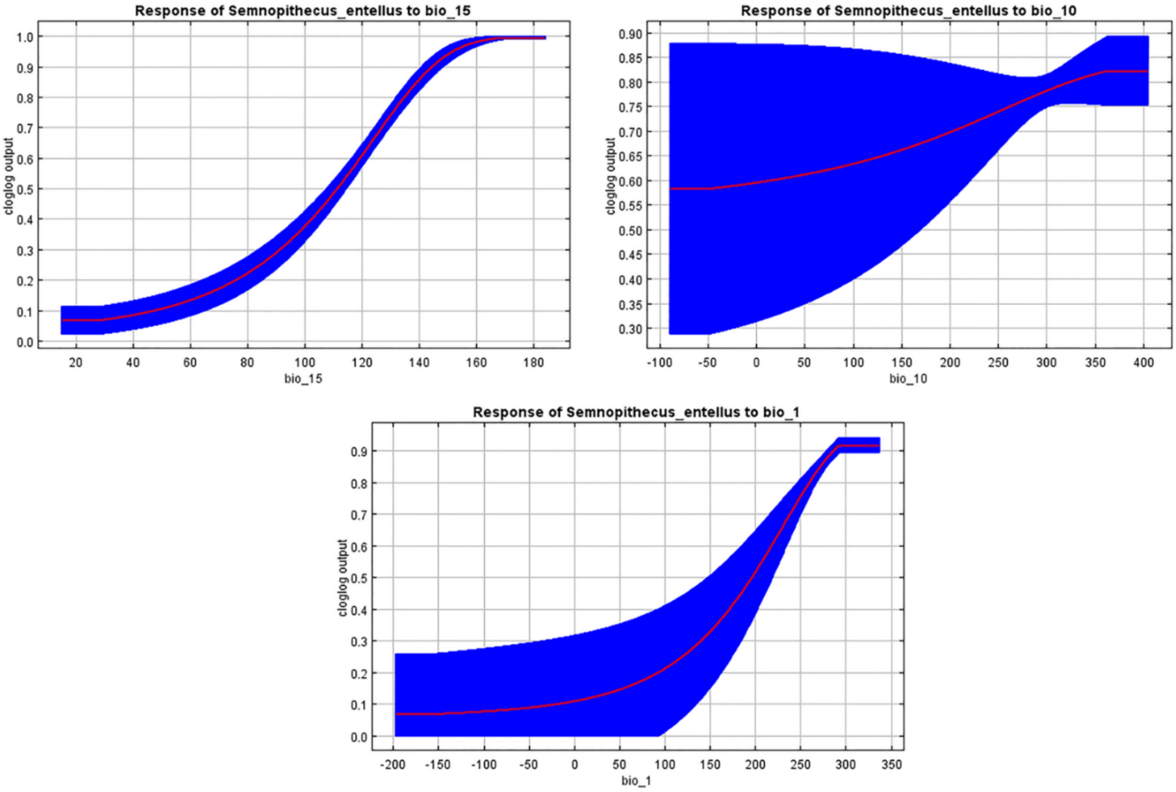
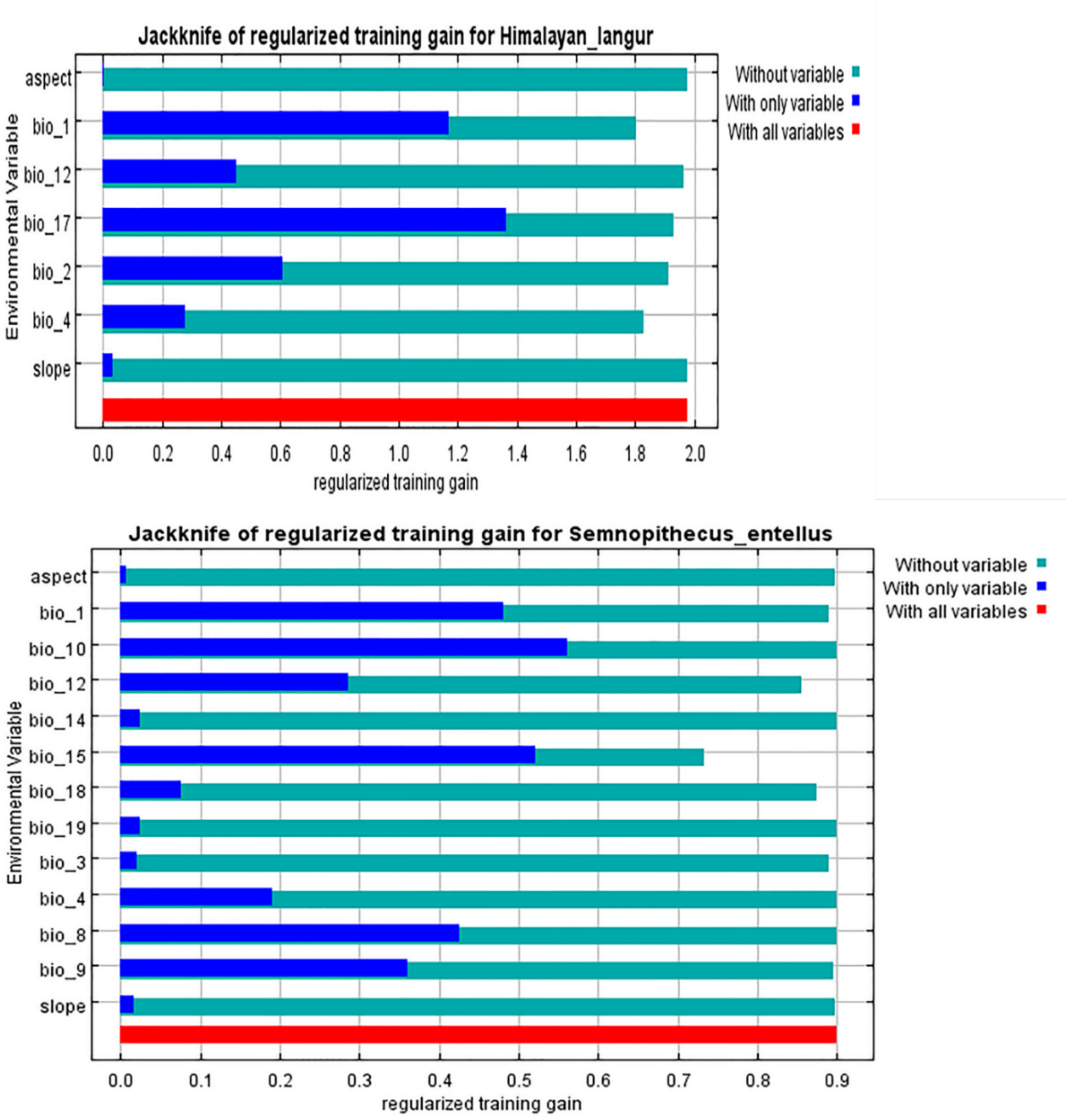


Fig 3.5: Jackknife test of regularised gain



3.7 Tables

Table 3.1: Different environmental layers used in this study. Each layer is of 30arcsec resolution and is clipped to the region from 68 °E to 97.4 °E and from 6.7 °N to 37 °N.

Layer	**Variable**	**Reference**
Bio 1	Annual Mean Temperature (°C*10)	http://www.worldclim.org
Bio 2	MeanDiurnalRange (Mean (period max-min)) (°C*10)	http://www.worldclim.org
Bio 3	Isothermality (Bio 2/Bio 7) (°C*10)	http://www.worldclim.org
Bio 4	Temperature Seasonality (SD*100)	http://www.worldclim.org
Bio 5	Max Temperature of Warmest month (°C*10)	http://www.worldclim.org
Bio 6	Min Temperature of Coldest month (°C*10)	http://www.worldclim.org
Bio 7	TemperatureAnnualRange (Bio 5-Bio 6)	http://www.worldclim.org
Bio 8	Mean Temperature of Wettest Quarter (°C*10)	http://www.worldclim.org
Bio 9	Mean Temperature of Driest Quarter (°C*10)	http://www.worldclim.org
Bio 10	Mean Temperature of Warmest Quarter (°C*10)	http://www.worldclim.org
Bio 11	Mean Temperature of Coldest Quarter (°C*10)	http://www.worldclim.org
Bio 12	Annual Precipitation (mm)	http://www.worldclim.org
Bio 13	Precipitation of Wettest Period (mm)	http://www.worldclim.org
Bio 14	Precipitation of Driest Period (mm)	http://www.worldclim.org
Bio 15	Precipitation Seasonality (Coefficient of Variation)	http://www.worldclim.org
Bio 16	Precipitation of Wettest Quarter (mm)	http://www.worldclim.org
Bio 17	Precipitation of Driest Quarter (mm)	http://www.worldclim.org
Bio 18	Precipitation of Warmest Quarter (mm)	http://www.worldclim.org
Bio 19	Precipitation of Coldest Quarter (mm)	http://www.worldclim.org
DEM	Digital Elevation model	USGS, EROS centre, Hydro1k for Asia
Aspect	Direction of slope	USGS, EROS centre, Hydro1k for Asia
Slope	Difference between two neighbouring cells elevation	USGS, EROS centre, Hydro1k for Asia

Table 3.2: Correlation matrix between the 22 variables used in this study. Numbers below diagonal are for *S. entellus* and the numbers above diagonal shows values for the Himalayan langurs. Values in red indicate high correlation between two variables.

