

# Conservation of the Red Panda in the Eastern Himalayas



# Conservation of the Red Panda in the Eastern Himalayas:

*Insights from the Indian  
Himalayan Region*

By

Supriyo Dalui, Mukesh Thakur  
and Lalit Kumar Sharma

**Cambridge  
Scholars  
Publishing**



Conservation of the Red Panda in the Eastern Himalayas:  
Insights from the Indian Himalayan Region

By Supriyo Dalui, Mukesh Thakur and Lalit Kumar Sharma

This book first published 2024

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Copyright © 2024 by Supriyo Dalui, Mukesh Thakur  
and Lalit Kumar Sharma

All rights for this book reserved. No part of this book may be reproduced,  
stored in a retrieval system, or transmitted, in any form or by any means,  
electronic, mechanical, photocopying, recording or otherwise, without  
the prior permission of the copyright owner.

ISBN: 978-1-0364-0782-7

ISBN (Ebook): 1-0364-0782-9

# TABLE OF CONTENTS

List of Tables.....	viii
List of Figures.....	ix
List of Photos.....	xii
Chapter I.....	1
A Comprehensive Overview of a Unique and Mysterious Himalayan Animal: The Red Panda	
1.1 The Red Panda: An Endangered Eastern Himalayan Species.....	1
1.2 The Basis of Our Investigation: Investigating the Rationale for Our Proposed Work.....	4
1.3 Charting the Course: Goals and Objectives .....	7
1.4 Investigating the Literature: A Comprehensive Review of Existing Studies .....	8
1.5 Exploring the Mountains and Forests: An Overview of the Study Area .....	11
1.6 The Scientific Methodology: Data Collection and Analysis Procedures.....	13
1.6.1 <i>The Red Panda Quest: Investigating Approaches                 to Occurrence Data Collection</i> .....	13
1.6.2 <i>Methods and Insights Regarding Non-Invasive DNA                 Sample Collection</i> .....	14
1.7 Species Identification Using Mitochondrial DNA: Gene Amplification and Sequencing.....	16
1.8 From Sample to Data: A Comprehensive Guide to Microsatellite Genotyping for Red Pandas.....	18
Chapter II.....	23
Uncovering the Evolutionary Mysteries of Red Pandas in the Eastern Himalayas: Impact of Landscape, Climate, and Human Activities on Demographic History	
2.1 Chapter Overview .....	23
2.2 Introduction.....	24

2.3 Methodological Strategies.....	25
2.3.1 <i>Red Panda Phylogeny</i> .....	25
2.3.2 <i>Red Panda Phylogeographic Structure</i> .....	26
2.3.3 <i>Red Panda Population Demographic History</i> .....	26
2.4 Insights into the Study Results: Detailed Findings .....	27
2.4.1 <i>The results of the phylogenetic analysis</i> .....	27
2.4.2 <i>Red panda phylogeography and population genetics</i> .....	31
2.4.3 <i>Red Panda Demographic History: Key Findings</i> .....	37
2.5 A Detail Discussion of the Research Findings.....	39
2.5.1 <i>The Phylogeographic Structure of Red Pandas in the IHR</i> . 40	
2.5.2 <i>Red Panda Population Demographic History and Divergence</i> .....	41
2.6 Wrapping it Up: The Conclusion .....	43
Chapter III .....	44
Exploring the Genetic Secrets of Red Panda Populations in the Eastern Himalayas: Genetic Diversity, Inbreeding, Population Genetic Structure, and Gene Flow	
3.1 Chapter Overview .....	44
3.2 Introduction.....	45
3.3 Methodological Approaches .....	47
3.3.1 <i>Red Panda Ecological Habitat Modelling</i> .....	47
3.3.2 <i>Red Panda Landscape Connectivity Analysis in KL and Arunachal Pradesh</i> .....	50
3.3.3 <i>Population Genetic Assessments</i> .....	51
3.4 Insights into the Study Results: Detailed Findings .....	53
3.4.1 <i>Red Panda Habitat Predictor Variables in IHR</i> .....	53
3.4.2 <i>Red Panda Habitat Suitability and Landscape Connectivity in IHR</i> .....	61
3.4.3. <i>Red Panda unique genotype identification and genetic variability evaluation</i> .....	65
3.4.4 <i>Red Panda Population Genetic Structure in IHR</i> .....	69
3.4.5 <i>Migrants and Gene Flow in Red Panda Populations</i> .....	71
3.5 A Detail Discussion of the Research Findings.....	74
3.5.1 <i>Habitat suitability and corridor connectivity</i> .....	74
3.5.2 <i>Genetic diversity and inbreeding</i> .....	76
3.5.3 <i>Population Genetic Structure and Gene Flow of Red Panda in IHR</i> .....	76
3.5.4 <i>Functional Connectivity and Conservation Priorities</i> .....	80
3.6 Wrapping it Up: The Conclusion .....	81

Chapter IV .....	83
A Race Against Time: Mapping the Current Distribution, Status, and Looming Threats to Red Panda Survival in the Majestic Eastern Himalayas	
4.1 Chapter Overview .....	83
4.2 Introduction.....	84
4.3 Methodological Approaches .....	86
4.3.1 <i>Climate change modelling</i> .....	86
4.3.2 <i>Future land cover simulation in IHR</i> .....	88
4.3.3 <i>Questionnaire data collection and analysis</i> .....	89
4.4 Detailed Findings: Insights into the Study Results .....	91
4.4.1 <i>Climate change impacts</i> .....	91
4.4.2 <i>Future land cover change impacts</i> .....	96
4.4.3 <i>Socio-demographic and socio-economic profile                 of households</i> .....	102
4.5.1 <i>Future climate change impact on red panda habitat</i> .....	108
4.5.2 <i>Land cover change effect on red panda habitat</i> .....	110
4.5.3 <i>Impact of indigenous hunting on red panda</i> .....	110
4.6 Conclusion and recommendations .....	111
Chapter V .....	113
Management Recommendations, and Way Forward	
5.1 Conservation Management Plans.....	113
5.2 The Sympatric Species Conservation (SSC) Programme .....	114
5.3 Community Conservation Areas and Participatory Monitoring..	114
5.4 Conservation Education and Awareness.....	115
5.5 International Species Conservation Strategy.....	115
5.6 Human Resource Development and Strengthening .....	116
5.7 National Level Importance.....	119
5.8 Law Enforcement and Regulations .....	119
5.9 Knowledge Upgradation and Establishment of a Regional Database .....	119
5.10 International Red Panda Conservation Coordination.....	120
5.11 Initiation of Transboundary Conservation Efforts .....	120
5.12 Biodiversity Peace Park .....	121
References .....	122

