Progress Report

Blending fine-scale exploration, socioecology and conservation genetics to conserve enigmatic Assam macaques in Nepal

37648-B - Progress Report
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1. Background

Assamese macaques (*Macaca assamensis*) are the members of the *sinica*-species group of macaques. They are distributed in Southeast and South Asia. The species is known to have two distinct subspecies- the eastern Assamese macaque (*Macaca assamensis assamensis*) and the western Assamese macaque (*M. a. pelops*). Brahmaputra River is believed to demarcate the spatial distribution of the two species (Choudhury, 2022). The Nepal population of Assamese macaques is morphologically different from the population in eastern India and Southeast Asia (Molur et al., 2003). A recent genetic assessment also suggested the distinct species status of the population (Khanal et al., 2021).

The Assamese macaque population is distributed in the mid-hills of Nepal (Chalise, 2013) and it is a habitat specialist requiring a broad-leaved riverine forest (Khanal et al., 2019). Genetic analysis suggested that in response to the Late Quaternary climatic fluctuations, Assamese macaques experienced a range shift in the past (Khanal et al., 2018) and ongoing anthropogenic climate change could have significant effects on the future survival and distribution of the population (Khanal et al., 2023). More than half of the population currently resides outside protected areas (Khanal et al., 2019, Khanal et al., 2023) and incidents of human-macaque conflict, especially driven by crop-raiding, are high. Habitat loss and alteration as well as retaliatory killings continue to threaten this small population of Assamese macaques. Therefore, given its low genetic diversity, phylogenetic distinctiveness, small population size, sporadic distribution, fragmented habitat, and ongoing anthropogenic pressure the Assamese macaques in Nepal require a high conservation priority (Khanal et al., 2021).

This study aims to assess the fine-scale geographical survey of the Assamese macaques in previously unsampled areas and collect information such as their population status, distribution, and conservation threats. Besides, it also aims to collect faecal samples for the conservation genetic analysis. Additionally, the project aimed at conducting awareness campaigns in the areas where Assamese macaques and humans are involved in negative interactions.

2. Fieldwork and findings

Fieldwork was conducted in the eastern Nepal from 15 October to 12 November 2023. Assamese macaque surveys were conducted in Jhapa, Ilam, Panchthar, Tehrathum and Bhojpur districts of eastern Nepal. Whenever a troop of Assamese macaques was encountered during the survey, a detailed population census was carried out. Local people in the area were interviewed about the level of human-macaque conflict. From each troop, faecal samples were collected separately for DNA analysis and gastrointestinal parasitic analysis.