Layers	*Bio 1*	*Bio 2*	*Bio 3*	*Bio 4*	*Bio 5*	*Bio 6*	*Bio 7*	*Bio 8*	*Bio 9*	*Bio 10*	*Bio 11*	*Bio 12*	*Bio 13*	*Bio 14*	*Bio 15*	*Bio 16*	*Bio 17*	*Bio 18*	*Bio 19*	Altitude	Aspect	Slope
Bio 1	1.000	0.566	0.595	-0.601	0.934	0.983	-0.357	0.914	0.956	0.958	0.980	0.733	0.763	-0.366	0.715	0.759	-0.096	0.577	-0.179	-0.951	0.085	-0.307
Bio 2	-0.112	1.000	0.709	-0.378	0.557	0.494	-0.031	0.568	0.489	0.538	0.570	0.178	0.278	-0.437	0.473	0.295	-0.399	0.214	-0.414	-0.493	-0.032	-0.192
Bio 3	0.218	-0.320	1.000	-0.877	0.341	0.650	-0.714	0.677	0.462	0.387	0.713	0.534	0.594	-0.773	0.752	0.616	-0.618	0.707	-0.642	-0.439	-0.041	-0.113
Bio 4	-0.195	0.733	-0.744	1.000	-0.293	-0.707	0.908	-0.711	-0.506	-0.349	-0.746	-0.700	-0.750	0.785	-0.841	-0.753	0.455	-0.804	0.510	0.457	0.026	0.138
Bio 5	0.172	0.828	-0.605	0.716	1.000	0.866	-0.011	0.796	0.932	0.992	0.851	0.551	0.585	-0.118	0.511	0.576	0.097	0.312	0.010	-0.937	0.106	-0.320
Bio 6	0.604	-0.748	0.554	-0.858	-0.499	1.000	-0.510	0.924	0.931	0.900	0.992	0.780	0.803	-0.421	0.752	0.799	-0.117	0.657	-0.208	-0.912	0.086	-0.277
Bio 7	-0.214	0.913	-0.671	0.901	0.888	-0.842	1.000	-0.478	-0.258	-0.094	-0.519	-0.611	-0.598	0.639	-0.625	-0.606	0.400	-0.775	0.434	0.212	0.010	0.004
Bio 8	0.602	0.098	-0.090	0.401	0.152	-0.036	0.114	1.000	0.856	0.822	0.937	0.718	0.760	-0.494	0.764	0.753	-0.208	0.632	-0.291	-0.838	0.069	-0.273
Bio 9	0.373	0.238	-0.027	0.241	0.273	-0.076	0.211	0.433	1.000	0.943	0.921	0.666	0.707	-0.272	0.658	0.695	0.027	0.471	-0.061	-0.922	0.106	-0.307
Bio 10	0.517	0.635	-0.519	0.680	0.879	-0.287	0.699	0.555	0.436	1.000	0.884	0.600	0.620	-0.144	0.534	0.614	0.058	0.382	-0.022	-0.949	0.112	-0.304
Bio 11	0.651	-0.560	0.634	-0.854	-0.362	0.958	-0.735	-0.090	0.005	-0.205	1.000	0.779	0.818	-0.497	0.801	0.815	-0.191	0.676	-0.274	-0.903	0.065	-0.288
Bio 12	-0.128	-0.264	-0.212	-0.331	0.004	0.259	-0.135	-0.592	-0.348	-0.219	0.266	1.000	0.961	-0.427	0.772	0.971	-0.111	0.879	-0.186	-0.673	0.035	-0.177
Bio 13	-0.300	-0.098	-0.072	-0.295	0.028	0.103	-0.037	-0.718	-0.285	-0.266	0.165	0.857	1.000	-0.571	0.895	0.995	-0.209	0.862	-0.306	-0.701	0.022	-0.230
Bio 14	-0.312	-0.006	-0.430	0.076	0.184	-0.098	0.166	-0.459	-0.183	-0.020	-0.138	0.457	0.287	1.000	-0.790	-0.577	0.810	-0.655	0.842	0.266	0.086	0.202
Bio 15	-0.156	0.457	0.242	0.277	0.135	-0.402	0.298	0.112	0.281	0.123	-0.300	-0.637	-0.171	-0.465	1.000	0.887	-0.443	0.770	-0.530	-0.629	-0.021	-0.281
Bio 16	-0.233	-0.067	-0.210	-0.235	0.135	0.102	0.030	-0.684	-0.298	-0.163	0.161	0.947	0.960	0.402	-0.376	1.000	-0.245	0.884	-0.334	-0.696	0.018	-0.225
Bio 17	-0.410	-0.094	-0.490	0.040	0.098	-0.119	0.124	-0.486	-0.398	-0.159	-0.179	0.768	0.550	0.706	-0.673	0.681	1.000	-0.468	0.961	0.022	0.092	0.105
Bio 18	-0.028	-0.791	0.215	-0.432	-0.749	0.429	-0.694	0.101	-0.255	-0.529	0.230	0.056	-0.067	-0.103	-0.341	-0.121	0.063	1.000	-0.512	-0.473	0.008	-0.054
Bio 19	-0.389	0.013	-0.601	0.220	0.212	-0.251	0.265	-0.349	-0.027	-0.010	-0.305	0.626	0.428	0.702	-0.579	0.562	0.873	-0.031	1.000	0.118	0.116	0.135
Altitude	-0.807	0.323	-0.298	0.201	0.187	-0.516	0.391	-0.767	-0.408	-0.227	-0.480	0.262	0.427	0.497	0.134	0.408	0.440	-0.310	0.380	1.000	-0.015	0.337
Aspect	-0.291	-0.047	0.153	-0.228	-0.139	0.014	-0.094	-0.438	-0.107	-0.310	0.060	0.247	0.447	-0.105	0.132	0.348	0.148	0.014	0.031	0.323	1.000	-0.021
Slope	-0.166	0.218	0.085	0.026	0.143	-0.121	0.153	-0.261	-0.074	0.024	-0.056	-0.140	0.095	0.014	0.376	0.014	-0.154	-0.312	-0.154	0.348	0.154	1.000

Table 3.3: Maxent results showing the most important variables ranked on the basis of the amount of variation they explain in predicted distribution of *Semnopithecus entellus* and the Himalayan langur along with the permutation importance for the variables.

Semnopithecus entellus			**Himalayan langur**		
Variable	**Percent contribution**	**Permutation importance**	**Variable**	**Percent contribution**	**Permutation importance**
Bio 15	35.5	39.6	Bio 17	42.1	5.9
Bio 10	26.5	7.2	Bio 1	20.6	48.2
Bio 1	20.7	19	Bio 2	15.5	16.5
Bio 12	11.7	19.9	Bio 4	13.7	23.7
Bio 18	2.5	5.7	Bio 12	8.1	5.7
Bio 3	1	5.4	Slope	0.1	0
Bio 8	0.8	0	Aspect	0	0
Slope	0.3	0.1			
Bio 9	0.3	1.7			
Aspect	0.3	0.7			
Bio 14	0.2	0.6			
Bio 19	0.1	0			
Bio 4	0.1	0			

Table 3.4: Model selection for Maxent analysis: The table shows AUC values for different models. AUC values in bold shows the features and RM values selected for respective taxa.

Features	**RM values**											
	0.5	**1**	**1.5**	**2**	**2.5**	**3**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
	Himalayan langur						***Semnopithecus entellus***					
Auto	0.962	0.958	0.968	0.966	0.963	0.963	0.861	0.869	0.877	0.859	0.867	**0.88**
L	0.893	0.872	0.877	0.867	0.878	0.888	0.837	0.844	0.838	0.849	0.856	0.846
LQ	0.957	0.958	0.955	0.955	0.958	0.958	0.846	0.862	0.86	0.846	0.858	0.859
LQP	0.961	0.961	0.963	0.965	0.961	0.965	0.87	0.842	0.848	0.847	0.846	0.851
LQPH	0.964	0.966	0.964	0.965	0.964	0.963	0.873	0.877	0.862	0.868	0.862	0.867
LQPTH	0.958	0.968	0.964	0.964	**0.969**	0.966	0.876	0.871	0.87	0.861	0.849	0.868
Q	0.862	0.851	0.853	0.855	0.871	0.863	0.844	0.847	0.849	0.846	0.837	0.847
T	0.961	0.96	0.961	0.963	0.958	0.958	0.867	0.868	0.857	0.845	0.832	0.819

L = Linear; Q = Quadratic, P = Product, H = Hinge; T = Threshold; RM = Regularisation Multiplier

Chapter 4

Phylogeographic patterns and barriers to dispersal

4.1 Introduction

Phylogeography deals with elucidating the principles and procedures of geographical distribution of genealogical lineages (Avise, 2000), generally applicable to the patterns at intraspecific levels. It aids in explaining the link between the micro and macroevolutionary processes that gave rise to the contemporary geographical distribution of the species. It also helps in identifying the roles of historical geological and climatic processes that might have influenced the distribution of different lineages. A typical phylogeography study involves collection of samples from across the distribution range of a species and generating molecular data by sequencing DNA loci. This data is then used to generate evolutionary relationships among different populations and individuals of a species. This evolutionary relationship when combined with geographical information explains the correlation of genetic lineages and landscape. Thus, phylogeography is a useful tool to understand the processes that have shaped the genetic variation among and distribution of populations.

Accumulation of genetic variation within different populations of a species is shaped by temporal changes in geography, climate and population demographic history via a combined effect of genetic drift, gene flow and selection (Hewitt, 1999; Geffen et al., 2004; Thanou et al., 2017). Geographical barriers such as rivers and mountain ranges which are created as a result of geological activities, can prevent gene flow among populations, and over time, creates population genetic structures. Apart from physical barriers, certain non-physical barriers such as environmental gradients as well as behavioural aspects of the organisms such as philopatry, dispersal ability, dispersal patterns, etc. can lead to reproductive isolation and disruption in gene flow leading to population structure (Eriksson et al., 2004; Macfarlane et al., 2016; Khanal et al., 2018b).

Climatic fluctuations have been one of the major drivers of phylogeographic patterns. Many species have been known to shift ranges in response to climate change. During the ice ages, most part of the high latitudes were covered by ice sheets. This led to shifting of species ranges from high latitude to low latitudes as these lower latitudes had favourable climate and served as refugia. These species later expanded in the higher latitudes with the retreat of ice sheets during the interglacial period (Hewitt, 2000, 2004; Horreo et al., 2018). Corroborating this hypothesis, there have been numerous studies in Europe and in North America on various plants and animal taxa (Gutiérrez-Rodriguez et al., 2017; Harrington et al., 2017; Loera et al., 2017; Horreo et al., 2018; Livraghi et al., 2018). These studies pointed out that certain places in Europe, among many, acted as refugia e.g. the Italian peninsula, the Balkan-Greece, the Iberian peninsula (Hewitt, 2004; Gutiérrez-Rodriguez et al., 2017; Horreo et al., 2018). Similarly, in north America too there were places, for e.g. southern Rocky and Appalachian mountains, Baja, Mexican plateau, Cascade-Sierra, etc. which acted as refugia for species during different glacial periods (Harrington et al., 2017; Loera et al., 2017; Livraghi et al., 2018). In the tropics the glaciation had wide-ranging effects; first, the glaciation resulted in the fluctuations in sea level which occasionally caused connections between previously isolated land masses which allowed movement of organisms across (Voris, 2000; Shelley et al., 2020). Second, during glaciation, the species from the high mountain tops receded in the low-lying areas which acted as refugia (Hewitt, 2000, 2004; Khanal et al., 2018b). Third, glaciation resulted in reduction of tropical forests and formation of drier areas with range contraction, division and refugia formation especially for wet zone and fresh water taxa (Weir et al., 2016). Fourth, fluctuation in Sea level formed land barriers which restricted movements of marine taxa (Ludt and Rocha, 2015). In the Indian context too, there have been a few studies trying to investigate the effects of Pleistocene glaciations on the diversification and distribution of various taxa (Iyengar et al., 2005; Vidya et al., 2005; Robin et al., 2010, 2015; Srinivasan et al., 2014; Vijaykumar et al., 2016; Nair et al., 2019;)

Mountain regions and river valleys are perceived as barriers to gene flow among natural populations and therefore are important for speciation and divergence (Hewitt, 2001; Li et al., 2008, 2019; Sanchez-Montes et al., 2018). The ability of rivers to act as a genetic barrier depends on a number of characteristics such as the size of the river, the amount of water, speed of the flow of water; also, the size and dispersal ability of the organism play an important role (Boubli et al., 2015; Lecompte et al., 2017). Many studies, especially in the tropics, have shown the effect of rivers as barriers to geneflow in different species of plants, birds, amphibians, reptiles and primates (Ayres and Clutton-Brock, 1992; Eriksson et al., 2004; Arora et al., 2010; Naka et al., 2012; Ribas et al., 2012; Kawamoto et al., 2013; Nater et al., 2013; Fernandes et al., 2014; Boubli et al., 2015; Ram et al., 2016; Nazareno et al., 2017, 2019; Godinho and da Silva, 2018; Khanal et al., 2018b; Oliveira et al., 2018). Ayers and Clutton-Brock (1992) showed that the similarity between primate faunas on the opposite banks of the river, decreases with the increase in the size and the annual discharge of the river. Outside of tropics, many studies have analysed the effects of rivers as genetic barriers for various species such as European wildcats (Hartmann et al., 2013), common vole (Stojak et al., 2016), bank vole (Gerlach and Musolf, 2000), red fox (Mullins et al., 2014), *Lomatia* spp. (Milner et al., 2012). Mountain ranges too, such as Alps, Rockies, Andes and Himalaya, have known to restrict species movements (Brumfield and Capparella, 1996; Hewitt, 2004; von Oheimb et al., 2013; Qiong et al., 2017; Machado et al., 2018; Salgado-Roa et al., 2018). Apart from these, other mountain ranges such as Hsueshan and central mountain ranges in Taiwan (Li et al., 2019) and the Pyrenees mountains (Pujol et al., 2017), also acts as barriers to gene flow.

Indian subcontinent is a mosaic of many different types of habitat (Boivin et al., 2013) ranging from tropical rainforests in the south and the east, grassland and savannahs in central India to desert in the west and temperate and subalpine forests in the north. This diversity in habitats does support a large range of organisms. But it also prevents movements of organisms, adapted to a specific habitat, over to a different habitat; giving rise to different genetic patterns. There are very few studies addressing intraspecific genetic patterns in the Indian subcontinent (Mukherjee et al., 2010; Robin et al., 2010, 2015). Most of these studies are restricted to the species found in the tropical forests of Western Ghats and northeast India. Many different groups in the Indian subcontinent show a disjunct distribution, like the wet zone species that are restricted to the tropical forests of Sri Lanka, Western Ghats and northeast India. These species however are not found in the central Indian dry zone (Mani, 1974; Karanth, 2003). Few studies on the Western Ghats taxa have showed the effect of valleys and high mountains as barriers to gene flow (Robin et al. 2010; 2015; Vijaykumar et al., 2016; Nair et al., 2019). Major rivers such as Brahmaputra also act as a barrier to gene flow in many species of reptiles, small mammals and amphibians (Ngamriabsakul et al., 2000; Bell and Donoghue, 2003; Veron et al., 2006; Pawar et al., 2007).

