The Rufford Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Prajwol Manandhar
Project title	Developing cost-effective molecular tools to identify wild felids around Kathmandu valley through non-invasive genetic sampling
RSG reference	25359-1
Reporting period	July 2019
Amount of grant	£5,000
Your email address	Prajwol.f.manandhar@gmail.com or prajwol.manandhar@intrepidnepal.com
Date of this report	20 Oct. 2019

1. Please 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
Development of PCR based molecular tools for species identification				PCR based species identification assays of leopard, jungle cat and domestic cat have been devised. Due to unavailability of positive controls of leopard cat and clouded leopard, lab optimisation of these two felids are still remaining while in-silico primer design has been completed.
Baseline knowledge on distribution of wild felids				Distribution of leopard, leopard cat, jungle cat was mapped from the data obtained. The clouded leopard signs have not yet been detected, probably due to their rarity.
Evolutionary insights for felid species in Himalayan belt				We have performed DNA barcoding of the non-invasive genetic samples of all felid species. These data will be deposited soon in NCBI Genbank repository and thus will contribute as the regional data of Nepal in the global database.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

During fieldwork, there were rainstorms that affected some field days. Apart from these, there were not any major unforeseen difficulties that hampered our work in any significant way. However, due to relatively few scat samples found of leopard, a proper in-depth population size cannot be evaluated.

3. Briefly describe the three most important outcomes of your project.

I. Distribution of carnivores

This study represents first comprehensive scientific survey of leopards (*Panthera pardus*) across hills of Kathmandu valley. Geo-spatial distribution of leopards along with two small felids, leopard cats (*Prionailurus bengalensis*) and jungle cats (*Felis chaus*), throughout major hill forests of Kathmandu valley (Figure 1) were mapped. Apart from these, we were also able to obtain some incidental data on yellow-throated marten (*Martes flavigula*), large Indian civet (*Viverricula zibetha*), Himalayan serow (*Capricornis sumatraensis thar*), Himalayan goral (*Naemorhedus goral*), a shrew using non-invasive genetic samples.

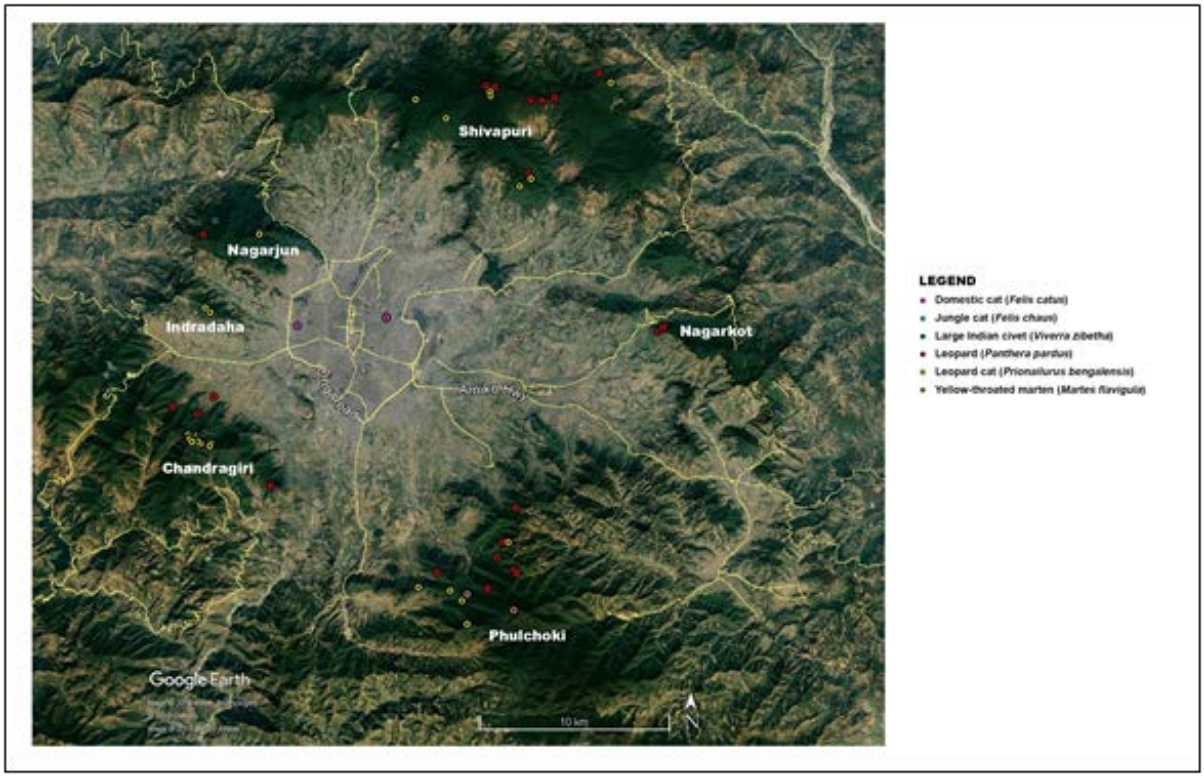

Figure 1. Distribution map of carnivores identified using non-invasive genetic samples.