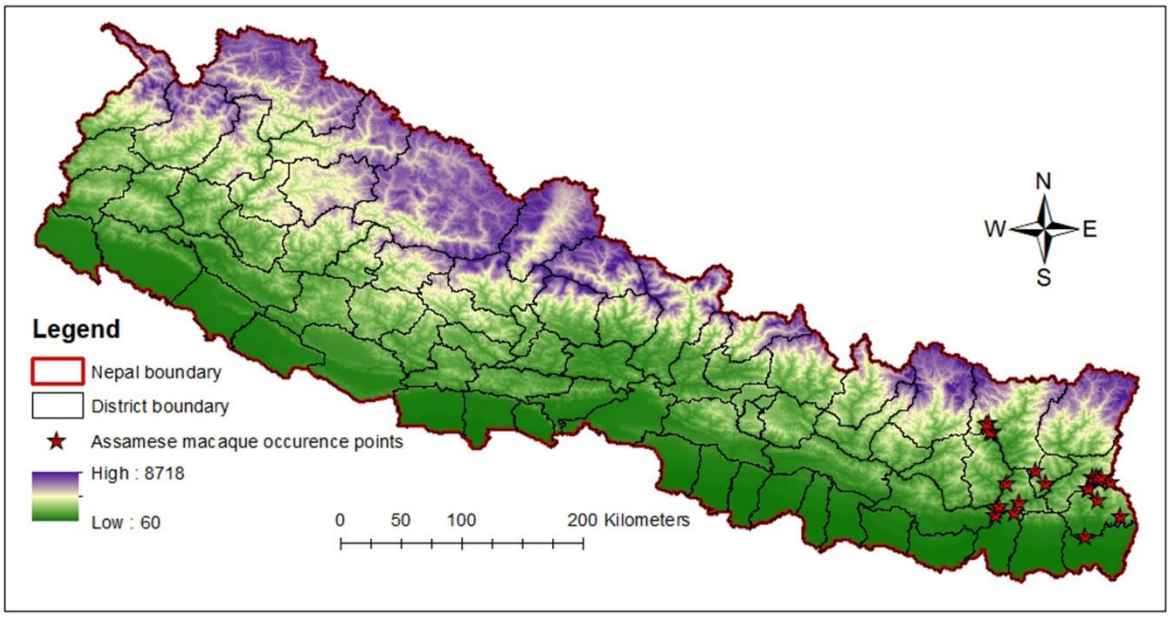


Figure 1. Map of Nepal showing the Assamese macaque occurrence points in eastern Nepal based on our recent surveys.

A total of 39 faecal samples of Assamese macaques have been collected from seven different troops for DNA extraction purposes. Fresh faecal samples that still retained moisture, were collected for further genetic analysis following the methods of Khanal et al. (2018). Faecal samples were collected in sterilized plastic vials with 2ml of lysis buffer (White & Densmore, 1992). Sterilized cotton swabs were used for the collection of faecal samples. A dry cotton swab was rolled on the surface of the faeces and dipped in lysis buffer to recover epithelial cells from the faeces. This process was repeated at least three times per faecal pellet. The faecal sample was turned over and similar swabbing was done using another cotton swab. The faecal samples were stored in the lysis buffer at ambient temperature and transferred to the lab of the Central Department of Zoology, Tribhuvan University for DNA extraction.

Besides that, 29 fresh faecal samples were also collected for gastrointestinal parasitic analysis. Macaques were systematically followed for the collection of faecal samples. Fecal samples of macaque were collected from Satashi Dham Temple of Jhapa (n =14) and the forest area around Ilam and Panchthar (n = 15) by purposive method. A faecal sample was collected from macaque without causing any harm or disturbance to animals and stored in a clean vial containing 2.5% potassium dichromate solution. The samples were properly labelled indicating the name of the collector, species, sample number and date of collection. A separate spatula was used to prevent contamination, and masks and gloves were used to protect the researchers and the samples as well as other precautions to maintain sample integrity.

3. Laboratory works

3.1 Laboratory processing of the samples for DNA extraction and PCR amplification

The faecal samples are being processed in the molecular laboratory of the Central Department of Zoology, Tribhuvan University. Total genomic DNA was extracted from faecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN, Germany). PCR amplifications of mtDNA fragments encompassing the entire control region (CR) and cytochrome B (CYTB) loci are

being done. The successful amplifications will be tested by electrophoresis in 1.5% agarose gel.

