

Final Evaluation Report

We ask all grant recipients to complete a project evaluation that helps us to gauge the success of your project. This must be sent in **MS Word and not PDF 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 – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please DO NOT fill in and submit this form until the project has been completed.

Complete the form in English. Note that the information may be edited before posting on our website.

Please email this report to jane@rufford.org.

Your Details	
Full Name	Carlos Abraham Castillo Morales
Project Title	Evolutionary History of Two Leatherback Turtle Lineages in the Eastern Pacific: Insights From Genomics, Morphometry and Local Ecological Knowledge
Application ID	43723-2
Date of this Report	30 January 2026

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
Take morphological measurements of leatherbacks turtles				<p>The original idea was to take photographs to analyse geometric morphology, and measurements to assess linear morphology. Geometric morphometrics was discarded due to the difficulty of obtaining high-quality photographs in the field (same angle and scale), given the irregular surface of the beach and low light conditions, since surveys are conducted at night and the use of flash photography is not permitted for this species. However, linear morphometrics was successfully carried out: I measured the length and width of the front and rear flippers, the curved carapace length, the curved carapace width, the tail length, the neck length, and the skull length. The size of the eggs was also measured, and a photo was taken of each carapace specimen where the coloration can be observed.</p>
Collect genetic samples				<p>We successfully collected 20 samples for genomic analyse.</p> <p>It took us about a month to reach this number. During that month, we went out every night on two different ATVs, which translate to approximately 30 nights per vehicle. Although the sampled beaches are two of the most important nesting sites for the leatherback turtle in the eastern Pacific, the number of nesting females is low.</p>

				<p>Even if we had remained on the beach longer, it was unlikely that new nesting females would appear. Instead, we began to observe the first females we had sampled returning to nest again.</p>
<p>Determine which variety each individual belongs to, according to the local ecological knowledge (Lek)</p>				<p>With the help of community experts, we were able to categorize 8 turtles according to the varieties identified by the lek. Each of these turtles was also measured, and a sample for genomics was collected.</p> <p>During the fieldwork, we went out every night to maximize the probability of reaching the established number of samples. The community experts accompanied us on the patrols whenever they were able to, based on their schedules and activities. These community experts are people who work in multiple trades. For example, one is now a farmer and when he was young, he was a fisherman. He has knowledge about sea turtles because he has participated alongside the turtle camp in efforts to conserve them in the community. He also lived during the time when the use of sea turtles was permitted.</p>
<p>Sequence 10 complete leatherback turtle genomes</p>				<p>Ten leatherback turtle genomes were sequenced using the whole genome sequencing method. We are now assembling the genomes and preparing them to run the analyses.</p>
<p>Sequence 40 genomes using GBS (Genotyping-by-Sequencing)</p>				<p>The genomes to be sequenced using GBS are still in the process of being sequenced. We are fully confident that all genomes will be included and will be ready before the results are published.</p>

2. Describe the three most important outcomes of your project.

a). Ten leatherback turtle sequences generated by Whole Genome Sequencing will be made public. Currently, only one genome has been published, so this represents a valuable contribution to the knowledge and conservation of this species. This will allow us to better understand its evolution by gaining insight into gene flow, inbreeding, natural selection, effective population size, among other factors. Ultimately, this will enable better management and conservation decisions, and, if necessary, allow us to define or modify evolutionarily significant units.

b). One of the objectives of this work is to address the question of whether more than one group of leatherback turtles exist. This has been a debate within the scientific community since the late nineteenth century. We aim to address this question which, although it has been discussed for a long time, has never been tested with robust empirical data. Although only one species is currently recognized, the necessary analyses to confirm or reject this hypothesis have not yet been conducted. Only comments and conclusions have been made on this matter, treating this hypothesis rather lightly.

c). We are using an innovative methodology that integrates genomics, morphometrics, and lek. This approach allows us to address the same question from different perspectives and to determine whether these three sources of information support or reject the hypothesis.

In addition, we consider it important to include the knowledge of the communities that inhabit the territories where these species nest. This knowledge is often ignored or dismissed simply because it is not part of formal academic education; however, it represents a valuable source of information that should be taken into account.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

One of the unforeseen issues during fieldwork arose when attempting to take photographs for geometric morphometrics. The difficulty lies in the fact that, for this type of analysis, each individual must be photographed in an almost identical manner. This requires positioning the individual parallel to the camera, which is very difficult to achieve on the beach due to its irregular surface and because turtles sometimes nest on dunes, adding an angle of inclination. In addition, nesting occurs at night, when ambient light is low, making it difficult to obtain high-quality photographs without using flash and without motion blur.

Because this proved to be practically impossible for us, we decided to use only linear morphometrics instead. There are studies in which this type of photography has been successfully carried out in other species of sea turtles; in those cases, the

turtles are removed from the beach and taken to a laboratory for photography. For us, this was not feasible, as such permits are not granted (in addition to the high level of stress this would cause to the animals), and because leatherback turtles are very large and heavy, which would greatly complicate logistics. Moreover, there are no laboratories near the nesting beaches; only turtle conservation camps are present.

