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	Maira Proietti
Project title	Identification and Genetic Characterisation of Immature Hybrid Sea Turtles in Brazil
RSG reference	16162-B
Reporting period	October 2014 – Mar 2017
Amount of grant	£9955
Your email address	mairaproietti@gmail.com
Date of this report	17 March, 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
Sampling of immature hawksbill turtles			■	143 new samples were obtained for analysis.
mtDNA analysis of samples			■	A total of 300 samples were analysed in terms of mtDNA.
nDNA analysis of samples		■		40 samples were analysed in terms of nDNA; we are currently finishing lab analyses.
Communication of results			■	Partial results were communicated in several ways – see item 6 of this report; final results will be reported as described below
Preparation and submittal of scientific paper		■		A final paper will be submitted by August 2017 to journal *Molecular Ecology*.
Advisory of two undergraduate students (Oceanography course)			■	Two undergraduate students were advised in their graduate work.
Advisory of one graduate student (Master's degree in Biological Oceanography)			■	One MSc candidate is currently finishing her dissertation on the subject of this project

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

We had difficulties in amplifying the nuclear DNA markers we had initially planned. After several months of failed or very poor PCR reactions, we had to change the markers (adopting those described by Vilaça et al. 2013), leading to more execution time since additional primers needed to be ordered, and PCR reactions needed to be redone. We also had problems with PCR contamination, which led to the complete interruption of lab activities for almost 3 months, until the contamination source was found and dealt with. We are currently finishing lab analyses and have partial nuclear results, which will be finalized and reported in a final paper submitted by August 2017 to journal *Molecular Ecology*.

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

We obtained and analysed 143 new samples from immature turtles morphologically identified as hawksbills (*Eretmochelys imbricata*) from: the northeast, at Ceará state (Almofala), Bahia state (Arembepe, Praia do Forte and Sauípe) and the Abrolhos National Marine Park; southeast Brazil, at Espírito Santo state; and south Brazil, at Rio Grande do Sul state (Cassino). The previous RSGF project conducted by this team detected four hybrids among 157 samples collected along the coast. Our updated sampled size of 300 immature hawksbills has led to the detection of 17 sea turtle hybrids using the mtDNA D-Loop marker, corresponding to approximately 6% of analysed turtles. Our previous work had encountered only hawksbill x loggerhead hybrids, with high frequency of occurrence in south Brazil. The updated analyses revealed more extensive hybridisation, including one hawksbill hybridised with an olive ridley (*Lepidochelys imbricata*) and one with a green turtle (*Chelonia mydas*). Preliminary nuclear DNA data has also shown that hybridisation between immature sea turtle species is more common than shown only through mtDNA – for instance, in the 40 samples with nDNA analysed, we detected one hybrid that was not identified through mtDNA, which displayed an unusual hybridisation pattern: hawksbill x loggerhead hybridisation with a backcross with olive ridley. This indicates that the process is much more complex than previously believed, and must be further investigated. Finally, increasing sample size confirmed that the highest hybrid frequency occurs with loggerheads in south Brazil, with 11 in 34 samples (over 34%) corresponding to hybrids instead of actual hawksbills. South Brazil is a region more common for loggerheads, indicating that these immature hybrids, which are morphologically similar to hawksbills, could be adopting behavioral traits more similar to loggerheads. Better understanding the distribution and ecology of sea turtle hybrids is essential for developing adequate management plans for these animals.

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

N/A

5. Are there any plans to continue this work?

Yes. We are currently increasing our sample size to include other regions along the Brazilian coast, and obtain a more accurate picture on hybrid distribution. Additionally, we aim to develop a genomic study of these hybrids, in order to evaluate their genomes in terms of functional genes and possible adaptive potential. We are also part of a network of projects on sea turtle hybrids, which involve multidisciplinary studies, and will continue to collaborate with efforts to order

to clearly identify the possible ecological impacts of hybridisation on endangered sea turtle populations in Brazil.

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

The results of this work have been shared in several ways. We have presented at several scientific conferences – such as the International Sea Turtle Symposium held in Peru in 2016 (presentation previously sent), and the Brazilian Symposium on Marine Biology in 2015. Additionally, I presented the results in the EURAXESS Science Slam 2015 competition in Brazil, in which I was selected as one of the five best videos, for presenting my research on sea turtle hybrids in an accessible manner. The competition video can be found on YouTube through the link https://www.youtube.com/watch?v=FGjuEDej_BU&feature=gp-n-y&google_comment_id=z12aedwwomu0zrnpp04cgzco0xnxtrojx0s - English subtitles are available.

In 2015 I was invited to present and discuss our partial results in the meeting of the National Action Plan for the Conservation of Sea Turtles, held in Regência, Espírito Santo state, where sea turtle and conservation experts met for 3 days to discuss the next steps in the Action Plan.

In January 2017 I presented my work with sea turtle population genetics and hybridisation at the Rufford Foundation Brazil Conference, where I received second place for best presentation. The abstract and a photo of the event are attached.

Additionally, we have produced two short notes during field expeditions, published in Marine Turtle Newsletter (attached), where the Rufford Foundation is also acknowledged.