# LIST OF TABLES

TABLE 1. DETAILS OF SEQUENCES RETRIEVED FROM GENBANK. ....	17
TABLE 2. CHARACTERISTICS OF THE MICROSATELLITES SCREENED FOR THE SPECIES RED PANDA.....	20
TABLE 3. GENERATION OF MULTIPLEX PANELS WITH SELECTED NINE MICROSATELLITE MARKERS.....	21
TABLE 4. NUCLEOTIDE VARIABILITY AMONG DIFFERENT HAPLOTYPES OBSERVED IN THE WILD COLLECTED SAMPLES OF RED PANDA IN THE PRESENT STUDY FROM IHR USING MITOCHONDRIAL CONTROL REGION (MTDNA-CR).....	29
TABLE 5. OBSERVED HAPLOTYPES IN RED PANDA ACROSS IHR AND CHINA .....	33
TABLE 6. SUMMARY OF MTDNA GENETIC DIVERSITY INDICES AND NEUTRALITY TESTS OF DEMOGRAPHIC EXPANSION OF RED PANDAS.....	34
TABLE 7. SUMMARY OF THE SPATIAL ANALYSIS OF MOLECULAR VARIANCE TEST (SAMOVA).....	35
TABLE 8. LIST OF FINAL VARIABLES USED IN HABITAT MODELLING AFTER COLLINEARITY TEST ALONG WITH THEIR DATA SOURCES.....	48
TABLE 9. BEST FIT MODEL SETTING SELECTED ON THE BASIS OF LOWEST AICc VALUE IN ENMEval FOR KL-INDIA .....	56
TABLE 10. BEST FIT MODEL SETTING SELECTED ON THE BASIS OF LOWEST AICc VALUE IN ENMEval FOR ARUNACHAL PRADESH.....	59
TABLE 11. DISTRICT AND PROTECTED AREA WISE HABITAT SUITABILITY IN KL AND ARUNACHAL PRADESH .....	62
TABLE 12. INDIVIDUAL IDENTIFICATION AND GENOTYPING ERROR IN RED PANDA POPULATION AT NINE MICROSATELLITE LOCI.....	67
TABLE 13. GENETIC DIVERSITY ESTIMATES OF HIMALAYAN RED PANDA POPULATION OF KL AND AP INDIA AND CHINESE RED PANDA POPULATION OF DIBANG IN EASTERN AP INDIA.....	68
TABLE 14. CONTEMPORARY MIGRATION RATE (> 5% AS SIGNIFICANT VALUE) AND $F_{ST}$ (WITHIN BRACKET) AMONG RED PANDA POPULATIONS IN KL-INDIA .....	73
TABLE 15. CONTEMPORARY MIGRATION RATE (> 5% AS SIGNIFICANT VALUE) AND $F_{ST}$ (WITHIN BRACKET) AMONG RED PANDA POPULATIONS IN ARUNACHAL PRADESH.....	73



# LIST OF FIGURES

FIGURE 1. STUDY AREA MAP. A) RED PANDA IUCN DISTRIBUTION RANGE IN INDIA WITH 5*5 KM GRID, B) KL-INDIA WITH PA BORDERS, C) ARUNACHAL PRADESH WITH PA BORDERS. ....	12
FIGURE 2. STUDY ARE MAP SHOWING THE SAMPLING LOCATIONS IN CENTRAL AND EASTERN HIMALAYAS .....	15
FIGURE 3. QUALITY OF DNA ISOLATED FROM FECAL SAMPLES .....	15
FIGURE 4. ELECTROPHEROGRAM SHOWING ALLELIC PROFILE OF MICROSATELLITE MARKERS. ....	19
FIGURE 5. BAYESIAN PHYLOGENETIC RECONSTRUCTION EMPLOYING MITOCHONDRIAL CONTROL REGION (NODE VALUES INDICATED MILLION-YEAR-OLD DIVERGENCE TIME, MEDIAN DIVERGENCE ESTIMATE). CLADE 1A REPRESENTED WESTERN AND CENTRAL ARUNACHAL, 1B KL AND TIBET, 1c KL, AND 2A, 2B, 2C DIBANG VALLEY (RED DOT) SAMPLES AND NCBI/GENBANK CHINESE RED PANDA SEQUENCES. ....	28
FIGURE 6. DISTRIBUTION OF HAPLOTYPES WITH RESPECT TO THE GEOGRAPHICAL ORIGIN OF THE SAMPLES IN INDIA AND CHINA. GREEN COLOUR DOTS REPRESENTED HIMALAYAN RED PANDA, YELLOW DOTS REPRESENTED CHINESE RED PANDA (SAMPLES ORIGINATED FROM THE ADMINISTRATIVE BOUNDARY OF CHINA), AND RED COLOUR REPRESENTED CHINESE RED PANDA OBSERVED IN THE DIBANG VALLEY, ARUNACHAL PRADESH, INDIA.....	32
FIGURE 7. BAYESIAN ANALYSIS OF POPULATION STRUCTURE (BAPS) OF A. FULGENS INTERPRETED THREE POPULATION CLUSTER 1 AND 2 IN GREEN AND BLUE COLOUR REPRESENTING KL-INDIA POPULATION AND CLUSTER 3 IN RED COLOUR REPRESENTING WEST AND CENTRAL ARUNACHAL PRADESH POPULATION OF HRP. THE VORONOI POLYGONS ON THE MAP ALSO REPRESENTED THE POPULATION CLUSTERS. (MAP GENERATED USING ARCGIS: .....	36
FIGURE 8. SAMOVA FCT VALUE REACHED A PLATEAU AT K=3 AFTER WHICH THE FCT VALUE INCREASES SLOWLY BUT SINGLE POPULATION GROUPS WERE STARTED TO APPEAR AND POPULATION STRUCTURE WAS DISAPPEARING. THUS K=3 HAS BEEN ACCEPTED AS THE FINAL GROUP OF POPULATION. ....	37