4. Describe the involvement of local communities and how they have benefited from the project.

In this project, we worked together with community experts—individuals who are or have been dedicated to the conservation of sea turtles. Some of these community experts recognize two varieties of leatherback turtles, so we collaborated with them in the field to categorize each turtle according to whether it belonged to variety 1 or 2. Community experts are a fundamental part of this project, as they contribute the lek information. I believe this work helps to make visible the knowledge held by local communities, which is important even though it is often ignored. Even if, in the end, morphometrics and genomics do not align with the lek, I believe it is important to include the lek as a valuable and relevant source of information.

On the other hand, this project aims to contribute to the conservation and management of the leatherback turtle, a species that is important to the communities where it nests. Thanks to the work of other people dedicated to the conservation of these animals, these communities have learned to appreciate the value of these living beings and therefore conserving them also brings benefits to the communities themselves.

5. Are there any plans to continue this work?

Yes, I am passionate about conservation and sea turtles, so I intend to continue along this line of research. Once the results are ready, it will be time to make decisions and determine the direction I want to take the research. Perhaps the next step will be to sample another region or to use the genomes to obtain additional information. We aim to determine whether these varieties correspond to distinct groups or if they are simply morphological variations. Depending on the results, I may continue to further investigate these leatherback turtle varieties in other regions.

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

I plan to publish my results in a scientific journal, preferably one with high impact. This project is a continuation of previous work whose results were published in Scientific Reports, which is part of the Nature Portfolio. In addition, this project forms part of my doctoral thesis, so the results will also be published in the dissertation. I am also in communication with the Laúd OPO network, which includes many key individuals

dedicated to the conservation of the leatherback turtle in the eastern Pacific. Once the results are ready, I will also share them in this network. Finally, I would like to return to the communities to carry out environmental education activities and to share and discuss the results obtained from this project with them.

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

The next steps will depend on the results obtained. If the existence of two leatherback turtle groups is confirmed, it will be necessary to adapt management, and conservation plans to reflect these new findings. The presence of two distinct groups would require independent conservation and management strategies for each group. If, on the other hand, these are only morphological variations, the characteristics of each variety will be described. It is important to highlight that even morphological variation represents a form of diversity worth conserving because of its evolutionary potential. Since natural selection favors certain phenotypes over others, each of these varieties represents an opportunity for the survival of this species.

8. 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?

In every talk I give about this project and my previous project (which was also funded by Rufford), I include the foundation's logo on the cover slide of my presentation and I mention that the project was funded by The Rufford Foundation and I also express my acknowledgements to you. I have had the opportunity to speak about my project in different venues, such as a talk organized by a conservation center called Reino Animal, civil associations, on a conference poster, during the classes I taught to students of reptile management and conservation, and in presentations of my research protocol and doctoral thesis, among others. In addition, they will be included in the acknowledgements and/or funding sections of the articles that arise from this work.

9. Provide a full list of all the members of your team and their role in the project.

M. Sc. Carlos Abraham Castillo Morales (myself): This project is part of my doctoral studies; therefore, I was the one who proposed its implementation (with the support and guidance of my entire team, who also serve on my doctoral committee). I have been responsible for all logistics and administrative procedures. I conducted the fieldwork to collect the data, carried out the DNA extractions, and obtained the necessary permits for the execution of the project. Likewise, I am currently working on the bioinformatics, both for the genomic and morphometric analyses, and I will be responsible for preparing the manuscript in which the results will be presented.

Dr. María Andrea Sáenz Arroyo de los Cobos: She is my thesis advisor and the person who helped me design the project from the beginning. She primarily advises me on the LEK component and has also assisted with administrative procedures. She reviews and edits all documents and manuscripts required for the execution and dissemination of this project. She will also collaborate on the manuscript.

Dr. Gabriela Castellanos Morales: She is the genomics expert. She has helped design the genomic component of the project and has provided guidance in this area. Together, we planned the type of sequencing and the analyses to be conducted in this project. Thanks to her advice, we were able to determine which analyses are necessary to answer our research questions. She is currently also assisting me with the bioinformatics component and will collaborate on the manuscript.

Dr. Alfonso Ángel González Díaz: He is the expert in taxonomy and morphometrics. He helped design the morphometric component of the project and advised on the methodologies and analyses required to address the research questions. He has also conducted some of the morphometric analyses and is my primary advisor on morphometrics. He will also collaborate on the manuscript.

Dr. Lorena Ruiz Montoya: She is the expert in genetics. She has helped design the genomic component of the project and the analyses needed to answer the research question. She will also collaborate on the manuscript.

There are other individuals who do not formally belong to my team, but who have been important to the execution of the project. M. Sc. Anahí Canedo Texón is the bioinformatics expert at ECOSUR (the research center where I am pursuing my doctorate). She was responsible for training me in bioinformatics and continues to assist and advise me in genome assembly and in running the necessary genomic analyses. Biol. Maricela García Bautista, head of the genetics laboratory at ECOSUR, has advised and assisted me with DNA extractions and even carried out some of them herself. During fieldwork, I was assisted by my doctoral colleague M. Sc. Filiberto Moisés González Martín del Campo, who carried out some of the measurements and sample collection. I was also accompanied and supported by the staff of the turtle conservation camps at Barra de la Cruz and Cahuitán in Oaxaca, as well as by the staff of the NGO Kutzari A.C.

10. Any other comments?

We are currently working on the analyses and the manuscript for the publication of the results of this work. We are confident that it will be published, or at least submitted, by mid-year. Once the publication is completed, I will share it with you.

ANNEX – Financial Report

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