Final Evaluation Report

Your Details	
Full Name	Hernando Rodríguez Correa
Project Title	Conservation of Endangered Quercus insignis Martens & Galeotti, 1843 (Fagaceae) under climate change
Application ID	29520-2
Date of this Report	18/09/23

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
Identification of valuable genotypes reflecting adaptation capacity throughout the species range to be used in future reforestation and restoration efforts.				We have obtained all samples and RAD-seq data. Analysis is being performedand results will be disseminated as a Master thesis and a later scientific paper.
Areas where future climatic conditions will not match the genetic capabilities of the current individuals.				
Improved understanding of the response potential of different populations to climate change through the study of functional traits.				Deeper understanding of potential response of the species to climate change has been achieved through the modulation of the ecological niche for current times and projected to different climatic change scenarios. Although in situ functional trait data from adult trees was gathered, due to the dimensions of the trees, and the variation of available light between sites, standardisation of the height in which leaves were taken was very difficult, and therefore could not be included in the analysis mentioned above. However, as will be described below, to understand differences in physiological strategies used by populations from different regions and environments, we have started a greenhouse experiment where seedling functional traits will be measured
Increased knowledge of the species demographic processes (survival, germination and growth).				Sampling locations in Mexico have been continuously monitored for 3 to 5 years.

Informed and empowered local communities participating in monitoring and experimental activities.				We conducted informal interviews with members of the local communities and incorporated their observations into our data analysis and interpretation. In light of the pandemic, formal interviews were suspended to minimise risk. However, we are currently testing a pilot interview instrument in Costa Rica with the help of one of our collaborators. We plan to conduct formal interviews using this approach during the next field season.
Conservation and management recommendations disseminated across Mexico and Central America.				We are in the final stages of completing and submitting to peer review a scientific manuscript that provides conservation recommendations based on the climatic suitability and landscape connectivity of the study area. We believe that this article will have a broad impact, reaching not only fellow scientists but also decision-makers who can use our findings to better protect cloud forest species like *Q. insignis*.
Creation and dissemination of technical reports and divulgation material for government agencies and educational institutions.				We have created various forms of dissemination material, including infographics, progress reports to The Rufford Foundation, and a Master's thesis. These materials have been shared through multiple channels, such as our lab's website, social media platforms, and the university's institutional thesis repository. Once all the analyses are complete, we intend to update the aforementioned materials and create a dedicated report that will be presented to all the communities where sampling was conducted.

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

The main results that can be highlighted from our project can be divided in three sections:

a). Characterisation of the genetic conservation status of *Q. insignis*

Firstly, our results focus on the characterisation of the conservation status of *Quercus insignis* through the estimation of genetic diversity, genetic structure, and gene flow patterns among different life stages of the species, as well as the potential threat of climate change throughout its distribution. Specifically, our study addresses the question of how much genetic diversity has been conserved over time by comparing genetic information from individuals in different life stages. Our main result suggests that, at the local scale, *Q. insignis* seems to be resilient, so far, to the loss of genetic diversity among life stages. However, at a regional scale, we identified a strong genetic structure among Mexican populations, indicating low genetic interchange among sites. This highlights the importance of conserving as many populations as possible, as they harbour different subsets of the genetic diversity of the entire species.

Moreover, we estimated the potential risk to suitable habitat and the connectivity of populations throughout the distribution range of *Q. insignis* using ecological niche modelling. This methodology allows for the identification of sites that currently have suitable climatic conditions for the species' development and enables the estimation of expected changes in these areas under different models of climatic change. The main results indicate that the current distribution of *Q. insignis* will probably be reduced by approximately 60-88%, and only a moderate percentage of the current suitable area, around 11-39%, will remain climatically stable. These stable areas are primarily concentrated in the southern regions of the Costa Rican montane forests. Currently, approximately 20% of the suitable habitats for this species are under protection in natural protected areas. However, although this strategy is more effective against habitat loss due to deforestation, it is likely vulnerable to the effects of climate change. Our results suggest that around 50% of the currently protected suitable habitat will be lost.

Finally, we identified regions in the species' distribution where the landscape and climatic conditions might favour connectivity. These regions coincide with climatically stable areas and are mainly concentrated in Central American countries, particularly to the south of the species' distribution.

b). Potential maladaptation to climate change.

Secondly, we expanded the scope of the study by including samples from Central American populations and utilising a genetic marker that allows sampling a larger portion of the genome, potentially enhancing the resolution of our results. Our findings indicate a regional division between two groups of populations: one consisting of sites in Mexico and Petén (Guatemala), and another composed of sites from southern Guatemala and Honduras. Furthermore, the results confirm a greater genetic structure among Mexican populations compared to Central American populations, as previously demonstrated using microsatellites.