FIGURE 9. THE DEMOGRAPHIC HISTORY OF RED PANDA POPULATIONS WAS ESTIMATED USING A BAYESIAN SKYLINE PLOT (BSP). (A) HIMALAYAN RED PANDA; (B) CHINESE RED PANDA; (C) HIMALAYAN RED PANDA—KL INDIA POPULATION; AND (D) HIMALAYAN RED PANDA—ARUNACHAL PRADESH POPULATION. THE SOLID LINE SHOWS THE MEDIAN ESTIMATES OF $N_e T$ ( $N_e$ = EFFECTIVE POPULATION SIZE; $T$ = GENERATION TIME), AND THE BLUE AREA AROUND MEDIAN ESTIMATES SHOWS THE 95% HIGHEST POSTERIOR DENSITY (HPD) ESTIMATE OF THE HISTORIC EFFECTIVE POPULATION SIZE. THE TIMING OF EVENTS WAS ESTIMATED ASSUMING A SUBSTITUTION RATE OF $1.2 \times 10^{-7}$ CALCULATED FOR RED PANDA. THE MISMATCH DISTRIBUTION CURVES OF THE PAIRWISE DIFFERENCES OF EACH RED PANDA POPULATION ARE SHOWN WITHIN THE CORRESPONDING BSP GRAPH. ....	38
FIGURE 10. MAXENT OUTCOME FOR KL-INDIA A) ROC CURVE, B) JACKKNIFE AUC, C) RESPONSE CURVES.....	54
FIGURE 11. MAXENT OUTCOME FOR ARUNACHAL PRADESH A) ROC CURVE, B) JACKKNIFE AUC, C) RESPONSE CURVE.....	55
FIGURE 12. BINARY HABITAT SUITABILITY MAP OF RED PANDA IN A) KL-INDIA, B) ARUNACHAL PRADESH .....	55
FIGURE 13. PREDICTED LANDSCAPE CONNECTIVITY MODEL OF RED PANDA IN A) KL-INDIA AND B) ARUNACHAL PRADESH. ....	64
FIGURE 14. INDIVIDUAL IDENTIFICATION IN RED PANDA WITH NINE LOCI. ....	66
FIGURE 15. POPULATION GENETIC STRUCTURE OF RED PANDA POPULATION IN KL-INDIA. A) POPULATION ASSIGNMENT USING STRUCTURE AT K3; B) MAP OF ESTIMATED CLUSTER MEMBERSHIP SHOWING SPATIAL DISTRIBUTION OF THE THREE INFERRED GENETIC CLUSTERS THROUGH GENELAND; C) SPATIAL PCA SHOWING CLUSTERS IN SPATIALLY DISTRIBUTED POPULATIONS; D) EIGEN VALUES OF PCA ESTIMATION SHOWING THREE CLUSTERS IN DAPC, EACH IDENTIFIED BY INDIVIDUAL COLOURS AND INERTIA ECLIPSES. ....	70
FIGURE 16. POPULATION GENETIC STRUCTURE OF RED PANDA POPULATION IN ARUNACHAL PRADESH. A) POPULATION ASSIGNMENT USING STRUCTURE AT K3; B) MAP OF ESTIMATED CLUSTER MEMBERSHIP SHOWING SPATIAL DISTRIBUTION OF THE THREE INFERRED GENETIC CLUSTERS THROUGH GENELAND; C) SPATIAL PCA SHOWING CLUSTERS IN SPATIALLY DISTRIBUTED POPULATIONS; D) EIGEN VALUES OF PCA ESTIMATION SHOWING THREE CLUSTERS IN DAPC, EACH IDENTIFIED BY INDIVIDUAL COLOURS AND INERTIA ECLIPSES. ....	71
FIGURE 17. PERCENTAGE OF MOLECULAR VARIANCE A) KL & B) ARUNACHAL PRADESH .....	73

FIGURE 18. QUESTIONNAIRE SURVEY VILLAGE LOCATION IN ARUNACHAL PRADESH .....	90
FIGURE 19. A) SSP 2-4.5 AND B) SSP 5-8.5 CLIMATIC HABITAT SCENARIO IN KL-INDIA BY 2040 .....	92
FIGURE 20. A) SSP 2-4.5 AND B) SSP 5-8.5 CLIMATIC HABITAT SCENARIO IN KL-INDIA BY 2080 .....	93
FIGURE 21. HABITAT CHANGE IN KL-INDIA BY 2040 AND 2080.....	93
FIGURE 22. SSP 2-4.5 AND SSP 5-8.5 CLIMATIC HABITAT SCENARIO IN ARUNACHAL PRADESH BY 2040 .....	94
FIGURE 23. SSP 2-4.5 AND SSP 5-8.5 CLIMATIC HABITAT SCENARIO IN ARUNACHAL PRADESH BY 2080 .....	95
FIGURE 24. HABITAT CHANGE IN ARUNACHAL PRADESH BY 2040 AND 2080.....	96
FIGURE 25. LULC CHANGE IN KL-INDIA BY 2040 A) LULC 2017 B) LULC 2040 C) CHANGE CLASSES .....	98
FIGURE 26. HABITAT CHANGE DUE TO LULC CHANGE IN KL-INDIA BY 2040 .....	99
FIGURE 27. LULC CHANGE IN ARUNACHAL PRADESH BY 2040 A) LULC 2017 B) LULC 2040 C) CHANGE CLASSES .....	100
FIGURE 28. HABITAT CHANGE DUE TO LULC CHANGE IN ARUNACHAL PRADESH BY 2040 .....	101
FIGURE 29. PROPOSED ARUNACHAL FRONTIER HIGHWAY MAP, ADAPTED FROM THE PRINT .....	101
FIGURE 30. SOCIO ECONOMIC CLASSES OF THE RESPONDENTS .....	103
FIGURE 31. TRIBE WISE RESPONDENTS .....	103
FIGURE 32. SOCIO ECONOMIC CLASS WISE HUNTING PRACTICE .....	104
FIGURE 33. RED PANDA KNOWLEDGE OF RESPONDENTS.....	104
FIGURE 34. SOCIO ECONOMIC CLASS WISE RED PANDA HUNTING .....	105
FIGURE 35. HUNTING PATTERN OF RED PANDA.....	105

# LIST OF PHOTOS

PHOTO 1. HIMALAYAN RED PANDA PHOTO CAPTURED IN TAWANG DISTRICT OF ARUNACHAL PRADESH. (PHOTO CREDIT: DST-INSPIRE RED PANDA PROJECT, CLICKED BY HIREN KHATRI) .....	2
PHOTO 2. IUCN RED PANDA DISTRIBUTION RANGE IN FIVE RANGE COUNTRIES. (PHOTO CREDIT: IUCN RED LIST OF THREATENED SPECIES) .....	3
PHOTO 3. RED PANDA HABITAT IN EASTERN HIMALAYA, INDIA (PHOTO CREDIT: DST-INSPIRE RED PANDA PROJECT) .....	6
PHOTO 4. RED PANDA IN ITS HABITAT, CAPTURED IN SINGHALILA NATIONAL PARK, WEST BENGAL (PHOTO CREDIT: DST-INSPIRE RED PANDA PROJECT).....	7
PHOTO 5. USE OF RED PANDA FUR AND TAIL AS CAP BY MEMBA COMMUNITY PEOPLE IN ARUNACHAL PRADESH, HUNTING EVIDENCES OF RED PANDA IN CENTRAL ARUNACHAL PRADESH, RED PANDA HUNTED FOR TROPHY COLLECTION IN ARUNACHAL PRADESH. (PHOTO CREDIT: DST-INSPIRE RED PANDA PROJECT).....	10
PHOTO 6. STUDY AREA DIFFERENT LANDSCAPES (FIRST ROW: FOREST, HIGH ALTITUDE MEADOW AND OLD GROWTH FOREST; SECOND ROW: GRASSLAND, TRIBAL HOUSE AND FARMLAND IN ARUNACHAL PRADESH; THIRD ROW: SNOW COVERED FOREST OF SIKKIM, TEA PLANTATION IN DARJEELING AND ROAD CUTTING (PHOTO CREDIT: DST-INSPIRE RED PANDA PROJECT) .....	13
PHOTO 7. FIELD SURVEY IN THE DIFFICULT TERRAIN OF ARUNACHAL PRADESH (PHOTO CREDIT: DST-INSPIRE RED PANDA PROJECT).....	21
PHOTO 8. FIELD DATA AND SAMPLE COLLECTION FOLLOWING DNA EXTRACTION, PCR, GEL ELECTROPHORESIS AND DNA QUALITY CHECKING IN THE LAB.....	22
PHOTO 9. LANDSCAPE GENETICS WORKFLOW FOR CORRIDOR MAPPING ....	51
PHOTO 10. QUESTIONNAIRE SURVEY WITH LOCALS IN ARUNACHAL PRADESH .....	91
PHOTO 11. EVIDENCE OF RED PANDA HUNTING BY LOCALS IN ARUNACHAL PRADESH .....	107
PHOTO 12. EMPOWERING TRIBAL COMMUNITIES AND LOCAL STUDENTS THROUGHOUT THE PROJECT JOURNEY .....	116
PHOTO 13. COLLECTION OF PRIMATE SKULLS BY TRIBALS FOR RELIGIOUS CEREMONY .....	117