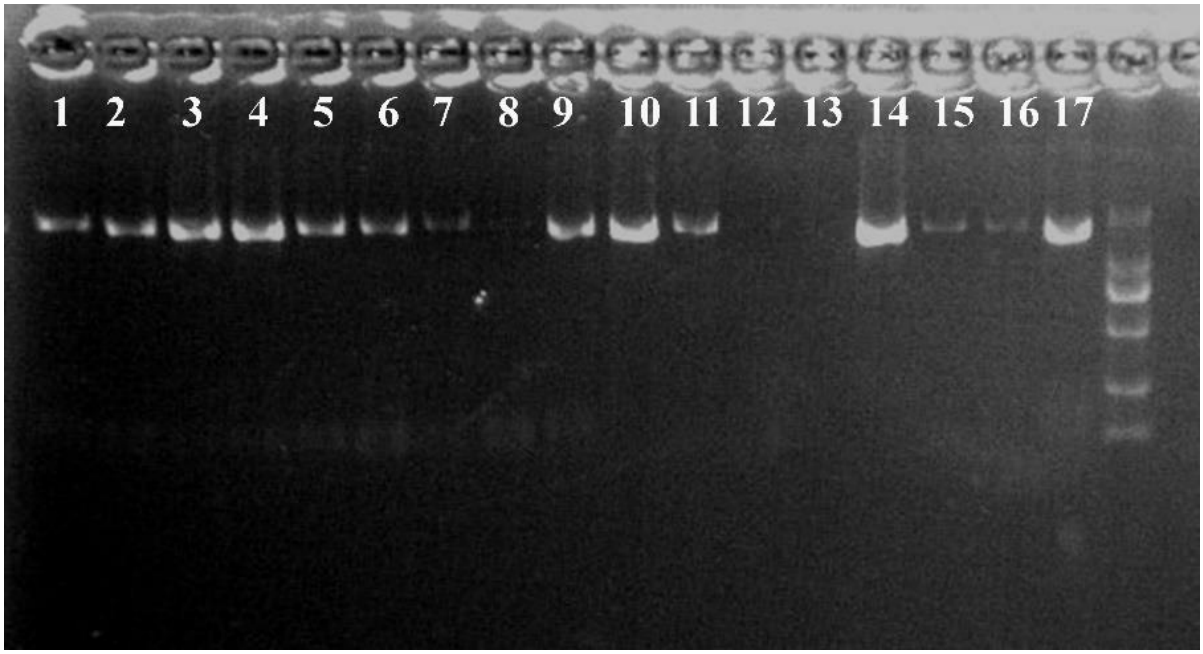


Figure 2. The image of gel electrophoresis for PCR products of the CytB gene (1140 bp) of the Assamese macaques. Seventeen samples with a negative control were used to run PCR on each batch. The second last column in the image is a 1500 bp DNA marker.

3.2 Laboratory analysis for gastrointestinal parasites

Microscopical examination of faecal samples was done for the identification of trophozoites, eggs, cysts and larval stages of GI parasites by direct smear method and concentration method. The direct smear method was used for the identification of different helminth eggs or larvae and protozoal cysts, oocysts, and trophozoites by wet preparation i.e. unstained smear preparation and stained smear preparation. The concentration method was based on two procedures: sedimentation and floatation. Sedimentation was used to isolate eggs of flukes, nematodes and tapeworms that do not float in common floatation solutions. The floatation method employed saturated salt solution as a fluid floatation medium. The slides were examined under a microscope at 10× and 40× magnification with Lugol's iodine (Zajac et al., 2012).

Of the 15 wild macaque samples, 26.67% (n=4) had parasite infection. Out of the 14 temple macaque samples, all 14 samples 100% (n=14) had an infection. One-way ANOVA test showed a higher prevalence of GI parasites in wild forest macaque than in Temple monkeys with a significant difference ($p<0.05$).

