

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	Naresh Kusi
Project title	Performing genetic analyses of wild yaks in Nepal, investigating their hybridization with domestic yaks and producing additional conservation material
RSG reference	22685-B
Reporting period	12 months
Amount of grant	£9871
Your email address	naresh.kusi@gmail.com
Date of this report	18 July 2018

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
To perform the genetic analyses of wild yaks				We could not extract good quality DNA from historical samples (10-20 years old) of bones and hairs despite multiple extraction attempts as they were exposed to sunlight, wind, rain etc. for a long time, making the genetic materials likely to degrade. However, we were able to extract DNA and sequence their D-loop mtDNA fragment of ~530 bp length from one hair and two dung samples collected in Humla. We are currently running sequence analyses on these.
To investigate the hybridization of wild yaks with domestic yaks				We have sequenced D-loop mitochondrial fragments (~530 bp) from dung and hair samples of domestic yaks and are running various analyses (phylogenetics, haplotype network). Reference data from studies in China's Qinghai Tibetan Plateau has also been utilised to perform these analyses to enable comparison in larger geographic scale. We will come to a conclusion by few months.
To produce wild yak related fables in the local Tibetan language				

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

Genetic analyses of wild yaks against domestic yaks is complex as their genetic makeup do not show species level divergence which has been clearly demonstrated by Wang et al (2010). Lots of secondary analyses such as haplotype network and phylogeographic analyses need to be performed to reach to any conclusion. We underestimated this aspect in the time scale. We are still performing these analyses with more reference sequence data from throughout the geographic range of wild yaks which will take at least a few more months.

The fieldwork for the project was conducted in July (in Mustang) and August-September 2017 (upper Dolpa and upper Humla) which correspond to the rainy season in Nepal. Since all the study sites are located in the transHimalayan belt, we had expected that rain will not affect us. But unlike our expectation, we had to work in rain for most of the times, especially in upper Humla and upper Dolpa, leading to a substantial decrease in our projected output.

Similarly, one of our field researchers got sick in Humla and it also affected the overall performance in field work.

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

- **Outcome 1: Preliminary insights into genetics of wild and domestic yaks**

We collected a total of 28 non-invasive samples (wild yak: six, domestic yak: 21 and unknown: one) on which we performed all genetic analyses (Table 1). Two dung and one hair sample of wild yaks generated D-loop sequences. Among the samples of domestic yaks, only one hair sample produced bad quality sequence. All the remaining useful sequences of wild and domestic yaks are being analysed for investigating the hybridisation footprints in the D-loop control region. All the sequence analyses are currently being carried out using AliView and MEGA software for alignment and haplotype identification. The haplotype network analysis is being performed using PopArt software platform. We will perform further phylogenetic analyses using maximum likelihood and Bayesian methods to infer phylogeographical links among the domestic and wild yak lineages. Preliminary haplotype network analysis has shown that there is moderate degree of hybridisation among the haplotypes identified in domestic and wild yak lineages. We will obtain concrete findings of these analyses in few months.

Table 1. Sample details and their sequence quality information.

S.N	Sample type	Location	Phenotype	Dloop PCR	Seq code	Seq quality
1.	Dung	Mustang	DY	+ve	YK01	Good
2.	Bone	Mustang	DY	+ve	YK02	Good
3.	Dung	Mustang	DY	+ve	YK03	Good
4.	Dung	Mustang	DY	+ve	YK04	Good
5.	Hair	Mustang	DY	+ve	YK05	Good
6.	Hair	Mustang	DY	+ve	YK06	Good
7.	Bone	Mustang	WY	-ve		
8.	Dung	Humla	DY	+ve	YK08	Good
9.	Hair	Humla	DY	+ve	YK09	Good
10.	Hair	Humla	DY	+ve	YK10	Good
11.	Bone	Humla	DY	+ve	YK11	Good
12.	Bone	Humla	WY	-ve		
13.	Dung	Humla	DY	+ve	YK13	Good
14.	Dung	Humla	UK	+ve	YK14	Good
15.	Hair	Humla	WY	+ve	YK15	Good

16.	Hair	Dolpa	DY	+ve	YK16	Good
17.	Hair	Dolpa	DY	+ve	YK17	Good
18.	Hair	Dolpa	DY	+ve	YK18	Good
19.	Bone	Dolpa	WY	+ve	YK19	Average
20.	Hair	Dolpa	DY	+ve	WF479	Good
21.	Hair	Dolpa	DY	+ve	WF480	Good
22.	Hair	Dolpa	DY	+ve	WF481	Good
23.	Hair	Dolpa	DY	+ve	WF482	Good
24.	Hair	Dolpa	DY	+ve	WF483	Bad
25.	Hair	Dolpa	DY	+ve	WF484	Good
26.	Hair	Dolpa	DY	+ve	WF485	Average
27.	Dung	Humla	WY	+ve	WF330	Good
28.	Dung	Humla	WY	+ve	WF331	Good

DY: Domestic Yak, WY: Wild Yak, UK: Unknown

- **Outcome 2: A more concentrated effort towards wild yak conservation**

We published two fables on wild yaks; one each on the ecological value and the cultural value of the species in the transHimalayan communities of Nepal. Since the fables contain texts in the local Tibetan language complimented by appropriate illustrations, they will effectively convey the important message on the urgency and significance of wild yak conservation in Nepal. As our focus has always been to ensure that our productions reach the target communities, we have already sent them to the places through our local friends. We are happy to realize that the fables will remain as a long-term reminder of wild yak conservation issue in the regions.

