The Rufford Small Grants Foundation

Final Report

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

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. 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. 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	Licia Ho
Project title	Conservation Genetics of the Endangered *Nasalis larvatus* in Sarawak, Borneo.
RSG reference	13073-1
Reporting period	January-December 2014
Amount of grant	£5981
Your email address	licia_ho@hotmail.co.uk
Date of this report	31st January 2015

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
Collection of DNA Data			√	Non invasive sampling was applied throughout the project sampling season. A total of 138 faecal samples were collected from six sampling sites in Sarawak and Sabah, Malaysia.
Genomic DNA Isolation and PCR Amplification			√	Of the 138 samples used in DNA extraction, a total of 70 PCR products were successfully amplified.
Study on Genetic Diversity of Proboscis Monkey		√		We believe that proboscis monkey has maintained considerable genetic variation in both Sarawak and Sabah populations studied here. Total 33 haplotypes with two separate haplo-groups were identified suggesting that the isolation might have been caused by historical events. High level of genetic divergence were also detected between two haplo-groups. Given that samples collection depends on the accessibility of the locations, thus some sampling sites are yet to be covered.
Population Expansion Analysis			√	Significant negative values were detected for both Fu and Li's D* and F* statistics indicating that populations of proboscis monkey in Malaysian Borneo have undergone population expansion.
Gene Flow and Population Partitioning Analysis			√	Three haplotypes were found shared between both Sarawak and Sabah populations, indicating there possible occurrence of gene flow. Population from Bako National Park in Sarawak and Labuk Bay in Sabah are possibly the ancestral populations; which is supported by low access of gene flow and high genetic distance.

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

A) Collection of Fresh Faecal samples

Nasalis larvatus, proboscis monkey is an endangered primate species and therefore direct darting or tranquillizing of this animal to obtain their blood sample for molecular study is unlikely. Thus,

collecting faecal sample is more applicable and ethical to study this species. However, searching faecal sample of *N. larvatus* using boats are limited by fluctuated tide timing and low accessibility in mangrove and peat swamp areas such as rivers blocked by fallen trees etc. Given that *N. larvatus* are free-ranging animals, they tend to flee from observers upon visual or auditory contact, which makes it difficult to get close to them for any observational purposes. Besides that, searching faecal samples on the forest floor was not easy, since the leaves and branches could affect our sighting chances of the faecal. To overcome this, we had been practising to detect and identify the faecal of *N. larvatus* based on odour and colour. Yet, more field assistants are needed in order to shorten the time for faecal searching and to cover larger areas.

B) Quality of faecal DNA
Genomic DNA extraction and amplification from stool samples was not as efficient compared to those extracted using blood or tissue samples. The purity and concentration of DNA from stool samples was low and potentially may contain other contaminants. These samples required more time to acquire better extraction products, and to subsequently optimize for PCR products. Besides that, the preservation and storing method of the faecal samples also can dictate the quality of the faecal DNA.

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

a) Enhance understanding on the genetic diversity of Bornean endemic primate, proboscis monkey. This study is the first attempt to apply non-invasive sampling technique in studying genetic diversity of proboscis monkey in Sarawak, Borneo. To date, we have collected important genetic data on proboscis monkey from few coastal areas around Sarawak, as well as in Sabah for comparison. After completion of my MSc thesis, I hope to publish my findings in scholarly journals.
b) Proboscis monkey is listed as Endangered Species in the IUCN Red List of Threatened Species due to their population trend that is decreasing and threatens from habitat destruction. Result from this study can provide fundamental information for the conservation of proboscis monkey. In this study, we predict that there are possibly at least three major proboscis monkey refugia in Borneo. The population divergence that is reflected through their genetics among proboscis monkey populations might result from geological formation, climatic barriers and vegetation change during the Pleistocene period.
c) During sampling at Samunsam Wildlife Sanctuary, we detected a group of leaf monkey which is suspected to be *Presbytis chrysomelas*. This species is one of the Bornean endemic primates and is considered as one of the rarest primates in the world. This species is now listed as Critically Endangered Species in the IUCN Red List of Threatened Species. The population trend of this species has been reported to reduce dramatically due to habitat loss, but data on this species are still lacking. We have reported this sighting to the staffs of Samunsam Wildlife Sanctuary for further investigation.

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

During the sampling period, we have hired local villagers to work with us as our boatman and guide. During the survey, we have showed and trained them as our assistant on how to search and identify the faecal samples of proboscis monkey. Besides, we also shared information on the purpose of this research, the importance of conserving animals, and the major threats to this animal especially to

proboscis monkey during our conversation with the local peoples. As I am not a local Sarawakian, they were really helpful by providing information about the sampling areas and help in sighting proboscis monkey along the rivers. They also showed interest when explained about how faecal can be useful in studying population genetic of this animal.

5. Are there any plans to continue this work?

Yes, our work in the field and lab will continue until August 2015. We will continue to work on other targeted gene and increase sampling coverage within the distribution range of proboscis monkey in Malaysian Borneo. Population distribution and estimation of proboscis monkey will continue to be studied by our department.

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

After completion of this project, data produced from this study will be presented at scientific conferences or seminars, and scientific manuscripts will be published in peer reviewed journals.

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

The Rufford Small Grant was awarded on December 2013. Funding were first used starting January 2014 until last year, December 2014. The RSG funding was used for travel expenses, sampling equipment and lab consumables. So far the project has completed two third of the actual duration allocated for the project and the remaining budget will be able to fund our research until August 2015.

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
Travelling, Transportation and Accommodations expenses	£2250	£2170.78	£79.22	Sampling for proboscis monkey depends on boat survey. Therefore most of the travelling costs were spent on petrol. Remaining amount of budget will be used until August 2015.
Salaries for porter and field assistants	£1217	£584.76	£632.24	Since some of the sampling sites hiring a porter is not necessary, which reduced the cost of salaries. The extra budget will used to cover the expenses for travelling to sampling sites.
Research materials and consumables	£2108	£1921.1	£186.9	Lab consumable and disposable items are mainly prepared in the university research lab. Remaining budget will be used for materials top-up in the future.

Research equipments	£203	£199.53	£3.47	We bought a Garmin GPS eTrex 30, Bushnell binocular 10-90x80 for the survey.
Publications	£203	£0.00	£203	Remaining budget will be used for final publication purposes.
TOTAL	£5981	£**4876.17**	£1104.83	(1 GBP= RM 5.33)

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

The most important step after this is to finalise and conclude the genetic information of *N. larvatus* populations in Malaysian Borneo. Those findings will provide better understanding on the genetic diversity of *N. larvatus* between populations, which can be used by Sarawak Forest Department for conservation management of this endemic species. Besides that, the non-invasive techniques used to study *N. larvatus* also can be applied for others endangered primate conservation throughout Sarawak, as well as Malaysia.

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

We used the logo in our departmental presentation. We are still working on several scientific manuscripts where the financial support of The Rufford Foundation will be acknowledged.

11. Any other comments?

I sincerely thank The Rufford Small Grant Foundation for funding this research project. I hope there will be more chance of engagement between The Rufford Foundation and my institution in the future.