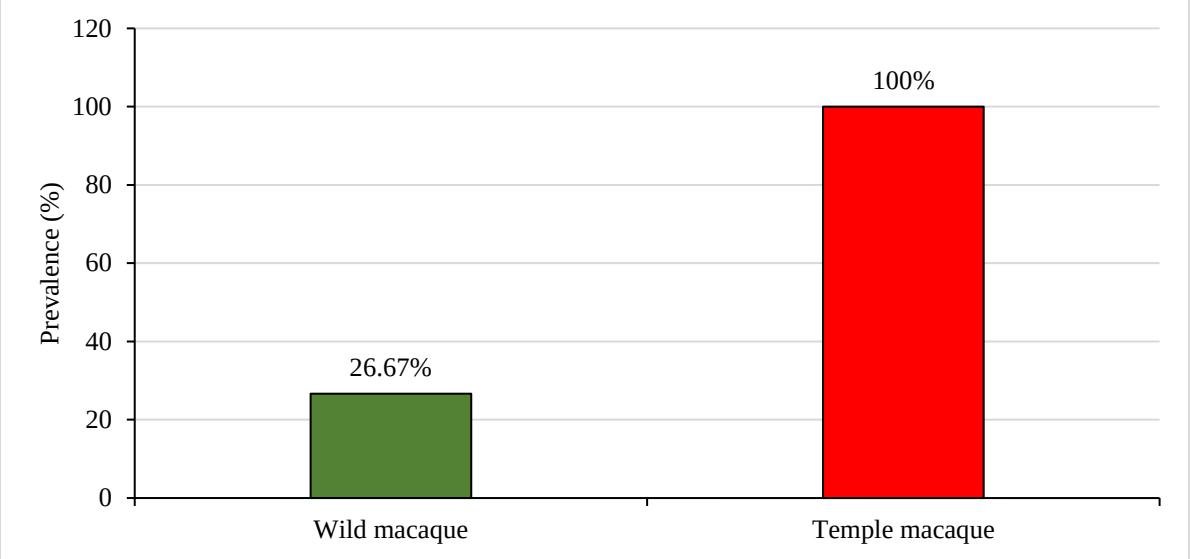


Figure 2. Prevalence of parasitic infestations in Assamese macaque of two areas.

The result indicated that Assamese Macaque suffered from single or multiple parasite infections, either protozoan or helminth parasites. The laboratory examination of the total 15 samples of wild macaque of Panchthar and Ilam showed that 73.33% (n=11) of the samples were found to be negative for the parasites. Out of 4 positive faecal samples, 6.67% (n=1) of the sample had a single infection, and 20% (n=3) had multiple parasitic infections. Similarly, 14 samples of temple macaque of Jhapa showed 100% (n=14) samples to be positive for the presence of at least one type of parasite. Among these 57.14% (n=8) of the samples were found to have a single infection and 42.86% (n=6) of them had multiple parasitic infections (Fig. 3).