PHOTO 14. INDIGENOUS HOUSEHOLD AND CULTURE .....	117
PHOTO 15. PEOPLE OF DIFFERENT COMMUNITIES OF ARUNACHAL PRADESH .....	118
PHOTO 16. LIFESTYLE OF LOCAL ADI TRIBE PEOPLE OF ARUNACHAL PRADESH .....	118



# CHAPTER I

## A COMPREHENSIVE OVERVIEW OF A UNIQUE AND MYSTERIOUS HIMALAYAN ANIMAL: THE RED PANDA

### 1.1 The Red Panda: An Endangered Eastern Himalayan Species

Cuvier in 1825 first described the red panda, a fascinating species larger than a domestic cat with a bear-like physique and thick russet hair. This endearing creature has magnificent reddish-brown soft upper body fur and black underbelly, as well as white cheeks, muzzle, brows, and inner ear margins, and a bushy tail with blackish-brown ring-like patterns to help in balance and provide warmth in winter (Roberts and Gittleman, 1984; Fisher, 2021) (Photo 1). Since the species lives in Himalayan old growth forest, the coloration helps it blend in with the crimson moss and white lichen-covered trees. Notably, the red panda's whole body is covered with longer and rougher guard hairs, but the undercoat is thick, woolly, and fluffier for thermoregulation (Roberts and Gittleman, 1984). Furthermore, the soles of the paws are coated with fur that assists the animal in walking effortlessly on snow and slick, uneven terrain (Fisher, 2021). The red panda has a small head with a short snout and triangular ears, as well as equally lengthened limbs (Roberts and Gittleman, 1984; Fisher, 2021). It has a head-to-body length of 51-63.5 cm (20.1-25.0 in) and a tail length of 28-48.5 cm (11.0-19.1 in). While the Himalayan red panda (*Ailurus fulgens fulgens*) weighs 3.2-9.4 kg (7.1-20.7 lb), the Chinese red panda (*Ailurus fulgens styani*) weighs 4.2-13.4 kg (9.3-29.5 lb) for males and 4-15 kg (8.8-33.1 lb) for females (Fisher, 2021), and there is no sexual dimorphism in size or coloration (Roberts and Gittleman, 1984). The red panda also has five curled fingers on each foot, each having curved semi-retractile claws that help in climbing (Roberts and Gittleman, 1984). The pelvis and hind limbs have flexible joints, adapted for an arboreal quadrupedal lifestyle (Makungu *et al.*, 2015). The red panda (*Ailurus fulgens*), an arboreal herbivore of the order Carnivora, feeds mostly on bamboo shoots and leaves (Yonzon and

Hunter, 1991; Wei *et al.*, 1999). Interestingly, both bamboo-feeding giant pandas and red pandas have a "false thumb" on their forepaws, which is a carpal bone called the radial sesamoid that has been expanded to act as an opposable thumb (Antón *et al.*, 2006). This flexible thumb and wrist enable improved dexterity and allow the animal to grab bamboo (Antón *et al.*, 2006). It is also worth mentioning that, despite being specialized herbivores, red pandas lack a ruminant stomach; instead, they have a carnivore-like digestive tract that is only 4.2 times the length of their body, with a simple stomach, no discernible boundary between the ileum and colon, and no caecum (Fisher, 2021).



*Photo 1. Himalayan Red Panda photo captured in Tawang district of Arunachal Pradesh. (Photo credit: DST-Inspire Red Panda project, clicked by Hiren Khatri)*

Red pandas have attracted the interest of scientists and wildlife enthusiasts alike due to their striking appearance and unique habitat. Their existence in old-growth forests in the mid and upper Himalayas is a vital indicator of the health of these ecosystems. These adorable creatures flourish in temperate conifer and cold broadleaf forests with extensive bamboo undergrowth, which may be found in the eastern Himalayas at elevations ranging from 1500 to 4800 meters (A. Choudhury, 2001), with a preferred altitudinal



range between 2300 and 4000 meter (Glatston *et al.*, 2015). Red pandas are found in five countries: Nepal, Bhutan, India (Sikkim, West Bengal, Arunachal Pradesh, and a possible population in Meghalaya), China (Yunnan and Sichuan provinces, as well as south-eastern Tibet), and northern Myanmar (Yonzon and Hunter, 1991; A. Choudhury, 2001; Pradhan, Saha and Khan, 2001; Dorji *et al.*, 2012; Kandel *et al.*, 2015; Wei *et al.*, 2022) (Photo 2). Despite their widespread distribution, their number has dropped dramatically over the last 20 years, with an estimated 50% reduction in their wild population. There might be just 2500 viable individuals remaining in the wild (Glatston *et al.*, 2015). Because of this substantial reduction, the red panda is now listed as "Endangered" on the IUCN Red List, and the Convention on International Trade in Endangered Species (CITES) has listed it under *Appendix I* to prevent any illegal trade of the species (Xu and Guan, 2018; Badola *et al.*, 2020). In order to safeguard this unique species, Wildlife (Protection) Act of India of 1972 designated the red panda as a *Schedule I* protected mammal (A. Choudhury, 2001) in India.

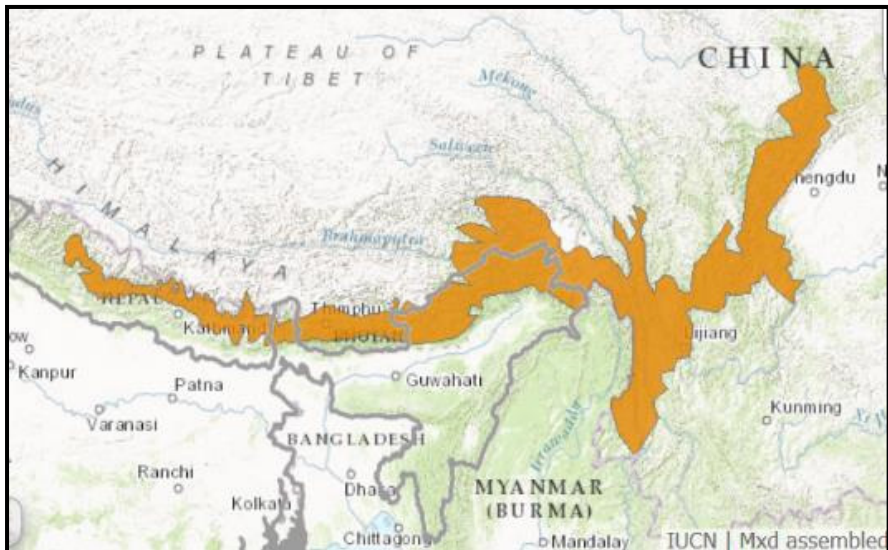


Photo 2. IUCN red panda distribution range in five range countries. (Photo credit: IUCN Red List of Threatened Species).

