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	HASINIAINA Irène Alida Frankline
Project title	Geographical variation in vocalization, behaviour, morphology and genetics of mouse lemurs in northwestern Madagascar
RSG reference	15472-1
Reporting period	April 2015 to November 2016
Amount of grant	£5932
Your email address	yasnalid@yahoo.fr
Date of this report	February 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
1st study site: Ankarafantsika National Park (ANP). • Inter - river system I (IRSI). • Species studied: *Microcebus ravelobensis.*				21 April to 17 June 2015. Collecting data methods: - Trapping. - Measurement. - Observation in cage experimentation and recording of the vocalisation. - 42 individuals of *M. ravelobensis* and 13 individuals of *M.murinus* were caught. - 12 pairs (six pairs male/female and six pairs male/male) of *Microcebus ravelobensis* were needed for making the cage experiment. - Morphometric data and vocalisation need to be analysed. - Status of reproduction defined. - Tissues and hair samples was taken for genetic analysis. - Ectoparasites (ticks and lice) and endoparasites (faecal) collected for parasite analysis. - All these data need to be analysed.
- 2nd Locality Marosely forest (MF) • Inter-river system II (IRSII). • Species study: Microcebus bongolavensis				01 July to 17 August 2015. - Two sympatrically species of mouse lemurs (*Microcebus murinus* and *Microcebus bongolavensis*) have been found during the trapping session. - 35 *M. bongolavensis* and five *M. murinus* were caught. - 11 pairs (six pairs male/female and five pairs male/male) of *M. bongolavensis* were studied on this

				site. - Status of reproduction defined. - Morphometric data and vocalisation need to be analysed. - Tissues and hair samples were taken for genetic analysis. - Ectoparasites (ticks and lice) and endoparasites (faecal) were collected for parasite analysis. - All these data need to be analysed.
- 3rd Locality: Bombetoka Forest (BF) • Inter - river system 0 (IRS0). • Species studied: *Microcebus myoxinus*				September and October 2015 - Two sympatrically species were found in BF. - 33 *M. myoxinus* and 18 *M. murinus* caught. - 12 pairs (six pairs male/female and six pairs male/male) of *M.myoxinus* were used for making the cage experiment. - Status of reproduction defined. - Morphometric data and vocalisation need to be analysed. - Tissues and hair samples was taken for genetic analysis. - Ectoparasites (ticks and lice) and endoparasites (faecal) were collected for parasite analysis. - All these data need to be analysed.
4th Locality: Lokobe National Park (LNP) • Inter-river system VI (IRS VI). • Species studied: *Microcebus mamiratra*				June to July 2016 - Distribution and density of *M. mamiratra* was defined in the whole part of LNP. We conducted our study in four different localities: (Ampasindava, CNRO, Ampasipohy and Ambalahonko). - 38 *M. mamiratra* caught. - 12 pairs (six male /female and six male/male) of *M. mamiratra.* - Morphometric data and vocalisation need to be analysed. - Tissues and hair samples were taken for genetic analysis.

				- Ectoparasites (ticks and lice) and endoparasites (faecal) were collected for parasite analysis. - All these data need to be analysed.
5th Locality Ankaramibe Forest (AF) • Inter-river system (IRS V). • Species studied: *Microcebus sambiranensis*				August to September 2016 - We conducted our study in four fragmented forest (Antanimbaribe, Antafondro I, Antafondro East, Andohany Ankaramy.) - 39 *Microcebus* spp. caught. - 12 pairs (6 male/female and 6 male/male) of *Microcebus* ssp. were observed. We supposed to work on *M. sambiranensis* but since we worked in a new area where there no research has been done before, we have to confirm the status of the species by doing genetic analyses. - Data need to be published for the distribution of mouse lemur in Ankaramibe forest. - Vocalisation and morphometric data need to be analysed. - Tissues and hair samples was taken for genetic analysis. - Ectoparasites (ticks and lice) and endoparasites (faecal) were collected for parasite - analysis. - All these data need to be analysed.
6th Locality: Anjiamangirana Station Forest (ASF) • Inter-river system III (IRS III). • - Species studied: *Microcebus danfossi*				October to November 2016 - Distribution and density of *Microcebus danfossi* defined. - 45 *M. danfossi* caught - 12 pairs (six male/female and six male/male) of *M. danfossi*. - Vocalisation and morphometric data need to be analysed. - Tissues and hair samples were taken for genetic analysis. - Ectoparasites (ticks and lice) and endoparasites (faecal) were

				collected for parasite analysis. - All these data need to be analysed.

