# 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** | Vania Carolina Fonseca da Silva |
| **Project title** | *Comparative Conservation Genetics of Giant and Neotropical Otters in Central Brazilian Amazon Basin* |
| **RSG reference** | 20201-1 |
| **Reporting period** | September 2016 – September 2017 |
| **Amount of grant** | £5000 |
| **Your email address** | vaniacfs@gmail.com |
| **Date of this report** | August 2016 |

**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 |
|---|---|---|---|---|
| Collect samples from giant and neotropical otters for DNA analysis, including tissue, fecal and mucus materials. | | | | Good progress was made at several key sites, however unavoidable delays and an early rainy season together with the consequent flooding had a negative impact since we had to go inside the igapo flooded forest in order to find the animals. It is much more difficult to work in the igapos, because the giant otters expand their territories, the vegetation is very dense, and our locomotion is slow (by paddling a canoe), so not really efficient conditions for this kind of sampling. This had further implications, since the DNA capture and sequencing needs to be undertaken when a full set of samples becomes available. |
| DNA extraction | | | | DNA extraction techniques have been worked out to provide good quality and yield. |
| Capture and sequencing DNA | | | | The bioinformatics required for bait capture design has been completed, facilitated by collaborations with researchers who have generated reference sequences for both study species. Work can be expected to progress quickly once the full sample set has been collected (planned for the upcoming field season). |

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

**-Delayed field campaign**

Due to legal complications my scholarship sponsors took more time than expected to issue the authorisation for my trip to Brazil, so my field season was delayed and by the time work commenced the wet season had started. This was further

complicated by January being a particularly rainy month that year. Some areas where I was supposed to work on started flooding, so I had to adjust my plans in order to be able to collect samples in suitable areas, which were: Amana Sustainable Development Reserve, Rio Pitinga and Uatuma Biological Reserve, Balbina reservoir.

**-Sampling and weather adversities**
Although the collection of biopsies was originally planned, flooding meant that the giant otters expanded their territories into the flooded forest area making it more difficult to find and follow them. Therefore I decided to focus on fecal/mucus sampling as it is less weather-dependent, and provides sufficient DNA for the planned analyses. In the end, the overall sampling was successful in spite of the inadequate weather conditions. Although due to weather restrictions I sampled at fewer and different areas than initially intended, this will be compensated for in the next field season. This also means that DNA capture and sequencing was delayed until this can be based on the full sample set.

**-Protocol for fecal DNA extractions**
Extracting DNA from non-invasive samples is always challenging as the DNA can be more rare or degraded than from fresh tissue, and additional compounds in fecal samples can affect efficiency. We initially tried the recommended commercial kit from Qiagen (DNA stool mini kit), which was successful in other studies, however the yield of DNA was low compared to phenol-chloroform extraction. We therefore focused on phenol-chloroform extraction, but this also required further titration to ensure good quality DNA (with low protein contamination). This has involved the inclusion of a purification step using CTAB. A series of alternative methods involving the use of salt for protein precipitation and spin columns were tested prior to settling on this preferred method.

Work on developing the bait capture protocol also progressed to completion, and we can now order the bait capture kit. This involved extensive bioinformatics analyses and population genetic modelling to select loci that would provide sufficient diversity to be detectable at the population level. It also involved the inclusion of a range of locus types, dominated by non-coding regions, but also including some key coding loci to permit tests for local adaptation. Baits will be designed matching both species, made possible by the provision by collaborators of reference genome sequences for each of our study species. It is most cost effective to build DNA libraries and undertake the sequencing runs once all samples are available, and so this will be done following the final field season (to commence in September this year).

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

First, in the last field season, in a total of 50 effective days of sampling, I collected 79 Neotropical otter samples and 54 giant otter samples. During fieldwork, I developed protocols to increase the chances of finding scat samples. I determined that in the hydroelectric reservoir of Balbina, there seems to be areas of higher concentration of Neotropical otter signs which appear distinct from the ones used by giant otters.

