

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	Randall Jimenez Quirós
Project title	Amphibian skin microbiome and its interaction with <i>Batrachochytrium dendrobatidis</i> : tools for the development of probiotic therapies to mitigate chytridiomycosis in Costa Rica.
RSG reference	19648-1
Reporting period	May 2016 - January 2018
Amount of grant	4748
Your email address	randall.jimenez@uni-ulm.de ; randall87@gmail.com
Date of this report	January 2018

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
Examine the skin bacterial community of <i>L. vibicarius</i> across development, site and time.				We completed all fieldwork. We are currently conducting laboratory analysis and bioinformatics to complete objective.
Evaluate the prevalence of chytrid fungus (Bd) in <i>L. vibicarius</i> .				Laboratory analysis is being conducted to complete objective.
Determine the effect human-induced landscape alteration in the skin bacteria of <i>L. vibicarius</i> , and the influence of these skin bacteria to host chytrid fungus-infection rate.				All samples have been collected and we are working on laboratory analysis to complete objective.
Establish an amphibian conservation program in the National Park Juan Castro Blanco with the support of local communities.				We had successful meetings, and presentations with local people to involve them as actors in our long-term conservation programme.

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

We had one setback during the development of this project. We originally planned to conduct two fieldwork seasons in 2016. Due to low rainfall in 2016 we were only able to conduct one field season. Therefore, we postponed our second field season for 2017.

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

1- We were able to gather samples for the skin microbiome and chytrid fungus from *Lithobates vibicarius*. The analysis of these samples are providing relevant knowledge for future amphibian conservation actions due to the relationship between the host-microbiome and host-health. Our results can be use as tools to

better understand how anthropogenic stressors and emerging pathogens (i.e., Bd) affect amphibians' health, to better protect threatened relict populations. We will have more knowledge about how to maintain amphibians with a good health in their habitat.

2- We rediscovered an extinct frog species, *Craugastor escoces*, during the fieldwork of this conservation project. The rediscovery of this species comes after 30 years without being observed and 12 years of being declared extinct by the IUCN. The last sighting of this species preceded the last sighting of the symbolic golden toad (*Incilius periglenes*) in the nearby cloud forest of Monteverde, Costa Rica by only 3 years. Furthermore, we recently found the frog *Isthmohyla rivularis*, which is currently categorised as Critically Endangered (CR) by the IUCN. These findings gives a higher conservation value to the National Park Juan Castro Blanco and urges the need to better protect these amphibians in their habitat.

3- We were able to develop and establish an amphibian conservation project, and involve local communities to better protect the habitat of *Lithobates vibicarius*, *Craugastor escoces* among others (e.g., *L. rivularis*). This programme is crucial because we detected: a) new reproduction sites of *L. vibicarius* that requires further monitoring and conservation; and b) new potential threats (exotic species, pesticides and habitat disturbance) to the population of *L. vibicarius* and *C. escoces*. The latter highlights the need of an evaluation about the actual impact to the survival of these amphibians.

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

We have been working with a community that have been involve in the conservation of natural resources, particularly water. An agreement between APANAJUCA (local NGO) has been established with the University of Costa Rica for continuous support in research and further conservation actions. Workers from APANAJUCA were involved during our fieldwork, whom provided assistance field assistance.

We were able to talk and do presentations about our project to local communities nearby the National Park Juan Castro Blanco. We also had the opportunity to explain about the role of amphibian in the ecosystems and the threats they are facing. We made them understand that conservation actions for amphibians and its habitat are crucial for the maintenance of water reservoirs.

In 2017, the national postal service of Costa Rica selected *L. vibicarius* and *C. escoces* for postage stamps to commemorate the 25th anniversary of the NPJCB. They were officially presented on August 28th 2017 during a ceremony in Ciudad Quesada, Alajuela, Costa Rica. Also, an amphibian documentary was released in May 2017 mentioning about the threats and conservation *L. vibicarius* and the rediscovery of *C. escoces* was also mentioned. The documentary was sponsored by the University of Costa Rica and it is now available on Youtube (link: <https://www.youtube.com/watch?v=PaGvhSwgaC4&t=1864s>).