- **Outcome 3: Enhanced knowledge data on more wildlife species**

Similar to the first and the second projects, we were able to gather new scientific knowledge on some of the non-target wildlife species during this project.

We obtained the first hand-held photograph of Eurasian lynx (*Lynx lynx*) in its natural habitat for Nepal from upper Mustang. The first specimen of this high altitude cat in Nepal was collected from Mustang in 1976. But the cat is so rare in the country that no one has ever been able to capture it in a camera. The first camera trap image was obtained only as recently as 2016. We have already submitted a comprehensive article on this for publication.

We also generated a distribution update for Nepal argus (*Paralasa nepalica*) butterfly from upper Humla. The first specimen of this butterfly was collected by Martens (1973) and later studied and described as a new species for science by Paulus (1982). This butterfly is a Nepal endemic and there has been only three observational records since its first collection. Our observation provides the fourth record from a new locality. The manuscript is under revision for final submission to a scientific journal.

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

As in the previous projects, we worked closely with the local communities. We involved the local villagers as field guides, mule keepers and cooks during the field research in all the study sites and also provided them with daily allowances.

We took a step further in this project by involving a local graduate student from upper Dolpa as a field researcher. Also, we continued our respect towards the local communities by inviting the local villagers from upper Humla during the launch of fables in Kathmandu, the capital of Nepal, where they publicized the fables among the public.

5. Are there any plans to continue this work?

Yes. We will continue our works on wild yaks and other high altitude wildlife in Nepal.

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

We shared our results through conservation talks, books, journal articles and documentary films in our previous projects.

With three year's data from field and the results of genetic analyses in progress, we are in a position to write a comprehensive journal article on wild yaks and share it to the public once it gets published.

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

We used the grant for 10 months from June 2017 to April 2018 as outlined in the project proposal.

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
Daily allowance for researchers (two people for 90 days @ 8)	1440	1440	0	
Daily allowance for local field guide and cook (two people for 90 days @ 8)	1440	1440	0	
AA batteries (20 pairs at 0.6)	12	12	0	

Silica gel (1kg @ 10), sampling vials (50 vials @ 0.5), surgical gloves (100 pairs @ 0.3) and cotton (1 pack @ 2)	67	67	0	
Bus fare (Kathmandu-Nepalgunj - Kathmandu (two people for four trips/Humla and Dolpa @ 22.5))	90	90	0	
Busfare (Kathmandu - Pokhara - Kathmandu (two people for two trips/Mustang @ 22.5))	45	45	0	
Busfare (Pokhara - Jomsom (Mustang) - Pokhara (two people for two trips @ 30))	60	60	0	
Airfare (Nepalgunj - Dunai (Dolpa) - Nepalgunj (two people for two trips @ 225))	450	450	0	
Airfare (Nepalgunj - Simkot (Humla) - Nepalgunj (two people for two trips @ 225))	450	450	0	
Genetic analyses	1100	770	+330	I got some discount.
Fables (two fables (1000 copies each @ 0.616))	1232	1485	-253	The printing cost was more than we expected.
Conservation booklet (1000 copies) @ 0.565))	565	0	+565	
Mules (two mules @ 8 for 90 days)	1440	2880	-1440	We had to hire double the no of mules than we had estimated.
Consumables (Four people for 90 days @ 4)	1440	1440	0	
Conservation workshop (hall charge)	35	0	+35	I did the workshop in the hall of the organization I work with and thus did not have to pay the charge.
Data sheets (lump sum)	5	5	0	
Total	9871	10462	(-763)	WWF Nepal helped me manage the deficit.

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

The important next steps are:

1. A study on the transboundary movement (between the Tibetan Autonomous Region of China and Limi valley) pattern of wild yaks in upper Humla.
2. Building community stewardship towards wild yak and other endangered mammals conservation in upper Humla.

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

Yes.

We used the logo in the fables where the Rufford Foundation is mentioned as one of the publishers.

We also used the Rufford Foundation logo in the banners of wild yak conservation talks.

RF received publicity during the conservation talks in universities and among the conservation stakeholders and also among the public during the fable launch event.

11. Any other comments?

We are grateful to the Rufford Foundation for its continued support. We believe that the foundation will keep motivating us to work more for the conservation of high altitude wildlife in Nepal.



Fable launch.