The main purpose of this section was to identify important environmental variables which explain the potentially adaptive genetic variability among *Q. insignis* populations and to identify regions in the distribution which might be vulnerable to climate change. Our preliminary results, firstly, suggest that genetic variation is explained by both geographic and historic factors (such as geographic distance and low levels of genetic interchange between populations) and environmental factors. In many of our models geographic variables were a very important predictor which could indicate that, either there is a great influence from demographic processes explaining the divergence in genetic information or there are unsampled selective pressures which are correlated with the spatial patterns which can explain the differences in allelic frequencies.

Our results, considering the complete set of sampled localities, suggest that precipitation seasonality and the minimum temperature of the coldest month were the most important climatic predictors, excluding geographic predictors. Specifically, we identified a significant change in allele frequencies in localities at the northern limit of the species distribution, where there is the highest variation in precipitation throughout the year. Furthermore, regarding temperature, our models revealed significant genetic differences between localities with higher temperatures during the cold season, such as those in northern Guatemala (Peten) and Costa Rica, and the remaining sampled localities.

One important caveat to note with these results is the presence of markedly different regional genetic structure patterns. This complexity complicates the differentiation of genetic signatures associated with natural selection and historical processes. However, within the localities in Guatemala and Honduras, which constitute one of the aforementioned regions, we observed low genetic structure but high environmental variation. The models constructed using data exclusively from these localities identified mean annual temperature and isothermality as the primary predictors.

The second objective of this study was to identify regions in the distribution of *Q. insignis* that may be more vulnerable to climate change. This vulnerability arises from the necessity for a larger change in allele frequencies to maintain associations with the environment, equivalent to those currently observed. Analyses for this aspect are still being conducted, and the results will be published first in a publicly available master's thesis and subsequently in a scientific article.

c). International and interdisciplinary collaboration network, and technical capacitation:

One of the most crucial aspects of conservation is collaboration between scientists of various disciplines and non-scientists, such as local communities and decision-makers. Throughout this project, a vast network of collaborators has formed around the conservation of *Quercus insignis*. It includes members from institutions of the scientific community in multiple countries such as Morton Arboretum (USA), INECOL (México), ENES Unidad Morelia (México), USAC University in Guatemala, El Zamorano and the UNAH University in Honduras, and OSA Conservation in Costa Rica. Local communities at the distribution sites of the species in Mexico, Guatemala, Honduras, and Costa Rica, as well as NGOs, are also part of this network. These collaborators have been working on investigating various aspects of the biology of *Quercus insignis* necessary for its conservation. For instance, field exploration to discover previously unregistered populations (this study), germination (Montes-Hernández & López-Barrera, 2013), survival, and dispersion experiments at specific sites (García-Hernández et al., 2023; Toledo-Aceves et al., 2023), planting techniques to use the species in restoration efforts (Rodrígue-Acosta & Coombes, 2017), and population genomic analyses to understand genetic structure and characterise the relationship between population allele frequencies and environmental gradients (this study). The process of generating this information has not only strengthened collaborations between researchers and institutions but has also led to technical and scientific training and awareness at all levels. We anticipate that the synthesis of this information will begin to consolidate into regional management recommendations led by interdisciplinary groups.

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

The main unforeseen difficulties and delays we faced during the execution of the project were the following:

- Covid-19 effects - The pandemic had two major impacts on the timeframe of our project. Firstly, our field trips were scheduled during the early stages of the pandemic, when vaccinations had just begun, resulting in varying numbers of cases between countries. As the safety of our participants and the people receiving us was our top priority, we decided to postpone both trips to Central America (Guatemala in November 2021 and Honduras in March 2022). Secondly, during the genomic data generation phase, sequencing centres were experiencing delays in sending back data for analysis due to the backlog accumulated during the lockdown period. Fortunately, we have quality data for all sampling locations.

- Laboratory work- High quality, concentrated, and pure DNA samples are essential for performing genomic analyses using high throughput sequencing protocols. Initially, we had planned to use commercial kits for DNA extractions, which are known for their fast completion time. However, early evaluations resulted in extractions of poor quality and concentration. To overcome this issue, we modified a homemade protocol based on (Doyle & Doyle, 1991).

- Additional collaborations needed in Honduras - Another unexpected challenge we encountered was the need for additional collaborations to expand our travel to more sites in Honduras. However, this difficulty turned out to be an opportunity for two reasons. Firstly, it allowed us to find two previously unreported populations of this species and secondly, to expand our network and potential for future collaborations.