II. Evolutionary history

Evolutionary insights of leopards in the Himalayan belt has never been investigated. This work is ongoing as it requires in-depth research and development. Some preliminary data have suggested us that the genetic lineage of leopards in this terrain is distinct from what is currently known of the genetic history of Indian leopard subspecies.

We also sequenced a region of cytochrome-b gene of other carnivores to confirm their species identification. Using the same gene sequence along with reference sequences from GenBank database, we reconstructed a phylogenetic tree to infer their evolutionary insights. This tree represents the taxonomic topology of Nepal's regional data in comparison to the global sequence data of those carnivores.

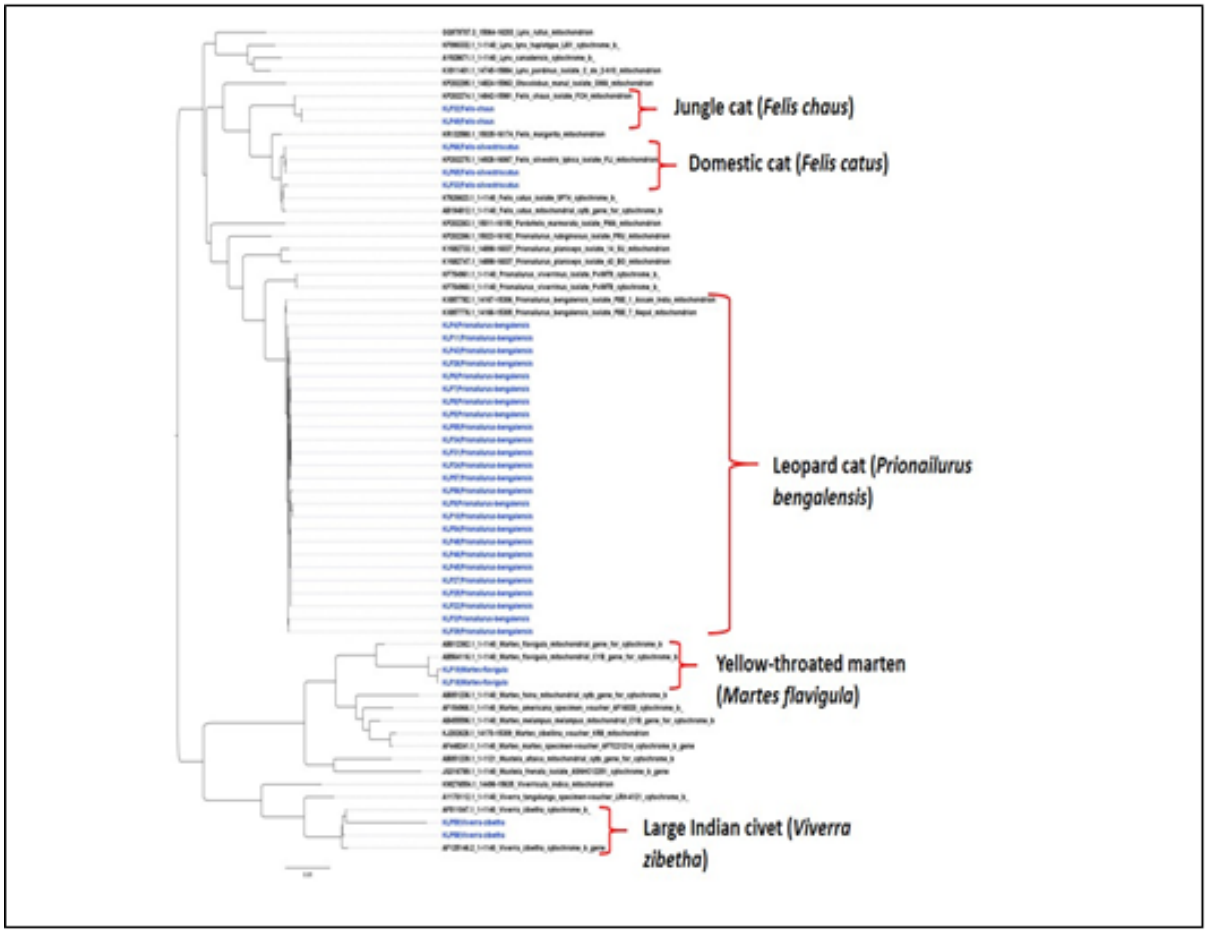

Figure 2. Phylogeny tree of small carnivores reconstructed using reference sequences of cytochrome-b gene downloaded from NCBI genbank along with sequences of jungle cat, domestic cat, leopard cat, yellow-throated marten and large Indian civet (all indicated as blue coloured taxa) found in this study.