Rivers also act as barrier for the dispersal of large mammals. Vidya et al. (2005) proposed that presence of an ancient river might have been the cause for genetic break in populations of Asian elephants along the Palghat gap. Within Indian subcontinent, the rivers Tapti and Godavari act as barrier to dispersal for the northern type (NT) hanuman langurs and southern type (ST) hanuman langurs (Roonwal, 1979, 1984; Karanth et al., 2010). Similarly, the rivers Manas, Sunkosh and Brahmaputra form a barrier preventing the dispersal of golden langurs *(Trachypithecus geei*) across these rivers (Wangchuk et al., 2008; Ram et al., 2016). However, very few studies have investigated the role of Himalayan rivers and mountains as barriers to gene flow (Khanal et al., 2018). The Himalayan landscape is interlaced with many different valleys – formed due to years of glacial retreat, erosion caused by the fast-flowing rivers (Lavé and Avouac, 2001; Scherler et al., 2017) and high mountains – caused due to the upliftment resulting from the collision of the Eurasian and Indian plates (Lave and Avouac, 2001).

For this chapter, I will be testing two different hypotheses to understand the effects of past processes on diversification and distribution of Himalayan langurs. For Hypothesis 1 (Fig. 4.1), I will test the effects of different river valleys in the Himalaya as potential barriers to gene flow. For this hypothesis, the samples will be split into five populations divided by four major rivers – Sutlej, Bhagirathi, Gandaki and Kosi. I will study the effect of these river valleys as barriers to gene flow between different populations of Himalayan langur (details are discussed in Methods; section 4.2.4).

A recent study by Khanal et al. (2018b) used samples from Nepal Himalaya and showed that during the last glacial maxima (LGM), the range of the Himalayan langur shrank and restricted to lower elevations in central Nepal. Then later, after the LGM ended, the langurs expanded their range to their current distribution. Furthermore, my niche modelling analysis (Chapter 3) shows a high probability of distribution of the Himalayan langurs in the western Himalaya, as compared to Nepal and the eastern Himalaya, which somewhat supports the post-LGM expansion theory proposed by Khanal et al. (2018b) and suggesting that at present, the western Himalayan habitat is more suitable. For this hypothesis, I would be testing if the Himalayan langur populations in the central and eastern Nepal acted as source population for post-LGM expansion of these langurs into the western Himalaya. Therefore, for Hypothesis 2 (Fig. 4.2), the samples will be divided into two populations, the eastern population (EP), containing the samples from central and eastern Nepal, and Sikkim; and the western population (WP) containing the samples from the states of J&K, H.P, Uttarakhand and western Nepal.

Furthermore, the WP also corresponds to the low divergent western Himalayan clade and the EP corresponds to the eastern lineages as seen in chapter 2, Fig. 2.4).

4.2 Methods

4.2.1 Sample collection

I collected 176 faecal samples from 46 locations (Fig. 2.3A, Table 2.4) across the distribution range of Himalayan langur in India with multiple samples collected from each location. Out of these, I was successfully able to amplify the desired DNA marker (mitochondrial cytochrome *b*) from 72 samples. I also downloaded 16 sequences from GenBank database (Table 4.1). DNA extraction, PCR amplification and sequencing methods are similar to Chapter 2, section 2.2.1.

4.2.2 Phylogenetic analysis

The sequence files obtained were viewed and edited manually in ChromasLite v2.01 (Technelysium Pty. Ltd.). The sequences were aligned using MUSCLE algorithm (Edgar, 2004) incorporated in MEGA v7 (Kumar et al., 2016). I used jModelTest 2.1.3 (Darriba et al., 2012) to pick the best model of sequence evolution. Phylogenetic reconstruction was performed using Maximum Likelihood (ML) and Bayesian methods. ML analysis was performed in RAxML7.4.2 incorporated in raxmlGUI v1.3 (Stamatakis, 2006). I used GTR+I model in RAxML as there is no provision to use other models. 1000 replicates were performed to assess support for different nodes. I used MrBayes 3.2.2 (Ronquist et al., 2012) to perform the Bayesian analysis with GTR+I nucleotide substitution model. Two parallel runs with four chains each were run for 5 million generations with sampling frequency every 1000 generations. Convergence between the two runs was determined based on standard deviation of split frequencies. The program Tracer v1.6 (Rambaut et al., 2013) was used to determine stationarity, an effective sample size (ESS) value of >200 for each parameter was used as a cut-off for run length. The first 25% of trees were discarded as burn-in.

4.2.3 Phylogeographic analyses

I performed different analyses for the two hypotheses mentioned above; For Hypothesis 1, I divided the sequences into five different populations corresponding to five regions in the Himalaya – WestSutlej (WS): sample locations west of the river Sutlej; Sutlej_Bhagirathi (SB): sample locations between the rivers Sutlej and Bhagirathi; Bhagirathi_Gandaki (BG): sample locations between the rivers Bhagirathi and Gandaki; Gandaki_Kosi (GK): sample locations between Gandaki and Kosi rivers and EastKosi (EK): sample locations east of the river Kosi. If this hypothesis is true, i.e. if the river valleys do act as barriers between the populations of Himalayan langurs, I expect to see five clusters corresponding to the five populations mentioned above.

For Hypothesis 2, I divided the populations from hypothesis 1 into two groups – Western population (WP) (containing the populations WS, SB and BG of hypothesis 1) and Eastern population (EP) (containing the populations GK and EK of hypothesis 1). These two groups, i.e. WP and EP also corresponds to the western clade and the eastern lineages, respectively as seen in Fig. 2.4. Also, as mentioned earlier, Khanal et al. (2018) showed that, during LGM, the Himalayan langur distribution was restricted to low elevation central Nepal and later started expanding into other areas after LGM ended. Therefore, if this hypothesis were true, I expect to see two clusters of populations corresponding to WP and EP, further I also expect to see signatures of recent expansion in WP and signatures of long-term stable population in EP. Hereafter, all the analyses were performed separately on both the hypotheses, unless mentioned otherwise. Fig. 4.1 and Fig. 4.2 graphically illustrates the two hypotheses.

Network Analysis

I used the median-joining (MJ) network (Bandelt et al., 1999) incorporated in PopART http://popart.otago.ac.nz/ (Leigh and Bryant, 2015) to build a haplotype network which graphically represents the relationship of each sample from different geographical locations. For this analysis, the individuals were grouped based on hypothesis 1.

Genetic diversity

Genetic diversity indices including number of polymorphic sites (s), haplotype number (H), haplotype diversity (Hd) and nucleotide diversity (π) were calculated using DnaSP v6 (Rozas et al., 2017).

Demographic history

I calculated two summary statistics: Tajima's D and Fu's F for each population described under both the hypothesis to understand the evolutionary history under different demographic scenarios using DnaSp v6 (Rozas et al., 2017). I also carried out the mismatch distribution analysis for all populations to estimate the trends of population growth in DnaSp v6 (Rozas et al., 2017).

Population genetic structure

Population pairwise F_{ST} was calculated using Arlequin v3.5.2.2 (Excoffier and Lischer, 2010) and the statistical significance was tested by performing 10,000 permutations. Further assessment of the genetic structure was performed using analysis of molecular variance (AMOVA) by grouping the five populations of hypothesis 1 into two geographical groups (west and east) corresponding to WP and EP, respectively.

Statistical Phylogeography

To compare concurrent colonisation histories, I carried out a model based hypothesis testing in a Bayesian framework using Approximate Bayesian computation (ABC) analysis implemented in DIY ABC v2.1.0 (Cornuet et al., 2014). ABC approximates posterior probabilities which it then uses to rank the different scenarios being considered. It first creates a prior distribution of parameter values by simulating large number of datasets under each scenario and then it uses a logistic regression method to estimate the posterior probability by picking scenarios, from among the simulated datasets, that are closest to the observed data (Fagundes et al., 2007). For this analysis I tested two scenarios (Fig. 4.3), in the first scenario the EP originates from the WP. This indicates that the WP is ancestral. Whereas in the second scenario, the EP is ancestral from which the WP originates and colonises the western Himalayan region. The prior distribution settings were kept default. I estimated four one sample and four two sampled summary statistics. 1 million datasets were simulated for each scenario.

4.2.4 Niche modelling using past climate layers

Khanal et al. (2018) showed that the Himalayan langurs were restricted to low elevation central Nepal during the LGM and later, started expanding after LGM ended. However, this study used samples from only Nepal Himalaya and lacked samples from the Indian Himalayan region (IHR). Therefore, here I included samples from both the IHR and Nepal Himalaya, and performed ecological niche modelling (ENM) using bioclimatic layers from LGM period as well as current layers. My aim here is to understand what was the distribution of these langurs during LGM and how it changed over time.

For this analysis, I used 217 occurrence records of the Himalayan langurs. Out of these, 104 records were from the field surveys conducted for this study, 58 occurrence records were obtained from previous studies (Minhas et al., 2012; Khanal et al., 2018; Minhas et al., 2018) and 55 occurrence records were downloaded from GBIF (Global Biodiversity Information Facility) database (www.gbif.org). I used the MaxEnt algorithm (Phillips et al., 2006) and 19 bioclimatic variables (www.worldclim.org) for the current (~1960-2000) and Last Glacial Maxima (LGM) (~22000 years before present, YBP). Spatial resolution of LGM was resampled to 30 arcsec to match current. These bioclimatic variables were clipped to the region from 68 °E to 97.4 °E and from 6.7 °N to 37 °N using ArcGIS 10.2.1. These clipped layers were then exported to ASCII format using QGIS 2.18.12. The 19 bioclimatic layers were tested for multicollinearity by calculating Pearson's correlation coefficient (r). The layers with $r \leq |0.8|$ were selected for further analysis.

Performance of Maxent depends on the choice of features and regularisation multiplier (RM) (Phillips et al., 2006). I tested 48 models, for the Himalayan langur dataset by employing different combinations of features and RM (Table 4.2) in MaxEnt v3.4.1 (Phillips et al., 2006). Maxent was run with some modifications as described in next section. Here, the output format was chosen as 'Raw'. The model (combination of features and RM) with the highest AUC value was selected as the best model.

ENM analysis was performed in Maxent v3.4.1 with the following modifications; Random test percentage was set to 30%, maximum number of background points was set to 10000 and the replicates were set to 10 with replicated run type changed to Subsample. 5000 iterations were performed with the convergence threshold set to 1×10^{-5}. Jackknife test was used to estimate the contribution of each environmental variable. The feature type was selected LQPTH with RM value 1. To overcome sampling bias, a bias file was created in R (v4.0.1) using the package ENMeval v0.3.0 (Muscarella et al., 2014). The output format was chosen as Cloglog (Phillips et al., 2017). AUC values were examined to check for the predictive ability of the model.

4.3 Results

4.3.1 Phylogenetic analysis

The final dataset contained 746 bp of 88 Himalayan langur sequences, out of these 72 were sequenced for this study and 17 sequences were downloaded from GenBank (Table 4.1) (Karanth et al., 2010; Khanal et al., 2018b). One *S. entellus* sequence was used as an outgroup. GTR+I substitution model was used for this analysis.

In both the Bayesian and ML tree, a well-supported subclade consisting of haplotypes from the western Himalaya (west from 28.07N, 83.26E), which was nested within samples from eastern Himalaya (east from 28.19N, 83.65E). Both, the Bayesian and ML trees, showed similar topology where in all the major clades were retrieved. In the Bayesian tree (Appendix 2), the 128_Nepal and 129_Nepal were placed within the clade containing samples from the Indian Himalayan Region (IHR), whereas in the ML tree (Fig. 4.4), these two samples were sister to the above-mentioned clade. The position of the sample 121_Nepal was also different in the Bayesian and ML trees.

