Final Evaluation Report

Your Details	
Full Name	Laxman Khanal
Project Title	Blending fine-scale exploration, socioecology and conservation genetics to conserve enigmatic Assam macaques in Nepal
Application ID	37648-B
Date of this Report	27 June 2024

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Assamese macaque population and distribution survey			■	Population and distribution surveys were done from all the planned study areas. Several new troops of macaques from previously unreported areas have been discovered.
Conservation genetic analysis		■		Fecal sampling and laboratory analyses have been completed. Two mitochondrial loci, CYTB 1140 bp and control region 1090 bp were sequenced for 95 samples of macaques. DNA sequence data analysis has been partially done and is ongoing.
Awareness campaigns			■	A total of six awareness campaigns were completed involving 123 locals from the

				areas of Assamese macaques.

2. Describe the three most important outcomes of your project.

i. Discovery of Assamese macaque troops from previously unreported areas in Nepal.

From this project, we have been able to know the detailed distribution of Assamese macaques in Nepal (Figure 1). Many new troops of Assamese macaques were discovered from previously unreported areas. New records of Assamese macaque troops in forests of eastern Nepal and temples of Ilam (Sano Pathivara Temple) and Jhapa (Shiva Satashi Dham) districts were noteworthy. An extensive field survey in the Gaurishankar Conservation Area in central Nepal identified several troops and sampled them for genetic analysis. For the first time, we reported the occurrence of Assamese macaques from the Bheri River Basin of western Nepal. The finding of new troops from western Nepal has been published in a peer-reviewed journal (https://doi.org/10.3126/njz.v8i1.67114).

ii. Fine-scale population genetic analysis of the Nepal population of Assamese macaques and its integration with the DNA sequence data from other parts of the Himalayas to understand the historical phylogeography of the sinica-group of macaques.

The newly generated sequences were integrated with homologous sequences from GenBank submitted by the previous studies (Khanal et al. 2018, Khanal et al. 2021) and analyzed for the population genetic structure.

A total of 242 sequences of the mitochondrial control region (1090 bp) of the Assamese macaques were analysed. The population genetic analysis revealed 58 unique haplotypes of Assamese macaques based on the mitochondrial control region sequences. The haplotype diversity was high (0.971 ± 0.04) but nucleotide diversity was low (0.0098 ± 0.0003). The genetic structure of the subpopulations was not clearly delineated, however, the haplotypes from Ilam, Panchthar, and Jhapa of eastern Nepal and far-western Nepal were isolated from the rest of the population as there was no haplotype sharing. Therefore, those populations qualify for the genetic management units (MUs). In the TCS haplotype network (Figure 4), the haplotypes from Koshi River basin of eastern Nepal and the Gandaki River basin of central Nepal were interwoven indicating a recent diversification and dispersal. The maximum likelihood (ML) phylogenetic tree among the 58 haplotypes (Figure 5) also supported the results of the TCS network. These findings further clarified the hypothesis of Khanal et al. (2018) about the glacial refugia of Assamese macaques in the river valleys of central Nepal and the post-LGM expansion of the population in Nepal Himalaya.

By integrating the DNA sequence data generated during this project with the sequences from previous studies and recent sampling from Tibet by our Chinese collaborators, we analysed the phylogeography of the *sinica*-group of macaques. The analyses revealed that the Assamese macaques currently considered as the western subspecies, is a distinct species. The manuscript with the phylogeographic analysis of the genetic data is currently under review in an SJR Q1 journal.

iii. Community awareness campaigns

We conducted six awareness campaigns during this project, two each in eastern, central and western Nepal. The areas for the awareness campaigns were selected based on the observation of the Assamese macaques in the wild habitats and records of human-macaque conflicts.

In eastern Nepal, awareness campaigns were conducted in the Sandakpur Rural Municipality of the Ilam District and Falelung Rural Municipality of the Panchthar District. A total of 49 local people (26 and 23) participated in the campaigns.