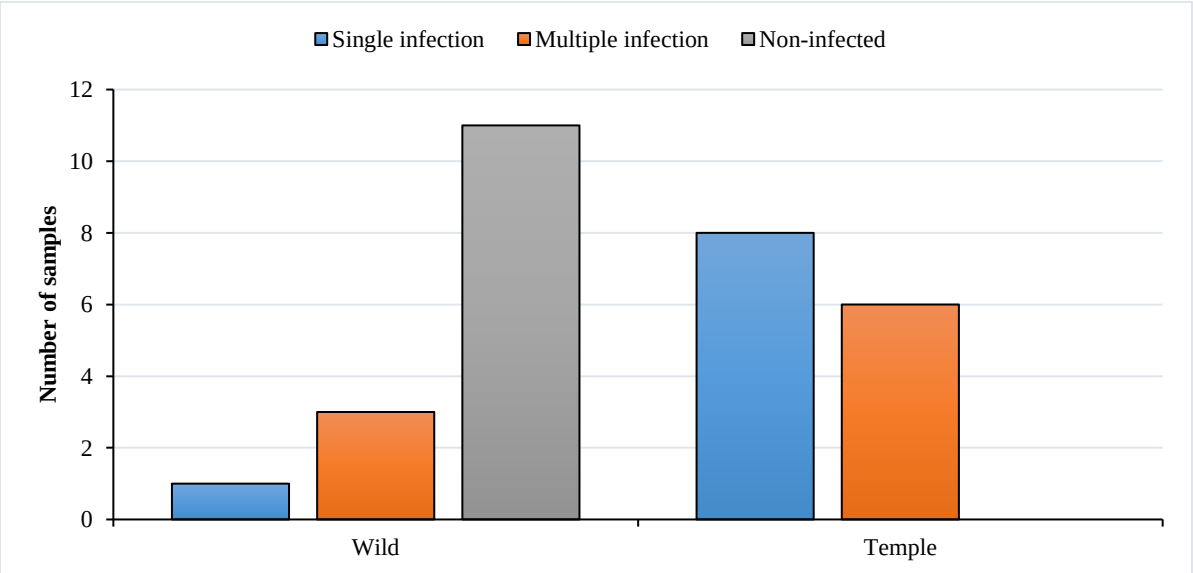
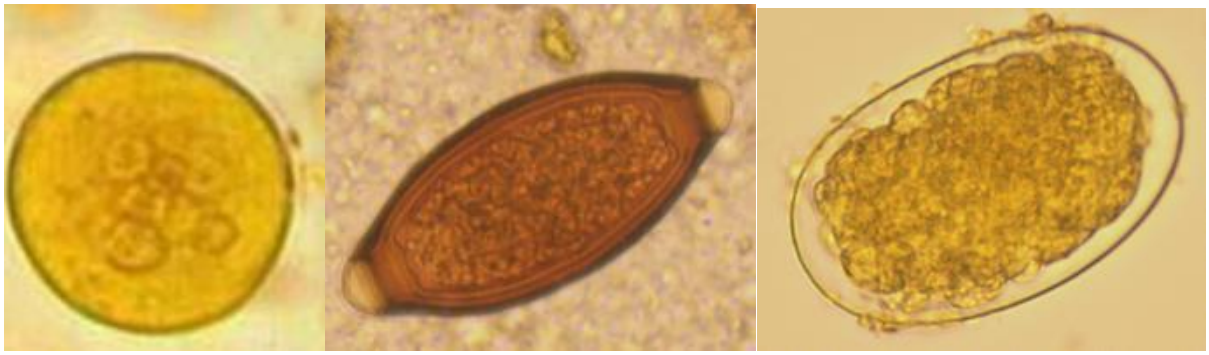
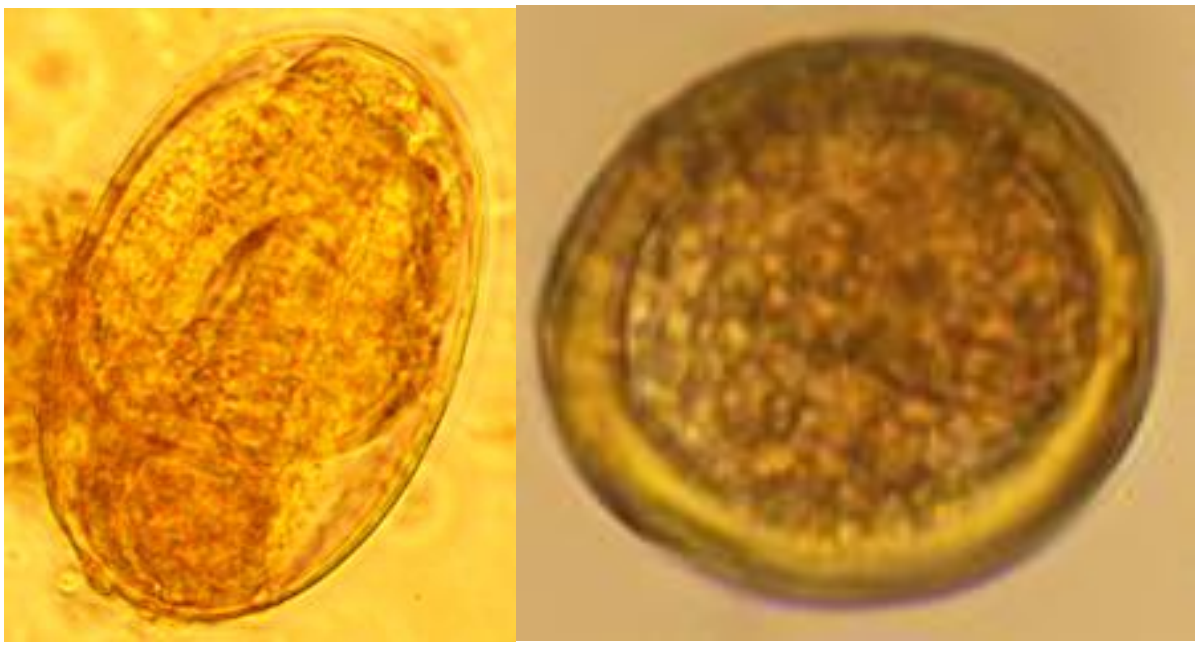


Figure 3. Intensity of intestinal parasites (Based on the type of parasites).