4.3.2 Phylogeographic analyses

Network analysis

The network analysis for all the sequences yielded 22 haplotypes across the distribution zone of the Himalayan langurs (Fig. 4.5). Two clusters can be observed in the network, one consisting of the Western population i.e., all the sequences from the western Himalayan region (west from 28.07N, 83.26E) and the other consisting of the Eastern population i.e., all the sequences from the eastern Himalayan region (east from 28.19N, 83.65E). Within the Western population one high frequency haplotype was observed which consisted majority of the individuals from the western region. Whereas, the sequences from the Eastern population formed haplotypes with frequencies of one or two. This pattern is similar to the results from the Bayesian and ML analyses and this might suggest that the Western population might have undergone a recent bottleneck event or it might have recently colonised the western region. Further, none of the river valleys seem to act as barriers to gene flow since we cannot see any structure across any of the river valleys. Exception to this is the river Gandaki which seems to have caused the clustering of the Western population and the Eastern population.

Genetic diversity

Both haplotype diversity (Hd) and nucleotide diversity (π) for the EP (0.93 and 0.015, respectively) were higher than the WP (0.81 and 0.003, respectively) (Table 4.3). Among the five populations of hypothesis 1, the GK population had the highest Hd and π values (0.95 and 0.015) which are comparable to EP. The two populations on the extremes, WS (Hd - 0.54, π - 0.00087) and EK (Hd - 0.6, π - 0.0036), had the lowest Hd and π; however, the low values of EK could also be an artifact of low sample size (n = 6) (Table 4.3).

Demographic history

The mismatch distribution analysis yielded a multimodal distribution of the observed data for the EP populations indicating a stable population (Fig. 4.6, G). On the contrary, the WP showed a unimodal distribution indicating exponential population growth (Fig. 4.6, F). Out of the five populations under the Hypothesis 1, the GK population (Fig. 4.6, D) showed a multimodal observed distribution which is comparable to the EP population. Whereas, the populations WS (Fig. 4.6, A) and SB (Fig. 4.6, B) shows a unimodal distribution with the peak closer to the Y-axis. Population BG shows a bimodal distribution and EK population show a unimodal distribution.

The WP yielded a significantly negative Tajima’s D (-1.935, $P < 0.05$) indicative of recent demographic expansion. Fu’s F value, although not significant, is also negative (-3.31) (Table 4.3). The Tajima’s D and Fu’s F were not significant for the remaining populations, except for EK where Tajima’s D is positively significant (2.006, $P < 0.05$) which is an indication of either balancing selection or sudden population contraction, however it is important to note here that the sample size for this population was less ($N = 6$).

Population Genetic Structure

The pairwise F_{ST} values were low among populations that were geographically closer (Table 4.4) whereas F_{ST} values were higher in distant populations. The Highest F_{ST} value was between WS and EK (0.94596), these are the two geographically extreme populations; the lowest F_{ST} value was between WS and BG (0.07428). Assessment of genetic structure by AMOVA partitioned 74.59% of variation among groups and the variation among populations within groups was very less, 7.04% (Table 4.5). The fixation index among populations within groups is also very less (F_{SC} - 0.27728). These results indicate that most of the river valleys, except for Gandaki, does not play any role in genetic differentiation.

Statistical phylogeography

In the ABC analysis, second scenario was selected as the scenario of choice, where the WP diverged from the eastern population and subsequently, colonised the western Himalaya (Fig. 4.3, scenario 2). The posterior probability for scenario 2 using the direct approach was 0.46 and by logistic approach it was 0.93.

4.3.3 Niche modelling using past climate layers

The AUC values for the training and test data for the Himalayan langur dataset were 0.9772 and 0.9702, respectively, indicating that the potential distribution of the Himalayan langurs fits well with our data. Precipitation of the driest quarter (Bio 17) had the highest contribution to the model (45.2%) followed by annual mean temperature (Bio1; 26.2%) and precipitation seasonality (Bio15; 8.2%). The response curves reveal that Bio17 value of above 200, Bio 1 value between 80 and 120 and Bio 15 value in the range of 115-121 indicates ideal habitat for Himalayan langurs. According to the palaeodistribution model, the distribution of Himalayan langurs in the LGM, as compared to the current, expanded towards the south especially in the lowland Terai region of central Nepal and parts of northern India; however, the probability of distribution was moderate. The probability of distribution of these langurs in the western Himalaya was lower in the LGM as compared to their current distribution (Fig. 4.7).

4.4 Discussion

Effects of past glaciation events

The effects of past climatic events, such as Pleistocene glaciation, on the distribution and demography of a species includes range contraction, range fragmentation, local extinction and resultant bottleneck (Hugall et al., 2002; Gaubert et al., 2016; Khanal et al., 2018a, 2018b). On the contrary, the habitats of taxa adapted to cold and dry conditions expanded, or persisted, during glaciation events (Holder et al., 1999; Loehr et al., 2006; Tian et al., 2010; Yan et al., 2013; Leite et al., 2016). In the former case, after the glaciation ends, species may undergo range expansion and exponential population growth (Excoffier et al., 2009; Unmack et al., 2012).

In this study, I found that all the Himalayan langur samples, when pooled together, have a high haplotype diversity (H = 0.853) as compared to another temperate colobine *Rhinopithecus brelichi* (H = 0.457) and a low nucleotide diversity (π = 0.012) which is comparable to *R. brelichi* (π = 0.014) (Yang et al., 2012). This combination of high haplotype diversity and low nucleotide diversity could be the result of population expansion after a recent bottleneck event or population expansion after a recent colonisation event (Avise, 2000). When the Himalayan langur individuals were divided into different populations (Table 6) according to the two hypotheses, I found that the populations in the east (GK in hypothesis 1 and EP in hypothesis 2) showed high haplotype and nucleotide diversity indicative of a long term stable population (Barrow et al., 2017; Khanal et al., 2018b) whereas the populations from the western Himalaya (WS and SB populations in hypothesis 1; WP in hypothesis 2) showed low haplotype and nucleotide diversity.

Here I used Tajima's D and Fu's F which are two commonly used statistical tests to detect population expansion. Negative values of Tajima's D and Fu's F indicates a recent population expansion (Fu, 1997; Tajima, 1989). My analysis showed that Tajima's D was significantly negative for the Western population (WP), Fu's F value, although was negative, but not significant. These two tests differ slightly in their approach which could explain the difference in the significance (Ramos-onsins and Rozas, 2002). The mismatch distribution analysis shows a multimodal graph for the GK (hypothesis 1) and EP (hypothesis 2) suggesting long term population stability, such pattern could be found in populations that survived in refugia during glaciation (Benke et al., 2009; Khanal et al., 2018b). The WS (hypothesis 1) and WP (hypothesis 2) populations show a unimodal curve, which suggests exponential population growth after recovering from a bottleneck event (Bernatchez, 2001; Hewitt, 2004). In the ABC analysis, the model that proposes colonisation of Western population (WP) from the Eastern population (EP), was supported by higher posterior probability. This is also supported by the results of the phylogenetic analysis (Fig 4.3) where a low divergent western clade is nested within the eastern lineages. The palaeodistribution modelling result showed that the Himalayan langurs were distributed in the low elevation central Nepal and adjoining parts of India, with a reduced distribution in the western Himalaya. And after LGM ended, the Himalayan langur distribution moved northwards from the low elevation areas and the post LGM expansion actually facilitated the movement of these langurs from central Nepal into western Himalaya where a high probability for the distribution of these langurs can be seen (Fig. 4.7). Thus, results from the niche modelling analysis corroborate the genetic study, which also shows a demographic expansion in the west. A recent study on the population genetic structure of these langurs in the Nepal Himalaya found that the Nepal population shows signatures of long-term stability, however, there results were not significant (Khanal et al., 2018b). They also showed that the low elevation central Nepal acted as refugia during the last glacial maxima. My analysis here shows that the Hiamlayan langur were indeed distributed in the low elevation central Nepal and adjoining parts of India, however this study also shows the westward population expansion of Himalayan langurs post LGM.

Do Himalayan rivers act as barriers?

The results of the haplotype network analysis show two distinct clusters corresponding to the WP and EP of hypothesis 2. The western cluster shows a star-like phylogeny which suggests shallow genetic structure and recent demographic expansion (Slatkin and Hudson, 1991). Rivers have been shown to act as barriers to gene flow across different taxa (Ayres and Clutton-Brock, 1992; Hewitt, 2004; Harcourt and Wood, 2012), however the ability of river to act as barrier depends on various factors such as its size, speed of the flow of water, body size of the organism, dispersal ability of the organism, etc. (Ayres and Clutton-Brock, 1992). The Himalayan rivers valleys does not seem to prevent gene flow among different populations of Himalayan langurs, except for the river Gandaki in central Nepal which seems to have caused the east-west clustering. The AMOVA analysis partitioned 74.59% of variation among groups suggesting that most of the river valleys did not play any role in genetic differentiation.

4.5 References

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4.6 Figures

Fig. 4.1: Diagram illustrating the grouping of individuals as per Hypothesis 1 (see Methods section). Multiple samples were collected from each location.

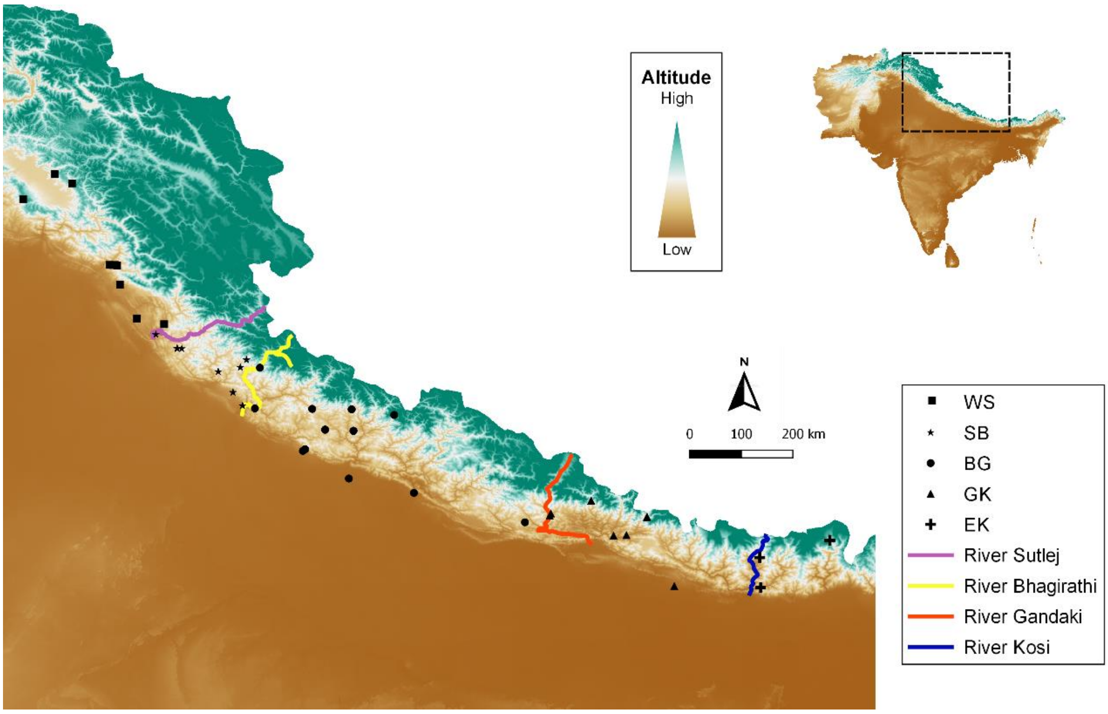


Fig. 4.2: Diagram illustrating the grouping of individuals as per Hypothesis 2. For details see methods section. WP – western population; EP – eastern population.