I have successfully completed data recording on all six proposed study sites (see Figure 1), as suggested in my project proposal, so that we now have a first comparative data set on individualised acoustic signalling behaviour (2090 GB), reproduction and social behaviour, tissue/hair samples for genetic analysis, ectoparasite and faecal samples for further parasitological analysis as well as morphometric data of 24 individual mouse lemurs per study site (in total 72 individuals). Studied individuals most probably belong to six different rufous-coloured mouse lemur species, further to be verified by genetic analysis. Four of these species are described as endangered (*Microcebus sambiranensis*, *Microcebus danfossi*, *Microcebus ravelobensis* and *Microcebus bongolavensis*), one species critically endangered (*Microcebus mamiratra*), one species consider as vulnerable (*M. myoxinus*) according to IUCN criteria (www.iucnredlist.org), so that my project will help to enhance our knowledge on the status and biology of these barely known species. All my field data have now to be analysed according to current state-of-the-art methods and to be prepared for presentations and publications to guarantee dissemination. Further, I will further qualify with this study for my PhD in Zoology, since I am accepted as DAAD fellow (Deutscher Akademischer Austauschdienst: German Academic Exchange Service) and enrolled now into the PhD training programme "Veterinary Research and Biology" of the University of Veterinary Medicine (TiHo) under the supervision of my principal supervisor Prof. Dr. Elke Zimmermann (Director of the Institute of Zoology, TiHo). My supervisor group consist further of PD Dr Sabine Schmidt (expert in bat bioacoustics and orientation, Zoology, TiHo), PD Dr Heike Hadrys (expert in molecular genetics, Animal Ecology, TiHo) and Prof. Dr Miguel Vences (expert in research on Malagasy biodiversity and evolution, Zoology, University Braunschweig), so that I will get major support from diverse areas of research to successfully perform and complete data analysis.

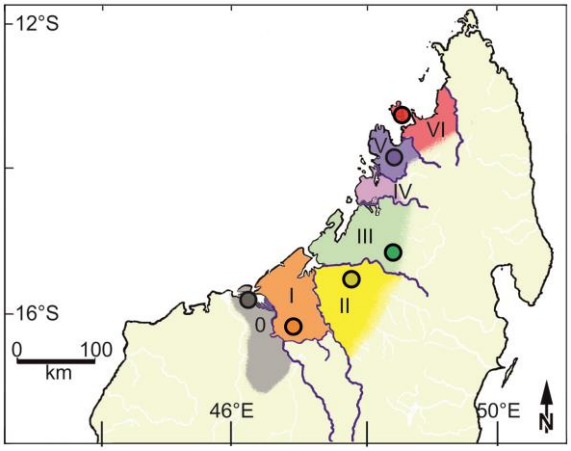

Figure 1. Map showing the six study sites = Inter-River Systems (IRS) in north and north western Madagascar (Source: S. Von den Berg). Colours code for the study sites: IRS 0: Bombetoka forest, BF (in grey), IRS I: Ankarafantsika National Park, ANP (in orange), IRS II: Marosely Forest, MF (in yellow), IRS III: Anjiamangirana, AF, (in green), IRS V: Ankaramibe Forest, AF (in violet), IRS VI: Lokobe National Park, LNP (in red).

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

There were several unforeseen challenges that arose during the project. In the following I will describe them and emphasise how these challenges were tackled.

1. Remote study sites: Five of the six study sites were not accessible by paved roads and study sites were about 115 km to 200 km apart from each other. Case of Marosely forest, MF (2nd study site) and Anjiamangirana Station Forestiere, ASF (6th study site), to reach the study sites, we used a charrette to transport all the equipment and we walked by foot until the Camp. To reach the study sites Lokobe National Parc LNP and Bombetoka forest, BF we had to cross the sea by boat (Ambanja to Ampasipohy, LNP: 3 hours and Mahajanga to Bombetoka, BF: 1 hour). In the case of Ankaramibe forest AF, our camp was in the mountain which is inaccessible for all transport vehicles so we payed porters to carry the equipment and all the team walked by foot.

2. Access to water and energy for recharging electronic equipment:
    a. At Marosely Forest, AF, our camp was situated up the hill where there was no water source nearby. To solve this problem we had to buy water from the nearest village (12 km from the camp) and also rent a charrette to bring the water up into our camp once in a week.

    b. The problem of electricity was a main difficulty during our field study. Since, the study focussed on the recording of the vocalisation, electronic equipment was necessary to accomplish the task. However, only one (ANP) among the six localities had access to electricity. For transferring data into the hard disk, we needed electricity to charge the computer. Thus, we have to go to town every ten days to make the data transferring. We had to stay there overnight because in 10 days we collected around 200 GB of data files.

