Update Report 2 October 2025

Tracing Roots of Himalayan Biodiversity: Discovery, Description, and Conservation of Bats in Nepal

Basant Sharma

Division of Biology, Kansas State University, Manhattan, Kansas 66506, USA

Update summary

We made four significant progress this year: field sampling and preservation, laboratory work, museum archives, and training and awareness activities.

A. Field sampling and preservation

Sampling was conducted mainly in caves, with additional efforts at other roosting sites and through netting in forested and agricultural lands. In total, sampling covered 24 caves, 16 netting sites, and 3 roosting sites (Figure 1 & Image 1). Most sites were in tropical and subtropical regions, with a few extending into temperate zones at elevations of up to 2300 m a.s.l.

Bats were captured using scoop nets (in caves and other roosting sites during the day) and mist nets (at cave entrances, forests, and agricultural lands in the evening or night after emergence). Altogether around 20 species of bats were capture during the study period (Image 2). Individuals were handled with leather gloves, and morphometric measurements including forearm, tail, ear, tibia, and body weight, were recorded (Image 3). For genetic analyses, 2 mm wing tissue punches were taken from both wings. These were supplemented, when possible, with buccal swabs and fecal samples (collected only if a bat defecated while resting in a sterile bat bag before processing or during processing). Additionally,

ectoparasite samples were collected from heavily infested individuals. Echolocation calls were also recorded for some bats before release.

Samples were stored in either in 1.5 ml microcentrifuge tubes or 2 ml cryogenic vials using a combination of preservation methods. Wing tissues were dried with 0.5 gm silica gel packet; swabs were preserved in either in 0.5 gm silica gel packet or DNA/RNA Shield; and fecal and ectoparasite samples were preserved in 95% ethanol solution.