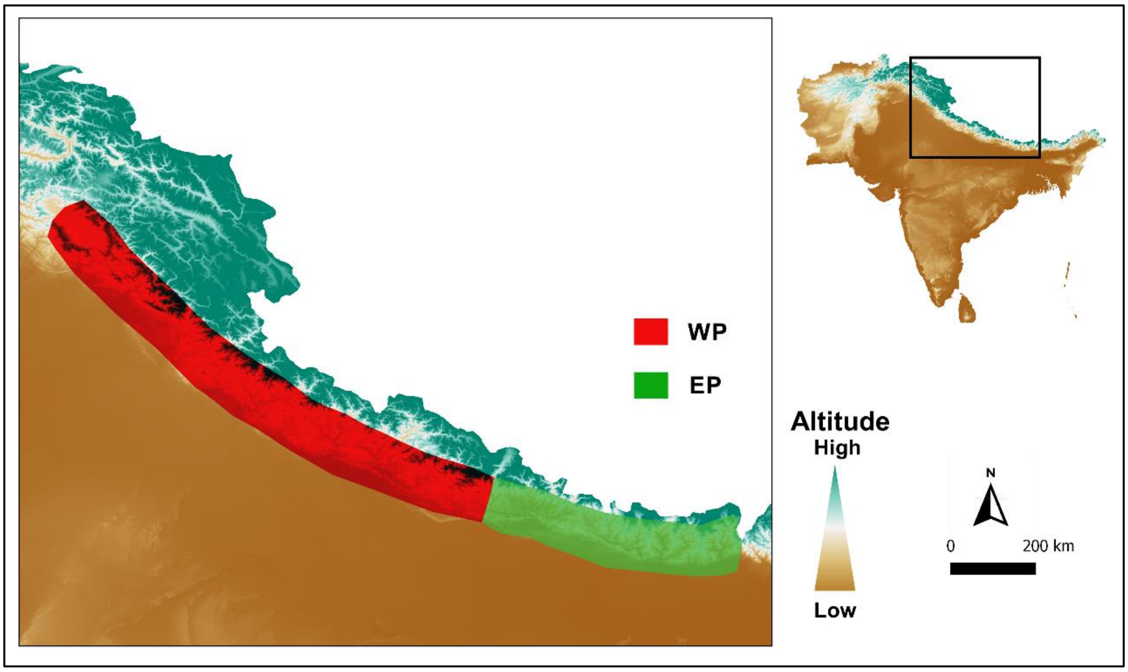


Fig. 4.3: The evolutionary scenarios used for comparison under the hypothesis testing framework in the ABC analysis. The scenarios are shown here as trees, where one population is branching of from another. The red and green lines consist of individuals collected from the western population (WP) and the eastern population (EP), respectively. Maps with distribution of each population is shown next to each scenario, the black arrow indicates the mode of colonisation.

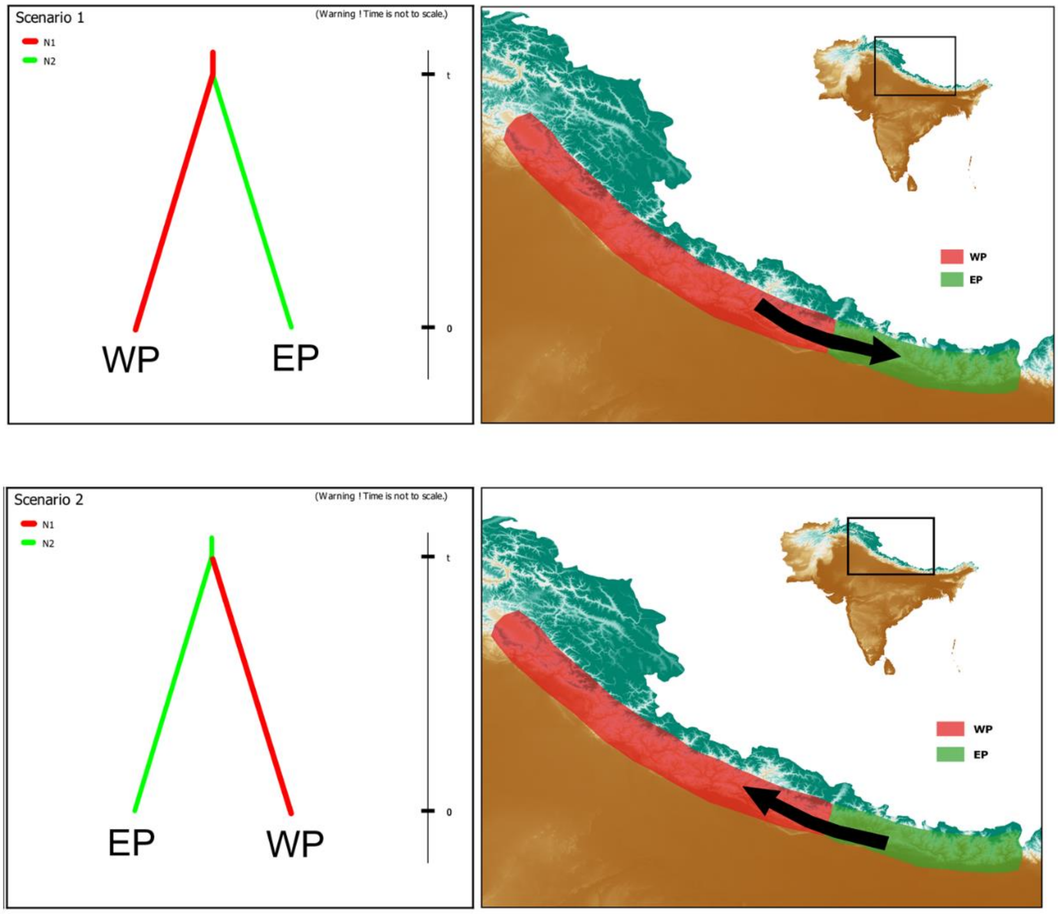


Fig. 4.4: Maximum likelihood (ML) phylogeny of Himalayan langur for mitochondrial cytochrome *b* (Cyt-b) gene. Numbers at the node indicate ML bootstrap (MLBS)/Bayesian posterior probability (BPP) values. Node support values >80/0.8 are shown. Node support values are shown only for major clades. ***** indicates MLBS/BPP < 0.8.

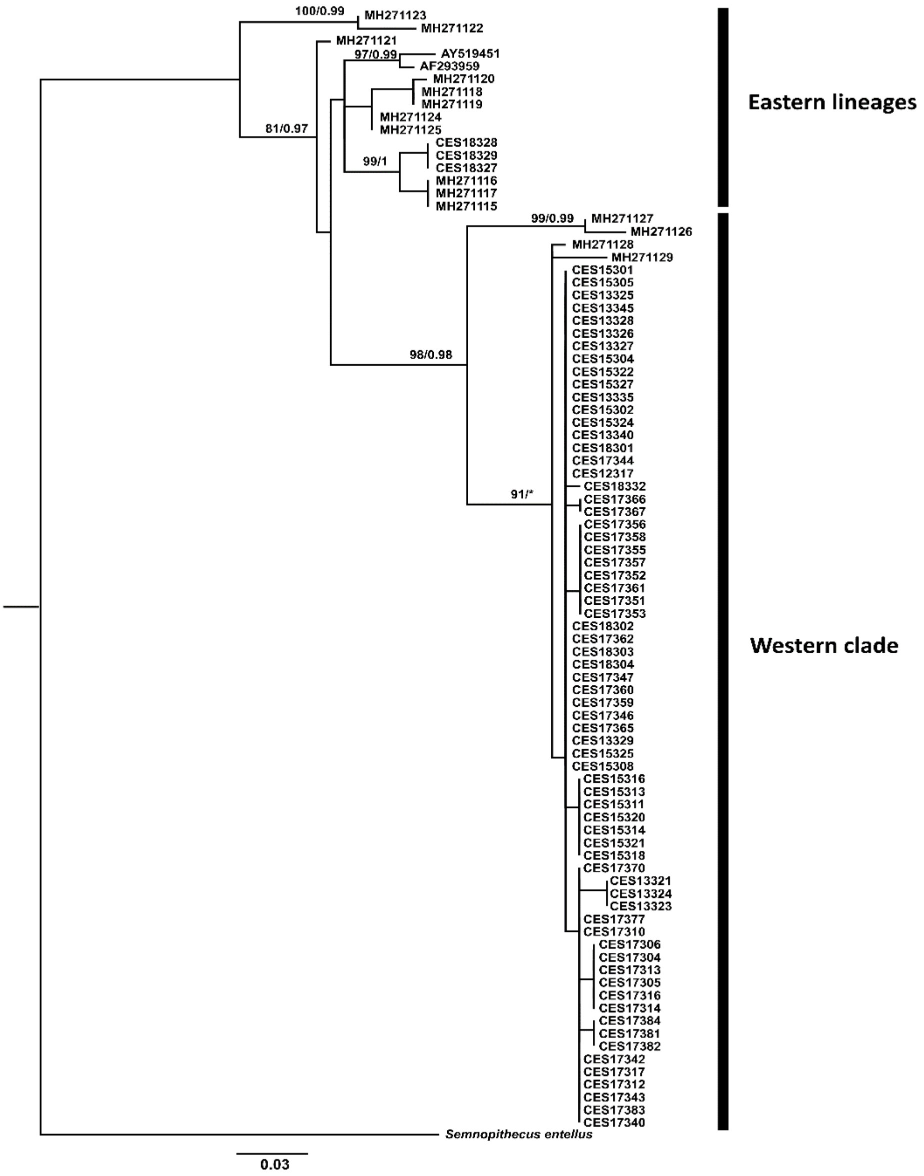


Fig. 4.5: Haplotype network for the Himalayan langurs using Cyt-*b* sequence. The circles represent extant haplotypes while the polygons represent missing haplotypes. The sizes of the haplotypes are proportional to their frequency i.e. the number of individuals that constitute those haplotypes. This network is built for the Hypothesis 1 where the populations are segregated as per the four river valleys listed in the methods section. The bars on the link between the circles stand for the number of substitutions between those haplotypes. While the length of the link is somewhat proportional to the number of substitutions

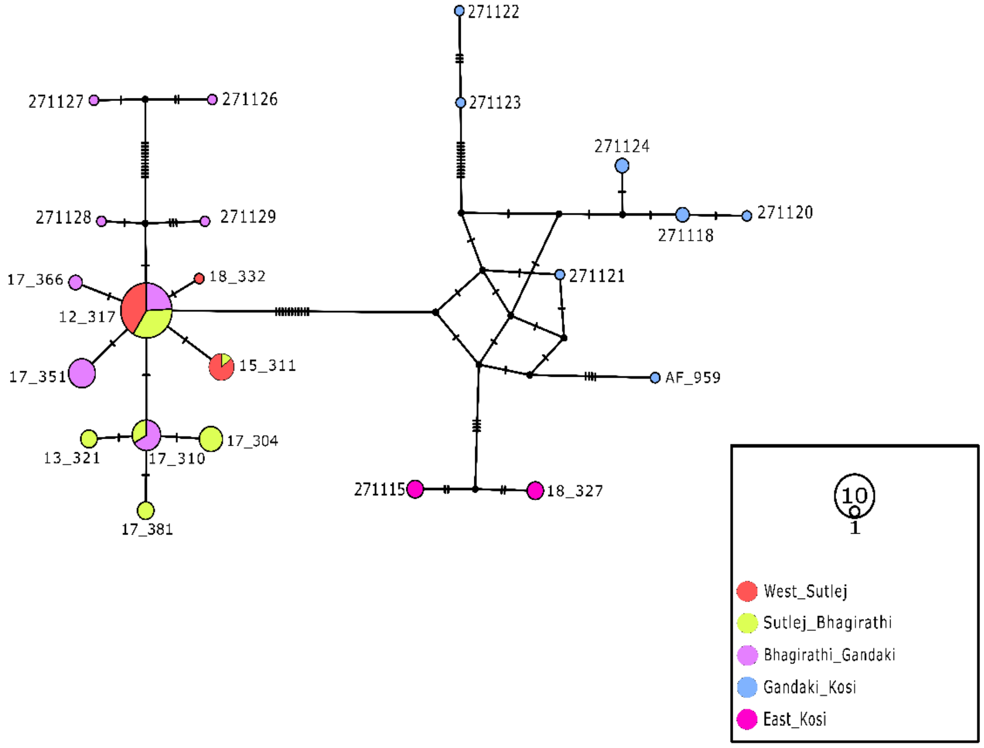


Fig. 4.6: Results of mismatch distribution carried out on Cyt-*b* data obtained from the Himalayan langurs. The graphs show pairwise differences between sequences (X-axes) plotted against the frequency of those differences to generate the distributions. Red box contains graphs for populations divided according to hypothesis 1, green box shows graphs for populations divided according to hypothesis 2, yellow box shows mismatch distribution graph for all the individuals together.