Red pandas are also considered living fossils, having an ancestral fossil distribution record stretching from China in the east to Britain in the west (Mayr, 1986), and a fascinating evolutionary history that has long been debated by scientists. It was formerly included in the same evolutionary lineage as Ursidae, but further genetic research have put it in its own family Ailuridae within the clade Musteloidea, which also contains Procyonidae (raccoons), Mustelidae (weasels), and Mephitidae (skunks) (Sato *et al.*, 2009). Further, Red pandas were believed to exist in two subspecies, *i.e.* *Ailurus fulgens fulgens* (Himalayan subspecies) and *Ailurus fulgens styani* (Chinese subspecies) based on morphological evidences (Glatston, 1994; Wei *et al.*, 1999; Li *et al.*, 2005; Liu *et al.*, 2005; Liang *et al.*, 2007; Hu *et al.*, 2011). However, a new high-depth sequencing data-based taxonomic categorization of Red Pandas revealed two separate phylogenetic species, the Himalayan Red Panda (HRP) *Ailurus fulgens*, and the Chinese Red Panda (CRP), *Ailurus styani*, which split via the Yalu Zangbu River (Hu *et al.*, 2020). Furthermore, the research revealed that CRP has better genetic diversity and experienced historic population expansion in China, but HRP has historically experienced three bottlenecks, resulting in reduced genetic diversity and higher genetic load, raising the concern of timely conservation activities. Unfortunately, no active monitoring or genetic evaluation of the species has been done in India in order to build conservation action plans or devise site-specific interventions for the species' long-term survival in the Indian Himalayan Region (IHR). To conserve this unique and endangered species, dedicated conservation efforts are urgently required in the identified suitable habitats within the distribution area of red panda populations in the IHR.

## **1.2 The Basis of Our Investigation: Investigating the Rationale for Our Proposed Work**

Studies conducted in all range countries have found that many factors pose a threat to red pandas. These include the destruction and degradation of their forest habitat, poaching, the illegal trade of their pelt, the unsustainable harvesting of medicinal and herbal plants from their habitat, forest fires, killing by guard dogs, tourist disturbances, bamboo flowering, a lack of regulatory law enforcement, and inadequate public awareness (A. Choudhury, 2001; Pradhan, Saha and Khan, 2001; Mallick, 2010; Thapa, Hu and Wei, 2018). Additionally, many Himalayan tribal and underprivileged people depend on hunting for subsistence, cultural practices, and supplementary revenue (Xu and Guan, 2018; Badola *et al.*, 2020; Bista *et al.*, 2020). Unfortunately, ineffective law enforcement and a

lack of knowledge in these areas hamper conservation efforts. Red pandas are very sensitive to habitat disturbance and degradation because they are a habitat-specific species with a transboundary range (Bista *et al.*, 2022). Red panda habitat will soon be greatly affected by the ongoing infrastructure projects in the Himalayas, including the "Belt and Road's Trans-Himalayan Economic Corridor," the "China-Nepal Railway Corridor," and the recently proposed "Trans-Arunachal Highway" and "Arunachal Frontier Highway" project of Indian Government (Mann *et al.*, 2021; Li *et al.*, 2022). Deforestation caused by infrastructure projects leads to the fragmentation of ecosystems, resulting in the formation of smaller patches or forest "islands." This phenomenon has a significant impact on the mobility of many arboreal species, such as red pandas, leading to inbreeding and a reduction in genetic diversity due to restricted movement. Previous research has warned that red panda distribution is disjointed and existing populations may not be genetically contiguous (Dalui *et al.*, 2020; Hu *et al.*, 2020), raising concerns about assessing the genetic makeup of red panda populations across their range. In addition, the study of red pandas in India is limited due to their elusive behaviour, preference for solitude, and diurnal activity patterns (Choudhury, 2001; Pradhan, Saha, & Khan, 2001; Ghose *et al.*, 2011; Chakraborty *et al.*, 2015). Red pandas are found in the Eastern Himalayas in India, specifically in mountainous regions of Sikkim, northern West Bengal (limited to the Darjeeling and Kalimpong district), and Arunachal Pradesh (Photo 3 & Photo 4). There is a possibility of a separate population in Meghalaya, although this has not been confirmed (Choudhury, 2001). The overall range of red pandas in IHR covers approximately 170,000 square kilometres, but their actual occupied area is much smaller, estimated to be around 25,000 square kilometres, and supports approximately one-third of the global red panda population (Choudhury, 2001; Glatston *et al.*, 2015). A genetic assessment of this population is required to learn more about their genetic makeup, population structure, and gene flow. These insights would provide conservationists with the means to ascertain the most efficient techniques for the conservation of these creatures. To date, there has been a lack of genetic research undertaken in India on crucial elements such as the genetic structure of the population, gene flow, population demographics, and the effectiveness of forest corridors as habitat links for red pandas in the Eastern Himalayas. The absence of thorough research signifies a substantial deficiency in information, which impedes our capacity to execute efficient conservation strategies. In contrast, extensive research has been conducted in China regarding the genetics of red pandas. This includes the development of molecular markers (Li *et al.*, 2005; Liu *et al.*, 2005; Liang

et al., 2007; Hu et al., 2011; Yang et al., 2019), non-invasive genetics-based population estimation (Hu et al., 2011), phylogeography (Su, Fu, Wang, Jin, et al., 2001; Li et al., 2005), demographic history, and population genetic structure (Hu et al., 2011, 2020). Further, recent genome-based high-throughput sequencing data have shown that red pandas diverged into two separate phylogenetic species roughly 0.22 million years ago during the Late Pleistocene (Hu et al., 2020). It has been postulated that species diversified during Pleistocene glacial cycles through the direct separation of populations into distinct allopatric refugia, a phenomenon also known as the Pleistocene species pump (Avice, 2000; Hu et al., 2020). However, additional research is necessary to determine whether the phylogenetic speciation of the red panda is a consequence of the 'Pleistocene species pump' or has a more ancient and profound basis. Therefore, the objective of this present study was to examine the genetic composition of the red panda in the IHR in relation to its evolutionary history. Additionally, the study aims to assess current threats, including human-induced changes to natural habitats, climate change, and illegal hunting of red pandas for trade. Finally, the study proposes conservation and management strategies to safeguard red pandas in the IHR.



