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	ALI Cheha
Project title	The use of Non-Invasive Genetic Monitoring of the Livingstone's flying-fox populations in the Comoros islands to inform a relevant conservation plan
RSG reference	19010-1
Reporting period	April 2016 to June 2017
Amount of grant	4 990£
Your email address	cheha_ali@yahoo.fr
Date of this report	April 2017

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
Evaluate the habitat suitability, ecological niche, and geographic distribution range for the Livingstone's flying fox				A paper for this part is completely finished and currently in submission on **Biological conservation** international journal
Develop individual monitoring protocol to model population dynamics using non-invasive method				A paper for this part is completely finished and currently in submission on **Mammalian Biology** international journal
To assess genetic diversity, population structure of the species				All Lab analyses and statistical possess are finished, we are currently in the paper writing
Assess in collaboration with local people a relevant management plan for the species				Analyses in progress
Provide a basic guiding and scientific field work to local guides and Comorian students. Such knowledge will be useful for a long-term monitoring based on these approaches.				
Successful completion of my Master thesis in France				

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

Field work in remote regions of Anjouan during the night time in highly inaccessible and dense forests is always complicated. Some locations were more difficult to reach than we expected and some field constraints significantly differ from our expectations. First, sample collection was carried out during the wet season and mostly under rains. To collect sample we placed a plastic awning below each roost to collect fresh faecal samples but the plastic awning is often washed by rain since it rained almost every day in these rain forests. In most cases, it was necessary to wait

below dormitories under rains in night time to collect fresh sample just after defecation that minimize contamination risk.

Second, our samples were maintained at room temperature for a long time (4 to 6 months in Comoros) before lab process due to administration protocol related to sample exportation. Samples exposed to wet environment in rain season for a long duration may be affected quickly to moisture and DNA degradation. Samples are often degraded but also present many PCR protein inhibitors. To increase the probability of positive PCR and genotyping and minimize cost and time consuming, we amplified firstly our DNA with a 460 bp of mtDNA cytochrome b and at last, only the positive PCR obtained with mtDNA are amplified with microsatellite loci. This process allows us making sure to amplify samples with non degraded DNA.

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

1: <u>educational and training aspect</u>
The first outcomes of this project relate to the educational and training aspect: Basic guiding, scientific field work, and basic conservation training have been provided to our local guides from all localities as well as a complete training for our Comorian Masters student (Dhurham Said Ousseni). The training courses included population density estimate models, basic computing knowledge, ecological niche modeling, basic statistics using the freely available R statistical language, Statistica software and simple Geographical Information System (GIS) tools. These courses were conducted by **Mohamed Thani** and allowed the Master student to improve their data analyses and acquire many experiences on the conservation biology aspects.

<u>2: Habitat selection and ecological geographic distribution</u>
This study highlight that the Livingstone's flying fox and the Comorian flying flying fox species use different habitat types thus ensure different ecosystem services. The two species are not replaceable each other because each one ensure specific role in the ecosystem functioning which is crucially needed for maintaining global ecosystem dynamic. Livingstone's flying fox seems to be highly threatened at relatively short term due to its low population abundance, its small spatial range and the high rate of habitat loss in the Comoros islands. Nevertheless, population of this species is located on altitudinal forests within steep slopes and where the access by human is difficult. In addition, according to our interview with local population, this species is not hunted by comorian people. On the contrary, the comorian flying fox seems to be less threatened due to a large population abundance and large spatial distribution. Yet, its dependence on anthropized habitats and the high rate of hunt pressure and roost disturbance by local people can rapidly shift its situation depending to human demographic evolution, to agriculture practices and to the economy of local people. Conservation measures must be taken by the comorian government to ensure the viability of both species (paper submitted in **biodiversity and conservation**).

<u>Non-invasive genetic method</u>
Using both microsatellites (7 polymorphic loci) and mitochondrial (460 bp cytochrome b) markers, we successfully amplified faecal samples for fruit bats

species stored in 96° ethanol and silica gel with high rate of genotyping success and low PCR errors. Samples were stored for a duration ranging from 4 to 6 months in tropical region. This study confirms the usefulness of noninvasive methods to produce genetic and demographic data on tropical bats including flying fox which highly needed in order to establish relevant management and conservation actions. This method allows reducing cost and effort deployed with traditional methods such as capture recapture approaches for long-term population surveys. However, the reliability and the accuracy of results in noninvasive genetic method depend on some field and lab condition such as faecal sample conservation methods minimizing DNA degradation, choosing the appropriate material for DNA extraction allowing minimizing PCR error rate and genotyping success.