In central Nepal, two awareness campaigns were conducted in Gaurishankar Rural Municipality of Dolakha District and Bhotekoshi Rural Municipality of Sindhupalchowk District involving 46 local people. In western Nepal, one awareness campaign was conducted in among 27 locals from Barekot Rural Municipality and another one involving 21 locals in Aathbishkot Municipality of West Rukum District.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

Several unforeseen difficulties arose during the project. Some of the major issues have been listed below.

i. Delay in project beginning: The project start was delayed due to a delay in releasing the project funds to the organisation's account. The Government of Nepal enforced a rule that non-governmental organizations receiving funds from abroad should get approval from the Social Welfare Council before receiving the funds. Because of this, the project was started 1 month after the original plan.

ii. Earthquake in western Nepal delayed the fieldwork: Our team had planned fieldwork in Jajarkot District, western Nepal from the middle of November to the first week of December 2023. Unfortunately, an earthquake of 5.7 Richter scale struck Jajarkot District, Karnali Province, Nepal, at 23:47 NPT (18:02 UTC) on 3 November 2023 killing almost 200 people and injuring almost 400. Therefore,

we had to postpone our fieldwork to March-April 2024. In March and April of 2024, we conducted fieldwork and awareness campaigns in Jajarkot and West Rukum districts.

iii. Faecal samples processing for DNA extraction and PCR: Our genetic analyses are based on the DNA sequences from the non-invasively collected faecal samples. The DNA retrieved from fecal samples is degraded and contains low concentrations. Additionally, it contains PCR inhibitors which prevent the PCR and sequencing. We employed multiple methods to enhance the DNA retrieval with some modifications in the protocol of the DNA kit used. Finally, we were able to retrieve the DNA sequences from 77.23% of the fecal samples.

4. Describe the involvement of local communities and how they have benefitted from the project.

Community awareness campaigns were carried out during the project (as explained above). Six such campaigns were conducted involving the locals from the areas where Assamese macaques were recorded and had some negative interactions.

In all the fieldwork, field assistants were hired from the local communities who helped in the Assamese macaque population and distribution survey as well as during the awareness campaigns.

The involvement of local people in the campaign helped them understand the diversity of macaques; their conservation and legislative status; causes, effects and prevention techniques of human-macaque conflicts; the importance of biodiversity conservation for human welfare, etc.

5. Are there any plans to continue this work?

We plan to explore conservation threats to Assamese macaques further and contribute scientifically to their management. During this project, some troops of Assamese macaques were observed in human habitats including temples in Ilam, Jhapa, and Sindhupalchowk districts. Those macaques were aggressive, begging for food from people, and snatching food. Many individuals in each troop were injured, often cleft leaps, broken legs, amputated tails, etc. (Figures 2a–c). They were seen foraging on the anthropogenic wastes (Figures 2d–f), and even snatching food off the visitors and local stores. From preliminary analyses of gastrointestinal parasites in fecal samples of these macaques, we found that the temple macaques have a higher prevalence of protozoan and helminth parasites than the wild ones (Figure 3). The risk of interspecific parasitic transmission between the Assamese macaques and humans is high in those shared environments. Additionally, we found the temple macaques more

aggressive to humans and free-ranging dogs. Therefore, it is important to explore the type of interaction of those macaques with local people and other fauna including domestic and stray dogs. We plan further studies on Assamese macaques in anthropogenic environments and their behavioral and parasitic dynamics.

6. How do you plan to share the results of your work with others?

We plan to share the findings of this project in three major forms- scientific publications, local newspaper articles and workshops. We have analysed the data and prepared two manuscripts. One scientific paper about the new distribution records of Assamese macaques in the Bheri River basin has been recently published in a peer-reviewed journal (Nepalese Journal of Zoology; https://doi.org/10.3126/njz.v8i1.67114). Another manuscript based on genetic analysis is under processing in a reputed international journal. We will prepare newspaper articles from our findings and publish them in national daily newspapers. We organised one workshop at Tribhuvan University and shared the major results among the students of wildlife biology at Tribhuvan University and wildlife biologists in Kathmandu.

