

Final Evaluation Report

Your Details					
Full Name	José Manuel García Enríquez				
Project Title	Are the isolated distribution of the American Horseshoe Crab in the Yucatán Peninsula, Mexico, and current anthropogenic impacts a threat for its conservation?				
Application ID	39193-1				
Date of this Report	17 – December - 2024				



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
Determine the diversity and genetic structure of the American Horseshoe Crab				We analysed a total of 154 individuals from 8 localities throughout its Mexican distribution (Fig. 1). We detected moderate values of genetic diversity, highlighting the low diversity in the localities of the northern coast of Yucatan and the Caribbean. With respect to genetic structure, we confirmed the presence of two genetic populations through nuclear DNA; however, mitochondrial DNA provided different information, highlighting the lack of clear phylogeographic differentiation. This is probably due to a relatively recent origin of these populations, possibly originated from a founder event in the recent past.
Analyze methylation patterns in populations of the American Horseshoe Crab				We developed the first section of methodology to analyse the methylation patterns of genes involved in cellular detoxification (<i>i.e.</i> GST and MT) in this species. We have had difficulties in the amplification section of these genes and are currently developing alternatives to carry it out.
Carry out environmental education campaigns in areas where the				We conducted face-to-face and virtual environmental education campaigns, in addition to sharing our project progress in two international congresses. We conducted 100 interviews in 7



populations of this species are most at risk		localities of the study area to address the perception of local communities towards this species, in addition to carrying out awareness activities and sharing information about the ecology and the main risks this species
		faces. The interview data are currently being analysed with the intention of sharing the results through a scientific journal.



Fig. 1 Locations sampled within the Mexican distribution of *L. polyphemus*: Laguna de Términos (LT) and Champotón (CH), Campeche; Ría Celestún (RCL), Chuburná (CHU), Chelem (CHE) and Ría Lagartos (RL), Yucatán; Yum Balam (YB) and Sian Ka'an (SK), Quintana Roo.

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

a) We analysed the diversity and genetic structure of the American Horseshoe Crab throughout its distribution in the Yucatan Peninsula, Mexico. We detected moderate overall values through the mitochondrial DNA and low values for localities in the northern coast of Yucatan through both nuclear and mitochondrial DNA. We confirm the presence of two genetic clusters or populations (by means of SSRs) in this



section of the species distribution, and the presence of 16 haplotypes arranged in a star-shaped haplotype network (Fig. 2). The information generated may be useful for conservation management proposals.

b) We addressed the social perception, through semi-structured interviews (Fig. 3), towards this species throughout its distribution in Mexico, considering three main axes: knowledge, attitudes and practices. This allowed us, in the first instance, to detect critical regions where the species is exploited as a fishery resource, and where conservation and environmental education efforts should be focused.

c) We disseminate information about our project and the importance of conserving this species through different media and activities such as environmental education events, dissemination of educational material through social networks, and physical information material (Fig. 4) in local communities, aimed at authorities, park rangers, fishermen and local people.



Fig. 2 a) Structure analysis for the Mexican distribution of the American Horseshoe Crab (in yellow 'Cluster 1' and in green 'Cluster 2'); b) Haplotype network and distribution of haplotypes in the different localities sampled. Location code: Laguna de Términos (LT), Champotón (CH), Ría Celestún (RCL), Chuburná (CHU), Chelem (CHE), Ría Lagartos (RL), Yum Balam (YB) and Sian Ka'an (SK).





Fig. 3 Handing out outreach material and interviews with local people. © Catalina Ocampo.



Fig. 4 Front and inside view of L. polyphemus information brochure.

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

1) One of the main difficulties we faced during the development of the project was the postponement of several of the planned campaigns due to climatic events such as tropical storms and hurricanes, given that the area in which the study area is located is a prone region due to its location in the Caribbean and the Gulf of Mexico. In addition, the time of greatest abundance of the study species overlaps with the time with the highest rate of these climatic events. Therefore, it was necessary to extend the originally planned sampling period.



- 2) We have encountered technical problems in the amplification of specific genes for the analysis of methylation patterns. In this section we developed de novo primers specific for the GST and MT genes, responsible for cellular detoxification and regularly used in the analysis of contaminant impact at the genetic level, but we have not yet been able to standardize the amplification conditions. Our alternatives have been to design primers that increase the efficiency of amplification. This section is still under development, as part of my doctoral project.
- 3) During the edition of the electropherograms of the COI gene sequences we found different nucleotide positions that presented uncertainties with respect to the polymorphisms, probably due to a misreading of the sequencer. The solution was to purify the PCR amplified products using the "Gel Extraction Kit (Qiagen ®)" and resequencing to verify these uncertainties.

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

In the sampling campaigns, the participation of local people was indispensable, particularly fishermen with whom we worked hand in hand and whose knowledge of the area was fundamental to successfully carry out the sampling. Their valuable support in the field work was remunerated economically according to the time and/or days we worked together.

