Conservation of the Red Panda in the Eastern Himalayas

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Insights from the Indian Himalayan Region

By

Supriyo Dalui, Mukesh Thakur and Lalit Kumar Sharma

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TABLE OF CONTENTS

List of Tables

LIST OF FIGURES

LIST OF PHOTOS

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.

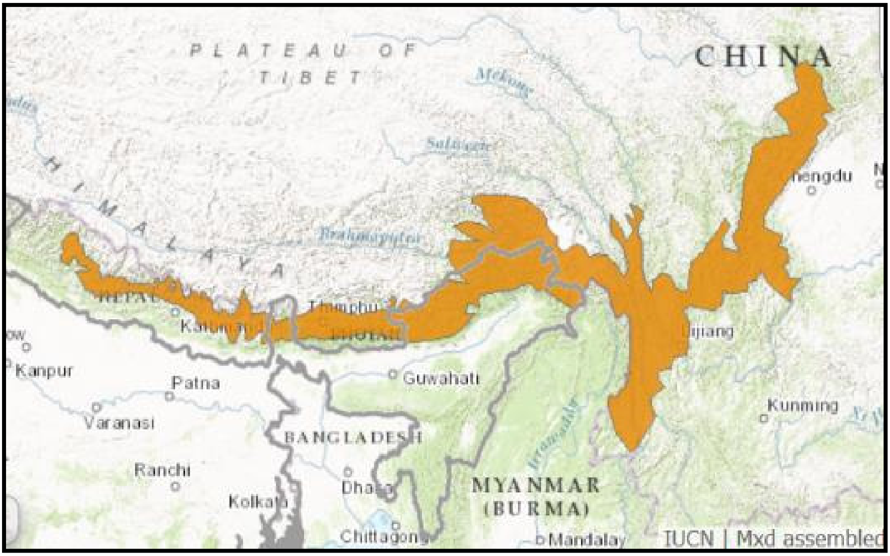

Photo 2. IUCN red panda distribution range in five range countries. (Photo credit: IUCN Red List of Threatened Species).

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 (Avise, 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 20th 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).

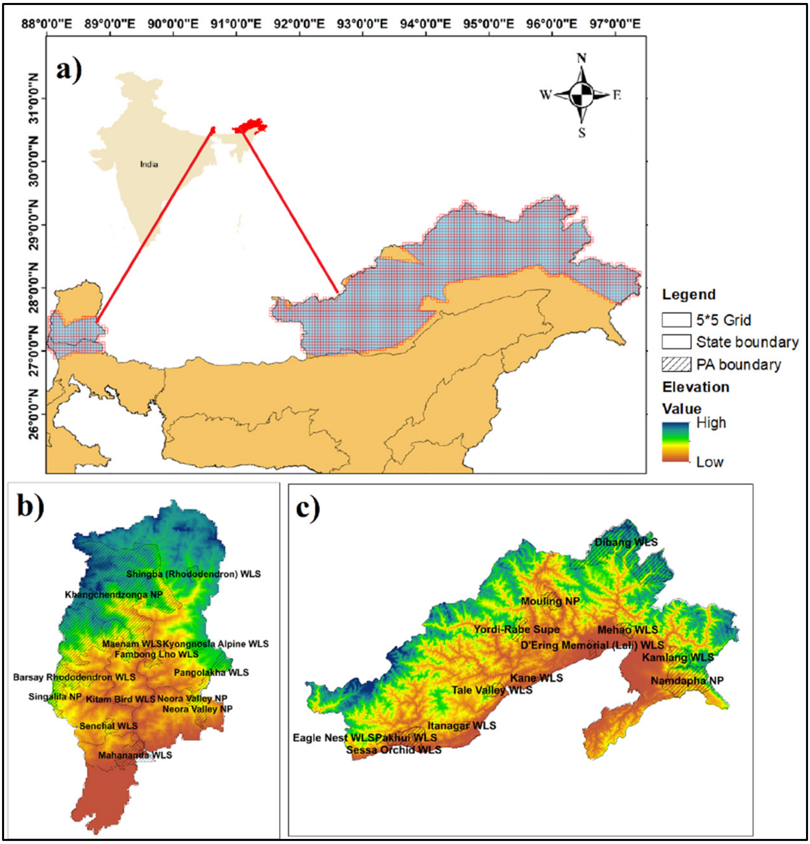

Figure 1. Study area Map. a) Red Panda IUCN distribution range in India with 5*5 Km grid, b) KL-India with PA borders, c) Arunachal Pradesh with PA borders.