Finally, the main paper reporting the final results of the project is currently being written, and will be submitted by August 2017 to the high-impact journal *Molecular Ecology*.

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

October 2014 to July 2017, representing more time in terms of the anticipated length of the project. This occurred since we had difficulty in amplifying the nuclear DNA markers we had initially planned. Due to this difficulty, we had to change the markers, leading to more execution time since additional primers and PCR reactions were necessary. We also had problems with PCR contamination, which led to the complete interruption of lab activities for almost 3 months until the contamination

source was found and dealt with. We are currently finishing lab analyses and have partial nuclear results, which will be finalized and reported in a final paper submitted by August 2017 to journal *Molecular Ecology*.

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
Veriti 96-well thermocycler	6570	6795	+225	Due to currency exchange, the cycler cost slightly more than anticipated
DNA extraction kit (tissue)	522	522	-	DNA extraction numbers were as anticipated
Primers for PCR	306	510	+204	Due to failed PCR reactions additional primers needed to be ordered
dNTPs for PCR	252	252	-	dNTPs were sufficient for all extra reactions
Ultrapure water	98	98	-	Ultrapure ater was sufficient for all extra reactions
Taq polymerase for PCR	367	470	+103	Due to failed PCR reactions additional Taq needed to be ordered
GELRED gel stain	350	0	-350	GELRED was acquired by the Biological Oceanography Graduate Program
PCR purification kit	560	560	-	No extra purification kit was requited
Sequencing and genotyping	930	930	-	Final sequencing will be done by August 2017
Total	**9955**	**10137**	**+182**	Difference in budget was met by the Biological Oceanography Graduate Program

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

The next steps ate to continue analysis of nuclear DNA markers, which are revealing an even higher frequency of sea turtle hybrids in Brazil. These markers are also showing unusual hybridisation patterns, such as hawksbill x olive ridley and hawksbill x green turtle hybrids, and hawksbill x loggerhead hybrids backcrossed with olive ridley. This indicates that the process is extremely widespread and must continue to be investigated, along with other ecological and genomic characteristics, in order

to better understand the possible effects and negative impacts of hybridisation on the behaviour, distribution, adaptation and survival of endangered sea turtle populations. If negative impacts are identified, we must work to propose adequate management strategies for decreasing this process. We are also part of a network of projects on sea turtle hybrids, which involve multidisciplinary studies, and will continue to collaborate with efforts to order to clearly identify the possible ecological impacts of hybridisation in Brazilian sea turtles.

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?

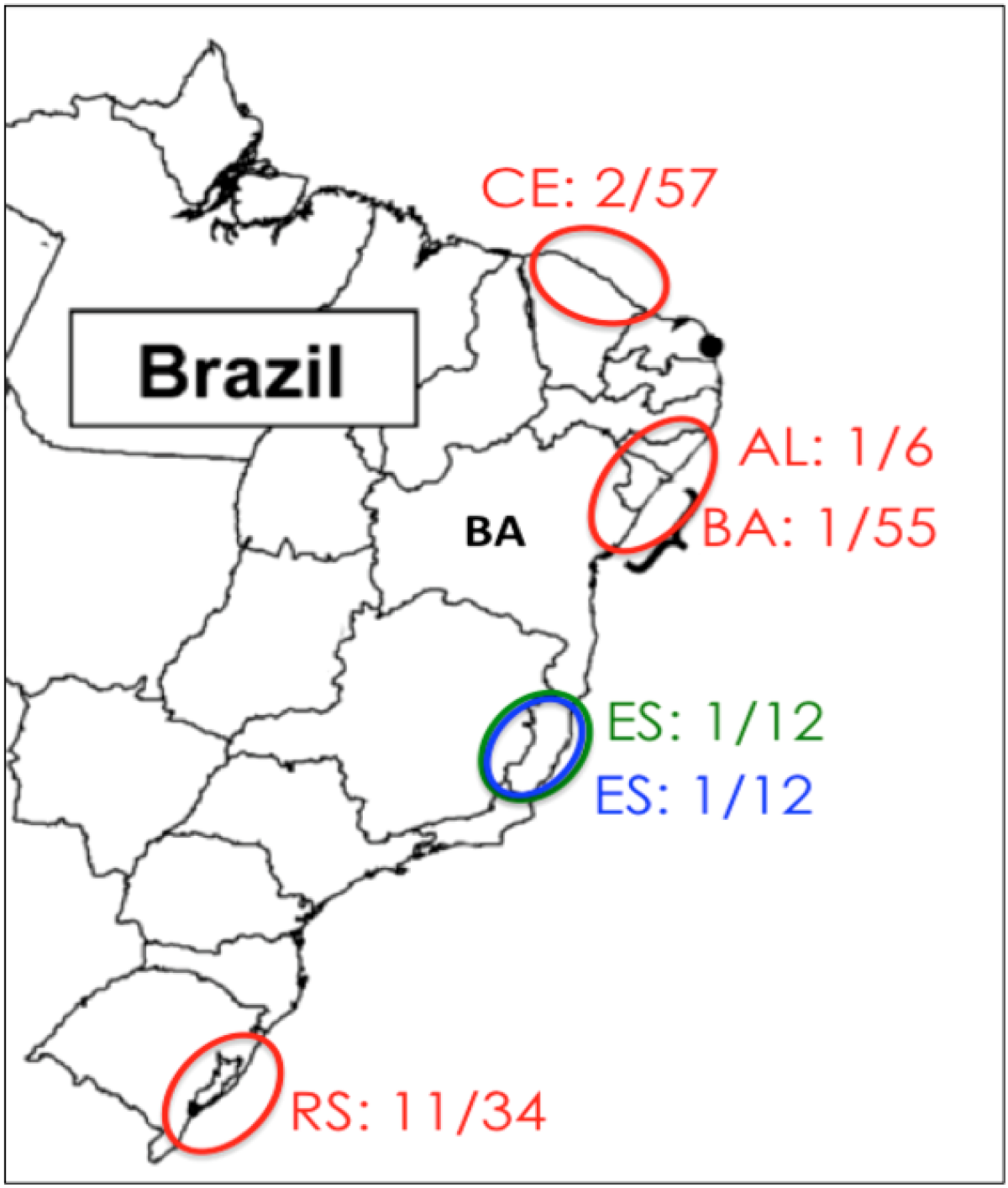
Yes, all materials produced received the Rufford Foundation logo. The logo was used in presentations (conferences and lectures), t-shirts, and the Foundation was mentioned in all papers and materials produced.

