

## Final Evaluation Report

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Your Details	
<b>Full Name</b>	José Daniel Ramírez Fernández
<b>Project Title</b>	An overlooked endangered endemic small cat? Redefining the taxonomic status of the oncilla ( <i>Leopardus tigrinus oncilla</i> ) in the highlands of Costa Rica.
<b>Application ID</b>	29081-1
<b>Grant Amount</b>	£5500
<b>Email Address</b>	<a href="mailto:josed-rf@hotmail.com">josed-rf@hotmail.com</a>
<b>Date of this Report</b>	December 19 <sup>th</sup> , 2021

**1. 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
<p>To characterize the genetics of <i>Leopardus tigrinus oncilla</i> in the highlands of Costa Rica.</p>				<p>A diverse set of biological samples have been collected through different strategies. The samples include: 1) skin and bones from museum specimens under the approval and inspection of the National Museum of Costa Rica; 2) non-invasive (faeces) samples obtained directly from enclosures of animals sheltered in rescue facilities such as Toucan Rescue Ranch and La Paz Waterfall Gardens; 3) non-invasive (faeces) samples collected in the field with the assistance of a trained dog in alliance with the US non-profit Working Dogs for Conservation (WD4C); and 4) opportunistic collection of samples (road kills and specimens preserved by members of rural communities at the highlands of Costa Rica, mostly).</p> <p>This mixed strategy allowed us to collect 16 samples to date, which is the biggest biological sample set of the species in Costa Rica to our knowledge. The collection of biological samples available for analysis is very suitable as a starting point for the characterisation of the Costa Rican oncillas, since the samples were collected from diverse geographical locations through the habitat range of the species, and it also includes references from the past biodiversity of the species (samples from deceased and preserved animals).</p> <p>The faecal samples have been processed at LABGENCON, at the University of Costa Rica, to obtain isolated DNA and mitochondrial molecular markers (ctyb) have been</p>

			<p>successfully amplified using the extracted DNA to confirm the viability of DNA to perform subsequent genetic analysis (STR amplification) and assign a preliminary taxonomic identity of the samples. Tissue samples (skins and bones) are pending of DNA extraction, which is scheduled for January 2022.</p> <p>The isolated DNA of the complete set of samples will be amplified using a set of STRs in order to generate the genotypes necessary to describe the genetic diversity and the population structure of the oncillas in the highlands of Costa Rica. We expect to conclude Objective 1 in the first trimester of 2022.</p>
<p>To determine if the Central American populations of tigrina (Leopardus tigrinus oncilla) represent an endemic and different species from South American populations.</p>			<p>The current pandemic situation has resulted in constant unexpected closures of the laboratory, which has led to some delays in the execution of the project.</p> <p>In order to achieve Objective 2, the whole set of genetic information (genotypes) must be complete, and our team is still working in the sample processing and genotyping.</p> <p>At the same time, is also necessary a set of data from the South American populations of tiger-cats as a reference to determine the taxonomic status of the Costa Rican populations. We are cooperating closely with PhD Eduardo Eizirik and his team from Pontificia Universidade Católica do Rio Grande do Sul (PUCRS) in Brazil. Eduardo Eizirik has more than 15 years working with South American cats and he is a member of the IUCN/SSC Cat Specialist Group, Small Carnivore Specialist Group and Conservation Genetics Specialist Group.</p> <p>Once we finish the genotyping of the Costa Rican samples, the direct comparison and analysis of both set of data will be conducted. We expect to conclude Objective 2 during the first half of 2022.</p>

To develop a conservation action plan for oncilla and its habitat in Costa Rica.				The conservation action plan is waiting for the genetic and taxonomic results to be completed.
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**2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.**

The first difficulty for the development of the project was due to delays caused by the pandemic situation. Both with respect to the receipt of funding and laboratory work, with which not much could be done but to be patient waiting for the health situation in the country to improve and to follow the indications of the health authorities.

Another situation that took us by surprise was a small misunderstanding with one of our potential collaborators, the tracking dog suppliers, who more than collaborating were selling their services and at the same time wanted to profit from the results obtained without collaborating with monetary expenses. In this aspect we requested the collaboration of the organization Working Dogs for Conservation based in the USA, who collaborated enormously, and even covered the cost of their air travel to Costa Rica and also incurred certain expenses in the country, in addition to providing a dog specifically trained to track our species of interest.

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

- Previous analyses around the taxonomic status of *Leopardus tigrinus* have been performed including only one sample from the Central American subspecies, with this project we have already collected 16 samples.
- An important network of national and international collaborators has been formed, who have expressed their interest in continuing to collaborate with the Oncilla Conservation project in 2022.
- Oncilla Conservation's project is positioning itself as the number one small wild cat conservation project in Costa Rica.

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

Parallel to this project, we have been developing a process of educational workshops with the communities of San Jerónimo de San Pedro, Herradura de Rivas, and Cerro de la Muerte, neighbours of the oncilla's habitat within the Talamanca Mountain Range. These workshops have focused on presenting the particular taxonomic situation of the oncilla to the participants, as well as understanding its ecology, the different conservation conflicts with small cats that exist in the community and seeking threat mitigation measures to ensure coexistence between communities and wildlife.