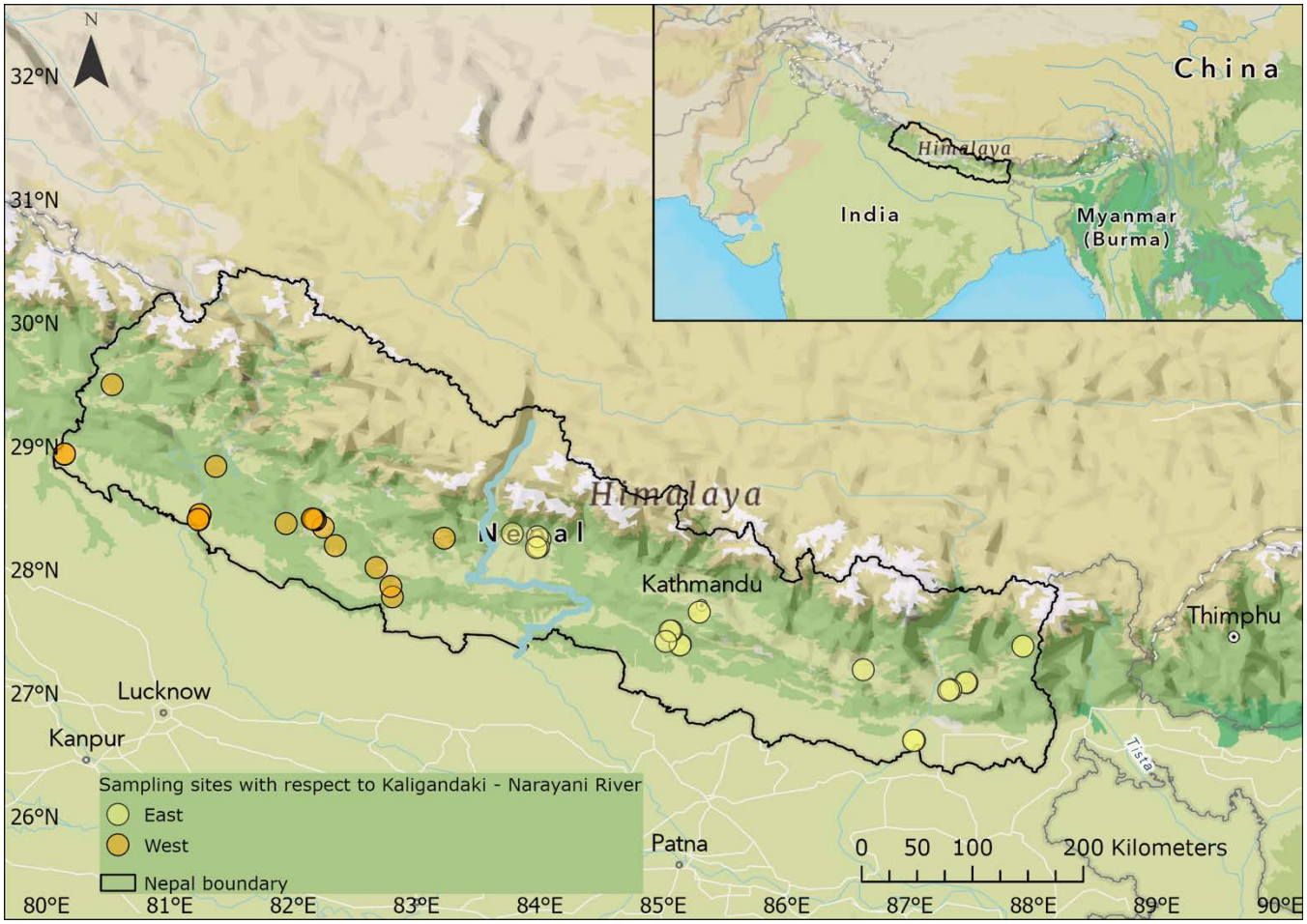

Figure 1: Map of study area (Nepal) showing bat sampling sites, including caves, roosting sites other than caves, and bat netting areas in the forest and agricultural lands, with respect to Kaligandaki – Narayani River i.e. consisting portion of both eastern and western Himalayas.

Image 1: Some of the caves visited during field data collection from April to August 2025. Top (left: Patal Bhuwaneshore Cave in Baitadi, mid: Chameri Cave in Teherathum, right: Malarani cave in Surkhet), Middle (left: Chadani Cave in Salyan, mid: Chameri cave in Salyan, right: Siddha Chameni cave in Dang), Bottom (left: Karuna bat cave in Baglung, mid: Bhairav cave in Khotang, right: Chamero cave in Taplejung).

Image 2: Some of the bat species captured during field visit from April to August 2025. Top (left: *Pipistrellus sp.*, mid: *Miniopterus fuliginosus*, left: *Scotophilus heathii*), Middle (left: *Cynopterus sphinx*, mid: *Myotis sp.*, left: *Hipposideros gentelis*), and Bottom (left: *H. armiger*, mid: *Rhinolophus. pusillus*, right: *Lyroderma lyra*).

Image 3: Field activities, including setting nets, handling live bats, and collecting samples with the field crew, during data collection from April to August 2025.

B. Laboratory work

Total DNA was extracted primarily from wing tissues, and for some individuals, swabs were also used. Extractions were carried out using three methods: New England Biolabs' Monarch nucleic acid purification kits, Qiagen DNeasy Blood and Tissue Kits, and the standard salt extraction procedure. DNA concentration was measured with an Invitrogen Qubit 4 Fluorometer, and only samples meeting the required concentration thresholds were selected for library preparation and subsequent sequencing. Library preparation was performed on the Oxford Nanopore Technology (ONT) platform using the Ligation Sequencing Kit V14 and the Native Barcoding Kit 24 V14. Sequencing was conducted on the ONT's MinION MK1D device using MinKNOW software. Adaptive sampling was applied using all available bat reference mitochondrial genomes from NCBI to sequence from our samples. All laboratory work, from DNA extraction to sequencing, was conducted in the newly established laboratory setup at Tribhuvan University, Institute of Forestry, Pokhara Campus (IoF PC) (Image 4).

C. Museum archive development

As this project is part of the doctoral research, we have so far only used wing tissues and a few buccal swabs. However, there is a wealth of additional material available, including swabs, fecal samples, and ectoparasites, that can be used for various research purposes. All these resources are preserved at –18 °C in the laboratory at IoF PC and are open for use by anyone interested in pursuing related research. Despite our best efforts to minimize harm during field sampling, a few casualties did occur, particularly when using scoop nets to capture bats. In addition, we also encountered dead bats during cave visits. All these specimens have been collected and preserved at the IoF PC lab for future research and teaching purposes. This laboratory was established through collaboration between the Kansas State Biorepository (Division of Biology, Kansas State University, Manhattan, Kansas, USA) and IoF PC, with additional financial support from the U.S. Fulbright Program. Although still in its early stages of development, the lab is expected to expand in the future. All resources will be digitized and made publicly available

through Arctos, a collaborative, research-grade collection management system and community that supports natural history, cultural, geological, and other types of museum collections.