3. Trapping enough animals for data collection: During the second part of our field study, 2016, we worked in the tropical rain forest of LNP and in the transitional forest of AF. The rate of trapability over several days at these two study sites was very low by using Sherman traps. Thus, we changed our capture strategy and decided to adopt another method (catch by hand) which needed more people. We supposed to have only one guide, however to practice this new technique, we have needed at least five people. Consequently, we added two other guides which increased the number of guide to three. In that case we had in total three guides at all.

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

According to my view, there will be three major outcomes of my project:

Scientific outcome

My project will help enhancing our present knowledge on the species status and the biology of six newly described and barely known lemur species which are considered as endangered, critically endangered or vulnerable according to the IUCN criteria. My bioacoustics study will help to evaluate new bioacoustics tools to diagnose these species non-invasively and rapidly. A vocal repertoire of these target species will be established as well as the context and some behavioural characteristic of each species. Then, the evolution of social dominance of these species will be described as the result of my colleague (Rina Evasoa Mamy PhD student at University Veterinary of Hannover, Germany).

Educational outcome

My project has included the field work of my colleague (Rina Evasoa Mamy PhD student at University Veterinary of Hannover, Germany) on the evolution of social dominance of *Microcebus* spp. in the north and north western of Madagascar. Four Master students from the University of Mahajanga, Madagascar (2015: Mahatoly Ursulla Laura Zita supervised by Dr Romule Rakotondravony, and Andriamendrikaja Hasinirina Angelo Stefan supervised by Prof Solofonirina Rasoloharijaona, Topic: Activity budget of caged mouse lemurs and implications for conservation management and ecotourism: effects of species, sex, body mass and parasite load . 2016: Ratsimbazafy Sandra Paule supervised by Dr Romule Rakotondravony and Etangie Radelin supervised by Prof. Solofonirina Rasoloharijaona , Topic: Study of *Microcebus* spp. from Northwestern and Northern Madagascar: Ethology, density, threats and implication for conservation) have benefitted from a training course including techniques of mouse lemur trapping, handling, measurement as well as the censusing to determine the density of the population on the respective sites and also helping us during behavioral observations.

Conservation outcome

Further, basic conservation training has been provided to our local guides from all localities because three of the locality where we conducted our research is not a protected area. Thus, we convince them to induce the villagers to protect the biodiversity and to preserve the environment. Beside of that, we paid a salary to the local guide as well and the cost depends in general on the study site (5000 to 16000Ar per day). We also paid for the cooker which is also among the villagers.

The negotiation of conservation issues with the rural population at the different study sites (in particular the head of the villages) has contributed to focus the attention of

the rural population to conservation issues. A focus group with the head of the villagers and somes villagers was made at the beginning of our stay. This meeting focussed especially to explain the aim of our study and also the benefits of conservation (e.g. ecotourism, help of researchers etc.) which could create more jobs for the villagers.

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

During our stay in the field, we noticed that the rural population at five study sites have no knowledge about the relationship between destroying the natural environment and the longterm effects of that to their daily life. We thus introduced not only our project to them, but also discussed a lot with the respective local community and the local head of the villagers at the beginning of each field study upon the costs and benefits of conserving natural environments, e.g. forests or protected areas. We thereby mentioned that the conservation of the nature is really important and everyone is responsible of his/her act. We educated them to preserve the biodiversity by explaining them the advantages that they could have for protecting the biodiversity. We also convinced them to create a small association to preserve the nature on their area. We suggested to them to stop making bushfires, charcoal and logging, since there will be a lot of people coming to enjoy nature as it is e.g. the case for ecotourists, or researchers like we, since this nature is unique on the whole world. Only when the nature is protected and there will be no further logging, no bushfire and no lemur hunting, they will benefit by being employed.

We informed the responsible of Madagascar National Parc, MNP and Madagascar Aye Fund, MAAF (which are an organisation who managed the protected area in Madagascar) about the preliminary results of our field study by reports about the situation at each study site and the information that we collected during our field study. These reports should help them to raise attention to conservation issues and to integrate the rural community in conservation actions.

For the case of not protected area BF, MF and AF we first suggested them to conserve the biodiversity in the respective area by starting to work together with some of them as a local guide. Beside of that, we given advice to them on how to create an association to protect the biodiversity on the not protected area and also how to get financial support?

5. Are there any plans to continue this work?

To date, I am working on the transcription of handwritten and dictaphone protocols to Excel tables. Besides of that, I am working by screening audio recording file with a respective programme (Audacity) for call analysis.