7. Looking ahead, what do you feel are the important next steps?

Assamese macaques are legally protected in Nepal. However, most of their habitats lie outside of the protected area system. In many places, they are close to people, and human-macaque conflict incidents are common. Therefore, assessing the level of negative interactions between humans and Assamese macaques is inevitable for their conservation and management.

The Assamese macaque populations in Nepal have a low level of genetic diversity and shallow genetic structure. They have a disjunct distribution in fragmented forest patches. The subpopulations in far-eastern Nepal (Ilam, Taplejung, and Jhapa districts) and far-western Nepal (Karnali-Mahakali River basin) do not share haplotypes with those of central Nepal. Those subpopulations have genetic uniqueness, bear small troop sizes and fragmented distributions, and are genetically distinct from others. Therefore, subpopulations of Assamese macaque in far-eastern Nepal and western Nepal should be treated as management units (MUs).

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

Yes, in banners used for the awareness campaigns. We acknowledged the support of The Rufford Foundation for this project in all the campaigns and shared the information with the local field assistants and participants. The foundation received publicity during the implementation of the project. The funding support of the Rufford Foundation has been acknowledged in the published paper and will be done in upcoming papers too.

9. Provide a full list of all the members of your team and their role in the project.

Principal investigator Laxman Khanal: Dr. Khanal, the principal investigator of this project performed field surveys, sampling, laboratory analysis of the samples, analysis of the data, and ran the conservation awareness programs. He guided the young members of the team about the primate field survey techniques, identification of the macaques and their age-sex categories, non-invasive collection of fecal samples from the macaques, laboratory processing of the samples for DNA extraction, polymerase chain reaction (PCR), DNA sequence assembly and analyses, etc.

Co-investigator Naresh Pandey: Mr. Naresh Pandey, an MSc in Zoology (Ecology) from Tribhuvan University was involved in field survey, fecal sampling and conservation awareness campaigns.

Co-investigator Asmit Subba: Mr. Asmit Subba, an MSc in Zoology (Ecology) from Tribhuvan University was involved in field surveys, non-invasive sampling, conservation awareness campaigns, and laboratory analysis of the fecal samples. He has learnt the basic tools for genetic analysis from the PI and assisted in the analysis of mitochondrial DNA sequences.