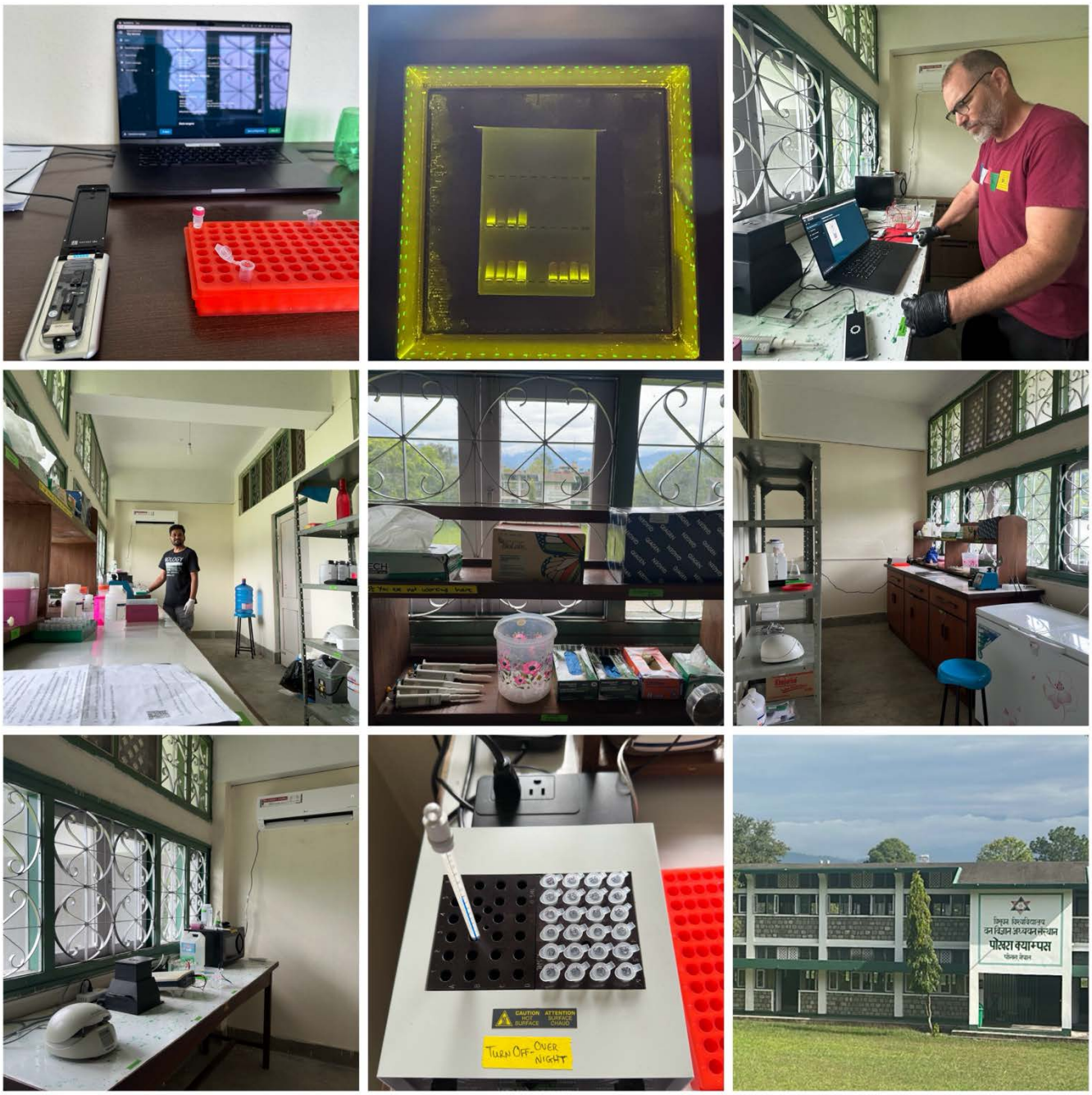

Image 4: Lab activities, involving DNA extraction and sequencing using ONT's platform, in the newly established genomic lab at Tribhuvan University IoF PC.

D. Training and awareness

Similar to the previous field session, this year we also provided field-based training on bat capture, handling, identification, and genetic sample collection and preservation to several undergraduate students at IoF PC (Image 5). In addition to field activities, laboratory-based training on DNA extraction using the standard salt extraction method was provided to four students.

To raise awareness, we held group discussions with residents near caves as well as with cave management committees throughout the field sampling period. These discussions focused on assessing participants' current knowledge and attitudes toward bats, while also providing information on bat diversity, evolutionary history, dietary differentiation, the species found in their local caves or surroundings, and their ecological roles. This has enhanced local understanding of bats and encouraged positive attitudes and greater motivation toward conserving bats and their habitats.

Future plans

Almost all project activities have now been completed. The main research objectives were to collect bat samples from across Nepal, extract DNA, and conduct sequencing. Samples were successfully collected from multiple regions of the country, except for high-elevation sites. These areas were difficult to access during the summer due to monsoonal rainfall, transportation challenges, and remoteness. In terms of laboratory progress, DNA has been extracted from all wing tissue samples, and sequencing has been carried out, establishing a functional molecular laboratory within Nepal. The remaining tasks include bioinformatic analyses, result generation, and manuscript preparation for the completion of the PhD degree, followed by publication. These activities will be pursued over the next two years. Beyond these commitments, the project has successfully achieved and completed the activities proposed under the Rufford Foundation for the past two years.

Image 5: Training involving cave field visits, mist netting, bat handling, and genetic sample collection and preservation methods with undergraduate students at IoF PC.