The study showed the presence of one species of protozoa which includes *Entamoeba coli* 18% and helminths include four different species which are *Ancylostoma* sp. 9%, *Ascaris* sp. 26%, *Strongyloides* sp. 26% and *Trichuris* sp. 21%. Among these, *Strongyloides* sp. and *Ascaris* sp. were found to have the highest (26%) prevalence rate whereas *Ancylostoma* sp. with the lowest (9%) prevalence rate.



Entamoeba coli *Trichuris* sp. *Ancylostoma* sp.



Strongyloides sp. *Ascaris* sp.

Figure 4. Microscopic photographs of gastrointestinal parasites observed from the Assamese macaques.

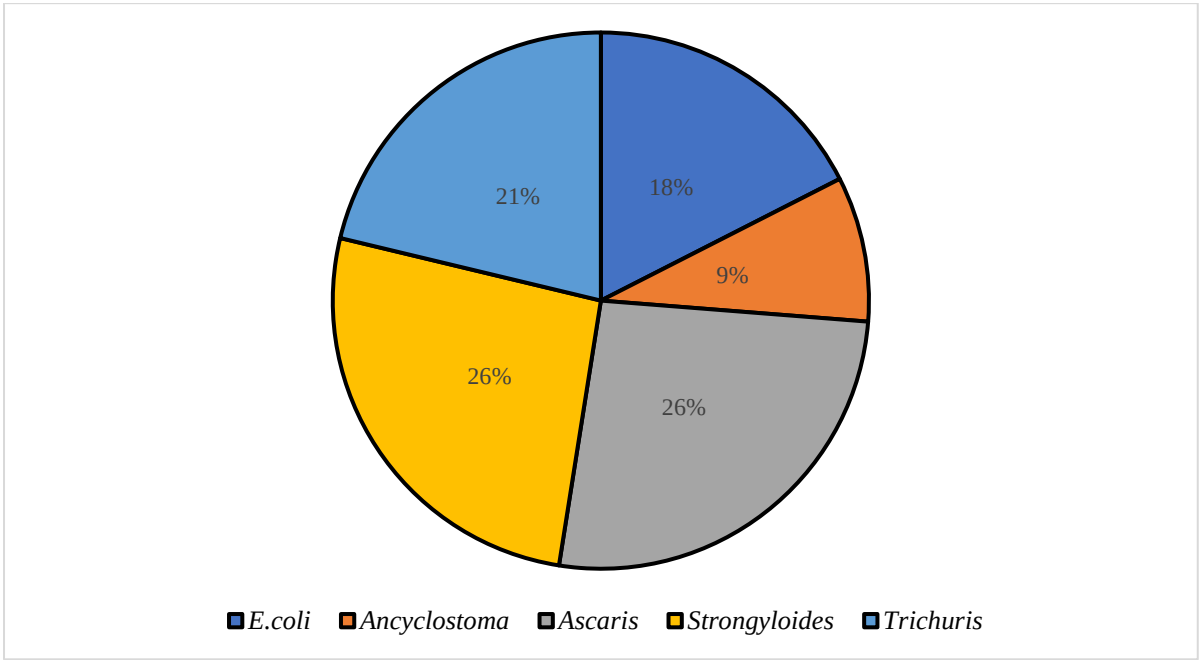


Figure 5. Prevalence of specific intestinal parasites in collected samples.

4. Awareness campaigns

Our team conducted two separate awareness campaigns during the field surveys in eastern 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.

The first campaign was conducted in the Sandakpur Rural Municipality of the Ilam District. A total of 23 local people from different wards of the Sandakpur RM participated in the one-day awareness campaign organized in the Deurali area.

The second awareness campaign was conducted in the Falelung Rural Municipality of the Panchthar District. A total of 26 participants from different villages of the rural municipality participated in the campaign organized in the Goruwale Bhanjyang.