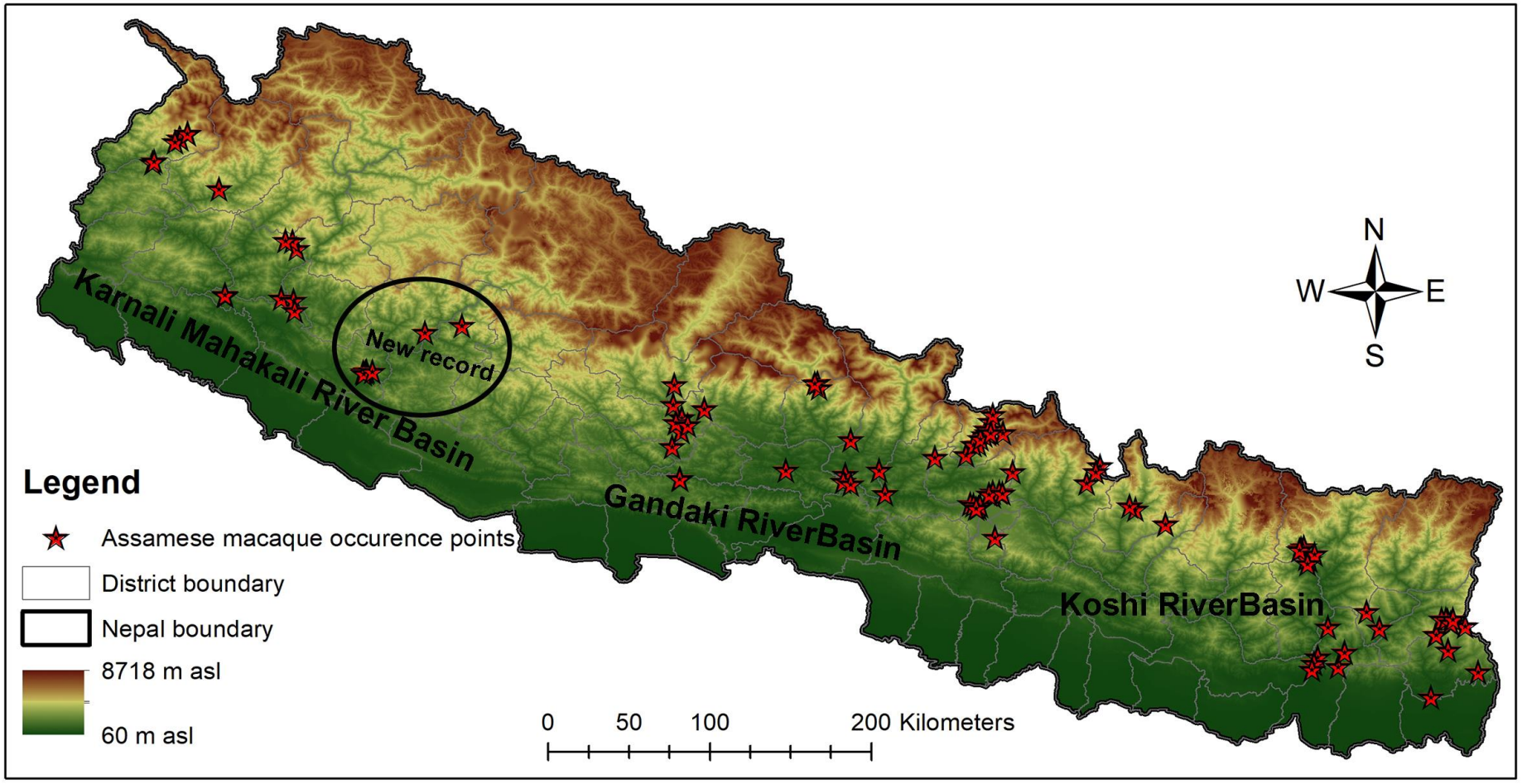
Field and laboratory assistants: Laxmi Prasad Upadhyaya, Sapana Ulak and Minu Silpakar were involved in the field survey, laboratory analysis and data analysis.