I also participate in PhD training programme and courses at the Institute of Zoology which help me to have a good knowledge for managing my analyse of data. During my PhD study, I have to show my results step-by-step by presentations at the Institute once or twice in a year, talk and poster presentation to the Graduate School at the TiHo and Seminar scientific which is also mandatory for the PhD Student.

A manuscript about lemur communities at the different study sites will be submitted to be published in Lemurs News soon.

No scientific paper has been submitted yet to an international journal but several papers are planned: 1) Divergences in the vocal repertoire of genetically distinct species of mouse lemurs; 2) Agonistic calls discriminate cryptic species in nocturnal primates; and 3) Is social role reflected in vocal behaviour in phylogenetically basal primates?

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

After analysing my comparative data set, I plan to present and discuss findings in posters or talks at different scientific meetings and conferences, to get further suggestions, to prepare scientific manuscripts and to submit these manuscripts for publication in peer-reviewed international journals. Furthermore, I plan to publish some findings to local journals in Madagascar. Major parts of the project will thus be accessible to the local and international audience. I also always discuss my data with my supervisor Prof. Dr Elke Zimmermann and other experts and scientific colleagues in my field. Thus, I have to present and defend my findings step by step in seminars at the Institute of Zoology (1-2/year), in seminars of the PhD training programme (1-2/year) and at German, European or International Conferences in my field (at least 1/year).

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 2015 to October 2015 (6 months) for the first part and from June 2016 to November 2016 for the second part of my field data collection in Madagascar, which is now part of my PhD. My personnel support was coming from

the Institute of Zoology at Hannover during the first field period and was then coming from a fellowship of the DAAD. The DAAD will support the data analysis in Germany and the preparation of my PhD thesis (planned to be submitted in about two and a half years).

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
Travel expenses	1329,4	1353,84	+24,84	The transportation costs were higher than anticipated. We had to book a car, boat and charrette to safely transport our equipment. At some point we had to move several times, trying to find a place with a high density of mouse lemurs (ANP (3 times) and ASF (1 time: instead of Anjajavy we changed to the study site to Anjiamangirana).
Field assistant salary	882,3	923,07	+40,77	Each assistant was paid 100000Ar/month. 3 assistants were paid
Local guides fees	661,7	612,56	-49,14	The guides' fees varied between the studies sites (16000 to 5000Ar/per day). For LNP, ASF and AF we increased the number of the local guides because we need more people for the new methods that we used.
Daily expenses	2647	2461,53	-185,4	The daily expenses covered food and accommodation for the project responsible and the assistant. Transportation of water is

				included in this as well.
MNP Fees MAAF Fees Research fee for the commune of Anjiamangirana	7,05	142,30	+135,26	The expenses for MNP fees were significantly higher than previously budgeted. This is because we were supposed to work only on one site managed by the MNP. However the schedule was changed and we worked in 2 sites (ANP, LNP) managed by the MNP and also 1 site (ASF) managed by MAAF (Madagascar Aye aye Fund). Besides of that, we were accompanied by 4 Masters students and also paid the fees for them. In ASF, we also paid a communal tax to the Mayor of this site.
Consumables	404,8	512,2	+107,4	The majority of this money was spending on batteries that we needed for the equipment such as wildlife acoustic box, Maglite, headlamps, flashlight. Other expenses were cell phone credit for communication and information and some medicaments.
Total	5932,2	6005,5	+73,73	

Rate: 1 Livre sterling is around 3900Ariary

I highly appreciate the financial help by RSG without which my PhD qualification would not have been possible.

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

The next step is to focus on data analysis. Major important steps for me are the following

1. Analysing comparative data set of project and dissemination of findings.

2. Fulfillment of training requirements (lectures, courses) to further qualify for the submission of a PhD thesis, defence of thesis.
3. Return to Madagascar to search for a position in education, conservation or research to transfer my knowledge to the responsible authorities as well as local communities in Madagascar.

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?

The first part of my field study contributed to a poster presented during the 26th Congress of International Primatological Society (IPS) in Chicago in 2016 (see attached poster).

The Rufford small grant was mentioned as financial support for this project. The poster was entitled "The role of vocalisations in species diversity and evolution of lesser Mouse lemurs (*Microcebus* spp)" and was presented by Prof. Dr Ute Radespiel from University Veterinary of Hannover, Germany.

The RSGF logo has been used during my presentation for the first meeting of Supervisor group on 24 March 2016 at the University Veterinary Medicine of Hannover and at the Rufford Small Grants Recipients Conference, 16-17 January, Madagascar 2015.

The RSGF logo will be shown as a financial support to the Poster Presentation that my supervisor will present at the Etho 2017 conference in Bonn between 22nd and 24th February and that I will present at European Conference of Primatology in Strasbourg between 22nd and 27th August 2017.