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

The participation and awareness of local communities are crucial aspects of any successful conservation project. However, one common challenge faced in many scientific collaborations with local communities is the lack of continued interaction once sample collection is complete. Despite the significant geographic scale of the distribution of *Quercus insignis*, we have made an effort to continue visiting sampling locations, particularly in Mexico, to strengthen our relationships with the local communities. This sustained engagement has resulted in increased community member who typically accompanies us during fieldwork took the initiative to search for more populations of the species in the region and discovered a previously unknown location.

The knowledge and information provided by local communities, government conservation branches, and natural reserves through their direct observations of the species have greatly enriched our conservation project. In turn, our visits to the communities have provided them with a greater appreciation for the unique value of their forests, opportunities to participate in scientific processes, and a modest economic contribution. Additionally, the identification of new *Q. insignis* populations has increased the conservation value of the community's forests, with the potential for more tangible benefits in the future. Once our study is complete, we will share and explain all the knowledge generated about *Q. insignis* with the communities where we conducted our sampling. Finally, our collaborations have allowed us to enhance the involvement of local communities. The better example is our participation during the first "Taller Nacional para la Conservación de *Quercus insignis*". Such an event was a national workshop in Costa Rica where people from several communities, academics and NGO participated sharing experiences around the conservation of montane cloud forest and *Q. insignis*.

5. Are there any plans to continue this work?

Due to the ecological vulnerability of the species, the geographical isolation of its populations, and the threat of climate change, *Quercus insignis* is highly susceptible to population extirpation or extinction. These conditions highlight the need for a study focusing on the species' genetic adaptation and ecophysiological response within its populations. Our future plans centre on understanding the species' response from an adaptive perspective to contrasting environmental conditions of mountain cloud forest. To achieve this, we will conduct seed germination and seedling growth experiments using individuals from different provenances in a common garden setting. These seedlings will then be subjected to environmental stress experiments aimed at identifying potential adaptation strategies to drought and uncovering the genes associated with these responses. Additionally, the existing genetic adaptive diversity with candidate genes of the species will be evaluated. As the physiological and genetic response is characterised, it will be possible to determine effective conservation strategies for long-term preservation of *Quercus insignis* populations.

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

The initial findings of this project have been disseminated through various channels. One of them is a freely available Master's thesis on the Universidad Nacional Autónoma de México thesis portal. Additionally, we presented our results at scientific events such as the online Rufford's events in El Salvador and Cancún, Mexico, the 58th annual ATBC conference, and a community workshop in Costa Rica (Taller Nacional para la Conservación de Quercus insignis). A manuscript exploring the potential impacts of climate change on the distribution and connectivity of Q.

insignis is currently nearing completion. We have also shared summary infographics, progress reports, memories, photographs, and activities on our lab's website and social media platforms. As for the upcoming results, a Master's thesis and two scientific papers are expected to be finished by 2024 and will be made available to the public. Finally, we will prepare a document with all results to be disseminated and explained among the local communities that were visited throughout the development of the project.

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

There are two key areas that we consider important next steps:

Experimental validation:
The approaches we chose for this second phase of the project are correlative. It is a great way of providing a general overview of the potential climatic vulnerability and vulnerability to maladaptation in different areas of the species distribution and to narrow down hypotheses about which environmental factors might be driving local adaptation in the species' populations. However, it is necessary to complement this information with experimental evidence that proves that the proposed environmental factors are really causing an effect on traits related to survival or reproduction. Therefore, as mentioned above, a critical next step is to design greenhouse experiments that: i) measure differences in germination, growth and survival of seedlings from different parts of the species distribution; and ii) test the response of seedlings originated in populations with different environments to regimes of drought and measure their survival capacity as well as their physiological strategies to deal with stress.

Translation of biological knowledge into conservation recommendations:
Currently, our focus has been on establishing adequate scientific knowledge to comprehend current and historical aspects of the species. In subsequent stages of the project, our aim is to translate this knowledge into suitable conservation measures. Specifically, we would like to update the current IUCN assessment to incorporate the new regional information. Moreover, we would also like to start evaluating conservation actions using the Green List IUCN framework. This framework is an approach to periodically assess species recovery and conservation success, focusing on evaluating progress in the recovery process of a species in different dimensions: viability, functionality, and representation. It includes specific metrics that assess the impact and potential of conservation actions, such as the impacts of current conservation efforts, the dependence on conservation actions, the expected gains from conservation actions, and the species' recovery potential(Akçakaya et al., 2018) .