10. Any other comments?

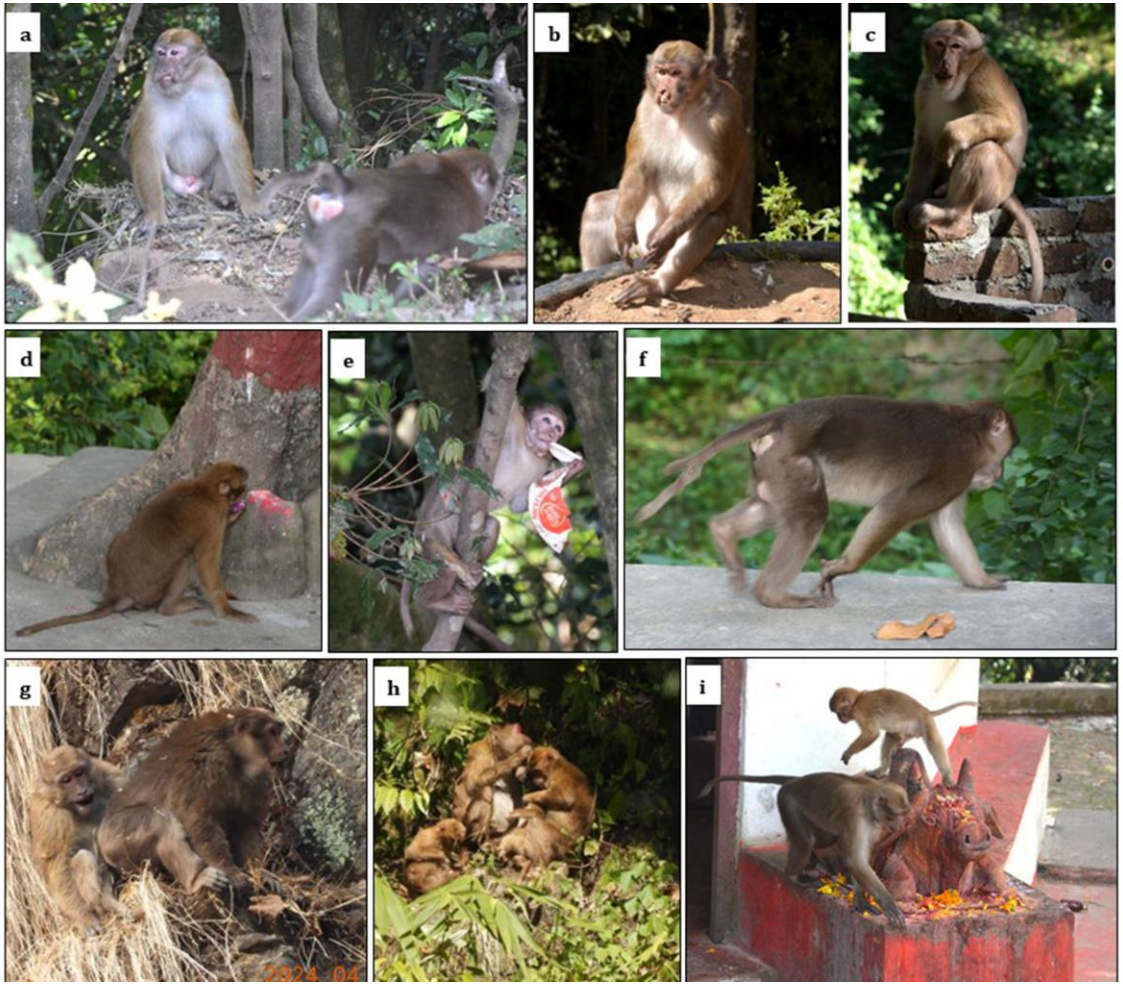
We would like to thank The Rufford Foundation, UK for the project grant. We express our sincere thanks to the local field assistants who provided invaluable support in field surveys and awareness campaigns. We extend our appreciation to Kamal Rai, Temba Sherpa, Ram Sherpa and Resh Bir Rai from Ilam and Panchthar districts; Man bahadur Lama, Gyaljen Lama, Badri Prasad Wagle

and Ganesh Thapa from Gaurishankar Conservation Area; Gobinda Bahadur Singh, Bhabilal Thapa, Bijay Chand, Dhrubraj Magar, Dhan Bahadur RC from Jajarkot and West Rukum districts for their support in the field surveys and awareness campaigns. We are thankful to all the participants of the awareness campaigns for their active participation. Thanks are due to local people who participated actively in the awareness campaigns.