Community members have become aware of the particularity of the oncilla as an endemic and unique species in the world and have become interested in taking conservation actions to reduce the threats it faces in these areas. This has positioned the oncilla as a flagship species for the area, where the communities themselves recognize it as an opportunity for rural growth, since their main economic income is tourism and charismatic and endemic species such as this one add value to the natural habitats surrounding the communities.

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

One more field trip to look for biological samples in-situ is already planned for the first half of 2022. This time we will focus on geographic areas of the country that were not previously covered, seeking to obtain samples throughout the distribution of the species in the country in order to have a better understanding of its genetic structure in the country. These samples will be added to our genetic database of the species to answer more elaborate questions that may have importance in its conservation status, such as understanding the level of connectivity among its populations, and determining if there is any degree of inbreeding

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

Social media has proven to be a powerful tool for communicating results in a way that is accessible to the general public, gaining a wider reach and reaching a more diverse audience, so we will keep on posting our work in virtual platforms such as Facebook and Instagram.

We will also communicate our results through at least one scientific publication, understanding the importance of presenting our results to the scientific audience for the implementation of further conservation actions based on research in the future.

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

In March 2020 we were informed that the funds had been obtained. As stipulated in the original proposal the idea was to begin execution in mid-March, however we received an email from the Rufford Foundation stating that due to the pandemic situation the funds obtained would be withheld until the effects of the pandemic were deemed over. In the second semester of 2020 we receive another email stating that if we could start executing the project spite of the pandemic, they will transfer the funds immediately. So finally, we receive the funds in December 2020. However, due to the pandemic situation, this deadline has been extended and we are currently in the last stage of execution, which will be extended for another 4-6 months, if no unforeseen events occur.

**8. Budget: 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. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.**

Item	Budgeted Amount	Actual Amount	Difference	Comments
Fuel for fieldtrips	265	191	-74	This item includes fuel expenses related to visits to ex-situ collections as well as field trips to natural habitats.
Rent a car	805	636	-169	
Food for fieldtrips	485	477	-8	
Lodging for fieldtrips	570	814	+244	The shortfall to complete the budget associated with this item was taken from the surplus of the following items: "fuel", "rent a car", "food", "dog & trainer costs", and readjusted with external financing (£40.98).
Dog and trainer costs for fieldtrips	404	392	-12	
Samples exportation	285	285		
Local transport		60	+60	Although this item was not accounted in the original proposal, on many occasions it was necessary to travel back and forth by local transportation (e.g., taxi, uber) to the ex-situ collection sites to coordinate and present the project to the suppliers and for sample collection. The budget for this item was taken from the "rent a car" item surplus.
Vacutainers w/EDTA	40	40		
Sterile spatula to collect the scats	32		-32	
RNAlater	424	424		
Gloves	20	20		
Cryovials	80	80		
Permanent markers	16	16		

Microcentrifuge tubes	24	24		
PCR tubes	333	333		
Stool DNA extraction & purification kit	400	400		
Micropipette tips	160	160		
Blood & tissue DNA extraction & purification kit	280	280		
Sterile 50mL tubes	80	80		
Master Mix for PCR	604	600	-4	
Regular primers	110	110		
BSA	16	15	-1	
TAE buffer	67	67		
<b>TOTAL</b>	<b>5500</b>	<b>5504</b>	<b>+4</b>	1£ = 714.46 CRC

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

First of all, to communicate our results in scientific publications, and social media to reach a wider audience. We will continue our field work with camera traps to monitor oncilla populations in the country. And most importantly we will keep on going with the educational workshops process in communities surrounding the oncilla habitat, taking conservation actions on the ground to mitigate its threats in the region.

**10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?**

The Rufford Foundation logo was used in the acknowledge section of all our virtual and in person presentations in respect to the Oncilla Conservation program since we receive this funding. Also in workshop informative banners, and in printed informative materials to be posted in the communities and in a collaborator rescue centre, which held the only living Central American oncilla in any ex-situ collection around the world to our knowledge. We have also made mention of The Rufford Foundation account in all our social media posts, both in Facebook and Instagram.

**11. Please provide a full list of all the members of your team and briefly what was their role in the project.**

**José Daniel Ramírez Fernández** – Project Coordinator: networking for collaborations, sample collection field trips logistics, field work, fund administration, permits, results reporting.

**Juan Carlos Delgado Carazo** – Genetics Coordinator: ex-situ sample collection, sampling kit preparation for in-situ collection, lab work (DNA extractions and molecular analysis), DNA sequences exportation.

**Patricia Blanco Murillo** – Communication Coordinator: design and development of workshops banners, informative posters, and social media materials to communicate about the project and the ongoing work. In-situ and ex-situ sample collection documentation through photos and videos.

**Fabiola Araya Chavarría** – Education Coordinator: design and implementation of an environmental education process around the oncilla particular situation with local communities.

**Valeria Gallardo Castro** – Genetics assistant: ex-situ sample collection, lab work.

**Valeria Aspinall Harvey** – Field assistant.

**Esteban Brenes Mora** – Field assistant.

**12. Any other comments?**