*Photo 3. Red Panda Habitat in eastern Himalaya, India (Photo credit: DST-Inspire Red Panda project)*



*Photo 4. Red Panda in its habitat, captured in Singhalila National Park, West Bengal (Photo credit: DST-Inspire Red Panda project)*

### **1.3 Charting the Course: Goals and Objectives**

- I. Uncovering the evolutionary mysteries of red pandas in the eastern Himalayas: impact of landscape, climate, and human activities on demographic history.
- II. Exploring the genetic secrets of red panda populations in the Eastern Himalayas: genetic diversity, inbreeding, population genetic structure, and gene flow.
- III. A race against time: mapping the current distribution, status, and looming threats to red panda survival in the majestic Eastern Himalayas.

## 1.4 Investigating the Literature: A Comprehensive Review of Existing Studies

In comparison with other high-altitude mountain-dwelling species such as the giant panda (*Ailuropoda melanoleuca*), snow leopard (*Uncia uncia*), and Himalayan musk deer (*Moschus leucogaster*), published information on the red panda (*Ailurus fulgens*) is limited. Unfortunately, most studies have been conducted in only a few countries, primarily China and Nepal, leaving significant gaps in the documentation. Previous surveys have revealed that more than 77% of suitable red panda habitats in Nepal are located outside of protected areas and are under anthropogenic pressure (Yonzon and Hunter, 1991; A. Choudhury, 2001; Kandel et al., 2015; Thapa, Hu and Wei, 2018; Shrestha et al., 2019). Similarly, surveys in Bhutan have confirmed the presence of red pandas in 13 districts (Dorji et al., 2012), whereas the distribution data of red pandas in Myanmar is underrepresented (Lin et al., 2021). Red panda distribution in China is limited to Sichuan, Yunnan, and Tibet, with CRP found in Sichuan and northeastern Yunnan provinces and HRP found in Tibet and southern Yunnan (Wei et al., 2022). Surveys in India have mapped the habitat and distribution range of the red panda, with Arunachal Pradesh in the eastern Himalayas having the most habitat (A. Choudhury, 2001; Pradhan, Saha and Khan, 2001; Mallick, 2010; Ghose et al., 2011; Chakraborty et al., 2015). Furthermore, the global potential habitat of the red panda has been estimated using various modelling-based approaches (A. Choudhury, 2001; Kandel et al., 2015; K. Thapa et al., 2020; Tobgay and Mahavik, 2020; Dong et al., 2021). However, the estimated potential red panda habitat area varies due to inconsistent methodologies. According to various studies on habitat ecology and suitability, different forest types such as evergreen forests, evergreen and deciduous mixed broad-leaf forests, deciduous forests, deciduous and coniferous mixed forests, and coniferous forests with associated bamboo thicket understories, dense canopy, fallen logs, bamboo density, elevation, slope, aspect, and distance to water are important environmental variables for red panda habitat (Yonzon and Hunter, 1991; Pradhan, Saha and Khan, 2001; Dorji, Vernes and Rajaratnam, 2011; Zhang et al., 2011; Zhou et al., 2013; A. Thapa et al., 2020; Wei et al., 2021). Due to the species' shy and secretive nature, as well as its crepuscular habits, only a few studies on behaviour (Pradhan, Saha, and Khan, 2001) and movement ecology (Zhang et al., 2011) have been conducted in the wild. Molecular genetic studies on the red panda have recently flourished across its range of countries. Non-invasive genetic research and the development of new genetic markers for red pandas have been successful (Liang et al., 2007; Yang et al., 2019). Analyses based

on mitochondrial DNA (mtDNA) and microsatellites revealed a high level of genetic diversity in Chinese red pandas (Su, Fu, Wang, Jin, et al., 2001; Li et al., 2005; Liu et al., 2005; Hu et al., 2011). Several previous studies attempted to investigate subspecies differentiation using mtDNA markers but failed to detect significant lineage divergence (Su, Fu, Wang, Jin, et al., 2001; Li et al., 2005; Liu et al., 2005; Hu et al., 2011). However, recent genome-based study (Hu et al., 2020) concluded that red pandas have diverged into two phylogenetic species. In comparison with CRP, HRP has a higher genetic load and a rapidly declining population trend, according to the same study.

Further studies have identified the most serious threats to the red panda as habitat degradation, loss, and fragmentation, deforestation, demand for firewood, logging (A. Choudhury, 2001; Mallick, 2010), illegal wood and forest products collection (Zhou *et al.*, 2013), and livestock grazing (Dorji, Vernes and Rajaratnam, 2011; Sharma, Belant and Swenson, 2014; Sherpa *et al.*, 2021). The second most important threat was identified to be poaching and illegal trading (Photo 5). Poaching and illegal trafficking were identified serious threats to red pandas in a recent study (Bista *et al.*, 2020) conducted in Nepal. Poaching was considered a threat prior to the establishment of the national parks in India (Pradhan et al., 2001b). The use of red panda fur to make hats and clothing in China (Wei et al., 1998), live trapping of the red panda for exhibition (Wei et al., 1999), and supply to zoos (Pradhan, Saha and Khan, 2001; Mallick, 2015) were common in the 20<sup>th</sup> century. One of the serious threat in Nepal, is the occurrence of red pandas being attacked and killed by guard dogs and predation (Yonzon and Hunter, 1991; Mallick, 2010; Sharma, Belant and Swenson, 2014). Human activities such as road construction (A. Choudhury, 2001; Pradhan, Saha and Khan, 2001; Acharya *et al.*, 2018; Dendup *et al.*, 2020) and forest fires for “Jhoom” cultivation (A. Choudhury, 2001; Mallick, 2010) have also been identified as threats to the red panda. Though red pandas are recognised to benefit local communities through tourism (Sharma, Belant and Shaner, 2019; Dorji *et al.*, 2022); however, a few of the articles reported threats to red pandas from tourism, such as the potential risk of habitat loss from the extraction of wood for cooking and heating purposes for tourists (A. Choudhury, 2001) and tourism-related developmental activities (Bista, Greg S. Baxter, *et al.*, 2022).

Overall, most research on the red panda is outdated, scarce, and unevenly distributed across its range countries. More research is needed to understand the ecology, behavior, genetics, and recent threats to this elusive and endangered species.