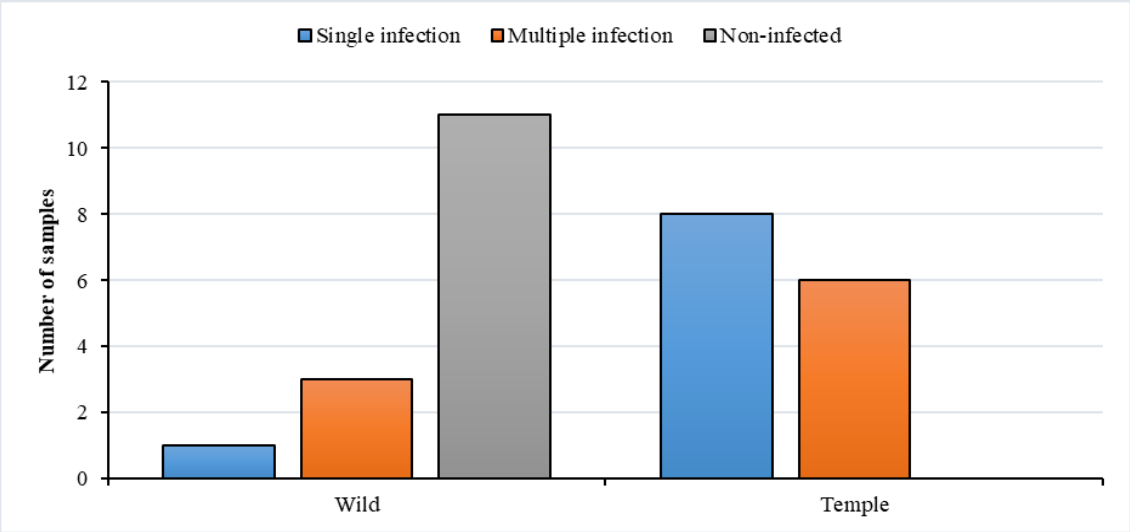
1. **An updated distribution map of Assamese macaques in Nepal.**



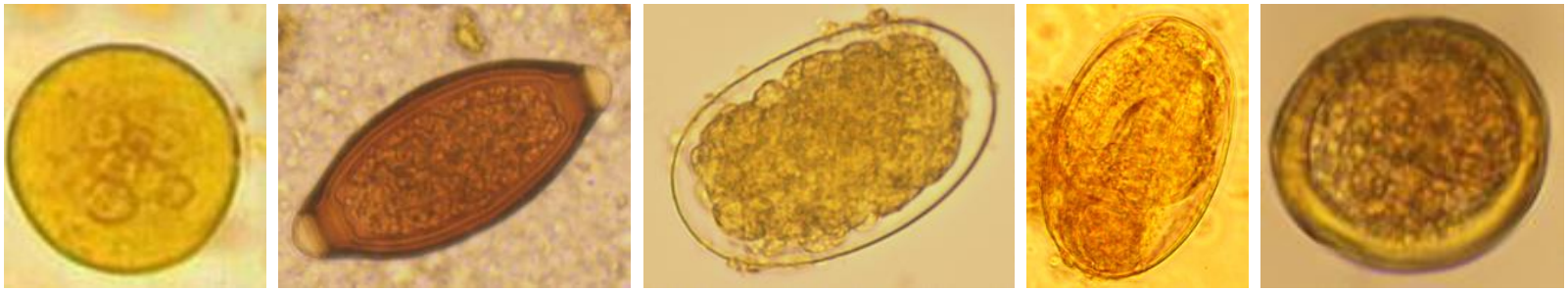
2. Some selected photographs of Assamese macaques from different parts of Nepal. Photos a–c depict injuries on macaques; d–f depict macaques foraging anthropogenic food; g–h show macaques in the wild; and 'i' shows macaques in the temple.



