

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	Yoamel Milián-García
Project title	Conservation Genetics and Molecular Systematics of Cuban <i>Crocodylus</i> (Crocodylia: Crocodylidae)
RSG reference	19318-B
Reporting period	March 2016-May 2017
Amount of grant	£10000
Your email address	yoamelmg@gmail.com
Date of this report	June 5 th , 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
To evaluate molecularly the systematics and taxonomy of the Cuban crocodiles based on mitochondrial and nuclear markers				

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

During the developing of the project we submitted a paper to an Open Access Journal. The paper focused on mating system of the Cuban crocodile passed two rounds of revisions, but it was not finally accepted for publication in the journal. It implied to reformat the paper and resubmit it to another journal. It consequently implied to reinitiate new rounds of revisions and a delay in the publication of the paper.

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

1. The genetic results revealed for the first time that the Critically Endangered *C. rhombifer* mating system is likely best characterised as promiscuous. This new knowledge of mating system is important for reintroduction, especially when considering optimal sex ratios and choosing males of high breeding potential. The results also have implications for *ex situ* management, as it indicates that imposing polygyny by enforcing a 1:2 male to female sex ratio in captive breeding pens is no longer justifiable. It consequently suggests that the standard practice of enforcing a 1:2 sex ratio should be altered in order to better maintain a demographically and genetically healthy population.
2. It was ratified the existence of a cryptic species of the American crocodile inhabiting Cuba using for the first time mitogenomic data. We generated for the first time a partial mitochondrial genome from a wild Cuban *Crocodylus acutus*. These results highlight the necessity of a review of the current taxonomy of the species of *Crocodylus* inhabiting Cuba which has also an impact in the order Crocodylia.
3. The genetic results generated were essential in the selection of the individuals released to the wild, as part of the first time recovering programme of the Cuban crocodile in the wild. A total of 100 individuals were released to the wild Zapata swamp and all of them were born in captivity from genetically characterized and ratified as non-hybrids breeders based on both, molecular and morphological markers.

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

Local community members have supported field expeditions serving as guides in the field. They have also taken part in experience interchanges while looking for getting them actively involved in the conservation of Cuban crocodiles. We have also completed regular interviews with local community members to explore their awareness on the Cuban crocodile population decline. They have been also the target of all the communication actions looking for diminishing the illegal hunting.

Local community members have been also invited to participate in workshops organised in the area. They have been focused on updating the situation of the Cuban crocodile. It has the goal the make them feel part of the studies and at the same time increase their knowledge about the importance of the species.

5. Are there any plans to continue this work?

Yes, there are. I would like to scale the study to a genomic level. At present, we have significantly increased the sampling area and the number of samples. In that sense, we have a broad representation of the main population of the species around the Cuban archipelago. We have also established a solid collaboration network with prestigious international institutions. It makes us possible to propose regional studies to answer scientific questions of common interest.

On another hand, none of the genomes of the Cuban crocodile species have been sequenced yet. I would like to initiate those studies in collaboration with the experienced international centres.

We have also completed a characterization of the current subfossil record of the Cuban *Crocodylus* (*C. rhombifer*) deposited at the American Museum of Natural History (AMNH). I would like to genetically characterize this material looking for signals of past hybridisation in the type locality of the Cuban crocodile.

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

I plan to share the results of the project through the publication of scientific papers and also taking part in both national and international workshops on species conservation. At the moment, we have already published a paper on a peer-reviewed scientific journal and a PhD thesis as a book by an international academic editorial. In addition, we have two other papers submitted to scientific peer-reviewed journals. Both of them are a result of graduate and postgraduate students' work completed under my supervision and during the development of the project.

On another hand, I will be sharing the results of the work through the organisation of scientific seminars to discuss methodologies and strategies to face new challenges on *Crocodylus* species Conservation in Cuba. I have also been invited to present the results generated during the project in an international workshop on the *Status of*

Crocodile Conservation in Cuba to be held in Playa Girón at Matanzas, Cuba. The Workshop dates are from 5th-9th June 2017.

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 Rufford Foundation grant used in a period of 1 year and 2 months.

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	1450		0	
Lodging	1200		0	
Gas	1200		0	
Communication	200	500	+300	Extra Internet access and cell phone communication needed/ International communication
Printing & toners	350		0	
Office materials	100	250	+150	It covered extra material used for postgraduate and graduate students
Rope	100	150	+50	Extra rope meters were gotten for field work
Backpacks	150		0	
Food	1500	1600	+100	Field expeditions involved in some cases more specialists or students than previously planned
Hiking shoes and boots	300		0	
Field gear	250		0	
Pipets	300	500	+200	A complete set of pipets was acquired for sample processing
Tubes for sample collection	100	200	+100	There was collected a high number of samples that needed to be stored
Tablet for data collection	250		0	
Kit for PCR	450	500	+50	Reagents were acquired accordingly to the number of samples collected in order to complete sample processing
Kit for DNA extraction	150	400	+250	Reagents were acquired accordingly to the number of samples collected in order to complete Lab processing
Headlamps and lanterns	90		0	
External hard drives and memory cards, batteries	310		0	

and recharger				
Publishing in Open Access Journal	1200	0	-1200	One paper was submitted to an Open Access Journal but it was not finally accepted for its publication in the Journal. The paper was resubmitted to another Journal and the funding was consequently reallocated for other items.
Handling and shipping to Cuba/Contingencies	350		0	
Total	10000		10000	

Average exchange rates across the year: 1£=1.23 CUC (Cuban Convertibles)
 1CUC=24 CUP (Cuban Pesos) 1CUC=0.87 USD (US Dollar)

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

With recent developments in DNA sequencing technology and bioinformatics tools, evolutionary biologists are today able to track and characterize the fundamental evolutionary processes of speciation, adaptation and introgression at a level of detail not attainable only a few years ago. Still, many conservation actions are today being taken without critical knowledge about these evolutionary factors and mechanisms: how unique is a given evolutionary taxon? What is the level of genetic variability in its genome (which correlates with risk of inbreeding depression)? Is hybridisation occurring, and – if so – is it a natural or a recent anthropogenic phenomenon? I would like to use modern genomic approaches to study these questions and processes in the regionally restricted and critically endangered taxon – the Cuban crocodile (*Crocodylus rhombifer*).

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 Rufford Foundation logo was used in a prominent position in all the posters related to the work and presented in both National and International meetings. The RSGF was also properly acknowledged in the acknowledgement section of all the papers and materials already published or submitted for publication.

11. Any other comments?

I would like to express my deep gratitude to the Rufford Foundation for kindly support our research on Cuban *Crocodylus* conservation. Its support has been a key component in generating the genetic information that has served as the basis for the conservation genetics of the Cuban crocodiles. It also set the baseline of future projects on conservation genomics of the Cuban *Crocodylus*.