III. Collaboration

The project's successful execution has opened doors for national and international collaboration for the future works. We are working in collaboration with Dr Claudia Wultsch (American Museum of Natural History) and Dr Alexander Ball (Royal Zoological Society of Scotland) on molecular based diet analysis of leopard and leopard cat scats to understand the dietary habits of these two felids in Kathmandu valley. Similarly, we are in process of collaboration for more in-depth research with Shivapuri Nagarjun National Park, Amy Fitzmaurice (Oxford University, WildCRU), Nepal Tiger Trust, Chandragiri Hills Ltd and ICIMOD Knowledge Park.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

We had to hire a local porter during our field work in Shivapuri, and he benefitted financially during field work.

5. Are there any plans to continue this work?

Yes. Extensive survey for scat collection and camera trap monitoring in the current field sites is necessary. Currently we are performing DNA fingerprinting/genotyping of leopard scats in order to estimate the total number of individual leopards that represent our leopard sample pool (n=27). In order to make this estimation more scientific, the sample pool needs to be higher than the current number. Hence, an extensive scat survey is necessary throughout the current field sites. Additionally, we also intend to expand survey into neighbouring forest patches that were not surveyed in this phase.

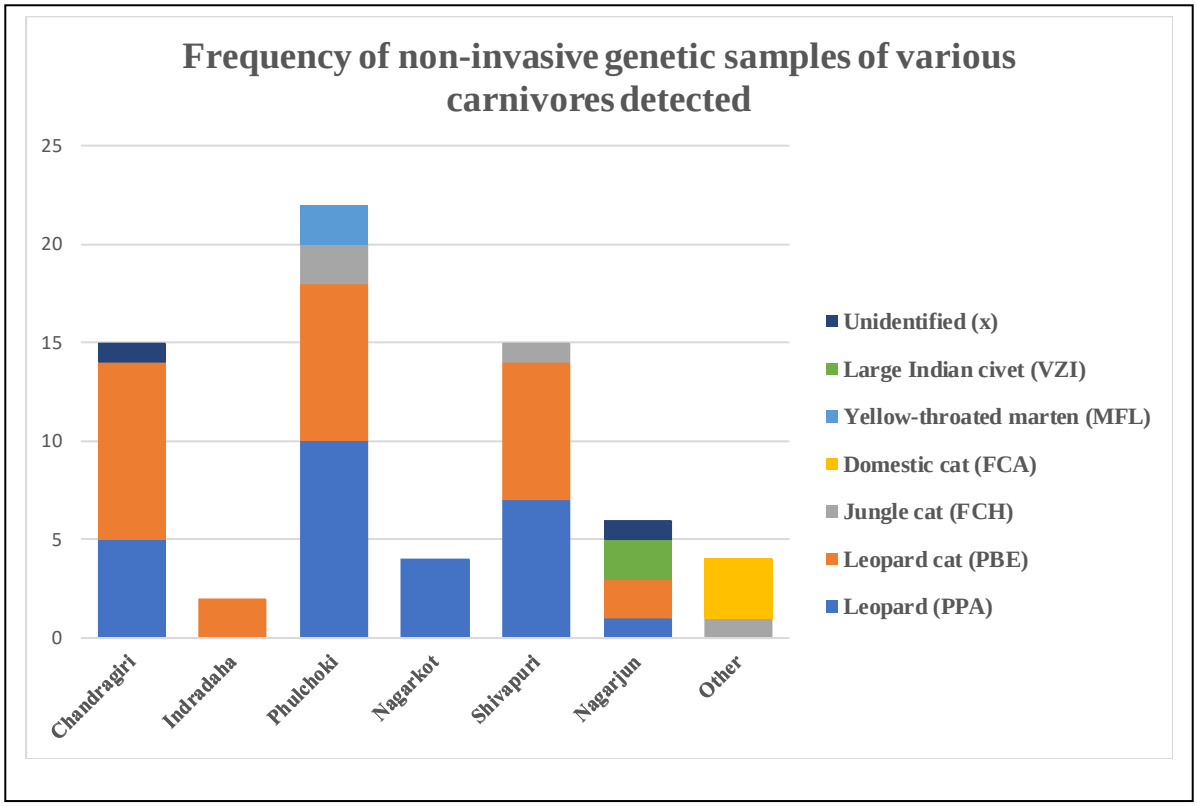

Figure 3. Frequency graph of carnivores identified using non-invasive genetic samples across different field sites.