3. Gastro-intestinal parasites prevalence in Assamese macaques from Nepal

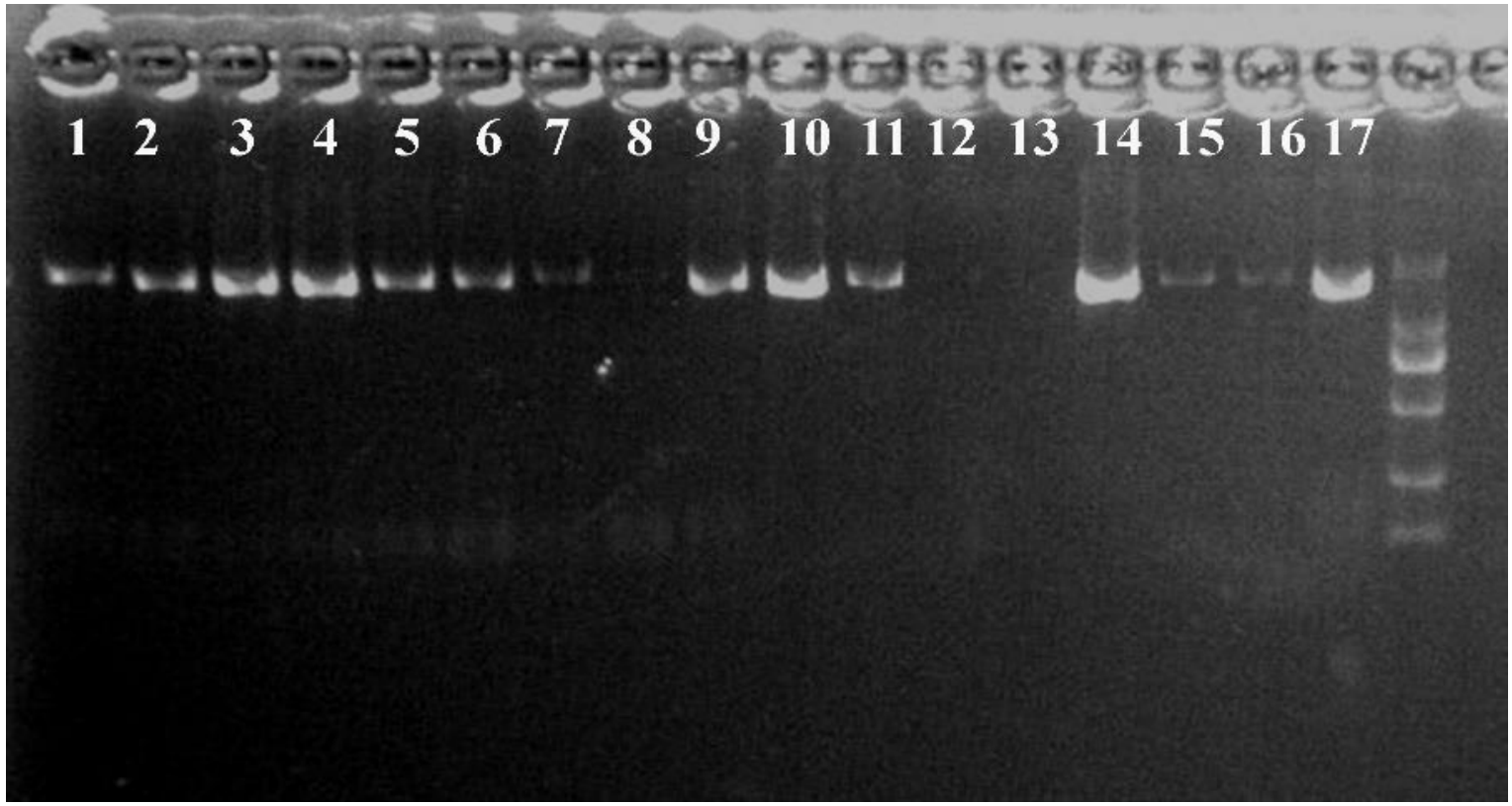


Prevalence of intestinal parasites in the wild and temple population of Assamese macaques (Based on the type of parasites)