Additionally, one of the long-term goals we aim to achieve with this project is to gather sufficient information to be able to submit a proposal for the inclusion of *Q. insignis* in the Mexican norm for the protection of wildlife (NOM-059). The Mexican Government periodically opens calls for scientists, activists, and universities to submit updates to the list of species under protection. This process involves analysing the risk of extinction using a framework called MER (Method of Evaluation of Extinction Risk of Wildlife Species in Mexico), which mainly includes a diagnosis of the baseline population status, ecological and social relevance, vulnerability to extinction, and the risk of no conservation measures being implemented (Sánchez et al., 2007).

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?

The Rufford Foundation logo has been used in scientific presentations, including Rufford's online events in El Salvador and Cancun, as well as the 58th ATBC conference and a community workshop in Costa Rica (Taller Nacional para la Conservación de *Quercus insignis*). In addition, it is displayed on our laboratory's website (https://www.biologianeotropical.com/) and project infographics, which provide a concise summary of the project's findings.

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

MSc. Lizeth Melissa Naranjo Bravo: PhD student at the Biological Sciences graduate program in the Universidad Nacional Autónoma de México. Her involvement in this phase of the project was focused on understanding the potential impact of climate change on the suitable habitat conditions and connectivity of Q. insignis.

Lic. Sofía Zorrilla Azcué: Master 's student at the Biological Sciences graduate program in the Universidad Nacional Autónoma de México. Her thesis project was focused on estimating potential vulnerability of Q. insignis populations to maladaptation under the effects of climate change.

Dra. Tarín Toledo: Researcher at the Instituto de Ecología A.C. (Mexico), specializing in cloud forest regeneration. We have collaborated with her by providing Q. insignis seeds to study the species' natural regeneration capacity in the Veracruz cloud forest.

Dra. Susana Valencia Ávalos: Worldwide authority in the Quercus species taxonomy and ecology working at Universidad Nacional Autónoma de México (México). Susana has advised us during the planning of the field stages and confirmed the collected Quercus species identity.

Dr. Jesús Llanderal Medoza: Laboratory technician at the Universidad Nacional Autónoma de México (México). He has advised us during the molecular markers amplification of the species.

PhD. Silvia Alvarez-Clare: Leader of Global Tree Conservation at the Morton Arboretum. She has been instrumental in promoting international collaboration and has provided invaluable advice throughout our project. Additionally, she accompanied us during our field expedition to Costa Rica and provided additional samples to enhance our research.

Victor Eduardo Henrriquez Aguilar: Technician at the Department of Ecology and Natural Resources at the Universidad Nacional Autónoma de Honduras. He provided valuable guidance and was a member of our field expedition team that collected samples in Honduras.

Ms. Manfredo Turcios Casco: Technician at the Instituto de Conservación y Vida Silvestre in Honduras. He provided us with invaluable advice on potential new locations for sampling. Additionally, he accompanied us during field sampling in Francisco Morazán, Honduras.

Rina Díaz: Collection manager in the Paul C. Stanley Herbarium in the Escuela Panamericana de Agricultura. She provided valuable information on specimens and their respective locations in Honduras.

Phd. Eric Van Den Berghe: Head of the Paul C. Standley Herbarium and Associate professor of Ecology in the Escuela Panamericana de Agricultura. He proved to be an invaluable asset during our sampling efforts, providing key recommendations for new locations and joining us in the field.

Maura Quezada: Curator of the Herbarium in the Universidad de San Carlos de Guatemala. She provided essential guidance and support for our field expedition to collect samples. She also played a critical role in organizing the trip and joined our team in the field.

Ing. Heiner Acevedo Mairena: Environmental Engineer, CEO at Agnathos Natura SRL (Costa Rica). He provided assistance in the ecological niche modelling and connectivity analysis for Costa Rican cloud forests. He also played a critical role in providing geographic and climatic information to evaluate our findings for Central American populations.

Kathia Lorena Arce Guzmán: Undergraduate student of Environmental Sciences and a collaborator in the lab. She has provided invaluable assistance with organizing and capturing data from all of the field collections performed in the lab.

Rodrigo Niniz Romero: Technician at the greenhouse collaborating with the Laboratorio de Biología Neotropical. He has helped to care and monitor growth of seedlings from all sampling locations in Mexico to be used in experiments in further phases of the project.

Raúl Álvarez Jara: Technician with the Laboratorio de Biología Neotropical during fieldtrips. He has helped to collect and monitor all sampling locations in Mexico to be used in experiments in further phases of the project.

10. Any other comments?

No additional comments. However, we would like to thank the support of the Rufford foundation.

We are pleasantly grateful for your patience and support during these years working together.

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