5. Are there any plans to continue this work?

Yes, while conducting this project, new questions emerged for future research projects with conservation approach that will help us preserve the populations of threatened amphibians in the NPJCB.

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

We are producing one English language manuscript with the results of our research work and we will target a peer reviewed scientific journal to publish.

We have been using our social networks to divulgate the development of our conservation programme. As soon as we publish our results of this project we will use our social networks and costarican local media to share our findings and future direction in the conservation of *L. vibicarius*.

We already produced an English language peer-reviewed short note about the rediscovery of *C. escoces* and got published in the Journal Amphibia-Reptilia (accepted on Apr 17, 2017): Jiménez, R.R. & Alvarado, G. (2017). *Craugastor escoces* (Anura: Craugastoridae) reappears after 30 years: rediscovery of an "extinct" Neotropical frog. Amphibia-Reptilia. We produced a short video about the rediscovery and its relevance to amphibian conservation in Costa Rica. We used our social networks to share the rediscovery of this "Lazarus species". This rediscovery caused a very high attraction in national and international public media.

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

We conducted two field seasons for this project within the original timeline of our proposal because of the low rainfall in 2016 that caused a low reproduction of *L. vibicarius*. During the second semester of 2017 we conducted our second fieldwork to finish collecting samples from *Lithobates vibicarius*. We are currently conducting the genomics and bioinformatics for the amphibian skin microbiome, and the analysis for chytrid fungus.

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. Budget in Pounds US \$1 = £0.6993

Item	Budgeted Amount £	Actual Amount £	Difference £	Comments
Food and lodging	2014	1300	714	Cheaper than budgeted
Local transportation to field sites	210	550	- 340	More expensive than budgeted.
Flight tickets	1399	1420	- 21	More expensive than budgeted.

Fieldwork equipment and supplies	1125	1484	- 359	More expensive than budgeted because of unanticipated supplies. These supplies will also be used for future fieldwork in our long-term conservation project.
Total	4748	4734	- 6	

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

Evaluate the gut bacteria of *L. vibicarius* across landscape with different level of disturbance to keep improving our understanding in host health and microbiome dysbiosis.

Achieve a long-term monitoring of the population and health status of *L. vibicarius* and *Craugastor escoces*, for further in situ and ex situ conservation efforts.

Determine habitat selection, and population parameters (e.g., population size, survival rate) of *L. vibicarius* in the NPJCB across years.

Evaluate the impact of pathogens (Bd, ranavirus), pesticides (herbicides), and climate change (changes in water temperature and pH) to the health of *L. vibicarius*, to help in the development and improvement of conservation strategies that maintain a good health of this endangered species.

Maintain the support of local communities nearby the National Park to better protect the reproduction sites of *L. vibicarius* and *C. escoces*.

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. We have shown the Rufford Foundation logo in several presentations of our project. We acknowledged the Rufford Foundation as a funding agency in the scientific note of the rediscovery of *Craugastor escoces* published in the journal *Amphibia-Reptilia*. We will still use the Rufford Foundation logo in presentations that address this project in the future, and keep acknowledging the Rufford Foundation in future scientific manuscript(s) and conference presentations related to this project.

11. Any other comments?

We are very grateful with the Rufford Foundation because with its generous support we were able to secure funding to achieve this conservation project.



Ceremony in Ciudad Quesada, Alajuela, Costa Rica, 2017
Presentation of postage stamp of *Craugastor escoces* and *Lithobates vibicarius*



Ceremony in Ciudad Quesada, Alajuela, Costa Rica. Photo by San Carlos Al Día, (local news).



Presentation of our amphibian conservation project to local community of Ciudad Quesada



Presentation of our amphibian conservation project to local community of Ciudad Quesada



People from local communities, members of APANAJUCA and SINAC



Fieldwork in the National Park Juan Castro Blanco



Fieldwork



Lithobates vibicarius (adult), National Park Juan Castro Blanco



Postage stamp of *Craugastor escoces* to commemorate the 25th anniversary of the NPJCB