*Photo 5. Use of Red Panda fur and tail as cap by Memba community people in Arunachal Pradesh, Hunting evidences of Red Panda in central Arunachal Pradesh, Red Panda hunted for trophy collection in Arunachal Pradesh. (Photo credit: DST-Inspire Red Panda project)*



## **1.5 Exploring the Mountains and Forests: An Overview of the Study Area**

The transboundary Kangchenjunga Landscape (KL) shared by India, Nepal, and Bhutan is one of the Central Himalayas' most biologically diverse regions, and it is home to the Himalayan Red Panda (HRP) (Figure 1 a, b). KL—India is located in the Central Himalayan biotic province, spanning between 26° 21' 40.49–28° 7' 51.25 N and 87° 30' 30.67–90° 24' 31.18 E, and includes the states of West Bengal (Darjeeling and Kalimpong district only) and Sikkim, which are classified as biogeographic province 2C (Central Himalayas), and the north of Sikkim as biogeographic province 1C (Trans-Himalayas-Sikkim). The terrain is mountainous, and the habitat types include tropical, subtropical, warm temperate, cool temperate, subalpine, and alpine forest types (Champion and Seth, 1968) (Photo 6). The study landscape contains ten protected areas (PAs), including Singalila National Park (SNP), Senchal Wildlife Sanctuary (SWLS), and Neora Valley National Park (NVNP) in North West Bengal, and Barsey Rhododendron Sanctuary (BRS), Maenam Wildlife Sanctuary (MWLS), FambongLho Wildlife Sanctuary (FLWLS), Pangolakha Wildlife Sanctuary (PWLS), Kanchenjunga National Park (KNP), Shingba Rhododendron Sanctuary (SRS), and Kyongnosla Alpine Sanctuary (KAS) in Sikkim. Although the majority of these PAs are relatively small in size, they collectively contribute approximately 3,112.21 square kilometres of land area (Figure 1b). The PAs in this landscape, on the other hand, are dispersed as 'Conservation islands,' lacking the connectivity required for species to thrive and sustain themselves, and natural corridors that were once intact are now deteriorating.

On the other hand, the largest known distribution of red pandas in India (approx. 78 percent) is in the state of Arunachal Pradesh, where the Dibang Wildlife Sanctuary of 4149 sq. km. area supports a population of red pandas that is possibly the largest in India (A. Choudhury, 2001). (Figure 1 a, c). Arunachal Pradesh (91°36'–93°13'E, 26°52'–27°58'N, 83,743 sq. km) is unique in its location in the transition zone between the Himalayan and Indo-Burmese regions (Mishra, Madhusudan, and Datta, 2006) and is one of the world's most important biodiversity hotspots and ecoregions (Myers et al., 2000) (Figure 1c). The state shares borders with Bhutan, China, and Myanmar, and it is home to a large number of endemic and endangered species (Myers et al., 2000; Hu et al., 2021). The state's altitudinal range extends from 100 m to over 6000 m asl, resulting in a wide variety of habitats and forest types. Lowland tropical evergreen and semi-evergreen

forests exist up to 1500 m asl., with temperate oak and conifer forests up to 3500 m asl. and alpine areas at the highest elevations (Photo 6).

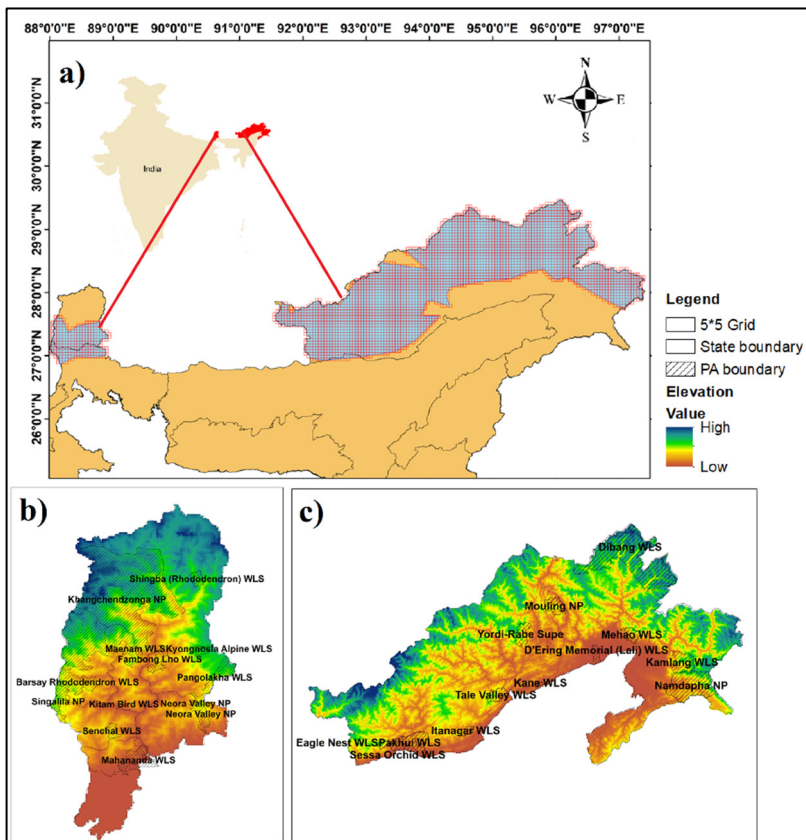


Figure 1. Study area Map. a) Red Panda IUCN distribution range in India with 5\*5 Km grid, b) KL-India with PA borders, c) Arunachal Pradesh with PA borders.

A mere 11 percent of the state is occupied by two national parks (Namdapha National Park & Tiger Reserve and Mouling National Park) and nine wildlife sanctuaries (Eagle-Nest WLS, Sessa-Orchid WLS, Pakke WLS, Itanagar WLS, Tale-Valley WLS, Kane WLS, Yorde-Rube Supse WLS, D'Ering-Memorial WLS, Dibang WLS, Mehao WLS, Kamlang WLS). Despite the fact that 23 percent of the state is above 3000 m, Arunachal's protected areas primarily cover low- and mid-elevation forests, leaving high-altitude habitats vulnerable. Along with its natural diversity, the state

is home to 26 major tribes and numerous subtribes, each with their own cultural and social identities as well as a distinct regional distribution. The communities practice hunting in various forms, and recent population increases, coupled with rapid changes in tribal communities' lifestyles and economies over the last few decades, have threatened the habitats of wild fauna in the landscape (Aiyadurai, 2011).



*Photo 6. Study area different landscapes (First row: Forest, high altitude meadow and old growth forest; Second row: Grassland, Tribal house and farmland in Arunachal Pradesh; Third row: Snow covered forest of Sikkim, Tea plantation in Darjeeling and road cutting (Photo credit: DST-Inspire Red Panda project).*

## **1.6 The Scientific Methodology: Data Collection and Analysis Procedures**

### **1.6.1 The Red Panda Quest: Investigating Approaches to Occurrence Data Collection**

The pursuit of elusive red pandas in the untamed wilderness proved to be a difficult task. We searched the terrain for signs of the elusive creature in the previously known distribution sites of West Bengal, Sikkim, and Arunachal Pradesh's Protected and Non-Protected Areas (PAs and non-PAs). We trekked through the wilderness, following transects and remote camera traps

to collect important data on the species' habitat and distribution (Photo 7). However, our investigation was not limited to the field. In red panda habitat range villages, we also conducted structured interviews and questionnaire surveys with community members and the forest department. The insights gained from these discussions assisted us in filling gaps in our data and providing a more comprehensive understanding of the red panda's range and locations (Pradhan, Saha and Khan, 2001; Alpizar-Jara, 2006). In addition, we combed the archives of the Global Biodiversity Information Facility (<https://www.gbif.org/>) for additional information on previous sightings of the species. We were able to build a more complete picture of the red panda's distribution by examining all available sources. We laid out a grid of 5 x 5 km sections in the red panda's habitat to ensure we covered as much ground as possible (Figure 1a). We surveyed the feasible grids that fell within this area every year, from March to May and October to December, combing the land for direct sightings and indirect signs like scat and feeding sites. Using the quadrat method, we documented vegetation and habitat characteristics, noting elevation, slope, and distance from human habitation at every interval of 150-250 metres. Following the methodology by (Pradhan, Saha and Khan, 2001), sampling plots, including animal central plots and random plots, were also established. We recorded GPS coordinates of each site where signs of red pandas were observed.