11. Any other comments?

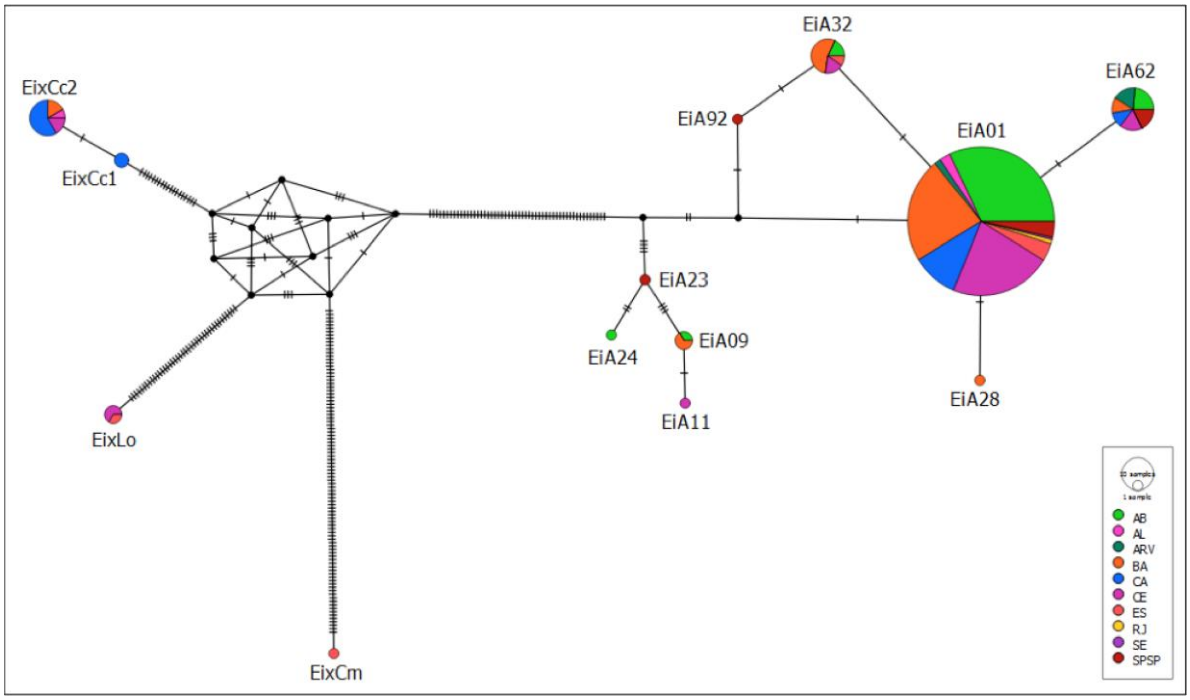
We greatly acknowledge the Rufford Foundation for its continued support in the development of studies on sea turtle ecology, genetics, and conservation.

Presenting our work on sea turtle population genetics and hybridisation in Brazil at RSG Brazil Conference 2017.

Certificate of 2nd place in presentations at the conference.



Distribution and frequency of immature sea turtle hybrids in Brazil. Red represents hawksbill x loggerhead hybrids; blue hawksbill x olive ridley; green hawksbill x green turtles.



Network of haplotypes found in immature hawksbill turtle samples along the coast of Brazil. Circle size represents haplotype frequency; colour represents location (AB=Abrolhos; AL=Alagoas; ARV=Arvoredo; BA=Bahia; CA=Cassino; CE=Ceara; ES=Espirito Santo; RJ=Rio de Janiero; SE=Sergipe; SPSP=Sao Pedro & Sao Paulo Archelago). Haplotypes EixCm, EixLo, and EixCc are respectively hawksbill x green, olive ridley, and loggerhead hybrids.