Neotropical otter scats were mainly found in the dead trees spread in the middle of the lake, away from land, whereas giant otters show their normal preference for high land banks present in the reservoir`s islands. That was an interesting finding since a recent study already reported that this lake constitutes a lower-quality habitat for giant otters despite increasing the available habitat area; so this may suggest competition between these two species in the area, and this could also affect their dispersal and thus gene flow.

Second, extensive titration using samples for which there was ample material has led to the development of a reliable method for the extraction of high quality DNA from the study samples, suitable for next generation DNA sequencing.

Third, a thorough bioinformatics analysis has led to the development of a DNA bait capture kit that will provide a large number of marker loci very likely to show informative levels of polymorphism. This will be available for others to use in future projects on these same species, broadening the geographic range of the wider study and the strength of inference.

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

In Amana Reserve, we shared the objectives of the study in meetings organised with the two villages involved in the sampling area. We explained the goals and methods to be used, with an emphasis on the biopsy darts, as it was something they have never seen, thus we had to make it clear what we were doing. We also asked their support in sharing recent observations of giant otters in the area. Another goal of the meeting was to identify the people who would want to work as field assistant with us, and make sure all the others agreed with that. The participants showed interest, raising questions about the project and about the otters. It was really helpful to have their support as they did share frequently information on otter sightings during the period of the study.

**5. Are there any plans to continue this work?**

Yes, this project represents my PhD study and is still ongoing (with 2 years remaining). The next steps will include a final field season during which sample sizes will be increased and the geographic range expanded. After returning to the lab work on DNA library construction and DNA capture for the target loci will commence. These data will then be analysed bio-informatically to extract data on the individual polymorphic loci, and to test hypotheses about population genetic structure, patterns of migration, levels of diversity, local adaptation, and to contribute to the effective conservation of diversity for these species.

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

One poster was already presented at Durham University`s Biosciences postgraduate conference. For next year, I plan to submit an abstract for the Student Conservation Conference in Cambridge and also participate in the next International Otter

Congress. My PhD program will be finished by October 2019 when I have to present my thesis, and I also expect to submit scientific papers in international peer-reviewed journals. Summary data will be presented online and directly to local and regional conservation agencies to help promote the better conservation and management of these species.

**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 grant was used mainly from November to April 2017 for fieldwork expenses. It was spent accordingly to what was proposed since the requested budget was focused on the fieldwork component of this project (with funds for the genetic work coming from other sources).

**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.** 1 £ sterling = 3.95 BRL

| Item | Budgeted Amount | Actual Amount | Difference | Comments |
|---|---|---|---|---|
| Boat Rental | 270 | 161 | +109 | I managed to borrow a boat from local collaborators in Uatuma Biological Reserve |
| Shipping of Samples | 180 | 180 | 0 | |
| Field assistant (dart sampling) | 3600 | 3450 | +150 | Less days than expected |
| Food | 400 | 400 | 0 | |
| Gasoline for trips between Tefe-Amana Reserve and Balbina Village-Field station, and for power generator | 550 | 600 | -50 | Increased price + currency rate changed |
| Extra supplies for sampling (tubes) | 0 | 15 | -15 | Not included in the estimate |
| Batteries for the GPS | 0 | 28 | -28 | Not included in the estimate |
| International Transfer charge | 0 | 52 | -51 | Not included in the estimate |
| Accomodation charge in Amana Reserve (1 person, 20 days) | 0 | 115 | -115 | Not expected upon budget estimation |
| **Total** | 5000 | 5001 | | |

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

The plan for the coming components of the study are outlined above. After this PhD study is complete, it will be important to work with regional stakeholders and agencies to help translate these data into more effective conservation policies. Our data (and the availability of our bait capture protocol) will also facilitate the extension of our understanding of population structure and dynamics for these species over a much wider geographic range.

**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, I did use it on the poster presented in Durham Bioscience Postgraduate conference and I also use it on my project blog and Facebook page. We will cite the support of the Rufford Foundation in all publications resulting from this work.

**11. Any other comments?**

We are very grateful for the support of the Rufford Foundation and convinced that these data will make a substantive difference to the conservation of these iconic regional species.