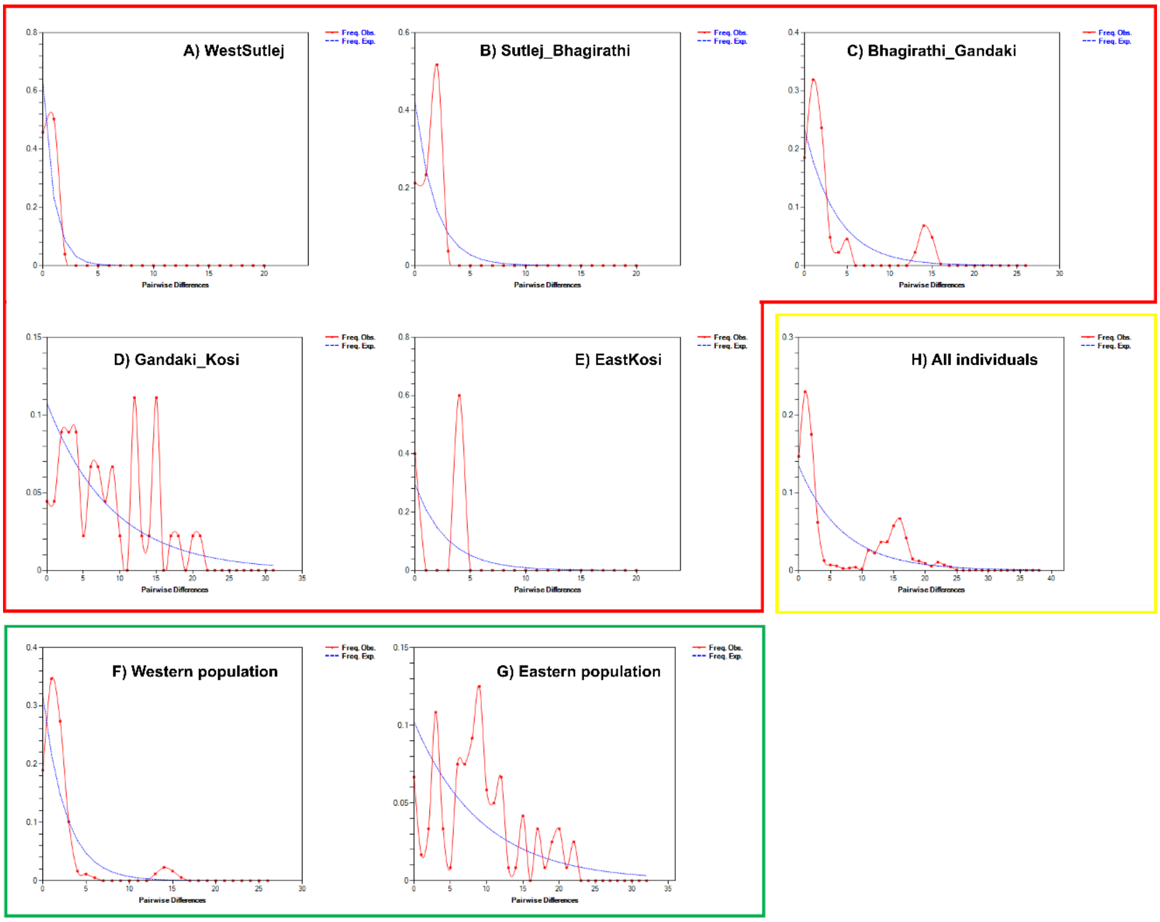
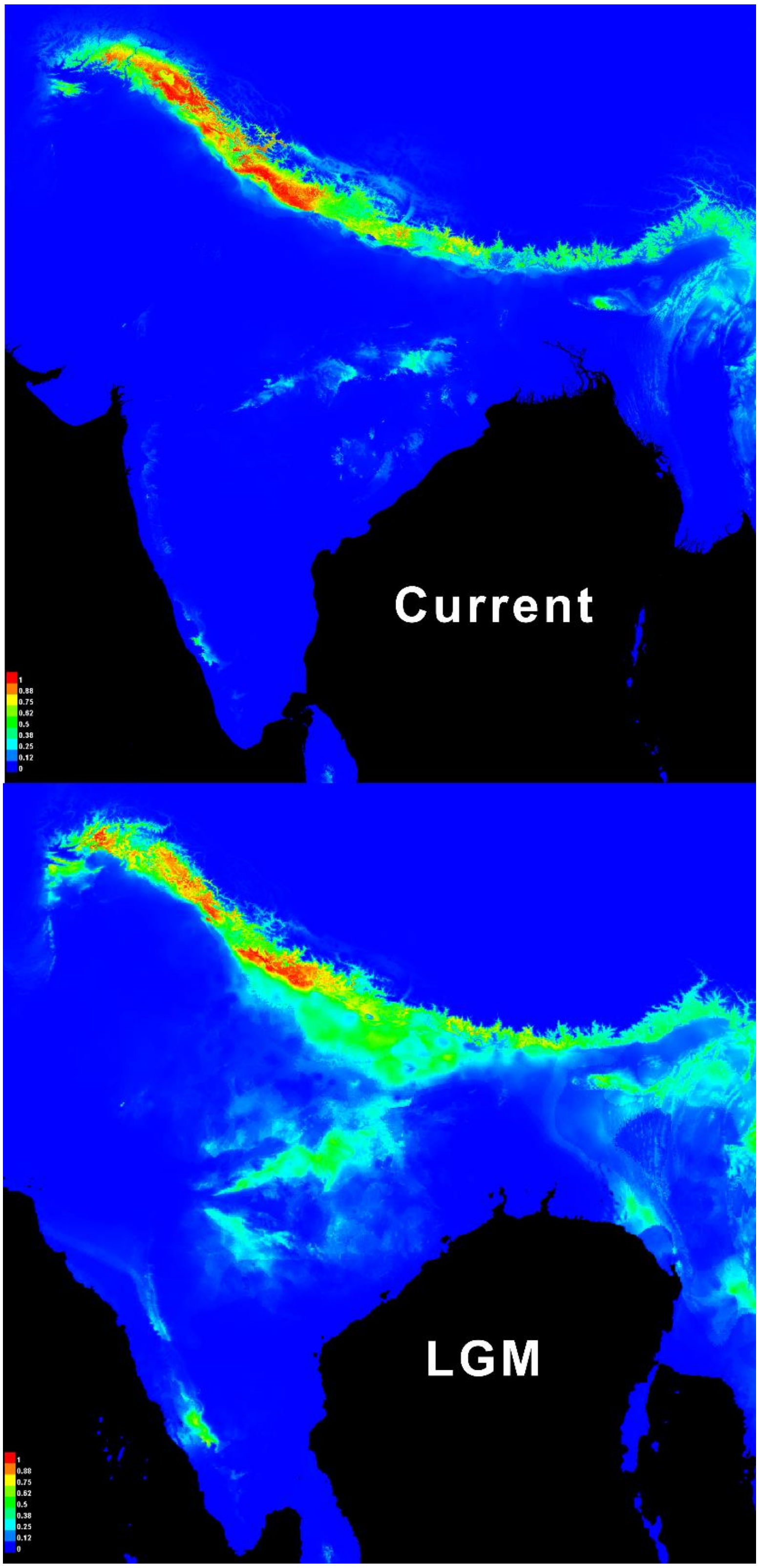


Fig. 4.7: Ecological niche modelling projections of the Himalayan langur, *Semnopithecus schistaceus* showing current distribution and potential distribution during LGM



4.7 Tables

Table 4.1: Samples used for molecular phylogenetic analysis and subsequent phylogeographic analyses. The samples column is same as the tip labels in Fig. 4.3. All the samples listed here were used for the phylogenetic analysis; only those samples that are marked with †, were used for all the phylogeographic analyses.

Samples	Latitude	Longitude	Accession Numbers
CES12317†	32.52777	76.0836	MT919053
CES13321†	30.10681	78.2954	
CES13323†	30.10681	78.2954	
CES13324†	30.10681	78.2954	MT919054
CES13325†	31.10409	77.1571	MT919046
CES13326†	31.10409	77.1571	
CES13327†	31.10409	77.1571	
CES13328†	31.10409	77.1571	
CES13329†	31.10409	77.1571	
CES13335†	32.19456	76.1321	MT919055
CES13340†	32.53511	76.0463	
CES13345†	32.53511	76.0463	MT919048
CES15301†	31.1035	77.1542	
CES15302†	31.1035	77.1542	
CES15304†	31.1031	77.1526	
CES15305†	31.1031	77.1526	
CES15308†	31.10026	77.2357	MT919045
CES15311†	31.34576	76.7839	MT919049
CES15313†	31.50311	76.9028	MT919050
CES15314†	31.50311	76.9028	
CES15316†	31.50311	76.9028	
CES15318†	31.60419	76.4305	
CES15320†	31.60419	76.4305	MT919051
CES15321†	31.60419	76.4305	
CES15322†	32.53807	75.9544	
CES15324†	32.53407	75.9731	MT919047

CES15325[†]	32.53407	75.9731	
CES15327[†]	32.5318	76.0176	MT919052
CES17304[†]	30.69999	77.8717	
CES17305[†]	30.69999	77.8717	MT919065
CES17306[†]	30.69999	77.8717	
CES17310[†]	30.90474	78.3651	
CES17312[†]	30.90474	78.3651	MT919066
CES17313[†]	30.77358	78.2573	
CES17314[†]	30.77358	78.2573	
CES17316[†]	30.77358	78.2573	MT919067
CES17317[†]	30.76847	78.5986	MT919068
CES17340[†]	30.05083	79.5099	MT919058
CES17342[†]	30.05083	79.5099	
CES17343[†]	30.05083	79.5099	
CES17344[†]	30.0316,	80.1665	
CES17346[†]	30.0316,	80.1665	MT919059
CES17347[†]	30.04284	80.1990	
CES17351[†]	30.04284	80.1990	MT919060
CES17352[†]	29.66763	80.2303	
CES17353[†]	29.66763	80.2303	
CES17355[†]	29.66763	80.2303	
CES17356[†]	29.66763	80.2303	
CES17357[†]	29.66763	80.2303	
CES17358[†]	29.66763	80.2303	MT919061
CES17359[†]	29.68749	79.7364	
CES17360[†]	29.68749	79.7364	
CES17361[†]	29.68749	79.7364	
CES17362[†]	29.68749	79.7364	MT919062
CES17365[†]	29.31781	79.3472	
CES17366[†]	29.34665	79.3856	MT919064
CES17367[†]	29.34665	79.3856	
CES17370[†]	29.34665	79.3856	
CES17377[†]	30.05882	78.5111	
CES17381[†]	30.33809	78.1288	

CES17382[†]	30.33809	78.1288	
CES17383[†]	30.33809	78.1288	
CES17384[†]	30.33809	78.1288	MT919063
CES18301[†]	33.68274	74.4442	
CES18302[†]	33.68274	74.4442	MT919056
CES18303[†]	33.95963	75.2961	
CES18304[†]	33.95963	75.2961	MT919057
CES18327[†]	27.75934	88.5385	
CES18328[†]	27.75934	88.5385	
CES18329[†]	27.75934	88.5385	MT919069
CES18332[†]	34.1211	74.9944	
MH271115[†]	27.4594	87.3161	MH271115
MH271116[†]	27.4594	87.3161	MH271117
MH271117[†]	26.93527	87.3322	MH271116
MH271118[†]	28.1725	85.3503	MH271125
MH271119[†]	28.1597	85.3480	MH271121
MH271120[†]	27.8508	84.9913	MH271124
MH271121[†]	27.8411	84.7663	MH271118
MH271122[†]	28.4513	84.3758	MH271120
MH271123[†]	28.4513	84.3758	MH271119
MH271124[†]	28.22694	83.6739	MH271123
MH271125[†]	28.19166	83.6519	MH271122
MH271126[†]	28.07333	83.2617	MH271127
MH271127[†]	28.5858	81.2850	MH271128
MH271128[†]	28.8358	80.1505	MH271126
MH271129[†]	29.9447	80.9408	MH271129
AF293959[†]	26.9625	85.8260	AF293959
AY519451	-	-	AY519451
Semnopithecus entellus	22.8822	88.3997	MT919070

Table 4.2: Model selection for Maxent analysis: The table shows AUC values for different models. AUC values in bold shows the features and RM values selected.