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).

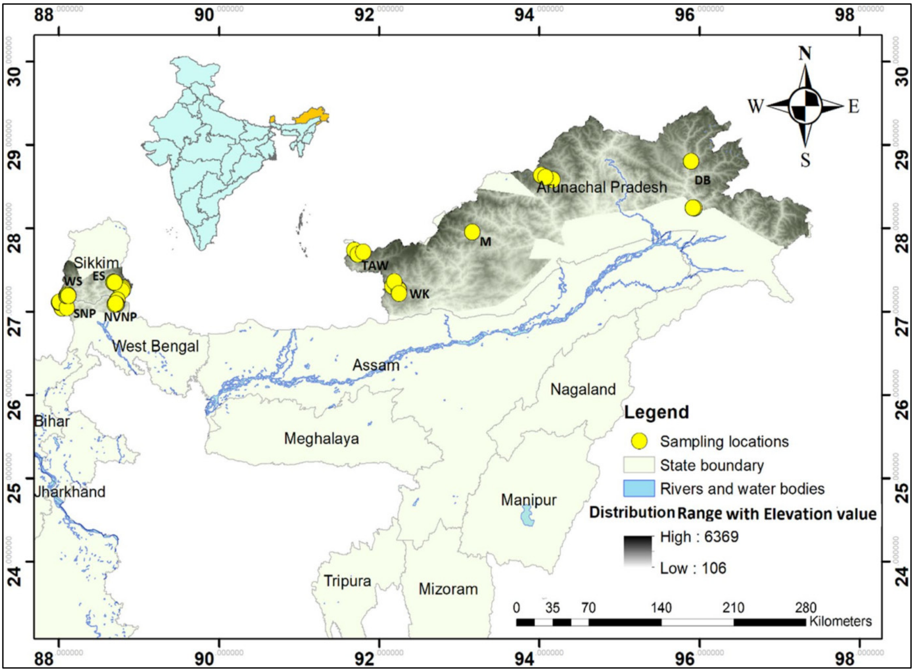

Figure 2. Study are map showing the sampling locations in Central and Eastern Himalayas

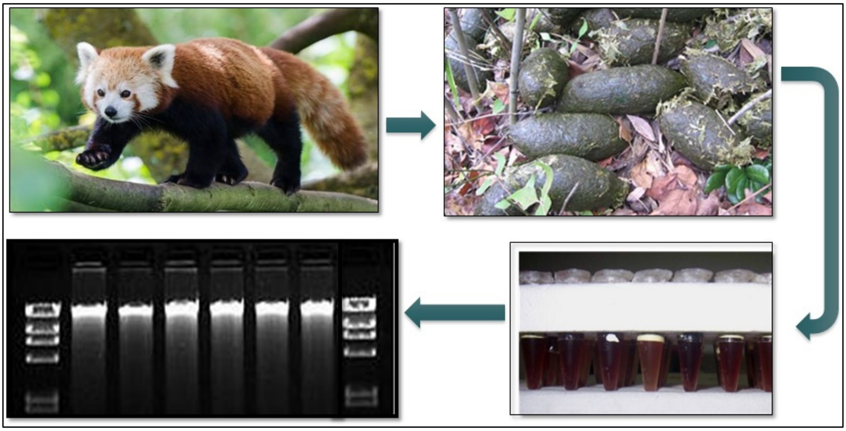

Figure 3. Quality of DNA isolated from fecal samples

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 MgCl2, 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).