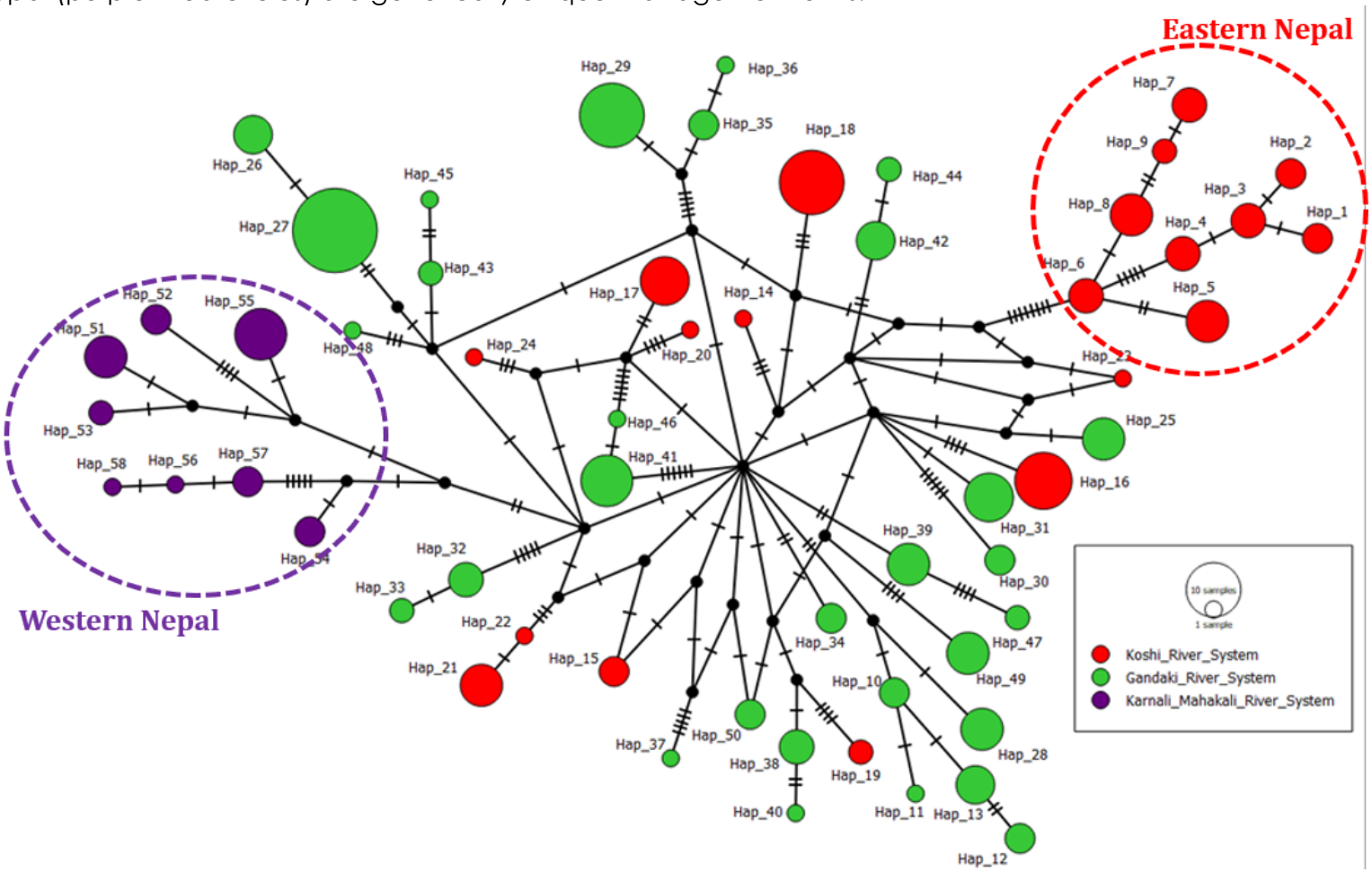


3. *Entamoeba coli* *Trichuris* sp. *Ancylostoma* sp. *Strongyloides* sp. *Ascaris* sp.

4. **The image of gel electrophoresis for PCR products of the CytB gene (1140 bp) of the Assamese macaques**. Seventeen samples with a negative control were used to run PCR on each batch. The second last column in the image is a 1500 bp DNA marker.



4. **TCS haplotype network of Assamese macaques of Nepal based on mitochondrial control region sequence haplotypes (1090 bp).** A total of 58 unique haplotypes were identified. The populations from eastern Nepal (red-filled circles) and western Nepal (purple-filled circles) are genetically unique management units.



6. **Maximum likelihood (ML) phylogenetic tree showing the phylogenetic relationship among the Assamese macaque haplotypes based on the control region sequences**. Colours correspond to the sampling localities as in Figure 4.