In parallel to the environmental education campaigns, we conducted a series of interviews in different localities within the study area, to address the perception that local people have towards this species. This was a great opportunity to learn from them, but also to share information. In our work in the communities, we seek to transmit knowledge about this species and its importance in the ecosystem, as well as its fragility. We were always pleased to find the willingness and sensitivity of the members of the local communities.

5. Are there any plans to continue this work?

This work is part of my doctoral project, so I will continue the points that have not yet been completed. Beyond that, I feel a deep commitment to the conservation of this species. The intention is to continue developing environmental education campaigns and, specifically, to develop training workshops aimed at tourist cooperatives that seek to reverse the impact on this species through ecotourism. We have found great interest and willingness in this regard and we believe that it can become a great activity that allows us to continue raising awareness about the importance of conserving this species but also to provide new opportunities or development alternatives for local communities.

On the other hand, it is of my total interest to fill information gaps about this species with respect to its evolutionary history, this through new tools and perspectives such



as genomics, in order to understand the evolutionary relationships between the Mexican and American populations of the American Horseshoe Crab.

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

We have shared preliminary results so far at two international congresses, the 53rd Annual Meeting of The American Arachnological Society and the XIX Latin American Congress of Genetics (Fig. 5). In addition, we plan to publish our results through scientific articles in peer-reviewed journals. One of them is about to be submitted to one of these journals (Fig. 6). We also consider it really fundamental to share the results with a less specialized, but equally important, public through infographics and scientific dissemination material.



Fig. 5 On the left, oral presentation made during the 53th Annual Meeting of The American Arachnological Society (© Jacob Huerta), and on the right, poster presentation at the XIX Latin American Congress of Genetics (©Luis E. Jiménez).



26 Acknowledgements

27 Thanks to the "Universidad Juárez Autónoma de Tabasco" (UJAT) Genomics Laboratory 28 team, the fishermen of Isla Aguada, Campeche, and Puerto Progreso, Yucatán, and Fabiola 29 Briceño ("El Colegio de la Frontera Sur") for their support in the field and sample collection. 30 Collection permissions were granted by the "Secretaría del Medio Ambiente y Recursos 31 Naturales" (Semarnat; permit number SGPA/DGVS/4239/19, SGPA/DGVS/07162/20, & SPARN/DGVS/04874/23). JMGE would like to thank the "Consejo Nacional de 32 Humanidades, Ciencia y Tecnologías" (Conahcyt) for granting a scholarship (grant number 33 34 955514) and The Rufford Foundation for awarding a conservation small grant (grant 35 number 39193-1).

Fig. 6 Acknowledgments section of the first article resulting from this project.

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

I consider two points to be priorities: (1) to generate a risk map for the species in its Mexican distribution, making use of the information that has been generated for the species, considering demographic, genetic and social aspects, in order to redirect conservation efforts to the most vulnerable areas; and (2) to propose, together with local authorities, the implementation of economic alternatives (e.g. ecotourism) that promote the conservation of this (and other) species, involving local people and influencing their quality of life.

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?

Yes, we have used The Rufford Foundation logo on all products generated in this project, including brochures (see Fig. 4), infographics (Fig. 7), stickers, posters and presentations (see Fig. 5), as well as t-shirts that were designed for our work team (see Fig. 3). Of course, we mention and thank The Rufford Foundation in all publications resulting from this project.





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Fig. 7 Infographic about the species L. polyphemus shared on social media (<u>https://www.facebook.com/photo/?fbid=969372708522978&set=a.5467584941177</u>37).



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

Dr. Salima C. Machkour M'Rabet is my thesis director and was essential in the realization of this project. She participated in the planning and execution of the project. She supervised the laboratory work and analysis of the results and participated in the elaboration of the dissemination material and in some of the Environmental Education activities. Together we worked on the drafting of the scientific articles resulting from this project.

Dr. Yann Hénaut is part of my doctoral committee and participated in some of the field campaigns. He has contributed substantially to the discussion of the results we have obtained. Later, he will contribute to the data analysis and discussion of the project results.

Msc. Raziel Lucio Palacio participated actively and enthusiastically in field work and environmental education events.

Msc. Jacob Huerta was part of the field work team. His skills were important to achieve the correct and efficient data collection.

Msc. Catalina Ocampo their support in the field was fundamental, specifically in the activities on social perception by collaborating in the interviews conducted.

10. Any other comments?

As part of the diffusion activities, part of the granted resource was used to pay for travel and registration to the XIX ALAG 2024 Congress. I am grateful for the support provided by The Rufford Foundation, which was fundamental for the realization of this project that seeks to contribute to the conservation of the American Horseshoe Crab, a species in danger of extinction in Mexico.