### **1.6.2 Methods and Insights Regarding Non-Invasive DNA Sample Collection**

It was a daunting task to survey the vast distribution range of the elusive red panda. It was impossible to cover every inch due to the rugged terrain and dense, inaccessible forests. Instead, we took a landscape approach, focusing on the species' most commonly reported habitats. From 2018 to 2022, our team conducted intensive field surveys in the Central and Eastern Himalayas, collecting both opportunistic and representative faecal samples of red pandas (Figure 2). In total, 298 faecal pellet samples were collected and stored in silica gel or 70% ethanol before being subjected to genomic DNA extraction using the QIAmp Fast DNA Stool Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol. We washed tissue and hair samples in Phosphate-Buffered Saline (PBS) before extracting DNA with the Phenol-Chloroform Isoamyl (PCI) method. We began PCR amplification after ensuring the quality of the extracted DNA using 1% agarose gel electrophoresis (Figure 3) (Photo 8).

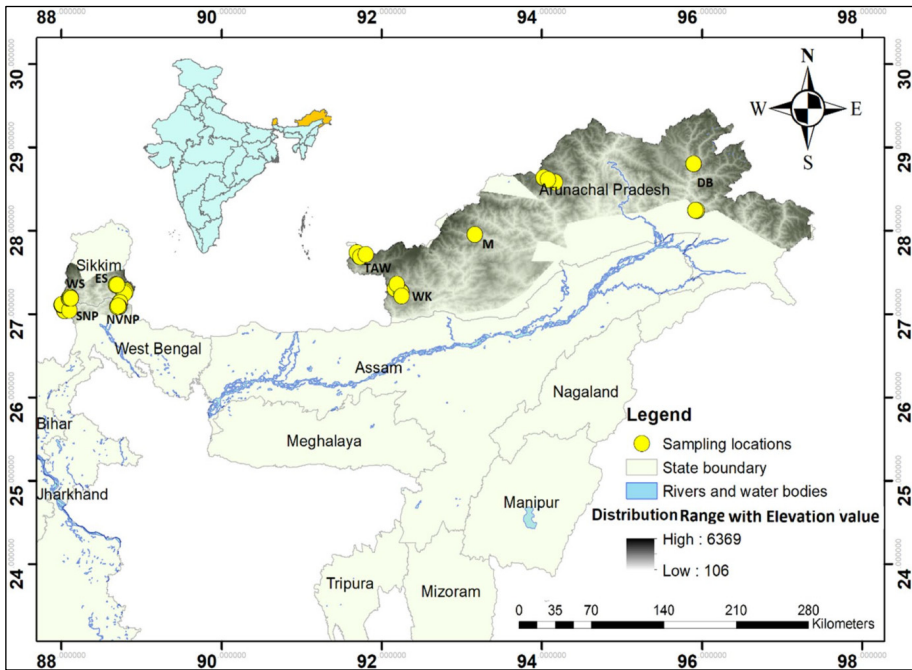


Figure 2. Study are map showing the sampling locations in Central and Eastern Himalayas

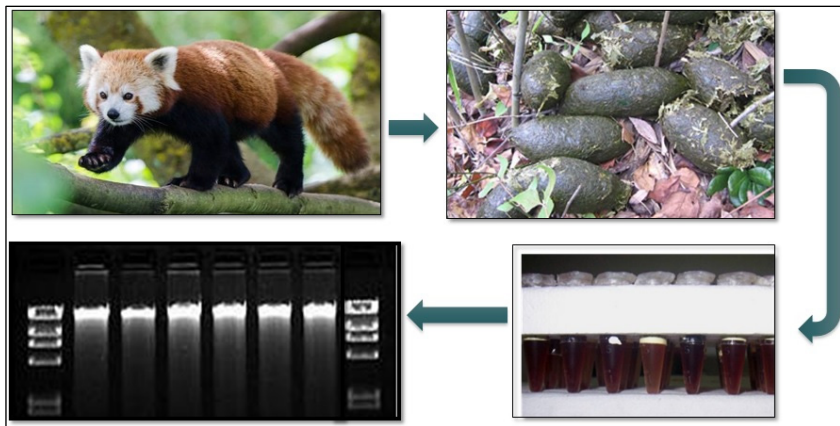


Figure 3. Quality of DNA isolated from fecal samples

## **1.7 Species Identification Using Mitochondrial DNA: Gene Amplification and Sequencing**

The investigation of red pandas was undertaken through the implementation of a polymerase chain reaction (PCR) technique using genomic DNA samples. By employing red panda-specific primers previously utilised in research conducted by Su, Fu, Wang, Jin, et al. (2001) and Li et al. (2005), we were able to effectively sequence a 440 base pair fragment of the control region (CR) of mitochondrial DNA. The Veriti thermal cycler (Applied Biosystems, USA) was employed by our research team to amplify the DNA. The amplification process involved the employment of a mixture consisting of 1U of Taq polymerase (Takara), 10x PCR buffer, 1 mM MgCl<sub>2</sub>, 2.5 mM dNTPs mix, 0.1 µM of each primer, and 0.1 µg/µL BSA. After conducting 35 cycles, the intended sequences were successfully obtained by employing meticulous thermal cycling settings. These conditions involved an initial denaturation step at a temperature of 94 °C for a duration of 5 minutes, followed by subsequent steps of 30 seconds at 94 °C, 45 seconds at 50 °C, and 1 minute at 72 °C for each cycle. The final extension step was conducted at a temperature of 72°C for a duration of 10 minutes.

In order to guarantee the precision of our results, we employed the Big-Dye Terminator Cycle Sequencing Kit 3.1 (manufactured by Thermo Scientific, USA) in conjunction with an ABI 3730 Genetic analyzer (manufactured by Applied Biosystems, USA). The sequences were subjected to manual verification using Sequencher v5.4.6 (Gene Codes Corporation, USA) in order to identify and rectify any base mistakes, as well as to remove poly-A tails. Additionally, each sequence was subjected to a nucleotide BLAST analysis in order to verify the species identity of the red panda. In order to further investigate the population diversity of red pandas, we obtained the consensus sequences of their control region from the online database/GenBank (Table 1). The MUSCLE algorithm (Edgar, 2004) used in the MEGA X programme (Kumar et al., 2018) was utilised to align the generated sequences with the provided sequences. This enabled us to conduct a phylogenetic analysis and make an estimation of population demography (Discussed in Chapter II).