	RM value				
Features	**1**	**1.5**	**2**	**2.5**	**3**
Auto	0.967	0.961	0.774	0.868	0.865
L	0.916	0.919	0.915	0.910	0.916
LQ	0.907	0.955	0.957	0.952	0.962
LQP	0.962	0.959	0.962	0.959	0.961
LQPT	0.962	0.959	0.957	0961	0.955
LQPTH	**0.968**	0.962	0.961	0.966	0.960
Q	0.921	0.908	0.902	0.917	0.913
T	0.959	0.950	0.952	0.951	0.949

Table 4.3: DNA polymorphism and Genetic diversity of different populations of the Himalayan langurs. Values in bold shows significance at $P < 0.05$

Populations	**Polymorphic sites (S)**	**No. of Haplotypes (H)**	**Haplotype Diversity (Hd)**	**Nucleotide Diversity** (π)	**Fu's F**	**Tajima's D**
Hypothesis 1						
WS (n = 18)	2	3	0.542	0.00087	-0.005	0.00096
SB (n = 26)	5	6	0.788	0.002	-0.903	0.147
BG (n = 27)	22	8	0.815	0.005	0.150	-1.526
GK (n =10)	23	8	0.956	0.015	-0.729	0.093
EK (n = 6)	4	2	0.600	0.0036	3.430	**2.006***
Hypothesis 2						
WP (n = 70)	27	13	0.810	0.003	-3.310	**-1.936***
EP (n = 16)	29	10	0.933	0.015	-0.040	-0.118
All individuals (n = 87)	56	22	0.853	0.012	-1.895	-1.397

WS – WestSutlej; SB – Sutlej_Bhagirathi; BG – Bhagirathi_Gandaki;
GK – Gandaki_Kosi; EK – EastKosi; WP – Western population; EP – Eastern population.
n = No. of individuals

Table 4.4: Comparisons of pairs of population samples – Population pairwise Fst (Below diagonal), average number of pairwise difference between populations (above diagonal) and average number of pairwise differences within populations (diagonal elements in bold) among the different populations (under Hypothesis 1) of Himalayan langurs calculated by distance method

	WS	SB	BG	GK	EK
WS	**0.58258**	1.44483	2.63301	18.05385	18.85704
SB	0.30075	**1.38240**	3.07972	18.77423	19.58576
BG	0.07428	0.09663	**4.16450**	19.18515	20.12862
GK	0.78184	0.79305	0.69431	**9.53952**	12.49178
EK	0.94596	0.91955	0.81127	0.47244	**2.40988**

Table 4.5: Analysis of molecular variance (AMOVA) of Cyt *b* (746 bp) sequences of Himalayan langurs for populations given under Hypothesis 1

Sources of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	171.549	6.23583 Va	74.59
Among populations within groups	3	36.832	0.58891 Vb	7.04
Within populations	83	127.405	1.53500 Vc	18.36
Total	87	335.786	8.35975	

Fixation indices: F_{SC}:0.27728, F_{ST}: 0.81638, F_{CT}:0.74594, $P < 0.01$

Chapter 5

Summary of findings and conclusion

In this thesis, my aim was to resolve the taxonomic status of the Himalayan langur population and to understand their current distribution in the light of past geoclimatic changes. Himalayan langurs are the northernmost population of the widely distributed Hanuman langur group (*Semnopithecus* spp.). For most part of the last century, the taxonomy of Hanuman langurs was in flux with multiple classification schemes proposed by various authors (Pocock, 1928, 1939; Hill, 1939; Ellerman and Morrison-Scott, 1966; Napier and Napier, 1967; Roonwal and Mohnot, 1977; Roonwal, 1984; Groves, 2001; Brandon-jones, 2004). These classification schemes were solely based on morphological characters which are variable, highly subjective and not many characters are available for delimiting different species/subspecies (Dayrat, 2005). However, recent studies were able to resolve the taxonomy of three species of the Hanuman langur group (Nag et al., 2011, 2014; Ashalakshmi et al., 2014) using an integrative taxonomy approach where, along with morphological data, they used molecular and ecological data to have a robust understanding of the species relationships.

Resolving the taxonomic status of the Himalayan langurs will help us to identify the number of species that constitutes the Hanuman langur group and what is the distribution of each species. Here I used an integrative approach by combining morphological, molecular and ecological data to understand how the Himalayan langurs are related to the rest of the species in the Hanuman langur group, and how many species or subspecies are present within the Himalayan langurs. The use of integrative approach to taxonomy have increased recently with studies on multiple taxa (Wiens and Penkrot, 2002; Steiner et al., 2005; Lanzone et al., 2007; Glaw et al., 2010; Joshi and Karanth, 2012; Melville et al., 2014; Lajmi et al., 2016; Jażdżewska and Ziemkiewicz, 2019; Venkatraman et al., 2019). An integrative approach where data from several disciplines is used, helps us go beyond simply naming the species and understand various processes responsible for species existence and survival (Desalle et al., 2005; Page et al., 2005; Padial et al., 2010; Schlick-Steiner et al., 2010).

Himalayan langurs are distributed throughout the Himalaya in India, Nepal and parts of Pakistan and Bhutan. Their altitudinal distribution ranges from the Himalayan foothills to 4270 asl (Bishop, 1977). Traditional taxonomists grouped Himalayan langurs either as a subspecies of the widely distributed *Semnopithecus entellus* (Pocock, 1928, 1939; Ellerman and Morrison-Scott, 1966; Napier and Napier, 1967; Roonwal and Mohnot, 1977; Roonwal, 1984; Brandon-jones, 2004) or as a separate species with multiple subspecies (Hill, 1939) or as multiple distinct species (Groves, 2001). In order to obtain a robust species hypothesis, I used molecular data in conjunction with morphological data. I also conducted hypothesis testing analysis where I constrained the molecular tree according to the three currently recognised species by IUCN (Groves, 2001) and compared the likelihood of this tree with the best tree recovered in the phylogenetic analysis. I was able to show that, both in the molecular and morphological analyses, the Himalayan langurs formed a lineage distinct from *S. entellus* of the northern plains. Further, the results did not show any support for splitting the Himalayan langurs into multiple species/subspecies. None of the three currently recognised species were recovered in both the analyses. Within the Himalayan langur clade, in the molecular phylogeny, a low divergent western clade can be seen which is nested within individuals from the eastern Himalaya. This western clade does not correspond to any of the species/subspecies mentioned in any of the classification schemes.

Divergence along the ecological axis is another property which biologists use to delimit different lineages (de Queiroz, 1998). It emphasizes on the role of ecological niches in lineage separation (Van Valen, 1976). It has been proposed in the previous studies that the temperate climate of the Himalaya governs the distribution of the Himalayan langurs (Bishop, 1979), which is different from the tropical climatic conditions in the northern plains. By using ecological niche modelling I found that the Himalayan langur distribution is distinct from the distribution of *S. entellus* of the northern plains. Further, there is no overlap between the distribution zones of these two lineages. I also found that the distribution of the Himalayan langurs is mainly governed by the precipitation along with moderate temperatures. My results here corroborates the finding of a recent study which showed that precipitation plays a major role in the distribution of the Himalayan langurs in Nepal (Khanal et al., 2018). Langurs in the Himalaya inhabit broadleaf subtropical forest at lower altitudes and temperate broadleaf forest at higher altitudes (Sugiyama, 1976; Bishop, 1979; Curtin, 1982; Sayers and Norconk, 2008; Minhas et al., 2013). These forests receive high rainfall during the monsoon as well as precipitation in the form of snowfall in winter (Singh and Singh, 1987; Singh et al., 1995; Bhattarai and Vetaas, 2003).

This study showed that Himalayan langurs shows divergence along three lines of evidences, corresponding to different species concepts – the phylogenetic species concept (PSC) II (Donoghue, 1985; Mishler, 1985; de Queiroz, 1998), the phenetic species concept (Sokal and Crovello, 1970) and the ecological species concept (Van Valen, 1976). I propose to subsume all the currently recognised species and subspecies of the Himalayan langurs within a single species *Semnopithecus schistaceus*, Hodgson 1840. Among the plethora of the classification schemes, this study, along with the previous study (Nag et al., 2011), shows that the classification scheme proposed by Hill (1939) fits best for the Hanuman langur group.

The vast distribution of the Himalayan langurs (*Semnopithecus schistaceus*) traversed by deep Himalayan river valleys, compelled me to look into the role of these river valleys as barriers to gene flow among different populations of *S. schistaceus*. Rivers are a major physical barrier and the extent of the riverine barrier effect depends on the physical characteristic of the river such as the width of the river and the speed of the flow of water as well as the ecological and dispersal ability of the taxa (Ayres and Clutton-Brock, 1992; Boubli et al., 2015; Lecompte et al., 2017). I used mitochondrial cyt-*b* data from multiple individuals of *S. schistaceus* grouped according to the four major river valleys – Sutlej, Bhagirathi, Gandaki and Kosi. I found that these river valleys do not act as barriers to gene flow among different populations of *S. schistaceus* except for the river Gandaki which seems to have caused the structuring of different Himalayan langur individuals into western and eastern group (Fig. 12). The Bayesian phylogeny (Fig. 10), which shows a low divergent western clade nested within eastern lineages, also supports this result.

Past geoclimatic changes have been known to have varied effects on the contemporary distribution of many taxa (Hewitt, 2004). Processes such as dispersal, extinction and speciation governed by various biotic and abiotic processes results in the patterns of current distributions seen in different species. Here, in chapter 4, using a phylogeographic approach, I addressed the effects of Pleistocene glaciation on the distribution of intra-species genetic variation. The niche modelling analysis using paleoclimatic data showed that the distribution range of *S. schistaceus* during the last glacial maxima (LGM), in comparison to the current distribution, expanded to the lower elevations of central Nepal and adjoining parts of India. The probability of distribution in the low land central Nepal and adjoining northern India during LGM was moderate with pockets of high probability in the western Himalaya. During Pleistocene, there were many episodes of glaciation which could have facilitated the movement of langurs from central Nepal to western Himalaya. Khanal et al. (2018b) concluded that the Himalayan langur range contracted to lower elevation central Nepal. However, there sampling was incomplete since they did not have occurrence records for *S. schistaceus* from its entire distribution zone. In this study, High genetic diversity and the complex genetic structure in the eastern population of *S. schistaceus* suggests that the low elevation central Nepal and adjoining parts of north India supported long term stable populations as compared to the western region i.e states of J&K, H.P., Uttarakhand, and parts of Pakistan. Low genetic diversity and results from various demographic analysis suggests recent expansion of population in the western region. At present, a high distribution probability of *S. schistaceus* is seen in the western region encompassing the parts of Pakistan, the Indian states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand, and parts of western Nepal.

Conservation implication of taxonomic resolution

Species is the fundamental unit of biology and it is important to develop a robust method for identifying distinct evolutionary lineages (de Queiroz, 2007; Schlick-Steiner et al., 2010). Surprisingly, there is no consensus among biologists on how to define a species (de Queiroz, 2007, 1998). There are over 20 different (Mayden, 1997; Hey, 2006; Wilkins, 2009; Hausdorf, 2011) species concepts proposed to define a species. The subjective nature of the traditional taxonomy results in continuous lumping and splitting of taxa which can have immense practical ramifications on conservation planning and management (Agapow et al., 2004). Further, many fields of biology such as behaviour, development, ecology, evolution, genetics, molecular biology, palaeontology, anatomy, physiology, and systematics rely on accurate species designations, a confusion over alpha taxonomy could again have serious consequences on the inferences in these fields. Therefore, clearly defining species boundaries is crucial to basic research and conservation (Frankham et al., 2012; Fujita et al., 2012).

The current species extinction rates, due to anthropogenic activities, is estimated to be 1000 times higher than pre-human extinction levels (de Vos et al., 2014). Such an unprecedented crisis demands an immediate conservation and management measures at the global level (de Vos et al., 2014; Pimm et al., 2014; Ripple et al., 2017). However, in order to conserve, one needs to identify what is to be conserved; therefore, it is essential to defined and delimit species boundaries. Traditional morphology based taxonomy is sometimes inadequate and fails to delimit similar species. This could create confusion about the number and identity of species found in a particular region, which could prevent the species from getting the protection it would have been granted had they been accurately recognised. Molecular tools have given a new approach to delimit taxa (Hillis, 1996). However, it is another singular data type, like morphology. Therefore, to get a robust understanding of what constitutes a species, data from multiple and complimentary sources needs to utilised (Dayrat, 2005; Padial et al., 2010).