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

During our missions, local guides were hired to help carry out the fieldwork. These guides benefitted from courses in which we provided basic and theoretical training on flying fox populations monitoring, population density estimate models, basic computing knowledge, ecological niche modeling. At the same time we tried to favor exchange with the guides so as to better understand how they view their environment. These sessions had positive consequences for us. During field work interviews with local people were carried out in order to understand the natural resource utilization that conduct to habitat loss and disturbance and to incite local community participation for a conservation strategy.

Overall interviewed people are in favour to the conservation and all local people proved positive perception through this critically endangered and natural rain forest. However, our project does not provide direct benefit to local people but our objective in a near future will be to minimise forest disturbance, improve forest management, reforestation and wildlife conservation in the Comoros islands.

5. Are there any plans to continue this work?

The work that I carried out is part of a larger research project involving Mohamed Thani and my supervisors (Aurélien Besnard).

We are very interested in investigating reliable population size population dynamic but also the demographic history of this species using non invasive genetic methods because these field studies are unknown for Comorian fruit bats. We will thus continue for a long term population size and population demography monitoring using these non invasive genetic methods. I plan to apply for a 3 years PhD to pursue this study. The fieldwork will continue to explore the Comoros islands for the Livingstone's flying fox but also for the Comorian flying fox.

We intend to broaden our field study by including the comorian flying fox (*Pteropus seychellensis comorensis*) from other islands including the Grande Comoros and Mayotte.

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

A large part of this project was published through my Master thesis submitted and defended in the "Ecole Pratique des Hautes Etudes, France". This final Masters report is a part of the knowledge sharing. Moreover, to offer the findings of the study to a larger audience, two scientific papers are in submission on international journal and another scientific paper is in writing. Results are also communicated during some international conferences including one conference in France (Poster presentation, see Cheha et al. 2016) and one international conference in Canada (Oral presentation and abstract, Ibouroi et al. 2017). Moreover, information of project activities are communicated to some acquainted people through informal discussions. Personal communication will thus be a mode of sharing of the final results. Pictures of the study (mostly taken in the field) are also shared on the Facebook page of the team: https://www.facebook.com/groups/466224763543329/?fref=ts
This page was created during the project and is managed by most of the field members of the project.

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

The RSG was used from April 2016 to June 2017.

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	Budget Amount	Actual Amount	Difference	Comments
Field Allowance for personals (guide and students)	1150	1200	+50	
Travels from Locality to locality	300	450	-150	Higher costs than was anticipated
Food 15 month	2200	2400	-200	Higher costs than was anticipated
Sampling material	100	100	0	
Genetic analysis reagents				Aurelien Besnard payed all la prossess
Master student payments	120	650	-530	It was an error calculation in the initial budget
Battery Charger for head lamp	20	0	+20	We used lab materials proved by Aurelien Besnatd lab
Handling material (Bags and gloves)	50	0	+50	We used lab materials proved by Aurelien Besnatd lab
Cooker payments	500	200	+300	Less expensive than expected

Field Laptop netbook	500	0	+500	We used lab materials proved by Aurelien Besnatd lab
Field shoes	50	0	+50	We used lab materials proved by Aurelien Besnatd lab
TOTAL	**4990**	**5000**	-10	

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

The next steps of this project are to continue field work and collect faecal samples every year in order to estimate population size and population demography using non invasive genetic methods with capture mark recapture approaches. I plan to assess the demographic history of these species allowing assessing relevant conservation plan.

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?

The Rufford Foundation support is acknowledged in two scientific papers (in submission) and one scientific paper in writing. The RSGF logo has been used during my Master presentation in France, and during international conference in Canada. My final master reports and the poster presented during the conference in France have RSGF logo on the cover page.

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

ALI CHEHA
ALI CHEHA is the project Leader. He conducted field work including genetic data collection, habitat characterization, habitat data collection for ecological niche modeling and geographic distribution, lab process and some statistical analyses (DNA extraction, mtDNA amplification and statistical analyses)

Mohamed Thani IBOUROI
Mohamed Thani is a PhD student in the "Biogeography et Ecologie des Vertebrés". He conducted field works including genetic data collection, habitat characterization, habitat data collection for ecological niche modeling and geographic distribution and the training courses on master student. He contributes to lab process and statistical analyses (DNA extraction, mtDNA amplification, microsatellite characterisation and statistical analyses). He wrote the three first scientific papers.

Aurélien BESNARD
Besnard is senior lecturer in "Biogeography et Ecologie des Vertebrés". He continuously supervises the study from field to paper writing. He provide additional financial for the project including all travel from France to Comoros and laboratory analyze financials

Dhurham Said Ali Ousseni

Said is a Master student from Madagascar, he contributes to the field works including genetic data collection, habitat characterization and habitat data collection for ecological niche modeling and geographic distribution.