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

I have presented updates and outcomes at various places including local media houses. During World Environment Day, I was invited as a speaker in event organised by Women in Nature Network Nepal and Green Decisions Consulting Pvt Ltd to give talk about my Leopard research in Kathmandu valley where I presented updates of the project.

The project updates and outcomes have been covered in various media/blog articles. Links of those articles:

- News article on Arghakhanchi case: Two short news articles were covered in paper-based media of Kantinupur news of the human-leopard fatal incidence case where we handled DNA forensics portion of the case to solve the identification of actual leopard that killed human child.

- LinkedIn blog article: https://www.linkedin.com/pulse/local-conservationists-use-genetics-raise-leopard-adarsh-man-sherchan/

- Kathmandu Post article: https://kathmandupost.com/climate-environment/2019/06/21/this-is-why-leopards-are-entering-our-cities-20190621195755

- RZSS blog article: https://www.rzss.org.uk/news/article/16254/in-the-paw-prints-of-leopards-/

- News article in Nepali: https://www.dekhapadhi.com/news/461

In the near future, we are preparing for project dissemination, publishing detailed report, peer-review articles in scientific journals, and possibly presenting project results in national and international conferences.

7. Timescale: Over what period was The Rufford Foundation grant used? How does this compare to the anticipated or actual length of the project?

August 2018 – July 2019

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Human resource allowance	700	700		
Equipment accessories	215	150	-65	GPS device was contributed from CMDN. Other accessories like torches, headlights, batteries, tent etc. were bought
Travel/Lodging/Food	1645	1500	-145	Anticipated costs were lesser
Sampling accessories and miscellaneous	285	300	+15	Extra sampling kits had to be bought and sent to Arghakhanchi during fatal human-leopard conflict case for sample collection (mentioned in previous update

				report)
Lab cost	2155	2065	-90	Discount and R&D fund contribution from CMDN
Outreach materials (documentary, website)		400	+400	We had not planned this initially; we are currently in the process of making a short video documentary as an education outreach material addressing conservation importance of leopard in Kathmandu valley based on the findings from this project.
TOTAL	**5000**	**5115**	**-115**	**I am spending this extra amount for the documentary from my personal account.**

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

- Extensive survey for scat sampling and camera-trap monitoring.
- Involvement and capacity building of park rangers, students from local universities, and citizen scientists.
- Collaboration with right stakeholders for more research and conservation activities.
- Awareness and outreach materials for leopard conservation and research.
- More research into population, spatial ecology, dispersal behaviours, dietary habits of leopards is of high necessity.
- Website of the project is being developed mainly for communication and outreach networking with global community working on leopard conservation.

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

Yes.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Prajwol Manandhar: project lead, conduct field surveys, analyse genetic data, communicate and network.

Naresh Kusi & Sandesh Lamichhane: field survey design and plan, sample collection, data collection.

Jyoti Joshi, Hemanta Chaudhary & Rajindra Napit: lab protocol design, lab logistics, molecular works.

Laba Guragain & Suman Bhandari: logistic management in National Park, field survey, sample collection, data collection.

Nikita Pradhan & Adarsh M Sherchan: outreach, media, networking.

12. Any other comments?

We heartily thank the Rufford Foundation team for considering funding our project. This initiation has meant a lot for leopard research in the Kathmandu valley as well as for Nepal. And this has given us more motivation for taking this work ahead in the long run to achieve our ultimate goal of leopard conservation and policy strengthening in Nepal.

Some other notes from our field research:
Apart from our primary non-invasive genetic sampling work, we also had sightings of various wildlife including leopard, jungle cat, yellow-throated marten, barking deer, wild boar, Himalayan goral, squirrel, Kalij pheasant and numerous other birds.

Top row: Leopard resting up in tree in Tokha municipality, Leopard cub found inside an abandoned house in Nagarkot, Spotted owlet. Middle row: Yellow-throated marten, Leopard's pugmark, Himalayan Goral sighted in Shivapuri. Bottom row: The same leopard as in the top-row, Himalayan rock lizard, Scratch mark of leopard in Schima wallichii tree.