Currently, a third of the world's non-human primates are threatened with extinction (Estrada et al., 2017). Apart from having a close evolutionary history with humans, non-human primates play key role in various ecosystem functions and processes. Primates are known to be primary seed dispersers in many tropical forests (Heymann, 2011; Chapman et al., 2013), enhancing forest regeneration. Primates are valued as models for many biomedical and behavioural research which helps us to gain insights into the evolutionary history of humans and various other aspects of human behaviour and health (Hare, 2011; Phillips et al., 2014; Pontzer, 2015). Major threats to the non-human primate populations include, but not limited to, habitat destruction, deforestation, illegal trade and hunting (Estrada et al., 2017).

Molecular systematics promulgate the idea of conserving a species group rather than a single lineage (Karanth, 2010). Smaller populations of a widely distributed species might actually be distinct lineages adapted to local environmental conditions and therefore it is necessary to identify and delimit them. When a widely distributed species is split into two or more different species, the population size and distribution of the resulting species decreases. This warrants an assessment of conservation status of these newly described species and implementation of necessary conservation measures. Traditionally, the Hanuman langur was considered to be a single species and thus were accorded a low conservation status (Karanth, 2010). However, recent taxonomic studies (Nag et al., 2011, 2014; Ashalakshmi et al., 2014) have redescribed three species in peninsular India. These three species are evolutionarily distinct from each other and can be differentiated based on morphology and further, they occupy different ecological niches. Thus, these three species are delineated based on three different species concepts. This work suggested an urgent need to reassess the conservation status of these three species. The Himalayan langurs is the northernmost population of the Hanuman langur group. For most part of the last century, their taxonomic status has been unresolved with multiple classification schemes proposed. From the viewpoint of conservation, it is important to resolve their taxonomic status.

Himalaya is one of the biodiversity hotspots (Myers et al., 2000; Mittermeier et al., 2004) facing an immediate threat due to anthropogenic activities such as deforestation which is a direct result of ever-increasing human population and its demands (Roy et al., 2013). It is estimated that the net deforestation rate in the Himalaya is -0.54 (Reddy et al., 2013) which is attributed mainly to agricultural activities. In recent years, infrastructural developments have also been recognised as a major cause of deforestation (Reddy et al., 2013). This rampant destruction of habitat has led to the movement of langurs towards human settlements in search of food which has resulted in increased conflict mainly due to crop raiding by the langurs (Pers.

Obs.). In recent years, langur populations distributed along the major roads to pilgrim sites are fed by people leading to changes in behaviour and associated threats such as disease and potential increase in human-langur conflict in future. This conflict sometimes results in the people being hostile towards the langurs. There have been occasional incidents of these langurs attacking humans. There is a need to adopt and implement a robust conservation management and mitigation plan to reduce this human-langur conflict. Further, there is a need to devise a sustainable and holistic approach for human utilisation of the forest habitats.

In this study, by using an integrative taxonomy approach, I was able to categorise the Himalayan langurs i.e., *S. schistaceus*, as a species distinct from *S. entellus* of the northern plains and further comment on the population genetic structure and demographic history of *S. schistaceus* in the Himalaya. I also identified, among the plethora of classification schemes, the scheme that best describes the categorisation of *S. schistaceus*. Further research questions in this system can be formulated to understand the separation, and its underlying causes, between the western and the eastern population through fine scale sampling especially in the contact zone of these two populations. This approach and framework can be expanded to study taxonomic ambiguities in other widely distributed taxa in the Himalaya as well as in the rest of the Indian subcontinent.

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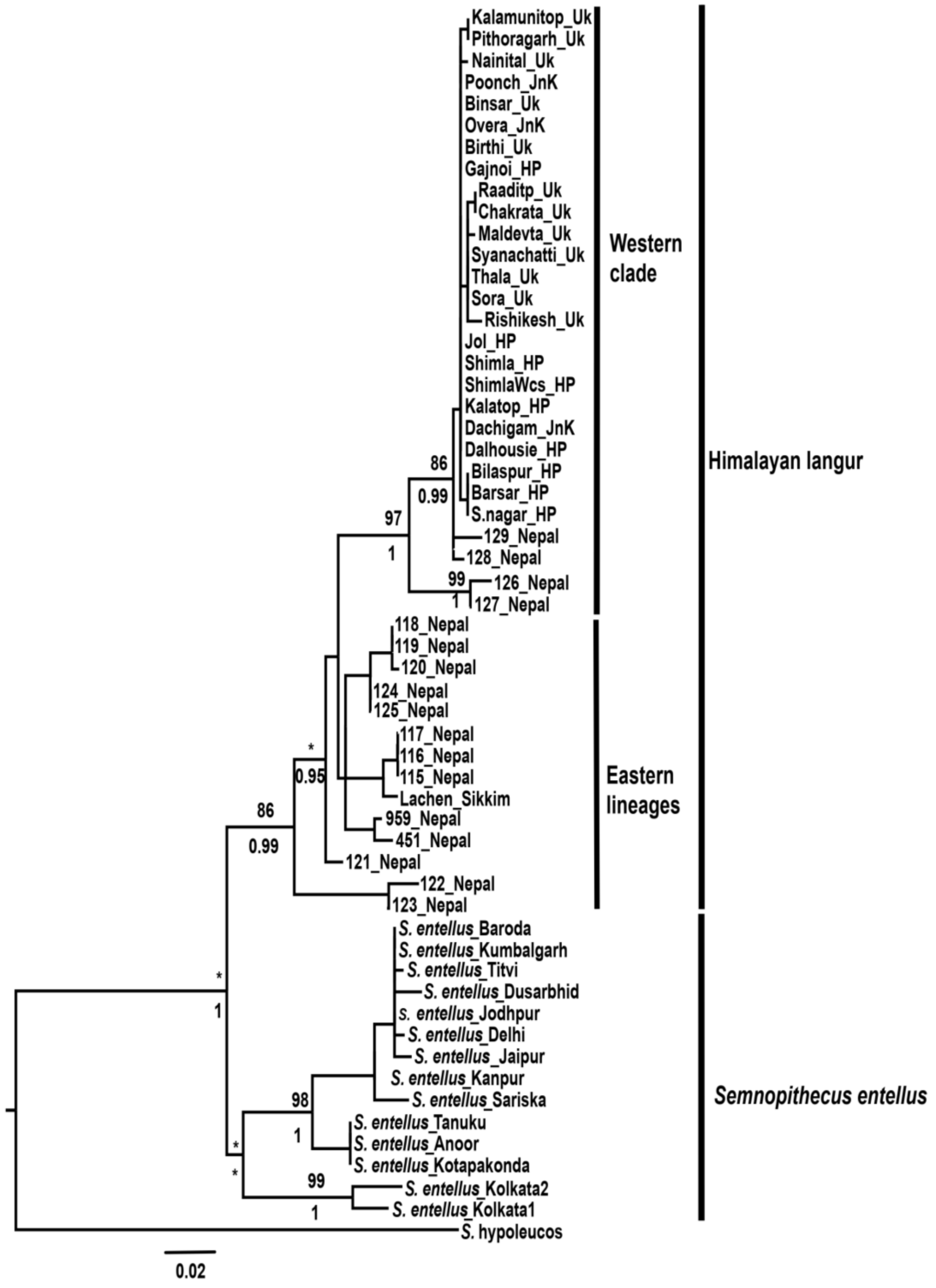
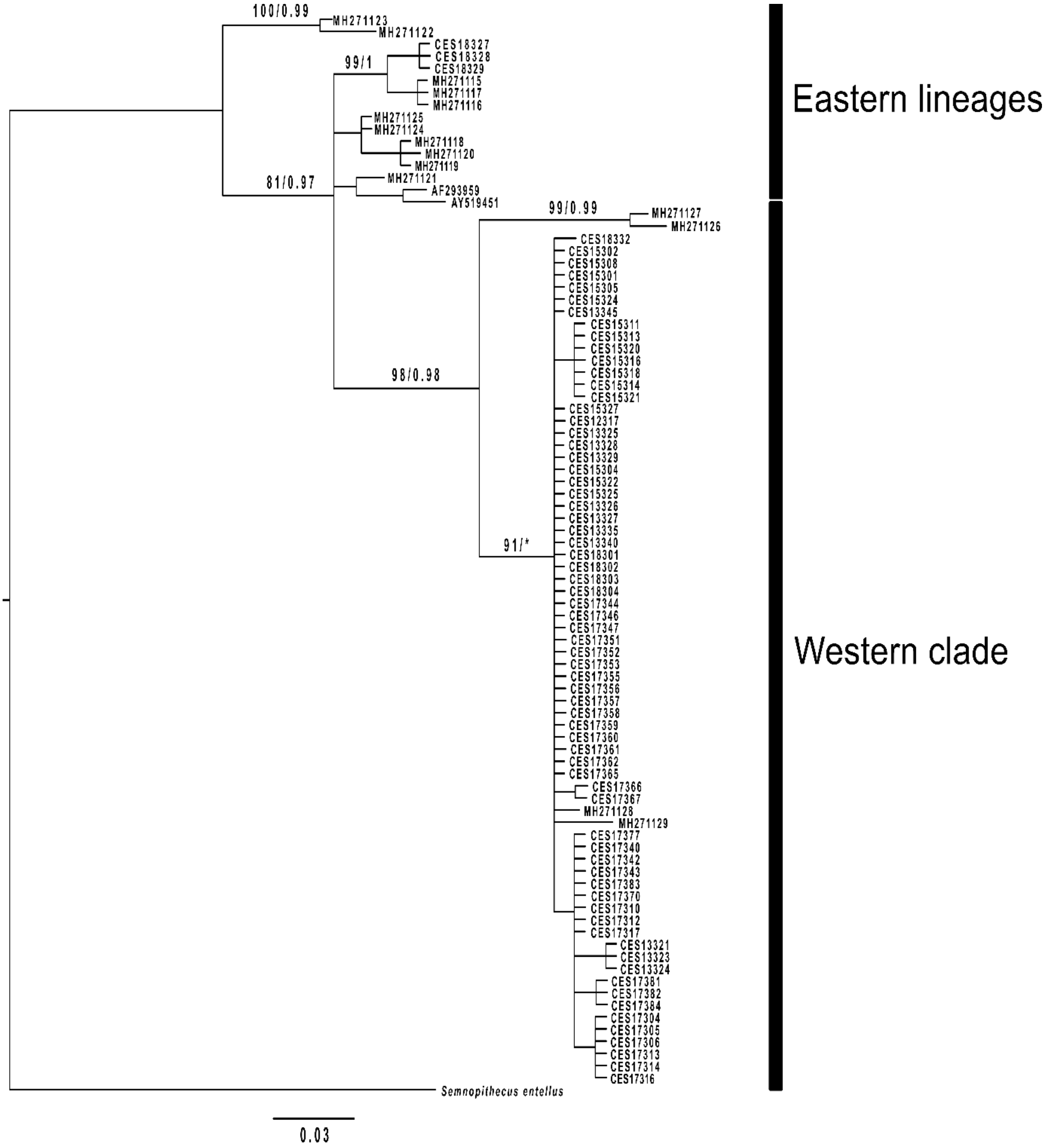
Appendix 1: Maximum likelihood tree of the Himalayan langurs and *Semnopithecus entellus* individuals reconstructed using mitochondrial cytochrome – b (Cyt – b) gene. Number above and below the nodes are the bootstrap support values and posterior probability values, respectively, for each node. * indicates node support value < 85 or < 0.95. This tree is reconstructed from samples used in Chapter 2.



Appendix 2: Bayesian tree of the Himalayan langurs reconstructed using mitochondrial cytochrome – b (Cyt – b) gene. Number at the nodes are the bootstrap support values and posterior probability values, respectively, for each node. * indicates node support value < 80 or < 0.80. Support values are shown only for major nodes. This tree is reconstructed from samples used in Chapter 4.



Publications

1. Arekar, K., Sathyakumar, S., & Karanth, K. P. (2020). Integrative taxonomy confirms the species status of the Himalayan langurs, Semnopithecus schistaceus Hodgson, 1840. *Journal of Zoological Systematics and Evolutionary Research*, 1–14. https://doi.org/10.1111/jzs.12437