5. Future plans

This project plans to conduct field surveys in the Gaurishankar Conservation Area at the central Nepal and Jajarkot District in western Nepal during March, April and May 2024. The field surveys will follow similar approaches to the previous field work done in eastern Nepal. A detailed population survey will be done, and fecal samples will be collected for DNA analysis as well as gastrointestinal parasitic analysis. Besides this, two awareness campaigns will be conducted in each of the survey areas in central and the western Nepal.

The mitochondrial control region (CR) and Cytochrome B (CytB) genes will be sequenced, and conservation genetic analysis will be performed.

6. Challenges in the project

Our team had planned fieldwork in Jajarkot District, western Nepal from the middle of November to the first week of December. 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 almost 200 people and injuring almost 400 (The Guardian, 2023; USGS, 2023). Most of the people lost their houses due to the earthquake. By the end of December 2023 also, temporary settlements are under establishment. We felt that the planned fieldwork and awareness campaigns would not be feasible at the moment. Therefore, we have postponed our fieldwork in Jajarkot to March and April 2024.

7. Acknowledgements

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8. References

Chalise, M. K. (2013). Fragmented primate population of Nepal. In L. K. Marsh & C. A. Chapman (Eds.), *Primates in fragments: Complexity and resilience* (pp. 329-356). Springer Science+Business Media https://doi.org/10.1007/978-1-4614-8839-2_22

Choudhury, A. (2022). A review of the Assamese macaque *Macaca assamensis* complex and its geographical variation. *Primate Conservation*, *36*, 203-231.

Khanal, L., Chalise, M. K., & Jiang, X. L. (2019). Distribution of the threatened Assamese Macaque *Macaca assamensis* (Mammalia: Primates: Cercopithecidae) population in Nepal. *Journal of Threatened Taxa*, *11*(1), 13047–13057. https://doi.org/10.11609/jott.4623.11.1.13047-13057

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

Khanal, L., Chalise, M. K., He, K., Acharya, B. K., Kawamoto, Y., & Jiang, X. L. (2018). Mitochondrial DNA analyses and ecological niche modeling reveal post-LGM expansion of the Assam macaque (*Macaca assamensis*) in the foothills of Nepal Himalaya. *American Journal of Primatology*, *80*(3), e22748. https://doi.org/10.1002/ajp.22748

Khanal, L., Upadhyaya, L. P., Pandey, N., Chand, D. B., Karki, M., Chalise, M. K., & Kyes, R. C. (2023). Projected distribution of the westernmost subpopulation of Assamese macaques (Macaca assamensis pelops) under climate change: conservation implications of a threatened population. *Frontiers in Conservation Science*, *4*. https://doi.org/10.3389/fcosc.2023.1235595

Molur, S., Brandon-Jones, D., Dittus, W., Eudey, A., Kumar, A., Singh, M., . . . Walker, S. (2003). *Status of South Asian Primates: Conservation Assessment and Management Plan (C.A.M.P.)* (Workshop Report 2003). Zoo Outreach Organisation /CBSG-South Asia, Coimbatore, India.

The Guardian (03 November 2023). Dozens killed and injured by powerful earthquake in Nepal. https://web.archive.org/web/20231103230606/https://www.theguardian.com/world/2023/nov/03/dozens-killed-and-injured-by-magnitude-64-earthquake-in-nepal

USGS National Earthquake Information Centre (2023). M 5.6 - 42 km S of Jumla, Nepal. https://web.archive.org/web/20231103182212/https://earthquake.usgs.gov/earthquakes/eventpage/us7000l8p5/executive

White, P. S., & Densmore III, L. D. (1992). *Mitochondrial DNA isolation*. Molecular genetic analysis of populations: a practical approach. Oxford: IRL Press, Oxford University Press. p. 29-58.

Zajac, A. M., Conboy, G. a., Greiner, E. C., Smith, S. a., & Snowden, K. F. (2012). Faecal examination for the diagnosis of parasitism. In Veterinary Clinical Parasitology.